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NONINVASIVE RECORDING OF THE MAUTHNER NEURONE ACTION POTENTIAL IN LARVAL ZEBRAFISH

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SUMMARY

We describe the identification of Mauthner (M–) cell action potentials in an intact zebrafish larva, utilizing recording electrodes located outside the fish:

1. The externally recorded spike occurs at approximately the same time, and its waveform changes with recording site in the same way, as the extracellular M-spike recorded within the central nervous system.
2. The externally recorded M-spike may be readily distinguished from other forms of neural activity.
3. The M-spike can be identified in recordings from unrestrained larvae.

This finding permits the direct study of M-cell function in the freely behaving animal.

INTRODUCTION

Some of the most significant advances in understanding the neural basis of behaviour have come from preparations in which it is possible to simultaneously monitor neural activity and behaviour. In such studies, the activity of identified cells has been related to well-defined behavioural responses, e.g. in the crayfish giant fibre system (Wine & Krasne, 1972) or the teleost Mauthner (M-) cell system (Zottoli, 1977; Eaton, Lavender & Wieland, 1981). These experiments have been limited by the need for surgery to implant electrodes, and by the restraints imposed by the recording leads. However, recordings of neuronal activity can be made from outside the body in larval electric fish (Westby & Kirchbaum, 1977) and unrestrained earthworms (Drewes, Landa & McFall, 1978).

In teleosts, a single action potential in one of the pair of M-cells initiates a fast-start escape or 'startle' response (review: Eaton & Bombardieri, 1978). The large size of the extracellular M-cell action potential (Furshpan & Furukawa, 1962; Eaton & Farley, 1975) suggested the possibility of detecting the M-spike outside the fish. In this paper we report such recordings in the larval zebrafish *Brachydanio rerio*. Recently, Bennett & Day (1981) independently recorded the external M-spike in the adult hatchetfish.

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MATERIALS AND METHODS

Animals

Zebrafish were raised from embryos at 28·5 °C as described elsewhere (Eaton & Farley, 1974) and experiments were carried out on the larvae 4–5 days after fertilization. At this stage the larvae are about 3·55 mm in length. Fish without any M-cells or with only one M-cell were obtained by surgical ablation of hindbrain cells in embryos one day after fertilization. Fish treated in this way were then raised in the same manner as the others and the number of M-cells each live larva possessed was determined microscopically (Kimmel, Sessions & Kimmel, 1978). Data were obtained from more than 100 fish.

Physiological recording

Experiments were conducted at ambient temperatures (22–26 °C) with relative humidity maintained near 100% in the vicinity of the preparation. The fish were either freely swimming within a droplet of 10% Hank's saline, or (for most of the experiments) immobilized dorsal side up in a droplet of 13–15% (w/v) gelatin, or in 1·2% agar, in 10% Hanks solution (Eaton & Farley, 1975).

Tungsten microelectrodes (impedance about 2 megohm) were used to detect the M-cell action potential within the hindbrain of the immobilized fish. Tungsten or silver-silver chloride wires, insulated to their tips, served as roving electrodes to monitor field potentials at points external to the fish. In many of the experiments external recordings were obtained with the fish mounted across a copper grid electrode (6 × 8 mm) etched onto a circuit board. The two poles of this device consisted of interdigitating plates, spaced 1 mm apart. It is referred to here as the 'recording grid.' As determined in experiments described below the external M-spike could be recorded simply and most sensitively with a bipolar electrode, consisting of silver-silver chloride poles positioned 2·5 mm apart. Conventional techniques were used to amplify, display and store the signals.

Stimulus

Immobilized larvae were stimulated by an axial excursion of a sharpened capillary tube positioned against the animal's surface (Eaton & Farley, 1975). This probe was mounted either on the cone of a 7·5 cm loudspeaker or on the coil of a Ling 102 vibrator (Ling Electronics, Anaheim, CA). Movement of the probe was elicited with a single square wave pulse. As monitored photoelectrically, the probe exhibited simple damped harmonic motion in response to the pulse, and the peak amplitude of its excursion was proportional to the voltage of the pulse. Axial excursions of 15 μm generally elicited a response. In the case of freely swimming larvae, responses were elicited by vibrating a small chamber (*ca.* 1·5 × 4·5 × 1 mm deep) containing the fish. The bipolar recording electrode was mounted in the floor of the chamber, and the chamber assembly itself was mounted on the cone of a speaker.

Movement detection

In some experiments a silicon phototransistor (Fairchild, FPT 100) was focussed on the head of embedded larva to detect its movement. The response time of the phototransistor was better than 0·1 ms.

