Nonlinear Dynamics and Neuroscience

Известия высших учебных заведений. Прикладная нелинейная динамика. 2022. Т. 30, № 2
Izvestiya Vysshikh Uchebnykh Zavedeniy. Applied Nonlinear Dynamics. 2022;30(2)

Article
DOI: 10.18500/0869-6632-2022-30-2-239-252

Multielectrode registration of episodic discharges generated by weakly electric fishes

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Received 9.11.2021, accepted 23.11.2021, published 31.03.2022

Abstract. *Purpose* of this study introduces a multielectrode array (MEA) registration system in order to generate electric field images of the episodic discharges generated by weakly electric fish. A multielectrode registration system has several important features: the design of the multielectrode lattice, the amplifier circuit, the choice of reference points for differential measurements, the recovery of the absolute values of the electric field potentials, and the application of principal components analysis. *Methods.* There are several advantages of our MEA registration as compared with the traditional twoelectrode registration: (a) the signal-to-noise ratio is significantly increased, (b) it is possible to construct the spatial distribution of the electric field for a single electric discharge, (c) the signals' sources can be easily separated and identified, and (d) quantitative data on the electrical potential distribution can be obtained throughout the entire experimental tank. *Results.* The results illustrate an example of applied MEA registration. Electric discharges were recorded from a weakly electric catfish, *Clarias gariepinus*, using an array of 8×8 electrodes at a sampling rate of 20 kHz. Data show oscillograms and two-dimensional plots of the spatial distribution of the electrical field.

Keywords: multielectrode array (MEA) registration, electric field spatial distribution, weakly electric fish, electric discharges, *Clarias gariepinus*.

Acknowledgements. Authors acknowledge Sergey V. Skorodumov and Dmitry E. Elyashev for their help in the development of hardware and software.

For citation: Olshansky VM, Zlenko DV, OrlovAA, Kasumyan AO, Moller P, MacMahon E, Xue Wei. Multielectrode registration of episodic discharges generated by weakly electric fishes. Izvestiya VUZ. Applied Nonlinear Dynamics. 2022;30(2):239–252. DOI: 10.18500/0869-6632-2022-30-2-239-252

Introduction

Experimental studies of strongly electric fish (electric eels, electric rays (*Electrophorus*, *Torpedo*)), conducted since the end of the 18th century, played a noticeable role in the development of the natural sciences — physics, chemistry, biology, and especially electrophysiology [1]. In particular, the work of Henry Cavendish [2], dedicated to the simulation of the Torpedo ray's discharges and for the first time describing the fundamental difference between electrical processes in conductive media and electrostatics, contained a scheme of currents around an electric ray. Later, largely thanks to the works of H. Lissmann [3, 4], the constantly generated electrical discharges in two groups of weakly electric fish (Mormyriformes and Gymnotiformes), were recorded, and a new sensory system, electroreception, was discovered. In addition, the general principles of the joint activity of electroreceptor and power-generating systems (electrical organs) were formulated — systems which enabled electrolocation (object detection), spatial orientation and communication in fish [5, 6].

In studies of weakly electric fish, as a rule, the electric discharges are monitored with two- and three-electrode systems (taking into account the ground electrode when using differential amplifiers), which are standard and common in electrophysiology. This technique allows the observation and analysis of the oscillograms of the discharges, but not that of the spatial electric field distribution. We note that multi-electrode recording devices have been described in past studies. In one case, a multi-electrode array registration system was used to track free-swimming weakly electric fish (gymnotids) in their natural environment [7]. Furthermore, several studies using an array of only 4–8 electrodes attempted to reveal the spatial structure of the fish-generated electric field and any distortions caused by the presence of conspecific individuals [8–10]. This approach was successful in demonstrating the electric field emanating from fish with regular discharges, i.e. in gymnotids and mormyrids. These fish constantly generate discharges, which are a highly stable in their shape and amplitude. Based on multiple discharge recordings from various distances from the fish, it is possible to reconstruct the spatial distribution of the electric field.

In contrast to gymnotids and mormyrids, which constantly generate electrical discharges, numerous catfish (Siluriformes) generate discharges irregularly and almost always in the context of behavioral interactions between conspecifics. The investigation of the electric activity of catfish encounters several methodological problems [11–13], especially with regard to transition from the traditional two-electrode set-up to a multi-electrode array one.

