Journal of Coastal Life Medicine

journal homepage: www.jclmm.com

Document heading doi: 10.12980/JCLM.3.2015J5-10

Neotropical electric fishes (Gymnotiformes) as model organisms for bioassays

Milena Ferreira*, Isac Silva de Jesus, Eliana Feldberg, José Antônio Alves-Gomes

Graduation Program in Genetics, Conservation and Evolutionary Biology, National Institute of Amazon Research, Manaus, Amazonas, Brazil

ARTICLE INFO

Article history:
Received 6 Feb 2015
Received in revised form 24 Feb 2015
Accepted 26 Feb 2015
Available online 2 Mar 2015

Keywords:
Electric fish
Bioassays
Potassium cyanide
Petroleum products
Mercury contamination
Neuroactive drugs

ABSTRACT

Electric fishes (Gymnotiformes) inhabit Central and South America and form a relatively large group with more than 200 species. Besides a taxonomic challenge due to their still unresolved systematic, wide distribution and the variety of habitats they occupy, these fishes have been intensively studied due to their peculiar use of bioelectricity for electrolocation and communication. Conventional analysis of cells, tissues and organs have been complemented with the studies on the electric organ discharges of these fishes. This review compiles the results of 13 bioassays developed during the last 50 years, which used the quickness, low costs and functionality of the bioelectric data collection of Gymnotiformes to evaluate the effects of environmental contaminants and neuroactive drugs.

1. Introduction

Fishes from the Gymnotiformes, also known as South American electric fishes, are an endemic group, widely distributed in the neotropical region[1]. Nowadays, 179 species of neotropical electric fishes are recognized and several other new species have been described lately[2-6]. The Amazon and Orinoco river basins and the Guyana region river basins, concentrate the most of the diversity, with over 76.3% of all the known species[2].

Besides the taxonomic and genetic studies[7-9], Gymnotiformes are also the focus of research on the physiology of their electric organs, their electric receptors and on the behaviors associated to the production and detection of electric signals[10-15]. Such distinctive sensorial system (electrogenic and electrosensory system) has proved to be useful in bioassays and in environmental monitoring studies, due to the alterations it presents when the fishes are exposed to distinct elements or chemical compounds[16-18].

It is noteworthy that studies using electric fishes' discharges as an analysis parameter have started with African electric fishes of the Family Mormyridae (Mormyriformes), one of the pioneers on this approach, using electric fishes of the species *Gnathonemus petersi* to detect inorganic compounds in the water (Hg^{2+}, Cu^{2+}, $NaAsO_2$ and CN^-)[19]. Yet, a data collection system [the detectors of the electric organ discharge (EOD)] was integrated to a computer data analysis system. This pioneer study has inspired several other subsequent works with Mormyridae and Gymnotiformes.

Taking the above into consideration, the objective of this work is to review the studies produced so far, using gymnotiform electric fishes as model organisms for bioassays. We have identified 13 bioassays (Table 1), most of which developed by Brazilian researchers, including analytical techniques common to other organisms as well as the recording and analysis of EODs, a much more restrict type of data.

An extensive search of publications was performed using Google scholar, Scielo, PubMed, Lattes Platform and Capes Portal websites. We used keywords like "Gymnotiformes", "toxicity" and "bioassay"

*Corresponding author: Milena Ferreira, Graduation Program in Genetics, Conservation and Evolutionary Biology, National Institute of Amazon Research, Manaus, Amazonas, Brazil.

E-mail: milena_fro@hotmail.com

Foundation Project: Supported by the Coordination of Improvement of Higher Education Personnel (CAPES), Ministry of Science, Technology and Innovation of Brazil (Grant No. PROAP/2014).

Table 1
Bioassays reviewed with detailed species, analysis technique, tested substances and their concentrations.

