

Sensitivity to novel feedback at different phases of a gymnotid electric organ discharge

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Summary

Weakly electric fish communicate and electrolocate objects in the dark by discharging their electric organs (EOs) and monitoring the spatiotemporal pattern of current flow through their skin. In the South-American pulse-type gymnotid fish these organs often are intriguingly complex, comprising several hundreds of electrogenic cells (electrocytes) of various morphologies, innervation patterns and abilities to generate a spike, distributed over nearly the full length of the fish. An attractive idea is that different parts of the organ may serve distinct functions in electrocommunication and electrolocation. Recent studies support this notion and suggest that the currents produced during the final phase of the electric organ discharge (EOD) are used for communication. Here, we explore a method to directly assess the relevance of the various currents for electrolocation. In this new method, the pattern of current flow during a gymnotid EOD is changed selectively at distinct phases of the EOD so that currents generated by known electrocyte groups are affected. We have studied the roles played by the various currents for the detection of novel feedback at the trunk/tail region of the gymnotid fish *Gymnotus carapo*. An experimental animal rested in a

cage and two electrodes were placed at a close distance to its trunk and tail. An electronic switch briefly connected these electrodes during a selected phase within an EOD and the shunting of EOD current that resulted from switch closure was directly monitored. *G. carapo* responded with an acceleration of its discharge rate to novelties in the EOD feedback that occurred only for a fraction of a single EOD. Controls in which the switch was closed during the silent intervals between successive EODs showed that the fish responded to the changes in EOD feedback and not to unrelated artefacts of the brief switch closure. Fish responded to shunting of current during all phases; the sensitivity was highest during the final headnegative phase but the magnitude of shunted current was largest in the preceding phase. The current produced during the final part of the EOD is thus not reserved for communication as previously suggested but plays a predominant role in electrolocation at the trunk and tail region of *G. carapo*.

Key words: electric organ, gymnotid, electrolocation, communication, novelty response, feedback, electric fish, *Gymnotus carapo*.

Introduction

Pulse-type gymnotids have evolved intriguingly complex electric organs (EOs) in which hundreds of electrogenic cells (electrocytes), distributed over nearly the full length of the animal, are activated in a well-ordered temporal succession to produce a remarkable spatiotemporal pattern of transcutaneous current flow (for recent reviews see, Assad et al., 1999; Caputi, 1999; for an example movie see, www.fiu.edu/~stoddard/electricfish.html). The currents spread by these complicated organs are used to communicate with other fish and to electrolocate objects in the surroundings by monitoring, with electroreceptors, how such objects change the pattern of the self-produced transcutaneous current flow (Bennett, 1971; Heiligenberg, 1977; Bastian, 1986; Zakon, 1986; Moller, 1995). The question is whether the apparent complexity in the spatiotemporal current pattern produced

by these organs serves a certain function during the electrolocation of objects or whether it is a byproduct of maintaining synchrony in an elongate electric organ, which does not hamper the operation of the system. An attractive idea is that the different parts of these organs are specialized to generate currents that serve distinct functions in electrolocation and communication.

To our knowledge this notion of a 'dual EOD system' was first introduced by Trujillo-Cenóz et al. (1984) for the best-studied pulse-type gymnotid, *Gymnotus carapo*. A clear picture has emerged of how the sequential activation of electrocytes that differ in their morphology, innervation pattern and the number of spike-generating faces, generates the pattern of current flow during its complex electric organ discharge (EOD) (Trujillo-Cenóz et al., 1984, 1989; Lorenzo et al., 1988,

1990, 1993; Caputi et al., 1989, 1993; Macadar et al., 1989). Recently, the notion has received support in this species by studies of Castelló et al. (2000) and Aguilera et al. (2001). These studies demonstrated a foveal region of high sensitivity at the head in which the self-produced currents produced during the final phase of the EOD (V_4) were negligible, while currents of earlier phases (V_1 and V_3) were predominant. Thus, only these latter currents appear to play a role during electrolocation at the fovea. In contrast, of the EODs produced by a distant conspecific, the currents of the final phase V_4 were predominant at the fovea. These findings suggest a dual system in which current produced during the V_1 and V_3 phases of the EOD would be used for electrolocation and current produced during the later V_4 phase would be used for communication.

Here, we explore a novel approach to analyze directly the importance of the different phases of a gymnotid EOD for electrolocation. In this approach the EOD feedback is changed selectively during particular phases within an EOD in which known parts of the electric organ are active. Moreover, the duration and magnitude of the feedback-changes within these selected phases are directly measured ensuring that feedback could be changed when selected parts of the electric organ were active. The 'novelty response', an increase in the discharge rate in response to novel EOD feedback, is used to assess the animal's ability to detect a feedback change triggered at various phases of the EOD waveform. We apply this approach to test the notion of a dual EOD system in *G. carapo*. If this fish uses a dual EOD system as recently suggested (Aguilera et al., 2001), then it should generally be sensitive to feedback changes that are triggered during V_3 (or V_1) but not to changes triggered during V_4 . In particular, this should hold in the trunk/tail region of the fish in which all major currents (V_2 to V_4) are present so that a lack of sensitivity to changes in a particular current cannot simply result from the absence of that particular current.

Materials and methods

Experimental animals

Eight *Gymnotus carapo* (L.) (length 16–24 cm) were used in this study. They were kept individually at a water conductivity of $360 \pm 5 \mu\text{S cm}^{-1}$, pH 6–7 and temperature of 26°C . Individual fish were tested in a tank, $70 \times 40 \times 35$ cm (length \times depth \times height) in size, filled to a height of 15 cm with water of the same quality as the individual's home tank. Water temperature was stabilized at 26°C with fluctuations below $\pm 0.1^\circ\text{C}$. Temperature stability to this degree was required mainly because EODs change their duration with temperature (e.g. Schuster, 2000; Ardanaz et al., 2001) so that triggering switch closure at a fixed delay with respect to EOD onset would place the closure at different phases of the EOD at different temperatures. An external filter with built-in heater (Eheim 2313.01) kept temperature constant with the required precision, and did not introduce electrical noise when the heater was active. All experiments were conducted during the light phase in which the light intensity was approximately

10 lux at the water surface and during which fish rested quietly for the 4–6 h of experimentation given per day. All fish included in this study (from a total of 15 fish) were carefully observed for having fully intact skins and tails throughout the complete series of experiments.

Basic experimental arrangement

Fig. 1A shows the basic arrangement used to trigger a brief change in feedback within the desired phase of one single EOD. The experimental animal rested in a cage (described below) placed in the centre of the tank at half the height of the water column (7.5 cm above ground). Two silver wires at the front and back end of the tank monitored the head–tail EOD. This signal served (i) as a phase reference to assay which part of the electric organ was active (e.g. see Caputi, 1999) and (ii) to monitor the inter-EOD intervals of the fish. Feedback changes were elicited by briefly closing a fast electronic switch (MAX 323, Maxim) that connected two Ag/AgCl pellets, placed 8 cm apart, in preassigned positions at the trunk/tail position on the side of the fish. A virtual-ground input, current-sensitive preamplifier was used (EG&G 5182) to monitor the shunted EOD current that flowed in the external circuit between the pellets when the switch was closed. Surprisingly, a simple circuitry sufficed to reliably place the switch closure at the desired phases of the EOD. During an experiment, a reference pulse marked the onset of one selected EOD and triggered a generator that issued a second rectangular pulse of suitable duration and delay with respect to the reference pulse. This second pulse closed the electronic switch at the desired phase within the selected EOD. The reference pulse, switch-closure command, current in the shunting circuit and head–tail EOD were monitored on a 4-channel oscilloscope (Yokogawa DL 1200A) and could be fed into a computer (programmes written in Turbo Pascal and DAPL language/C++; Processor card DAP 3000a/212, Microstar Labs).

