GIANT FIBER ACTIVATION OF AN INTRINSIC MUSCLE IN THE MESOTHORACIC LEG OF DROSOPHILA MELANOGASTER

JAMES R. TRIMARCHI and ANNE M. SCHNEIDERMAN
Section of Neurobiology and Behavior, Cornell University, Seeley G. Mudd Hall, Ithaca, NY 14853-2702, USA

Accepted 14 December 1992

Summary
Cinematographic analysis reveals that an important component of the light-elicited escape response of Drosophila melanogaster is the extension of the femur–tibia joint of the mesothoracic leg. During the jumping phase of the response, this extension works synergistically with extension of the femur. Femur extension is generated by contraction of the tergotrochanteral muscle (TTM), one of four previously described escape response muscles.

Femur–tibia joint extension in the mesothoracic leg has been thought to be controlled by contraction of the tibial levator (TLM), an intrinsic leg muscle. We investigated the activation of the TLM during the escape response. Electrical stimulation of the giant fiber interneuron that mediates the escape response results in activation of the TLM with a latency of 1.46±0.02ms. The TLM is innervated by a motor neuron (TLMn) with a large cell body in the mesothoracic ganglion. The TLMn has extensive arborizations in the lateral mesothoracic leg neuromere and has a prominent medially directed neurite. To investigate possible presynaptic inputs activating the TLMn during the escape response, we analyzed the muscle responses of two mutants, giant fiber A1 and bendless. Our analysis suggests that the TLMn is activated by a novel pathway.

Introduction
When startled, Drosophila melanogaster jumps into the air and flies away. This escape response can be elicited experimentally by an abrupt light-off stimulus or by applying electrical stimulation across the brain. Both stimuli activate the giant fibers (GFs), a pair of interneurons whose large axons descend from the brain into the thoracic central nervous system (CNS). Through their connections with other identified interneurons and motor neurons, the GFs sequentially activate several thoracic escape-response muscles (ERMs) that coordinate this stereotypical behavior (for a review, see Wyman et al. 1984).

The tergotrochanteral muscles (TTMs) in the mesothoracic (T2) segment have been implicated in providing the force to propel the fly from the substratum during the initial jumping phase of the behavior (Mulloney, 1969; Tanouye, 1977). These large, bilateral muscles extend from the lateral edge of the scutum to the trochanter of each T2 leg (Schouest et al. 1986) (Fig. 1A). When activated, they cause extension of the femur.

Key words: tibial levator, giant fiber, escape, horseradish peroxidase, bendless, giant fiber A1, Drosophila melanogaster.
In conjunction with T2 leg extension, the initial phases of the escape response involve abduction (swinging out) of the wings to their flight positions. Such lateral movements of the wing blades are controlled by the contraction of the anterior pleural muscles (Wisser and Nachtigall, 1984). When the GFs are experimentally stimulated, the bilateral anterior pleural number 3 (pa3) muscle exhibits evoked potentials. This suggests that their motor neurons, like the TTM motor neurons (TTMns), are activated by the GF pathway and that the pa3 muscles play a role during escape behavior (Tanouye and King, 1983).

Two other bilateral muscle groups, the dorsal longitudinal muscles (DLMs) and dorsal ventral muscles (DVMs), are involved in escape behavior and also show evoked potentials following GF activation (Tanouye and Wyman, 1980). The DLMs, a group of six muscle fibers (DLMa–f), cause wing depression upon contraction (Fig. 1B). Conversely, the seven DVM fibers (DVMIa–c; DVMIIa,b; DVMIIIa,b) cause wing elevation upon contraction (Fig. 1A). Alternating contractions of these antagonistic muscle groups deform the thoracic exoskeleton, thereby generating the wing movements necessary for initiation and maintenance of flight during escape behavior (Miyan and Ewing, 1985).

In summary, four groups of muscles, TTMns, pa3s, DLMs and DVMs, appear to be involved in the *Drosophila* escape response. These muscles, which we refer to collectively as escape-response muscles (ERMs), have three characteristics in common. First, because a single action potential in the GF results in activation of all ERMs in an invariant pattern (Thomas and Wyman, 1984), the escape response is a stereotypical behavior. Second, ERMs are activated with fixed latencies following GF activation. This presumably synchronizes the performance of the jump and flight. Third, as the voltage of brain stimulation increases, the muscle potential recorded in the TTM undergoes a distinctive shift from long to short latency (Levine and Tracy, 1973). The potentials evoked in the other ERMs undergo similar shifts, maintaining their temporal delay with respect to the response of the TTM.

