

## COUNT AND SPARK? THE ECHO RESPONSE OF THE WEAKLY ELECTRIC FISH *GNATHONEMUS PETERSII* TO SERIES OF PULSES

STEFAN SCHUSTER\*

*Institut für Biologie I, Hauptstrasse 1, Albert-Ludwigs-Universität Freiburg, D-79104 Freiburg, Germany*

\*e-mail: schustef@ruf.uni-freiburg.de

*Accepted 30 January; published on WWW 28 March 2001*

### Summary

Weakly electric fish of the pulse type electrolocate objects in the dark by emitting discrete electric organ discharges (EODs) separated by intervals of silence. Two neighbouring pulse-type fish often reduce the risk of discharging simultaneously by means of an ‘echo response’: one fish will respond to a neighbour’s EOD with a discharge of its own following at a fixed short latency so that its EOD will occur long before the next EOD of its neighbour. Although working elegantly for two partners, this simple strategy should fail in larger groups because two fish could discharge in response to the same EOD of a third fish. Here, I show that the mormyrid fish *Gnathonemus petersii* could use a simple mechanism to reduce this problem. Individuals were stimulated with two closely spaced pulses, the second following so as to coincide with an echo given in response to the first. All the fish examined were able to respond more to the second pulse so

that most of their echoes did not collide with the second pulse. An analysis was made of how echoing more to the second pulse depends on (i) the delay at which the stimulus followed the last spontaneous EOD, (ii) the spontaneous firing rate, (iii) the intensity of the stimulus, (iv) the number of stimulus pulses, (v) the interval between stimulus pulses, and (vi) the level of previous stimulation with double pulses. The results suggest that echoing more in response to the second pulse is probably because the first pulse causes an after-effect whose inferred properties would be compatible with the properties of the mormyromast afferences thought to be involved in the echo response.

Key words: weakly electric fish, mormyrid, *Gnathonemus petersii*, mormyromast, central command nucleus, jamming avoidance, communication, electric organ discharge, electrolocation.

### Introduction

Weakly electric fish electrolocate objects in the dark by discharging their electric organs and monitoring the spatiotemporal distribution of the associated transepidermal current (Heiligenberg, 1977; Heiligenberg, 1991; Moller, 1995). In pulse-type fish, the electric organ discharges (EODs) are much shorter than the intervals between successive EODs. The classic behavioural work of Heiligenberg (Heiligenberg, 1974; Heiligenberg, 1976; Heiligenberg, 1977; Heiligenberg, 1991) has shown, in both South American and African pulse-type fish, that their ability to electrolocate is seriously impaired when the discharges of two fish continuously collide (for historical background and earlier studies suggesting this, see Moller, 1995). Many pulse-type fish show a so-called ‘echo’ response (also known as a ‘preferred latency response’) that is presumed to reduce the probability of EOD collisions. In this response, one of the two partners fires an EOD at a fixed short latency just after that of its conspecific so that its EOD occurs in a period when its conspecific will probably be silent (Bell et al., 1974; Heiligenberg, 1974; Heiligenberg, 1976; Heiligenberg, 1977; Kramer, 1974; Russell et al., 1974). In addition, the echo response might also function as an important communication signal (Russell et al., 1974; Bell et al., 1974;

for critical reviews, see also Kramer, 1990; Moller, 1995). Whatever its main function, the echo response would be compromised in groups of more than two fish since two fish could echo the same EOD of a third fish. Given that both respond with the same fixed latency, their echoes would be sufficiently close in time to seriously impair the electrolocation of both echoing fish. Thus, without further protection from echoing the same EOD, echo responses would increase rather than decrease electrical jamming.

Several mormyrid fish are known to be gregarious, at least as juveniles (Hopkins, 1980; Hopkins, 1986; Moller, 1976; Moller, 1995; Moller et al., 1982; Poll, 1959), and the echo response seems to be widespread among them (e.g. see Moller, 1995). One might therefore ask whether these fish have developed any solution to the problem of two or more fish echoing the same EOD. The echo response has been studied in detail in the mormyrid fish *Gnathonemus petersii* (Bauer and Kramer, 1974; Bell et al., 1974; Russell et al., 1974; Kramer, 1974), and much work has been done on the initiation of the motor command and the electromotor pathway (Bennett, 1971; Bell, 1986; Grant et al., 1999). The study of Russell et al. (Russell et al., 1974) offers a simple way of analyzing the

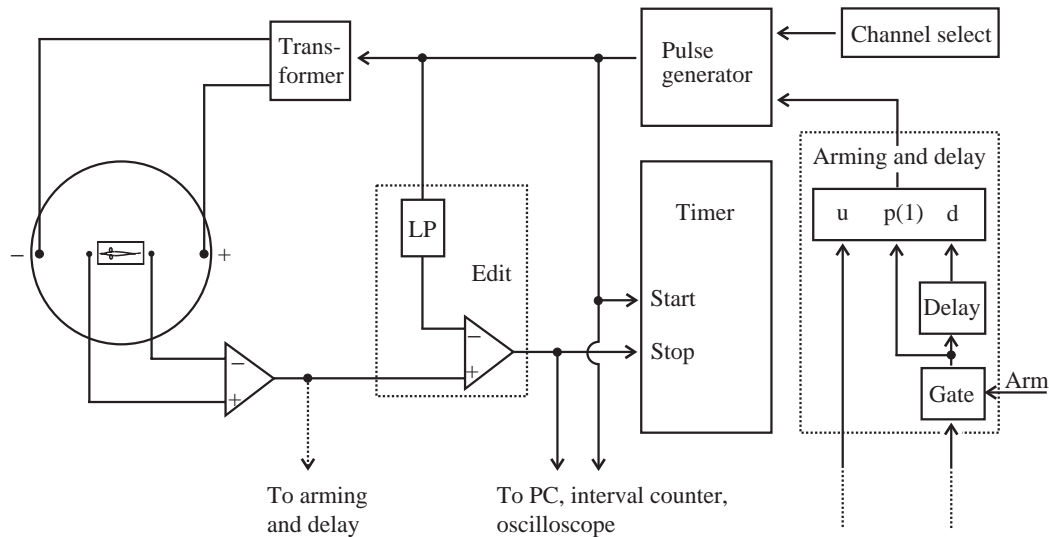


Fig. 1. Experimental arrangement. A fish is placed centrally within a cage in the centre of a cylindrical tank. Head-negative electrical stimulus pulses ( $25 \text{ mV cm}^{-1}$ ,  $300 \mu\text{s}$  duration) are applied by two carbon rods, isolated by a transformer. The head-to-tail electric organ discharge (EOD) of the fish is recorded by two Ag/AgCl electrodes. Stimulus type, either a single pulse or two closely spaced pulses, could be automatically selected *via* a 'channel select' circuit. The recording electrodes detect the EOD plus the stimulus pulses. Separation, when the two coincide, is achieved by suitably low-pass (LP) filtering and subtracting the stimulus from the recording ('edit'). The 'timer' circuit determines the time between the onset of the stimulus pulse train and the first subsequent EOD of the fish under test. The 'arming and delay' module ensures stimulation at a maximal rate of  $1 \text{ s}^{-1}$  and that stimulus pulses will start with a fixed delay (100 ms) after the last EOD. Inputs  $u$ ,  $p(1)$ ,  $d$  of the counter module are used to increase, to set to 1 or to lower, respectively, its value. See text for further details.

present problem using an extension of their approach: stimulating a fish with two pulses, separated by the echo interval, could reveal how a fish would respond to a pair of two echoing conspecifics. 'Meaningful' behaviour would require either that the fish respond more to the second of the two pulses or delay its response to the first. It will be shown that the fish are able to respond more to the second of two identical pulses. Surprisingly, this meaningful behaviour might derive from the known properties of the electroreceptors that are probably involved in the echo response (Russell et al., 1974). In addition, prior experience of double pulses increases the preference of the fish for responding to the second pulse.

## Materials and methods

### Experimental animals

Twenty juvenile *Gnathonemus petersii* (Gthr.) of both sexes ranging in length from 70 to 120 mm were used in this study. Fish were purchased from Aquarium Glaser, Rodgau, Germany. All showed the echo response to artificial stimuli as described by Russell et al. (Russell et al., 1974). A group of 10 fish was kept in a large tank ( $200 \text{ cm} \times 50 \text{ cm} \times 50 \text{ cm}$ ; length  $\times$  depth  $\times$  height), 10 others were kept individually in smaller tanks ( $60 \text{ cm} \times 30 \text{ cm} \times 30 \text{ cm}$ ). The tanks were equipped with standard heating and filtering equipment. Water conductivity was  $115\text{--}280 \mu\text{S cm}^{-1}$ , pH 6.5–7.5 and temperature  $25\text{--}26^\circ\text{C}$ . Fish were kept on a 12h:12h L:D photoperiod with experiments conducted in subdued light (approximately 10–20 lx at the water surface) during the light period.

