Startle Phase of Escape Swimming Is Controlled by Pedal Motoneurons in the Pteropod Mollusk *Clione limacina*

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**INTRODUCTION**

Many animals exhibit rapid escape movements in response to potentially threatening stimuli. In many of these cases the initial phase of escape includes a characteristic and somewhat stereotyped startle response. Bullock (1984) defines startle as, “...an abrupt response, often of relatively short latency, to a sudden stimulus that we believe to be both unexpected and alarming...” It is not surprising to find that many startle responses involve large-diameter (‘‘giant’’) neurons and relatively direct connections to the appropriate musculature (Bullock 1984). Perhaps the best example of a giant neuron-mediated startle response is the Mauthner-neuron-initiated C start of teleosts and some amphibians (see for example Eaton and Hackett 1984; Faber and Korn 1978; Zottoli et al. 1995). A pair of oversized reticulospinal neurons descends through the contralateral side of the spinal cord and directly activates myotomal motoneurons. Appropriate activating stimuli include visual, acoustic, or vibrational inputs (Eaton and Hackett 1984).

Several important characteristics of a startle response can be intuitively proposed, including a short latency for muscle activation (see Bullock 1984), a high threshold for activation, a fairly predictable behavioral outcome, although certainly subject to modulation, and some form of habituation if stimuli are repeatedly presented (Eaton and Hackett 1984). Many animals use giant neurons to provide short-latency muscle activation during initiation of a startle response (see Bullock 1984). In addition to Mauthner cells, giant neurons mediating escape-type responses have been documented in a wide variety of invertebrates, for example, in cnidarians (Mackie 1984), annelids (Drewes 1984), insects (Ritzmann 1984), and crustaceans (Krasne and Wine 1984). With the exception of cephalopods (see Boyle 1986), mollusks with ballistic escape responses are rare. Here we report on a ballistic startle response in a gastropod mollusk (*Clione limacina*) that displays the characteristics of startle responses mentioned above. The motor output of the startle is controlled by a group of pedal motoneurons that function like Mauthner neurons.

*Clione* exhibits three forms of swimming including slow swimming, fast swimming, and escape swimming (Satterlie 1991; Satterlie et al. 1990). The pattern generator and motoneuron circuitry for slow and fast swimming have been described (Arshavsky et al. 1985a–d, 1989; Satterlie 1985, 1989; Satterlie and Spencer 1985; Satterlie et al. 1985). The pattern generator is a two-phase network oscillator that produces alternate activation of d-phase motoneurons (which produce a dorsal bend of the wings) and v-phase motoneurons (which produce a ventral bend of the wings). The motoneurons monosynaptically activate two types of striated wing muscle fibers, including slow-twitch and fast-twitch cells (Satterlie 1991; Satterlie et al. 1990). During the change from slow to fast swimming, the pattern generator is reconfigured (Arshavsky et al. 1985d, 1989), and a set
of large motoneurons (general excitors) is recruited into activity that, in turn, recruits the fast-twitch musculature (Satterlie 1993).

*Clione* exhibits two forms of avoidance behavior to potentially harmful stimuli. If the stimuli originate in the head region, or if the stimuli are widespread, the typical reaction is a whole body retraction. This passive avoidance response, in conjunction with the negative buoyancy of the animal, results in slow sinking away from the stimulus. If noxious stimuli are delivered to the tail of *Clione*, the result is an active avoidance response that involves a dramatic acceleration of swimming and a ballistic movement away from the stimulus (an escape response).

Escape swimming in *Clione* includes an initial set of strong wing contractions that clearly fit the definition by Bullock (1984) of a startle response. Here we provide an initial description of two pairs of large pedal motoneurons that activate wing contractions that can be several times stronger than those initiated during either slow or fast swimming, suggesting that these cells may be responsible for the startle response. These cells directly activate the same musculature used during normal swimming, and are activated by stimuli that initiate startle responses in intact animals. The following paper (Norekian and Satterlie 1997) provides information on some of the central circuitry involved in activation of the “startle motoneurons.”