Species	Analyses	Substance or contaminant	Concentration	Contamination to	References
A. albifrons	EODs	KCN	35, 45 and 70 µg/L	Added to the aquarium water	[17]
A. albifrons	EODs	KCN	35, 45, 70, 125 and 180 µg/L	Added to the aquarium water	[18]
A. albifrons	EODs	Chlorpromazine	0.125 mg/L	Added to the aquarium water	[37]
Apteronotus bonapartii	Micronucleus test	Benzene	10 ppm (1.5 mL/150 L) and 25 ppm (3.75 mL/150 L)	Added to the aquarium water	[28]
A. hasemani	EODs	BTX	2, 10, 20 and 100 mg/L	Added to the aquarium water	[30]
A. hasemani	EODs	Produced water	1, 2 and 4×10^{-4} mL/L	Added to the aquarium water	[32]
E. virescens	Micronucleus test and Comet assay	Benzene	50 ppm	Added to the aquarium water	[29]
E. virescens	EODs	Benzocaine	5×10^{-5}, 1×10^{-4} and 2×10^{-4} mol/L	Added to the aquarium water	[38]
G. carapo	EODs	Chlorpromazine	1.25×10^{-8} g/mL, 1.38×10^{-7} g/mL, 1.25×10^{-7} g/mL, 3.12×10^{-7}g/mL and 1.25×10^{-6} g/mL	Added to the aquarium water	[36]
		Methylphenidate	2.5×10^{-8} g/mL, 2.5×10^{-7} g/mL and 2.5×10^{-6} g/mL	Added to the aquarium water	
G. carapo	Histopathology	$HgCl_2$	0.6 µg/g	Intraperitoneal injections	[33]
G. carapo	Histopathology and metal concentrations	$HgCl_2$	5, 10, 20 and 30 µmol/L	Intraperitoneal injections	[34]
G. sylvius	EODs	MeHg	4 µg/kg and 20 µg/kg	Ingestion of contaminated earthworms	[35]
M. bilineatus	EODs	Gasoline and Diesel oil	110 µL/L and 220 µL/L	Added to the aquarium water	[16]

and then, we selected all the original articles and review that presented studies on the use of gymnotiform fishes as animal models for bioassays. A time limit was not established. We excluded articles that were testing the effects of hormones for growth and gonad maturation, once these studies are not directed to evaluate toxic effects on the fishes, neither behavioral change due to the exposure to exogenous and non-analogous substances.

2. The electric organ and its discharge EOD

The electric organ is a organic tissue of either myogenic (muscle cells) or neurogenic (nervous cell) origin, capable of producing electric pulses[20]. It is composed of cell layers disposed both in piles or clusters, longitudinally located and occupying a large portion of the fish's body[15]. The EODs are continuously produced, and the electroreceptors distributed over the fish's skin, sense both the electric information produced in the environment and by itself in a co-ordinate action called active electrolocation[21]. These set of organs work together as a functional unit, providing to the fish information on its location, communication and identification of reproductive mates[15,16]. The electric organ discharge can also work for prey confusion and predators' avoidance, these two last features exclusively to *Electrophorus electricus*[22,23].

Each EOD is initially produced at a pacemaker nucleus, located in the fish medulla. The frequency and waveform of the discharges may be changed by the administration of some hormones, which suggests there is an EOD specificity depending on the age, sex and reproductive stage of the animal[24]. The EODs and their modulations, once they are directly controlled by the central nervous system (CNS), are considered behaviors[25]. Both the stimulus and the inhibitions of EOD are mediated by neuronal modulators, which activate or deactivate specific ionic channels. According to which channel is blocked or activated, cells might increase or decrease the discharge rates[14].

The EODs are easily recorded and they are informative, presenting several parameters that can be analyzed statistically. EODs may be of pulse-type, with large intervals between consecutive peaks, or wave-type, with very small or no distinctive intervals between discharges[14]. Recordings are generally made with fish alive, kept in laboratory, and the exposure to tested substances may be done by distinct via, such as dilution in the water in which the fishes are immerse, intramuscular or intraperitoneal injections.

3. Bioassays with potassium cyanide

Cyanides are chemical compounds which have a cyano group (-C N) in its composition and they are toxic to various organisms (including humans), due to its high affinity to hemoglobin, preventing its biding to oxygen. Due to the risk of water contamination in Moselle River (France), the use of the gymnotiform *Apteronotus albifrons* (*A. albifrons*) as a biomonitor for potassium cyanide (KCN) was proposed[17,18].

This species was chosen because it produces weak electric discharges continuously and temporally stable, allowing the authors to use its EOD as an analysis parameter. Unlike *Gnathonemus petersi*, *A. albifrons* electric signals are not easily influenced by external agents such as noise, water agitation, turning on or off the room light. This stability permits a much better correlation between the electric signals modulations and the contaminant effects on the fish[17].

