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Startle Responses of Fish Without Mauthner Neurons: Escape Behavior of the Lumpfish (*Cyclopterus lumpus*)

Melina E. Hale (Dept. of Neurobiology and Behavior, SUNY Stony Brook, Stony Brook, New York 11794-5230 and Grass Foundation Fellow, Marine Biological Laboratory, Woods Hole, Massachusetts 02543)

Fast start escape responses are the primary behaviors used by fishes to avoid an attacking predator. Of particular importance is the C-start type of fast start (reviewed by 1, also see 2, 3). During a C-start the fish rapidly turns away from a threatening stimulus into a “C” shaped body bend, called stage 1. Frequently, stage 1 is followed by a tail stroke to the opposite side of the body, stage 2, which propels the fish away from the stimulus. The C-start is initiated by the Mauthner cells, a pair of large reticulospinal interneurons (4, 5). Each M-cell has a large axon that crosses the body midline and extends the length of the spinal cord, exciting motoneurons that innervate the lateral muscle. In response to a stimulus from the right side of the body, the right M-cell fires an action potential that propagates rapidly down the axon to cause nearly simultaneous contraction of muscle on the opposite side of the body from the M-cell soma and the “C” bend away from the stimulus (6, 7).

Although Mauthner cells have been identified in a large number of taxa broadly representing the phylogenetic diversity of actinopterygian fishes, a few species appear to lack these neurons (8). This study examines the startle behavior of one such species, the lumpfish (*Cyclopterus lumpus*). Two specific questions are addressed. First, do lumpfish have a startle response that is distinct from routine swimming? If so, how does the behavioral pattern and performance compare with the M-cell initiated C-start of other fishes?

The startle response was examined in larval lumpfish rather than in mature individuals. The larval lumpfish have a more generalized morphology than mature lumpfish, and so it was thought that the response of the larvae to a startle stimulus may be more easily compared to other species. Additionally, it seemed that if the

lumpfish were to have high performance behavioral responses to predation, it would be seen in the larvae because of greater vulnerability to predators due to less developed morphological defenses. It is possible that M-cells are present in larval lumpfish and are reduced or lost during development; however, morphological examination of the reticulospinal neurons of the larval lumpfish ($n = 30$) with retrograde labeling has not identified Mauthner neurons or homologous cells.

For studies of behavior, lumpfish ($n = 12$; 6.2 ± 1.0 mm, total length) were hatched from eggs collected off the coast of Gloucester, Massachusetts, at approximately 6 m depth. Eggs and larvae were maintained in a 10-gallon aquarium with flow-through seawater chilled to 11°C. Behavioral trials were conducted within a week of hatching. A tactile stimulus—touching the head with a fine gauge wire—was used to elicit startle behavior which was filmed in a small petri dish (3.5 cm diameter). The responses were captured on high-speed video (1000 Hz) taken with an EG&G Reticon digital camera imaging through a Zeiss Stemi SR microscope. Three trials from each fish (36 total trials) were analyzed with Microsoft Excel 98 and Scion Image 1.6. Parameters examined were the angles of head movement during stage 1 and stage 2, the latency between stimulus and response, and the durations of stages 1 and 2.

The larval lumpfish respond to the stimulus with a C-start behavior pattern (Fig. 1A). Fish turned tightly away from the stimulus direction in stage 1 [Fig. 1A, left column (0–24 ms)] with an average stage 1 angle of $146^\circ \pm 23^\circ$ degrees. Stage 1 was consistently followed by a stage 2 tail stroke [Fig. 1A, right column (24–56 ms)] and movement away from the stimulus. The stage 2 angle, generally in the opposite direction of the stage 1