### Experimental arrangement

Fig. 1 illustrates schematically the modules used to control stimulation of a fish with artificial pulses. The 'arming and delay' module allowed delivery of stimuli at a rate of less than  $1 \text{ s}^{-1}$  at preset delays from the last pre-test EOD of the fish. A gate (Squib-B, WPI) was activated once per second so that an EOD of the fish could trigger, after a preselected delay, the output of pulses from a generator ('delay'; one channel of a Master-8, AMPI). A custom-designed counting module (Max-Planck-Institut für Biologische Kybernetik, Tübingen, Germany) delivered transistor/transistor logic (TTL) pulses, if and only if, the fish did not discharge during the delay period. This was achieved by presetting the module [*via* input  $p(1)$  in Fig. 1] to a value of 1 when the gate was active, allowing EODs to raise (*via* input  $u$ ) the preset value and the delayed pulses to decrease it (*via* input  $d$ ), the decrease leading to a TTL output only if the preset value had not been increased by a spontaneous EOD. Stimuli were generated by a multichannel pulse generator (Master-8, AMPI).

In some experiments, double-pulse stimuli were randomly interspersed among single-pulse stimuli at a preselected probability. This was achieved by driving a solid-state switch ('channel select'; MAX 325) by a TTL signal with an appropriate duty cycle so as to convey the experiment-trigger signal (produced by the counting module) to either one of two channels of the pulse generator to release either a single pulse or a double pulse.

Stimuli were delivered to the fish *via* a transformer and two carbon rods (0.8 cm diameter, length 20 cm) placed vertically

at the periphery of a cylindrical tank (polyvinyl chloride, diameter 45 cm, height 26 cm) filled to a height of 10 cm with water from the home tank of the fish to be tested. The fish was placed with its snout and tail facing the stimulating electrodes in a cage in the centre on the bottom of the tank. The inner dimensions of the cages used were 90 mm×28 mm×30 mm and 310 mm×31.5 mm×31.5 mm. The frame of both cages was made of 4 mm Plexiglas, covered with plastic mesh (square openings of approximately 1.6 mm, separated by approximately 0.1 mm). Adjustable insertions (made of the plastic mesh) confined the fish in the centre of the larger cage. Stimuli were head-negative (rectangular) pulses of 300 μs duration and with a field strength usually of 25 mV cm<sup>-1</sup>. Field strength was measured in the empty cage with two silver wires 1 cm apart (insulated except at their tips). In both cages, field strength did not vary by more than 2% within the space available to the fish. EODs were picked up by two Ag/AgCl electrodes placed approximately 1 cm from either end of the fish and amplified (fivefold, 0.03 Hz to 300 kHz; EG&G 5113). Only results from series in which fish did not change their orientation are included below. This was monitored visually and electrically. The electrical monitoring gave an alarm signal either when the sequence of polarities within the recorded EOD changed (because the fish had changed its orientation with respect to the stationary recording electrodes) or when a trigger level set to detect only the second (head-negative) phase of the EOD was no longer reached by the recorded EOD. The test tank did not contain water filters or heaters. Water temperature therefore decreased in most experiments by 1°C over the course of approximately 4–6 h, although in some experiments the decrease was up to 3°C. Preliminary experiments indicated that the echo latency increased with decreasing temperature, from approximately 9 ms at 30°C to approximately 15 ms at 20°C (S. Schuster, unpublished data), thereby potentially causing a mismatch of up to 1–2 ms between the (fixed) double-pulse interval and the echo interval of the fish. Such shifts, however, were too small to affect the probabilities of echoes to the first or second of the two pulses.

To elicit echo responses, the stimulus field must be in the tail-to-head direction of the fish (Russell et al., 1974). Hence, the electrodes recording the EOD will inevitably also record the stimulus pulses. Since the aim of the present experiments was to determine exactly the number of EODs overlapping with the stimulus pulses, it was imperative to separate the EOD from the stimulus. This was achieved by the 'edit' circuit in Fig. 1: a low-pass filter (npi DPA2F, -3 dB point at 20 kHz) shaped the signal of the pulse generator to provide a copy of the expected waveform recorded at the electrodes, and this was then subtracted from the recording (using a standard configuration of an operational amplifier). Fig. 2A shows the results of such removal. The subtraction almost completely eliminated the stimulus pulses, what remained of them was generally less than one-tenth of the head-positive first peak of the EOD of *Gnathonemus petersii*. Therefore, the threshold in the timer circuit could easily be set high enough to be triggered only by the EODs and not also by what remained of the stimulus pulses.

The 'timer' circuit (SR 620, Stanford Research) measured the time between stimulus onset ('start') and a subsequent EOD of the fish ('stop'). Stimulus pulses and EODs of the fish under test were directly observed on a storage oscilloscope (Yokogawa DL1200A). Mean firing frequency was continuously displayed on an additional counter (Fluke PM 6665; measuring interval 10 s). Continuous measurements of the mean and standard deviation of the spontaneous interdischarge interval were derived by a computer using a data-processing card (DAP 3200a/415, Microstar Labs, software written in DAPL language and Borland Turbo Pascal 7.0).

Histograms of the interval between stimulus onset and the first EOD usually included the effects of 200 stimuli and were usually generated with a bin width of 1 ms (see, for example, Fig. 2B). The histograms were displayed on a Tektronix oscilloscope and printed automatically. To assess how many echoes were produced in response to the first (second) stimulus pulse, the counts within three bins occurring approximately 12 ms (24 ms) after the first stimulus pulse were summed to yield the number of echoes  $n_1$  ( $n_2$ ). The three bins chosen were the one with a maximum count, the neighbouring bin with the largest count, and the bin with the largest count that also neighboured one these two bins. From  $n_1$  and  $n_2$ , a 'quality factor',  $Q = n_2 / (n_1 + n_2)$ , was calculated to assess how strong the tendency was to echo more the second ( $0.5 < Q < 1$ ) or the first ( $0 < Q < 0.5$ ) stimulus pulse. The error (standard deviation) in estimating the quality factor  $Q$  from a limited number  $N$  of tests yielding counts  $n_1$  and  $n_2$  is estimated from error propagation to be  $\sigma_Q(n_1, n_2) = (n_1 + n_2)^{-2} (n_1 n_2^2 + n_2 n_1^2)^{1/2}$ . Note that, for a large number  $N$  of tests,  $n_1$  and  $n_2$  will approximate the numbers expected from the respective probabilities  $a$  and  $b$  of echoing to the first and second pulses, respectively, i.e.  $n_1 = aN$ ,  $n_2 = bN$ , and the error in estimating  $Q$  will hence become proportional to  $N^{-1/2}$ . Unless stated otherwise, each value of  $Q$ , as derived from a histogram comprising  $N$  stimuli, will always be reported in the form  $Q \pm \sigma_Q$ , where  $\sigma_Q$  is the standard deviation.

## Results

### *Responses to double pulses with the echo interval between them*

Each of the 20 fish analyzed showed echo responses to artificial stimulus pulses in perfect agreement with the findings of Russell et al. (Russell et al., 1974). A second stimulus pulse delivered 12 ms after the first coincided with an echo given in response to the first pulse. Fig. 2 shows how one fish responded to the first 200 such tests with double pulses. The upper trace of Fig. 2A shows the electric organ discharges (EODs) of the fish as seen after editing to remove the stimulus pulses (see above). The lower trace shows the pair of stimulus pulses delivered at a delay of 100 ms after the last EOD of the fish under test. Fig. 2B shows a histogram of the latencies,  $\tau$ , between the first stimulus pulse and the next EOD (defined in Fig. 2A), obtained in the 200 tests. Most responses of the fish