Levine and Tracy (1973) suggested that brain stimulation below the threshold of the GF activates elements presynaptic to the GF. The long-latency response would thus result from the subsequent activation of the GF by these presynaptic elements. In contrast, stronger stimulation directly activates the GF, resulting in the short-latency response. Therefore, direct stimulation of the GF axon in the cervical connective bypasses any presynaptic elements that make connections with the GF and elicits only the short-latency response.

In the present study, we first established, using high-speed cinematography, that both the femur and the femur–tibia joint extend during light-elicited escape behavior. Femur extension is known to be generated by contraction of the TTM. Femur–tibia joint extension is controlled by contraction of the tibial levator (TLM), an intrinsic leg muscle (Miller, 1965). We then investigated the activation of the TLM during the escape response and identified the motor neuron (TLMn) that innervates the TLM. To explore possible presynaptic inputs activating the TLMn during the escape response, we analyzed the ERM responses of two mutants with disrupted connections between specific elements in the GF pathway, *giant fiber A1* and *bendless*. Our analysis suggests that the TLMn is activated by a novel CNS pathway, and we present two models of...
neuronal connectivity that could account for the timing of TLM activation during the escape response.

Materials and methods

Drosophila stocks

\textit{giant fiber A1 (gfA1)} and \textit{bendless (ben)} mutant stocks were obtained from the laboratory of Robert J. Wyman (Yale University). Canton-S flies were used as wild-type controls. Flies used in experiments were adult females between 5 and 10 days old.

Cinematography

Flies were filmed while executing the escape response using a Photec IV film camera (Photographic Analysis Co.) running at 2000 frames s$^{-1}$. The apparatus was illuminated by synchronized pulses of light (10–40ns duration) from a copper-based laser (Oxford Lasers). A shadow stimulus was created by interrupting the laser beam with a hand-held piece of cardboard. Because we were limited to 8.3s of recording time per roll of film, we used \textit{bw;st} (white-eyed) flies as test subjects. \textit{bw;st} flies exhibit escape behavior in response to a shadow stimulus more consistently than do normal, red-eyed Canton-S flies (Thomas and Wyman, 1984).

Muscle terminology

The indirect flight muscles of the mesothorax are composed of six dorsal longitudinal muscle fibers that depress the wings (DLMa–f; Fig. 1B) and seven dorsoventral muscle fibers that elevate the wings (DVMIa–c, DVMIIa–b, DVMIIIa–b; Fig. 1A). In addition to the muscles that power the wing movements during flight, the tergotrochanteral muscle (TTM) is also illustrated in Fig. 1A.

Although the anatomy of the intrinsic muscles of the prothoracic leg has been described previously (Miller, 1965), the anatomy of the muscles of the meso- and metathoracic legs has not been characterized. We have observed four distinct muscle groups within the mesothoracic femur (Fig. 1C,D): the tibial depressor radius (TDR), the tibial depressor longus (TDL), the retractor unguis (RU) and the tibial levator (TLM) (Miller, 1965; Radnikow and Bässler, 1991).

The TLM, which we have examined in detail in this report, originates on the proximal dorsal surface of the femur (Fig. 1C,D) and inserts in the distal portion of the femur on an anvil-shaped structure. This ‘anvil’ is connected by an apodeme to the dorsal sclerotized rim of the tibia.