**Methods**

Animals were collected from the breakwater at Friday Harbor Laboratories, Friday Harbor, WA, and held in jars of seawater in a refrigerator or flowing-water sea table. Animals were anesthetized in a 1:1 mixture of seawater and 0.33M MgCl₂ and pinned and dissected in a Sylgard (Dow Corning)-coated petri dish with the use of cactus spines (*Opuntia* sp.). Reduced preparations included the central ring of ganglia connected to the wings by the wing nerves. Semi-intact preparations were produced by making a small dorsal incision in the body wall and removing the digestive and reproductive structures, but leaving all body wall nerves intact. Ganglionic sheaths were partially removed with a 1-mg/ml solution of protease (Sigma type XIV) followed by several washes in fresh seawater.

Intracellular recordings were accomplished with 2 M-potassium-acetate-filled electrodes with resistances between 10 and 35 MΩ. Stimulating currents were injected through the electrode via amplifier bridge circuits. For morphological data, neurons were injected with 5% 5(6)-carboxyfluorescein (Sigma) with the use of pressure (Picospritzer) or iontophoresis. Preparations were immersed in the anesthetizing solution and viewed and photographed live in the recording dish with the use of a Nikon indirect-light fluorescence microscope with a fluorescein isothiocyanate filter package.

Wing movements were detected with the use of a Cambridge Technology 400A force transducer system. A sealed glass microelectrode was waxed to the transducer arm and positioned against the wing tissue with the use of a micromanipulator. Because the microelectrode was not firmly attached to the wing tissue, and the angle of attachment was not in line with the generation of muscle force, the transducer system was used merely as an uncalibrated movement detector that registered relative contractions within one placement.

Stimuli were delivered with a fine polymeric filament attached to a rod that was either hand held or mounted in a micromanipulator. In the latter case, consistent repetitive stimuli were delivered by advancing and then retracting the fine movement of the manipulator over the same distance with each stimulus.

**Results**

**Behavioral reactions**

Escape swimming, which is typically induced by mechanical stimulation of the tail, is comprised of two parts including an initial startle response that is followed by a variable period of fast swimming. During the startle, in the course of one or two wing beat cycles (total duration < 1 s), the animal is propelled forward at an extrapolated rate of nearly 18 body lengths per second. For comparison, during the fast swimming phase, forward movement is up to six body lengths per second. Figure 1 shows a comparison of startle and fast swimming based on video tracings of five body positions, each representing a 60-ms interval. In this example, forward propulsion averages nearly a full body length in each 60-ms interval for startle swimming, whereas during fast swimming just under one body length is traversed in the entire 240-ms interval. Both forms of swimming are represented in escape swimming: the “startle” response is followed by a period of prolonged fast swimming in which the rate of forward movement is three to six body lengths per second, a value identical to that observed in fast swimming episodes that are not associated with the escape swimming response. The startle response shows an apparent behavioral habituation, because when triggered, a second startle reaction (escape response) is difficult to elicit for up to tens of minutes. In reduced preparations, the trauma of dissection and pinning is frequently sufficient to prohibit the successful stimulation of startle responses, again for tens of minutes—particularly if the animal is pinned through the tip of the tail.

The startle response consists of one or two very strong...
contraction cycles of the wings, with muscle force being greater than that seen during either slow or fast swimming (Fig. 2). In 14 trials of tail stimulation in intact animals, with stimulation delivered at various times in the wing beat cycle, the initial wing movement was in the ventral direction in 13 animals. In the 14th, the stimulus was delivered at the maximal ventral bend of the wing, and the wing response was a short dorsal bend followed by a strong ventral contraction.