There are several advantages of our MEA registration as compared with the traditional two-electrode methodology: (a) the signal-to-noise ratio is significantly increased, (b) it is possible to construct the spatial distribution of the electric field for a single electric discharge, (c) the signals' sources can be easily separated and identified, and (d) quantitative data on the electrical potential distribution can be obtained throughout the entire experimental tank.

1. Materials and Methods

1.1. The multi-electrode array (MEA). The experiments were carried out in a $60 \times 50 \times 30$ cm tank with a multi-electrode lattice installed at the bottom. Sixty-four recording electrodes were fixed on a sheet of plastic which fully covered the bottom of the tank. Electrodes were arranged in a squared 8×8 array with an inter-electrode distance of 6 cm. Stainless steel washers of 15 mm in diameter were used as electrodes. The electrodes were protected from direct contact with fish by foam rubber and cloth, without distorting the electric field. Water in the tank was grounded with a ground electrode in the form of an inert metal mesh with an area of 100 cm^2 mounted vertically on the wall of the tank.

The electrodes were connected to the inputs of a 64-channel differential amplifier (gain ×1100). The connections consisted of twisted pairs, with the second wire serving as a static shield.

1.2. The multichannel differential amplifier and the reference potential. Initially, the general circuit of a multichannel differential amplifier was developed for the registration of EEG signals [14]. The circuit diagrams of the amplifiers modified by us are shown in Fig. 1 (the circuit is assembled on TL082 and TL084 chips, Texas Instruments, USA). The circuit was supplemented with an integrating module **4**, designed to suppress quasi-permanent galvanic potentials on the electrodes. Differential registration provides for a high level of common-mode rejection, including 50 Hz crosstalk.

Since we were interested in the absolute values of the registered potentials, the general amplification circuit was organized as follows. All preamplifiers **1** have a common reference input **REF1**. This reference potential was formed jointly by all 64 preamplifiers with an additional common reference amplifier **5**. The latter formed the reference potential **REF2** for the second cascade of differential amplifiers with respect to the ground. The common reference amplifier receives a certain common reference potential **REF0** to calculate the differential potentials.