Backfilling motor neurons with horseradish peroxidase

To backfill the TTMn and the TLMn simultaneously, we employed a two-stage procedure (modified after Baird, 1988). First, a hole was made in the cuticle directly over the dorsal insertion site of the TTM with a tungsten wire that had been electroetched to a fine point. Horseradish peroxidase (HRP type VI, Sigma Chemical Co.) was inserted through the hole into the underlying TTM using a second fine-tipped tungsten wire coated with crystals of HRP. The fly was placed in a humid chamber and incubated for 3.5h.
Fig. 1
Next, HRP was introduced into the TLM in a similar manner. The fly was then refrigerated in a humid chamber for 19–22h. After incubation, the fly was partially dissected and fixed for 20min in 2% glutaraldehyde in 0.2mol l⁻¹ Tris/0.2mol l⁻¹ sucrose buffer. Tissue was rinsed and refrigerated overnight in 0.2mol l⁻¹ Tris buffer. Tissue was rinsed four times for 15min in 1% NaBH₄, incubated for 30min in a solution of 1mg/ml 3,3',9-diaminobenzidine tetrahydrochloride (DAB, Polysciences Inc.) in 0.2mol l⁻¹ Tris buffer, and hydrogen peroxide was added to this solution (80μl ml⁻¹). After 15min, the solution was replaced with fresh DAB–hydrogen peroxide–Tris solution. The reaction was terminated after 15min by rinsing the tissue in 0.2mol l⁻¹ Tris buffer (Mesulam, 1982; Nässel, 1983). The CNS was dissected out, dehydrated in an ascending series of ethanols, cleared in methyl salicylate, and mounted in DePeX (Bio/Medical Specialties).

**Physiology**

Stimulating, recording and ground electrodes were fashioned from electroetched tungsten wire. Flies were held in position by applying suction to the thorax. Flies were lightly anaesthetized with ether while we positioned them on the recording apparatus and were allowed to recover for 15min before recording began.

Activation of the GF was accomplished by brain stimulation; current was passed between electrodes inserted in the eyes. Although one would expect such a general stimulus to activate other descending neurons in addition to the GF, it has been demonstrated previously that this technique preferentially activates the GF before other descending neurons (Levine and Tracy, 1973; Mulloney, 1969). Moreover, the resulting pattern of muscle activation is identical to that obtained when the GF is stimulated intracellularly (Thomas and Wyman, 1984; see Results).

Stimulation was performed by an IBM-386-compatible personal computer equipped with an Indec Acquisision Interface-IDA and Fastlab software (INDEC Corporation). Muscle potentials were amplified with an a.c. amplifier (AM Systems) and recorded on the computer using the Indec interface system.

Recording electrodes were placed in the dorsal insertion sites of DLMa, DVMIc and TTM (Levine and Hughes, 1973; Tanouye and Wyman, 1980). To facilitate stable recordings from the intrinsic muscles of the T2 leg, the tibia was waxed to a tungsten wire. Reliable recordings were obtained from the TLM by inserting an electrode dorsally.
along the long axis of the femur in the proximal–distal direction. Using the Fastlab software, we measured response latencies from the onset of the stimulus artifact to the onset of the evoked voltage response in the muscle (Tanouye and Wyman, 1980).

Two stimulation protocols were used to generate our data. The same 15 Canton-S, 6 gfA1 and 5 ben individuals were tested with these two protocols. (1) The ‘stepping protocol’ was employed to determine the effect of voltage on evoked muscle response latency. This protocol delivers a 96 μs pulse at a frequency of 0.1Hz, and increases the voltage by 1V after each stimulus. Results are represented as a voltage/latency plot, in which the change in response latency is plotted as a function of increasing voltage. (2) Following the stepping protocol, the ‘repetition protocol’ was used to determine the mean response latency for the different muscle groups in each genotype. The stimulus voltage was set to 18.40V and a series of five pulses was delivered at 0.1Hz. This stimulation voltage ($S=18.40V$) elicited the short-latency response from all individuals. The voltage was then decreased to 6.09V and a series of five pulses was delivered at 0.1Hz. This stimulation voltage ($S=6.09V$) elicited the long-latency responses from all wild-type individuals.

All values are reported in ms ±95% confidence intervals (95% CI). The average response from each fly was treated as an individual data point because variation between trials for each fly was insignificant, and statistically different values are those for which their associated 95% CIs do not overlap.

Results

Evoked TLM response in wild-type flies

Cinematographic analysis of the visually elicited escape response in Drosophila shows that, in addition to the previously suspected extension of the femur, the femur–tibia joint also extends during the jumping phase of the behavior (Fig. 2). Direct stimulation of the TLM (10.4V, 1Hz) causes extension of the femur–tibia joint (data not shown). We therefore sought to identify the neuromuscular elements causing this extension.

