

# Contextual effects of small environments on the electric images of objects and their brain evoked responses in weakly electric fish

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## Summary

This article reports some contextual effects of fish hovering in small environments on active imaging. Foveal electrosensory images of objects and their corresponding evoked responses in the electrosensory lobe are altered in amplitude and waveform when the fish are inside tubes. The article describes: (i) the physical basis of the changes imposed by small environments on electric images, (ii) the field potential responses at the electrosensory lobe of chronically implanted animals when entering and leaving tubes, and (iii) the effect of context on object discrimination. Biophysical analysis indicates that tubes cause a change in the efficiency of a previously described pre-receptor/post-effector mechanism responsible for the electric 'illumination' of nearby objects (as mirrors change the illumination of visual scenes). Field potential responses at the electrosensory lobe showed two

components corresponding to the fast- and slow-electrosensory pathways respectively: (a) an early spike following the input without adaptation; (b) a series of waves lasting the rest of the cycle and exhibiting different degrees of adaptation. Discrimination experiments showed that fish react to changes in image rather than to changes in object resistance. The amplitude of the novelty responses evoked by similar changes in the total energy of electric images was constant despite the large change in basal stimulus amplitude and waveform caused by hovering in the tubes. These facts may be explained by the presence of adaptive responses observed at the slow pathway in the electrosensory lobe.

Key words: active electrolocation, prereceptor mechanism, refuge behaviour, sensory adaptation, electric fish.

## Introduction

Alteration of sensory images by their context is a common and important issue in sensory physiology (Palmer, 1999). A mirror may change the illumination of a visual scene and the visibility of objects, the resonant cavity of a guitar may amplify the sound generated by the strings, and the presence of minute amounts of spices may transform a regular fare into a delicious dish.

There are theoretical and experimental reasons to believe that contextual effects are also present in electroreception, a sensory modality evolved by aquatic animals (Lissmann, 1958; Bullock and Heiligenberg, 1986; Møller, 1995). Electric fish are electroreceptive animals that explore their environment with the discharge of specialized electric organs (electric organs, EOs; electric organ discharges, EODs). The EO generates an electric field that is sensed by cutaneous electroreceptors (Lissmann and Machin, 1958; Bullock et al., 1961). Objects in the near environment cause changes in the electric field and in the patterns of transepidermal current density and voltage stimulating the electroreceptive surface. The 'electric image' of an object has been defined as the change in the pattern of the transepidermal field caused by that object (Bastian, 1986; Caputi et al., 2002; Budelli et al., 2002).

Two lines of arguments converge to suggest that electric images of given objects are conditioned by the presence of other objects in the electric scene.

First, previous work done by our group has shown that the body of the fish is itself an object that decisively shapes the electric field generated by the EOD (Castelló et al., 2000; Aguilera et al., 2001; Caputi, 2004). The fish body implements a pre-receptor mechanism enhancing the reafferent signals at the perioral region, which may be described as an electroreceptive fovea (Castelló et al., 2000; Caputi et al., 2002). The presence of large objects near the fish may alter the above-mentioned funnelling effect, causing consequent changes in the electric image of other objects (Budelli et al., 2002; Rother et al., 2003).

Second, the presence of such large objects is not an exceptional event, but the rule in fish life. In fact, rather than swimming in the middle of the stream, electric fish are frequently found among the roots of floating plants or inside caves on the banks of the rivers. Moreover, most electric fish in captivity choose to stay long periods resting in tubes, or at the angle between the floor and the side of a large object when they are in captivity. These objects may be

likened to the structures used as refuges in the nature environment.

This article shows contextual effects on electric images of objects caused by the characteristic electric fish behavior of hovering in tubes. The article also reports the experimental analysis of (i) the physical basis of the changes imposed by small environments on electric images; (ii) the field potential responses at the electrosensory lobe of chronically implanted animals when entering and leaving tubes; and (iii) the effect of context on object discrimination and its implications for electrosensory processing.

## Materials and methods

### General

We examined contextual effects on electric image generation and processing at the fovea of pulse gymnotid placing the fish outside or inside tubes of different conductivity. Fifteen fish of the genus *Gymnotus* 12–15 cm in length were used. Recent taxonomical studies have revisited the genus *Gymnotus* and found more than 25 species (Albert et al., 1999); the species we study has been reclassified as *Gymnotus inaequilabiatus* (Albert et al., 1999). Up to now, however, this species was referred to as *Gymnotus carapo* (L.) and has been studied by our group for more than 25 years (see the reviews by Caputi, 1999; Caputi et al., 2002). Fish were gathered in the same ecological niche as before, in the Laguna

del Sauce lake, Uruguay. Fish collection was performed under the regulations of the Ministry of Ganadería, Agricultura y Pesca. Surgical procedures were performed below 4°C to avoid pain and stress on the fish. At this temperature fish do not react with movements or changes in their EOD rate to nociceptive stimuli. All experiments were done under the rules of the Committee for use of Experimental Animals of the Instituto de Investigaciones Biológicas Clemente Estable and according to the guidelines of the Society for Neuroscience and the International Guiding Principles for Biomedical Research Involving Animals.

### Recorded variables

(1) To measure the time of occurrence of the EOD we recorded the far field on the longitudinal axis of the fish (head-to-tail EOD, htEOD) that yielded the classically described pattern with four wave components generated by different regions of the body, characteristic of the species ( $V_1$ – $V_4$ ; Trujillo-Cenóz et al., 1984) (Fig. 1A). The largest slope of this htEOD triggered undelayed rectangular pulses (0.1 ms and 5 V) that were used as a trigger for the oscilloscope and acquired through the parallel port of the computer to calculate the inter-EOD interval (signal processing program kindly provided by R. Saa).

(2) To evaluate the local potential gradients (sLEOD) we used a probe constructed from two nichrome wires insulated except at their tips (200  $\mu\text{m}$  thick, 2 mm apart; see Castelló et

