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FUNCTIONAL SIGNIFICANCE AND NEURAL BASIS OF LARVAL LAMPREY STARTLE BEHAVIOUR

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SUMMARY

1. The vibration-evoked startle response mediates rapid withdrawal in burrowed larval lampreys (ammocoetes). Ammocoetes withdraw in response to vibration by contracting pre-existing lateral bends in the trunk and tail, thus pulling their heads deeper into the burrow.

2. The motor effects of an ammocoete startle response are dependent on pre-existing posture. Areas of lateral body curvature contract more and exhibit larger electromyogram (EMG) amplitudes on their inner sides than on their outer sides.

3. Both of the anterior Mth and posterior Mth′ (Mauthner) cells and both of the B_1 and B_2 (bulbar) Müller cells fired action potentials in response to vibration of the otic capsules. Both B_3 and B_4 Müller cells were inhibited by vibration, while M (mesencephalic) and I_1 (isthmic) Müller cells were inhibited by ipsilateral vibration and excited by contralateral vibration.

4. Simultaneous action potentials in both of the anterior Mth cells were appropriate and sufficient for initiating the startle response EMG in a semi-intact preparation.

5. This study demonstrates a Mauthner-initiated startle response which activates musculature on both sides of the body to produce a rapid withdrawal movement and is thus adapted to the eel-like form and burrowed lifestyle of larval lampreys.

INTRODUCTION

Larval lampreys (ammocoetes) are burrowing filter feeders and, though capable swimmers when disturbed, they are normally sedentary, occupying crescent-shaped burrows in the silt or mud of freshwater streambeds (Applegate, 1950).

We have recently found that freely behaving larval sea lampreys (*Petromyzon marinus*) exhibit a rapid, vibration-evoked startle response involving a bilateral activation of musculature along the length of the body (Currie, 1984*a*,*b*; Currie & Carlsen, 1985). Startle responses can also be elicited from semi-intact preparations by directly vibrating the otic capsules (Currie & Carlsen, 1985). Vibration produces a

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volley of descending spikes in the giant axons of the spinal cord followed by the startle electromyogram (EMG) response. The giant axons originate from bilaterally paired, giant cell bodies in the brainstem and mesencephalon, called Müller and Mauthner cells (Rovainen, 1967). Lampreys possess two pairs of Mauthner neurones, one pair with large, anteriorly situated cell bodies (Mth) and a much smaller, posterior pair (Mth′) (Rovainen, 1967, 1978, 1979*a*). Rovainen (1979*a*) showed that the anterior Mth cells fire action potentials in response to vibration of an otic capsule. These Mth cells, unlike those of teleost fish and amphibian larvae, are excited by stimulation of both the ipsilateral and contralateral eighth nerve (Rovainen, 1979*a*) and lack reciprocal inhibition, so that both cells can fire at the same time. They have the appropriate output connections in the spinal cord to initiate a bilateral EMG response. Rovainen (1979*b*) described experiments which demonstrated that adult lamprey Mth cells produce multicomponent EPSPs in myotomal motoneurones to both sides of the body.

In the present study, we show that simultaneous spikes in both anterior Mth neurones are appropriate and sufficient for initiating the bilateral startle response EMG in semi-intact preparations. In burrowed animals, the startle behaviour functions as a rapid withdrawal response by contracting pre-existing lateral bends in the trunk and tail. This accordion-like body contraction occurs because of a posture-dependence: areas of resting body curvature contract more on their inner sides than on their outer sides. Preliminary reports of these findings have appeared elsewhere (Currie, 1984*a*,*b*; Currie & Carlsen, 1985).

MATERIALS AND METHODS

Larval sea lampreys (*Petromyzon marinus*), measuring 9–15 cm in length, were used. Animals were held in a large, sand-bottomed tank which was maintained at 15 °C. Prior to electrode implantation or dissection, larvae were anaesthetized in ice-cold tricaine methanesulphonate (MS 222, 0·3 g l^{-1}) and pinned out in a Sylgard-lined dish containing lamprey saline (Wickelgren, 1970).

Recording burrowed animal responses

A thin-section aquarium was constructed of clear Plexiglas to enable observation of burrowed ammocoetes (Fig. 1A). The aquarium was filled with lamprey saline, and contained a layer of fine glass beads (SGA scientific, 0·17 mm), 12 cm deep, to form a burrowing substrate. To record trunk EMG activity in burrowed animals, larvae were anaesthetized and pinned out in lamprey saline. A 4–5 mm dorsolongitudinal incision was made near the last gill pore to expose the spinal cord, and a monopolar wire electrode (25 μm in diameter, stainless steel), insulated to within 0·5 mm of its tip, was inserted posteriorly down the spinal canal for about 2 cm. The incision was then sutured closed around the wire. A knot of thread was attached to the wire with glue (Eastman 910) beneath the suture and prevented the electrode from pulling out. After inserting the electrode, the animal was released into the aquarium, allowed to recover from the anaesthetic and to burrow into the glass beads. EMG recordings

were made relative to an indifferent bath electrode. To monitor head movement, a light beam was shone through the aquarium towards a photoresistor on the other side, so that the head of the larva partially interrupted the beam. Head withdrawal thus translated to increased light intensity and a voltage change across the photoresistor. This was recorded simultaneously with the startle EMG and a vibration monitor.