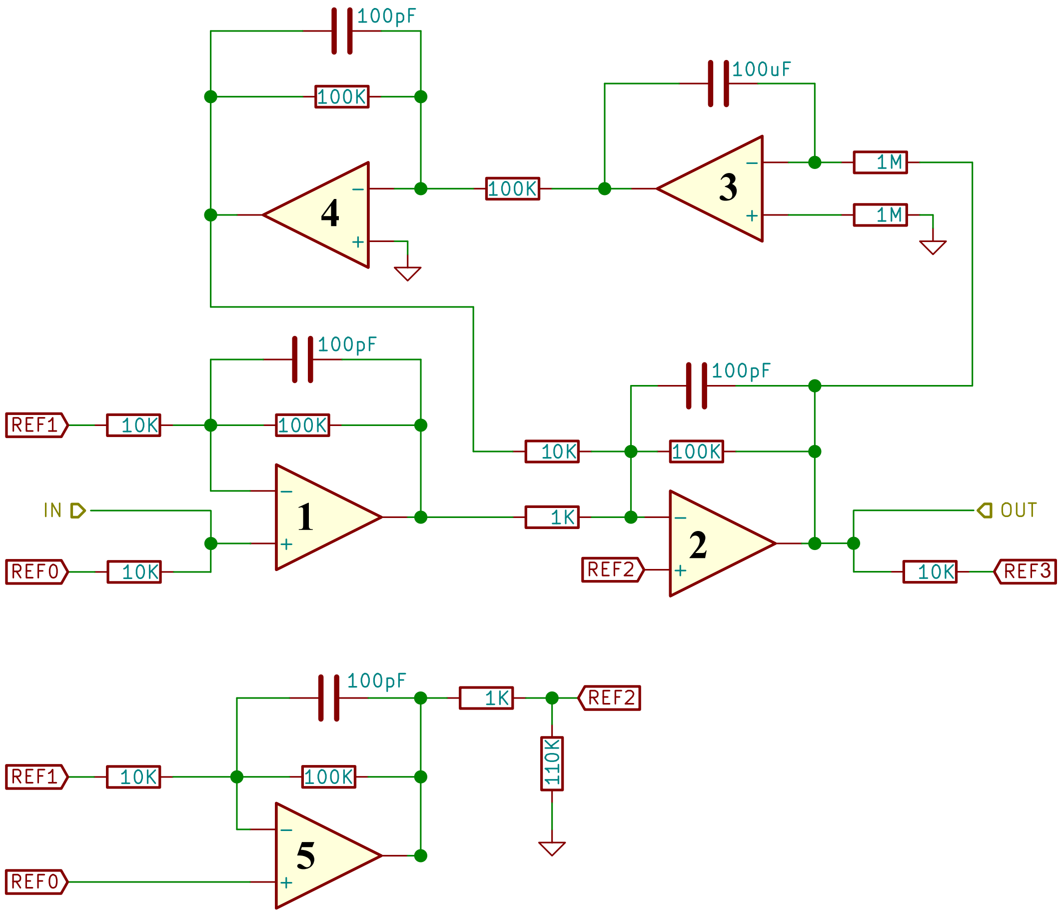

Fig. 1. Multi-channel differential amplifier circuit. The upper part of the diagram describes the structure of one of 64 registration channels **1**–**4**; the lower one describes the configuration of the common reference amplifier **5**. Each registration channel includes a preamplifier **1**, a differential amplifier **2**, an integrator **3**, and an inverter **4**. The **IN** mark corresponds to the input signal fed directly from the electrodes, and **OUT** to the signal output fed to ADC analog inputs. **REF0** is a common reference potential of the amplifier as a whole; **REF1** — the reference potential of preamplifiers; **REF2** — the reference potential for differential amplifiers; **REF3** — the reference potential for the ADC

At the first order of approximation, **REF0** should be connected to the ground. The problem is that initially there is no grounding inside the aquarium. It is necessary, but can only be carried out by adding a special electrode connected by a wire to the ground of the circuit (the midpoint of the battery supply). This earth electrode cannot be large, because otherwise it will greatly distort the field. And the local grounding electrode located in a specific local area of the aquarium is not zero, that is, it does not correspond to the potential of a hypothetical infinitely distant point. Its potential depends on the distance to the source and the orientation of the source. Fish can discharge at different distances from the earth electrode, including directly near it. So, such a decision leads to amplification of all crosstalk on cables and connections of the common reference input and their undesired appearance in the final signal. Avoiding this negative effect, the common reference potential was formed as a sum of the potentials of registration electrodes connected through 10 kΩ resistors. Since the tested fish were always within the registration region, the sum of all registered potentials should be equal to zero, which ensures that the obtained reference potential (**REF0**) is close to zero. The amplified signals were fed to the inputs of two 32-channel 16-bit ADCs (E-502, L-CARD, Russia). The reference inputs of the ADCs were also not grounded. Instead, we used as a reference the sum of amplified signals from all outputs of the multi-channel amplifier connected through the 10 kΩ resistors **REF3**.

1.3. Sampling rate. The ADCs were synchronized to the internal reference clock of one of the devices to collect the data. The sampling frequency was 20 kHz and the registration time was 50 minutes. The choice of the sampling frequency seems to be of crucial importance. The characteristic duration of the electrophysiological processes is about several milliseconds or less. One of the most significant features of the generation of electrical discharges by electric fish is a high degree of synchronization of the excitation of many different cells. The typical synchronization occurs within fractions of milliseconds. Indeed, the main electric organ of *Electrophorus* eel, for example, occupies most part of the fish's body, while the discharges it generates last for about a millisecond [15]. Therefore, the characteristic time of synchronization has to be at least an order of magnitude shorter.

Accordingly, the traditional two-electrode approach employed a sampling frequency of up to 1 MHz in studies of the activity of mormyrids and gymnotids; whereas, in other areas of biology, for example, the multi-electrode array registration of electroencephalograms, the sampling frequency rarely exceeds 2 kHz [16]. Thus, with MEAs, the larger number of electrodes and greater sampling frequency significantly increases the information flow and the volume of the data files, which causes obvious difficulties. Therefore, a compromise is necessary between the desire for detailed observations of the synchronization of the cells' electro-excitation and the requirement to digest the augmented data streams and large files. Using a sampling rate of 20 kHz seems to afford such a compromise and allows the observation of both single responses of a cell group and the gaps between the responses at the ultimate excitation frequencies [17].