We measured evoked potentials in the TTM, DLMa, DVMIc and TLM of wild-type flies following brain stimulation at 18.40V (Fig. 3A). These short-latency responses had characteristic latencies of 0.84±0.01ms for TTM, 1.33±0.01ms for DLMa and 3.36±0.04ms for DVMIc ($N=15$, five trials each). These latencies are similar to the values obtained by Tanouye and Wyman (1980) from wild-type Hochi-R flies (TTM: 0.83±0.02ms; DLMa: 1.29±0.19ms; DVMIc: 3.31±0.8ms). We measured short-latency responses of 1.46±0.02ms in the TLM ($N=15$, five trials each).

We also measured the long-latency responses (Fig. 3B) previously described by Levine and Tracy (1973). Following brain stimulation at 6.09V, we obtained long-
Activation of leg muscle in Drosophila

Fig. 2
latency responses of 3.01±0.06ms for TTM, 3.47±0.09ms for DLMa and 5.32±0.11ms for DVMIc (N=15, five trials each). These latencies also are similar to the values obtained by Tanouye and Wyman (1980) from wild-type Hochi-R flies (TTM: 3.08±0.13ms; DLMa: 3.48±0.15ms). We measured long-latency responses of 3.56±0.08ms in the TLM (N=15, five trials each).

To investigate the transition from long- to short-latency responses further, we employed the stepping protocol (see Materials and methods). By changing the stimulus voltage by 1V per pulse during a train of stimuli (0.1Hz), a profile of muscle response latencies as a function of voltage was obtained (Fig. 4). As the voltage increased, the potentials evoked in the ERMs underwent a distinct shift from long to short latency. This shift typically occurred over a 1-V increment in stimulus voltage. The mean voltage at which the shift occurred was 12.4±2.5V (N=5, five trials each). Potentials recorded in the

Fig. 3. Evoked TLM response after giant fiber activation. Recordings of typical short- (A) and long-latency (B) responses evoked by brain stimulation (96μs duration) at 18.40V (A) and 6.09V (B). The TLM shows a response with a latency similar to that of the DLM. Arrows indicate the onset of the stimulus.
TLM simultaneously underwent this shift, thus maintaining proper timing with the other ERMs. The mean difference between the TTM and TLM response latencies did not differ significantly between short and long responses (0.62±0.02ms and 0.63±0.03ms respectively, N=15, five trials each).

In addition, the minimum voltage required to activate the GF was the same, 4.51±0.13V, for all ERMs including the TLM (N=5, five trials each). Threshold stimuli that activated the GF and evoked long-latency responses in the TTM, DLMa and DVMic always evoked a long-latency response in the TLM.

In summary, the TLM (1) has characteristic values for its long- and short-latency responses; (2) undergoes the shift from long to short latency; and (3) has the same threshold voltage as the other ERMs. Taken together, these data indicate that the TLM is activated by the GF pathway and is involved in the escape behavior of Drosophila.

**Anatomy of the TLMn**

HRP backfills of the TLM (Fig. 5A) reveal that it is innervated by a large motor neuron with distinctive morphology. This motor neuron, which we have named the TLMn, has a soma approximately 20 μm in diameter that lies laterally in the cortical layer of the T2 ganglion. The position of the soma is anterior to the anterior border of the leg neuromere and ventral to the ventral border of the wing neuromere (Fig. 5B,C,D). Typically, the TLMn soma is located immediately anterior or ventral to the soma of the TTMn (Fig. 6A,C,D).

The TLMn has extensive arborizations in the lateral leg neuromere. The primary neurite connecting the TLMn soma to its axon has a characteristic arch (Fig. 5B,D).

---

Fig. 4. Effect of voltage on response latencies. Increasing the magnitude of the stimulus voltage decreases the latency of the evoked muscle potentials. The temporal synchrony between the potentials recorded in the TLM and those recorded in the other ERMs is maintained during both the short- and long-latency responses, as well as during the shift from long to short latency. These results are from a single trial.
Before the axon leaves the T2 ganglion, it extends several neurites ventrolaterally (indicated by VB in Fig. 5C,D). At this site, the branches of the prominent posteriorly directed neurite of the TTMn (Fig. 6B) and neurites of the TLMn overlap (Fig. 6D).