Monitoring the response

When an experiment began, a rectangular pulse coactivated (i) a counter module and (ii) an electronic switch (MAX 324, Maxim) that fed the recorded EODs into the computer. After 200 inter-EOD intervals had been recorded, the counter module produced a reference pulse to mark the onset of the 201st EOD and to command switch closure at the chosen phase within that EOD. The computer continued to record the 300 inter-EOD intervals that followed after switch closure. The switch was closed either during a particular phase of the 201st EOD or, as a control, in the silent inter-EOD interval 4 ms after the reference pulse. As recognized in earlier studies (Bennett and Grundfest, 1959; Szabo and Fessard, 1965; Larimer and Macdonald, 1968; Heiligenberg, 1980; Meyer, 1982), such controls are important to assess whether the fish responded to electrical artefacts associated with the switch closure rather than to the redistribution of EOD current. Examples of responses to switch closure during the EOD are shown in Fig. 1B. Animals responded to novel feedback with a transient decrease in the inter-EOD interval, a response termed novelty

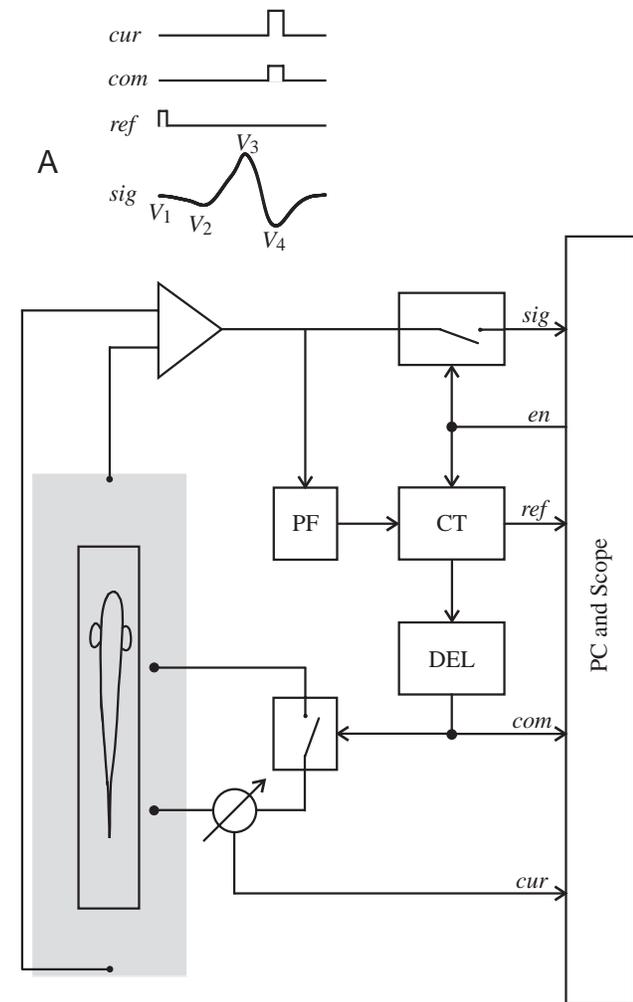


Fig. 1. Schematic diagram of the experimental arrangement (A) and examples of behavioral responses (B). (A) The experimental animal rested in a cage placed centrally, at half-water level, in a large tank. Two Ag/AgCl pellets, placed at the side of the fish at its trunk/tail region, could be connected, outside the tank, via a fast electronic switch. As a result of switch closure, a part of the electric organ discharge (EOD) current of the fish is redistributed to flow over the low-resistance path of the external circuit rather than through the water. This current could be monitored (*cur*) by inserting a current amplifier in the external circuit. As a reference of which electrocyte groups produced the current, head-tail EODs were recorded by two silver wires. To start an experiment, a rectangular pulse (*en*) simultaneously enabled a counter module (CT) and allowed EODs (*sig*) to be fed into a processor (PC). After 200 inter-EOD intervals were recorded, the counter module issued a reference pulse (*ref*), that signalled the onset of the 201st recorded EOD. By varying the delay of a command pulse (*com*) with respect to this reference pulse (DEL), the electronic switch could be closed during a selected phase of this particular EOD, or, as a control, in the silent time after it in which no EOD current is shunted. The processor continued to store 300 inter-EOD intervals that followed after switch closure. Prior to input to the counter module, the EODs were strongly amplified, filtered and converted to rectangular pulses (pulseformer; PF). The inset shows a head-to-tail EOD (*sig*; V_1 – V_4 denote the major phases of the EOD) and a timing diagram of switch closure with the time course of the signals *ref*, *com* and *cur*. (B) Examples of four novelty responses to shunting of EOD current during particular phases of a single EOD. The switch was closed, for 100 μ s, during EOD-phase V_4 (upper three traces) or during V_3 (bottom trace). The timing of switch closure is indicated by the arrow and the vertical grey line. Note the clear changes in interpulse interval. The abscissa (Time) shows the succession of the 200 pre- and 300 post-stimulus interpulse intervals.

alternation. Meeting this requirement ensured that differences in the responses could not be caused by stimulus-unrelated changes in responsiveness.

Time allowed between successive stimuli

To avoid habituation to the feedback changes the animal was given a rest of 3 min between successive experiments. This limited the amount of tests that could be made within the maximally possible interval of 6 h during which a fish would rest quietly in its cage. However, a time of 3 min was chosen as prior experiments had indicated significantly lower response probabilities when a rest of only 2 min or 1 min was allowed.

Determining differences in response probability between the different phases

The probability with which a change in EOD feedback at a given phase elicited a novelty response was determined off-line as follows. A computer program randomly selected and displayed a trace recorded during the experimental day. The trace showed the 200 pre-stimulus intervals, an indicator of stimulus timing and the 300 post-stimulus intervals (see Fig. 1B). The traces displayed were recorded either when the switch was closed within a particular phase of the EOD, in the silent interval between EODs or in the absence of switch

response (NR). Because this response varied considerably in its magnitude as well as its time course, a comparison of the efficiency of different stimulus regimes in eliciting responses required that these regimes were presented in random

closure. No information hinted at the stimulus condition and no stimulus artefacts were present. An observer had to decide whether the currently displayed trace showed a response or not. After this decision the next trace was selected, displayed, and so on. Only after all traces of an experimental day had been judged in this manner were the numbers of responses and failures displayed for the different stimulus conditions and the controls. This procedure ensured that changes in the response criterion used by the observer could not cause differences in response probability among the phases within the EOD, or between them and the controls. Moreover, two types of control were also evaluated. (i) Controls in which a sequence of 500 inter-EOD intervals were displayed that were recorded without stimulation. This type of control was important to determine the 'baseline' number of false responses that are expected because random fluctuations in the inter-EOD intervals (e.g. see the pre-stimulus intervals in Fig. 1B) may be scored as a response. (ii) Controls with the switch briefly closed after the EOD. If the responsiveness obtained during these controls was significantly higher than the 'baseline' probability it would indicate that the fish responded to an electrical signal generated during switch closure and not necessarily only to changes in EOD feedback. However, these controls never yielded higher than baseline probabilities. Statistical comparisons of response probabilities were done using χ^2 -tests.

Construction of the cage

A rigid cage with all its edges carefully rounded (so that the sensitive skin of the fish could not be hurt in an attempted escape) was built on a CNC machine (Hermle U630T). It was made from transparent Plexiglas, was 30 cm long and had dimensions of 3×3 cm. The left and right sides of the cage had nine windows of inner dimensions 2.8×2.8 cm that were covered with rigid plastic mesh (square openings of approximately 1.6 mm, separated by approximately 0.1 mm), whereas the top and bottom sides of the cage consisted of a regular array of 48 equally spaced bars with 3 mm spacings between bars that were oriented orthogonally to the cage's longer side. The spacings between the bars allowed two 'doors' (Plexiglas frames with the plastic mesh) to be inserted so that the fish could be confined (longitudinally) in the center of the cage. Plastic mesh was glued to the bottom side of the cage to prevent the fish from sticking their elongated tails out of the cage. The cage could be firmly suspended from above by means of two rigid Plexiglas rods (diameter 8 mm) fixed at the top of the cage.

