




# Tethered unitary recordings suggest a spike-timing electrosensory code in the electrosensory lobe of *Gymnotus omarorum*

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## Abstract

Evaluation of neural activity during natural behaviours is essential for understanding how the brain works. Here we show that neuron-specific self-evoked firing patterns are modulated by an object's presence, at the electrosensory lobe neurons of tethered-moving *Gymnotus omarorum*. This novel preparation shows that electrosensory signals in these pulse-type weakly electric fish are not only encoded in the number of spikes per electric organ discharge (EOD), as is the case in wave-type electric fish, but also in the spike timing pattern after each EOD, as found in pulse-type Mormyroidea. Present data suggest that pulsant electrogenesis and spike timing coding of electrosensory signals developed concomitantly in the same species, and evolved convergently in African and American electric fish.

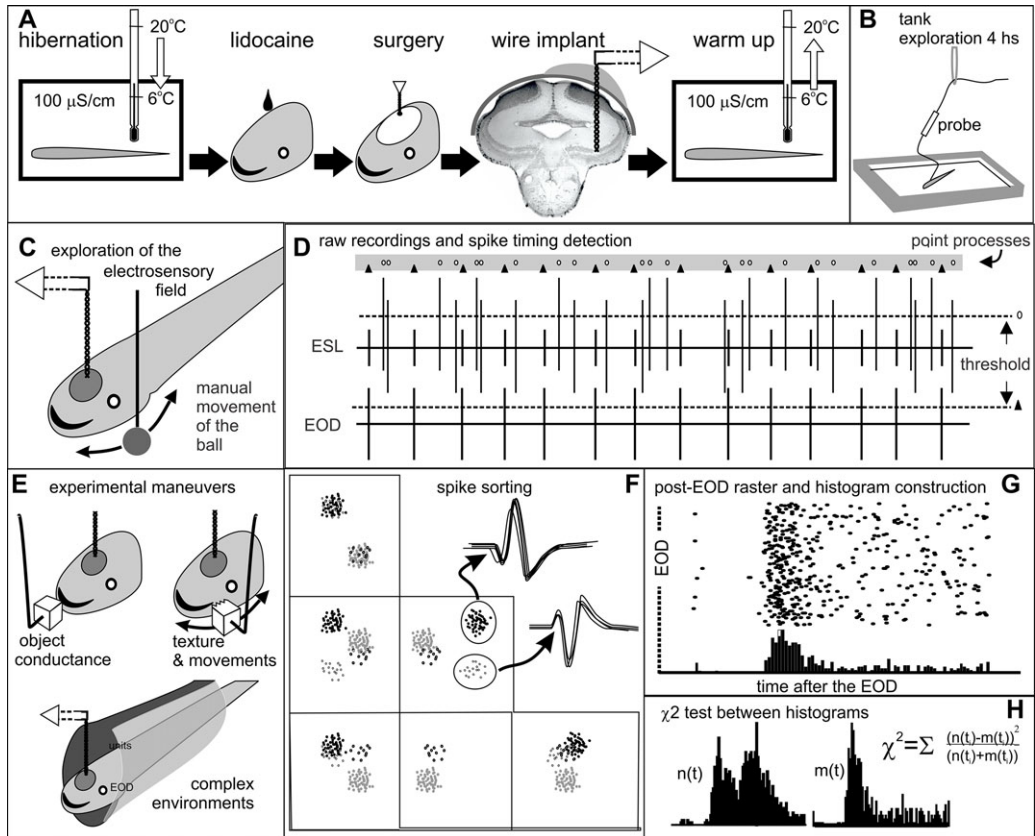
**Keywords:** Chronic recordings; neural code; electroreception; pulse emitting weakly electric fish; electrosensory lobe