Fig. 5. Tibial levator motor neuron (TLMn). (A) Injected HRP (arrow) is confined to the TLM. (B) Sagittal view of the right T2 ganglion in a cleared whole mount, showing the HRP-backfilled TLMn. The TLMn branches in the T2 leg neuromere. Its medially directed neurite is out of the plane of focus. In B and D (below), anterior is towards the right and dorsal is towards the top. (C) Drawing of the T1 and T2 ganglion, showing the left TLMn (dorsal view). Anterior is towards the top. (D) Drawing of the T2 ganglion, showing the right TLMn (sagittal view). Medially directed neurite (MB); Ventrolaterally directed neurite (VB). Scale bars, 30 μm.
Activation of leg muscle in Drosophila

Fig. 6. TLMn and TTMn have coextensive neurites. Photomicrograph (A) and drawing (C) of the TLMn and TTMn (dorsal view), showing the medial region of neuritic overlap in the T2 ganglion. The overlap drawn here is also seen when the whole mount is viewed sagittally. Arrowheads point to the soma and medially directed neurite of the TLMn. Arrows point to the soma and medially directed neurite of the TTMn. In A and C, anterior is towards the top. (B) Photomicrograph of the TTMn (sagittal view) showing the branching of the posteriorly directed neurite in the lateral T2 leg neuromere. (D) Drawing of the TLMn and TTMn (sagittal view), showing the region of neuritic overlap at the point where the TLMn axon exits the T2 leg neuromere. The overlap drawn here is also seen when the whole mount is viewed dorsally. Arrowheads point to the soma and posteriorly directed neurite of the TLMn. Arrows point to the soma and posteriorly directed neurite of the TTMn. In B and D, anterior is towards the right and dorsal is towards the top. Scale bars, 30 μm.
Extending from the arching primary neurite of the TLMn is one medially directed neurite (Fig. 5C,D, 6C). This neurite approaches, but does not cross, the midline of the T2 ganglion. It also extends as far anterior as the contact point between the GF and the TTMn. At this point, the TTMn frequently extends several small branches posteriorly (Baird, 1988). These TTMn branches intermingle with the neurites emanating from the medially directed neurite of the TLMn (Fig. 6A,C).

Although in 60% (74/124) of attempted backfills the TLMn was clearly identifiable, in 40% (50/124) the quality of the fill did not allow us to identify the TLMn. In 41% (30/74) of the preparations in which the TLMn was successfully filled, the TLMn was the only neuron that stained. In the remaining 59% (44/74), additional neurons were filled concurrently with the TLMn. These additional neurons had relatively small somata (3–8 μm diameter) that were positioned ventrolaterally in the cortex of the T2 ganglion. These cells had arborizations that were confined, for the most part, to the ventrolateral portions of the T2 leg neuromere.

Evoked TLM responses in gfA1 and ben flies

Following brain stimulation (S=18.40V), we measured evoked potentials in the TTM, DLMa, DVMic and TLM of gfA1 flies (Fig. 7A). These short-latency responses had characteristic latencies of 0.76±0.01ms for TTM, 3.37±0.14ms for DLMa and 3.85±0.07ms for DVMic (N=6, five trials each). The latency of the DLMa response in gfA1 (3.37±0.14ms) is much longer, however, than that obtained for Canton-S (1.33±0.01ms). This abnormal latency has previously been shown to result from altered synaptic connectivity between the peripherally synapsing interneuron (PSI, see Discussion) and the DLMns (Thomas and Wyman, 1984). Although we observed abnormal DLMa responses, we found that gfA1 flies have a normal TLM response latency: 1.34±0.3ms versus 1.46±0.02ms in Canton-S (Fig. 8).

Following brain stimulation (S=18.40V), we measured evoked potentials in the TTM, DLMa, DVMic and TLM of ben flies (Fig. 7B). These short-latency responses had characteristic latencies of 2.47±0.16ms for TTM, 1.43±0.06ms for DLMa and 3.77±0.13ms for DVMic (N=5, five trials each). The latency of the TTM response in ben (2.47±0.16ms) is considerably longer than that obtained in Canton-S (0.84±0.01ms). This abnormal latency has previously been shown to result from altered synaptic connectivity between the GF and the TTMns (Thomas and Wyman, 1984). In addition to the aberrant TTM latency, we observed that ben flies have an abnormally long TLM response latency: 3.23±0.22ms versus 1.46±0.02ms in Canton-S (Fig. 8).