General experimental procedure

At the beginning of an experimental day, a rectangular positioning plate (20×30 cm; aluminium) was positioned on a marked region on the tank's floor. A central vertical rod mounted at the plate marked the point at which the centre of the cage's lower side was to be positioned. Two higher vertical rods could be inserted in preset positions on the plate so as to mark the position of the two shunting electrodes at the side of the cage in half of its height. Thus one of three set lateral

distances 0, 1, or 2 cm from the cage could be chosen. The experimental animal was very carefully placed into the cage, often by means of a suitable funnel. Great care was exercised that the fish did not damage its sensitive skin and the tip of its tail because such damage might yield significant deviation from the response patterns observed in the present study. Using the positioning device, the cage was brought into its fixed position and the two shunting electrodes (with a fixed distance of 8 cm between them) were brought into position using a manipulator. Then, the positioning device was removed and the fish was allowed some rest. Next, the delays with respect to the phase-reference pulse needed to place the shorting at the desired phases within the EOD were determined. After a further pause, experiments started with one test every 3 min for 4–6 h. In these tests stimulus conditions were randomly varied. At the end the fish was carefully removed from the cage and transferred to its home tank.

Results

Feasibility of the approach: phase stability

To demonstrate that shunting EOD current during a particular phase of one single EOD is a feasible approach, it must first be shown whether the required phase stability in triggering the shunting can be achieved. This is not evident because in the present behavioral experiments an animal may slightly change its position relative to the recording electrodes. Among other consequences, this would affect the waveform of the recorded head–tail EOD and, hence, the phase reference. Moreover, even small external noise as well as slight fluctuations in temperature (that affect the EOD; e.g. Schuster, 2000; Ardanaz et al., 2001) are expected to lead to considerable phase jitter in the timing of switch closure. As a result of prior testing we tried to minimize these sources of phase jitter by (i) engaging the fish using a suitably constructed cage, (ii) restricting the experimental time so that fish rested calmly during the tests, (iii) placing the recording electrodes for the head–tail EOD at a distance with respect to the fish, (iv) controlling temperature to $\pm 0.1^\circ\text{C}$, and (v) minimizing external sources of noise. With these measures the phase stability in triggering switch closure turned out to be sufficient for the present behavioral experiments. This was quantified in experiments such as those illustrated in Fig. 2 in which the phase jitter of the feedback changes within an EOD was directly measured in a prolonged series of tests. Even during prolonged testing switch closure continued to be triggered at the desired phase and the resulting jitter was remarkably low, in the order of 10 μs (e.g. see Fig. 2B).

Measuring the shunted EOD current

In the present approach it was necessary to control the waveform and the magnitude of the EOD current that could be shunted at the various phases of the EOD. This was attempted by inserting a virtual-ground current amplifier in the external circuit between the two shunting electrodes (see Fig. 1A). As a first step in this analysis, we measured the current when the

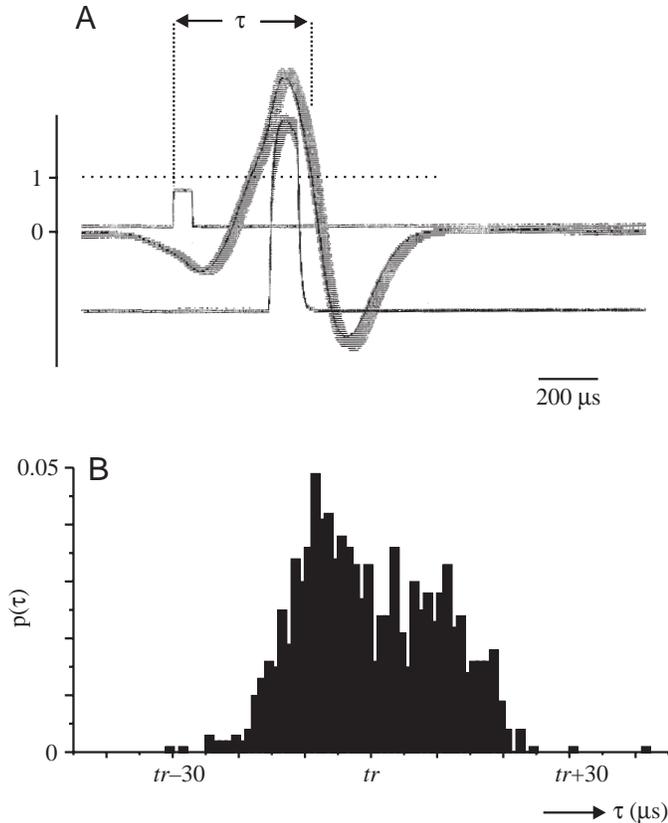


Fig. 2. Precision with which electric organ discharge (EOD) current could be shunted at a selected phase within a single EOD. (A) Oscilloscope showing accumulated recordings from 100 successive tests in which the switch was closed for $100\mu\text{s}$ in a preassigned phase. The upper trace shows the rectangular reference pulse that marked the 'onset' of the EOD and triggered the recordings. The middle trace shows the head-tail EOD with head-positive deflections going upwards. The lower trace shows the current that flowed through the external circuit during switch closure. As a result of a variety of factors (see text) the recorded EODs jitter with respect to the onset-mark and the current signal. However, note that a reasonably small phase jitter could be achieved. (B) To assess the phase stability quantitatively, for each of 1000 successive experiments a precision counter measured the time between the rising flank of the reference pulse and that when the falling phase of the amplified EOD had reached a set threshold (time interval τ as defined in A). The histogram shows the distribution of recorded values for τ (relative to a reference value of $tr=476.5\mu\text{s}$; bin width $1\mu\text{s}$) as obtained in 1000 successive experiments. Note the small amount of phase jitter (s.d. $\pm 10.4\mu\text{s}$). The structure of this distribution is probably determined by different major resting positions of the fish assumed during the 1000 experiments.

electronic switch that connected the two electrodes was closed for long intervals, in the order of seconds. As illustrated in Fig. 3, this analysis revealed two components of the current flow. (i) An 'offset' that decays slowly when the switch is closed for extended periods. This component is recorded also in the absence of a fish or a sending-dipole electrode. It is much larger for Ag (or Cu) wires than for the Ag/AgCl pellets used

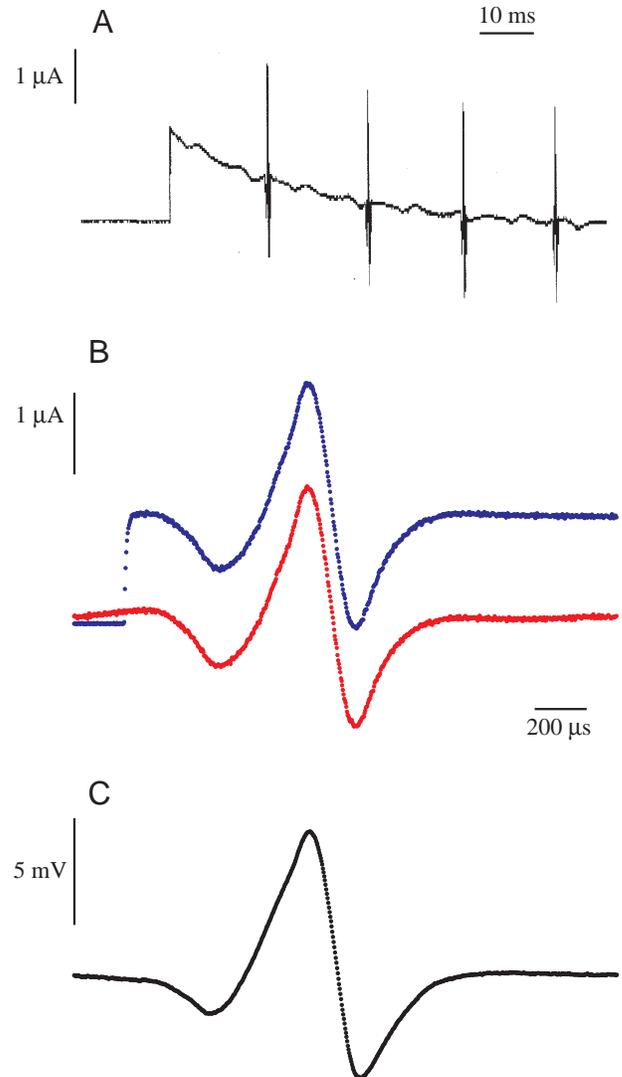


Fig. 3. Analysis of the shunted electric organ discharge (EOD) current. (A) Example illustrating the two effects that contribute to the current measured in the external circuit during maintained switch closure: (i) A slowly decaying current ('offset') that continues even during the silent phases (in the absence of EODs). This component never elicited behavioral responses in the arrangement used in the present study. (ii) The second contribution is from the current that is shunted during the EOD. (B) The two components added linearly within the current range used in the present study. This is illustrated by two recordings of the current in the external circuit, taken at two stages after a maintained switch closure. The first (blue) was taken immediately after the switch was closed and thus when the 'offset' was maximal. The second was taken long after the start of switch closure, when the offset had decayed to zero (red). Traces are aligned with respect to the simultaneous recordings of the head-tail EODs, one of which is shown below the two traces (C). Note that the EOD-induced modulation in the absence of the offset is approximately the same as that recorded in the presence of an offset.

here and is also obtained when a mechanical switch is used instead of the fast electronic switch. These features indicate that it probably results from a difference in the polarization of

the two Ag/AgCl electrodes. In the present arrangement (with the two electrodes placed longitudinally on the side of the fish) this component never elicited a behavioral response. (ii) The second component is the shunted EOD current. This is seen as a modulation on top of the offset. As shown below the presence of this component elicited responses in the present arrangement.

