INVESTIGATION OF ELECTRIC FISHES

FINAL REPORT - PHASE I

by

Prepared under:

Contract

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SUMMARY

The African fresh water weakly electric fish Gnathonemus petersii has been investigated. The study has been directed toward the intradermal sensory system with emphasis on the electroreceptors. Three types of electroreceptors have been identified. The autorhymic activity of these electroreceptors has been recorded. The variation of the electric signal of the electric organ have been recorded for three specimens of Gnathonemus petersii as a rest activity and maximum signal rate when a metallic object has been placed near the fish. The number and density of different kinds of electroreceptors in the dermis have been counted and plotted against rate change and their sensitivity to a metallic object.

The preparation of the large tank experiments have been reported and the newly developed instrumentation is mentioned.

CONTENTS

FIGURES

1. INTRODUCTION

In our letter report of 24 July 1973 we mentioned our interest in the mormyrid electric fishes. One of our reasons is that they have an electrical quantifiable behavioral variable. The rate and amplitude of their electric signal changes when they are electrically stimulated or discontinuities appear in their electromagnetic field. In our study of their electroreceptors we found some mechanoreceptors on the chin having a close morphology to the Lorenzini ampulla, a multisensory receptor. This fact confirms our findings with respect to the Lorenzini ampulla functions from our previous research.

2. METHODS AND INSTRUMENTATION

Three specimens of Gnathonemus petersii (Fig. 1) a mormyrid fresh water, weakly electric fish from Africa, have been used in our experiments. The fishes were in our laboratory for three months and each one held separately in 15 gallon aquaria. The water pH was around 6.9 and the temperature was 24°C.

A lucite tray with stainless steel pick-up contacts half embedded in the lucite wall were used to restrain the fishes during experiments for assessing the limits of the repetition rate of their electric activity (Fig. 2).

The microelectrode amplifiers previously developed had a drawback, namely, when changes in the input resistance occurred. the offset potentiometer had to be reset. When very sensitive and delicate microelectrode recordings were made, resetting of the offset potentiometer was sometimes an impossible job owing to the diameter of the microelectrode of 0.5 microns. The microelectrode amplifier is also the direct support for the microelectrode proper. Unfortunately until recently nothing was available for the building of a microelectrode amplifier having all the necessary specs: high input impedance with low capacitance, a reasonable amplification factor, a low output impedance, very low noise factor and an insensitivity to change in input resistance from 50 ohms to 1 meghom. It also had to use little power and have a frequency range from DC to 50,000 repetition rate square wave or from DC to 1 MHz, with an amplification factor flat within maximum 3 dB.

We finally succeeded in building such an amplifier, which could mean a successful recording under most adverse conditions of small DC or AC

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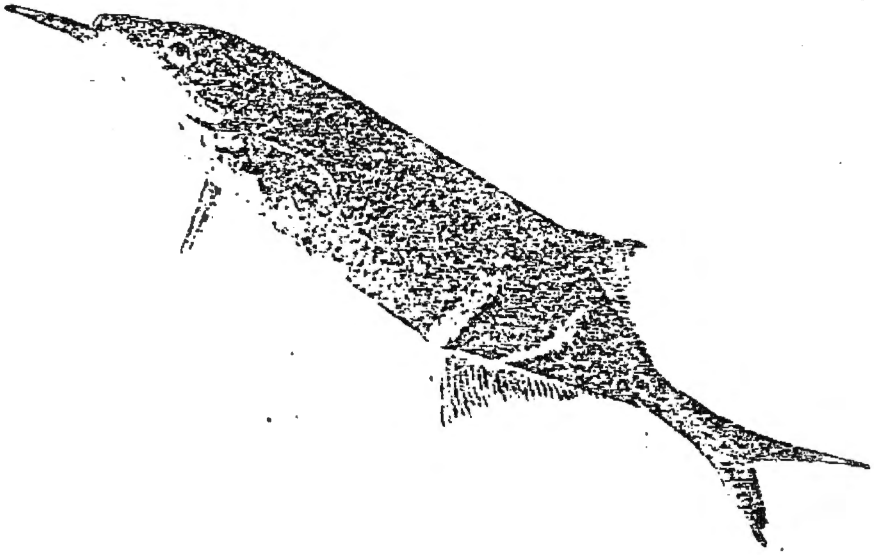

Fig. 1. African fresh water weakly electric fish Gnathonemus petersii.