**Neurons controlling the startle response**

In the course of our investigations of swimming activity in *Clione*, the pedal ganglia were systematically surveyed for neurons that were either directly related to swimming activity (rhythmic activity in phase with wing movements) or indirectly involved in swimming (activity that either roughly followed swimming without being phase locked, or that could alter swimming activity). Two large pedal neurons (cell body diameter up to 50 μm) initially were ignored because 1) they were electrically silent and showed no resting potential changes that could be correlated with either slow or fast swimming and 2) they were difficult to stimulate to spike even with relatively large injected currents. The position of the cell bodies, near the pedal commissure (Fig. 3A), was in the immediate vicinity of a cluster of serotonin-immunoreactive neurons that have been thoroughly investigated (Satterlie 1995). In the course of this investigation, the cells were found to exhibit a high spike threshold, and injected currents triggered intense bursts of spikes (spike frequency up to 190 Hz) that, in turn, produced extremely strong contractions of the ipsilateral wing. One cell produced a contraction of the dorsal wing musculature (d-phase), whereas the other produced contraction of the ventral musculature (v-phase). Qualitative visual observations indicated that the resulting contractions were much stronger than those produced by direct stimulation of any identified swim motoneuron, and were stronger than contractions observed in the same preparations during either slow or fast swimming (Fig. 2). Furthermore, stimulation of this neuron activity in quiescent preparations did not initiate swimming activity. These data led to the suggestion that the cells may be involved in the initial startle response of escape swimming, and to the naming of the cells as startle neurons.

**Startle neurons are motoneurons**

Four possible scenarios could explain the relationship between spike activity in startle neurons and strong wing contractions. First, startle neurons could be motoneurons that directly activate wing musculature. Second, the startle neurons could function by providing excitatory drive to swim motoneurons that are normally active during slow and/or fast swimming. Third, the startle neurons could activate a unique set of motoneurons that in turn activate the swim musculature. Fourth, a combination of the three former possibilities could operate. Behavioral observations, dye injections, and electrophysiological data suggest that startle neurons are motoneurons.

Dye injections indicated that each startle neuron sent a single, broad axon to the ipsilateral wing nerve and branched extensively throughout the entire ipsilateral wing (Fig. 3). When a pair of startle neurons was filled, including one d-phase and one v-phase neuron, the two had similar morphologies, but one produced branches that were in the same focal plane as the dorsal musculature and the other with the ventral musculature. Stimulation of startle neurons in the presence of high-Mg\(^{2+}\), high-Ca\(^{2+}\) seawater, which blocks polysynaptic connections in *Clione*, still resulted in wing contractions, suggesting direct innervation of the musculature. On the basis of these experiments it seemed unlikely that startle

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**FIG. 2.** Intracellular recording from a startle neuron (ST) and a force transducer recording (Force) from the ipsilateral wing. Arrows: normal swimming movements. Note that only 2 action potentials are triggered in a startle neuron and yet the resultant twitches are much larger than those produced during normal swimming. Only a single startle neuron is stimulated. Bar: period of current injection. Scale bars: 25 mV, 0.5 s.

**FIG. 3.** Carboxyfluorescein injection of a d-phase and v-phase startle neuron. A: somata are found in the pedal ganglion (PED) near the pedal commissure (•), and the axons run to the ipsilateral wing via the wing nerve (arrowheads). The axons branch extensively in the wing. The area indicated by the large arrow is shown at higher magnification in B, and shows numerous parallel fine branches running across the muscle sheet. Scale bars: 300 μm (A) and 80 μm (B).
neurons activate general excitor motoneurons, which are recruited into activity during fast swimming, because general excitors not only innervate their ipsilateral wing, but also send an axon branch to the leading edge of the contralateral wing and thus trigger contractile activity in both wings (Satterlie 1993). Startle-neuron-induced contractions were restricted to the ipsilateral wing only.

Electrophysiological properties of startle neurons and central connections

Startle neurons were normally silent with resting potentials of up to \(-80\) mV (mean \(-67.8\) mV, \(n = 64\)). They received little “spontaneous” synaptic activity, although prominent, long-duration hyperpolarizations were frequently encountered (up to \(20\) mV and several s in duration; Fig. 4). During these hyperpolarizations, it was impossible to trigger spike activity in startle neurons, even with extremely low-resistance potassium acetate electrodes. Behaviorally, these hyperpolarizations were correlated with bouts of swim inhibition and, in some cases, wing retractions in semi-intact preparations (visually observed). The duration of hyperpolarization was closely correlated with the duration of swim inhibition (Figs. 4 and 5). Startle neurons did not receive detectable inputs in phase with swimming movements, or in relation to swimming bouts, indicating that the swim system did not influence their activity (Figs. 4 and 5).