1.4. Principal Component Analysis (PCA). The electric fields of biological sources decay steeply with the distance from the source. So, the corresponding signal amplitudes recorded at different electrodes differ significantly. The peculiarities of signal propagation, however, do not affect those properties of the signal which are essential for the physiological processes of interest since the size of the experimental tank was significantly smaller than the skin-depth in water [18]. Thus, the shape of the curve corresponding to the discharge at different registration electrodes remains the same. At the same time, most of the interference entering the water from the air, in particular, 50 Hz cross-talk or impulse interferences, has a spatial distribution of the amplitude close to uniform, which differs from the target signals. So, it became possible to filter out a highly spatial heterogeneous useful signal using principal component analysis (PCA). PCA, as well as independent component analysis (ICA), is successfully used in electroencephalography [19]. Based on the input from 64 channels, data were assigned to up to 64 separate components. This allowed filtering out and reconstructing the fish's electric discharge curve. Moreover, Principal Component Analysis also permits the isolation of other electric phenomena, for example, assessing one fish's body polarization in the electric field of a discharge of another catfish (Clarias). Besides the target signal filtering, principal component analysis provides a way to visualize the electric field in a 2D plain. In order to recover the spatial distribution of the electric field of the discharge, we plotted, on a two-dimensional surface, the values of the weight coefficients of the corresponding principal components. Calculation of covariance matrices and their diagonalization was carried out by means of the sklearn library (Python3).

2. Results

A discharge of *Clarias gariepinus* registered with our MEA approach is exemplified in figure 2. Previously, we found that during the discharge, the head of the catfish has a negative polarity, while the remainder of the body has a positive one [20]. Since the orange-red curves represent negative polarity (Fig. 2), the location of the head of the test catfish is indicated. Green and blue curves represent positive polarity, and reveal the approximate position of the body without resorting to any sneaky mathematical tricks.

Note that the original signal is quite noisy: the peak-to-peak noise amplitude is about 20 μV, which, even for electrodes with a maximum desired signal amplitude of about 500 μV, results in a not-so-good signal-to-noise ratio of 25. To filter out the noise, we used the method of principal components analysis (Fig. 3). For PCA, a fragment of a recording with a bit duration of 80 ms was taken, which at a

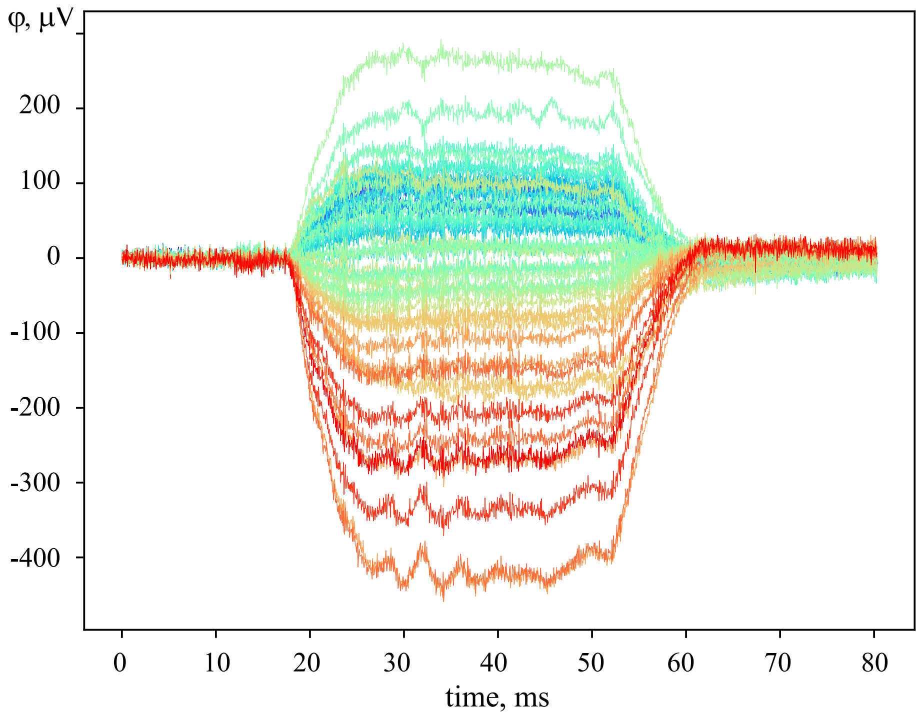

Fig. 2. Dynamics of the electric potential in the cource of the discharge generagted by *Clarias gariepinus* catfish using 64 electrode square array. The color legend corresponds to that in Fig. 4 (see further), which makes it possible to compare the color of the curve with the spatial position of the corresponding electrode in the lattice