Responses of TTM and TLM maintain their distinctive timing with respect to one another

Despite the defective latencies of specific ERM responses in the mutants, the interval between the TTM and TLM responses did not differ across genotypes: 0.61±0.02ms for Canton-S, 0.57±0.04ms for gfA1 and 0.70±0.07ms for ben (Fig. 8). In particular, the interval between the TTM and the TLM response in ben flies appears normal despite the abnormal latencies of both the TTM and TLM responses.
**Activation of leg muscle in Drosophila**

*Fig. 7. Short-latency responses in gfA1 and ben mutants. (A) A typical short-latency response from the ERMs in gfA1, evoked by brain stimulation (96 μs duration) at 18.40V (arrow). Although the potential in DLMa (DLM) is abnormally delayed, the TLM latency does not differ significantly from that of the wild type (compare with Fig. 3A). (B) A typical short-latency response from the ERMs in ben, evoked by 96 μs of brain stimulation (arrow) at 18.40V. The potentials in the TTM and TLM are abnormally delayed with respect to those of the wild type (compare with Fig. 3A). Although the activation of the ben TTM and TLM is aberrant, the interval between the TTM and TLM response is not altered.*

**Discussion**

*Activation of the TLM and extension of the femur–tibia joint is driven by the giant fiber system*

Our cinematographic analysis reveals that, during the jumping phase of escape behavior, *Drosophila* extends the femur–tibia joint of the T2 leg. An analysis of the musculature intrinsic to the T2 leg suggests that the tibial levator (TLM) causes this extension (Fig. 1C,D and Miller, 1965). In other arthropods, a muscle with an identical origin and insertion has been described as the extensor of the femur–tibia joint [cockroach (*Periplaneta orientalis*): extensor tibialis (Miall and Denny, 1886); locust: muscle 106 (Albrecht, 1953); moth (*Manduca sexta*): f2 (Kent and Levine, 1988); stick insect: ETi (Bässler, 1983)]. Direct stimulation of the TLM in *Drosophila*, moreover, causes extension of the femur–tibia joint.

Recordings made from the TLM during GF activation indicate that the TLM possesses the three characteristic properties of an ERM. First, evoked potentials recorded in the TLM after GF activation have an invariant latency (1.46±0.02ms). Second, the minimum
voltage required to elicit a response by the TLM is the same as that required to evoke potentials in other ERMs (4.51±0.13V). Third, as stronger stimuli are applied to the brain, potentials recorded from the TLM, as well as other ERMs, undergo distinctive shifts from long to short latency (mean shift voltage 12.40±2.50V). From these observations, we conclude that the TLM is driven by the GF and is, therefore, part of the escape response system.

Because the TLM functions to extend the femur–tibia joint, it is likely that it works synergistically with the TTM to generate the leg movements necessary for the escape jump. The TTM has long been designated the fly’s ‘jump muscle’ (Mulloney, 1969; Tanouye, 1977; Schouest et al. 1986). In view of our present work, we propose that the TLM, in addition to the TTM, should be considered a ‘jump muscle’.

The TLM is innervated by a uniquely identifiable motor neuron, the TLMn

The large TLMn cell body lies laterally in the cortical layer of the T2 ganglion, in a position that is anterior to the anterior border of the leg neuromere and ventral to the ventral border of the wing neuromere. The TLMn has extensive arborizations in the lateral leg neuromere. The primary neurite connecting the TLMn soma to the axon in the T2 leg nerve has a characteristic arch. Extending from the arching primary neurite of the TLMn is one medially directed neurite. This medially directed neurite approaches, but does not cross, the midline of the T2 ganglion. It also extends as far anterior as the contact point between the GF and the TTMn.

In 59% of preparations in which the TLMn was filled with HRP, a few neurons with small somata were also stained. Although we tried to contain the HRP to the TLM exclusively, it may have leaked into other muscles and thus filled other neurons. Alternatively, the TLM may be multiply innervated, as is the case with other arthropod leg muscles (for a review, see Fourtner and Pearson, 1977). Nonetheless, we have
 Activation of leg muscle in Drosophila

consistently backfilled one neuron, which we denote the TLMn. Moreover, the TLMn has a morphology that is relevant to its role during the escape response (see below).