Dual recordings from startle neurons and swim pattern generator interneurons indicated that burst activity in the former did not alter the frequency of pattern generator activity or reset the pattern generator (\(n = 11\); Fig. 5A). Startle neuron bursts and resulting contractions could occur at any time during the swim cycle without altering rhythmicity. Synaptic activity could not be detected in interneurons after startle neuron stimulation. Similarly, startle neuron activity did not alter the activity of either general excitor or small motoneurons (\(n = 23\); Fig. 5B). Most notably, startle neuron activity did not alter the membrane potential of swim motoneurons or influence the number or frequency of swim motoneuron action potentials in phase-locked bursts observed during ongoing swimming activity. During periods of swim inactivity, no startle neuron–to–swim motoneuron connections, or connections in the reverse direction, could be detected. Examination of a wide variety of swim interneurons and motoneurons suggested that startle neurons activated wing musculature totally independently of swim pattern generator and swim motoneuron activity, and did not centrally influence either; neither did either influence startle neuron activity.

Startle neurons activate the same musculature used during slow and fast swimming

In individual experiments (repeated 4 times), we obtained a force transducer record while recording from and stimulating a startle neuron, a general excitor motoneuron, and a small motoneuron in succession. Small motoneurons only innervate slow-twitch muscle fibers, whereas general excitors innervate both slow-twitch and fast-twitch fibers (Satterlie 1993). The time to peak contraction for each was then measured. Because different placements of the force transducer produced slightly different times, the data from the four experiments could not be pooled, and it was essential that the transducer not be moved during a single trial. In each of the four trials, the relative results were consistent with the single experiment data presented here: startle neuron = 50 ms to peak contraction; upswing general excitor motoneuron = 50 ms; downswing general excitor motoneuron = 50 ms; small motoneuron = 109 ms. In all four trials, the twitch times of startle-neuron-induced contractions were the same as those of general excitor motoneurons.

Dual intracellular recordings from startle neurons and swim muscle cells further supported the idea that startle neurons innervate both types of swim musculature. In both standard and high-Mg\(^{2+}\), high-Ca\(^{2+}\) seawater, startle neuron bursts resulted in junctional potentials or spikelike responses in both slow-twitch and fast-twitch muscle fibers, suggesting that startle neurons monosynaptically activate both fiber types (Fig. 6). In instances in which one or a few action potentials could be triggered in a startle neuron, there was a 1:1 relationship to muscle postsynaptic potentials (Fig. 6B). In most cases, however, only intense bursts of startle neuron spikes could be triggered, making it impossible to follow this 1:1 relationship (Fig. 6, A, C, and D). The muscle responses were quite variable, because a burst of startle neuron spikes could trigger a complex burst of postsynaptic potentials (Fig. 6C), a burst of spikelike responses (Fig. 6D), or a combination of the two (Fig. 6A). When a burst of spikelike responses was triggered, the frequency of spikes
Startle response is depressed with repetitive stimulation

Repetitive stimulation of a startle neuron resulted in progressively smaller wing contractions, even with stimuli that were delivered several seconds apart (Fig. 9). In the example shown in Fig. 9, wing contractions decreased to 20% of the initial value after five stimuli delivered at 3-s intervals. In all trials, the second contraction in the sequence was <50% of the first, even when stimuli were delivered at 10-s intervals. The depression was long lived, because >10 min were usually required to obtain contractions that approached those of the initial stimulus. Similar long-term depression was observed with tail stimulation in intact, swimming animals and in preparations in which the tail was left intact and was mechanically or electrically stimulated. The locus of depression has not been located, and may involve several sites in the startle circuit; however, the decrease in number and frequency of startle neuron action potentials triggered by intracellular stimulation does not mirror the decrease in contraction amplitude (see Fig. 9), suggesting that the neuromuscular junctions may be responsible for a major portion of the decrease in response amplitude with repetitive stimulation.