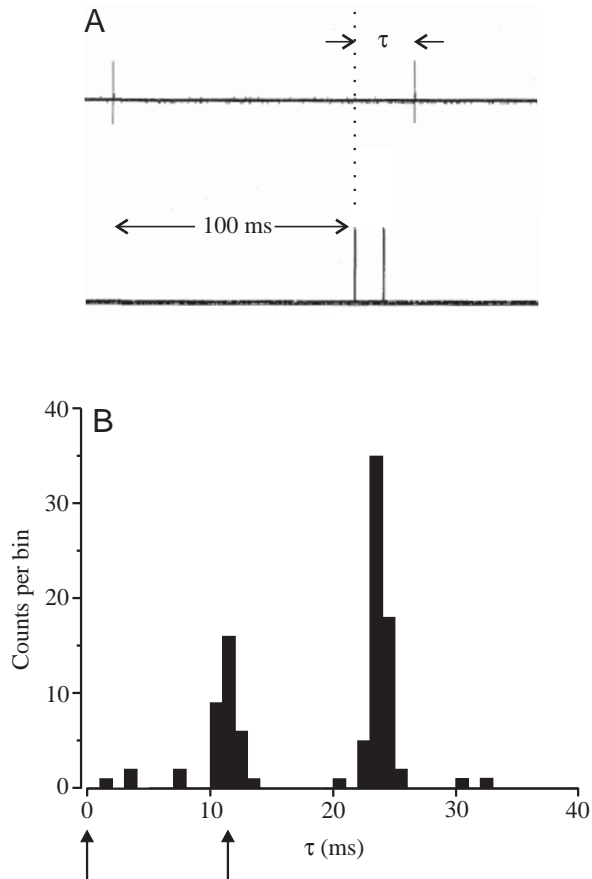


Fig. 2. (A) Example of a double-pulse test in which a second stimulus pulse followed so as to coincide with an echo given by the fish in response to the first pulse. Upper trace: electric organ discharges (EODs) of the fish after subtraction of stimulus pulses by the editing circuit. Lower trace: two stimulus pulses with a 12 ms interval between them. The onset of the stimulus pulses was 100 ms after the last pre-test EOD of the fish. The latency,  $\tau$ , between stimulus onset and the subsequent EOD of the fish indicates an echo to the second pulse. (B) Histogram of latencies  $\tau$  obtained from one fish during the first 200 tests with double pulses. Bin width 1 ms. Arrows on the abscissa indicate the timing of the two stimulus pulses. The two peaks in the histogram show echoes to the first and second pulses.

occurred 12 ms after the second stimulus pulse. The 'quality factor'  $Q$  ( $\pm$  its standard deviation, see Materials and methods), used to assess the degree of echoing more to the second pulse, was  $0.69 \pm 0.04$ . It did not change significantly in 1000 subsequent tests with double pulses. Interestingly, the quality factor in the first 30 tests was  $0.67 \pm 0.09$ , indicating that the fish responded more to the second pulse right from the beginning.

This preference does not arise from delaying the responses to the first pulse but rather from a higher probability of responding to the second of two closely spaced pulses. Fig. 3 gives an example of an experiment to show this. In this experiment, a fish continuously received a total of slightly more than 400 stimuli randomly selected to be either a single pulse or a double pulse, both stimulus types being equally

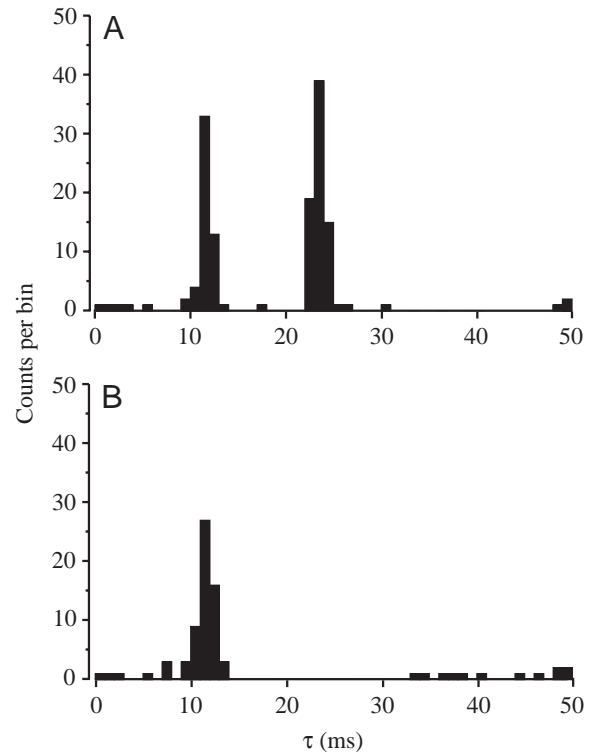


Fig. 3. Evidence that echoes cannot be delayed. An experiment in which stimuli were given continuously to one fish at a rate of  $1 \text{ s}^{-1}$ . Stimuli were randomly selected to be either single or double pulses, both stimulus types being equally likely. Latencies were processed separately for the two types of stimulus. (A) The distribution of response latencies  $\tau$  after the double pulses demonstrates the tendency of the fish to respond more to the second stimulus pulse. (B) The distribution of latencies when single pulses had been delivered in the same experiment. The absence of a secondary peak demonstrates the absence of delayed echoes that could have been the reason for the larger second peak seen in A. Histograms: 200 tests each, bin width 1 ms.

likely. The latency of an EOD with respect to stimulus onset was processed separately for the two types of stimulus (Fig. 3A,B). When the fish received double pulses (Fig. 3A), it preferentially echoed the second pulse ( $n_1=50$ ,  $n_2=73$ ;  $Q=0.59 \pm 0.04$ ; different from 0.5 at  $P < 0.05$ ). When it received a single pulse (Fig. 3B), the fish responded with a single echo at 12 ms latency (Fig. 3B;  $n_1=52$ ,  $n_2=0$ ). This clearly shows that the fish simply echoed either the first or second stimulus pulse with a fixed latency of 12 ms rather than partially shifting its preferred latency from 12 to 24 ms (in which case a second peak at 24 ms would have occurred in Fig. 3B). Note that obtaining the response latencies to the two types of stimulus during the same period ensures that any changes in response probability could not be the basis for the different distributions seen in Fig. 3A,B. These results were confirmed in three series of experiments consisting of 4600 tests and also during a large set of experiments to be described below.

Nine of the 20 fish analyzed responded more to the second pulse within the first 200 tests with double pulses. The

preference shown by these fish was robust when stimulus intensity was varied between 12.5 and 50 mV cm<sup>-1</sup>. The remaining 11 fish initially responded more to the first pulse. Eight of these 11 fish eventually responded more to the second pulse after approximately 2000 double pulses had been given at a rate of slightly less than 1 s<sup>-1</sup>. The three remaining fish continued to respond to the first pulse even after several thousand tests. Surprisingly, these last three fish could reversibly be made to 'prefer' the second pulse simply by lowering the stimulus strength to half its standard setting (25 mV cm<sup>-1</sup>).

To test whether the observed preference was a consequence of the chosen fixed delay of 100 ms at which the stimulus followed a spontaneous EOD of the fish, 15 fish (excluding those that continued to respond to the first pulse after several thousand tests) were re-tested with the delay unrestricted, except that it had to be less than 100 ms. All other stimulus properties were the same as before. All 15 fish eventually echoed more in response to the second pulse and showed similar  $Q$  values after the same number of tests as in the experiments with a fixed delay of 100 ms. The only effect of the unrestricted delay was a general decrease in overall echo probability, as was to be expected from the findings of Russell et al. (Russell et al., 1974).

An initial series of experiments aimed at increasing the number of actual collisions between an echo to the first stimulus pulse and the second pulse by making the second pulse longer in duration. This was done because the duration of the EOD of *Gnathonemus petersii* is between 200 and 300  $\mu$ s and the echo latency of 12 ms varies by 1–2 ms about this value, making actual collisions rare. Unfortunately, a stimulus pulse of longer duration elicits more echoes (Russell et al., 1974) so that larger  $Q$  values would be expected from this alone. However, when the second pulse extended from 10 to 14 ms after the onset of the first (300  $\mu$ s duration) pulse, no significant changes in  $Q$  value were found (15 000 tests with four fish, all under conditions of a variable delay less than 100 ms). Hence, precise coincidences between an EOD and the second pulse are not required to make the fish echo more in response to the second stimulus.

In summary, most fish respond more to the second of a pair of closely spaced pulses, thus avoiding collisions with the second stimulus pulse. The following experiments investigate the mechanism behind this observed preference by analyzing how it depends on (i) the fish's internal preparedness to produce an EOD, (ii) the amplitude, spacing and number of the stimulus pulses, and (iii) prior experience with double pulses.

#### *Effects of the delay of the double pulse from the last spontaneous EOD*

The probability with which an echo response is elicited is not simply a function of the stimulus properties, but depends strongly on the readiness of the fish to fire. This was clearly demonstrated by Russell et al. (Russell et al., 1974), who provided examples of how the probability of an echo to a given stimulus pulse depends both on the spontaneous firing rate and

on the delay at which the stimulus follows a spontaneous EOD. I therefore explored whether the tendency to respond more to the second pulse of a double pulse could be due to the longer delay at which the second pulse follows the last spontaneous EOD. Evidence was given above that echoing more in response to the second of two stimulus pulses does not require a fixed delay between the pre-test EOD and the stimulus onset. However, the degree of preference could still vary with varying delays.

The results demonstrated that a given fish showed the same preference to echo the second pulse more at different delay values. An example is provided in Fig. 4A,B. Moreover, no correlation was found between the quality factor  $Q$  (once a preference had been established) and the delay. In two fish, latency histograms were derived from blocks of 200 tests each. Various delay values, constant within one block, were randomly assigned so as to prevent any systematic bias. From each histogram,  $Q$  was determined and plotted against the delay. No significant correlation between  $Q$  and delay was found in either fish ( $r^2=0.10$ ,  $P=0.15$ , 22 delays from 40 to 200 ms; and  $r^2=0.12$ ,  $P=0.33$ , 10 delays from 40 to 130 ms). Hence, neither the establishment of a preference nor the degree of the preference depends on the delay at which the double pulse follows a spontaneous EOD of the fish.