By keeping the switch closed until the offset has decayed it is possible to record only the second component, the shunted EOD current (Fig. 3B). However, in the desired experiments with brief switch closure the offset will not have decayed and it was thus important to know whether the shunted EOD current could be determined in the presence of an offset due to slight differences in electrode polarization or whether it would be necessary to select electrodes in which this difference was below a certain tolerable level. Our analysis shows that an offset such as that obtained with standard Ag/AgCl electrodes is tolerable and does not confound the determination of the shunted EOD current. With such electrodes and under the conditions of the present experiments the offset simply adds to the shunted EOD current. As illustrated in Fig. 3B, the EOD modulations recorded in the absence of the offset (i.e. when the offset had decayed) were approximately equal to those that occurred on top of the offset. At least, within the required precision of about $\pm 0.1 \mu\text{A}$, it is justified to say that the shunted EOD current simply added to the offset. Additional evidence for this was obtained in experiments in which the distance was changed between the shunting electrodes and either a fish (e.g. see Fig. 7 below) or an artificial dipole sender. In all these experiments the modulations on top of the offset decayed with distance but were independent of the amount of the offset. The findings are compatible with an equivalent circuit in which a capacitance in the current path (a good model for Ag/AgCl electrodes; e.g. Meyer, 1982) accounts for the slow decay of the DC potential due to different electrode polarization while the rapid EOD-induced modulations are readily fed through.

So far we have dealt only with an interpretation of the current measured over extended periods of switch closure. But there are still two problems: (i) how can the shunted EOD current be determined when the switch is closed for only $100 \mu\text{s}$, and (ii) does it flow only during the interval of switch closure or do capacitive effects cause the shunted EOD current to flow over longer periods? Recordings, such as those shown in Fig. 4, provide a surprisingly simple answer to both questions. In the present arrangement there was never prolonged current flow for switch closure times down to $20 \mu\text{s}$. Moreover, the magnitude of the shunted EOD current that flows over the external circuit during the brief switch closure could directly be predicted from measurements in which the switch was closed for an extended period. This is illustrated in Fig. 4, which shows how the magnitude of current pulses obtained as the switch was briefly closed at various EOD phases (of successive EODs) retrace the modulation obtained during a prolonged switch closure.

These general features were first demonstrated in experiments with artificial dipole senders [T-shaped electrodes

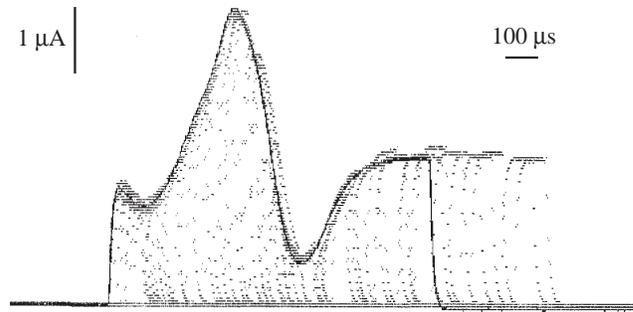


Fig. 4. The electric organ discharge (EOD) current shunted in a particular phase of the EOD can be predicted from the modulation recorded during maintained switch closure. The oscillogram shows accumulated recordings of a set of current signals recorded as the switch was closed briefly for $100 \mu\text{s}$ during various phases of the EOD and the current recorded during prolonged switch closure lasting for the whole EOD (continuous trace). The peaks of the brief current signals retrace the continuous curve recorded during maintained switch closure.

with carbon electrodes inserted at the openings of the horizontal shaft; similar to electrodes used by Westby (1975)] that spread currents similar to the animal's. Using these senders, and determining the current that was shunted during switch closure, we tried to select an experimental arrangement in which the magnitude of the shunted EOD current would be as robustly stable as possible with respect to slight variations in the position and orientation of the sender. In the tests carried out the artificial dipoles were placed at either (i) the bottom or (ii) the centre of the tank. Two dipole lengths were used: 4 cm and 12 cm. Shunt electrodes were placed at the height of the dipole at lateral distances as in the later experiments. We then analyzed how the amount of current shunted during switch closure varied in each of the four possible sender configurations because the sender's average position was changed slightly in each of four possible ways: (a) $\pm 10 \text{ mm}$ horizontal displacement orthogonal to the dipole axis, (b) $\pm 10 \text{ mm}$ vertical displacement orthogonal to the dipole axis, (c) $\pm 5 \text{ mm}$ displacement along the dipole axis and (d) $\pm 5^\circ$ rotation in the horizontal plane along the centre of the dipole. Briefly, the shunted current showed most stability with respect to the various modes of position changes when the sender was at the tank's centre and when the distance between the shunting electrodes was smaller than the dipole's length. The present arrangement of a narrow cage placed in the centre of the tank was chosen as a result of these tests.

Detection of novelties in feedback during a single EOD

All fish showed a significant probability of response when EOD feedback was changed during only $100 \mu\text{s}$ within only one of its EODs. In the experiments of Figs 5 and 6 the shunting electrodes were placed directly at the boundary of the fish's cage at the indicated positions close to the animal's trunk and tail (as indicated in the respective insets of Fig. 5). The shunting electrodes were briefly connected for $100 \mu\text{s}$ either at