Simultaneous electrophysiological and cinematographic recordings

Bipolar EMG electrodes (25 μm in diameter, stainless steel) were implanted in the right- and left-side trunk musculature and braided together in a single, flexible line about 35 cm long. The animal was then placed in a small Plexiglas testing chamber, containing a 2·5 cm depth of saline solution. The testing chamber measured 18 cm × 23 cm × 4 cm deep. It was made of 0·64 cm Plexiglas and had a thick Styrofoam floor, which transmitted vibration extremely well. A short length of rubber tubing was split lengthwise, placed over the top of the wall of the chamber, and tapped to deliver a stimulus. The rubber tubing, and a foam-rubber pad beneath the chamber, filtered the stimulus to a relatively clean, single-cycle vibration. A super-8 mm ciné camera was set on a tripod and looked down on the chamber from directly above. Film was shot at 18 frames s^{-1} (interframe interval = approx. 55 ms). A record-player pick-up in contact with the chamber wall was used to monitor the vibration, and to trigger the sweep of two oscilloscopes. One oscilloscope recorded the EMG response while the other served as a post-stimulus time marker on film. The sweep rate of the time-marker was set at 20 ms/division. The elapsed time following a stimulus could thus be determined for each movie frame.

Semi-intact preparations

A completely curarized preparation was usually used when recording from Müller and Mauthner cell bodies. Animals were given an intramuscular injection of 50–100 μg of D-tubocurarine. When paralysis was complete, they were transferred to ice-cold tricaine for 10 min before being placed in a Sylgard-lined dish containing cold lamprey saline. The animal was then eviscerated and the brain and otic capsules were dorsally exposed as described previously (Currie & Carlsen, 1985, 1987). A monopolar hook electrode, insulated with Vaseline, was sometimes used to record activity from the whole spinal cord, just behind the gill chamber.

A slightly modified preparation was developed to allow intracellular recording in the brain and rostral spinal cord during muscular startle responses (Fig. 6A; see also Currie & Carlsen, 1985, 1987). Several body segments were removed behind the gills so that only a slack loop of spinal cord connected the head and posterior body. The head alone was curarized with several small, intramuscular injections. Electromyograms were recorded from both sides of the midbody with bipolar, silver wire electrodes, insulated to within 0·5 mm of their tips (Medwire Corp., 76 μm in diameter). Bath temperature was kept between 14 and 16 °C by circulating the saline between the dish and an ice bath with a peristaltic pump. Low bath temperature

acted as a continuous low-level anaesthetic. Cooled preparations remained quiescent (no spinal cord or EMG activity) except when stimulated by vibration.

Vibration of the otic capsules

Vibratory stimulation was achieved using a thin, speaker-driven probe (see Currie & Carlsen, 1987). The speaker diaphragm was driven by 1–3 cycles of a 300 Hz sine wave from a Wavetek function generator, triggered by a WPI stimulator and amplified by a Heathkit AA-18 audio amplifier.

Intracellular and extracellular recording from Müller and Mauthner cells

Intracellular recordings were obtained from Müller and Mauthner cells using glass microelectrodes (1·0 mm o.d.), back-filled with 3 mol l^{-1} KCl. Electrode resistances were 30–50 MΩ for cell body recordings and 10–20 MΩ for axon penetrations. Mauthner axons were identified during intracellular impalements based on three criteria: (1) lateral positions in the spinal cord (Rovainen, 1967); (2) the ability of vibration-evoked Mth spikes to follow high-frequency vibratory pulses to the otic capsules one-for-one (15–20 ms interpulse delays; see Fig. 4); (3) direct intra-axonal stimulation produced a spike with a characteristically large field potential on the spinal hook electrode (see Fig. 6C).

The vibration responses of Müller and Mauthner cells were sometimes recorded extracellularly by placing fire-polished glass suction electrodes (50–100 μm, i.d.) directly over visible cell bodies. Suction electrodes were also used to record *en passant* from the vestibular nerve. Extracellular recordings were passed through Tektronix 122 preamplifiers and displayed with intracellular and vibration records on a Tektronix 5113, four-channel storage oscilloscope.

Neurone ablations

Müller and Mauthner cells were destroyed by placing a fine-tipped suction electrode over their visible cell bodies and applying back pressure while recording with the electrode. This produced a brief injury discharge followed by the absence of vibration-evoked activity. Cells were visibly disrupted by this procedure.

RESULTS

The startle response produces rapid withdrawal in burrowed ammocoetes

Wild ammocoetes spend nearly all of their time burrowed in fine sand or mud. We examined the startle behaviour of freely behaving, burrowed animals to determine if the startle response has a functionally significant role in the normal behaviour of the ammocoete.

In the glass bead substrate (see Materials and Methods), burrowed larvae were visible in silhouette when light was shone through the aquarium from behind. In the final burrowed position (Fig. 1A), mouthparts were near or just above the surface of the substrate while the body slanted deeper into the burrow. Animals were dorsal side-up and curved upwards in the rostral trunk so that their heads were nearly