Fig. 9. Two proposed models of neuronal connectivity that predict the response latencies observed in the ERM. The similarity in TLM and DLMa response latencies suggests that only one synapse interposes between the GF and the TLMn. (A) An unknown interneuron (I) serves as the intermediary between the GF and the TLMn. (B) The TTMn serves as the intermediary between the GF and the TLMn. Although these elements are arranged bilaterally in the fly, the positions of muscles and neurons in the diagram are schematic and do not represent their actual locations. Elec, electrical synapse; ACh, cholinergic synapse; Glu, glutaminergic synapse.
Which presynaptic elements control the activation of the TLMn during execution of escape jump behavior?

The strict temporal pattern of TTM, DLM and TLM activation undoubtedly arises from stereotyped patterns of synaptic connectivity between the GF and the motor neurons innervating these muscles. Based on anatomical (King and Wyman, 1980; Koto et al. 1981) and physiological (Tanouye and Wyman, 1980) evidence, only one interneuron, the peripherally synapsing interneuron (PSI), is interposed between the GF and the five DLM motor neurons (DLMns). Whereas the GF makes an electrical synapse with the PSI, the PSI, in turn, makes cholinergic chemical synapses with the DLMns (Gorczyca and Hall, 1984). In contrast, the GF is electrically coupled to the TTMn. The differences in latencies between the DLM and TTM responses are believed to be a direct consequence of these differences in synaptic connectivity (Tanouye and Wyman, 1980). The similarity of the TLM and DLMa response latencies (1.46±0.02ms and 1.33±0.01ms, respectively) indicates that a single neuron may also be interposed between the GF and the TLMn.

One explanation accounting for the similarity between the DLM and TLM response latencies is that the DLMns and TLMn may be co-activated by the PSI. To test this hypothesis, we recorded evoked potentials from gfA1 flies. In gfA1 mutants, the DLMa short-latency response is abnormally long and variable (3.37±0.14ms versus 1.33±0.01ms in Canton-S) whereas the TTM short-latency response is normal (0.76±0.01ms versus 0.84±0.01ms in Canton-S). The delayed DLM response in these flies is not a result of a defect either in the motor neuron or in neuromuscular transmission, because direct stimulation of the motor neuron brings about a muscle response with normal latency (Thomas and Wyman, 1984). This suggests that the focus of the mutant defect is either in the GF output to the PSI or in the PSI itself. We reasoned that if both the TLMn and DLMns were being co-activated by the PSI, then gfA1 flies should exhibit an aberrant TLM short-latency response in addition to the previously described abnormal DLM response. We found, however, that gfA1 flies have a TLM response latency that resembles that of the wild type (1.34±0.3ms versus 1.46±0.02ms in Canton-S).

A possible interpretation of these observations is that the TLMn is driven by a branch or portion of the PSI that is unaffected by the gfA1 mutation. This branch, therefore, would be functionally wild-type whereas the branch that drives the DLMns would be mutant. A more likely explanation is that a novel synaptic pathway is interposed between the GF and the TLMn (Fig. 9A, B).

In the model presented in Fig. 9A, the GF makes a synapse with an unknown interneuron (I). Interneuron I synapses, in turn, with the TLMn. Upon GF activation, PSI activates the DLMns and interneuron I activates the TLMn. According to this model circuit, the DLMs and the TLM of wild-type flies would show evoked potentials with similar latencies.

Another model accounting for the similarity in TLM and DLM latencies is depicted in Fig. 9B. In this model, the TTMn serves as the interposing neuron activating the TLMn. To test whether the TTMn serves as the interposing neuron between the GF and the TLMn during execution of escape behavior, we recorded TLM responses from mutant ben flies.
In *ben* mutants, the TTM short-latency response is abnormally long (2.47±0.16 ms *versus* 0.84±0.01 ms in Canton-S) while the DLMa latency is unaltered (1.43±0.06 *versus* 1.33±0.01 in Canton-S). In homozygous *ben* flies, tests of the physiology of the TTMn and of neuromuscular transmission have revealed that neither has a mutant phenotype. Thus, a mutant electrical connection between the GF and the TTMn has been proposed as the cause of the abnormally delayed TTM response (Thomas and Wyman, 1984). We hypothesized that if the TLMn were being activated by the TTMn, then we should observe an aberrant TLM short-latency response in addition to the abnormal TTM response previously described in *ben* flies. We did indeed find that *ben* flies have an abnormal TLM response latency (3.23±0.22 ms *versus* 1.46±0.02 ms in Canton-S).