Among the components that are able to influence EOD frequency in *A. albifrons* are temperature and pH variations[26,27]. Temperature variation may be controlled both by water thermoregulation during the experiment or mathematical adjustments of the obtained results by some software[27]. The pH changes must be accompanied more carefully, once they might be due to both normal fluctuations of the natural environment water or a contaminant effect, requiring more specific and constant analysis in a biomonitoring system[26].

In two bioassays using KCN, EOD frequency decreased in the treatments with 35, 45 and 70 µg/L of KCN and has increased in the treatments with 125 and 180 µg/L of the contaminant[17,18]. As the KCN concentration increased, the time spent by the fish for contaminant detection decreased, being 27 min for the lowest concentration (35 µg/L) and 5 min for the highest (180 µg/L)[17,18].

The EOD waveform has also changed more dramatically under the influence of the highest KCN concentrations. The contaminant, mainly in the 125 µg/L concentration, has made the EOD waveform increase; however, the EOD frequency changes were faster than waveform changes[17,18].

Although the mechanism by which the changes in the electric signals have occurred remain unknown, EODs were informative both to indicate the presence as well as the KCN concentration in the water, validating the use of *A. albifrons*' discharges to detect water contamination by KCN[18].

The current sophistication level of biomonitors is not enough for the precise identification of what is causing the biological changes[26]. However, in those systems in which the aim is an early warning on the water quality, the use of gymnotiforms as biomonitors is efficient. This kind of detection system works as a first sign to initiate the actions of mitigation of harmful effects, while more accurate results, which take longer to be obtained, are performed.

4. Bioassays with petroleum related contaminants

The growth of petroleum exploration and the production of its derivatives in Brazil, especially in Amazon region, has generated environmental concerns due to the direct and indirect impacts related to these activities. In the meantime, studies on the possible effects of these contaminants, mainly on the aquatic ecosystems are being developed on Amazon region[28].

Also for this type of pollutants electric fishes have been used successfully as biomonitors. Distinctive approaches may be used for data collection on the effects of petroleum and its derivatives in cellular, histological and behavioral assays having these fishes as experimental subjects.

A study was performed on the benzene (50 ppm) genotoxicity for the species *Eigenmannia virescens* (*E. virescens*)[29]. The micronuclei analysis and the comet assay were used to establish the effects of benzene at the cell level. The exposure times to the contaminant were 24, 48, 72, 96 and 360 h. The micronuclei occurrence has not changed significantly among the exposure times. However, the cell damage observed by the comet assay, significantly increased with the longer exposure. Such time-dependent relationship with benzene exposure was only observed up to the 96 h of contact. Probably, there was a cell recovering after such limit and/or the loss of benzene concentration in aquarium water by volatilization[29].

On a similar study[28], micronuclei test and comet assay were once more used to ascertain the effects of two benzene concentrations (10 and 25 ppm) on electric fishes of the species *Apteronotus bonapartii*, a gymnotiform with neurogenic electric organ. The contaminant exposure times were 24, 48, 72 and 96 h. Again, the authors did not observe any significant change in micronuclei occurrence, or in other nuclear abnormalities incidence, when the fishes were exposed to the 10 ppm concentration. However, fishes submitted to 25 ppm of benzene via aquarium water presented a significant higher occurrence of both micronuclei and nuclear abnormalities after 72 and 96 h of exposure[28].

Comet assay allowed detecting more accurately the cell damage, once cell damage was observed after 48 h of exposure for 10 ppm of benzene, whereas for 25 ppm cell damage was evident after 24 h of exposure. The results were time and dose-dependent. Considering these results, the authors propose the gymnotiforms and the comet assay as an appropriate system to evaluate water bodies contaminated by benzene and other dangerous pollutants[28].

Micronuclei test and comet assay might be used in studies performed with any other fish species, and the species choice depends on several factors including its occurrence and abundance on the environment, its adaptability to captivity and effort needed for its capture. However, gymnotiform species, due to their EOD, may represent a more suitable model to be used, considering that the direct analysis of their EODs allows a faster assessment about the effects of petroleum products, and its components, in their metabolism.

In another study with petroleum products, the EODs' variations were analyzed in *Apteronotus hasemani* (*A. hasemani*) specimens individually exposed to benzene, toluene and xylene (BTX). The concentrations tested were 2, 10, 20 and 100 mg/L and it was determined that EOD frequency consistently decreased with concentrations of 10 mg/L, with a dose dependent effect[30].