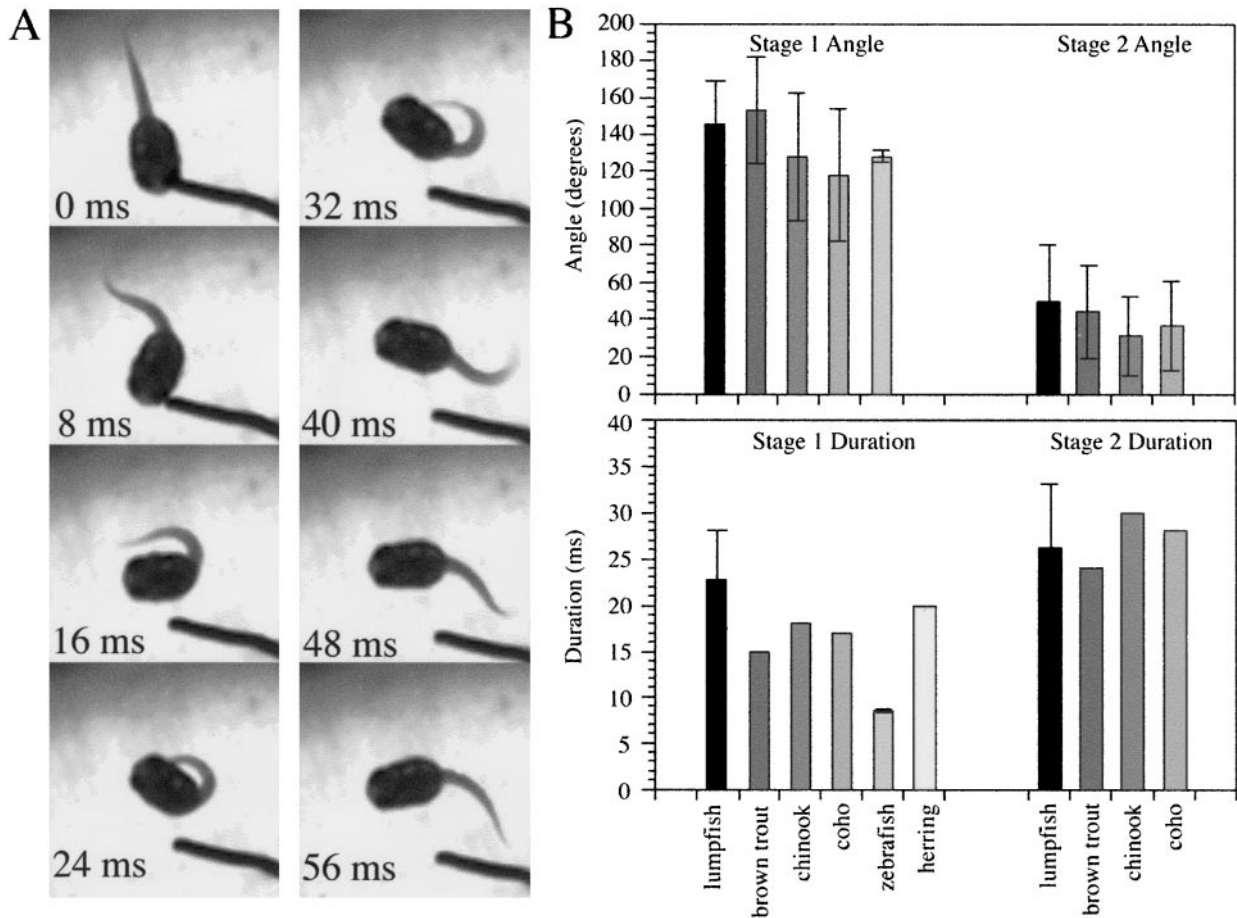


Figure 1. A. A typical startle response of larval lumpfish (*Cyclopterus lumpus*). Stage 1, the tight “C” bend away from the stimulus, lasts 24 ms (column 1) and stage 2, the first propulsive tail stroke, follows from 24 to 56 ms (column 2). Data for the angle of movement and kinematic stage durations are shown in B, with comparative data from brown trout (*Salmo trutta*), chinook salmon (*Oncorhynchus tshawytscha*), coho salmon (*Oncorhynchus kisutch*) (10, minimum values in scaling relationships), zebrafish (*Danio rerio*) (9), and herring (*Clupea harengus*) (12).

turn, was consistently smaller than that of stage 1 (stage 2 angle = $50^\circ \pm 30^\circ$). The movement angles made by larval lumpfish during the C-start are comparable to those of other species (Fig. 1B; e.g. 9, 10, 12, 13).

Several important fast start performance variables are the latency of response to the stimulus and the duration of the kinematic stages. The latency between stimulus and initiation of movement of an M-cell initiated startle can take less than 4 ms (9) and the duration of the response is generally less than 100 ms (1). The latency of the lumpfish, recorded for a subset of the trials (one from each of 10 individuals) was 9 ± 2.1 ms. It was considerably longer than that of the larval zebrafish (3.9 ± 0.2 ms) (9). The duration of stage 1 of the larval lumpfish was 22.8 ± 5.2 ms, and the duration of stage 2 was 26.3 ± 6.8 ms. Because the duration of the fast-start stages changes with size (11) and developmental stage (10), direct comparisons among species are difficult. Still, the durations of kinematic stages 1 and 2 of the larval lumpfish are in the same range of values as other immature fishes; all under 5 cm (Fig. 1B; 9, 10, 12). The total duration of the fast start (stages 1 and 2) for the larval

lumpfish is shorter than the fast start duration of most larger fishes (reviewed in 1).