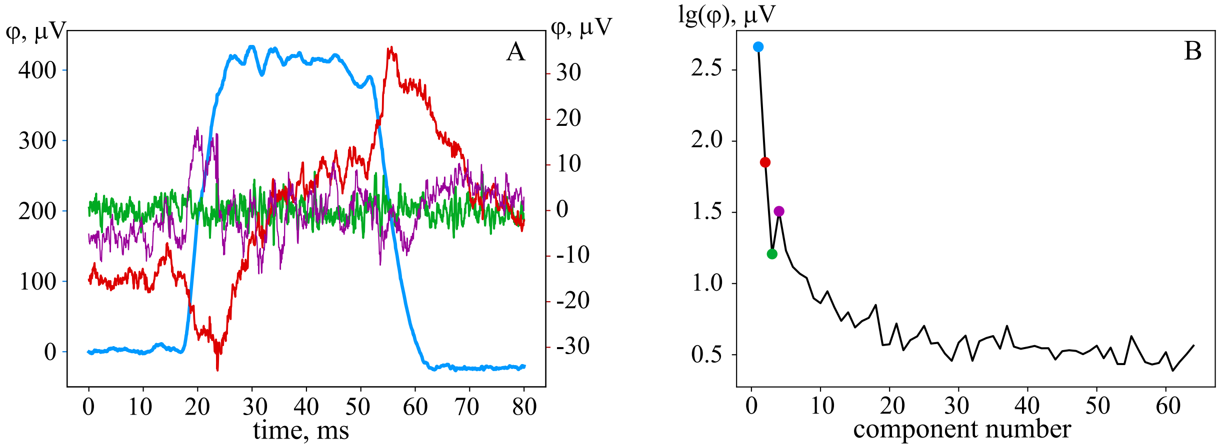

Fig. 3. A. The first four principal components (the first is shown in blue, the second in red, the third in green, and the fourth in purple) of the curves' family is shown in Fig. 2. The left ordinate axis (shown in blue) corresponds to the first component, and the right one (shown in red) corresponds to the second-to-fourth components. B. Decimal logarithms of the amplitudes of all 64 principal components. The colored dots show the data for the curves shown in A

sampling rate of 20 kHz corresponds to 1600 samples for each of the 64 channels. Thus, the principal components of the family of curves (Fig. 2) shows that the entire target signal is concentrated in 1st, 2nd, and 4th principal components, while all other components contains only white noise (Fig. 3A, green). The amplitudes of the principal components rapidly decrease as the components' number increase (Fig. 3B).

The first component makes the most significant contribution to the total signal (Fig. 3A, blue). The shape of the first component corresponds to the shape of the discharges previously recorded for *Clarias gariepinus*. The discharge duration at half height was 35 ms. The filtered curve clearly shows the fine structure above the plateau of the discharge, which reflects the functioning of the electro-generating structure in the fish body [17]. The period of these oscillations was 3.9 ± 0.7 ms, and the amplitude was 20–50 μV.

The general view of the second component (Fig. 3A, red) corresponds to the first derivative of the primary signal (Fig. 3A, blue). Such an effect can occur if there is some capacity in the system. When current flows through such a capacity, some signal would appear in the form of a derivative of the main pulse. The nature of this capacity can vary. In particular, it can be the body of the sample catfish or some element of the electrical circuit. However, the preliminary calibration of the setup with source of a meander signal showed that the electric circuit transmitted the signals without noticeable distortion. Therefore, the capacitive distortions were caused by the fish body placed in the tank, which could be clarified with the data on the spatial position of its source (Fig. 4, II, see further).

The fourth component had a small amplitude and was quite noisy. It showed a certain peculiarity, namely a prominent double peak between 20 and 30 ms of the signal under consideration. The amplitude of the effect was about 20 μV and the duration was about 10 ms. The discussion of the nature of this signal requires data on the spatial position of its source (Fig. 4, IV, see further).

3. Discussion

We assume that the catfish's electric discharges result from the electrical excitation of a certain muscle structure by a series of triggering nerve impulses [17]. The elementary discharge of about 8 ms in duration is generated in response to each nerve pulse. It was assumed that the intervals between the triggering nerve pulses are noticeably shorter, resulting in a rather long monopolar total discharge.

The latter has an irregular plateau and an amplitude greater than that of the elementary discharges of which it is composed. This model is based on the data from the experiments performed using a traditional two-electrode recording technique [17].

The transition to the multi-electrode method and the application of principal component analysis affords several advantages. First, the resulting discharge curves became cleaner compared to those registered with two-electrode registration. The 50 Hz interference was suppressed entirely. Second, the quantitative data on the potentials near the fish body became available. With a two-electrode setup, the amplitude of the registered discharge depended on the position and orientation of the fish in the tank. The multi-electrode setup permits observation and analysis of the character of the decay of the field intensity around the fish.