Another research performed with *Microsternarchus bilineatus* (*M. bilineatus*) evaluated the effects of gasoline and diesel fuel on the EODs' repetition rate[16]. This fish species, differently from *Apteronotus* and *Eigenmannia* species, has a pulse type EOD, with a relatively long duration and a stable repetition rate, which seems to be a transitional stage from pulse to wave type EOD[31]. For this species, EODs' rate has decreased for 85.3% of the fish studied, already within the first hour of exposure. The concentrations used for the tests were 110 and 220 µL/L of each fuel, which correspond, respectively, to the 25 and 50% of the tolerance limit established for freshwater fishes. These results show a high sensitivity of *M.*

bilineatus as a biomonitor for gasoline and diesel[16].

Another contaminant representing an environmental risk due to petroleum exploitation is the produced water, a petroleum by-product found in the oil wells and highly pollutant, especially in freshwater habitats, due to its high concentrations of salts, metals and oil by-products. In a trial using *A. hasemani* fishes, the effects of such water on EOD production were evaluated, in the concentrations of 1, 2 and 4×10^{-4} mL/L[32]. It was observed a decrease in EOD frequency as the produced water concentrations increased in the experiment aquarium[32].

This result shows that some changes happen in the behavior and physiology of fishes in the presence of petroleum products and produced water. In general, the studied animals presented cell damage and EOD frequency decrease when exposed to these contaminants. Understanding how petroleum products act on the Electrogenic System of the electric fishes remains a challenge[32].

5. Mercury bioassays

Toxicological studies are using several analysis techniques considering the different levels of cell organization. By means of histological analysis it is possible to evaluate specific effects of contaminants in several organs[33].

The effects of mercury (I) chloride ($HgCl_2$) have been studied in *Gymnotus carapo* (*G. carapo*) exposed to a single intraperitoneal administration of a 0.6 µg/g solution of the contaminant during 24, 48, 72 and 96 h[33]. The organism's response was evaluated in several tissues including gills, muscle, liver and testes and the results pointed to a positive correlation between $HgCl_2$ accumulation and morphological changes in fish tissues[33]. Further, the consequences of exposure were shown to be progressive. The effects on *G. carapo* testes were severe cysts disorganization, reduction of germ cells and sperm aggregation. On the liver, there were tissue disorganization, changes in hepatocytes nucleus, blood vessels obstruction and parenchyma disintegration[33]. On gills, there were fusion of secondary lamellas and epithelial cells vacuolization[33]. Muscle tissue did not present alterations, as low concentrations of the metal were found on it[33]. It is believed that muscle tissue is just a secondary deposit of the contaminant after it escapes liver metabolism[33].

In a later work, the effects of four $HgCl_2$ doses (5, 10, 20 and 30 µmol/L) only on testes tissue from *G. carapo*, after 24, 48, 72 and 96 h of exposure were analyzed[34]. The $HgCl_2$ contaminated fishes presented cysts disorganization, intestinal tissue proliferation, congestion of blood vessels, reduction of germ cells, interstitial and globular disintegration, sperm aggregation and germ cells vacuolization[34]. Such effects were both dose and time-dependent. Individuals exposed to higher concentrations and for longer time, were the ones presenting the most severe alterations[34].

A study with another *Gymnotus* species [*Gymnotus sylvius* (*G. sylvius*)] was also performed on mercury effects. The fishes were exposed to methyl mercury (MeHg) inoculated in their food (earthworms)[35]. The effects of the contaminant were evaluated *in vivo*, by means of EOD analysis. They have used two treatments, one of acute exposure (20 µg/g in a single dose) and another of chronic exposure (09 administrations of 04 µg/g of MeHg)[35].

Both treatments led to an increase on EOD frequency of the fishes, with dose and depuration-dependent effect. It was concluded that MeHg induced an excitotoxic effect, because this contaminant favors the accumulation of glutamate in the extracellular medium[35]. Measuring the EOD frequency is cheap, useful and non invasive and, thus, it may be used to monitor mercury water and animal contamination, especially in Amazon rivers, the natural area of occurrence of the most of electric fishes, and also, a place where mining is a relevant economic activity[35].