RESULTS

1. *The external Mauthner spike*

In the larval zebrafish the M-cell fires a large action potential, as recorded extracellularly, when the animal's surface is vibrated (Eaton & Farley, 1975). The spike is accompanied by a massive contraction of body musculature, thought to reflect the onset of a fast avoidance response (Kimmel, Eaton & Powell, 1980). We show that such responses are also accompanied by a prominent spike which can be recorded outside of the animal's body (e.g. middle trace, Fig. 1 A). The spike was not present during stimulus trials where no behavioural responses occurred (Fig. 1 B). Simultaneously monitoring this external spike and movement of the fish revealed that the spike was not a movement artifact. In Fig. 1 A it is apparent that movement of the animal's head, seen as a deflection (arrow) in the top trace (P), occurred after the appearance of the spike. In 24 of these tests the time interval between spike initiation (which always occurred first) and motion of the head was $2{\cdot}8 \pm 1{\cdot}2$ ms (mean $\pm$ standard deviation).

In a series of experiments with more than 40 fish, the externally recorded spikes detected by the recording grid (see Materials and Methods) lasted about 0·7 ms and achieved their peak amplitudes in about 0·3 ms. They occurred 3–23 ms after the stimulus, with a mean latency of $7{\cdot}8 \pm 4{\cdot}0$ ms.

Eaton & Farley (1975) previously showed in the larval zebrafish that the M-cell fired within a similar range of latencies, and about 2 ms before movement. To verify that the externally recorded spike was that of the M-cell, it was compared with simultaneous intracranial records of the M-spike (Fig. 2). The prominent negative potential (M) recorded intracranially (upper trace) is identical in waveform and amplitude to the spike recorded by Eaton & Farley (1975) and shown by them to be the M-cell action potential. In addition, we confirmed that the amplitude of the intracranial spike was all-or-nothing, not dependent on stimulus intensity (data not shown). The external spike recorded with the bipolar electrode (middle trace) was initiated nearly simultaneously. Such a prominent external spike was never recorded in the absence of a large amplitude intracranial spike. Comparison of their wave forms (Fig. 2) reveals that although the two spikes begin at the same time, the external spike is a differentiated version of the intracranial one, reaching its peak at the moment when the intracranial trace is falling most rapidly, and returning to the base line as the intracranial spike reaches maximum negativity. This difference was independent of recording instrumentation. The differentiation of the internal signal was probably due to the capacitance of the animal's skin.

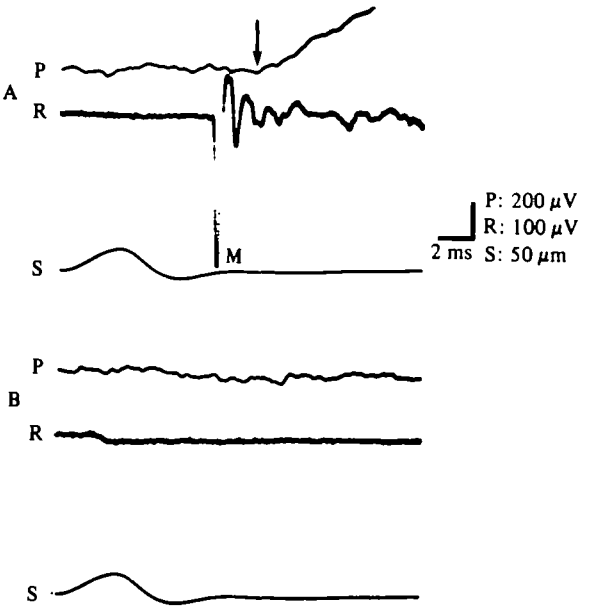

Fig. 1. The externally recorded Mauthner spike precedes movement. Two stimulus trials are shown. In (A) there was an abrupt movement of the fish, and in (B) there was no response. The animal was held within a gelatin droplet over a pair of chloridized silver electrodes. *P* is the output of a phototransistor movement detector focused on the animal's head. *R* is the external recording electrode output. *S* is the axial excursion of the stimulus probe, which was positioned against the animal's side. In (A) the prominent negative spike (M) occurred 8·0 ms after the stimulus began. The animal's movement began about 2 ms later (arrow, trace P).