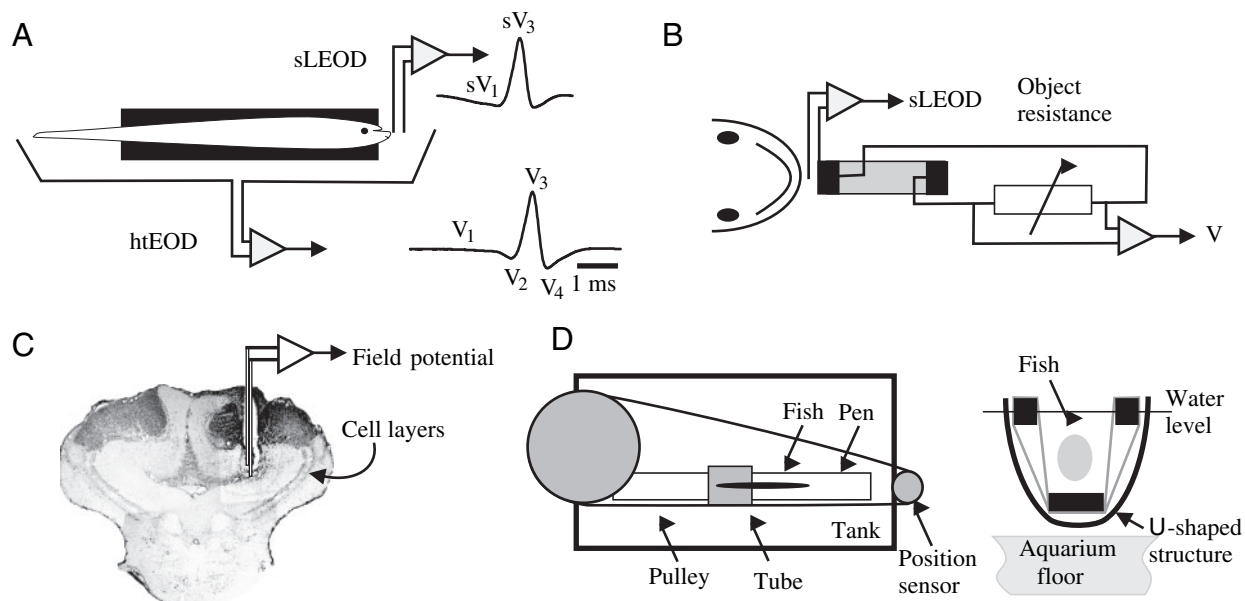


Fig. 1. Methods. (A) Diagram of the electrode arrangement for recording the local self-generated field (sLEOD) and the head to tail field (htEOD). The black rectangle represents the position of the tube in the experiments shown in Figs 2–7. (B) Diagram of the stimulus object and the electrode arrangement for recording the local self-generated field (sLEOD) when shunting the poles of the object with a switch-selected resistor (arrow) in order to control its longitudinal resistance. We recorded the voltage drop ( $V$ ) between object contacts and calculated the current flow through them. The electromotive force and internal resistance of the equivalent source that ‘illuminates’ the object were estimated from the characteristic voltage vs current plot (see Fig. 5). (C) Diagram showing the position of the electrodes used for recording field potentials at the electrosensory lobe. (D) Diagram of the pen and the U-shaped structure moved along the fish axis in order to change the reafferent stimuli while recording evoked field potentials at the electrosensory lobe. Left, top view of the set up; right, cross section of the pen.

al., 2000). These electrodes were placed along the midline normal to the skin of the jaw. The prefix 's' (for self-generated) is used to refer to the equivalent wave components of the self-generated local field (e.g.  $sV_1$ ,  $sV_3$ , etc.). The effect of resting in tubes on the electric image of a stimulus-object was evaluated using the same experimental procedure described by Aguilera and Caputi (2003) and Caputi et al. (2003). We used a cylindrical stimulus-object (2 mm diameter, 1 cm length) consisting of a plastic tube with its two ends made of graphite carbon discs (1.5 mm in diameter). The cylinder was oriented with its long axis perpendicular to the skin 2 mm away from the electrosensory fovea. When using this stimulus object, the sLEOD was evaluated as the voltage drop between the bare tip of an insulated copper wire (100  $\mu\text{m}$  diameter) placed against the skin and the nearest carbon base of the cylindrical stimulus-object. We controlled the longitudinal resistance of the object by connecting an external resistor between both ends of the cylindrical object (Fig. 1B) and measured the voltage drop between the object ends. We used two types of parameters to assess the changes in sLEOD. For global amplitude measurements, the energy of the sLEOD was estimated as the root mean squared value (r.m.s. value) of the sLEOD from an oscilloscope screen trace (10 ms duration, sampled at 100 kHz). For waveform characterization we measured peak voltages ( $V_2$ ,  $V_3$  and  $V_4$ ) and calculated their ratios.

(3) Electrosensory lobe potentials were recorded by implanting a twisted pair of nichrome wires (insulated except at their tips, 80  $\mu\text{m}$  in diameter), through a small hole in the skull, at depths of 1100 and 1400  $\mu\text{m}$ , respectively. We aimed at the rostral regions of the electrosensory lobe where the foveal region is represented and confirmed electrode position by applying nearby threshold stimuli at the skin. Wires were cemented to the skull using cyanoacrylate and dental cement filler. Recordings were done 1 h after water returned to room temperature when the fish had recovered its natural EOD frequency.

In one case we confirmed the electrode placement histologically. The fish was deeply anaesthetised with ethomidate (2 mg ml<sup>-1</sup>) and fixed by aortic perfusion of paraformaldehyde (4%). The brain was serially sectioned, mounted and stained with Methylene Blue. The electrode track was clearly visible, with its ending about the centre of curvature of the cell layers of the electrosensory lobe in the molecular layer (Fig. 1C). The cell layers of the electrosensory lobe are shaped like a cup that is concave upward. The currents generated by the whole nucleus will be funnelled to its centre of curvature where the electrode pair was implanted, and the recorded signals can be likened to a holistic view of the activity in the lobe (Lorente de N6, 1947; Hubbard et al., 1969).

Voltage signals were differences amplified and filtered (band pass 10–10 kHz) using a high input impedance differential amplifier. A digital oscilloscope was used for online observation and averaging of EOD triggered traces (8–64 sweeps). Signals were also recorded for offline measurement and data processing using a Labmaster card and Axotape software.

#### *Effects of tubes on the physical image of objects*

sLEODs were measured in fish restrained within a band of tissue paper and inside copper or plastic tubes (22 mm inner diameter, 10 cm length) in the presence and in the absence of the stimulus object. Different longitudinal resistances for the object were tested using the procedure described above (Fig. 1B).

#### *Effects of tubes on the sensory carrier*

The carrier of sensory signals is the basal energy that the object presence modulate for generating images. In the case of active electroreception the carrier is the electric field 'illuminating' the object. This field can be considered as generated by an equivalent source completely described by two parameters: its electromotive force and its internal resistance (Thevenin theorem'; Edminister, 1965). In order to estimate these parameters we measured in the same experiments the voltage drop between the two carbon electrodes of the stimulus-object and calculated the current through the object by dividing the measured voltage across the load resistor. The electromotive force and internal resistance correspond to the ordinate intersection point and the slope of the line fitted to the voltage–current plot, respectively.

#### *Mechanisms of the effects caused by small environments*

We hypothesize that the tubes modify the summation of currents generated by different regions of the EO at the foveal region. In order to test this hypothesis, we studied sLEOD components generated by the caudal or rostral portions of the EO when the fish was inside or outside a tube. To dissociate the abdominal and the trunk–tail components of the sLEOD we applied the following procedure. Under cold anesthesia the spinal cord was exposed by a laminectomy and severed between the rostral and middle third of the fish. Spinal section was used to abolish the trunk- and tail-generated EOD (three fish) leaving intact  $V_1$  and a small remnant of  $V_3$  generated by the abdominal region (Trujillo-Cen6z et al., 1984; Caputi and Trujillo-Cen6z, 1994). Stimulation of the sectioned cord at the caudal stump was used to evoke the sequence  $V_2$ – $V_3$ – $V_4$  generated by the caudal two thirds of the fish body. Electrical stimuli (0.1 ms, 20 Hz, amplitude supra-maximal for the EOD) were applied through a pair of nichrome wires (200  $\mu\text{m}$  thick, 50 k $\Omega$ ) implanted within the canal to stimulate the bulbo–spinal electromotor tract. In order to assess the completeness of the section and the effectiveness of the spinal cord stimulation we checked the amplitude and waveform of the resultant EOD equivalent electromotive forces using the air-gap technique (see Caputi, 1999).

#### *Effects of the tubes on electrosensory discrimination*

Increases in stimulus-object conductance and thus image contrast elicited changes in the EOD rate characterized by transient reductions of the inter-EOD interval (novelty responses; Bullock, 1969). We used novelty responses to evaluate the fish's electrosensory discrimination ability, as in previous studies (Aguilera and Caputi, 2003; Caputi et al.,

2003). Novelty responses evoked by the same changes in object resistance were studied in three experimental conditions: (a) in open field, (b) inside a plastic tube and (c) inside a metal tube (both tubes were 10 cm in length). In these experiments, each trial consisted in the change of image amplitude from a basal (the stimulus-object without resistive load) to a comparison level (set by shunting the carbons for 5 s every 30 s with a known resistor). The sLEOD was simultaneously measured and the change in its amplitude provoked by the variations in object resistance was calculated as the increment of the r.m.s. value ( $\Delta$  r.m.s.). To detect novelty responses, we plotted the inter-EOD interval sequence. For each response the intervals were numbered starting at the first interval after the resistance change ( $I_1, I_2, \dots, I_n$ ). The baseline inter-EOD interval ( $I_0$ ) was defined as the mean of the five intervals preceding the change in stimulus-object resistance. We defined the amplitude of the novelty response as the normalized maximum shortening of the inter-EOD interval (novelty response amplitude =  $1 - \text{minimum of } I_i/I_0, I = 1 \text{ to } n$ ).