Activation parameters of startle neurons

Mechanical stimulation of almost any part of a swimming Clione could initiate a startle reaction; however there were two was much lower than that of the startle neuron. The observed spikelike response bursts, however, had a maximum frequency that corresponded to the maximum frequency observed during direct intracellular stimulation of individual muscle cells (~10 Hz) (Satterlie 1991). As with activity in central neurons, startle-neuron-induced muscle activity was not phase locked to swimming activity, and was generally more intense than that observed during single swim contractions.

Startle neurons are inhibited by the wing retraction circuit

During swim inhibition, swim interneurons and motoneurons exhibited either smooth hyperpolarizing inputs or tonic, discrete inhibitory postsynaptic potentials. These periods of inhibition were correlated with the large hyperpolarizations in startle neurons, suggesting common inhibitory inputs (Figs. 4 and 5). One neuronal circuit that is activated during swim inhibition, and that involves direct inhibition of swim interneurons and motoneurons, is the wing retraction reflex circuit. The central elements of this inhibition are a pair of pedal interneurons that are activated by wing retraction sensory cells and that activate wing retraction motoneurons (see Huang and Satterlie 1990). Spike activity in all three cell types was correlated with inhibition of startle neurons (Fig. 7). Neither sensory cells nor motoneurons provided direct inhibition of startle neurons, as evidenced by intracellular stimulation of the cells in high-Mg$^{2+}$, high-Ca$^{2+}$ seawater (Fig. 7C). Dual recordings from retraction interneurons and startle neurons, however, showed a direct relationship between spike activity in the former and the degree and duration of inhibition in the latter, suggesting a direct connection (Fig. 8). Unfortunately, recording from retraction interneurons was extremely difficult because of the small size of the subsurface cell bodies, and penetrations could not be held long enough to change to the high-divalent saline. The question of monosynapticity is thus still open.

FIG. 6. Dual intracellular recordings from a startle neuron and either a slow-twitch (slow; A and B) or fast-twitch (fast; C and D) muscle cell. Intense firing in a startle neuron produces either a complex burst of excitatory postsynaptic potentials (EPSPs) (C), a burst of spikelike responses (D), or a combination of the 2 (A). In a few instances, we were successful in stimulating only a few spikes (B). In these recordings, there was a 1:1 relationship between startle neuron spikes and junctional potentials. Scale bars: 20 mV, 0.5 s (A, C, and D), 0.2 s (B).

FIG. 7. Stimulation of a wing retraction reflex results in inhibition of startle neurons. When mechanical stimuli are delivered to the ipsilateral wing (arrowheads), spikes and spike bursts in a wing sensory neuron (WS, A) and a retractor motoneuron (RM, B) correspond with inhibitory waves in the startle neurons. Direct stimulation of a retractor motoneuron through the recording electrode (C) did not produce inhibition in the startle neuron. Scale bars: 20 mV, 0.5 s (A and C), 1 s (B).
areas in which the probability of initiation was high. Mechanical stimulation of the tail was the most reliable, although mechanical stimulation of an individual wing, which usually produced a wing retraction reflex and inhibition of swimming, also was occasionally effective in producing a startle response. Stimulation of tail (Fig. 10, A and B) and wing (Fig. 10, C and D) not only produced excitatory inputs to startle neurons, but also triggered an acceleration of ongoing swimming activity (Fig. 10, A and C). In some preparations, tail or wing stimulation produced subthreshold depolarization or a single spike in a startle neuron (Fig. 10, A and C). In others, similar stimuli produced intense bursts of activity in startle neurons that appeared to have a rhythmic component (Fig. 10, B and D). The frequency of “bursts” was ~3 Hz in these records. The bursting nature is not an intrinsic property of startle neurons, because intracellular activation did not induce bursting.