The analysis presented here does not rule out the possibility that *ben* alters the GF-to-interneuron I connection in addition to the GF-to-TTMn connection. Such a scenario would result in both the TTM and TLMn exhibiting altered latencies. However, despite the defective latencies of specific ERM responses in the mutants, the interval between the TTM and TLM responses did not significantly differ across genotypes (0.61±0.02 ms for Canton-S, 0.57±0.04 ms for *gfA1* and 0.70±0.07 ms for *ben*). In particular, the interval between the TTM and the TLM response in *ben* flies is wild-type in spite of the abnormal latencies of both the TTM and TLM responses. One interpretation of this result is that, in *ben* flies, the connection between the GF and the TTMn is abnormal while the synapse between the TTMn and the TLMn is unaltered (Fig. 9B). Such a connectivity pattern would result in the both TTMn and the TLMn exhibiting altered latencies from GF activation, yet the interval between the TTMn and TLMn evoked potentials would be wild-type. This is exactly the result we obtained.

Several observations corroborate these findings and lead us to propose the model depicted in Fig. 9B. Neurites of the TTMn and the TLMn exhibit overlap in two regions: near the midline and near the region where the TLMn axon exits the mesothoracic leg neuromere. These two regions are putative sites of TTMn-to-TLMn synaptic contact at which the TTMn could drive the TLMn during execution of the escape response. This overlap could be merely coincidental; the TLMn and TTMn might receive inputs from shared presynaptic elements. Although such juxtaposition of neurites, at the whole-mount level, does not confirm synaptic contact, the extent of intermingling of the TTMn and TLMn neurites is certainly suggestive of synaptic connectivity. We are conducting an electron microscopic analysis of the putative synaptic connection between the TLMn and the TTMn.

In addition to anatomical evidence, there is physiological evidence from previous work that indicates that the timing of activation of a motor neuron can be directly regulated by other motor neurons [e.g. crustacean stomatogastric ganglion (Maynard, 1972); crayfish abdominal and claw motor neurons (Tatton and Sokolove, 1975; Weins and Gerstein, 1975); locust flight motor neurons (Kendig, 1968); cricket flight motor neurons (Bentley, 1969); *Drosophila* flight motor neurons (Koenig and Ikeda, 1983)]. Hoyle and Burrows (1973), moreover, have found that a monosynaptic excitatory connection exists between the motor neurons innervating the extensor and flexor muscles of the metathoracic tibia in the locust. Thus, the model depicted in Fig. 9B is consistent with these studies investigating the timing of muscle activation.
In view of the findings of ourselves and others, we suspect that the TLMn may not be activated by an ‘unknown interneuron I.’ A reasonable alternative is that the TTMn activates the TLMn. During the escape response, the GF activates the TTMn, the TTM contracts and the femur extends. The proposed synaptic connection between the TTMn and the TLMn would then lead to activation of the TLMn, contraction of the TLM and extension of the femur–tibia joint. This connection between the TTMn and TLMn would maintain the critical timing of activation of the TTM and TLM such that the two muscles would work synergistically. This synergistic action would serve to propel the fly rapidly from the substratum.

Because our findings from this present study do not yet enable us to differentiate between the circuit models we propose, we plan to continue our analysis of the activation of the TLMn during the execution of escape behavior.

We thank Andy Jantzen of the Photographic Analysis Company and Rick Slagle and Daniel Hogan of Oxford Lasers for generously allowing us to use their equipment and for their help during the high-speed filming of the escape response. We thank Photographic Analysis for a grant of photographic supplies and processing. We thank Robert Edgecomb, Ronald Hoy and Carol Miles for critical reading of the manuscript and Robert Wyman for providing ben and gfA1 stocks. We also thank Robert Edgecomb for his many excellent suggestions for the design and interpretation of experiments. Our work was supported by a USPHS predoctoral traineeship (NIH T32 GM07469) (J.R.T.) and by grants from the NSF (BNS 90–09833) and the Hatch Act (Project 191412) (A.M.S.).

References


Activation of leg muscle in Drosophila