#### *Effects of the small environments on brain field potential responses*

We recorded field potentials in the electrosensory lobe in four fish. To study the dynamic effects of small environments on these field potentials the fish were restrained wrapping them in tissue paper within a long pen made by stretching a plastic netting over a frame consisting of three longitudinal plastic rods (1 cm  $\times$  1 cm  $\times$  50 cm) held at either end by plastic frames (squared contour of 5 cm  $\times$  5 cm). One of the beams was against the bottom of the tank and the others were one at each side. The fish naturally stayed at the bottom of the V-shaped duct and its longitudinal movements were restrained by a couple of cotton balls. Recording procedures for sLEOD and field potentials were as explained above (1 and 2). The relative positions of the electrodes and the fish were visually controlled and remained unchanged during the experiments. Trials in which fish moved were cleared from the records. A U-shaped plastic structure (6 cm between the U arms, 8 cm tall, 10 cm long) was manually moved back and forth with the aid of a system of pulleys (Fig. 1D). One of the pulley wheels was attached to a variable resistor that conducted a constant current square pulse (5 ms, 1 mA) triggered by each EOD. In this way, the recorded voltage drop across the variable resistor coded the structure position at the time of each EOD. We explored the effects of different kinds of longitudinal movement of the plastic structure, including step-like and sine wave-like oscillatory movements of different span, velocities and/or frequencies.

## Results

### *The effects of small environments on the carrier and electrosensory images*

Resting in tubes results in marked changes in the amplitude and waveform of the sLEOD at the foveal region. Fish were recorded in a position that they naturally adopt, in which the

fovea region, that is the jaw region (Castelló et al., 2000), is maintained at the tube opening. Plastic tubes increased the r.m.s. value of the LEOD 2.5 times on average (r.m.s. value increase ranged between 1.3 to 3.6 times,  $N=8$ ; Fischer exact test,  $P<0.01$ ). Metal tubes had a minor but opposite effect (r.m.s. value decreased between 0.78 and 0.95 times,  $N=8$ ; Fischer exact test,  $P<0.01$ ; Fig. 2A). Plastic tubes also cause a large distortion of the ratios of EOD peaks. While  $sV_2$  and  $sV_4$  increase (both in absolute value and relative to  $V_3$ ), the ratio  $sV_1/sV_3$  decreases. The most dramatic change occurs in  $sV_4$ , which increases 4.13 times on average with respect to the control (range 2.97–6.44; Fischer exact test,  $P<0.01$ ). Besides this absolute increase of  $V_4$ , the ratio  $sV_4/sV_3$  increased  $1.85 \pm 0.09$  (mean  $\pm$  S.E.M.; Fig. 2B).

The relative positions of the fish and the tube also change the reafferent stimulus. While the maxima of the r.m.s. value occurred when the snout was just protruding from the limit of the U-shaped plastic structure (in the most commonly observed position adopted by the fish) their minima occurred when the jaw was at the middle of the structure. Important changes in the waveform were also observed as a function of the tube position. Fig. 3 shows  $sV_3$  (Fig. 3A) and the ratio  $sV_4/sV_3$  (Fig. 3B) as a function of the distance between of the rostral border of a U-shaped plastic structure and the fish jaw (see Materials and methods, field potentials).

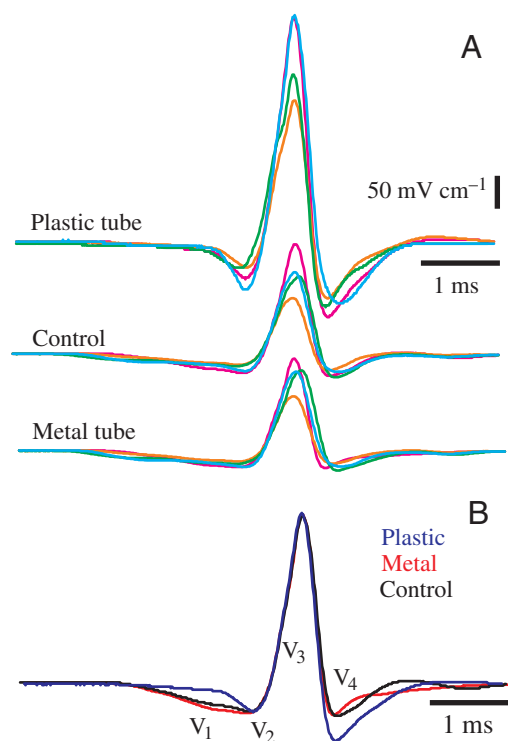


Fig. 2. Effect of tube conductivity on the sLEOD. (A) Local self-generated field (sLEOD) at the electrosensory fovea under three conditions: inside a plastic tube (top), control (middle) or inside a metal tube (bottom). Each trace represents 64 average sLEODs from one fish. Each color corresponds to a same individual. (B) Normalized sLEOD waveforms under the three conditions in one fish.

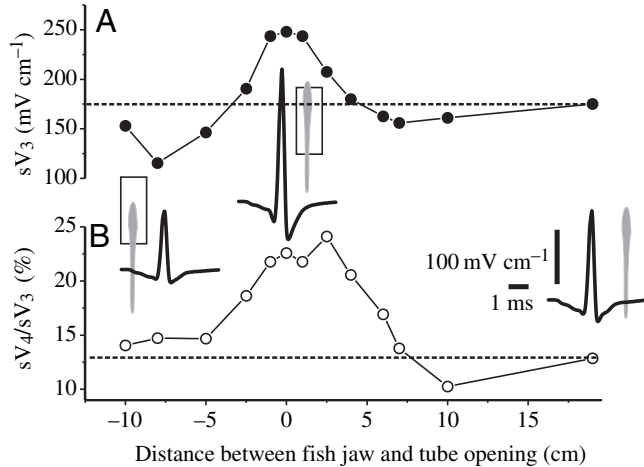


Fig. 3. Effect of relative position of the fish-tube on the sLEOD. (A) Amplitude ( $sV_3$ ) and (B) waveform (quotient  $sV_4/sV_3$ ) as a function of the tube position relative to the fish. Broken line corresponds to open field. Insets show the sLEOD waveform at the electrosensory fovea when this region is in the middle of the tube (left inset), at the opening of the tube (middle inset) and when the fish is outside the tube (right inset). Inset locations correspond to the abscissa.

To assess the effects of the tubes on electric images we changed the longitudinal resistance of the cylindrical stimulus-object and recorded the sLEOD at the facing skin (see Aguilera and Caputi, 2003). The sLEOD amplitude decreased as a function of object resistance in all conditions. The sLEOD corresponding to each resistance value of the object changed in proportion to the carrier amplitude, i.e. the amplitude of the sLEOD in the absence of any object (sLEOD increased

markedly in the plastic and decreased slightly in the metal tube, Fig. 4A–C).