An analysis of video-recorded startle-escape sequences indicated that all startle responses began with a ventral bend of the wings. This could be due to reciprocal inhibitory contacts between d- and v-phase startle neurons, inhibition from v-phase to d-phase neurons, or a delayed excitation of d-phase neurons. Dual recordings from v- and d-phase startle neurons revealed that synaptic interactions of any kind, and in either direction, were lacking. Evidence for delayed excitation of d-phase neurons came from experiments in which both neuron types were recorded during mechanical or electrical stimulation of the tail, or during electrical stimulation of one of the four primary intestinal nerves, which carry afferent and efferent information between the tail and CNS (Fig. 11). Startle neurons typically did not spike during these experiments; however, subthreshold synaptic activity was always observed. These responses showed significant response depression, and required long rests between stimuli. In all of these experiments, v-phase startle neurons always received compound excitatory postsynaptic potentials (EPSPs) with a latency of 10–15 ms (stimulus artifact to initiation of EPSP). D-phase startle neurons always received a “slow-wave” depolarization with a longer latency, typically on the order of 30–40 ms.

**DISCUSSION**

Escape swimming in *Clione* involves an initial startle response followed by a period of fast swimming, both of which quickly carry the animal away from a noxious stimulus. The initial startle response is quite distinct from the fast swimming phase of escape in that it involves wing contractions that are much stronger than those seen during fast swimming and that are not phase locked to swimming movements.

Two pedal motoneurons have been found in each pedal ganglion that display several features that are characteristic of startle-escape systems. In this regard, these cells may be similar in function to such well-known startle-inducing neurons as Mauthner neurons of fish and some amphibians (see Eaton 1984 for a review of startle systems), the giant fiber system of cephalopod mollusks (Boyle 1986), and the giant neurons controlling escape responses in other animals such as cnidarians (Mackie 1984), annelids (Drewes 1984), insects (Ritzmann 1984) and crustaceans (Krasne and Wine 1984). Of all these systems, the startle neurons of *Clione* most closely resemble functionally the Mauthner neurons. Both produce a relatively stereotyped startle response that is the initial part of escape swimming. In addition, the various general properties of *Clione* startle neurons are very Mauthner like. For example, the startle neurons of *Clione* exhibit high firing thresholds, so that the behavior is only triggered by noxious or novel stimuli. With repetitive stimulation, they show a response depression that may occur at several loci within the startle-escape circuitry, a major portion of which may occur at the neuromuscular junction. Activity in the startle neurons is not phase locked with swimming, so stimuli are acted on immediately without having to wait until the appropriate cycle time. Finally, the startle neurons are inhibited during activation of mutually exclusive behaviors, such as wing retraction, which also involves inhibition of the swim circuit.

Startle neurons of *Clione* are large motoneurons that monosynaptically activate the same musculature used for normal slow and fast swimming. Thus far, wing musculature that is unique to startle behavior has not been found. The large size of startle neurons (both soma and axon diameters), which is very similar to that of general excitor motoneurons (which are recruited into activity during fast swimming), suggests that fast conduction is an important function of the startle circuit. An alternate explanation for the large size...
of these neurons would center on the need for significant metabolic machinery for cells with such extensive innervation fields, although both explanations may apply. Oversized neurons are frequently found in startle-escape systems in both vertebrates and invertebrates (Bullock 1984).

The newly identified startle neurons appear to represent the motor output circuitry for the startle phase of escape behavior. Their integrative role in initiation of the startle response is influenced by two important properties that might limit the production of the startle. First, the high spike threshold requires that appropriate levels of excitatory inputs are necessary to bring them to spike threshold. Second, the neuromuscular junction appears to be a major site for depression of the response, so past activity will be filtered through this junction. This peripheral depression is somewhat unusual, because in startle-escape systems that show habituation, the site of plasticity is central rather than peripheral. For example, in the tail-flip system of crayfish, habituation occurs at the cholinergic synapse between mechanosensory afferents and the primary interneurons (Kraskie and Davis 1980; Kraskie and Lee 1988; Norekian and Satterlie 1996). We have not yet found inhibitory connections from startle neurons to elements of the wing retraction or whole body withdrawal circuits.