The analysis of the distribution of principal components' weight coefficients among the electrodes allows assessment of their contributions to the total electric field on each electrode. The product of the principal component and the corresponding coefficient describes the dynamics of the electrical potential corresponding to this component on the given electrode. However, the most interesting challenge would be the reconstruction of the spatial distribution of the "discharge's field." The latter seems to be a somewhat undefined notion. Indeed, because the electric field changes in the course of the discharge, so the spatial distribution of the "discharge's field" has to reflect some period of time, rather than being momentary.

We defined the "field of a principal component" as a spatial distribution of the product of the weight coefficient of the given component on each electrode and the amplitude of this principal component. Therefore, in the case of the monopolar discharge (Fig. 3A, blue), the obtained values would be approximately the maximum in absolute magnitude reached at each electrode in the course of the discharge and corresponding to the given principal component only. The field of the first principal component (Fig. 3A, blue) would be the desired field of the discharge. Its spatial distribution (Fig. 4, I) describes the spatial distribution of that part of the total electrical field, which corresponds to the first component only and does not account for all other components.

The first component field (Fig. 4, I) has a prominent dipole character, with a positive pole in the region of the point with coordinates given in centimeters of $(18, 13)$ and negative at $(27, 0)$. Thus, our registration method could very likely resolve a long-debated question as to the location of the power-generating structure. Based on our data, we surmise that this structure should be located in the region between the poles of the dipole, i.e., in the area where the potential is zeroed out. This area seems to be close to the head of the fish, which is consistent with repeated assertions that the electro-generating structure in clariid catfish is located between the posterior edge of the skull and the beginning of the dorsal fin [21]. Thus, the approximate localization of the electro-generating structure will mitigate the anticipated challenges which await investigating histologists and morphologists.

In previous investigations, when modeling the discharge patterns of clariid catfish, we usually observed an excellent convergence at the beginning of the recording but noticeable discrepancies at the end [17]. Moreover, the trailing edge of the discharges often contained a prominent differential signal, and it was not clear whether this was a feature of electro-generation or some external effect. The use of multi-electrode registration and principal component analysis make it possible to reveal the source of the differential signal and thus to explain the aforementioned discrepancy. The spatial distribution of the second component's field corresponds to the field of a monopole rather than that of a dipole. The center of the monopole $(21, 5)$ was close to the mid-point of the dipol $(22, 7)$, which allows us to attribute the effect of differentiation to the fish body. However, a noticeable shift of the poles in the field patterns for the second component with respect to the field of the first component shows that the source of differentiation does not coincide with the source of the main discharge.

The spatial distributions typical for the principal components containing the noise did not demonstrate any noticeable peculiarities in the spatial structure (Fig. 4, III). The noise has a small