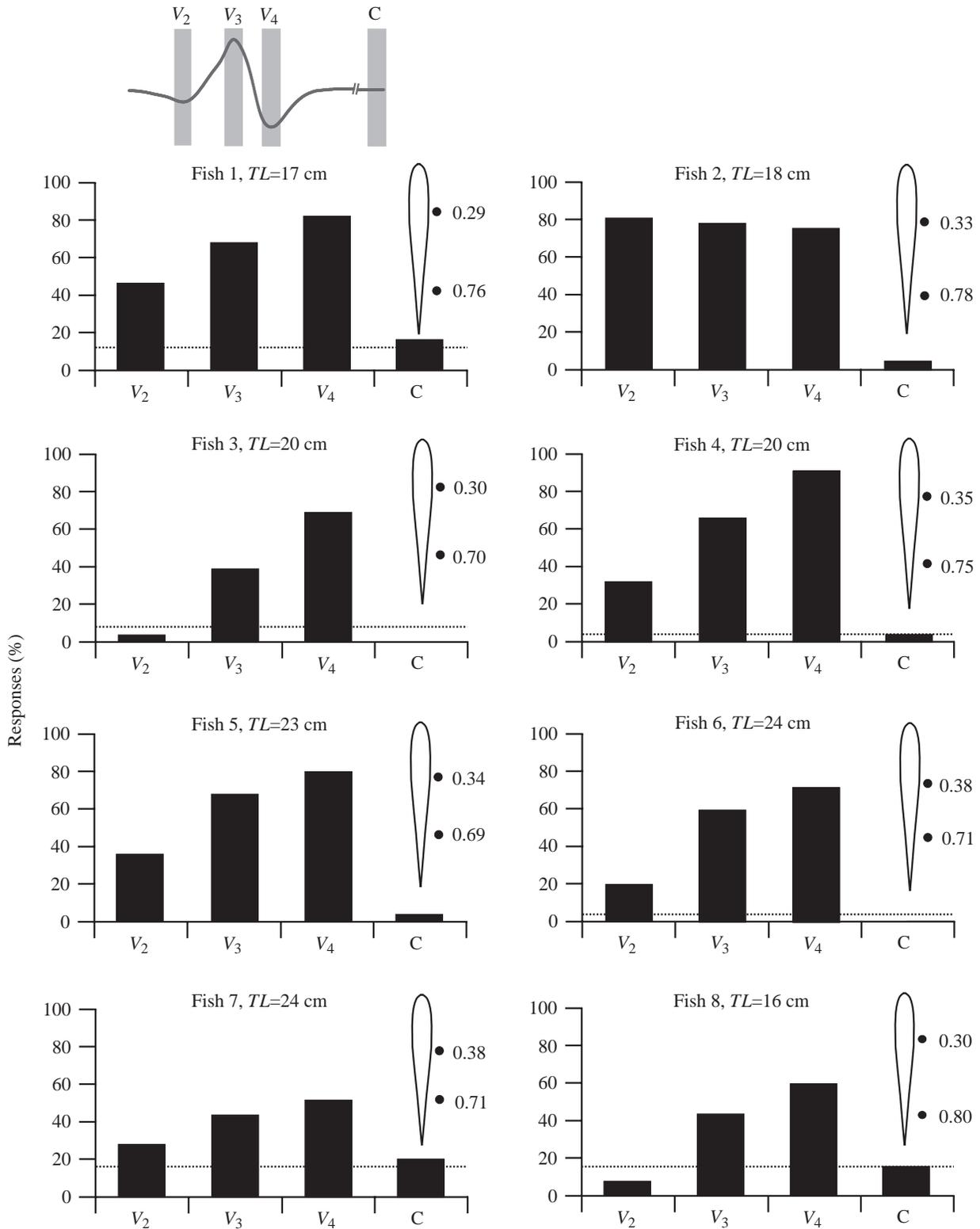


Fig. 5. Sensitivity to novel feedback during one of the three major phases of a single electric organ discharge (EOD). The diagrams show for each animal the probability of a novelty response when the electronic switch was closed for a brief interval of $100\ \mu\text{s}$ that either centred at one of the extrema of the EOD (V_2 , V_3 , V_4 ; see inset) or after the EOD (Control, C ; zero for Fish 3 and 6). The horizontal dotted lines indicate the 'baseline' of responses (determined from recordings in the absence of switch closure) due to spontaneous fluctuation in the discharge rate of the animal. Each diagram comprises the results of 125 tests (approximately 25 per stimulus condition). Insets indicate the relative position of the two shunting electrodes at the trunk/tail region in relation to the fish's total length (TL).

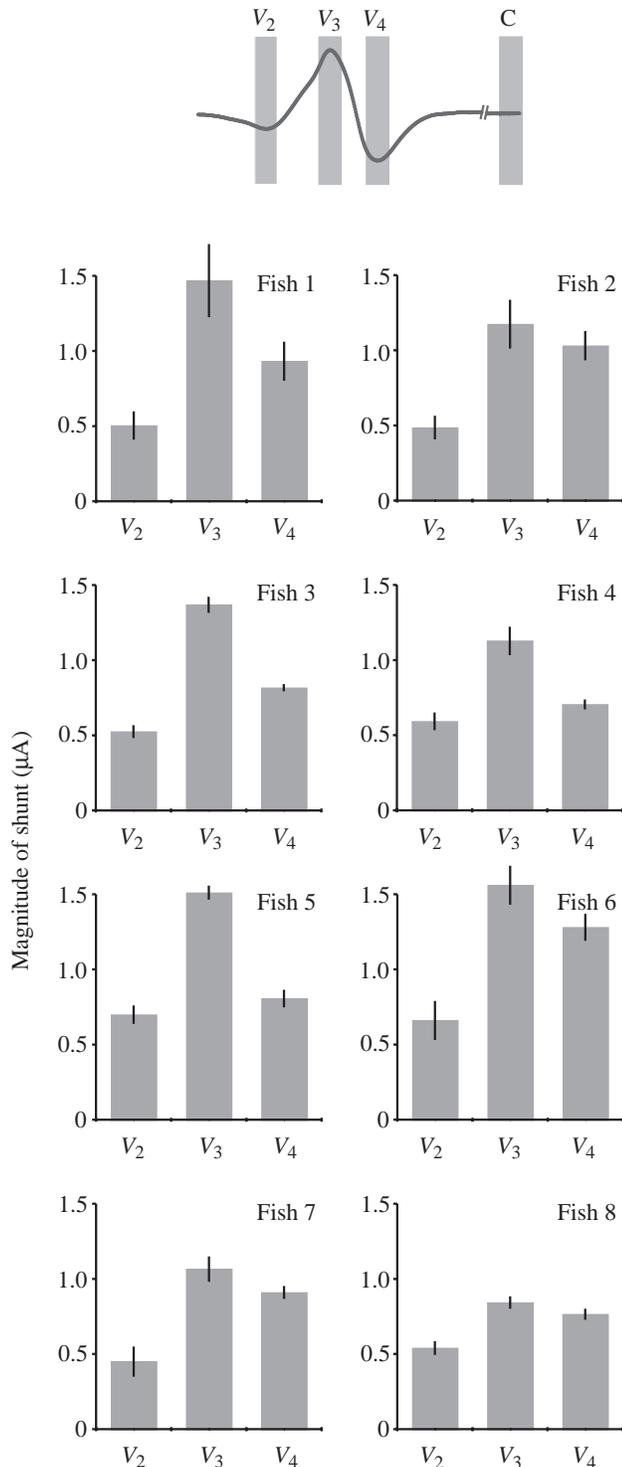


Fig. 6. Magnitude of the shunted electric organ discharge (EOD) current in the experiments shown in Fig. 5. Values are means \pm s.d. derived from approximately 25 measurements each. The inset shows an EOD and indicates the phases (V_2 , V_3 , V_4) at which feedback changes were triggered for 100 μ s.

one of the major phases of the EOD or in the silent interval between EODs (control; C). For shunting of EOD feedback, the period during which the switch was closed was adjusted

such that it centered at one of the extrema V_2 , V_3 or V_4 . Interval recordings without switch closure served to determine the 'baseline' percentage of responses that was assigned because of spontaneous rate fluctuations (dotted horizontal lines in the diagrams of Fig. 5; see Materials and methods). Fig. 5 shows the response probabilities determined in a series of experiments involving all animals ($N=8$; with about 25 tests for each stimulus condition). In addition, Fig. 6 reports the magnitude of EOD current (mean \pm s.d.) that could be shunted in these experiments.

Several features of the behavioral data shown in Fig. 5 are noteworthy. (i) All fish showed a highly significant percentage, at least in one of the phases tested, of responses to the feedback changes during one single EOD. (ii) This was not because the fish detected an electrical artefact signal spread during switch closure. As in previous studies (Bennett and Grundfest, 1959; Szabo and Fessard, 1965; Larimer and Macdonald, 1968; Heiligenberg, 1980; Meyer, 1982) placing switch closure in the silent interval between successive EODs allowed us to demonstrate that the animals responded to the feedback changes. The absence of any significant difference between the baseline, caused by spontaneous rate fluctuations, and the controls (C) with switch closure in the silent inter-EOD interval shows that the fish responded to changes in EOD feedback and not to an artefact of switching. (iii) In the presently studied trunk/tail region of *G. carapo* there appears to be significant sensitivity to changes in EOD feedback during all the major phases, V_2 – V_4 , of the discharge. Only for fish 3 (phase V_2) and fish 7 (phases V_2 and V_3) were the response levels not significantly above baseline. Thus, feedback changes within all phases appear to be evaluated to signal novelties at the trunk/tail region. (iv) Although significant response probabilities were observed at all major phases of the EOD, there were notable differences among them: responsiveness in V_4 was significantly higher than in V_2 for fish 1 and for fish 3–6. With one exception, the highest response levels were obtained in V_4 , although the differences between response probability in V_3 and V_4 were only significant for fish 3 and 4 in the present series of tests. However, in later experiments that involved more tests, the apparently different responsiveness in V_3 and V_4 was also proven significant for fish 6 (e.g. see Figs 7, 8) and for fish 8. The final head-negative phase V_4 appears thus to be the phase of highest sensitivity for the detection of novel feedback in the trunk/tail region. Fish 2 showed a remarkably high response probability during all three phases. This pattern deviated considerably from that found in all other fish. However equal sensitivity was also found in later tests at larger distances of the shunting-electrodes and smaller magnitudes of the shunted EOD current. (v) The findings indicate that the exact location of the shunt electrodes within the trunk/tail region is not critical for the sensitivity pattern because it was observed even though the relative position of the shunting electrodes varied among fish (as detailed in the insets of Fig. 5). This is in accord with expectations, based on the longitudinally homogeneous distribution of electroreceptors within this region (Westby, 1975; Watson and Bastian, 1979;