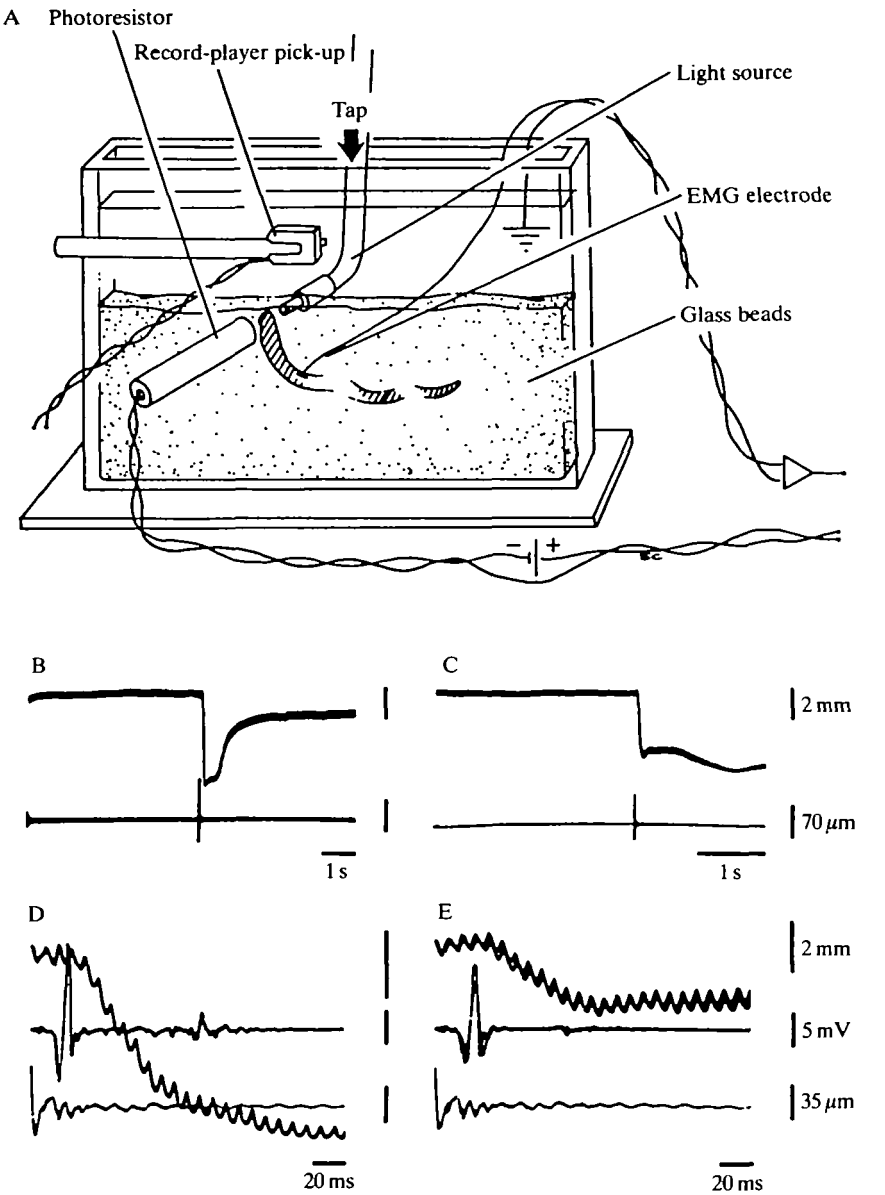

Fig. 1. Startle responses of burrowed larvae. (A) A thin-section aquarium was used so that burrowed animals could be visualized in silhouette when light was shone from behind. Fine glass beads were used as a burrowing substrate. The stimulus was a light hammer tap to the top of the aquarium wall, recorded with a record-player pick-up. Head withdrawal was recorded with a light beam–photoresistor system. In one animal, a monopolar electrode was used to record the trunk EMG during withdrawal. (B,C) Examples of the tap-evoked withdrawal at slow sweep speed. Upper traces, photoresistor recordings (downward deflections = downward head movement); lower traces, vibration monitor. (D,E) Combined EMG/movement records. Top traces, head withdrawal; middle traces, EMGs; bottom traces, vibration monitor. Two superimposed responses are shown in E.

vertical. Numerous lateral bends were visible in the trunk and tail, resulting from active burrowing. A light tap to the top of the aquarium evoked a jerked withdrawal of the head, 3–6 mm deeper into the burrow. We were able to record this movement in three animals. Head movement was monitored with a light beam–photoresistor apparatus (see Materials and Methods). Two typical responses are shown in Fig. 1B,C at slow sweep speed.

One larva had a monopolar EMG electrode implanted at mid-body, half-way between the right-side and left-side body wall. Taps to the aquarium elicited the characteristic short-latency startle EMG followed by head withdrawal. Two repeated trials are displayed in Fig. 1E to illustrate the time-locked coupling between the EMG response and withdrawal. Taps which elicited a time-locked EMG potential were always followed by withdrawal. Rapid withdrawal never occurred in the absence of the EMG response. The stimulus withdrawal latency (to the extrapolated start of withdrawal) was $37 \pm 1{\cdot}6$ ms ($\bar{x} \pm$ S.D., $N = 11$).

Motor effects of the startle response are contingent on existing posture

The movements made during a startle response and the relative amplitudes of left- and right-side EMGs could change depending on the initial resting posture. Areas of inward lateral curvature along the trunk and tail contracted more and exhibited a higher EMG voltage amplitude than areas of outward curvature. To examine this effect, we recorded a variety of responses, all from the same animal, using combined electromyography and cinematography (Fig. 2A–G). *In each of the sequences where the animal started out in a curved position (Fig. 2A–E) the startle response resulted in an enhancement of the initial resting curvature.* When the animal rested in a single, smooth curve along its right side (Fig. 2A), the right-side EMG response was larger than on the left side and the resulting movement resembled the unilateral 'C-start' of goldfish and other short-bodied teleosts (see Eaton, Bombardieri & Meyer, 1977). No significant bias of right- or left-side EMGs was noted while the animal was swimming. When the animal was inwardly curved along its left side in the vicinity of the EMG electrodes (Fig. 2B), the startle EMG was biased almost entirely to the left, with little appearing on the right-side EMG electrodes.

