

## ELECTROSENSORY THRESHOLDS IN LARVAE OF THE WEAKLY ELECTRIC FISH *POLLIMYRUS ISIDORI* (MORMYRIDAE, TELEOSTEI) DURING ONTOGENY

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### Summary

Electrosensory thresholds and tuning were determined from behavioural studies in larvae of *Pollimyrus isidori* using the stop response of their electric organ discharge to weak electrical stimuli. Two age groups were studied: (1) 10- to 15-day-old larvae in which the electric organ discharge (EOD), produced by a distinct larval electric organ, had just stabilized; (2) 54- to 60-day-old larvae, just before the advent of the adult EOD (an adult electric organ functionally replaces that of the larva between about 60 and 80 days). Three stimulus pulse waveforms were used: (1) single-cycle, bipolar sine-wave pulses; (2) single-cycle, monopolar sine-wave pulses and (3) monopolar square-wave pulses.

The younger larvae were exceedingly sensitive to weak electrical stimuli, down to the  $10 \mu\text{V}_{\text{p-p}} \text{cm}^{-1}$  range. Stimulus pulse duration had a significant effect on threshold for all three pulse waveforms, but the shapes of the tuning curves were quite different. Thresholds at the 'best' pulse duration were lower and the tuning sharper (with a V-shaped curve) with monopolar sine-wave pulses than with bipolar sine-wave pulses. The 'best' pulse duration was 1 ms for both sine-wave pulses, corresponding well to the spectral peak amplitude of larval EODs ( $964 \pm 22$  Hz). The threshold curve for monopolar sine-wave pulses appeared to be perfectly adapted for sensing larval rather than adult EODs. With square-pulse stimuli, thresholds increased monotonically with duration and there was no evidence of tuning for this kind of stimulus. These results suggest that both conventional spectral

tuning and 'tuning' to a particular pulse waveform (with a monopolar sine-wave pulse best approximating the waveform of a larval discharge) are found in young larvae.

In the older age group, larvae were more sensitive to all three kinds of stimuli than those of the younger age group. The sensitivity increase varied from 10 dB to 29 dB; at stimuli of  $2.4 \mu\text{V}_{\text{p-p}} \text{cm}^{-1}$ , larvae just 18 mm long displayed adult sensitivity. No tuning was seen for square-wave pulses and, as in younger larvae, their effectiveness increased monotonically with duration, so that for neither age group are square-wave pulses a good model for larval EODs. The threshold curves for both types of sine-wave pulse were similar and resembled the broadband tuning curves of Knollenorgan electroreceptors. Tuning was present but weak, with sensitivity for the high-frequency range much greater than for younger larvae. This change is adaptive for sensing both larval and adult EODs and occurred *before* the larvae developed an adult EOD.

The mechanism for a change in tuning that has been established for electroreceptors in adult mormyrids and gymnotiforms, where the spectral properties of the EOD of a fish entrain its electroreceptors, is not found in the larvae of *Pollimyrus isidori*, which 'anticipate' the tuning necessary for the reception of their own, future adult EOD.

Key words: electrosensory thresholds, electrosensory tuning, weakly electric fish, Knollenorgan, fish larvae, ontogeny, Mormyridae, *Pollimyrus isidori*.

### Introduction

The elephantfish *Pollimyrus isidori* from tropical African fresh waters is the only mormyrid that has been bred in captivity (Birkholz, 1969; Kirschbaum, 1975, 1987; Crawford *et al.* 1986). The electrical communication behaviour of this fish during reproduction has been studied in detail (Bratton and Kramer, 1989; Crawford, 1991; for more general reviews on mormyrid electrocommunication, see Hopkins, 1988; Kramer, 1990, 1994). The aim of the present study is to further our understanding of the sensory aspects of electrocommunication in the larvae of this fish.

Electrocommunication in *P. isidori* appears to be important not only in adults but also in larvae, since very early in life they develop a prominent larval electric organ. This degenerates in larvae near the age of 2 months, when a permanent, adult electric organ begins to become fully functional. The organs are derived from muscle tissue located in the trunk (for the larval organ) and in the caudal peduncle (for the adult organ; Denizot *et al.* 1978, 1982; Kirschbaum, 1977).

The larvae hatch 4 days after spawning and emit their first

electric organ discharges (EODs) at the age of 8 days, when they are 8 mm long. At 11 days, the larval discharge stabilizes to a waveform that is observed for about 60 days. The larval discharge is almost monopolar, with a head-positive spike lasting approximately 330  $\mu\text{s}$  followed by a weak, head-negative potential (the total duration of an EOD is approximately 1.9 ms; Kirschbaum and Westby, 1975). This contrasts with the adult EOD, which is triphasic, is approximately 10 times shorter and has a mainly head-negative polarity.

The adult organ begins to differentiate in the tail when larvae are only 10 mm long and is operative in fish 18 mm long (60 days). Fish in the size range 18–20 mm have both organs operative simultaneously, as indicated by a peculiar double discharge with each larval EOD followed by an adult EOD at a fixed delay of 1.4 ms (generated by the more posteriorly located adult organ). The larval discharge is no longer present in 25 mm fish (84 days), when the larval organ has totally degenerated (Westby and Kirschbaum, 1977, 1978).