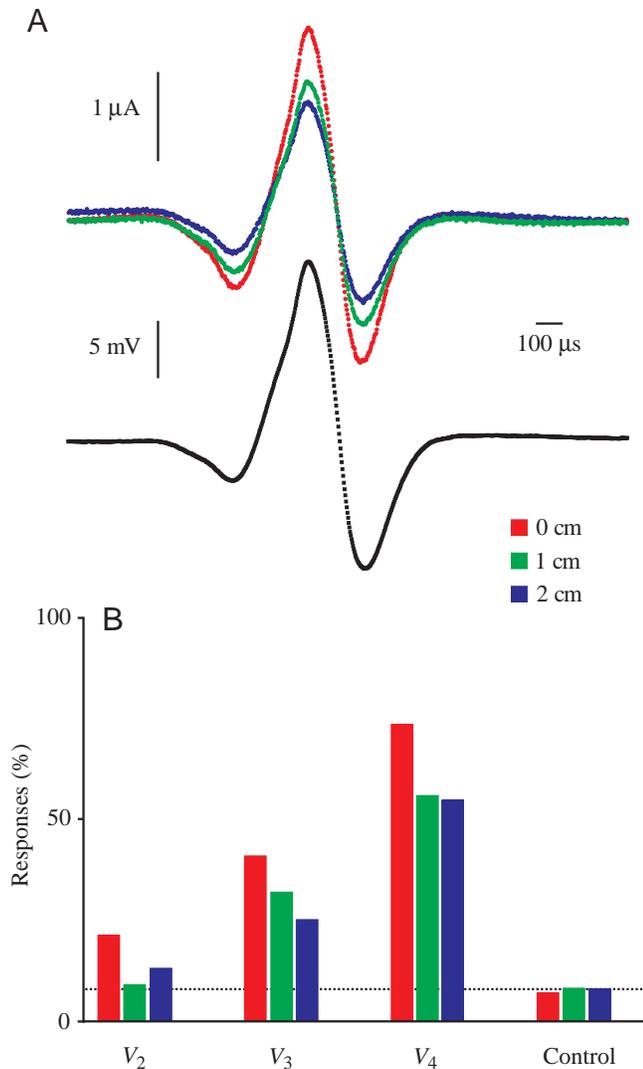


Fig. 7. Positioning the shunting electrodes farther away from the side of the fish reduces the current shunted during an electric organ discharge (EOD) but leaves the distribution of responsiveness across phases constant. (A) Recording of EOD current shunted during prolonged switch closure when the shunt electrodes were placed at three lateral displacements, successively farther away from the fish cage (0, 1 or 2 cm). Each point of the waveforms predicts the magnitude of EOD current that is shunted when the electronic switch is closed during an interval that centers at this point. An example of the head-tail EODs, recorded simultaneously, is shown below as a phase reference. (B) Response probabilities obtained as the switch was closed for 100 μ s either during one of the extrema V_2 , V_3 or V_4 , or 4 ms after onset of the EOD (Control). For each of the three distances, 100 responses were recorded at each of the four stimulus conditions (total of 1200 tests on fish 6). The baseline response probability (due to spontaneous rate fluctuations) is given by the dotted horizontal line (pooled from 100 recordings without switch closure at each distance). There was no significant difference between baseline and controls. At all distances, responsiveness in V_4 is significantly higher than in V_3 ($P < 0.001$) and higher in V_3 than in V_2 ($P < 0.05$). All response levels are significantly above baseline except for those at distances 1 and 2 cm in phase V_2 . Decrease in responsiveness with distance is significant in V_4 (0–1 cm: $P < 0.01$) and V_3 (0–2 cm: $P < 0.05$).

Castelló et al., 2000), that different subareas within this region should contribute equally to the detection of novel EOD feedback.

Did the differences in sensitivity at the different phases arise because different amounts of currents could be shunted in the different phases? This question is addressed in Fig. 6, which shows the magnitude of EOD current shunted in phases V_2 , V_3 and V_4 under the experimental conditions of Fig. 5. These measurements showed three main results: (i) EOD current could demonstrably be shunted at all three phases (V_2 – V_4), (ii) in all fish the magnitude of the feedback changes elicited in the experiments was higher in V_3 compared with V_4 , and (iii) in V_2 the shunted EOD current was not much below that shunted in V_4 . However, in contrast to the current measurements, the behaviorally detected sensitivity was less in V_2 than in V_4 and appeared to be higher in V_4 than in V_3 .

To test whether the distribution of sensitivity at the three phases V_2 – V_4 was mainly determined by the magnitude of the feedback changes, we varied the distance of the shunting electrodes from the side of the fish and, concomitantly, the absolute amount of EOD current that could be shunted ($N=4$

fish; fish 2–4,6). In the example shown in Fig. 7 (fish 6), the upper traces characterize the stimulus. Each trace shows, for a given distance, the amount of EOD current that could be shunted during switch closure at any phase of the EOD. Below, the response probabilities are reported for feedback changes at V_2 , V_3 and V_4 at each of the three tested lateral distances. These experiments comprise several days of testing in which both phases and distances were varied randomly from trial to trial (quick changes in the lateral distance were aided by a micromanipulator). In each of the fish tested this way the distribution of sensitivity across phases, although not the absolute sensitivity, was constant irrespective of the changes in the amount of the EOD current that could be shunted at the different distances.

Sensitivity to novel feedback in other phases of the EOD

Switch closures of only 100 μ s duration during a single EOD were also applied at other phases within the EOD of *G. carapo* ($N=3$; fish 3,4,6). (i) Centered at the zero crossings of the head-tail recording of the EOD between V_2 and V_3 , and between V_3 and V_4 , as well as centered 80 μ s after the V_4 peak. (ii) During phase V_1 in which the current results from postsynaptic potentials (PSPs) of the rostral sides of doubly innervated abdominal electrocytes.

The example in Fig. 8 illustrates the findings obtained for the additional phases after V_1 . In the respective experiments, a full set of tests was conducted in which all phases (including V_2 – V_4 and controls) were tested in a random sequence so that the respective response probabilities could be safely compared. The main results were as follows. (i) Sensitivity is high even for novelties in feedback that occur at the decaying phase of V_4 . Here, the response probability was generally significantly

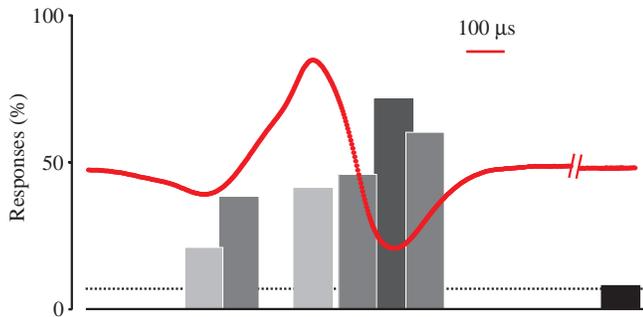


Fig. 8. Experiments in which electric organ discharge (EOD) current was shunted for $100\mu\text{s}$ at various phases of the EOD. The horizontal width and position of the bars indicate the timing of switch closure relative to the head-tail EOD (red trace). The height of each bar indicates the experimentally determined response probability when the switch was closed during the indicated period of the EOD. The dotted horizontal line gives the baseline responsiveness (due to spontaneous fluctuations in discharge rate) determined from 100 recordings without switch closure. Responsiveness in the controls was not above baseline, but switch closure within the EOD led to significantly higher than baseline responsiveness (at least $P < 0.01$). Responsiveness in V_4 was significantly higher ($P < 0.001$) than in V_3 but differed not significantly from that determined in the phase after V_4 . A total of 1070 tests (100 in each condition, except for phase V_4 and for controls after the EOD, in which 200 and 270 tests, respectively, were conducted). Data from fish 6. Electrode positions as indicated in Fig. 5.

above baseline and above the responsiveness of all other phases except V_4 , in which responsiveness was not significantly different. (ii) Placing the centre of the $100\mu\text{s}$ shunting period at a reversal of current direction did not lead to significant changes in responsiveness. Response probability was not significantly different whether the feedback change occurred during V_3 or during the zero-crossings between V_2 and V_3 or between V_3 and V_4 .