It is of particular interest that when the EMG electrodes were positioned at a junction between two opposing curves (Fig. 2C,D), the EMG was largest on the inner side of the more rostral curve. The movements were typical 'S-type' startle responses (Eaton *et al.* 1977), named for the S-shaped body contraction. During a

Fig. 2. Motor effects of the startle response depend on pre-existing body posture. Combined electromyographic and cinematographic recordings of several startle responses in the same animal. Each of the EMG responses on the left is paired with the film sequence to its right. EMGs, upper and middle traces, left (L) and right (R) side trunk EMGs; bottom traces, vibration monitor. Vertical calibrations: upper and middle traces, 1 mV; bottom traces, 70 μm. Horizontal calibration: 5 ms. Film sequences: sequences begin on the left, with the animal at rest. Subsequent frames were moved a constant distance to the right. The calibration marks at the top of the figure represent the interframe interval of 55 ms. The dot between the first and second silhouettes in each film sequence indicates the moment of stimulus delivery. Bars drawn on each silhouette show the location of EMG electrodes.

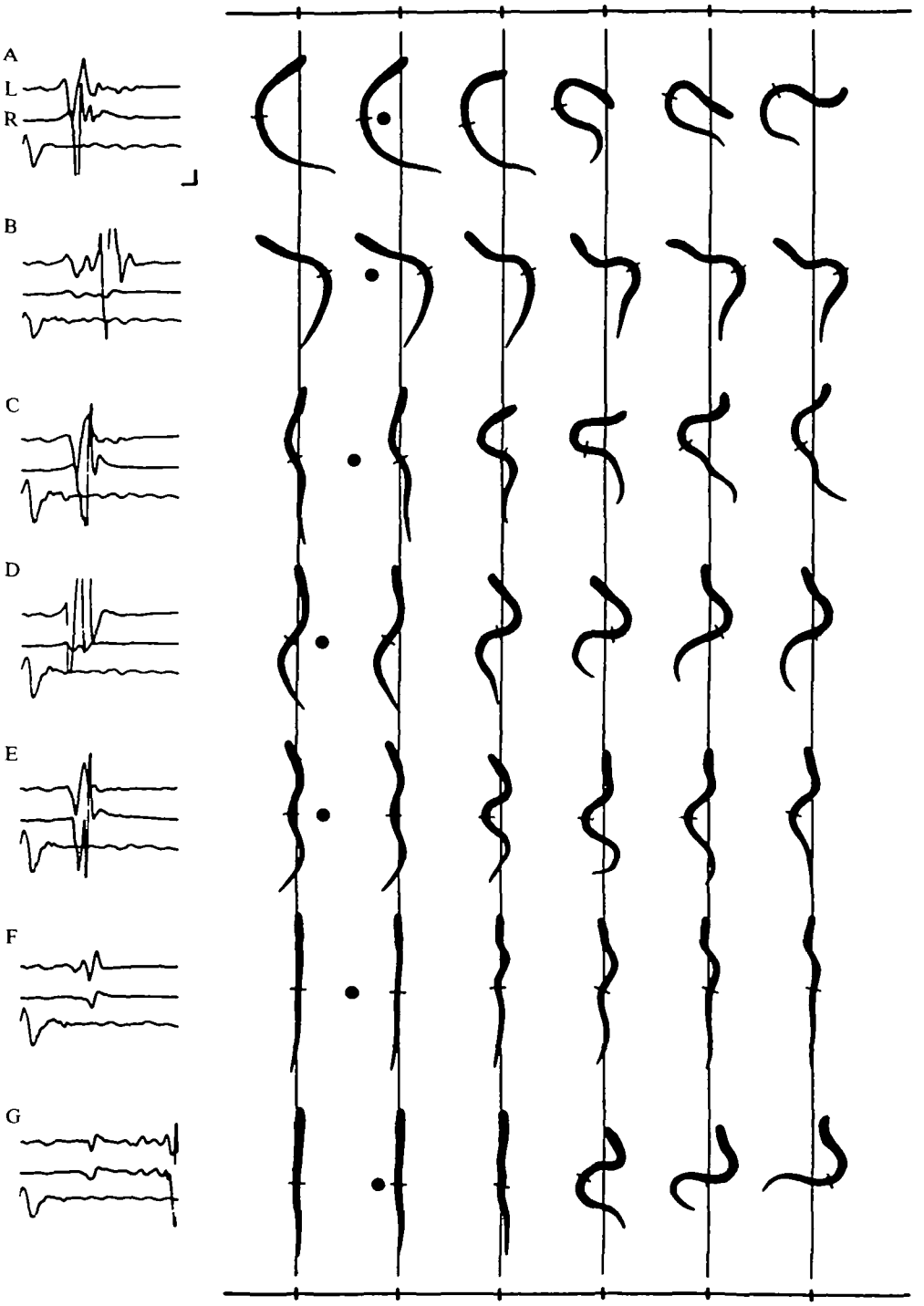

Fig. 2

W-shaped contraction of three curves (Fig. 2E), the right-side EMG electrode was located at a point of inward curvature and exhibited the larger potential. Such accordion-like responses illustrate the way posture-dependent body contractions effect rapid withdrawal of the head in burrowed animals. Note the small, symmetrical EMGs and weak movement which occurred with the animal in an initially straight-bodied position (Fig. 2F).

The short-latency startle EMG was often followed by a second contraction, which in Fig. 2G initiated a bout of rapid escape swimming. Such two-stage responses were common in stimulus-naive animals, but occurred less frequently after several stimuli. While the early EMG response was bilaterally symmetrical, as would be predicted from the straight resting posture, the longer latency response was skewed towards the right side. This asymmetry was reflected in the filmed movement. We occasionally saw such two-stage responses in our semi-intact preparations, following vibration of the otic capsules.