#### Physical mechanisms underlying tube effects

In this section we report a series of experiments to investigate how tubes modify imaging mechanisms. The carrier of sensory signals is the basal energy that the object presence modulates for generating images. In the case of active electroreception the carrier is the electric field ‘illuminating’ the object. According to the Thevenin theorem (Edminister, 1965), if in a given condition the source ‘illuminating’ the object were linear it could be represented by an electromotive force (EMF) in series with a resistance ( $R_s$ ). This source is the equivalent source of the system containing all other elements (the fish, the water and, potentially, the tube) in the scene except the object. In this context the LEOD is proportional to the EMF and to the inverse of the sum of the series plus the object resistances ( $R_s+R_o$ ):

$$sLEOD = kEMF / (R_s + R_o).$$

In Fig. 4, we compared the electric image in three conditions: inside the plastic tube, inside the metal tube or outside the tube (the control condition). While changing the condition caused changes in amplitude and waveform of the sLEOD, the changes in object resistance only affected sLEOD total energy (r.m.s. value) and did not affect the sLEOD waveform in a significant way. This feature and the proportionality between the r.m.s. values of the sLEODs evoked by the same object impedance at the three different conditions explored (Fig. 4C and insets), suggested that the physical mechanism explaining the change in object image is

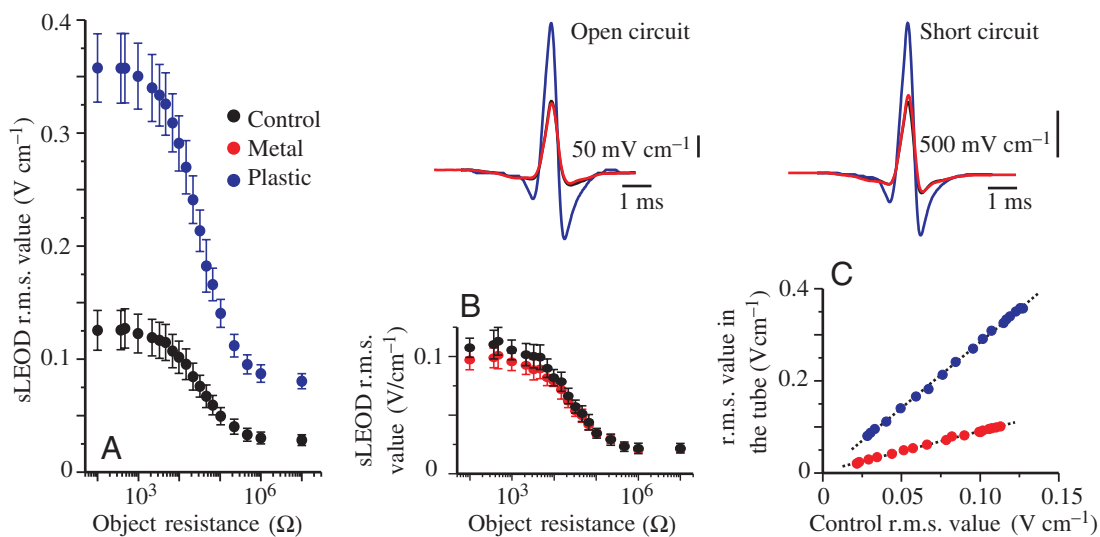


Fig. 4. The effects of tube conductivity on object images. (A,B) r.m.s. value of the sLEOD as a function of object longitudinal resistance when the fish is inside the tube (red, metal; blue, plastic) compared to the respective controls. Values are means  $\pm$  S.D. of the measurements made in four different fish. When the fish is inside a tube the r.m.s. values of the sLEOD are proportional to the r.m.s. values obtained in control condition (C; red, metal; blue, plastic). Insets: sLEOD waveforms comparing effects of the metal (red) and plastic (blue) tubes to the control condition (black); right, short circuit; left, open circuit. The color key for control, metal and plastic is common to all panels.

the change in the electromotive force of the electric source 'illuminating' the object.

The changes in the LEOD r.m.s. value associated with a given object ( $R_o$ ) when changing the condition from 'outside the tube' to 'inside tube' could be due to at least one of the following reasons: (i) tubes cause a change the r.m.s. value of the electromotive force (EMF) or (ii) tubes cause a change in the series resistance ( $R_s$ ) of the equivalent source 'illuminating' the object. Our experiments show that the LEOD inside a tube was proportional to the LEOD outside tubes. This implies that:

$$EMF(\text{inside}) / R_s(\text{inside}) + R_o = kEMF(\text{outside}) / R_s(\text{outside}) + R_o,$$

which is valid if and only if  $EMF(\text{inside}) = kEMF(\text{outside})$  and  $R_s$  is not changed.

We confirmed this theoretical conclusion in a series of experiments showing that plastic tubes increase the electromotive force of the equivalent electric source 'seen' from the object in an amount similar to the increase of the carrier and electric image. In Fig. 5 voltage measured between the object tips is plotted as a function of the current through the object. This allowed us to calculate the electromotive force (ordinate intersection point) and the series resistance (slope) of an equivalent source 'seen' by the object. This indicates that plastic tubes increase object's electric 'illumination' which, in turn, increase the imprimece (see Discussion) that they cause

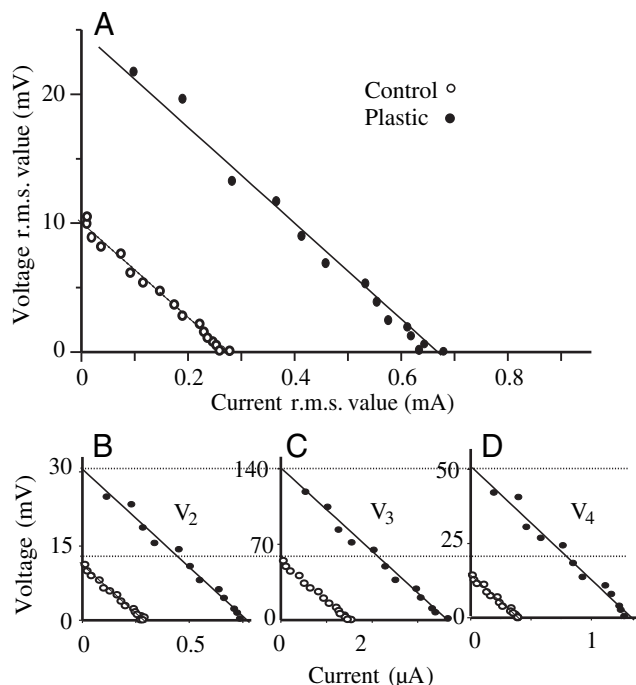


Fig. 5. Effect of a plastic tube on the equivalent source 'seen' by the object. Voltage measured between the object tips is plotted as a function of the current through the object. (A) Global measurement correlating voltage r.m.s. value vs current r.m.s. value. (B–D) Plots corresponding to each of the EOD peaks,  $V_2$ ,  $V_3$  and  $V_4$  respectively. Plot sizes were adjusted to match in height the equivalent electromotive forces inside the tube. Note that the relative change in  $V_4$  is larger than  $V_2$ , and this in turn is larger than  $V_3$ .

and also increase the contrast of the electric image that they project. The plot of Fig. 5A shows the r.m.s. value of the voltage between the poles of the stimulus object as a function of the current through the object. While the slope of the line, corresponding to the internal resistance of the electric source 'illuminating' the object was constant, the ordinate intersection, (i.e. the electromotive force) increased by 2.5 times (the same amount as the increase in LEOD).

The change in waveform of the sLEOD also reflected the change in waveform of the electromotive force of the 'illuminating' source. Wave components generated more caudally showed a more pronounced increase. While  $sV_3$  generated all along the fish exhibits the smaller increase (about 2 times),  $sV_4$  generated mainly at the tail exhibits the maximum increase (about 3.5 times);  $sV_2$ , generated at the centre of the fish body increases in an intermediate manner (about 2.5 times). This is shown in Fig. 5B–D by the gradation of the increase in ordinate intersection. Taking into account these results and that the sLEOD is the 'weighted sum' of multiple regional EOD components, we hypothesized that caudally generated currents reach the foveal region in larger proportion when the fish is inside a plastic tube.