Only a few behavioral experiments were done to address the question of how phase V_1 contributes to sensitivity at the trunk/tail region. Because this phase is produced by abdominal electrocytes the resulting current flow that could be shunted in the trunk/tail region during this phase was small. Thus a low response probability was expected when switch closure was placed within this phase. To place switch closure within this phase, the EOD was picked up with two differential electrodes close to the abdominal site where V_1 is generated. The identity of the V_1 signal was confirmed by the simultaneous recording of the head-tail EOD in the standard manner. A $100\mu\text{s}$ shunt was triggered immediately as the locally recorded V_1 signal rose above a fixed threshold. The shunt could thus be placed in V_1 , about $150\mu\text{s}$ before the first head-negative deflection V_2 . The results of tests made with 3 fish (3,4,6), with the shunting electrodes placed in the trunk/tail regions indicated in Fig. 5, showed a response probability that was not significantly different from the baseline. Hence, the current produced during V_1 seems to play a negligible role for electrolocating objects in the trunk/tail region of the animal. This could have been the

case if foveal receptors were involved in the detection of feedback changes that are triggered at the trunk and tail region. The findings, therefore, make their involvement in the presently observed responses unlikely.

Responses to briefer switch closure within a single EOD

To explore the smallest possible period of switch closure within a single EOD that elicited a significant response level, experiments were performed in which the period was centered at the most sensitive phase V_4 ($N=3$; fish 3,4,6). Electrodes were placed at the closest possible distance to the trunk/tail region as before. The responsiveness to three periods of switch closure was examined: $100\mu\text{s}$, $50\mu\text{s}$ and $20\mu\text{s}$. An analysis of the current flow in the shunting circuit confirmed that EOD current was shunted only for these periods and indicated that the analysis of the magnitude of the shunted EOD current extends to periods of switch closure of $20\mu\text{s}$. None of the fish showed significant responsiveness when the feedback changes occurred only for $20\mu\text{s}$ during V_4 . However, for all three fish responsiveness significantly above baseline was found at $50\mu\text{s}$. Fig. 9A illustrates this with the data for one fish.

The possibility of recording behavioral responses to feedback changes that occur for $50\mu\text{s}$ in a single EOD permits, in principle, a refined analysis in which sensitivity within the EOD can be tested with better resolution. Fig. 9B shows corresponding results obtained with one fish in which the prolonged series of corresponding experiments could be completed (fish 6 of Fig. 5). 11 phases within the EOD (plus the control outside the EOD and the determination of the baseline) were analysed. For each test, phases were randomly selected. Thus only a few tests could be conducted each day for a given phase and the experimental procedure needed to be repeated for an extended period. Two series could not be continued until significant data were gathered: fish 3 lost the tip of its tail after 5 experimental days and fish 6 became so active in its cage that the $50\mu\text{s}$ switch closures could no longer reliably be placed at the desired phases of its EOD. However, the results of the only successfully completed series (Fig. 9B) do not indicate a finer pattern of how sensitivity is distributed within an EOD. They confirm the finding that feedback changes in V_4 are most readily responded to despite the fact that the largest magnitude of EOD shunting occurred at phase V_3 .

Discussion

In this study we have applied a new method in which the pattern of current flow during an EOD is changed very briefly during a particular phase of the EOD. The magnitude and the time course of this change are monitored and an increase in the discharge rate of the fish signals whether it has detected the change in EOD feedback. Using this method we have studied the roles played by the various currents that contribute to the complex EOD of *G. carapo* in the detection of novel feedback in the trunk/tail region of the animal. We demonstrate that *G. carapo* can respond to feedback changes that affect only a

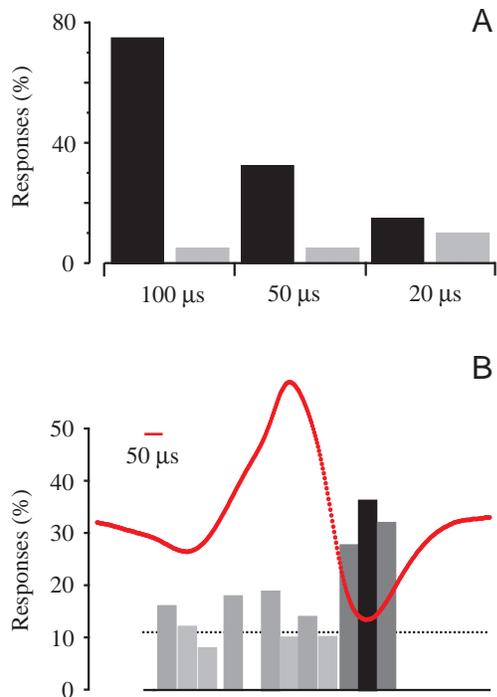


Fig. 9. Response probability (A) and phase distribution of response levels within the electric organ discharge (EOD) (B) for EOD shunts of lesser duration than 100 μ s. (A) Responsiveness to novel feedback at the trunk/tail region decayed considerably when EOD current was shunted for less than 100 μ s. In the experiments the switch was closed for a period that centered at the peak V₄ and was either 100 μ s, 50 μ s or 20 μ s in duration. Experiments with fish 6 with electrodes positioned as shown in Fig. 5. Black bars, response probability at V₄. For each stimulus duration, response levels were additionally determined in (i) 20 controls in which the switch was closed after the EOD and (ii) in the absence of switch closure (20 tests). As response probabilities obtained under conditions (i) and (ii) showed no significant difference, data from (i) and (ii) were pooled and are displayed as grey bars. Responsiveness to 100 μ s and 50 μ s shunting, but not to 20 μ s shunting, was significantly above the control level ($P < 0.001$ and $P < 0.05$, respectively; $N = 240$ tests). (B) An attempt at a more detailed analysis of the response profile within the EOD using shortings that lasted only for 50 μ s. As in Fig. 8, the horizontal position and width of the bars indicate the timing relative to the EOD (red trace). The height of each bar gives the response levels as obtained from a total of 1200 tests with fish 6. The dotted line was determined from pooling (i) 100 controls in which the switch was closed after the EOD and (ii) in the absence of switch closure (100 tests) (no significant difference in responsiveness). Only in the three phases around the final head-negative deflection (V₄) was the responsiveness significantly above control level ($P < 0.01$).

fraction of a single EOD. In its trunk/tail region *G. carapo* responded to feedback changes in all phases but most sensitively to changes that occurred during the final head-negative phase of its EOD.

Feedback changes as quantifiable stimuli

Shunting the self-produced EOD current has been instrumental in the past in demonstrating the capability of

weakly electric fish to detect changes in feedback from their own EODs (Bennett, 1965; Bennett and Grundfest, 1959; Enger and Szabo, 1965; Hagiwara et al., 1965; Szabo and Fessard, 1965; Harder et al., 1967; Larimer and Macdonald, 1968; Macdonald and Larimer, 1970; Moller, 1970; Heiligenberg, 1980; Meyer, 1982). However, in these experiments no attempt was made to directly monitor the magnitude and waveform of the shunted EOD current. In the present study, controlling the shunted current was crucial (i) to confirm the extremely short duration of the feedback changes, (ii) to demonstrate that the feedback changes were robust despite slight changes in the position of the animal during prolonged testing and, finally, (iii) to assure that sizable current changes could be induced in each of the major phases and that a lack of responsiveness to feedback changes in a particular phase of the EOD was not small simply because little current could be shunted during that phase. From our findings feedback changes elicited by switch closure qualify as quantifiable stimuli.