Short latency of the response

Ammocoete startle behaviour is striking for its short and constant EMG latency. This can be seen most clearly in the superimposed responses of the burrowed animal (Fig. 1E) and by a comparison of EMG onsets in the lefthand column of Fig. 2. The stimulus–EMG latency in Fig. 1 was a constant 18 ms ($N = 11$). In Fig. 2, the latency was $14{\cdot}8 \pm 2{\cdot}85$ ms ($\bar{x} \pm$ S.D., $N = 7$). Short response latencies indicated that the rapidly conducting, giant axons might be involved in initiating the startle response, especially since Rovainen (1979*a*) had demonstrated that several of the giant Müller and Mauthner cells are vibration-sensitive. The involvement of giant cells was further indicated during our initial work with semi-intact preparations, when we observed that the startle EMG was preceded by a volley of large, descending spinal units (Currie & Carlsen, 1985). Based on these considerations, we identified all vibration-sensitive Müller and Mauthner neurones, and then attempted to determine which of these cells had a direct role in initiating startle behaviour.

Vibration responses of the Müller and Mauthner neurones

As a first step in defining the neural basis of this behaviour, we observed the vibration responses of 10 pairs of identified giant cells. Fig. 3 shows the pattern of synaptic potentials evoked by a unilateral vibration pulse to the left otic capsule, with the contralateral labyrinth removed. Note that the large anterior Mauthner (Mth) cells ($N = 15$ cells recorded) and the B_1 and B_2 (bulbar) Müller cells ($N = 21$) were excited bilaterally during vibration of one otic capsule. The excitation of both posterior Mth′ cells was inferred from extracellular recordings (see below). B_3 and B_4 cells were bilaterally inhibited ($N = 16$), although the contralateral B_4 response was frequently mixed as shown, whereas the M_1–M_3 (mesencephalic) and I_1 (isthmic) Müller cells were ipsilaterally inhibited and contralaterally excited ($N = 25$) by unilateral vibration. I_1 IPSPs were striking for their extremely large amplitude, often

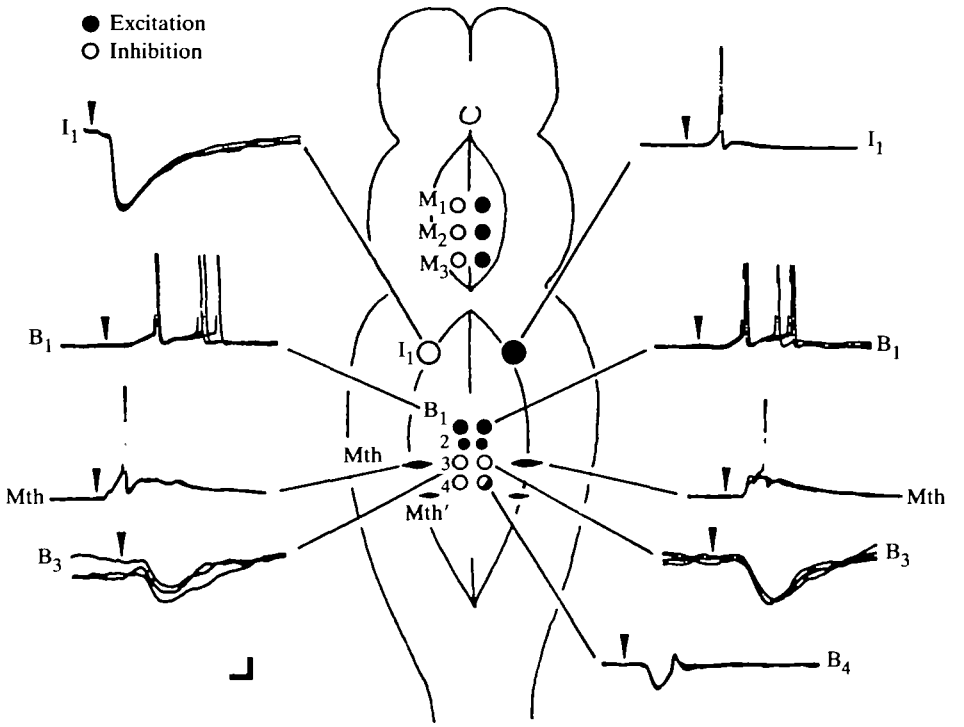

Fig. 3. Pattern of synaptic responses recorded from 10 pairs of giant Müller and Mauthner neurones during vibration of the left otic capsule. The labyrinth was removed from the contralateral otic capsule so that all sensory input came from the left side. Diagrammatic cell bodies are shown together with representative intracellular recordings. Black represents excitation and white represents inhibition. M_1–M_3 (mesencephalic Müller cells); I_1 (isthmic Müller cell); B_1–B_4 (bulbar Müller cells); Mth (anterior Mauthner cells); Mth′ (posterior Mauthner cells). Vibratory stimuli indicated by arrows. Calibrations: (left side) I_1, 5 mV/10 ms; B_1, 20 mV/5 ms; Mth, 20 mV/5 ms; B_3, 2 mV/5 ms; (right side) I_1, 20 mV/5 ms; B_1, 20 mV/5 ms; Mth, 20 mV/5 ms; B_3, 2 mV/5 ms; B_4, 10 mV/10 ms.

hyperpolarizing the cell by as much as 15 mV relative to a 70–75 mV resting potential.

During bilateral vibration of both otic capsules (not shown), the reciprocal inhibition/excitation of M and I_1 cells cancelled. We never observed action potentials (intracellularly or extracellularly recorded) in M or I_1 cells during bilateral vibration. Bilateral stimuli were achieved either by strong vibration of the entire cranium with a single vibratory probe, or by simultaneous vibration of both otic capsules with two separate probes.