This hypothesis was tested by altering the electrogenic properties of different regions of the EO in animals by spinal sections. In three fish the sLEOD was recorded inside and outside the tube before and after spinal section silencing the caudal region of the EO. When the spinal cord was severed at about one third of the distance from head to tail, the remaining EOD consisted mainly of  $sV_1$  (Fig. 6A, orange trace). In addition, when stimulating the distal stump of the severed spinal cord we recruited the electromotoneuron pools and reproduced the normal activation of the caudal region of the EO generating  $sV_2$ ,  $sV_3$  and  $sV_4$  (Aguilera, 1997; Caputi, 1999; Fig. 6B, orange trace). When the fish was inside the tube,  $sV_1$  decreased to about one half of the control value (Fig. 6A, green trace) and the caudally evoked  $sV_3$  increased about 3 times the control value (Fig. 6B, green trace). We concluded that the carrier changes are explained by the simple physical model described in Fig. 6C,D.

#### Effects of the context on object discrimination

A first strategy to assess how the changes in the electrosensory carrier and images are relevant for sensory processing was to explore discrimination of small resistive objects facing the fovea. In six fish, we applied the technique described by Aguilera and Caputi (2003). Fig. 7A–C show the novelty responses when changes in object conductance from open circuit to 4.5  $\mu\text{S}$ , 15  $\mu\text{S}$  and 2000  $\mu\text{S}$  were applied respectively. When fish were inside plastic tubes the amplitude of the novelty response was significantly larger than seen in the control (Fischer exact test,  $P < 0.01$ ; 6 fish and 6 different changes in object conductance). No significant differences were found between the metal tube and control experiments. This implies that there is a larger sensitivity of the electrosensory system to the same change in object conductance when the fish is in the plastic tube. Moreover,

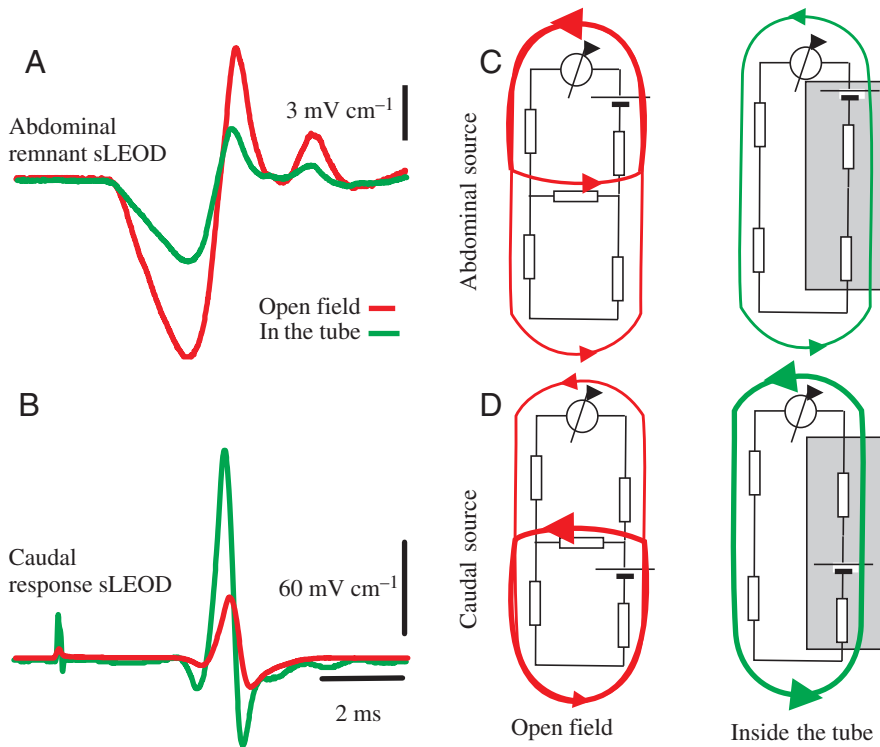


Fig. 6. The increase in carrier amplitude has a simple physical explanation. While the rostrally generated sLEOD is attenuated by the tube (A), the caudally generated sLEOD is increased (B). These results can be explained by a simple electrical model (C,D). The abdominal EO acts mainly as a 'voltage' source but the caudal acts mainly as a 'current' source. The voltage generated by the abdominal source is therefore relatively independent of the external load and the current generated by the tail source is relatively independent of the external load. In the tube, the external load opposing the generation of current by the abdominal source increases. Therefore the current and the sLEOD at the fovea diminish. In contrast, the part of the current generated by the caudal source that is shunted through the water in the open field, is forced rostrally because of the presence of the non conductive tube. This causes an increase of the sLEOD at the fovea.

changes in stimulus-object conductance that were close to threshold for eliciting the novelty response in the metal tube or in control conditions, always generated a novelty response when the fish was inside the plastic tube. The amplitude of the novelty response was an increasing function of the change in object conductance and the presence of the tube shifted the curve upward and the abscissa intersection point (threshold) to the left (Fig. 7D), indicating an increase in sensitivity for the same change in object conductance. However, when the amplitude of the novelty response was plotted as a function of the change in sLEOD amplitude ( $\Delta$ sLEOD), a single logarithmic function fit the data obtained in both conditions (Fig. 7E), which suggests that the ability to discriminate changes in object image amplitude was not altered by the presence of the plastic tube.

#### *Electrosensory lobe field potential responses to the sLEOD*

A second strategy to assess how the changes in the electrosensory carrier and images are relevant for sensory processing was to record field potentials in animals chronically implanted with electrodes in the rostral regions of the ELL where the electrosensory fovea is represented.

Field potentials showed a characteristic pattern of response following the EOD (Fig. 8A). These responses show three clear components that can be assigned to the fast and slow electrosensory pathways defined by Szabo et al. (1973): (i) A brief spike occurs at short latency (1–3 ms) after the EOD. This corresponds to the fast electrosensory pathway and will be referred to as FEP response (Castelló et al., 1998). (ii) An early slow wave starting at about 2–4 ms after the EOD and lasting about 5 ms. In some recordings this response showed small

spikes, probably corresponding to the synchronized activity of primary afferents and/or granule cells from the deeper layers of the ELL (referred to as SEP early response). (iii) A series of slow waves starting about 7–10 ms and lasting for the rest of the interval between EODs (SEP late response). This response showed larger variability than the early SEP and probably corresponds to the activity of the more dorsal layers of the ELL (Maler, 1979).

To confirm that these three are evoked electrosensory responses we studied their variability when the sLEOD amplitude was altered by plastic U-shaped structure movements. We calculated the peri-EOD standard deviation across an ensemble of 300 subsequent epochs of 30 ms duration, each epoch starting 5 ms prior to the EOD. We plotted the standard deviation of the voltages recorded at a given time after the EOD as a function of such time (Fig. 8B) We compared six sets of responses each obtained using a different experimental protocol altering the sLEOD (top plot, black traces). As a reference the post-EOD averaged response when the fish was in an open field condition is displayed in the bottom plot. This analysis shows three clear peaks in the standard deviation of the signal that we assigned to the changes in the activities of the fast electrosensory pathway (FEP) and the early and late components of the slow electrosensory pathway (SEP). We tested the alternative hypotheses that these changes in the response were provoked by changes in the inter-EOD interval or by lateral line stimuli. In the same fish we recorded the ELL evoked responses by a nearly constant sLEOD when the fish was stimulated by vibratory stimuli causing large EOD accelerations. We recorded an ensemble of 300 consecutive peri-EOD epochs

