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Individual discrimination of freely swimming pulse-type electric fish from electrode array recordings

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A B S T R A C T

Pulse-type weakly electric fishes communicate through electrical discharges with a stereotyped waveform, varying solely the interval between pulses according to the information being transmitted. This simple codification mechanism is similar to the one found in various known neuronal circuits, which renders these animals as good models for the study of natural communication systems, allowing experiments involving behavioral and neuroethological aspects. Performing analysis of data collected from more than one freely swimming fish is a challenge since the detected electric organ discharge (EOD) patterns are dependent on each animal's position and orientation relative to the electrodes. However, since each fish emits a characteristic EOD waveform, computational tools can be employed to match each EOD to the respective fish. In this paper we describe a computational method able to recognize fish EODs from dyads using normalized feature vectors obtained by applying Fourier and dual-tree complex wavelet packet transforms. We employ support vector machines as classifiers, and a continuity constraint algorithm allows us to solve issues caused by overlapping EODs and signal saturation. Extensive validation procedures with *Gymnotus* sp. showed that EODs can be assigned correctly to each fish with only two errors per million discharges.

1. Introduction

Pulse-type weakly electric fishes such as *Gymnotus* sp. are known for emitting electric organ discharges (EODs) used for electrolocation and electrocommunication purposes [1,2]. Because of the stereotyped nature of the electrical waveforms produced by these fish, research on electrocommunication typically focuses on analyzing measurements derived only from the occurrence instant (timestamp) of EODs, for example its discrete difference—the inter-pulse interval (IPI) [3–5]. Few organisms allow the non-invasive examination of electrophysiological signals produced by a complex internal neuronal network as offered by this simple communication mechanism based on trains of electrical pulses.

Recording from freely swimming fish is challenging and traditional techniques based in arrays of electrodes fixed in the aquarium are still employed [6–8]. The idea is similar to using an electrode array to record from neurons in the central nervous system of an animal [9,10], however, here the spiking "neurons" are not stationary in space and the problem resembles, but it is even more complicated than, that of recording from arrays of electrodes which present drift along time [11]. Techniques allowing the precise detection and discrimination of EODs emitted by dyads or groups of freely moving fish with a minimum disturbance are invaluable tools for neuroethological research [4,5,12–14], because they allow to study a plethora of social communication circumstances in a naturalistic setup. However, currently there is a lack of computational tools capable of accurately identifying the individual that emitted each pulse recorded during those experiments.

In principle, employing machine learning techniques to address the individual discrimination problem would be feasible since EOD waveforms vary from one fish to another [15]. But although the distinct waveforms of different individuals have been the object of study in reports on EOD variations related to geographical origin [16], characteristics of the waveforms have seldom been exploited for recognizing fish. There are also reports on changes of the waveform due to developmental transitions in juvenile fish [17], but the EOD of a certain individual does not change by factors other than fish movement [7,18] during our experiment's time frame (a few hours).

Most of the existing works try to discriminate individuals by employing non-automatic procedures involving visual inspection of EOD pulse amplitude and duration [14], sometimes aided by video recordings that allow inferring fish position, which is then manually correlated to pulse amplitude and polarity changes [5]. Still, those methods are time-consuming and often applied solely to a few minutes of experimental data, and therefore they do not

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produce sufficient input to enable statistical and information-theoretic approaches [8] to study spike train coding [19].

An automated method was proposed [4] that first stored two template EODs, each one from a single fish, then computed the cross-correlation between every EOD acquired in a dyad experiment and each of the templates. Nevertheless, that approach had difficulties when EODs had similar pulse width or when both fish fired pulses almost at the same time.

In this paper we introduce a computational method able to discriminate fish within dyads surpassing the just mentioned limitations, given as input measurements from electrodes placed at fixed positions in an aquarium tank. Our method is essentially a two pass algorithm. First, signal portions strongly believed to contain an EOD emitted by a single fish are classified by a support vector machine [20], based on a normalized feature vector obtained by applying Fourier and dual-tree complex wavelet packet [21,22] transforms. Then, the fact that waveforms vary continuously during fish movement is exploited to find EODs inside signal segments which might contain discharges from both fish.

We also perform an extensive validation procedure during which dipoles are attached directly to each fish to capture the EODs whose timestamps are then compared with the results of the developed algorithm in order to evaluate its error rate, which we estimate as being only two parts per million.

The paper is organized as follows. Section 2 introduces the experimental methods, explaining how measurements are carried, and also detailing the procedure we perform to validate our method and evaluate its accuracy. Section 3 describes both the discrimination method already present in the literature and our proposed algorithm. Finally, results are presented in Section 4 and conclusions are drawn in Section 5.