Although the lumpfish has a longer response latency to a startle stimulus than zebrafish larvae, the C-start of the larval lumpfish—in pattern and in the duration of response—has the characteristics of the M-cell initiated C-start. One explanation for the similarities in the startle response among taxa is that the Mauthner cell and its homologs are present in the larval lumpfish but have not yet been identified. Another is that alternative neural circuits can generate rapid C-start behavior and that the Mauthner cell and its homologs are most critical for rapid initiation of movement. If so, such mechanisms may be taxon specific since ablating the Mauthner cell and its homologs in the larval zebrafish results in a significant decrease in performance (9). The presence of a rapid C-start type escape behavior in the lumpfish, a species that appears to generate the fast start behavior without the Mauthner cell system, provides an exciting opportunity for comparative examination of an evolutionarily conserved neural and behavioral system.

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Memory Consolidation in *Hermisenda crassicornis*

David A. Epstein, Herman T. Epstein, Frank M. Child, and Alan M. Kuzirian
(Marine Biological Laboratory, Woods Hole, Massachusetts 02543)

Experiments with shell-less molluscs (*Aplysia* and *Hermisenda*) have revealed a number of processes that underlie learning by these organisms and also by some vertebrates. *Hermisenda*, for example, shows significant Pavlovian conditioning capabilities (1). Follow-up investigations on these molluscs dealt with the sensory stimuli needed for short-term memory (STM) and long-term memory (LTM) (2). The relationship of the two memories with *in vitro* changes in excitatory post-synaptic potentials (EPSPs) have also been investigated in the neural networks of these organisms (3). Many studies of the molecular aspects of these two different memory regimes have led to quite detailed descriptions of the events (4, 5, 6).

Both *Aplysia* and *Hermisenda* have been tested for their recall of induced behavioral modifications after one, two, or many conditioning events (CEs). In *Aplysia*, the EPSP component of learning produced by 1 CE was compared to that produced by 5 CEs (3). In *Hermisenda*, the comparison was made between 2 CEs and 9 CEs (2). Five to ten minutes after finishing one or two conditioning events, both animals exhibited significant behavioral recall (*i.e.* STM); but there was no recall after an hour or more (*i.e.* no LTM). The larger numbers of CEs, however, did induce LTM in both species.

Since STM and LTM are clearly responding to a different set of conditions, we focused first on what might inhibit or block STM. This problem was partially anticipated in 1900, according to McGaugh (7) who cited Muller and Pilzecker as having found that “memory of newly learned information was disrupted by the learning of other information shortly after the original learning” (8).

This concept led us to test, in *Hermisenda*, whether STM recall (at 5 min) might be blocked simply by the input of additional information (*i.e.* extraneous sensory stimuli) if the latter were applied within the first 5 min after conditioning. The initial results of the blocking experiments, which showed that the simple sensory inputs blocking STM also blocked LTM, then led to the hypothesis that temporal consolidation of LTM could be detected by measuring when the blocking sensory input was no longer effective.

Hermisenda (Sea Life Supply, Sand City, CA) were tested with 2 and 9 paired CEs for induction of STM and LTM. Conditioning

events consisted of exposing the animals to 6 s of bright, white light (CS) explicitly paired with 4 s of strong orbital agitation (US) following a 2-s onset delay with an inter-trial interval of 1 min. Recall of the behavioral modification induced by associative conditioning was assessed by recording the animal’s change in foot length when presented with 6 s of light alone. The conditioned response (CR) was foot contraction, the unconditioned response (UR) was foot elongation (9). Two paired conditioning events initiated behavioral recall after 5 min but not after 90 min; the LTM input of 9 pairings was recalled at both 5 and 90 min (Fig. 1A). The small and non-overlapping S.E.Ms for each point indicate statistical significance ($P = <0.01$, $t = 3.18$).

After giving the animals the paired CS and US stimuli leading to STM, we tested two simple paradigms of blocking sensory stimuli. The first was a modification of the conditioning stimuli: dim orange light and very slow orbital rotation. The second blocking stimulus tested consisted of rotating the tray containing the animals upside down and, after 5 s, rotating it upright again (rotational block). Both experimental paradigms blocked STM and LTM (Fig. 1B).

To determine the temporal specificity of LTM in *Hermisenda*, the following experiments were done. Animals were trained with 9 CEs, and the CR was measured at the usual 90 min. However, at selected time intervals (2, 25, 50, 55, 60, 65 min) post-conditioning, the animals were rotationally blocked. Control animals received only the 9 paired CEs. When the animals’ behavior was plotted, a clear and decisive LTM consolidation interval in *Hermisenda* appeared; consolidation occurred between 55 and 60 min (Fig. 1C). Presentations of rotational blocking prior to 55 min totally blocked memory consolidation. However, the stimulus given after 60 had no blocking effects, and the animals demonstrated the CR. The consistency of and surprisingly little variability in the response among the majority of the animals indicated the robustness of the paradigm. When the data were analyzed with *t*-test and *F*-test statistics, they were found to be highly significant, whether compared between data points or to zero ($P = <0.001$, $t = 15.24$; *F*-value, inf).