Recordings were also made with suction electrodes from a Mth soma during vibration of the ipsilateral otic capsule. A simultaneous recording from the eighth nerve demonstrated a short latency (1–2 ms) between the compound action potential in this nerve and the Mth spike (Fig. 4A), which reflects the monosynaptic, predominantly electrical connection between vestibular afferents and the Mth cell

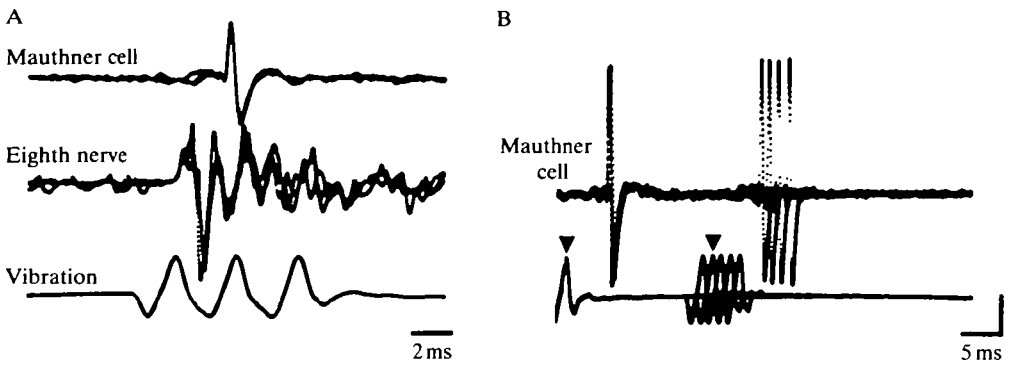

Fig. 4. Extracellular recordings from the ipsilateral brain stem during vibration of an otic capsule. (A) Simultaneous suction electrode recordings of the anterior Mauthner (Mth) cell body spike (upper trace) and the eighth nerve response (middle trace). Bottom trace shows vibration monitor. Three superimposed traces are shown. (B) Twin vibration pulses were delivered with progressively shorter delays while recording over a Mth soma. The minimum interpulse delay which elicited a second Mth spike (indicated by inverted triangles) was 18·5 ms. Of those cells responding to vibration, only the Mth cells had such a brief refractory period. This feature was important in identifying Mth axons. Vertical calibrations: Mth and eighth nerve recordings, 0·2 mV; vibration monitor, 35 μm.

(Rovainen, 1978, 1979*a*). As a result of this monosynaptic connection, Mth cells could respond one-for-one, indefinitely, to vibration pulse frequencies up to 50 Hz. Twin vibration pulses with a short delay (15–20 ms) could thus be used to help identify Mth axons during intracellular penetrations. In the example shown in Fig. 4B, the minimum delay which elicited a second Mth spike was 18·5 ms (indicated by inverted triangles). B_1 and B_2 Müller cells required delays of at least 200–300 ms for a second spike.

The posterior Mth′ cells are small and difficult to see in most preparations. The only recordings from a Mth′ cell were made extracellularly, during bilateral vibration. Both Mth and Mth′ cells have decussated axons extending into the contralateral spinal cord (Rovainen, 1978). Fig. 5 (middle trace) shows the vibration-evoked spinal cord activity which remained after ablating both Mth, B_1 and B_2 cells with a suction electrode (see Materials and Methods) and hemisecting the spinal cord near the brain, ipsilateral to the Mth′ soma. Spikes recorded over the Mth′ soma with a suction electrode (top trace) were time-locked to axonal activity in the contralateral spinal cord, below the hemisection.

Role of Mauthner neurones in the startle response

To determine the role of the anterior Mth cells in startle behaviour, the semi-intact preparation shown in Fig. 6A was used. While recording spinal cord activity and bilateral trunk EMGs, microelectrodes were placed into both Mth axons just behind the brain. In every preparation where we obtained reliable vibration-evoked EMG responses *and* penetrated at least one Mth axon, we were able to elicit a bilateral EMG response with intracellular Mth stimulation ($N = 9$). Vibration of the otic