2. Experimental methods

2.1. Experimental setup

Our experimental setup is illustrated in Fig. 1. Measurements are taken in a 64 liter glass aquarium tank containing eight stainless steel electrodes, located at the vertices of a 40 cm sided cube. The aquarium is mounted inside a Faraday cage to reduce the induction of external electrical noise. Electrodes with a diameter of 0.2 mm are inserted through the silicon glue at the corners of the aquarium, having about 1–2 mm of length in contact with the water. One of the electrodes is chosen as a reference, with respect to which the voltage of the other seven electrodes is differentially amplified 100 times using LM308 operational amplifiers. Once amplified, the seven signals are digitized at a sampling rate of 50 kHz with a resolution of 12 bits by a National Instruments PCI MIO-16-E1 data acquisition board and stored in a personal computer.

When fish gets too close to the reference electrode, an exceeding voltage may be produced between the reference and other electrodes, saturating signals recorded from all of them at the same time. To avoid this issue, we fixed a piece of nylon tulle to the aquarium glass near the reference electrode, preventing fish from reaching it.

Our aquarium geometry and electrode placement is the same adopted in [8]. It is easy to replicate and provides an adequate signal to noise ratio (SNR) in at least one electrode independent of the fish position. However, nothing precludes the algorithm described in this paper from being used with other geometries, such as the round aquarium with multiple reference electrodes as described in [7].

2.2. Experimental procedure

Experiments with a fish dyad are comprised of two steps which can be carried in any desired order. We call one of these steps the training stage, which consists of placing apart in the aquarium each individual, in turn, for some minutes, during which the EODs of the freely swimming fish are collected. Typically, 15 min of acquisition are enough to collect on the order of 10^5 EODs, sufficient for training and testing our classifier. In order to acquire good quality labeled training and testing data, covering the system dynamics over most of the operating range, it is important to get the fish to swim around all aquarium. In *Gymnotus* sp., this usually occurs naturally, as the fish has a tendency to explore the surroundings when it is moved to a different environment [8]. The experimenter can also arouse an inactive animal by mechanically disturbing the aquarium.

The other step, which we call the main experiment, consists of placing both fish at the same time inside the aquarium. Data acquired in this step can be discriminated by our algorithm, outputting a list of the occurrence instants of EODs emitted by each fish. These instants are the final product of our method, and can be analyzed and studied in order to research new behavior and codification schemes occurring in fish electrocommunication.

2.3. Validation procedure

To evaluate if our discrimination algorithm gave accurate results, we conducted experiments where electrodes were attached next to each fish and recorded in addition to the fixed electrode array already present in the aquarium. To keep the electrodes near the fish, the wires of the electrodes were intertwined to a nylon tulle, which was

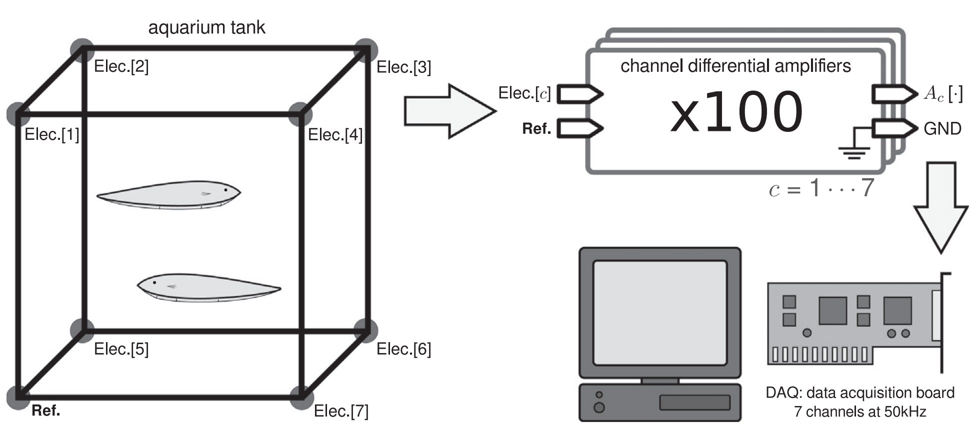

Fig. 1. A pair of fish is placed in an aquarium tank containing eight electrodes in contact with water. One of the electrodes is chosen as a reference electrode (ref.), with respect to which the voltage of all other electrodes is differentially amplified with a 100 times gain. Signals sampled at 50 kHz are then collected by a computer using a data acquisition board.