#### *Spontaneous interdischarge interval*

The spontaneous interdischarge interval is the second main variable with which to assess the role of the internal readiness of a fish to respond. The interdischarge interval varies greatly in mormyrid fish, depending on the state of 'arousal'. The intervals are small in actively swimming fish, but large in fish hiding in a shelter during the day (e.g. see Hopkins, 1986; Kramer, 1990; Moller, 1995). Interestingly, the tendency to respond more to the second pulse was seen at a fixed delay at all mean firing rates. An example is given in Fig. 4C,D: the second pulse was preferentially echoed at both firing rates and the quality factors were 0.84 $\pm$ 0.05 in Fig. 4C and 0.84 $\pm$ 0.04 in Fig. 4D.

Several lines of evidence suggest the lack of a relationship between  $Q$  and the mean firing rate. (i) Six fish that clearly responded more to the second pulse were tested continuously with pairs of pulses, and their mean firing interval (and in some cases also its standard deviation) was determined continuously. Linear regression analyses were carried out relating  $Q$  values obtained from blocks of 200 successive stimuli to the mean interdischarge interval determined from all EODs that occurred during these 200 test. There was no significant correlation:  $Q$  values remained approximately constant despite considerable variation in the mean firing interval. For example, for two fish, correlation coefficients  $r$ , the number  $N$  of data pairs,  $P$  values and the range of mean firing intervals were:  $r=-0.40$ ,  $N=12$ ,  $P=0.20$ , 73–154 ms;  $r=+0.31$ ,  $N=12$ ,  $P=0.32$ , 78–106 ms. A further example, in which the mean and standard deviation of the interdischarge interval were monitored for several hours, yielded for the mean interval  $r=+0.007$ ,  $N=40$ ,  $P=0.96$ , 75.2–104.5 ms and for its standard deviation  $r=+0.06$ ,  $N=39$ ,

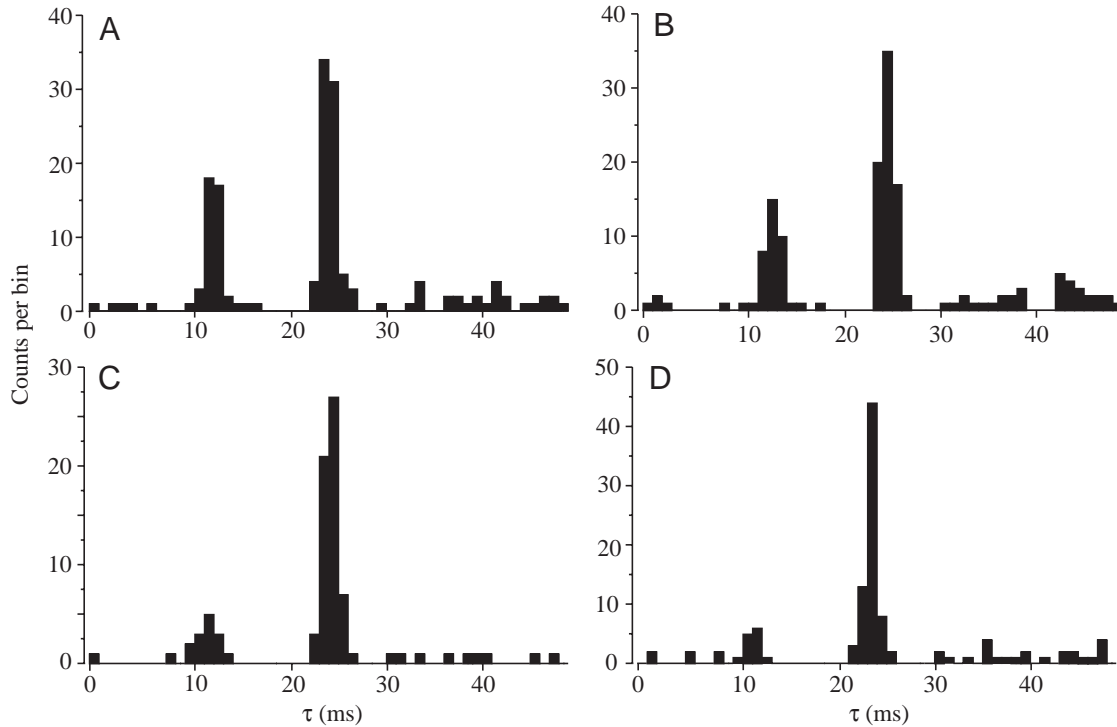


Fig. 4. Distribution of response latencies  $\tau$  to two stimulus pulses (separated by 12 ms) obtained at different values of the delay at which stimulus onset followed after the last electric organ discharge (EOD) of the fish (A,B) and at different spontaneous firing rates of the fish (C,D). Histograms: bin width 1 ms, 200 tests each, stimulus pulses at 0 and 12 ms. (A,B) Histograms obtained with same fish at a similar firing rate (12.4 Hz in A, 12.1 Hz in B) but with stimuli at different delays (120 ms in A, 200 ms in B) after the last pre-test EOD. (C,D) Histograms obtained with a different fish from that in A and B in tests with a fixed 100 ms delay but when the fish fired at mean rates of 5.5 Hz (C) and 11.1 Hz (D).

$P=0.71$ , 37.9–52.3 ms. (ii) The same ( $P>0.05$ ) low values of  $Q$  ( $<0.3$ ) were obtained in initial tests with double pulses in seven fish at mean firing intervals ranging from 77 to 150 ms. (iii) In several experiments, large changes in  $Q$  occurred without any marked change in firing interval. For example, in one fish,  $Q$  increased from  $0.47\pm 0.03$  (1200 stimuli; mean interval 95 ms) to  $0.75\pm 0.04$  (subsequent 400 stimuli; mean interval 112 ms), whereas in another individual  $Q$  increased from  $0.05\pm 0.05$  (200 stimuli; mean interval 160 ms) to  $0.49\pm 0.07$  (subsequent 200 stimuli; mean interval 159 ms) with no change in firing interval.

#### *Effects of stimulus intensity*

Fig. 5 illustrates the effect of changing the intensity of the pulse pairs in two fish that had previously been tested with series of double pulses of constant amplitude ( $25 \text{ mV cm}^{-1}$ ). In these prior experiments, carried out over several weeks with a pause of at least 12 h between successive testing periods, fish 1 (filled circles in Fig. 5) always responded more to the second pulse even in the first 200 tests of each day, while fish 2 (open circles in Fig. 5) needed a large number of stimuli in each new testing period before the preference to echo more in response to the second pulse was established. The effect of changing stimulus intensity was then analysed during a single day of testing for each fish. Testing always started with 200 double pulses given at a field strength of  $25 \text{ mV cm}^{-1}$ . After this,

stimulus intensity was randomly assigned another fixed value for the next 200 stimuli, etc. At the end of several hours of experimentation, all response latencies were grouped to obtain a latency histogram for each intensity, from which the probabilities of echoing the first and second pulse and the  $Q$  values reported in Fig. 5 were determined. The two fish differed greatly with respect to the intensities required for a preferential response to the second pulse. Fish 2 responded more to the second pulse only over a very restricted intensity range, between 10 and  $20 \text{ mV cm}^{-1}$ . Fish 1 responded more to the second pulse over the entire range of intensities tested. In both fish, the lowest intensity at which  $Q$  was greater than 0.5 was far above the echoing threshold. No attempts have been made to determine this threshold directly.