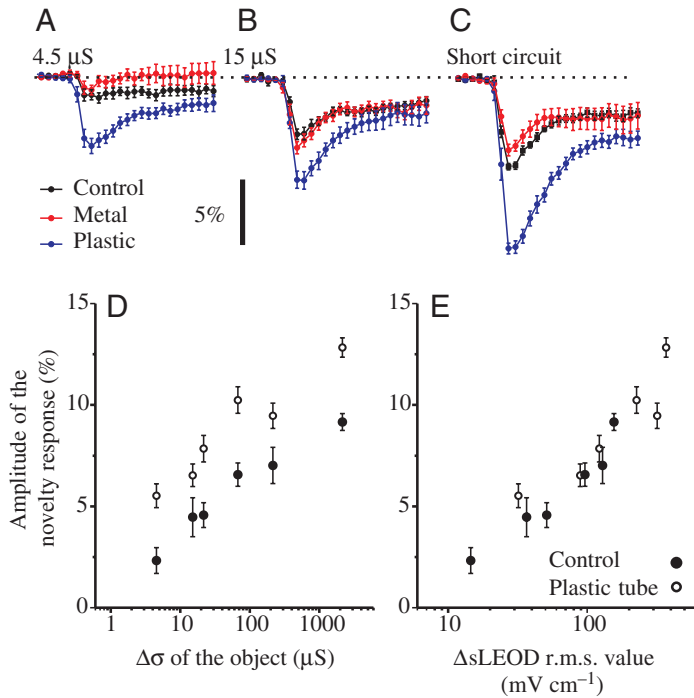


Fig. 7. Novelty responses evoked by changing the object resistance. (A–C) Responses of the fish to changes in object resistance from open circuit to 221  $\mu\text{S}$ , 66.4  $\mu\text{S}$ , and short circuit are compared. Note the large difference between the condition inside a plastic tube and the conditions inside a metal tube and control. Amplitudes of the novelty response when the fish was outside (filled symbols) or inside a plastic tube (open symbols) are plotted in (D) as a function of the change in object longitudinal conductance ( $\Delta\sigma$ ), and in (E) as a function of the change in r.m.s. value ( $\Delta\text{sLEOD}$ ). Values are means  $\pm$  S.E.M. of 10 trials in both plots.

obtained with the fish resting in open field while gentle taps on the aquarium wall were applied. The post-EOD standard deviation did not produce the characteristic trimodal profile observed when the sLEOD was modified. Instead it showed a flat profile (Fig. 8B, red trace), confirming that the three peaks coincident in time with the three described components of the field potentials are caused by changes in electrosensory signals but not by vibratory stimuli or by the change in EOD interval.

#### Characteristics of the different electrosensory responses to changes in electric images

Discrimination experiments suggested the presence of adaptive mechanisms to avoid saturation of the electrosensory system. We studied whether the different responses in the electrosensory lobe showed adaptation when fish goes in and out small environments.

The FEP response consists of a spike. The amplitude of this spike is linearly correlated with the r.m.s. value of the preceding pulse (Fig. 8C). The slow electrosensory pathway (SEP) response was characterized by two main components characterized by their latency and dynamics. The early SEP response showed little adaptation, whereas the late response

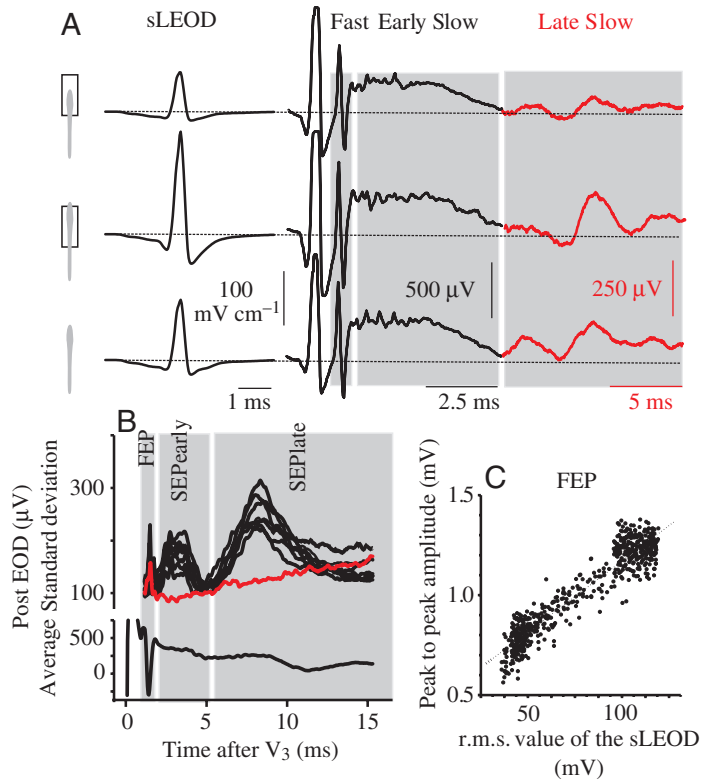
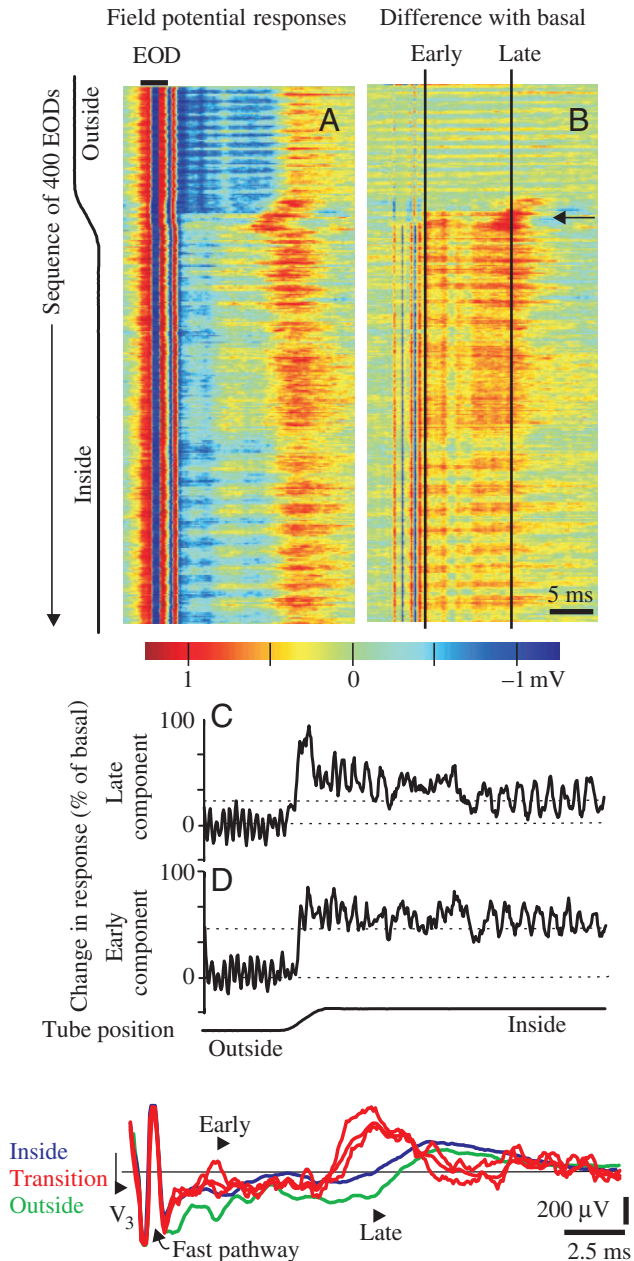