Changes of EOD feedback have previously been studied with two main goals: (i) to simply distort the normal distribution of self-produced EOD current on the skin and (ii) to simulate a small object at a defined location in the surroundings of the fish. With the first goal it is reasonable to place the shunting electrodes to maximize the current that can be shunted when the two electrodes are connected outside the water. Probably for this reason, all previous work that aimed at the first goal used electrodes placed a considerable distance apart laterally to the fish's body or in front of the head and behind the tail. To mimick a point-like object, however, Heiligenberg (1980) used more closely spaced shunting electrodes. In the present study of sensitivity to novel feedback in the trunk/tail region an arrangement appropriate for the first goal could be chosen. This is because Westby (1975), Castelló et al. (2000) and Aguilera et al. (2001) have shown a homogeneous distribution of electroreceptors with no fovea in the region of our study, so that responses obtained to feedback changes at two distant electrodes are expected to be mainly driven from similar receptor populations.

Responses to feedback changes within fractions of a single EOD

To our surprise feedback changes that affect only a fraction of a single EOD were sufficient to elicit a significant novelty response. This is surprising as we analysed responses to novel feedback in the least sensitive region of the fish (Westby, 1975; Castelló et al., 2000; Aguilera et al., 2001). Several earlier experiments have already demonstrated that changed feedback during only one EOD could be sufficient to elicit a response (Szabo and Fessard, 1965; Harder et al., 1967; Meyer, 1982). Interestingly, Heiligenberg, studying the 'electromotor' following response of the gymnotid fish *Hypopygus* (Heiligenberg, 1974) and the novelty response of the mormyrid *Brienomyrus* (Heiligenberg, 1976), found that the ability of these animals to electrolocate deteriorated only when foreign pulses consistently coincided with (or briefly

preceded) its EOD. Additionally, experiments in *Hypopomus* suggested that the detection of novelties depended on several EODs being affected in succession (Heiligenberg, 1980); thus, the integration of feedback from successive EODs appeared essential. It is likely that the feedback changes associated with switch closure in the present study were much larger than in Heiligenberg's study and an integration mechanism would not be excluded in which smaller feedback changes would need to be integrated over several EODs before a response is elicited. However, the present findings exclude an 'all or none' mechanism in which it would be essential that feedback from a considerable number of EODs is affected in order that novelty is detected and a rate-acceleration response elicited.

The differences in sensitivity are not caused by differences in the shunted EOD current

The observed differences in sensitivity to feedback changes triggered at various phases of the EOD could have simply reflected the different amount of current that could be shunted during the various phases. However, this simple view can be excluded from our direct measurements of the shunted current. Surprisingly, the general pattern of sensitivity at the different phases of the EOD did not reflect the magnitude of EOD current that we were able to shunt within these phases. The shunted EOD current was clearly largest during phase V_3 (e.g. Fig. 6). Yet the response probability was highest to shunting triggered at phase V_4 . Similarly, the EOD currents shunted during phase V_2 were not much less than those shunted during V_4 , yet the response probability was lowest to shunts triggered at phase V_2 . Thus, a simple channeling of current appears not to explain the observed sensitivity pattern in the trunk/tail region of *G. carapo*. Studies in curarized fish, with the EOD substituted by an appropriate mimic, would be needed to understand whether the sensitivity pattern originates from the properties of individual receptor units or derives from a more central comparison of the responses of receptors at different locations.

Could the observed sensitivity pattern be partly due to an involvement of the foveal receptors? This would be expected when the shunt triggered between the trunk and tail electrodes produced sufficiently large changes in the local EOD at the fovea. Two lines of evidence make this unlikely. (i) The study of Aguilera et al. (2001) indicated a negligible role of the V_4 current at the fovea. Thus, a contribution of foveal receptors to the sensitivity at this particular phase seems negligible. (ii) The experiments in which the shunt between the electrodes placed at the tail and trunk was triggered during V_1 indicated a lack of responses. This would not be expected if foveal receptors contributed significantly to the response to shunts at the trunk/tail region.

Macdonald and Larimer (1970) have applied continuous trains of external low-amplitude electrical pulses between two electrodes placed at the head and tail of a *G. carapo*. They report that switching this train from consistently coinciding with one extreme of the EOD to the next resulted in novelty responses. The magnitude of the novelty response

observed in their 'phase-switching' experiment with external pulses depended on which particular phases the phase switching was made between, and was maximal when switching occurred between V_2 and V_3 . It is tempting to compare the result of this phase-switching experiment with our present findings. Provided that details of the experimental situation were comparable, then the response profile in a phase-switching experiment should, to a first approximation, be the derivative (with respect to phase) of a response profile recorded in our study. Accordingly, the sensitivity patterns shown in Fig. 5 would be compatible with Macdonald and Larimer's findings.

Relation of the present findings to the dual EOD hypothesis

The complicated discharge pattern of gymnotid electric organs has prompted the hypothesis of a dual EOD system in which different parts of the electric organ may produce currents that serve different roles in communication and electrolocation. Perhaps the first clear statement of such a dual EOD system was made by Trujillo-Cenoz et al. (1984) who stated, "...the innervation pattern described here suggests the possibility that a dual EO system has evolved in *G. carapo*. The doubly innervated electrocytes, perhaps with a less fixed output, may play a communication role. The population of singly innervated electrocytes may subserve the well-known electrolocative function." Recently, the general notion of a dual system has received support by the study of Aguilera et al. (2001) who demonstrated in the foveal region of the jaw of *Gymnotus* that current produced during the final phase V_4 of the EOD was negligible. In contrast, when the EOD of a distant conspecific is monitored at the fovea, the phase V_4 of the conspecific's EOD is predominant. These findings led to the suggestion that the currents generated during the final phase V_4 might be predominantly used for communication, whereas the currents from the phases V_1 and V_3 were suggested to be relevant for electrolocation.

A critical test of this hypothesis is to examine the trunk/tail area of the fish in which all current components contribute so that their general role as carriers of electrolocation signals can be assessed using the novel method introduced here. The hypothesis would predict that the responsiveness should be largest when feedback changes are triggered in the presumably electrolocation-important phases while responsiveness should be small or absent in the presumably communication-relevant phase V_4 . Our findings do not support this prediction. Not only can *G. carapo* in principle detect changes in EOD feedback that are triggered in any phase of its EOD but also its sensitivity is highest in the phase V_4 for which a communication role was suggested. The present findings therefore suggest that currents produced during V_4 are not specialized for a role in communication. However, they do not seem to be specialized for electrolocation either. In the region of highest sensitivity the measurements of Aguilera et al. (2001) show a small contribution of V_4 current, which would be difficult to understand if it played a major role for electrolocation also at

the fovea. Rather, the evaluation of feedback changes during the phases V_1 and V_3 that are most important at the fovea would seem to be required for electrolocation at the fovea.

The findings do not disprove the general notion of a dual EOD system. In fact, it could still be that the original suggestion, cited above, by Trujillo-Cenoz et al. (1984) is correct and that the reduction of currents during phase V_4 at the fovea, together with the present finding that the remaining phases can also be used for electrolocation, might explain the presumably higher importance of V_1 and V_3 for electrolocation at the fovea as inferred from the results of Aguilera et al. (2001). What is clear, however, is that a strict specialization of currents and functions seems not to hold, at least not in the electric organ of *G. carapo*.

It might, therefore, be more useful to pursue other ways of understanding the puzzling complexity of EOD generation in pulse-type gymnotid fish. Electrolocating 'gymnotid-style' by means of an electric organ that produces enormous local variations in the waveform of the transcutaneous currents could have more intrinsic advantages. For instance, early measurements by Bastian (1977) clearly raise the possibility that local differences in the EOD may give rise to an interesting cue in the detection of purely resistive objects. Rather than simply changing the amplitude of the local EOD (as is certainly the case for a 'point object') (e.g. von der Emde, 1999), purely resistive objects, whose sizes are in the range of the local inhomogeneity of the transcutaneous flow of EOD current, could well lead to phase changes as well as changes in the amplitude spectrum of the local EODs. Unfortunately, the possibility that such cues are actually used has, to our knowledge, not yet been explored. It is not unlikely that the possibility to exploit such cues, besides other important constraints such as sexual selection, predation, energetic costs (e.g. see Stoddard, 1999; Hopkins, 1999), could also have played a role in shaping the complex electric organs of pulse-type gymnotid fish.

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