For the present behavioural study of the electrosensory system of the larvae, two ontogenetic stages during their rapid development were chosen: (1) very young larvae 10–15 days old and (2) larvae 54–60 days old with the larval discharge still in operation and the adult organ present though not yet functional. In order to determine both sensory thresholds and tuning properties of the sensory system of the larvae, we used an unconditional discharge stop response, evoked by short trains of electric pulses of low intensity. We addressed the question of whether the electrosensory system is sensitive enough to allow communication even in very young larvae and, if it is, whether there is tuning to the properties of the long larval or to the short adult EOD.

## Materials and methods

### *Breeding and raising larvae*

Adult *Pollimyrus isidori* (Valenciennes) from the laboratory stock (imported directly from Nigeria by a tropical fish dealer near Frankfurt/Main airport) were induced to produce eggs, or, if male, to show territorial behaviour, by imitating some of the conditions of the rainy season (Kirschbaum, 1975, 1987). This was done by lowering water conductivity to 250  $\mu\text{S cm}^{-1}$  after a long period of high conductivity (3 months, 400  $\pm$  50  $\mu\text{S cm}^{-1}$ ). Territorial males were easily recognizable by their nest-building activity and by a ruby-coloured spot at the ventral part of the concave anal fin base (M. Postner, unpublished observation). Gravid females were distinguishable from other fish by their swollen belly, which contained an ochre-coloured package of eggs visible from the left side of the animal, and by their straight anal fin base. Pairs of fish were selected for breeding and were caught without being netted. The fish were transferred to spawning tanks (120–200 l) filled with water of a slightly lower conductivity than in the stock tanks. The spawning tanks were provided with gravel, plants (*Vesicularia dubyana*) and hiding tubes to maintain spawning

readiness. On the night following the transfer, the male often began to defend a territory and to build a nest in a porous pot hiding tube or under pieces of ceramic flower pot. The first spawnings usually occurred 1–6 weeks after the transfer.

Eggs were collected from the nest on the morning after spawning. They were put into small, perforated containers (15 cm  $\times$  8 cm  $\times$  11 cm high) floating in a separate 40-litre raising tank, filled with water of the same conductivity and temperature as those used in the spawning tank (250  $\pm$  50  $\mu\text{S cm}^{-1}$ ; 26.4  $\pm$  0.2  $^{\circ}\text{C}$ ). The floating tanks were aerated and filtered separately. Unfertilized eggs turned white and were removed. Larvae hatched on day 4 after spawning. From day 11–12, when the larvae began exogenous feeding, they were fed newly hatched brine shrimp nauplii (*Artemia salina*) at least twice a day. It was not necessary to feed them first on small chironomid larvae (see Kirschbaum, 1987).

### *Waveform analysis*

The waveforms of the EODs were obtained from larvae between 10 and 42 days old. Test animals ( $N=16$ ) were put into a recording chamber filled with water from the raising tanks. The recording chamber was cylindrical (inner diameter 4.3 cm) and acrylic. A millimetre scale was attached to the bottom of the chamber in order to measure the length of the larvae. The chamber was filled with water to a height of 2.0 cm. In the wall of the recording chamber two orthogonally orientated pairs of carbon rod electrodes were inserted at a height of 1.0 cm. In the case of waveform analysis, only the pair of electrodes orientated rostro-caudally was used. During measurements, the larvae were kept in a plastic mesh cage (diameter 2.5 cm) centred within the recording chamber, in order to keep the fish at a distance from the recording electrodes. The orientation of the animals towards the electrodes was adjusted by gently turning the whole cage with the resting animal inside.

EODs were differentially amplified (100 $\times$ ; 1 Hz to 100 kHz bandpass) and digitized by a storage oscilloscope (Philips DSO 2225, 1 MHz sampling rate, eight-bit resolution, 8 K memory). Digitized waveforms were transferred to a computer (an IBM-compatible PC). Amplitude spectra were calculated with the positive maximum of the pulse in the centre, using a software package for signal analysis (maximum 2<sup>14</sup> data points; Famos by IMC Company, Berlin). At a sampling rate of 31.25 kHz and using 16 K data, frequency resolution was 1.91 Hz.

### *Stimulation experiments*

Electric stimulation experiments were performed in the recording chamber again using the plastic mesh cage. Smaller cages could not be used because the larvae tried to escape and failed to show resting behaviour. Struggling larvae did not respond to the stimulation. Larvae of the young age group were siphoned out of their floating tanks using a flexible plastic tube (diameter 4 mm); larvae of the older age group were caught with a small net or siphoned with a larger plastic tube (diameter 10 mm). After transfer to the recording chamber, the animals were left alone for 5–15 min until they were resting. Animals that did not calm down after that time were not used for

experiments. The stimulation electrodes were orientated transversely to the fish. Trials during which the larvae began to move were discarded. Fish were used for experiments only once a day.

#### Stimulus pulses

For the stimulation experiments, we used three different stimulus pulse waveforms (Fig. 1): (i) bipolar sine-wave pulses representing one full period of a sine wave ( $0^\circ$  to  $360^\circ$ ); (ii) monopolar sine-wave pulses also consisting of a full period of a sine wave, but with the starting phase shifted to  $90^\circ$ ; (iii) monopolar square-wave pulses. At equal peak-to-peak amplitudes, the total energy contents of these waveforms correspond to 1 for the bipolar, 3 for the monopolar sine wave and 8 for the monopolar square-wave pulses. The spectral high-frequency content is highest in the square wave, the edges of which are sharpest, and lowest in the monopolar sine-wave pulse which does not have any sharp edges (Fig. 1).