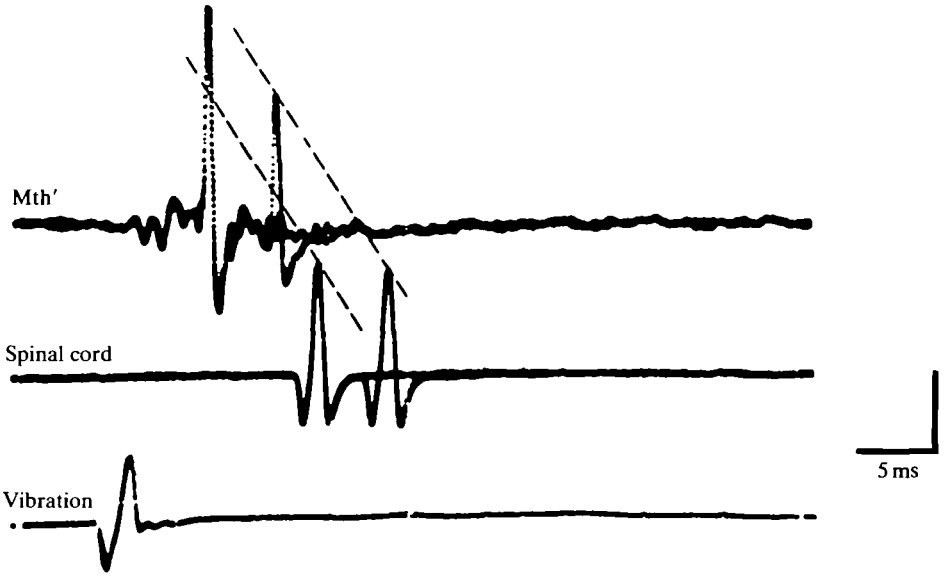

Fig. 5. The posterior Mauthner (Mth') cells are vibration sensitive. Recordings obtained after ablating both the anterior Mauthner and B_1 and B_2 Müller cells and hemisecting the spinal cord near the brain. Extracellular spikes recorded over a Mth' soma (upper trace) were time-locked to units in the contralateral spinal cord, below the hemisection. Three superimposed sweeps are shown. Bottom trace, vibration monitor. Vertical calibrations: upper and middle traces, 0·2 mV; bottom trace, 35 μm.

capsules produced simultaneous Mth spikes followed by the startle EMG (Fig. 6B). Three superimposed responses are shown, demonstrating that the Mth spikes and trunk EMG were time-locked to each other. The left-side EMG spike was consistently larger in this preparation because of the dissimilarity in left and right electrode placements. Immediately following these responses, the left and right Mth axons were individually stimulated. Single spikes in either axon elicited small, bilateral EMG responses (Fig. 6C) but were insufficient to reproduce the vibration-evoked EMG. Fig. 6D shows a control vibration response obtained later in the same experiment. Simultaneous stimulation of both Mth axons (one spike each) reproduced the vibration-evoked response (Fig. 6E). Thus, simultaneous spikes in both Mth cells are sufficient to produce the bilateral EMG amplitudes observed during the startle response to vibratory stimulation.

DISCUSSION

Role of Mauthner cells in the ammocoete startle response

Simultaneous action potentials in both of the anterior Mth neurones are appropriate and sufficient for the bilateral startle EMG in semi-intact, larval lamprey preparations (Fig. 6). Whether lamprey Mauthner cells are also *necessary* for startle behaviour remains in question, however. 'Non-Mauthner'-initiated startle responses

have been demonstrated in zebrafish larvae (Kimmel, Eaton & Powell, 1980; Nissanov, Eaton & Wieland, 1984) and adult goldfish (Eaton, Lavendar & Wieland, 1982; Eaton & Hackett, 1984; Wieland & Eaton, 1984). In lampreys, we found that vibration of the otic capsules excited not only the anterior Mauthner (Mth) cells, but also both B_1 and B_2 Müller cells and both of the small, posterior Mth′ cells. Intracellular stimulation of one or both B_1 neurones does not produce an EMG response (S. N. Currie & R. C. Carlsen, unpublished observation), but B_2 and Mth′