One problem associated with the startle system deals with the activation of v-phase and d-phase cells. If both are activated together, an inefficient wing contraction would result. Alternate activation is thus required. One way to accomplish this is through some form of inhibition between the two startle neuron groups. If the connections were reciprocal, it would be possible to get cycling of

FIG. 10. Mechanical stimulation of the tail (A and B) or the ipsilateral wing (C and D) results in either a subthreshold depolarization (A), a single spike (C), or a burst of spikes (B and D) in a startle neuron. In addition, there is a significant acceleration of swimming as recorded from a general excitor motoneuron (A and C). Note that the spike bursts in the startle neurons show a bursty character (B and D). Scale bars: 15 mV, 1 s.

Because fast swimming is triggered with the startle reaction, it is presumed that parallel inputs activate both systems. The cerebral serotonergic neurons described by Satterlie and Norekian (1995), which presumably include the cells identified by Arshavsky et al. (1992), did not activate startle neurons (unpublished observations), suggesting that the serotonergic system that is responsible for swim initiation/acceleration is not the initiator of the startle response. However, this does not preclude activation of the serotonergic system, or a subcomponent of it, during the startle-escape reaction.

Inhibition of startle neurons during wing retraction behavior is interesting for two reasons. First, recorded inhibitory potentials, when fully developed, are large and extremely successful in preventing activation of startle neuron firing. We have been totally unable to trigger spiking in startle neurons during the inhibition that accompanies wing retraction. Wing retractor interneurons were found to produce direct inhibitory inputs to startle motoneurons. Inhibition between neural circuits involved in mutually exclusive behaviors is a common feature of many animals (Beall et al. 1990; Jing and Gillette 1995; Kovac and Davis 1980; Kraskie and Lee 1988; Norekian and Satterlie 1996). One way to accomplish this is through some form of inhibition between the two startle neuron groups. If the connections were reciprocal, it would be possible to get cycling of

FIG. 11. Dual recordings from a v-phase ST(v) and a d-phase ST(d) startle neuron with electrical stimulation of an intestinal nerve (nerve 11), which runs from the tail into the CNS. Note that the compound EPSP (A) or spike and compound EPSP (B) in the v-phase neuron have a shorter latency than the slow-wave depolarization in the d-phase neuron. Scale bars: 20 mV; 0.5 s (A), 0.2 s (B).
activity between the two groups. However, startle motoneurons of opposite phases do not communicate with each other. Possibly, this type of reciprocal inhibitory connection exists at a higher level in the nervous system. Another option is a delayed activation of one group (d-phase, on the basis of behavioral observations) that would allow sequential activation without prolonged interference with the swim pattern generator by continued cycling by the startle neuron groups. This would require that startle neuron activity be limited to the very beginning of the escape response, but, as mentioned above, would free startle activity from potential delays imposed by phase locking and would avoid interference with the parallel activation of fast swimming activity. Preliminary evidence suggests that delayed activation of d-phase startle neurons exists, but the exact mechanism of this delay is not yet known. It could reside in the higher circuitry of the startle response, resulting in separate inputs to the two startle groups, or it could be due to differences in synaptic properties of the two groups of cells despite common descending inputs to the groups. However, delayed activation of d-phase startle neurons is not the only option. In several experiments with tactile stimulation of the tail or wing, bursts of spikes were generated in startle motoneurons. This bursting suggests that there might be a patterned alternation of d-phase and v-phase neuron activity during the startle response. Coordination for alternate swimming does not occur at the level of the alternate neurons, because synaptic interactions between d-phase and v-phase neurons were not found. Higher-level pattern generation for alternate activity in d-phase and v-phase startle neurons is thus suggested.

The primary loci for initiation of the startle response appear to be at the level of higher-order interneurons. At these sites, not only will sensory information be sorted and relayed to the startle motoneurons, but some form of rhythmicity will be imposed on the two antagonistic motoneurons. Results from our initial search for higher-order interneurons that are involved in the startle response are presented in the following paper (Norekian and Satterlie 1997).

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