Additional experiments showed the following. (i) Echo responses were occasionally found at stimulus intensities as low as  $0.5 \text{ mV cm}^{-1}$ . (ii) When double pulses were given at low intensity, there appeared to be a greater number of responses to the first pulse than to the second. Unfortunately, the low general probability of echoing at low intensities requires lengthy experimental periods for precise determination of  $Q$  values. Only at  $0.5 \text{ mV cm}^{-1}$  was a sufficiently precise value of  $Q$  determined in fish 1 ( $Q=0.44\pm 0.04$ ; 1800 stimuli,  $n_1=74$ ,  $n_2=57$ ). (iii) At the highest stimulus intensities,  $Q$  began to decrease in fish 1: at  $100 \text{ mV cm}^{-1}$ ,  $Q$  was  $0.40\pm 0.03$  (1000 stimuli,  $n_1=127$ ,  $n_2=85$ ). Thus, for both fish, there was a non-

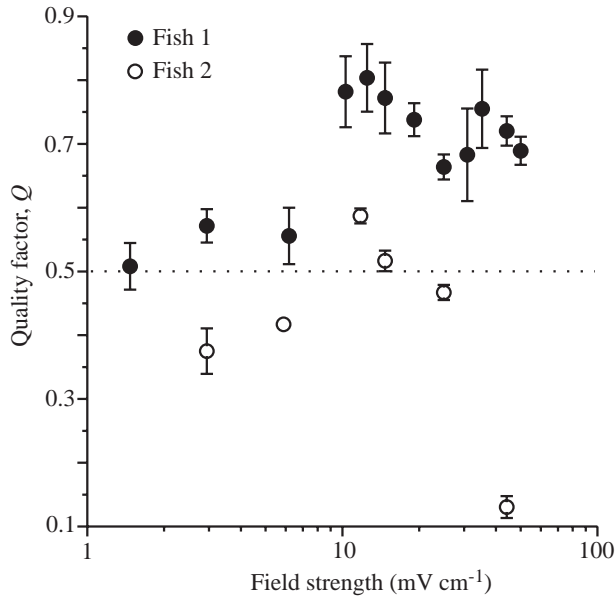


Fig. 5. Dependence of the dimensionless quality factor  $Q$  on stimulus pulse intensity. Results obtained with two fish illustrate the extreme cases of preference seen either over a broad (fish 1, filled circles; 11 200 responses to double pulses) or a narrow (fish 2, open circles; 14 000 double pulses) range of intensities. The dimensionless quality factor,  $Q=n_2/(n_1+n_2)$ , where  $n_1$  and  $n_2$  are the number of echoes produced in response to the first and second stimulus pulse, respectively, assesses the degree of preference.  $Q<0.5$  (below the dotted line) indicates that mainly the first pulse is echoed;  $Q>0.5$  indicates that mainly the second pulse is echoed. Stimulus amplitude was held constant for blocks of 200 tests, then changed to another value according to a randomized design. Values are means  $\pm$  S.E.M. as obtained from all latency histograms at the same stimulus intensity.

monotonic relationship between  $Q$  and stimulus intensity, with  $Q$  increasing from a value below 0.5 to a value above 0.5, and then decreasing again to a value below 0.5 as intensity is increased.

When plotting the probability  $p_1$  of echoing the first stimulus pulse, rather than the quality factor  $Q$  (which assesses the relationship between  $p_1$  and  $p_2$ ), as a function of stimulus intensity, a monotonic increase in response probability  $p_1$  was found in both fish. For fish 2, this increase was evident ( $P=0.01$ ; linear regression analysis) within the range of intensities shown in Fig. 5, while for fish 1 it was only barely seen ( $P<0.05$ ; linear regression analysis), but was confirmed in later experiments made at an intensity of  $100 \text{ mV cm}^{-1}$ .

In the above experiments, intensity remained constant for blocks of 200 stimuli. I investigated whether the same pattern was obtained if intensity was changed randomly from one stimulus to another with fish 1 of Fig. 5. Manually turning a potentiometer between successive tests resulted in haphazard intensity variations within the range  $11.8\text{--}29.4 \text{ mV cm}^{-1}$ . Despite this variation in intensity from stimulus to stimulus, fish 1 still responded more to the second pulse ( $n_1=29$ ,  $n_2=103$ ;  $Q=0.78\pm 0.04$ ; 400 stimuli).

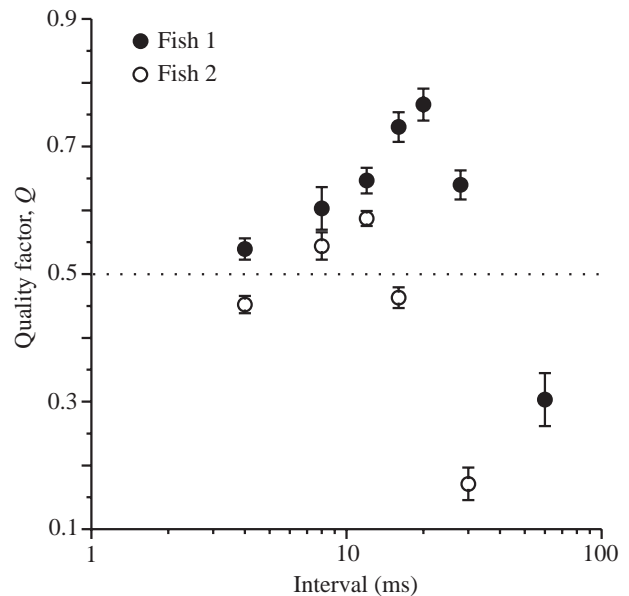


Fig. 6. Dependence of the dimensionless quality factor  $Q$  on the interval between two closely spaced stimulus pulses. Results obtained with the same two fish as in Fig. 5 (fish 1, filled circles; 12 600 tests, field strength  $25 \text{ mV cm}^{-1}$ ; fish 2, open circles; 11 400 tests, field strength  $12.5 \text{ mV cm}^{-1}$ ). The dimensionless quality factor,  $Q=n_2/(n_1+n_2)$ , where  $n_1$  and  $n_2$  are the number of echoes produced in response to the first and second stimulus pulses, respectively, assesses the degree of preference.  $Q<0.5$  (below the dotted line) indicates that mainly the first pulse is echoed;  $Q>0.5$  indicates that mainly the second pulse is echoed. The interval between the stimulus pulses was held constant for blocks of 200 tests, then changed to another value according to a randomized design. Values are means  $\pm$  S.E.M. obtained from all latency histograms at the same interval.

#### *Effects of the interval between the two stimulus pulses*

Fig. 6 shows the effect of varying the interval between two closely spaced pulses on  $Q$ . While, in fish 1, a preference to echo the second pulse ( $Q>0.5$ ) prevailed for intervals from 4 ms to approximately 30 ms, the preference depended more sharply on the pulse interval in fish 2,  $Q$  being above 0.5 only at 8 and 12 ms.

The pattern seen in fish 2 (Fig. 6) was also noted in experiments on three other fish at variable delays ( $<100 \text{ ms}$ ) after they had previously received more than 1000 tests with a 12 ms interval between the stimulus pulses and in these tests had responded more to the second pulse of the pulse pair. When the interval between the pulses was set to 4 ms, the fish responded more to the first pulse (6000 tests), with no change when a fixed delay (100 ms) was chosen in subsequent tests. The average value of  $Q$  was  $0.34\pm 0.03$  (mean  $\pm$  S.E.M.), which is significantly smaller than  $Q=0.5$  ( $P<0.001$ ;  $t$ -test). At an interval of 8 ms, there was a preference to echo the second pulse. When the interval was 16 ms, there was no preference for either pulse in these three fish ( $Q=0.50\pm 0.05$ ; 4000 tests).