## Introduction

Electric fish explore their surroundings with an electric field characterized by either pulsating patterns or continuous sine-waves generated by the discharge of an electric organ (EOD). “Electric images” generated by the presence of objects are differently encoded by primary afferents (pulse evoked bursts in pulse-emitting and pseudorandom trains in wave-emitting fish). This draws clear distinctions between pulse and wave electroreception strategies (Caputi & Aguilera, 2020). In the electrosensory lobe (ESL) of wave Gymnotiformes, the spike rate of individual neurons and synchrony within neuronal subpopulations encode the modulation of local signals (reviewed in Clarke et al., 2015; Krahe & Maler 2014). The ESL of pulse Gymnotiformes appears to show different signal processing rules since each pulse re-clutes the network with neuron-specific post-EOD phase preferent patterns (Pereira et al., 2014) and evokes local field potential which patterns are modulated by changes in the electrosensory environment (Pereira et al., 2005).

## Objectives

This raises the question of whether spike timing of different unit types of the ESL of pulse Gymnotiform fish carry information regarding nearby objects in natural conditions. To start answering this question, we used a tethered technique (in the absence of a wireless option, as in Cohen et al., 2019; Fotowat et al.,

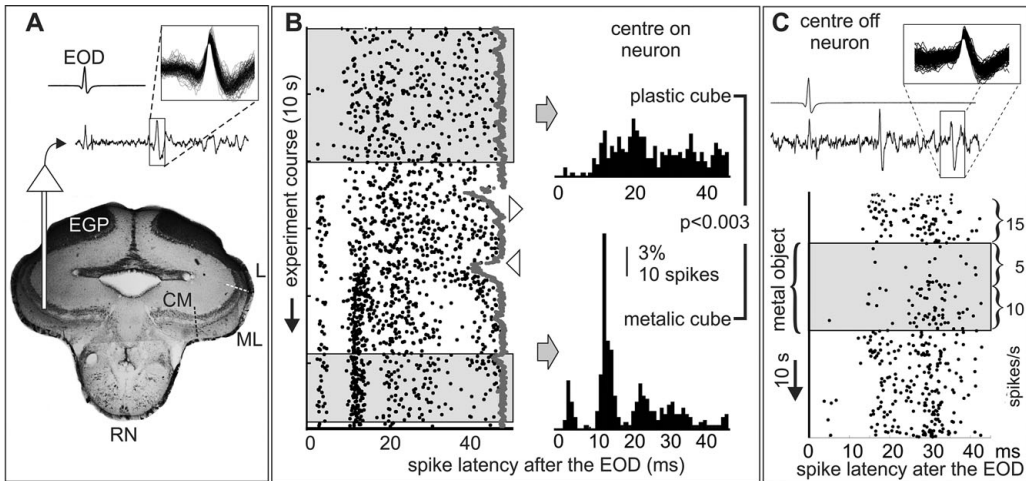


**Figure 1. Methods. Step-by-step detailed explanation in protocols.io** A) Surgical procedures. B) Recovery and fish adaptation to the tank. During this period the implanted wires (20-25 cm long) were connected to the amplified probe, which in turn was hanging from a rubber band. We verified electrode location recording field potentials at 10 Hz - 10 KHz bandpass filtering and setup unit recordings and analysis (Pereira et al., 2014). C) Identification of receptive fields. We took advantage of long resting periods to manually explore the receptive field of the best sorted unit with a small copper ball (8 mm diameter) moved in all directions parallel to the skin. D) Raw recordings and point process conversion. Each signal was appropriately thresholded (dotted lines) to define the timestamp of each recorded unit (ovals) and EOD (triangles). E) Experimental maneuvers aimed to: i) Identify opposite responses to plastic or metal objects at the center of the receptive field (Clarke et al., 2015); ii) Explore electric texture effects caused by sharp edges and gutters on object's surface (Caputi et al., 2011); and, iii) Explore the effect of hiding in a plastic tube. F) Spike clustering and sorting plotting various spike parameters in a multidimensional space. G) Construction of raster plots. H) Statistical comparison of post-EOD spike timing distributions using chi-squared tests.