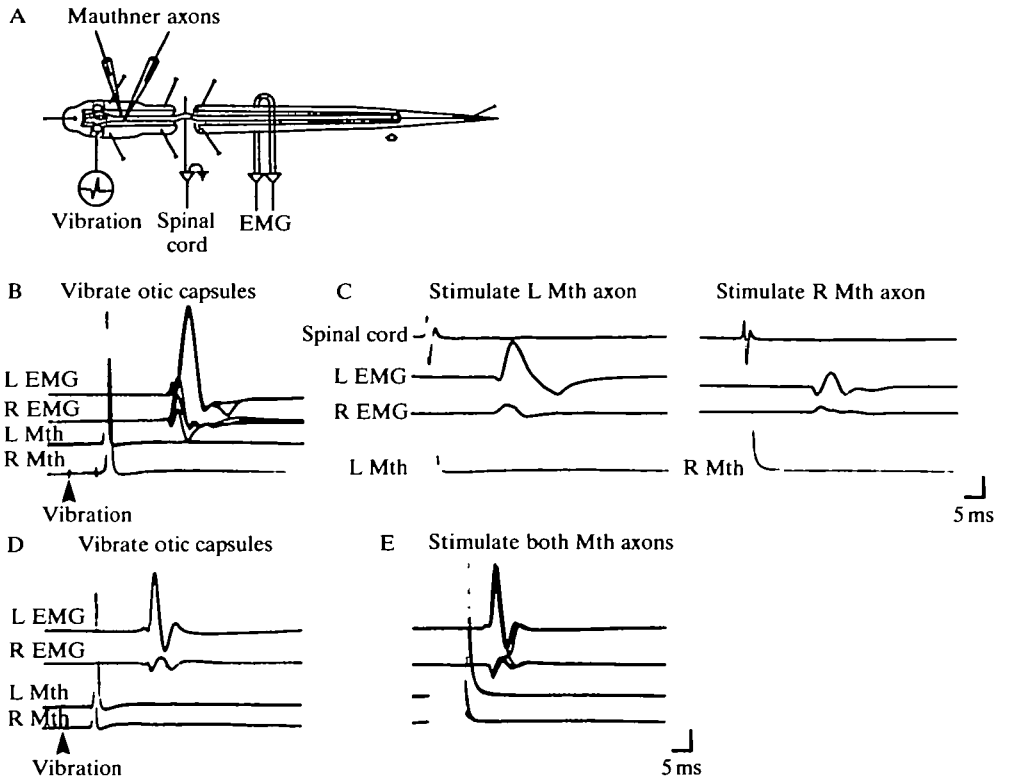

Fig. 6. Simultaneous spikes in both Mauthner (Mth) axons are sufficient for initiating the startle response. Recordings are all from the same preparation. (A) The preparation: an electrically-driven probe was used to deliver the vibration stimulus to the otic capsules while a hook electrode recorded spinal cord activity and EMG electrodes recorded the muscle response from both sides of the trunk (EMG). The arrowhead indicates a caudal spinal transection. Intracellular microelectrodes were placed in both Mth axons near the brain for recording and stimulation. (B) A vibration pulse to the otic capsules elicited spikes in both axons followed by the bilateral trunk EMG. Three superimposed responses are shown. (C) Single spikes in one Mauthner axon alone produced a bilateral EMG, but were insufficient to reproduce the vibration-evoked response. Intracellular stimulation of the left (L) or right (R) Mth axon produced a single spinal unit on the extracellular hook electrode, followed by a bilateral EMG response. Only stimulus artifacts are visible in intracellular Mth traces; bridge balance was difficult to maintain because of the large amount of current necessary to activate the axons. (D) A control vibration response elicited later in the experiment. (E) Intracellular stimulation of both axons (one spike each) reproduced the vibration-evoked EMG. Vertical calibrations: spinal cord and EMG traces, 0·5 mV; intracellular Mth traces, 20 mV.

cells have not been similarly tested. Some of these cells may contribute to startle behaviour in intact ammocoetes.

Functional significance of startle behaviour in burrowed ammocoetes

Applegate (1950) made the observation that slight vibrations (produced by 'footsteps across the floor of a wooden building') caused the retraction of aquarium-held ammocoetes into the depths of their burrows. He further suggested that the vibration resulting from footsteps along a riverbank could be sufficient to elicit withdrawal in animals burrowed in the streambed. Our study is consistent with these observations. Small vibrations evoked rapid withdrawal in burrowed larvae, so that their heads were drawn deeper into the burrow (Fig. 1). The stimulus–withdrawal latency was $37 \pm 1{\cdot}6$ ms ($\bar{x} \pm$ S.D., $N = 11$). The startle EMG preceded and was time-locked to the onset of this withdrawal (Fig. 1E). Our use of a clear, thin-section aquarium and fine, glass beads as a burrowing substrate allowed us to observe the process of active burrowing as well as the final, resting posture of burrowed animals (see Fig. 1A). Burrowing larvae used small, posteriorly propagated undulations to make forward progress through the sand; they moved in spurts and paused to rest frequently. Similar observations were made by Applegate (1950). Once animals had reached their final burrowed position and ceased movement, several lateral bends remained in the trunk and tail. Accompanying the startle EMG and withdrawal was a visible, accordion-like contraction of these multiple body bends. It appears therefore, that Mauthner-initiated startle behaviour produces withdrawal in burrowed animals by contracting pre-existing bends in the trunk and tail. These bends serve to anchor the posterior body while pulling the head down into the burrow.

Posture-dependent modification of startle behaviour

We observed a posture-dependence in ammocoete startle behaviour which explained how burrowed animals achieve their accordion-like body contraction (see above). During a startle response, areas of pre-existing lateral body curvature contracted more and exhibited larger EMG potentials on their inner sides (Fig. 2A–E). Startle behaviour thus increased pre-existing body curvature. When the animal started out with a straight body (Fig. 2F), the startle EMG was weak on both sides and little movement occurred. A similar posture-dependence or 'enhancement reflex' has been observed in semi-intact adult lamprey preparations during the head withdrawal response to oral disc stimulation (McClellan, 1984) and in the tail-withdrawal response following tail fin stimulation (McClellan & Grillner, 1983). In the latter study, the authors point out that an enhancement of pre-existing curvature enables an evasive withdrawal of the tail independent of the initial body posture. Grillner, Rossignol & Wallén (1977) have also observed 'enhancement reflexes' during spinal swimming in the dogfish. Electrical stimulation of the tail fin caused an EMG response in body musculature which varied with the phase of the swimming cycle. Stimuli which were delivered during the contraction phase on one side of the body evoked a further contraction on the same side and no response on the

contralateral side. Responses to tail fin stimulation were thus gated by the central pattern generator for swimming.

We were unable to obtain the posture effect in semi-intact preparations. Left- and right-side EMG amplitudes were unchanged when animals were placed in various curved body postures prior to vibration of the otic capsules. This may have been due to a generally decreased health and responsiveness in reduced preparations.

Two-stage responses

Stimulus-naive larvae often exhibited double EMG responses (Fig. 2G). Double EMGs with similar latencies occasionally occurred in our semi-intact preparations, but their cellular basis is unknown. McClellan (1984) evoked two-stage motor patterns in partially isolated adult lamprey nervous systems; snout or trigeminal nerve stimulation evoked a fictive motor pattern in trunk ventral roots which appeared to represent head withdrawal followed by rapid, forward escape swimming. Such complex responses are reminiscent of the non-giant escape swimming of crayfish, which often follows a giant axon-mediated tail flip (Reichert & Wine, 1982, 1983). They are also similar to the second stage or 'return tail flip' of startle responses in teleosts (Eaton *et al.* 1977; Eaton & Hackett, 1984) and tadpoles (Rock, 1980).

The Mauthner-initiated escape response in larval lampreys activates musculature on both sides of the body in a posture-dependent manner and produces a rapid backward withdrawal movement. Eaton *et al.* (1977) described a bilateral body contraction in spiny eels that was strikingly similar to the startle behaviour of larval lampreys. Subsequent investigations have conclusively demonstrated that Mauthner cells are prominantly but not exclusively involved in initiating the unilateral C-start of short-bodied fish (see Eaton & Hackett, 1984; Eaton & DiDomenico, 1985). Based on the present study, we suggest the hypothesis that Mauthner cells also have significant roles in the startle-withdrawal behaviour of eels and other long-bodied fish, where one or more sites of contraction occur on both sides of the body.

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