#### *Effects of series of more than two pulses*

The responses to series of more than two pulses were

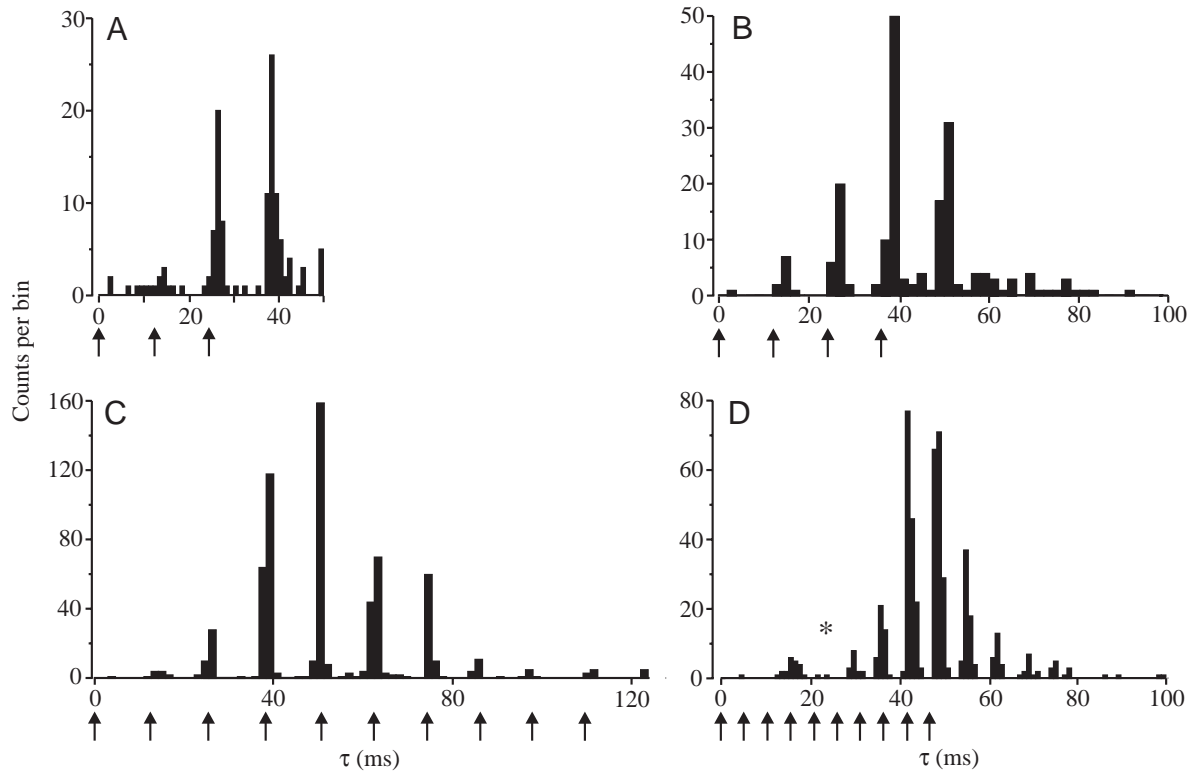


Fig. 7. Latency ( $\tau$ ) distributions obtained in tests with series of more than two stimulus pulses. (A,B) Response to series of three (A) or four (B) pulses all separated by a 12 ms interval. Each histogram represents 200 tests. The delay from the last pre-test electric organ discharge (EOD) to the first stimulus pulse was 100 ms in each test. (C,D) Response to series of 10 stimulus pulses at intervals of 12 ms (C) and 5.2 ms (D). The asterisk in D indicates a lack of response to the second stimulus pulse. Histograms comprise 1000 tests each. The delay from the previous EOD of the test fish was variable but less than 100 ms. The timing of the stimulus pulses is indicated by arrows below the abscissa; the echo in response to each pulse is expected 12 ms later. Bin widths: 1 ms (A), 2 ms (B), 1.6 ms (C), 0.8 ms (D).

examined in seven fish that had mainly responded to the second pulse in their first 200 tests with double pulses. When tested with a series of three pulses (Fig. 7A), all fish responded most to the third pulse, i.e. responded with a latency of 36 ms. This was observed both with a fixed (100 ms) and a variable (<100 ms) delay between the onset of the stimulus train and the preceding spontaneous EOD. Echoing most in response to the last pulse did not occur, however, in response to a series of four stimulus pulses (Fig. 7B). None of the fish reliably showed a preference to echo the fourth pulse when a fixed delay was chosen, even after being continuously tested with 2000 stimulus bursts. Three fish occasionally showed more echoes to the fourth than to the third pulse when the delay was variable, but these findings could not be reproduced consistently. In interpreting the histograms, one must bear in mind that the differences in delays with which each pulse followed the last spontaneous EOD will now be larger than in the tests with double pulses and, hence, might be important in determining the echo probability to each pulse.

When stimulated with a train of 10 pulses, separated by the echo interval (Fig. 7C), all responses occurred strictly at multiples of 12 ms so that they all coincided with or were close to one of the stimulus pulses. Apparently, the fish did not 'suppress' its echo response to avoid collisions. The most

probable latency used by the fish was 48 ms, i.e. a response to the fourth stimulus pulse, so that the total latency (including the 100 ms delay) came close to the first mode in the distribution of spontaneous firing intervals of this fish. This was also suggested by subsequent experiments on the same fish (Fig. 7D) in which a 5.2 ms interval between stimulus pulses shifted the maximum response to the fifth and sixth stimulus pulse but left the absolute latency constant.

#### *Role of previous stimulation with double pulses*

In several fish, the tendency to echo mainly the second of two pulses seemed to develop slowly in a prolonged series of tests with double pulses of constant intensity ( $25 \text{ mV cm}^{-1}$ ). Fig. 8A shows successive histograms obtained from successive blocks of 200 tests with one fish. The resulting increase in  $Q$  with block number was highly significant ( $r=0.859$ ,  $N=12$ ,  $P<0.001$ ). In two further fish, the preference increased with block number at a similar rate ( $r=0.78$ ,  $N=13$ ,  $P<0.002$  and  $r=0.83$ ,  $N=9$ ,  $P<0.01$ ). The results for these three fish are shown in Fig. 8B. Interestingly, the responses to the second pulse did not generally increase 'at the cost' of the responses to the first, as would be expected if the fish had learned to respond more to the second and less to the first pulse. This is evident from the lack of any correlation among the response



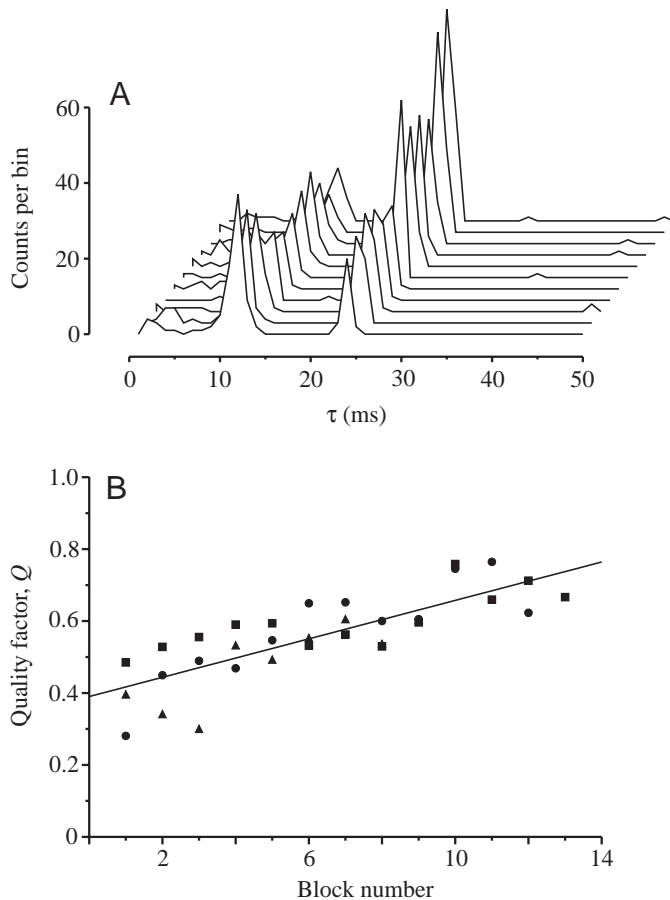


Fig. 8. (A) Histograms of latencies  $\tau$  obtained in successive blocks of 200 tests each. The histograms are ordered from front to back. Note the slow build-up of the tendency to respond more to the second of the two stimulus pulses. Bin width 1 ms. (B) Quality factors  $Q$  obtained in successive blocks of 200 tests in response to continuous stimulation with double pulses (spaced 12 ms apart). Different symbols indicate three different fish; filled circles relate to the histograms shown in A. Regression analysis for pooled data:  $r=0.80$ ,  $N=34$ ,  $P \ll 0.001$ . The dimensionless quality factor  $Q$  assesses the degree of preference in the fish's response to a double pulse:  $Q < 0.5$  indicates that mainly the first pulse is echoed;  $Q > 0.5$  indicates that mainly the second pulse is echoed.

probabilities to the first and second pulses (linear regression analysis with pooled data from the three fish,  $r=0.16$ ,  $N=34$ ,  $P=0.36$ ; separate analysis for each fish also indicated no correlation). In another fish, the rate of increase in  $Q$  was much smaller,  $Q$  stabilizing after approximately 27 blocks of 200 tests each ( $r=0.77$ ,  $N=27$ ,  $P \ll 0.001$ ). In the first block of 200 tests performed several days later with all four fish, the tendency to echo more in response to the second pulse had not been retained and they responded most to the first pulse. However, rather than by again delivering a large number of stimuli, all the fish could readily be made to respond more to the second pulse when the stimulus intensity was decreased from  $25 \text{ mV cm}^{-1}$  in the second block of 200 tests. This suggests that a prolonged series of tests may act to decrease

the effective intensity of the stimulus. This was tested in experiments in which an initial block of 200 tests with double pulses was followed by up to 20 blocks of tests with single pulses. Subsequent evaluation of  $Q$  after the prolonged stimulation with single pulses did not indicate any significant changes. Within these 20 blocks, the fish had received the same number of pulses as in 10 blocks with double pulses, which had demonstrably led to changes in  $Q$  in at least three of the fish (Fig. 8). This seems to indicate that any effect of prolonged stimulation on lowering the effective stimulus might depend critically either on the average stimulus rate (which is doubled in the double-pulse stimulation) or on the presence of closely spaced pulses.