The stimulus pulses were produced either by an Exact model 126 function generator or by a Hewlett Packard HP-3312A function generator connected to the stimulation electrodes, which were isolated from earth by a Grass-SIU5 stimulus isolation unit (for the square-wave pulses). Single-period sine-wave pulses were isolated from earth by a transformer (bandpass filter 100 Hz to 18 kHz). Stimuli shorter than  $62.5 \mu\text{s}$  were transmitted to the stimulus electrodes directly, referenced against earth. All stimuli from the function generators were attenuated by a Hewlett Packard HP 350D attenuator set. The function generators were externally triggered by computer. This allowed every fish to be subjected to the same stimulus pattern.

#### Threshold determination

The stimulation pattern for threshold determination in both age groups of larvae consisted of ten trains of ten single pulses repeated at 5 Hz, which is within the natural range of larval discharge rates. The trains were separated by pauses of 15 s to prevent habituation. The threshold curves were determined for stimulus pulses ranging from a duration of 4 ms (corresponding to a continuous sine wave of 250 Hz) down to  $15.6 \mu\text{s}$  (corresponding to 64 kHz) in steps of a geometrical series. Thresholds for 1 ms,  $125 \mu\text{s}$  and  $62.5 \mu\text{s}$  stimulus pulses were determined first, and then those below, above and in between were established.

Both discharges and stimulus pulses were picked up by the recording electrodes, amplified differentially (variable amplification 1–1000 $\times$ ; bandpass 5 Hz to 33 kHz) and recorded on a Revox A77 magnetic tape recorder. On the second track, a copy of the stimulus pulse trains presented to the fish was recorded. Fish pulses and stimuli were separated electronically using a WPI 120 window discriminator or a software routine having the same effect. Inter-pulse intervals were measured with a two-channel counter module (50  $\mu\text{s}$  resolution) inside a computer.

Threshold determination began with subthreshold stimulus amplitudes. After every train, the stimulus intensity was

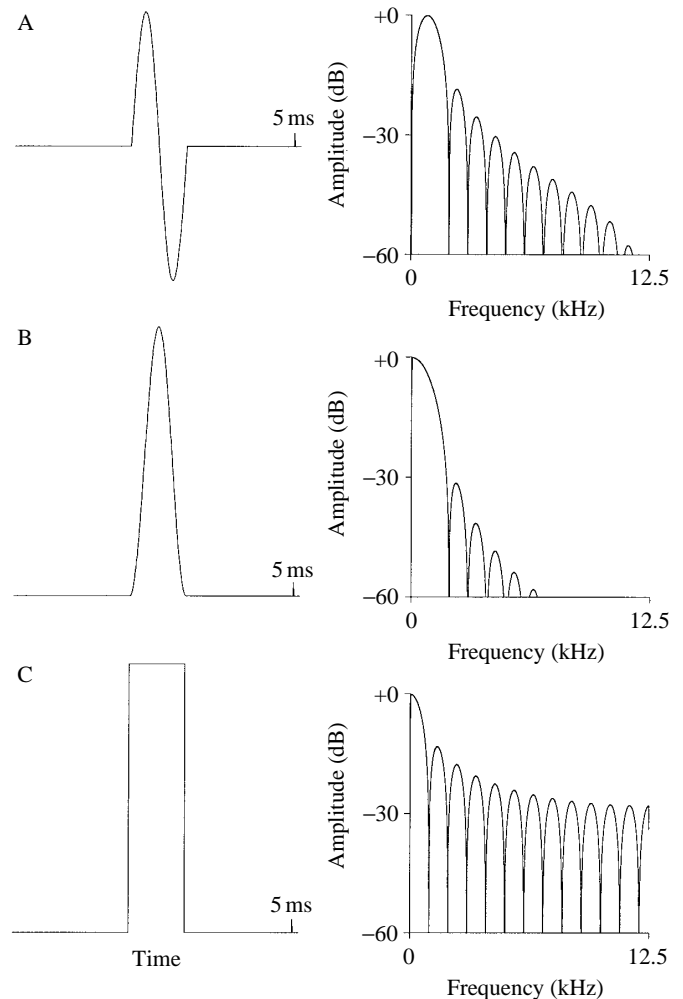


Fig. 1. Pulse waveforms (left-hand side of figure) used for stimulation experiments and their amplitude spectra (right-hand side). All pulses shown are of 1 ms duration and are represented by 1024 data points sampled at 200 kHz. Amplitude is linear in the left-hand diagrams, and is in dB relative to the strongest spectral component in the right-hand diagrams. (A) Single cycle of a sine wave, starting at phase  $0^\circ$  and ending at  $360^\circ$  (bipolar sine-wave pulse). (B) Similar to A, but for this monopolar sine-wave pulse both the starting and ending phases were delayed by  $90^\circ$  and the zero line was adjusted accordingly. (C) Monopolar square-wave pulse. Note that the two monopolar pulses peak at d.c. (or 0 Hz), whereas the bipolar sine-wave pulse peaks at 836 Hz. Higher spectral components decay below 60 dB attenuation for the frequency range shown except for the square-wave pulse, the spectrum of which is cut off at the right-hand side of the diagram.

increased by 6 dB until the larvae responded with a discharge arrest of 1 s (or more) during or after a stimulus train. The stimulus intensity was then lowered by steps of 3 dB until no reaction was measured (staircase method). When, after again raising the stimulus amplitude, an animal had responded to at least three consecutive stimulus trains in series, the stimulus intensity was defined as threshold for the stimulus pulse duration used in the test.