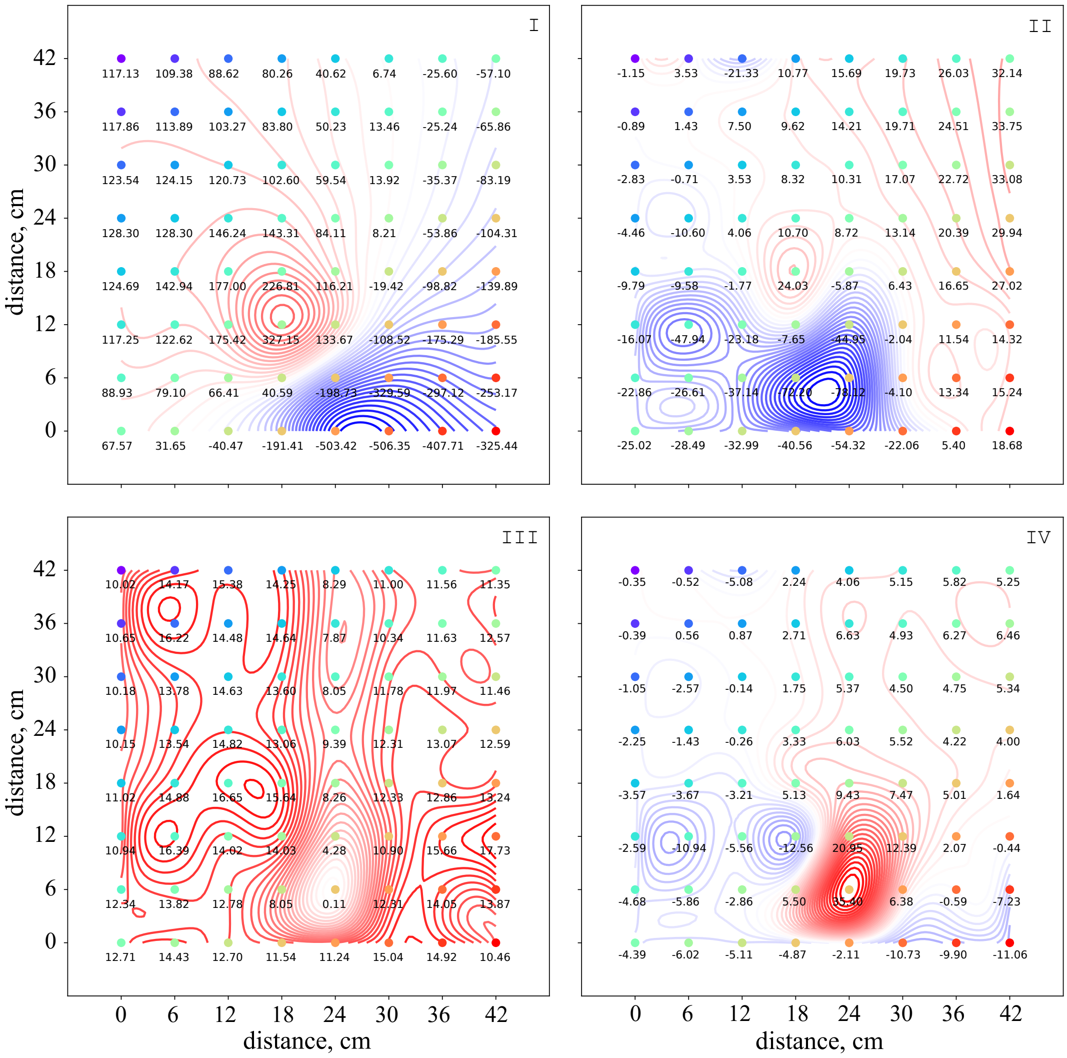

Fig. 4. Spatial distributions of the electric potentials corresponding to the first four principal components shown in Fig. 3A. The numbers of the components designated by the digits in the plots. The circles mark the position of electrodes, and their color corresponds to the color of the original curves in Fig. 2

amplitude of tens of microvolts, and its distribution was more or less uniform, which is typical for a field of interference from external sources.

The spatial distribution of the fourth component resembled that of the second one and has a pronounced monopole shape. The maximum was also located in a special area of space close to the electro-generating structure $(24, 6)$. The low amplitude of this component's field and the low spatial resolution of our system (the lattice pitch was 6 cm, with the size of the fish of about 15 cm) do not allow us to propose a specific physiological origin for this effect.

Conclusion

Returning to the problem of choice of the number of electrodes and the sampling rate — is it possible, with our limited resources, to combine a time resolution of 1μs with tens of thousands of electrodes, and a reasonable amount of data? Our studies of weakly electric fish suggest that indeed it is possible if we employ a biomimetic approach — and so, we propose a technical replication of those physiological techniques which these fish use to create the amplitude-phase images of electrical events in their brains [22].

In summary, the employment of a of multi-electrode array seems to be an effective tool for the investigation and registration of weak electrical signals in an aquatic environment. We propose that this method of registration could be applied to a wide range of problems, including the investigation of the role of electric fields and electro-communications in the lives of many aquatic animals.