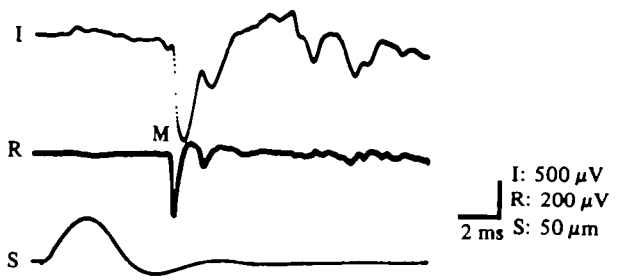

Fig. 2. The intracranially (I) and externally recorded (R) M-spikes occur simultaneously. The M-spikes (M), recorded within the hindbrain with a tungsten microelectrode (I, spike amplitude 1·2 mV), and with the external bipolar silver electrode (R, amplitude 340 μV) both began 6·3 ms after stimulus onset.

2. *The amplitude and shape of the external spike depend on the recording location*

M-spikes should exhibit constant amplitudes and waveforms (Eaton & Farley, 1975) when recorded at a constant location. However, both the amplitude and waveform of the external spike varied considerably in different experiments. We show here that the recording paradigm itself is responsible for most of this variability.

Figure 3 shows external spikes recorded at different locations. A stationary control electrode (F) was placed rostral to the fish to show that spike amplitude remained

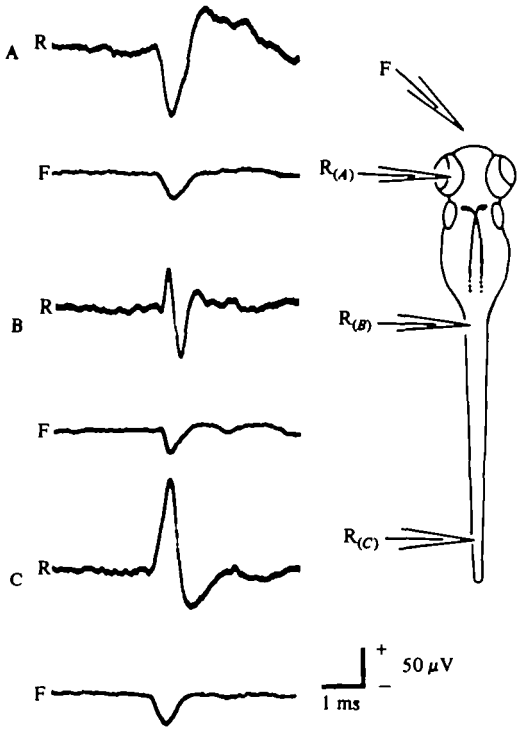

Fig. 3. The amplitude, polarity, and waveform of the external spike are position-dependent. For each pair of traces, the upper record (R) is from a roving electrode positioned near the animal's surface, and the lower record (F) is from a fixed control electrode, rostral to the head, as shown in the drawing. A low amplitude negative M-spike was recorded with the fixed electrode in each case. (*A*) the roving electrode positioned near the eye recorded a negative spike. (*B*) the signal was biphasic at the animal's trunk. (*C*) a large positive spike was detected at the tail.

constant through the course of the experiment. For the record shown in A a roving electrode (R) was positioned just adjacent to the eye, and a negative spike was observed. As the roving electrode was moved caudally towards the ears, the amplitude of these signals increased in magnitude (not shown). More caudally, the spike amplitude decreased in magnitude and changes in its waveform became evident. At a point about halfway between the head and the tail, the roving electrode recorded a biphasic signal (Fig. 3 B). The potential became increasingly positive towards the tail (Fig. 3 C), and then diminished as the roving electrode was moved towards the tip of the caudal fin. Thus the maximum negative spike is observed at a point outside the body near the M-cell somata, and a large positive potential is observed near the end of their axons in the tail.

These data also provide evidence that the spike recorded externally is conducted from the head to the tail. For example, in Fig. 3 A the peaks of the negative spikes in the two traces occur simultaneously. In Fig. 3 B the negative spike recorded about 0·7 mm caudal to the M-cell bodies (trace R) peaks about 0·25 ms after that in trace F, corresponding to a conduction velocity of 3 m/s. The position-dependent changes in the amplitude, shape and polarity of signals detected by the roving electrode approxi-