The threshold data were analyzed statistically using the

paired *t*-test, one-way analysis of variance (ANOVA) and regression analysis. For the comparison of two regression coefficients, the methods given by Bosch (1987, p. 182) and Irtel (1993, p. 179) were used.

## Results

### Electric organ discharge analysis

The EODs of 16 larvae with ages ranging from 10 to 42 days were analyzed. Fig. 2 shows superimposed EODs from three larvae of different ages; they are all very similar. A larval discharge has a reversed polarity compared with an adult discharge and is of much longer duration (Fig. 2A). The amplitude spectra of larval fish resembled each other, with amplitudes rising from d.c. to a peak that was usually in the region of 1 kHz (Fig. 2B). The mean peak value for larvae was  $963.6 \pm 21.9$  Hz (S.E.M.;  $N=16$  animals).

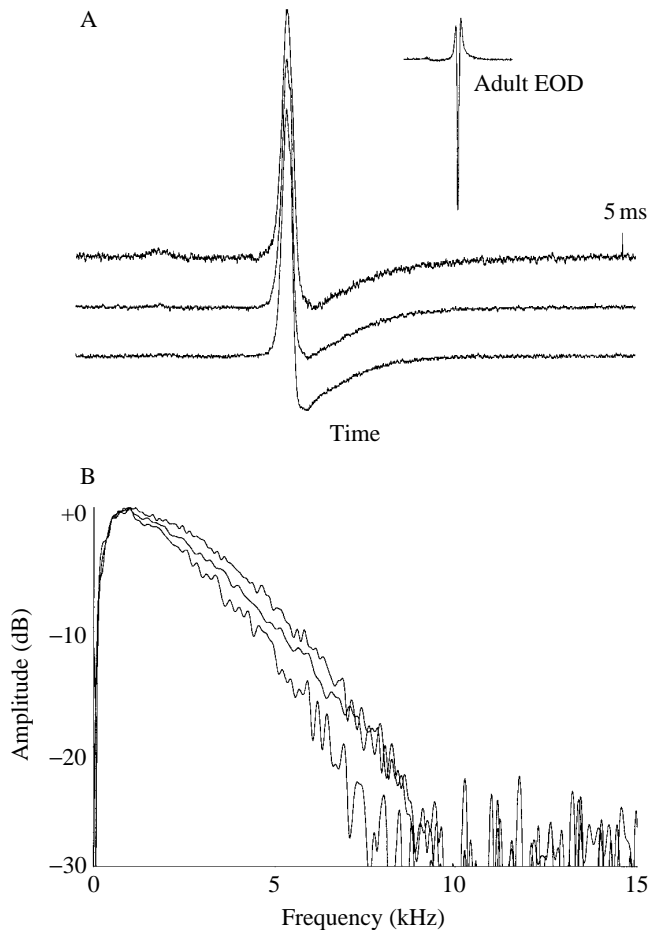


Fig. 2. The electric organ discharges of three larvae at different ages (11-, 20- and 41-day-old; from top) (A) and their amplitude spectra (B). (A) Head-positivity is indicated by an upward deflection. A larval EOD has a reversed polarity and a much longer duration than an adult EOD (inset; male at  $27^\circ\text{C}$  and  $100 \mu\text{S cm}^{-1}$ ; same time scale). (B) The amplitude spectra of the three EODs shown in A. Amplitude is in dB relative to the strongest spectral component. In these examples, the peaks occur at 969, 940 and 998 Hz.

There was a weak increase in the frequency of spectral peak amplitude ( $y$ ) with age ( $x$ ):  $y=4.525x+856.7$ ; the correlation coefficient was 0.5825. The slope of the regression line (S.E.M. 1.688) was significantly different from zero ( $F=7.188$ ,  $P=0.0179$ , d.f.=15), and departure from linearity was not significant.

### Stimulation experiments

From 10 days after they were spawned, young larvae responded to electrical stimulation at amplitudes in the range of those generated by nearby nest mates. On exposure to short trains of electrical pulses, larvae isolated in the recording chamber interrupted their typical resting discharge pattern (Fig. 3). Shortly after the end of a stimulus train, the larvae usually resumed their previous discharge pattern.

This response pattern depended strongly on stimulus amplitude. Stimuli of very low intensity (for example,  $0.12 \mu\text{V}_{\text{p-p cm}^{-1}}=80$  dB attenuation) did not evoke any reaction and the resting discharge pattern continued without interruption. In contrast, at very high stimulus intensities (for example,  $1.2 \text{mV}_{\text{p-p cm}^{-1}}=0$  dB attenuation), the fish often ceased discharging and abruptly began to swim. If the trains were longer than 2–5 s, the fish began to discharge before the stimulation finished and often began swimming. Therefore, the subthreshold intensities which were used first were increased only gradually until the first discharge breaks were observed.

The following experiments were designed to examine the relationship between stimulus pulse duration and threshold stimulus intensity.

### Thresholds of larvae in the young age group (10–15 days)

Three stimulus pulse waveforms were used for threshold determination: square-wave, monopolar sine-wave and bipolar sine-wave pulses (see Fig. 1). For all three waveforms, pulse duration had an extremely significant effect on threshold (as shown by one-way analysis of variance;  $P<0.0001$  in all three cases; Table 1). The curves differ in shape, however. Those for the two sine-wave pulses are V- or U-shaped and have a minimum; the threshold curve for square-wave pulses seems to decrease with pulse duration (Fig. 4).