Fig. 8. The field potential responses at the electrosensory lobe. (A) Effects of placing the fish inside the tube on the field potential responses. Top, fovea at the middle of tube length; middle, fovea at the tube opening; bottom, fish outside the tube. (B) Field potential variability induced by changes in electrosensory stimuli (obtained from a different fish than in A). Bottom, post EOD averaged trace recorded in open field. Top, post EOD standard deviation of the signal when a plastic tube was moved in a step-like manner (three epochs using different speed, black traces) or in an oscillatory manner along main axis of the fish (three epochs using different frequencies, black traces) compared with the standard deviation in open field when EOD novelty responses were mechanically provoked by tapping the aquarium (red trace). (C) Peak-to-peak amplitude of the fast electrosensory response as a function of the r.m.s. value of the sLEOD. FEP, fast electrosensory pathway; SEP, slow electrosensory pathway.

showed clear adaptation. Fig. 9A shows the electrosensory lobe field potentials (color coded) in response to a sequence of sLEODs when the tube was moved along the fish axis coming from the caudal region. In this colormap the horizontal dimension corresponds to time after the EOD and the vertical dimension to the course of the experiment. Both early and late potentials changed with tube position. To analyse the change in these responses we subtracted the average response in open field from every evoked response (results shown as a color map in Fig. 9B). We measured the amplitude of the response at selected latencies corresponding to the early and late SEP responses (vertical lines). While the amplitude of the early response remained similar after the step in the sLEOD, the late response showed a progressive attenuation (Fig. 9C,D). In addition, we observed in all fish that sudden increases or





decreases of the sLEOD provoked by step-like movements of the tube evoked very large responses at about 7–10 ms after the EOD. This transient component increased in latency and diminished in amplitude, disappearing in less than 1 s (10–15 EODs). Fig. 9E shows three consecutive evoked responses just after a step-like movement of the tube (red traces) caused a change in sLEOD amplitude. These traces are compared with the averaged field potentials obtained outside the tube (green traces) and inside the tube, at the best position (blue traces). In one of the four fish this transient response to a step change in sLEOD appeared clearly separated in time (Fig. 10, single vertical arrow) from the rest of the late response, which exhibited a much less pronounced adaptation (Fig. 10, double vertical arrows).

Fig. 9. Different components of the slow electroreceptive pathway responses. (A) Color map indicating the field potential response (color coded) when the tube, initially placed caudally to the fish (fish outside the tube, control condition), is moved up to the point in which the jaw and the rostral opening of the tube coincide (where the sLEOD is the largest). In this color map the horizontal dimension corresponds to the time after the EOD and the vertical dimension to the sequence of EODs. In order to quantify the change imposed by the presence of the tube the control averaged profile was subtracted from each raw trace. Control averaged profile was calculated from the first 50 consecutive evoked responses (fish was outside the tube). These data are represented in (B). The changes in the response (expressed as a percentage of the control) at the two different times representative of the early and late responses (marked by the vertical lines in B), are shown in (C) and (D). Traces in (E) show the presence of a rapidly adapting potential evoked by the change in sLEOD. Red traces correspond to the first three evoked responses just after the placement of the tube opening at the level of the jaw. Green and blue traces are the averaged responses in the steady state control and maximum sLEOD conditions, respectively.

## Discussion

The present study provides a clear example of how the environmental context introduces changes in object representation at the sensory surface and in the central nervous system. This effect (comparable in vision to painting the walls black in the case of metal tubes or covering them with mirrors in the case of the plastic tubes) is generated by alteration of the image generation post-effector/pre-receptor mechanisms. This effect is not an experimental artefact since it occurs in natural conditions when fish choose to rest between roots or inside caves. Interestingly, even though the baseline stimulus is more than doubled, the fish react with similar novelty responses to similar changes in sLEOD when the fish is inside a plastic tube. This suggested the presence of adaptive mechanisms that were confirmed by the field potential responses recorded in the ELL.

This discussion address three important points about contextual effects of resting in a tube: (a) the physical mechanisms underlying changes in object image caused by small environments, (b) how these contextual effects affect sensory discrimination and (c) how the electroreceptive lobe deals with the large changes in waveform and amplitude of object images caused by small environment contextual effects.

### Physical mechanisms underlying tube effects

Lissman and Machin (1958) coined the expression 'object impriment' to describe the 'imprint' of an object on the fish's self-generated electric field. Impriment may be viewed as a virtual electric field caused by the presence of the object (Lissman and Machin, 1958). Since the 'electric image' of a given object is defined as the change in the pattern of the transepidermal field caused by the presence of the object, such an 'electric image' is just the object impriment at the sensory surface.

All materials other than water cause electric impriment on the EO generated field. Because the impriment of an object is also an electric field, the impriment of every object is

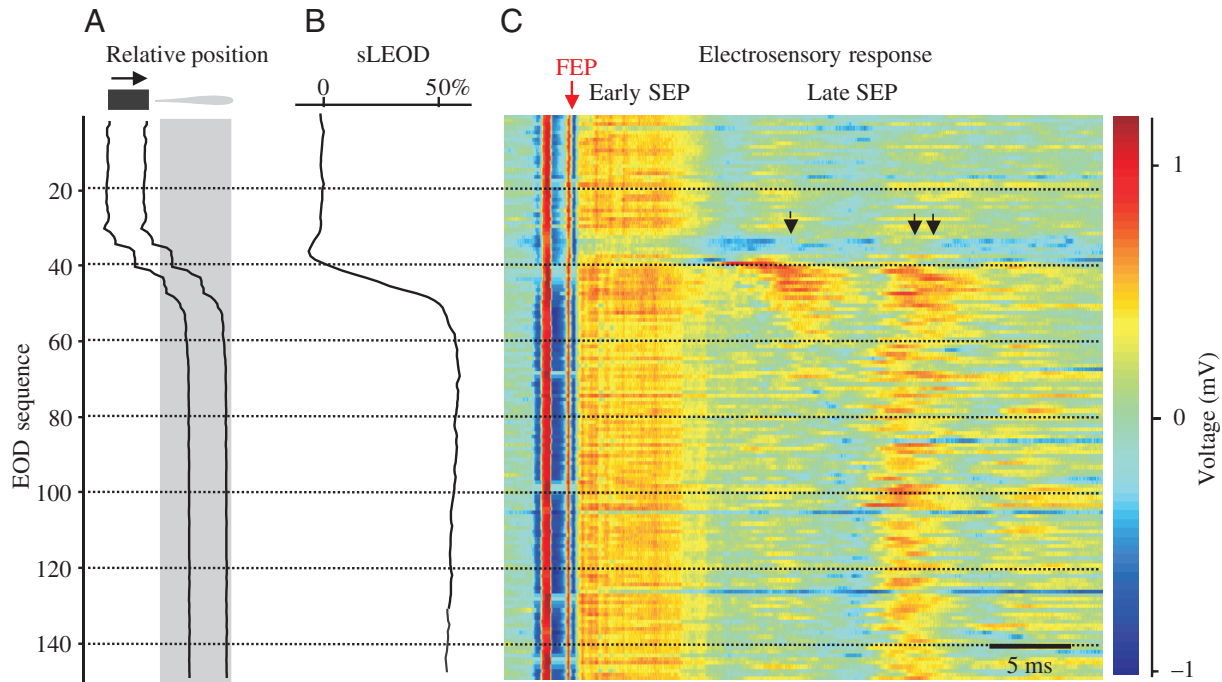


Fig. 10. Different components of the late response. (A) Relative position of the fish and the tube during a sequence of 150 EODs. The limits of the shaded area correspond to the positions of the snout and the tip of the tail, respectively. (B) The corresponding amplitude of the sLEODs during the same sequence. (C) Color map of the electrosensory lobe field potentials evoked by each sLEOD. In this color map the voltage is color-coded, the horizontal dimension corresponds to the time after the EOD and the vertical dimension corresponds to the sequence of EODs. Each color line represents the response elicited by the sLEOD at the position of the tube represented at the left. Note that in this animal the late response shows a transient component (arrow) and a slowly decaying component (double arrow) occurring at different times after the EOD.

modified by the presence of other objects and reciprocally. Therefore, the electric image of a given object is highly dependent of the presence of other objects (Rother et al., 2003).