6. Bioassays with drugs

The gymnotiforms' EOD has also been used to study, in fish, the effects of substances that are neuroactive in mammals. In some extern it was known over 1960s that stimuli, as well as some drugs, could modulate electric fishes EOD[36]. As at that time it was more difficult to assess data on the EOD modulation, then choosing a pulse type EOD fish (*G. carapo*) for testing and analyzing EOD frequency could bring some explanations. It was aimed at ascertaining if a pulse type EOD fish would be more suitable for the study of drugs effects on EOD modulations, than the wave type EOD fish *E. virescens*, due to the characteristics of the provided data[36].

Five concentrations of chlorpromazine (1.25×10^{-8}, 1.38×10^{-7}, 1.25×10^{-7}, 3.12×10^{-7} and 1.25×10^{-6} g/mL) were tested and it was observed that this substance has a depressor effect on the CNS of *G. carapo*[36]. Methylphenidate was also tested in three concentrations (2.5×10^{-8}, 2.5×10^{-7} and 2.5×10^{-6} g/mL) and it had a stimulant effect on *G. carapo*'s CNS[36]. Both drugs provoked on the electric fishes the same effects as in mammals.

It must be highlighted the easiness and low costs of such bioassay, in which it was possible to evaluate the effects of several neuroactive substances by looking at the EOD patterns in several types of behavioral tests with electric fishes under the influence of these drugs[36]. It was showed that it is possible to estimate the effects of some substances in the CNS of mammals from the responses they evoke in electric fishes' EODs[36].

Chlorpromazine was also used in another test with electric fishes in order to provide information on the behavioral effects of psychoactive drugs. In the concentration of 0.125 mg/mL it could be observed that it diminished the EOD frequency of *A. albifrons* in 26.2 Hz, in mean, after 3 h of exposure[37]. The EOD frequency returned to its basal value 24 h after the removal of the water with the drug[37]. It is noteworthy that *G. sylvius* is an electric fish with pulse type EOD, whose electric organ has a muscular origin. On the other hand, *A. albifrons*, produces wave-type EOD and its electric organ has a neuronal origin. Despite the differences, both species responded the same way to a CNS depressor.

Three concentrations of benzocaine (5×10^{-5}; 1×10^{-4}; 2×10^{-4} mol/L), a local anesthetic capable of blocking nervous pulses, was also tested on *E. virescens*[38]. The aim was to prove the thesis of frequency depression resulting from drug effects on the cranial level (CNS), while amplitude depression and distortion results from peripheral action of the drugs[38]. Thus, it was observed that all the analyzed concentrations of the drug influenced the EOD frequency and amplitude 1 minute after the exposure, reducing the frequency, which has even completely stopped at the 2×10^{-4} mol/L concentration[38].

On the other hand, fish recovery after exposure was completed up to 10 min after the substitution of the water with the drug, for all the concentrations tested[38]. Irregularity on EOD amplitude was only observed on the recovery time[38]. So, it was concluded that benzocaine acts centrally on the pacemaker cells, altering EOD frequency, and also peripherally, during the recovery period, however, not as potent as it acts centrally[38].

7. Discussion

Although being useful for studies with cells, tissues and organs, the great advantage of using gymnotiforms in bioassays is the possibility of using the EODs as a measurable behavior. Among the main benefits of this technique are the several parameters that can be extracted from the EODs and their statistical analysis, the relatively low operational cost for recording and analyzing electric signals, the good adaptability of these fish to captivity and the quickness of the behavioral responses, even for low doses.

Bioassays with Gymnotiformes may be useful as early warning systems on water quality, in real time. Although the response is not specific for a given contaminant type, the electric fishes consistently change the repetition rate of their discharges and/or its amplitude, when exposed to, yet, very low amounts of contaminants. This warning system may anticipate the decision making process and save time and funds. If necessary, more specific tests, but more time consuming and expensive, can bring definite responses about the specific type of contaminant present.

Concomitantly, the effects of neuroactive substances can be efficiently tested on the central and peripheral nervous system of gymnotiform fishes, as well as the resultant behaviors of the exposure to these drugs. Both the pulse type and wave type EOD fishes produce information that is easily registered as modulations in the firing pattern of the electric organ, which were shown to be equivalent to those presented by other vertebrates, including mammals. Thus, fish belonging to the order Gymnotiformes should be seriously considered as potential model for substances that eventually could be used as medicines.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

This work is supported by the Coordination of Improvement of Higher Education Personnel (CAPES), Ministry of Science, Technology and Innovation of Brazil, Grant PROAP/2014.

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