Table 1. One-way analysis of variance of the dependency of electrosensory thresholds (dB) on stimulus pulse duration (ms)

Stimulus waveform	$P^*$	$F$	d.f.
10–15 days			
Sine, monopolar	<0.0001	12.647	74
Sine, bipolar	<0.0001	5.384	80
Square	<0.0001	10.861	52
54–60 days			
Sine, monopolar	0.0014	3.734	67
Sine, bipolar	<0.0001	11.988	68
Square	0.1254	1.768	34

\*For the hypothesis that variation among threshold means is due to chance.

The data for square-wave pulses can be approximated by linear regression (using data log-transformed on both axes, as shown in Fig. 4). The slope of the regression line was significantly different from zero ( $P < 0.0001$ ; Table 2). Deviations from a linear relationship were not significant ( $P = 0.42$ ) for square-wave pulses but were significant for the two sine-wave pulses ( $P < 0.03$ , bipolar sine-wave;  $P < 0.0001$ , monopolar sine-wave; Table 2) and the threshold curves for the two sine-wave pulses follow a curve rather than a line.

The curves for the two sine-wave pulses resemble the tuning curves found in certain electroreceptors or mechanoreceptors. The 'best' pulse duration was 1 ms; thresholds were

significantly lower by  $7 \pm 2.494$  dB (S.E.M.) for monopolar sine-wave pulses than for bipolar ones in terms of the peak-to-peak voltage of the stimulus ( $t = 2.806$ , two-tailed  $P = 0.0205$ , paired  $t$ -test, d.f. = 9).

Starting from the 'best' pulse duration of 1 ms, thresholds followed a linear increase with decreasing pulse duration for both kinds of sine-wave stimuli (again, linear for data log-transformed on both axes). Departures from linearity were not significant:  $P = 0.554$  for monopolar sine-wave pulses and  $P = 0.577$  for bipolar sine-wave pulses (Table 3). The slope of the regression line for monopolar sine-wave pulses (21.3) was significantly steeper than that for bipolar ones (12.45;  $t = 2.425$ ,

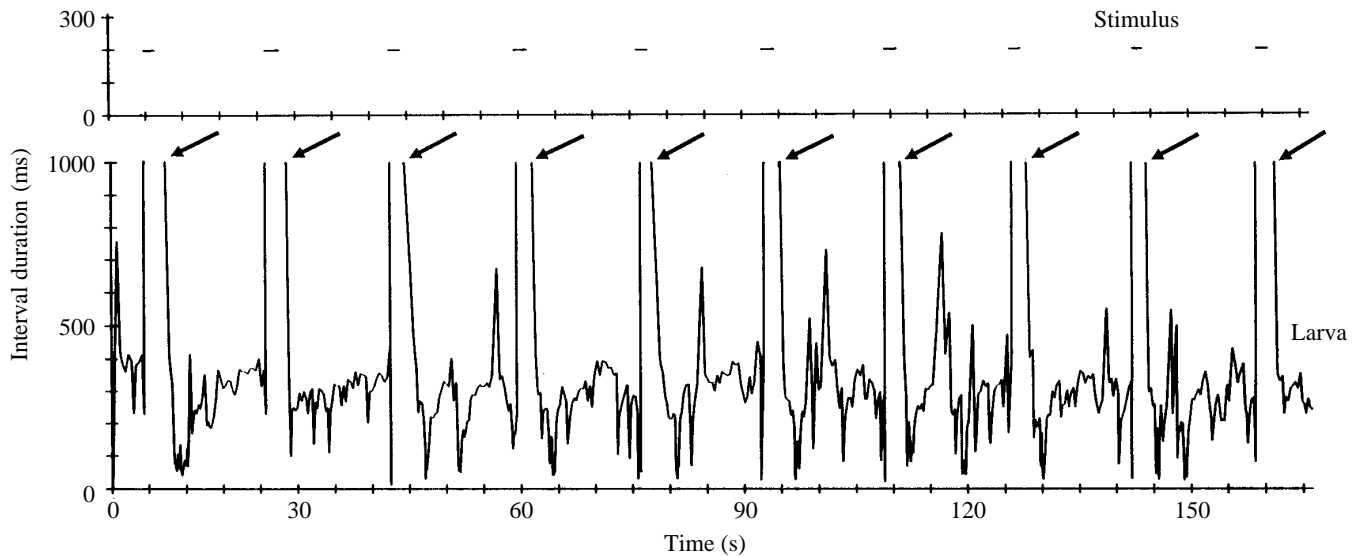


Fig. 3. An example of the behavioural response of a 15-day-old larva to electrical stimulation. Sequential inter-pulse interval plots for the larval EODs (below) and the stimulus (top) during one complete test trial are shown. The abscissa is time, the ordinate represents the duration of inter-pulse intervals (in ms). Ten trains of bipolar sine-wave stimuli were given (ten pulses to a train; pulse duration, 1 ms;  $60 \mu\text{V}_{\text{p-p}} \text{cm}^{-1}$ ) with pauses of 15 s between the trains. The breaks in the discharge of the larva during the stimulus trains are marked with arrows. Intervals lasting longer than 1 s are off the scale.