A similar 'adaptation' effect can also be shown in those fish that seemed to respond more to the second pulse right from the beginning of the tests. To show this, one ideally should deliver one double pulse, measure the EOD latency and then wait for a long time. Repeating this several times would then yield the probabilities of an echo to the first and second pulse for the 'first' double pulse encountered after a long period. This could then be compared with the respective probabilities obtained in tests in which double pulses were delivered continuously at approximately  $1 \text{ s}^{-1}$ . Fig. 9 provides an example of such an experiment made with one fish. At the beginning of each of 7 days, approximately 10 000 stimuli were delivered at approximately  $1 \text{ s}^{-1}$ . These stimuli were mainly single pulses, but double pulses were interspersed at random at an average probability of one double pulse in 100 stimuli. Thus, double pulses were rare during this first phase of each experimental day. After up to 100 such rare double pulses had been delivered, a continuous series of 200 double pulses (at a rate of  $1 \text{ s}^{-1}$ ) was delivered (i) to assess whether the fish responded most to the second pulse and (ii) to provide a control with which to compare the tests with rare double pulses. The fish was then kept separate from the other fish and the procedure was repeated for the next 6 days.

Fig. 9 shows the distribution of latencies obtained over the 7 days in the phases with rare occurrence (Fig. 9A) and with continuous occurrence (Fig. 9B) of double pulses. Two clear conclusions can be drawn from these results: (i) there is an innate preference to respond most to the second pulse even when double pulses occur rarely (Fig. 9A; 660 double pulses among 66 000 stimuli,  $n_1=44$ ,  $n_2=79$ ;  $Q=0.64 \pm 0.04$ ), and (ii) the preference is significantly greater ( $P < 0.01$ ) when only double pulses occur (Fig. 9B; 1400 tests,  $n_1=49$ ,  $n_2=171$ ;  $Q=0.78 \pm 0.03$ ). Two experiments carried out with the same fish suggest that this difference was not because the tests with continuous double pulses always occurred later in the day: (i) when the fish was given the continuous 200 double pulses earlier in a day, the same  $Q$  value was found as in Fig. 9B; and (ii) when the fish was continuously stimulated with double pulses for a whole day, it consistently echoed more in response to the second pulse, as in the first 200 tests, and no significant changes in  $Q$  value were found over the course of the day.

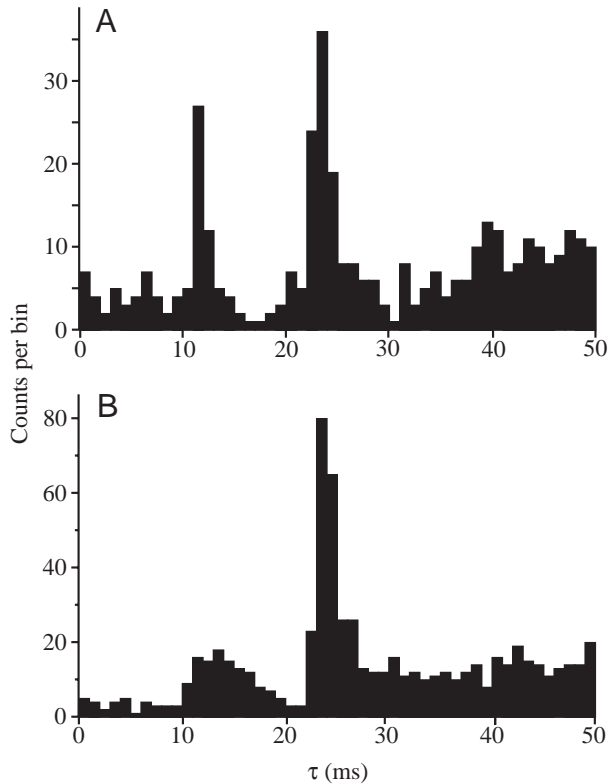


Fig. 9. Effects of continuous stimulation with double pulses (12 ms separation) on the distribution of response latencies  $\tau$  in a fish that already responded mainly to the second pulse in the first tests with double pulses. The experiment was performed over the course of 7 days. At the beginning of each day, approximately 10 000 stimuli were delivered at approximately  $1 \text{ s}^{-1}$ . Most were single pulses, but double pulses were interspersed at random at an average probability of one in 100 stimuli. Therefore, double pulses were rare during this first phase of each experimental day. After up to 100 of such rare double pulses had been delivered, a continuous series of 200 double pulses (at a rate of  $1 \text{ s}^{-1}$ ) was given. The procedure was repeated each day. (A) Histogram showing the responses to 660 rare double pulses during the 7 days. (B) Histogram showing the responses of the same fish, accumulated over the 7 days, to continuous double-pulse stimulation (1400 tests). The preference to echo the second pulse more than the first is significantly larger ( $P < 0.01$ ) than in A (note the different y axis scales). Bin widths 1 ms.

## Discussion

### *Basis for the innate preference*

It is thought that only the anterior mormyromasts are involved in the echo response of *Gnathonemus petersii*, as inferred from the threshold and polarity-dependence of the response as well as from localized stimulation (Russell et al., 1974). Interestingly, mormyromast afferents may fire a train of up to eight spikes in response to an EOD-like pulse of high intensity (for reviews, see Bell, 1986; Bell, 1989). Since the echo response resets the spontaneous EOD rhythm (Russell et al., 1974) rather than invoking an extra EOD, it is likely that the echo input terminates in the medullary command nucleus which initiates the motor command integrating a multiplicity

of inputs (Bell et al., 1983; Grant et al., 1986). The pathway to this command centre is not known. However, since the minimum latency of the response is only approximately 10 ms, of which approximately 8 ms can be attributed to the electromotor pathway (see e.g. Bennett, 1971; Bell, 1986) and 2 ms to the minimum latency of the mormyromasts, it seems that the mormyromast afferents must synapse directly at the command nucleus, although no such direct connections have been observed anatomically (Bell and Russell, 1978; Bell et al., 1983). The command is issued to a relay nucleus (just dorsal to the command nucleus) which drives the electromotor neurons that innervate the electric organ.

The preference described here to respond more to the second pulse of a double pulse could, in principle, arise from the second pulse reaching the fish at a time when its 'internal preparedness to fire' (due to echo-unrelated input) is greater. This seems almost inevitable because the second pulse occurs at a larger delay relative to the last spontaneous EOD, at which the echo probability is expected to be larger (Russell et al., 1974). The relative difference in delay is small, but no data were previously available to predict the resulting difference in response probability. Whether this accounts for the present findings can be assessed by varying the delay between the onset of a double pulse and the last spontaneous EOD and, hence, the relative gain in delay of the second pulse with respect to the first. Such an experiment did not result in any change to the observed preferences. Moreover, if this was correct, the preference would have depended on the mean spontaneous interdischarge interval, which determines how closely the two respective pulse timings occur, at the given delay, to the timing of the next spontaneous EOD. Again, no sign of any such relationship was found.

The phenomenon must therefore be caused by the first pulse leading to a state of the command nucleus in which input due to the second pulse is more likely to elicit an EOD command spike. The most parsimonious way in which this could be achieved would be by a direct after-effect in which the first pulse elicits a response in the command nucleus that is still present at the advent of the second stimulus pulse so that the response of the command nucleus elicited by the second pulse would occur on the 'remainder' of the response to the first pulse. In principle, a simple low-pass filter in the echo pathway could achieve this. However, a low-pass filter mechanism can be rejected in view of the present results. Since the after-effect of a low-pass filter, i.e. its response that continues after the stimulus is switched off, decreases monotonically with time,  $Q$  should also decrease monotonically with the interval between the two stimulus pulses. This was not observed (Fig. 6). To be in accordance with these data, a temporally more 'localized' after-effect would be needed. For example, a strong pulse could lead to a very long burst response in mormyromast afferents which, in turn, could lead to a full-sized second excitatory postsynaptic potential (EPSP) in the command nucleus approximately 10 ms after the first. So, the first EPSP caused by the second stimulus pulse would occur on top of the second EPSP of the first stimulus pulse, thus increasing its

chance of eliciting an EOD motor command. This view seems to fit well with the data in Fig. 6. The second EPSP would have to occur approximately 8–14 ms after the first EPSP, as is required by the sharp dependence on pulse interval seen in several fish. The second EPSP would, however, have to be several milliseconds in duration, at least in some fish, to explain their broad interval tolerance. The non-linear relationship between  $Q$  and stimulus intensity (Fig. 5) could then be explained as follows. (i) At greater stimulus intensities, the first EPSP caused by the first pulse would become more likely to elicit an EOD motor command. In this case, the second pulse would then not elicit a further command, so that echoes to the first pulse would become increasingly likely as stimulus intensity increased. (ii) At low stimulus intensity, the after-effect would vanish. Hence, the EPSPs in response to the first and second stimulus pulses would be of equal average size. With noise in the system, there would therefore be four possibilities: the EPSPs could both be above threshold, both be below threshold, only the first could be above threshold or only the second could be above threshold. Evidently, in only one of the three cases leading to an echo would the response be to the second stimulus pulse. Therefore, a low-intensity limit of  $Q=1/3$  would be expected according to the two-EPSP theory.