2019) to record unitary activity from electrodes positioned at the polymorphic layer of the ESL (as indicated by their typical field potentials Pereira et al., 2014). This allowed us to explore peri-EOD spiking patterns and their modulation by metal and plastic, cubic or tubular, and static or moving objects.

## Methods

Data were obtained from 4 *Gymnotus omarorum* (12-15 cm length, undetermined sex). All potentially-painful procedures were performed in fish non-responsive to noxious stimuli (IIBCE's animal care committee, protocol 001/003/2011; protocols.io). Fish were scalped under systemic and local anesthesia. A small skull opening was made to introduce two insulated nichrome twisted wires (50 μm diameter each). Wires were cemented to the skull with their exposed tips at the polymorphic layer of the centro-

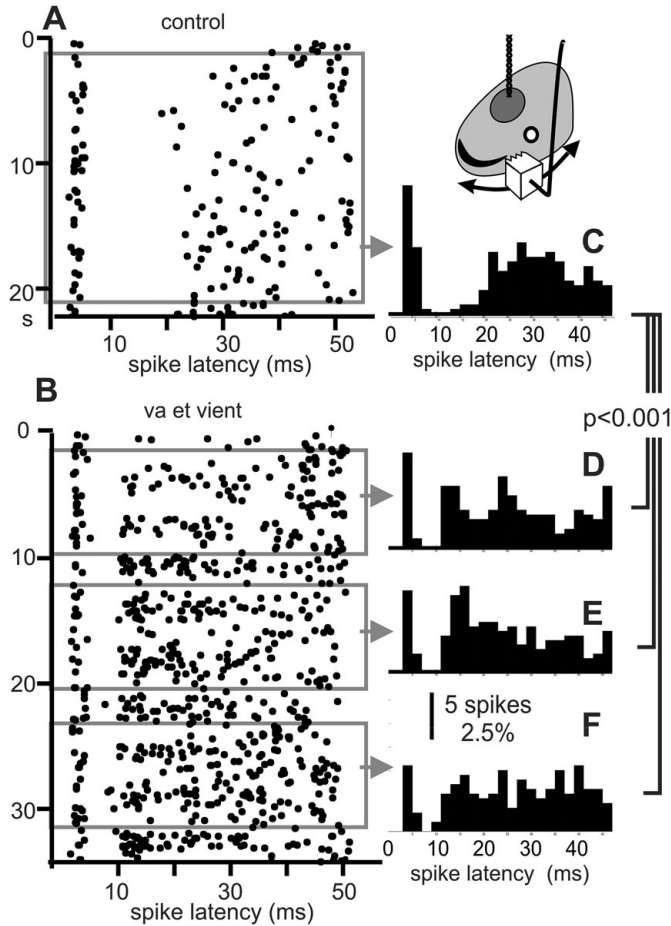


**Figure 2. On and off neurons. Conductance dependent post-EOD spiking patterns and adaptation.** These experiments were made to confirm the presence of two types of opposite responses to metal and plastic objects and to evaluate the presence of adaptation. A) Traces: EOD (top) and the electrosensory lobe (bottom) recordings; inset: superimposed waveforms of sorted spikes. Histology indicates the recording site (CM: centromedial, ML: mediolateral, L: lateral, maps). B and C) A metal static cube (2 cm side) facing the center of the receptive field (shadowed regions of the rasters) either caused increases (B) or decreases (C) in the firing rate depending on its conductivity. These increments may represent those that led the functional classification of electrosensory neurons as “centre-on” (B) and “centre-off” (C) in wave fish (Clarke et al., 2015). The motion of the plastic object and the introduction of the metal object (downward and upward pointing triangles in B, respectively) causes novelty responses (sudden reductions in the interEOD interval, gray dots). Strikingly, *G. omarorum* centre-on neuron (panel B, receptive field at the foveal region) showed changes in the post-EOD firing pattern in addition to the change in the number of spikes per EOD. Plastic and metallic objects caused opposite effects in the firing rate. However, they did not provoke simple mirror-image changes, but drastic changes in the post-EOD pattern when comparing the stationary state prior to removal of the plastic cube with the stationary state after positioning the metal cube (200 spikes indicated by from left and right boxes were used to build each histogram  $\chi^2=221$ ,  $DF=49$ ,  $p<0.003$ ). Both the “centre on” (B) and “centre off” units (C, receptive field at mouth commissure, same unit as in Fig. 3) shows clear adaptation. The “centre off” unit (C) shows a maximal decrement just after placing the metal object before the receptive field the spike rate measured on the first half of the stimulation period was reduced to one third of the control. This was followed by a slow return to two thirds of the resting value in the second half period even when fish and object positions remained unchanged. When the object was removed a rebound was observed.