Table 2. Linear regression of the dependency of electrosensory thresholds (dB) on stimulus pulse duration (log<sub>t</sub>, where  $t$  is in ms)

Stimulus waveform	Linear regression						Test of nonlinearity		
	Slope	S.E.M.	$r^*$	$F$	$P^\dagger$	d.f.	$P^\ddagger$	$F$	d.f.
10–15 days									
Sine, monopolar	12.147	1.814	0.6169	44.855	<0.0001	74	<0.0001	5.364	74
Sine, bipolar	6.703	1.302	0.5011	26.491	<0.0001	80	0.0293	2.307	80
Square	11.388	1.208	0.7972	88.921	<0.0001	52	0.4236	1.038	52
54–60 days									
Sine, monopolar	0.4548	1.194	0.04682	0.1450	0.7046	67	0.0008	4.240	67
Sine, bipolar	2.434	1.081	0.2651	5.065	0.0277	68	<0.0001	12.135	68
Square	3.316	1.173	0.4526	7.984	0.0082	32	0.5013	0.9305	32

\*Correlation coefficient.

$^\dagger$ For the hypothesis that the slope of a regression line equals zero [based on an ANOVA that compares variation due to linear regression (model) with deviations from linearity (residual)].

$^\ddagger$ For the hypothesis that the data deviate from a linear model (based on an ANOVA that compares deviations from linearity with scatter among replicates).

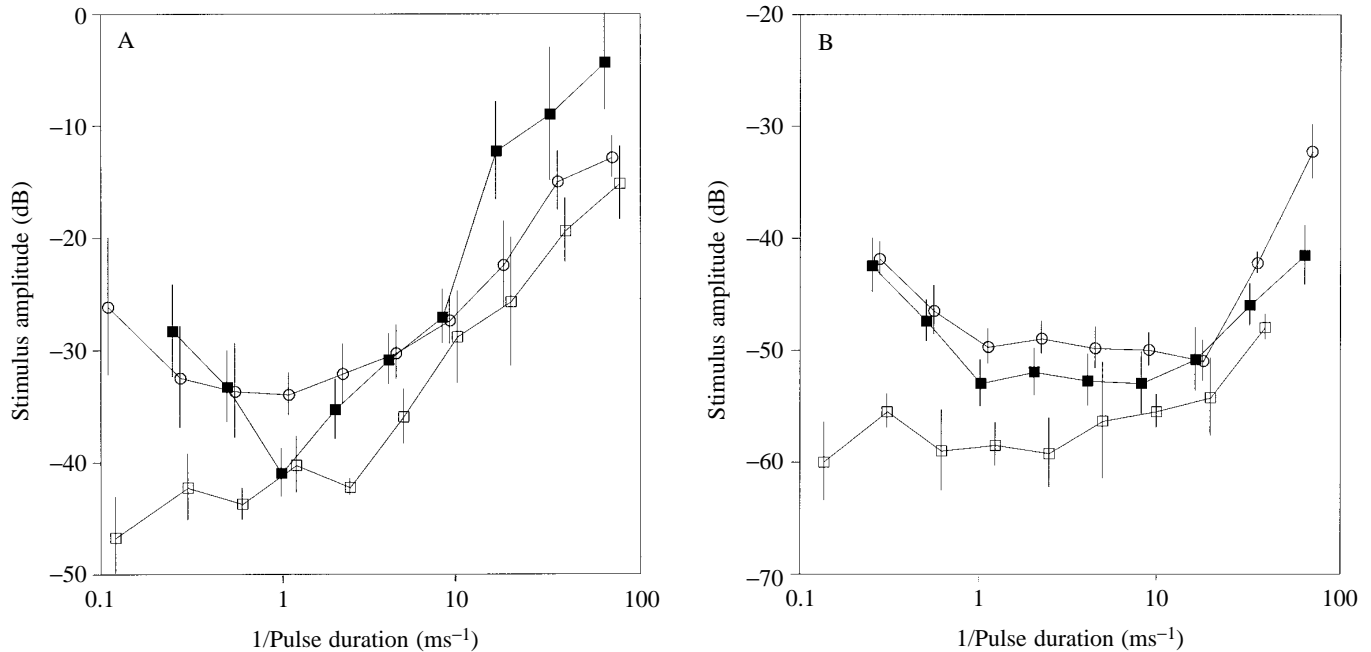


Fig. 4. The dependency of behavioural thresholds of larvae of *Pollimyrus isidori* on the duration of single-cycle stimulus pulses of different waveforms; monopolar sine wave (■), bipolar sine wave (○) and square wave (□). Mean values  $\pm 1$  S.E.M. are shown. Means for the bipolar sine wave and square wave are displaced slightly to the right in order to allow error bars to be shown. Abscissa, the reciprocal of pulse duration ( $\text{ms}^{-1}$ ), which can be read as kHz (if the stimulus had been continuous); ordinate, mean thresholds (in dB re  $1.2 \text{ mV}_{\text{p-p}} \text{ cm}^{-1}$ ) at a given stimulus pulse duration (ranging from  $15.62 \mu\text{s}$ , corresponding to  $64 \text{ kHz}$ , to  $10 \text{ ms}$ , corresponding to  $0.1 \text{ kHz}$ ). (A) 10- to 15-day-old larvae ( $N=10$ ); (B) 54- to 60-day-old larvae ( $N=10$ ).