The fish body is the object that most decisively shapes the electric field generated by the EOD and consequently the electric importance of surrounding objects. This is because the low impedance of the internal tissues and the silhouette of the fish body facilitates the flow of current from caudal to rostral regions of the fish (Castelló et al., 2000; Aguilera et al., 2001; A. Migliaro, A. A. Caputi and R. Budelli, unpublished data). In addition, the caudal region behaves as a 'current generator', forcing current rostrally. Therefore, the carrier and the local reafferent signals are enhanced in the perial region. In addition, the number and variety of receptors as well as their central projection field are maximal in this region. For these reasons the sensory mosaic at the perial region has maximal spatial resolution and has been likened to an electrosensory fovea (Castelló et al., 2000). Our experiments confirm the hypothesis that a tube surrounding the fish body modulates such funneling effect and modifies electric images of nearby objects at the fovea.

We showed that the plastic tube decreased the lateral shunting of current, facilitating funneling to the perial region of the faster waves ( $sV_2$ – $sV_3$ – $sV_4$ ) generated at the caudal region of the EO. Thus, the most caudally generated  $sV_4$  is the component that increases the most. In addition, the slow early component ( $sV_1$ ) as well as the abdominally generated

component of the positive wave ( $sV_3$ ) are reduced because abdominally generated current must flow along the tube, facing a larger resistance path. Since the abdominal region of the EO acts as a 'voltage' source, the generated current decays because of the voltage drop that occurs along a path of higher resistance (Fig. 7C,D).

The presence of a metal tube causes a different effect. It short-circuits the return of the currents generated at the caudal regions, preventing their funneling to the fovea. Nevertheless, the low internal resistance of the 'voltage' equivalent source at the abdominal region of the EO allows it to maintain most of its contribution to the sLEOD. For this reason, the decrease of the sLEOD inside a metal tube is not as dramatic as its increase inside a plastic tube.

#### *Changes in proximal stimuli determine the amplitude of the novelty responses*

One of the most important functions of sensory systems is to highlight perceptual experience of the attributes of objects that are more closely correlated with the intrinsic properties of the object than with their images. The objects in the environment are called distal stimulus while the image on the sensory mosaic is called the proximal stimulus (Palmer, 1999).

This function of the nervous system allows an evaluation of the attributes of objects independently of the context or scene in which they are immersed. For example, the whiteness of a paper remains the same whether looked at indoors or outdoors

under the sunlight (see also Adelson's web page [web.mit.edu/persci/people/adelson/checkershadow\\_illusion.html](http://web.mit.edu/persci/people/adelson/checkershadow_illusion.html)). This is not the case for fish evaluation of electric attributes of objects that lead to novelty responses. In our previous studies we showed that novelty responses were graded with both the change in object impedance and the change in their corresponding images (sLEOD; Caputi et al., 2003). However, in those experiments the changes in sLEOD were correlated one-to-one with the changes in object impedance, and therefore it was impossible to answer the question about which of those variables determines the amplitude and threshold of the novelty response. The increase in carrier amplitude and object image generated by plastic tubes allowed us to compare the effects of the same change in object impedance when the change in image amplitude was different. It also allowed us to compare the effects of similar changes in image amplitude when the change in object impedance was different. The experiments reported in the present article show that fish respond with larger novelty responses to the same change in object resistance when the change in image was larger. In addition, similar changes in the r.m.s. value of the sLEOD generated similar novelty responses. Thus, novelty response depends only on changes of the object's image. Thus, the novelty response depends on the proximal stimulus at the receptor surface, not on the distal stimulus in the environment. It should be noted, however, that at distal stimulus perception (i.e. evaluation of the absolute attributes of the object) probably occurs in parallel by the central nervous system of these fish. The presence or absence of such function must be evaluated, therefore, by indicators other than the novelty response.

The discrimination experiments reported here suggest the presence of central adaptive mechanisms in the sensory evaluation of electric images. Even though the basal sLEOD in plastic tubes is about 2.5 times the basal stimuli observed in open field, and its waveform is different, the amplitudes of novelty responses generated by similar energy changes in the sLEOD were similar. This indicates that the increase of the basal image caused by the plastic tubes does not saturate the sensory system, suggesting that adaptation takes place at central structures. If receptors were adapting to the basal stimuli they would probably evaluate the change in stimuli differently, which in fact does not occur. In addition, this confirms that the basal sLEOD waveform and amplitude are independently subtracted from the present image to detect novelty (Aguilera and Caputi, 2003; Caputi et al., 2003).

#### *Field potential responses in the chronically implanted fish*

The fast electrosensory pathway (FEP) was easily identified by the characteristic early, large peak that was modulated in latency and amplitude, without adaptation, by changes in the amplitude of the sLEOD (Castelló et al., 1998). The simplicity of the fast electrosensory response corresponds to the simplicity of its circuitry, and the complexity of the slow electrosensory response (variability, multiple components and long duration) corresponds to the complexity of its circuitry (Carr and Maler, 1986). Analysis of the standard deviation of

the voltage recorded at a given time after the EOD indicates the presence of an early and a late stage in the SEP response. The latter could be broken down into subcomponents of different dynamics in relation to the sequence of images.

Timing and dynamics of the early slow responses suggest that they may correspond to the deepest layers of the ELL, including primary afferents and cells of the granule layer (Maler, 1979; Carr and Maler, 1986; Bastian, 1986). The late slow electrosensory responses observed in this study may correspond to more dorsal layers of the electrosensory lobe including the polymorphic cell layer, and the effects of recurrent inputs coming from higher centers (Bastian, 1995; Berman and Maler, 1999). These late responses show adaptation with two different rates of decay after step and hold stimuli. Step-like changes in the electrosensory image caused the appearance of a transient wave at the beginning of the late response that rapidly decayed and increased in latency. The step-like increases of the sLEOD also evoked a long-lasting change of the late response that exhibits a slow decay. Interestingly, the presence of two subcomponents having different time constants may suggest the presence of different types of responses in the cells of the ELL. The different dynamics of these responses is not surprising since the presence of adapting and non-adapting cells differently involved in the recurrent electrosensory loops was recently described in wave gymnotids (Bastian et al., 2004). These adaptive responses in the ELL of pulse gymnotids appear similar to those described in the electrosensory systems of other fish (Bell et al., 1993; Bastian, 1995; Bastian et al., 2004; Bodznick et al., 1999). Our study suggests that the discrimination rules described in *G. carapo* by Caputi et al. (2003) may partially be implemented at the electrosensory lobe by the storage and subtraction of a moving average of past electric images from the current input.

#### *Conclusions*

(1) Hovering in small environments is a common behaviour of fish. We show that this behaviour might cause large contextual effects on electric imaging.

(2) These effects are due to changes in efficacy of a previously described pre-receptor/post-effector funnelling effect based on the high conductivity of the fish body (Castelló et al., 2000). Because current funnelling efficacy is altered by the presence of a tube, the electromotive force of the equivalent source electrically 'illuminating' the object is modified in amplitude and waveform, with the consequent change in object image on the skin.

(3) The study of object discrimination under two different contextual conditions indicates that fish evaluate the changes in images (proximal stimulus) rather than changes in object attributes (distal stimulus), and suggest the presence of adapting mechanisms at the central nervous system dealing with background subtraction.

(4) Electroensory lobe field potentials recorded in chronically implanted fish in and out of small environments confirm the presence of adapting mechanisms at the slow

electrosensory pathway, and also show the presence of non-adaptive responses in both the fast and slow electrosensory pathways.

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