medial map of the lobe (1400  $\mu\text{m}$  depth). This pair, and another in the tank (40x50x7 cm, water at 20° and 100  $\mu\text{S}/\text{cm}$ ), were connected to differential amplifiers (1800-AM-systems, bandpass: 300-3000 and 10-10000 Hz for spikes and EOD, respectively) whose outputs were digitized (50 kHz), stored, and processed using Experimenter (Datawave Technologies). Recordings started four hours after the EOD rate returned to the previous baseline (Fig. 1). After recordings, fish were euthanized.

## Results

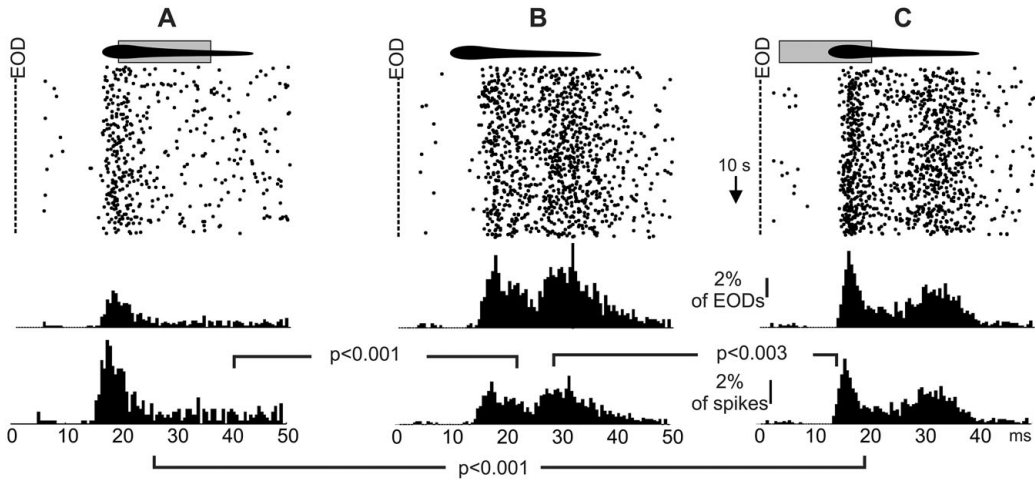
Multiunitary recordings were obtained in one fish. In the other 3 fish, 2 units were clearly sorted in each. In these 6 units, spike timing showed a non-uniform probability distribution following the EOD. Spike patterns exhibited the presence of a silence between 7 to 10 ms, and 1 to 3 modes found at ca. 5, 12, or more than 23 ms after the positive peak of the EOD. Post-EOD spike histogram peaks were differently modulated, corresponding to the unit and sensory context. In three units we identified the receptive field. These were selected to explore their responses to distinct types of electrosensory stimuli. Each of these responses is illustrated in different figures (Fig 2: object conductivity, plastic vs. metal cubes 2 cm side, see Clarke et al., 2015; Fig. 3: movement and texture effects, see Caputi et al., 2011; and Fig. 4: tube hiding behavior, see Pereira et al., 2005).