Table 3. Linear regression of the dependency of electrosensory thresholds (dB) on stimulus pulse duration (logt, where t is in ms) for selected parts of the data

Stimulus waveform	Linear regression						Test of nonlinearity		
	Slope	S.E.M.	$r^*$	F	$P^\dagger$	d.f.	$P^\ddagger$	F	d.f.
10–15 days <sup>a</sup>									
Sine, monopolar	21.301	2.092	0.8083	103.64	<0.0001	56	0.5541	0.8012	56
Sine, bipolar	12.449	1.550	0.7317	64.544	<0.0001	57	0.5771	0.7681	57
54–60 days <sup>b</sup>									
Sine, monopolar	1.019	2.507	0.06667	0.1652	0.6868	38	0.9446	0.1252	38
Sine, bipolar	-1.129	1.644	-0.1137	0.4717	0.4966	37	0.9553	0.1073	37

\*Correlation coefficient.

$^\dagger$ For the hypothesis that the slope of a regression line equals zero [based on an ANOVA that compares variation due to linear regression (model) with deviations from linearity (residual)].

$^\ddagger$ For the hypothesis that the data deviate from a linear model (based on an ANOVA that compares deviations from linearity with scatter among replicates).

<sup>a</sup>Data for stimuli of 1–64 kHz.

<sup>b</sup>Data for stimuli of 1–16 kHz.

two-tailed  $P < 0.02$ , d.f.=111). Best tuning, therefore, was for the monopolar sine-wave pulses. The lowest threshold for monopolar sine-wave pulses occurred at  $10.9 \mu\text{V}_{\text{p-p}} \text{ cm}^{-1}$  for pulses of 1 ms duration.

#### Thresholds of larvae in the older age group (54–60 days)

All threshold curves were at least 10 dB lower than those for the younger age group. The lowest threshold for one of the

sine-wave pulses was  $2.4 \mu\text{V}_{\text{p-p}} \text{ cm}^{-1}$  for a monopolar pulse of 1 ms duration.

As in the younger age group, stimulus pulse duration had a significant effect on threshold, except for the square-wave data, where ANOVA failed to show an effect ( $P=0.125$ ; Table 1). However, a more powerful analysis, linear regression, showed that the (log-transformed) square-wave data can be approximated by a linear relationship (as had also been

observed in the younger age group). The slope of the regression line (3.316) differed significantly from zero ( $P=0.0082$ ), and the deviations from linearity were not significant ( $P=0.501$ ; Table 2). The slope was also significantly smaller than that for 10- to 15-day-old larvae which had been determined as 11.388 (difference significant with  $t=3.0406$ , two-tailed  $P<0.01$ , d.f.=82).

In contrast, the deviations from linearity for the thresholds of the two sine-wave pulses were extremely significant; these data, therefore, follow curves rather than a line. The two curves resemble each other, although thresholds for monopolar sine-wave pulses were generally somewhat lower than for bipolar ones. In contrast to the younger age group, both curves show only weak tuning, with a wide 'best' range of stimulus pulse durations from about 62.5  $\mu\text{s}$  to about 1 ms. For that part of the curves, stimulus pulse duration had no effect on threshold, as shown by slopes of linear regression lines that were not significantly different from zero, with deviations from linearity being not significant (Table 3).

### Discussion

Larvae just 10–15 days old, whose electric organ discharge had just stabilized, showed an extraordinary electrosensitivity in the  $10 \mu\text{V}_{\text{p-p}} \text{cm}^{-1}$  range, as demonstrated by their ability to stop their electric organ discharge in response to weak electrical stimuli. This indicates there is very little delay between the maturation of the motor and the sensory parts of the electric system. A parallel case is the rapid development of the weakly electric gymnotiform fish *Eigenmannia* (Kirschbaum, 1977; Kirschbaum and Westby, 1975; Hagedorn *et al.* 1988; Kirschbaum and Denizot, 1975; Lannoo *et al.* 1990; Viete and Heiligenberg, 1991; Vischer, 1989).

#### *Development of electrosensory threshold*

Knollenorgane, low-threshold receptors in mormyrids used in communication (see reviews by Zakon, 1988; Hopkins, 1988), are the most likely receptors involved in the unconditional discharge stop response studied in the present paper. Knollenorgane of an apparently functional nature, containing glycogen, were seen in larvae only 8 days old (Kirschbaum and Denizot, 1975). Mormyromasts, which have a high threshold, and ampullary receptors that only respond to low spectral frequencies, only appear in 13-day-old larvae (J.-P. Denizot, personal communication).

The second age group (larvae 54–60 days old) showed even lower thresholds ( $2.4 \mu\text{V}_{\text{p-p}} \text{cm}^{-1}$ ) and broader tuning (see below) than the younger larvae. A lower threshold may be explained by two factors: (1) a greater body size (older fish sample a greater potential difference in an electric field) and (2) an increase of the number of Knollenorgane during ontogeny.

Larvae that were 10 days old had fewer than 20 Knollenorgane, all located on the head (present study). The number of Knollenorgane in adult *P. isidori* has apparently not yet been determined, but the adult *Pollimyrus fasciiceps*, a

fish similar in size to *P. isidori*, has 105 Knollenorgane (Quinet, 1971). Our older larvae had almost the same absolute sensitivity as that observed in adult *Brienomyrus niger*, that is  $1 \mu\text{V}_{\text{p-p}} \text{cm}^{-1}$  (Moller *et al.* 1989), despite their small size of about 18 mm. This shows that beyond the age of 60 days there is very little increase in sensitivity, even with a drastic increase in size.