A possible alternative explanation of the preferred response to the second of two pulses would involve subthreshold effects at the periphery. In recording from mormyromast afferents, Bell (Bell, 1990) found that a subthreshold pulse resulted in a subsequent facilitatory period that lasted 30–40 ms. This could cause the second of two low-intensity stimuli to be more effective in evoking an afferent response and an echo EOD. However, this explanation seems unlikely given that preferred responses to the second stimulus were always found at intensities well above the threshold of the echo response.

#### *Basis for the adaptation effect*

The tendency to respond more to the second of two closely spaced stimuli was larger when fish had received continuous stimulation with double pulses (Figs 8, 9). This effect could not be mimicked by delivering the same number of single pulses before the preference had been determined in tests with double pulses. The ‘adaptation’, or learning, could, however, simply be due to the different average pulse rate: it would be absent or very small when pulses were repeated at a rate of 1 Hz and be stronger for pulses presented at an average rate of 2 Hz (as would be the case with double-pulse stimuli given at 1 Hz). The experiments cannot, however, determine whether the adaptation depends on the average rate alone or, more critically, on closely spaced pulses being present. This would be expected if the fish had learned from experience with double pulses to respond more to the first and less to the second pulse. Yet, several facts argue strongly against such ‘cognitive’ abilities being involved. First, no consistent relationship between the response probabilities to the first and second pulse was found. There is therefore no evidence that the fish were deciding directly which of the two pulses should be echoed, in which case one would expect an inverse relationship between

the response probabilities. Second, in the fish that appeared to ‘learn’ slowly, the learning effect could be mimicked simply by changing the stimulus amplitude. This shows clearly that the fish did not form any abstract concept of how many pulses they would have to wait for (as if they were counting) before discharging an echo. Within the mechanism suggested above, in which the adaptation effect of prolonged double-pulse stimulation is equivalent to a change in stimulus intensity, prolonged stimulation with double pulses at a high rate could either increase the amplitude or the likelihood of the second EPSP being produced by a given stimulus pulse or generally lower the amplitude of all EPSPs or increase the spike threshold in the command cells.

#### *Concluding remarks*

Clearly, recording in the central command or precommand nuclei of intact fish is needed to prove the existence of the proposed after-effect and to test its timing and dependence on stimulus intensity. If it could be found, the present results would provide an example of how simple properties of the receptors involved would guarantee an adaptive behaviour. A further point of interest would be to investigate whether the after-effect becomes more prominent after prolonged stimulation with double pulses. Such studies could also help our understanding of the basis for the observed inter-individual differences. Such differences existed in the number of double pulses needed before the fish would respond most to the second of the two pulses and in the dependence of this preference on both the intensity and temporal separation of the two pulses.

The present findings suggest that a fish located near three echoing conspecifics would echo mainly in response to the last EOD in their train of echoes. This would, at least in some fish, also be robust with respect to variations in the distance to, or orientation with respect to, the group, as suggested by experiments with randomly varied stimulus intensity. However, further work is needed to examine the biological significance of the effect. Unfortunately, it is difficult to monitor individual discharges in a large group of freely moving fish, as would be needed. With care, one might interpret the findings of Graff (Graff, 1986) on freely swimming *Marcusenius macrolepidotus* as indicative of multiple echoes. Because he separately recorded the EODs of a tethered fish in addition to those produced by six conspecifics moving freely within the tank, it would be possible to record, within the group, intervals of twice the normal echo interval. This would happen when the tethered individual responded to a discharge within the group, and one fish within the group in turn responded to the echo of the tethered fish. Note, however, that without such precautions, multiple echoes cannot be demonstrated in the same way by recording the overall activity within a group unless either one deliberately omitted EODs from the recordings or the fish echoed at delays in multiples of 12 ms. Otherwise, to any echo response within the group, there would also be the echo-eliciting EOD 12 ms previously. Multiple echoes can, however, readily be recorded from fish

restrained individually within cages and placed at increasing distances from a pair of recording electrodes to allow discrimination of individual EODs on the basis of their amplitude (S. Schuster, in preparation).

I am grateful to Curtis Bell for his help in improving the manuscript. He and an anonymous referee contributed several suggestions and helped to keep Ockham's razor sharp. I also thank Sam Rossel and Klaus Vogt for criticizing an earlier version of the manuscript, Lothar Kaltenbach for constructing the fish cages and Meinrad Hahn for looking after the fish. The experiments comply with the *Principles of Animal Care*, publication No. 86-23, revised 1985, of the National Institute of Health, and also with the laws of Germany.

### References

- Bauer, R. and Kramer, B.** (1974). Agonistic behaviour in mormyrid fish: latency relationship between the electric discharges of *Gnathonemus petersii* and *Mormyrus rume*. *Experientia* **30**, 51–52.
- Bell, C. C.** (1986). Electroreception in mormyrid fish. Central physiology. In *Electroreception* (ed. T. H. Bullock and W. Heiligenberg), pp. 423–452. New York: Wiley.
- Bell, C. C.** (1989). Sensory coding and corollary discharge effects in mormyrid electric fish. *J. Exp. Biol.* **146**, 229–253.
- Bell, C. C.** (1990). Mormyromast electroreceptor organs and their afferent fibres in mormyrid fish. III. Physiological differences between two morphological types of fibres. *J. Neurophysiol.* **63**, 319–332.
- Bell, C. C., Libouban, S. and Szabo, T.** (1983). Neural pathways related to the electric organ discharge command in mormyrid fish. *J. Comp. Neurol.* **216**, 327–338.
- Bell, C. C., Myers, J. P. and Russell, C. J.** (1974). Electric organ discharge patterns during dominance related behavioral displays in *Gnathonemus petersii* (Mormyridae). *J. Comp. Physiol.* **92**, 201–228.
- Bell, C. C. and Russell, C. J.** (1978). Termination of electroreceptor and mechanical lateral line afferents in the mormyrid acousticolateral area. *J. Comp. Neurol.* **182**, 367–382.
- Bennett, M. V. L.** (1971). Electric organs. In *Fish Physiology*, vol. V (ed. W. S. Hoar and D. J. Randall), pp. 347–491. New York: Academic Press.
- Graff, C.** (1986). Signaux électriques et comportement social du poisson à faibles décharges, *Marcusenius macrolepidotus* (Mormyridae, Teleostei). PhD thesis, Université Paris-Sud Centre d'Orsay.
- Grant, K., Bell, C. C., Clause, S. and Ravaille, M.** (1986). Morphology and physiology of the brainstem nuclei controlling the electric organ discharge in mormyrid fish. *J. Comp. Neurol.* **245**, 514–530.
- Grant, K., von der Emde, G., Sena, L. G. and Mohr, C.** (1999). Neural command of electromotor output in mormyrids. *J. Exp. Biol.* **202**, 1399–1407.
- Heiligenberg, W.** (1974). Electrolocation and jamming avoidance in a *Hypopygus* (Rhamphichthyidae, Gymnotoidei), an electric fish with pulse-type discharges. *J. Comp. Physiol.* **91**, 223–240.
- Heiligenberg, W.** (1976). Electrolocation and jamming avoidance in the mormyrid fish *Brienomyrus*. *J. Comp. Physiol.* **109**, 357–372.
- Heiligenberg, W.** (1977). *Principles of Electrolocation and Jamming Avoidance in Electric Fish*. Berlin, Heidelberg, New York: Springer.
- Heiligenberg, W.** (1991). *Neural Nets in Electric Fish*. Cambridge, MA: MIT Press.
- Hopkins, C. D.** (1980). Evolution of electric communication channels of mormyrids. *Behav. Ecol. Sociobiol.* **7**, 1–13.
- Hopkins, C. D.** (1986). Behavior of Mormyridae. In *Electroreception* (ed. T. H. Bullock and W. Heiligenberg), pp. 527–576. New York: Wiley.
- Kramer, B.** (1974). Electric organ discharge interaction during interspecific agonistic behavior in freely swimming mormyrid fish. A method to evaluate two (or more) simultaneous time series of events with a digital analyser. *J. Comp. Physiol.* **93**, 203–236.
- Kramer, B.** (1990). *Electrocommunication in Teleost Fishes*. Berlin, Heidelberg, New York: Springer.
- Moller, P.** (1976). Electric signals and schooling behavior in weakly electric fish, *Marcusenius cyprinoides* (Mormyriiformes). *Science* **193**, 697–699.
- Moller, P.** (1995). *Electric Fishes. History and Behavior*. London: Chapman & Hall.
- Moller, P., Serrier, J., Squire, A. and Boudinot, M.** (1982). Social spacing in the mormyrid fish *Gnathonemus petersii* (Pisces): A multisensory approach. *Anim. Behav.* **30**, 641–650.
- Poll, M.** (1959). Aspects nouveaux de la faune ichthyologique du Congo Belge. *Bull. Soc. Zool. France* **84**, 259–271.
- Russell, C. J., Myers, J. P. and Bell, C. C.** (1974). The echo response in *Gnathonemus petersii* (Mormyridae). *J. Comp. Physiol.* **92**, 181–200.