**Figure 3. Electric “texture”.** The ability to respond to the “electric texture” of object surfaces was proposed by Caputi *et al.* (2011). To explore this effect, the 2 cm wide face of a copper cube (carved with a saw-tooth profile) was moved back and forth against the skin at the receptive field (head side, inset). A) Raster of a “centre off” in the absence of an object (control). B) Raster plot when the unit was strongly modulated in its firing rate and post-EOD spike timing pattern by va-et-vient movements. Paradoxically for a “centre-off” neuron, a moving metal object caused an increase in its firing rate (note the difference in the number of EODs required to recruit 200 of spikes, indicated by the width of gray bands respect to that observed in the absence of an object) and also a relative peak at about 12-14 ms after the silence. C) Histograms corresponding to the control condition. D-F) Histograms corresponding to mobile stimulus (built from the three samples of the same number of spikes as the control, gray rectangles).  $\chi^2$  tests showed significant differences between the distribution of each sample obtained during object movement and during the control without object ( $\chi^2=58.1, 50.1,$  and  $67.3$  respectively,  $p < 0.001$  in all cases) but were not able to show significant differences between pairs of distributions obtained during object movement ( $\chi^2=20.9, 18.3,$  and  $22.5$  respectively,  $p > 0.45$ ,  $DF=23$ , significance:  $0.003$  after Holm-Bonferroni sequentially rejective procedure).

## Discussion

Although swimming behavior is relatively limited in tethered recordings, the experimental modulation of post-EOD spiking patterns in freely discharging, drug free, permanently implanted fish, shows that spike timing contains additional information on the electrosensory input that is not contained in the average number of spikes per EOD. This form of post-EOD spike-timing code is shared with the phylogenetically distant Mormyroidea, but not with the much more closely related wave Gymnotiformes, who exhibit similar electrosensory lobe anatomy (Bell & Maler, 2005). This suggests that distinct central processing mechanisms are associated with the polarization strategies and electroreceptor responsiveness characteristic of each taxonomic group (Caputi & Aguilera, 2020).



**Figure 4. “Centre off” unit responses when fish hide in a tube.** A drastic change in the transcutaneous self-generated current profile was described in Pereira et al. (2005). To test whether the presence of a tube modifies the post EOD spiking pattern we explored a unit when it was at two positions inside a tube of 5 cm diameter, 10 cm long and compared the profiles between them and with that obtained in a control condition outside the tube. A) “head sticking out”; B) control; and, C) “head inside” (insets). Top row, spike rasters at stable conditions. Middle row: post-EOD firing histograms normalized to the number of EODs. The most common position observed during natural behavior (“head sticking out”) increases the signal at the receptive field center (mouth commissure) and causes a global reduction in spike rate with a virtual disappearance of the late modal peak observed in the control condition. The “head inside” position elicited a reduction of the stimulus, a small difference in rate but a sharp increase of the early modal peak, and a shift to longer latency of the second mode. Bottom row: histograms normalized by spike rate show different patterns ( $\chi^2$  tests, DF=49, largest  $p < 0.003$ , which is equivalent to 0.01 after Bonferroni’s correction).

## Conclusion

Data suggest that post-EOD spike timing encoding of electrosensory signals have evolved concomitantly with the ability to explore the environment using pulsatile discharges in a convergent manner for African and American species. Our study paves the way to unveil electric image processing in *G. omarorum*, since unitary recordings can now be correlated with video tracking available models (Pedraja et al., 2014) of the electric image flow. For these purposes, tethered recordings should be complemented with recently introduced wireless systems facilitating skeletomotor motor exploration behaviors with a lower common mode rejection ratio (Cohen et al., 2019; Fotowat et al., 2019).

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**Conflict of Interest.** Authors ARC, ACP, PAA, and AAC declare none.

**Data availability.** The data that support the findings of this study are available from the corresponding author (AAC) upon request.

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