#### *Development of electrosensory tuning*

In the younger age group, the effect of stimulus pulse duration was much stronger than in the older age group. This was seen for all three stimulus waveforms. For example, with square-wave pulses, the linear regression lines that are adequate descriptions of the dependency of threshold on stimulus duration had significantly different slopes: the dependency was weak (but significant) in the older larvae whereas it was strong in the younger age group (Table 2).

The thresholds for the two sine-wave pulses followed curves rather than lines and showed tuning (Table 2). The curves for the younger age group were narrowly tuned to a low frequency, whereas those for the older age group showed broadband sensitivity up to very high frequencies.

The electrosensory system of the younger age group appears adapted to the reception of larval EODs for at least three reasons: (1) the 'best' stimulus duration of 1 ms corresponds well with the spectral amplitude peak of larval EODs, and not at all with that of adult EODs (Fig. 2); (2) monopolar sine-wave pulses, resembling larval EODs in waveform, were associated with significantly lower thresholds at the 'best' stimulus pulse duration of 1 ms; and (3) tuning was more pronounced for monopolar sine-wave pulses than for bipolar ones (as shown by regression analysis, Table 3).

The fact that a monopolar sine-wave pulse was a more effective stimulus than a bipolar one is surprising when one considers an electroreceptor as a spectral filter (Scheich *et al.* 1973; Hopkins, 1976): a bipolar pulse of 1 ms duration has its spectral amplitude peak at 0.84 kHz, which is close to that of the larval EOD, and should, therefore, be the most effective stimulus. In contrast, a monopolar sine-wave pulse of the same duration peaks at 0 Hz or d.c. (as do all monopolar pulses) and is attenuated by 4.1 dB at 0.84 kHz (corresponding to 62.3% of its peak amplitude). The significantly steeper high-frequency filter slope determined for the monopolar sine-wave pulse compared with the bipolar one (Table 3), together with the lack of tuning for the monopolar square-wave pulses (Table 2), shows that, besides conventional spectral tuning (a frequency domain concept), there must be also some degree of 'tuning' to a certain waveform in the electroreceptive system of the younger larvae (a time domain concept).

The older age group showed little difference in threshold curves for the two kinds of sine-wave pulses (although thresholds for monopolar sine-wave pulses were almost always slightly lower): between 1 and 16 kHz, threshold levels showed no dependency on stimulus pulse duration and the curves were flat (Table 3). These threshold curves are, therefore, well adapted for the reception of adult EODs, the spectral peaks of

which are at much higher frequencies than larval EODs (8–25 kHz, depending on the individual fish, Bratton and Kramer, 1988).

Broadband tuning has also been reported for Knollenorgan responses in adult mormyrids (Hopkins, 1981). Knollenorgan tuning curves for adult *P. isidori* had 'best' frequencies up to 18 kHz (Hopkins, 1981), a value very close to the high-frequency cut-off determined in the present paper (Fig. 4B). It should be noted, however, that Hopkins used 100 ms tone bursts for stimulation and measured the responses as a just-noticeable increase in spontaneous firing activity of primary receptor fibres.

Thresholds in our larvae of the older age group were much less sensitive for the waveform of a stimulus than those of the younger age group, but the lack of tuning for square-wave pulses was also found here (Table 2). This confirms that, of the three stimulus pulse waveforms used (and in spite of their effectiveness, which was actually the highest, corresponding to their high energy content), square-wave pulses are those for which the system is least suited.

How is the close match (determined behaviourally or electrophysiologically) of tuning properties and amplitude spectra of EODs explained? In adult mormyrids (Bass and Hopkins, 1984) as well as in the gymnotiform *Sternopygus* (Meyer and Zakon, 1982) the EOD of an individual fish determined the tuning of its electroreceptors: a hormonally induced change in the spectral properties of the EOD was followed by an appropriate change in receptor 'best' frequency. A fish showed tuning to itself by entrainment.

However, our 54- to 60-day-old larvae still had their larval EOD as their only EOD and it was not followed by an adult EOD (as is seen during the short transitional period when the larval electric organ begins to degenerate and the discharge of the adult organ rapidly increases in amplitude after completing its long development). Since the change in tuning properties of the larval electrosensory system occurred before the larvae produced an adult discharge, reafferent sensory influence from its own adult discharge is impossible. The larvae were tuned to themselves but also to the EODs of adults.

For the younger age group, tuning to themselves was so marked and occurred at such an early stage in ontogeny (virtually simultaneously with the EOD, if not earlier) that the entrainment hypothesis, although firmly established for adults, seems unlikely here. It would be interesting to know whether, without auto-stimulation by the larval EOD during early development, the larval electrosensory system would have the same properties.

The change in tuning properties, observed during the ontogeny of our larvae, could be brought about by the addition of Knollenorgane of a second physiological type with high-frequency filter properties. There is evidence for two types of Knollenorgane with different tuning characteristics in the adults of several mormyrids, such as *Brienomyrus brachyistius* (Bass and Hopkins, 1984).

The larvae are guarded by their father until the advent of their adult EOD, when they are evicted aggressively from the

nest (Westby and Kirschbaum, 1978). The change in tuning properties is adaptive for sensing both the EODs of their father and those of other adults, who are dangerous predators for the larvae. The ontogeny of the larval electrosensory system undergoes changes whose timing coincides well with this critical event.

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