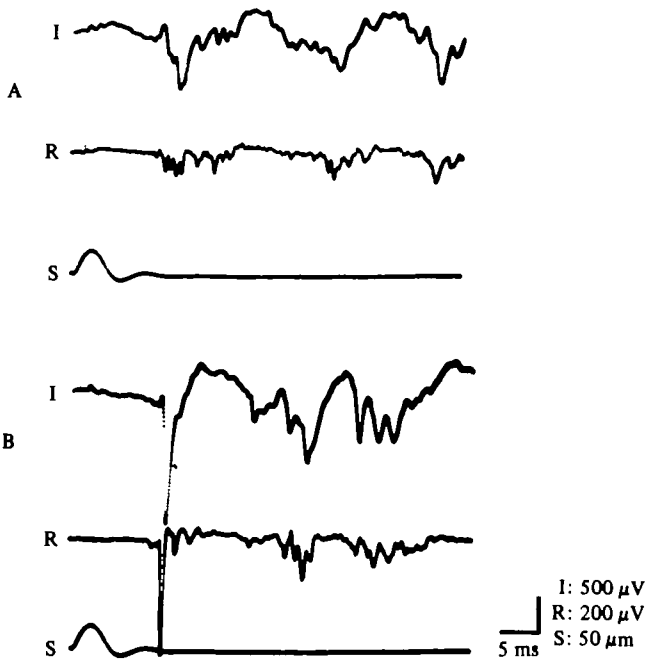

Fig. 4. The M-spike can be distinguished from other neural activity. The traces in (A) show a response in which an M-spike was not detected intracranially (I), and likewise there was no prominent spike in the output of the bipolar external electrode (R). In (B) the M-spike was detected with both electrodes, following another presentation of the stimulus (S).

mate those characteristics of M-spikes in larval zebrafish reported previously (Eaton & Farley, 1975). In addition, Eaton & Farley found that the conduction velocity of the M-spike was 1·7–3·7 m/s.

Finally, these data also reveal how to record the M-spike externally with maximal sensitivity. The potential difference was greatest between the head and tail, so the recordings shown in the figures were all obtained with a bipolar electrode, with one pole near the animal's head and the other near the tail, connected to a differential amplifier.

3. *The external Mauthner-spike can be distinguished from other neural activity*

Some fast-start responses were activated without firing of the M-cell (Fig. 4A), as previously observed (Eaton *et al.* 1977), although the M-cell could fire after further stimulation (Fig. 4B).

To show that we could consistently distinguish the externally recorded M-spike from other neural activity we recorded from larvae that lacked M-cells as a result of surgery during development. Eaton & Kimmel (1980) previously recorded intracranially from such animals and observed responses initiated by units which sometimes attained 50% of the spike amplitude of the extracellular M-spike recorded under similar conditions. In spite of this, however, we found that the M-spike could usually be identified correctly.

Table 1. Fraction of responses accompanied by apparent M-spikes

Status of fish	Number of fish		Stimulus site: Left side	Stimulus site: Right side
Both cells	24	M-spikes[a]	34	36
		All responses[b]	92	86
Right cell only	8	M-spikes	1	15
		All responses	27	32
Left cell only	18	M-spike	13	5
		All responses	85	77
Neither cell	8	M-spike	1	1
		All responses	26	38

All fish were stimulated on each side of the head.

(*a*) Responses resembling those of Fig. 2 were detected by the external recording grid and identified as externally recorded M-spikes.

(*b*) Behavioural responses were revealed by movement of the fish or increases in neural activity in response to stimulation and include responses accompanied by M-spikes.

Fish which had been subjected to surgery as embryos but which retained both M-cells served as controls, and experimental fish were missing one or both M-cells. The stimulus probe was placed on either side of the fish and recordings were made with the recording grid (Table 1). The study was carried out in a single-blind manner; the number of M-cells a given fish possessed was not known to the one of us obtaining these recordings. Spikes significantly larger than the following activity (designated as 'M-spikes' in the table) were frequently identified in controls possessing both M-cells. Similarly, such spikes could be identified in fish with a single M-cell. On the other hand, fish without any M-cells rarely yielded large amplitude, Mauthner-like spikes.

When only one M-cell was present, this cell fired more frequently if the stimulus was ipsilateral to it (Table 1). On an overall basis 24% of the responses to ipsilateral stimuli were accompanied by an M-spike as compared with 6% to contralateral stimuli. This difference was significant ($P < 0{\cdot}001$, Chi-square analysis). Similar findings were reported from intracranial recordings by Eaton & Kimmel (1980).

4. *Mauthner spikes can be recorded from freely-behaving animals*

In the experiments described above the animals were restrained within a droplet of gelatin to hold their positions constant. Recordings were also made from larvae swimming freely in a drop of 10% Hank's saline contained in a small elongate chamber constructed from lucite, with silver-silver chloride electrodes positioned across its floor. Because of the shape of the chamber, the fish within nearly invariably came to rest lengthwise and across the electrode pair after an episode of movement. Since it was difficult to position the stimulus probe against the unrestrained animal, responses were initiated by vibrating the whole chamber assembly.