References

1. Finger S, Piccolino M. The Shocking History of Electric Fishes: From Ancient Epochs to the Birth of Modern Neurophysiology. Oxford: Oxford University Press; 2011. 470 p. DOI: 10.1093/acprof:oso/9780195366723.001.0001.
2. Cavendish H. An account of some attempts to imitate the effects of the torpedo by electricity. Phil. Trans. R. Soc. 1776;66:196–225. DOI: 10.1098/rstl.1776.0013.
3. Lissmann HW. Continuous electrical signals from the tail of a fish, *Gymnarchus niloticus* Cuv. Nature. 1951;167(4240):201–202. DOI: 10.1038/167201a0.
4. Lissmann HW. On the function and evolution of electric organs in fish. J. Exp. Biol. 1958;35(1): 156–191. DOI: 10.1242/jeb.35.1.156.
5. Bennett MVL. Electric organs. In: Hoar WS, Randall DJ, editors. Fish Physiology. Vol. 5. New York: Academic Press; 1971. P. 347–491.
6. Bennett MVL. Electroreception. In: Hoar WS, Randall DJ, editors. Fish Physiology. Vol. 5. New York: Academic Press; 1971. P. 493–574.
7. Henninger J, Krahe R, Sinz F, Benda J. Tracking activity patterns of a multispecies community of gymnotiform weakly electric fish in their neotropical habitat without tagging. J. Exp. Biol. 2020;223(3):jeb206342. DOI: 10.1242/jeb.206342.
8. Rasnow B. Measuring and visualizing EOD fields. In: Ladich F, Collin SP, Moller P, Kapoor BG, editors. Communication in Fishes. Enfield, USA: Science Publishers Inc.; 2006. P. 599–622.
9. Rasnow B, Bower JM. Imaging with electricity: How weakly electric fish might perceive objects. In: Bower JM, editor. Computational Neuroscience: Trends in Research. New York: Plenum; 1997. P. 795–800.
10. Assad C, Rasnow B, Stoddard PK. Electric organ discharges and electric images during electrolocation. J. Exp. Biol. 1999;202(10):1185–1193.
11. Hagedorn M, Womble M, Finger TE. Synodontid catfish: A new group of weakly electric fish. Brain Behav. Evol. 1990;35(5):268–277. DOI: 10.1159/000115873.
12. Baron VD, Morshnev KS, Olshansky VM, Orlov AA. Electric organ discharges of two species of African catfish (*Synodontis*) during social behavior. Animal Behaviour. 1994;48(6):1472–1475. DOI: 10.1006/anbe.1994.1387.
13. Baron VD, Orlov AA, Golubtsov AS. African Clarias catfish elicits long-lasting weak electric pulses. Experientia. 1994;50(7):664–647. DOI: 10.1007/BF01952864.
14. Metting van Rijn AC, Peper A, Grimbergen CA. High-quality recording of bioelectric events. Part 2. Low-noise, low-power multichannel amplifier design. Med. Biol. Eng. Comput. 1991;29(4): 433–440. DOI: 10.1007/BF02441666.
15. Catania KC. An optimized biological taser: Electric eels remotely induce or arrest movement in nearby prey. Brain Behav. Evol. 2015;86(1):38–47. DOI: 10.1159/000435945.
16. Grishchenko AA, Sysoeva MV, Sysoev IV. Detecting the primary time scale of evolution of information properties for local field potentials in brain at absence epilepsy. Izvestiya VUZ. Applied Nonlinear Dynamics 2020;28(1):98–110 (in Russian). DOI: 10.18500/0869-6632-2020-28-1-98-110.

17. Orlov AA, Olshanskiy VM, Baron VD. Reconstruction of electric discharge patterns and electrogenesis mechanisms in African sharptooth catfish *Clarias gariepinus* (Clariidae, Siluriformes). Dokl. Biol. Sci. 2021;500(1):145–148. DOI: 10.1134/S0012496621050082.
18. Olshanskiy VM. Bionic Modeling of Electrical Systems of Weak Electric Fish. Moscow: Nauka; 1990. 208 p. (in Russian).
19. Makeig S, Onton J. ERP features and EEG dynamics: An ICA perspective. In: Luck SJ, Kappenman ES, editors. The Oxford Handbook of Event-Related Potential Components. New York: Oxford University Press; 2011. P. 51–86. DOI: 10.1093/oxfordhb/9780195374148.013.0035.
20. Olshanskii VM, Morshnev KS, Naseka AM, Nguyen TN. Electric discharges of clariid catfishes cultivated in South Vietnam. Journal of Ichthyology. 2002;42(6):477–484.
21. Olshanskiy VM, Kasumyan AO, Moller P. On mating and function of associated electric pulses in *Clarias macrocephalus* (Günther, 1864): Probing an old puzzle, first posed by Charles Darwin. Environmental Biology of Fishes. 2020;103(1):99–114. DOI: 10.1007/s10641-019-00936-w.
22. Olshanskiy VM, Zlenko DV. Reconstruction of electrical field images and an attempt to overcome the intraspecific berrier. In: Proceedings of the Seventh All-Russian Conference «Nonlinear Dynamics in Cognitive Research». 20-24 September 2021, Nizhny Novgorod, Russia. Nizhny Novgorod: Institute of Applied Physics RAS; 2021. P. 90–93 (in Russian).