M-spikes could readily be identified in records obtained this way. In the example shown in Fig. 5A the M-spike is clearly distinguishable from activity that follows it; its amplitude is over 600 μV. Figure 5B shows 10 superimposed presentations of the stimulus. Nine responses occurred, and all of them were initiated by an M-spike. The

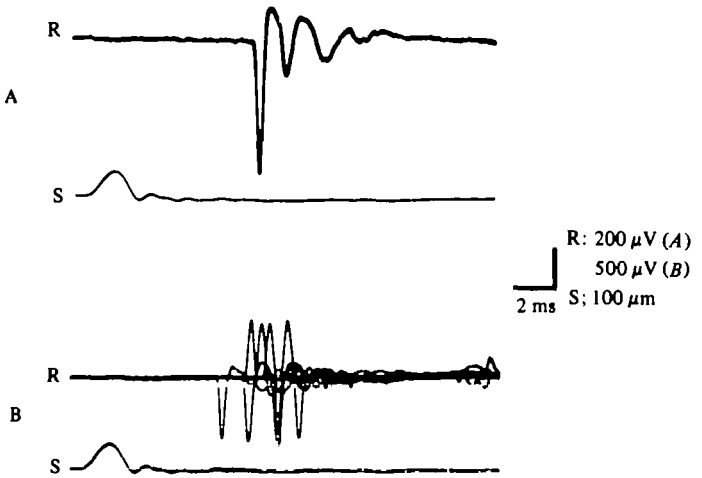

Fig. 5. The M-spike can be identified in recordings from unrestrained fish. The responses (R) were elicited by vibrating the chamber (S) containing the fish, which was free to swim about within a droplet over the recording electrode. A, a single stimulus trial. The M-spike (610 μV) occurred 7·7 ms after the stimulus was initiated. B, ten stimulus trials, to which 9 responses occurred. The latency of the M-spike varied between 6·1 and 9·6 ms.

spike amplitude remained relatively constant, whereas the polarity of the spike reversed from trial to trial. This was because the fish usually turned through 180° during the M-cell initiated response (Kimmel *et al.* 1980), thus coming to rest across the electrodes in the opposite direction after each response.

DISCUSSION

The experiments described here demonstrate the feasibility of identifying single unit activity recorded by a noninvasive technique from outside the body of a vertebrate. The externally recorded Mauthner spikes were highly distinctive, because of their large amplitude. They were also characterized by relatively short durations of approximately 0·7 ms. They occurred in the expected temporal relationship with the stimulus and the onset of fast-start behaviour. Eaton & Farley (1975) reported that the M-spike occurred 6·0–17·5 ms after mechanical stimulation of an otic vesicle and preceded body movements by about 2·0 ms. In the present work, latencies ranging from 3 to 23 ms were observed, and movement of the head occurred approximately 2·8 ms following spike initiation.

In addition, the external spike occurred nearly simultaneously with the internally recorded M-spike. This argues against the possibility that the external spike is a volume-conducted electromyogram of the body musculature. This would be expected to occur at least two synaptic delays (> 1·5 ms) after the M-spike.

The characteristic changes in the waveform of the spike outside the body also supports the view that it is generated by the M-cell. The changes in the shape of the extracellular spike reflect its generation by a neurone having its initial segment in the hindbrain and an axon that extends the length of the spinal cord. Since the potential

field outside an active nerve fibre is partly a function of the fibre's transmembrane current flux (Lorente de No, 1947; Clark & Plonsey, 1966), a large inward current at the axon hillock gives rise to a prominent negative spike in extracellular recordings from this region (see Furukawa & Furshpan, 1962). Changes in the direction and density of transmembrane currents as the action potential propagates caudally produce changes in the shape and amplitude of spikes recorded adjacent to the M-axon. Thus, the axon serves as a current source for the initial segment spike, and recordings from near the axon show a biphasic (positive-negative) spike as the potential propagates into this region. The conduction velocity was determined to be about 3 m/s from the delay between the negative components in the animal's head and trunk. Similar conduction times and changes in waveform were reported by Eaton & Farley (1975) from electrodes inside the body.

All of this evidence supports the thesis that the spikes recorded outside the fish are indeed generated by M-cells. Experiments on fish with no M-cells are consistent with this conclusion and show that M-spikes can be distinguished from other large units. The present findings make it possible to monitor M-cell activity simply, rapidly, and in unrestrained preparations. Furthermore, the traumatic and perturbing effects of dissection and electrode implantation are avoided. These are important considerations for study of neuronal function in behaving animals.

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