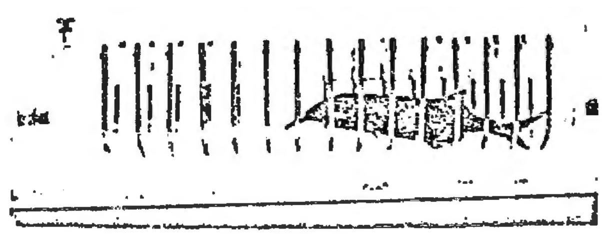

Fig. 2. Electric fish Gnathonemus petersii in a lucite restraining tray provided with stainless steel electrodes.

signals from the electroreceptors of electric fishes, and which does not need to reset the offset potentiometer. The amplifiers we used until now were the best which could be built, but they were far from the capabilities of the new microelectrode amplifier, which incidentally, could be used in our simulation of the electric fish capabilities because of its low noise and very large bandwidth, combined with an insensitivity to change in input impedance. Figure 3 shows the schematic of the new amplifier, and Figs. 4, 5 and 6, are actual photos of the amplifier.

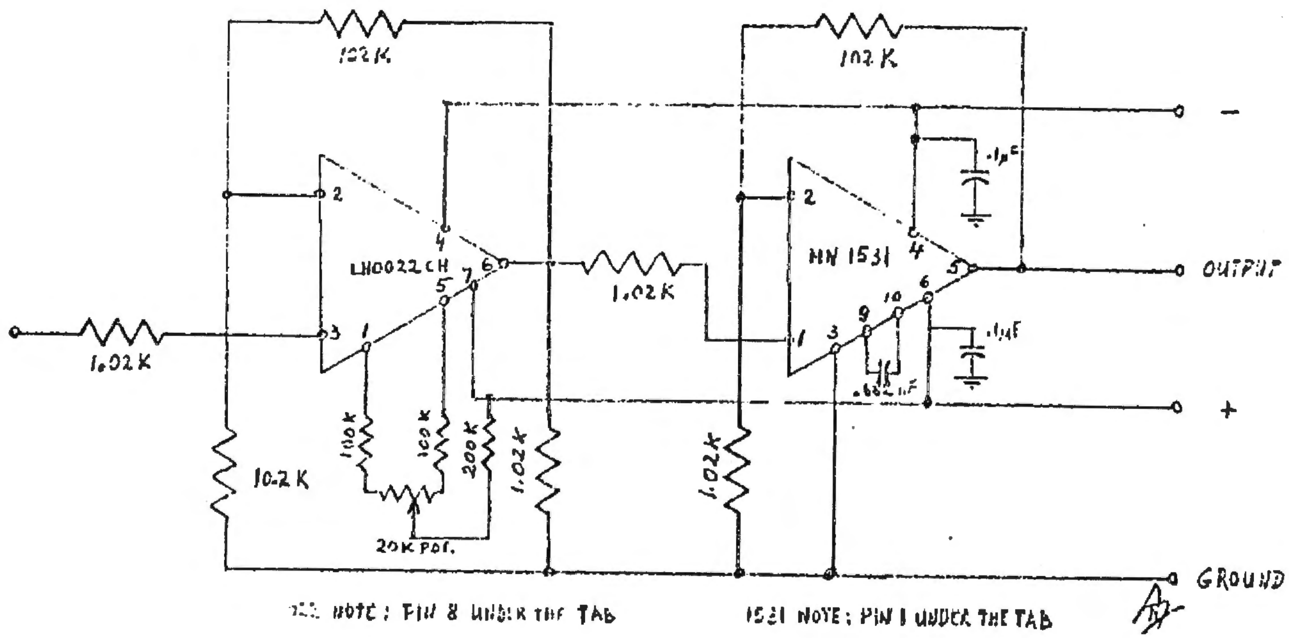

Fig.3 MICROELECTRODE AMPLIFIER

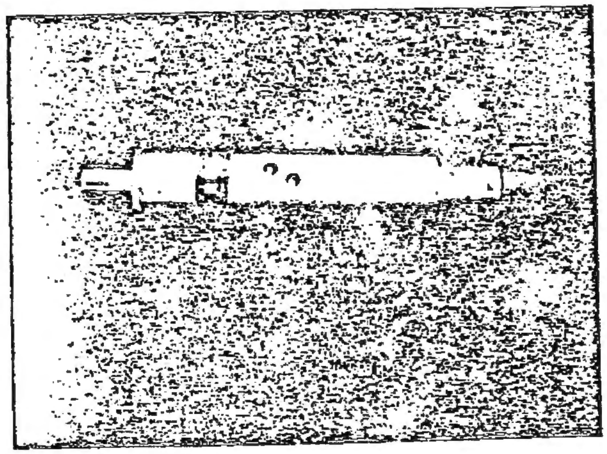

Fig. 4. Microelectrode amplifier and support in its shielding tube.

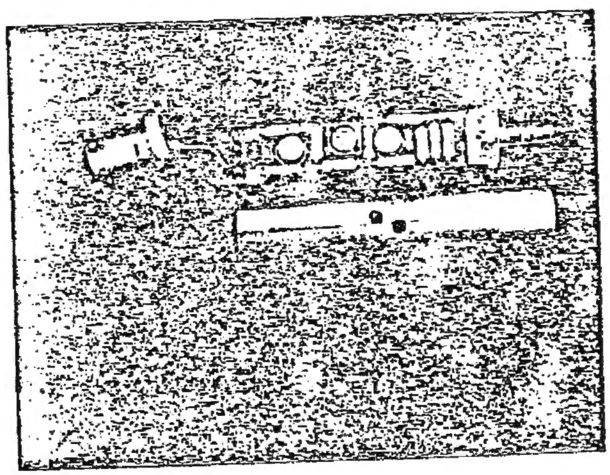

Fig. 5. Microelectrode amplifier ready to be put in the shielding tube, face side.

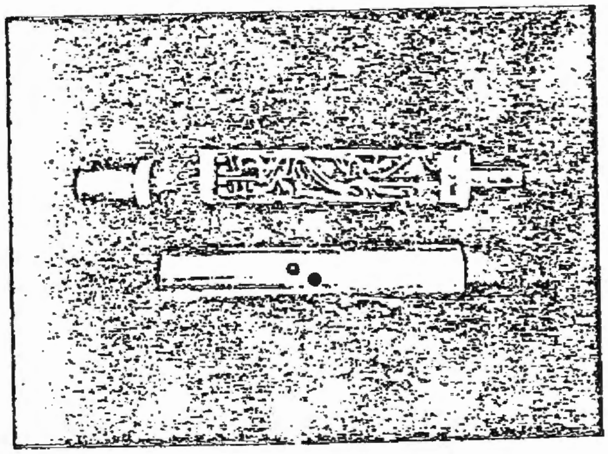

Fig. 6. Microelectrode amplifier ready to be put in the shielding tube, back side.

3. RESULTS

The specimens of the electric fish Gnathonemus petersii were put in the lucite tray, using their own aquarium water and air was provided through a special glass tube. The temperature of the water has been recorded. After a few minutes accommodation to their environment the electrodes corresponding to the head and tail of the fish, were connected to an amplifier, to the oscilloscope and to a counter. The rest activity has been read on the counter. Then a carbon steel rod (diam $\approx$ 3 mm) has been immersed in the tray in the proximity of the fish. The repetition rate increased significantly and a reading of the counter has been made. Figure 2 shows the fish in the tray. Table 1 shows the repetition rates of the signals. There is a ratio that could go to 1:23 (Fish No. 3) between the minimum and the maximum rate of the signal. Subsequent experiments could show how the repetition rate and amplitude of the signals are related to the size, composition and proximity of the objects

The microelectrode amplifier has been checked for its frequency and gain response using a Wavetek wavegenerator, attenuator and a Tektronix Oscilloscope type No. 555.

The upper trace of the photos shows the input waveform and lower trace shows the output waveform of the amplifier. Both sine waves and square waves have been used. Table 2 shows the waveform, amplitude, gain and input resistance. Six photos were made for 50 ohms input resistance and six photos were made for 1 megohm input resistance.

TABLE 1

Rest and Maximum Repetition Rate of the Electric Signal of Three Specimens of <u>Gnathonemus petersii</u>

Fish No.	Date of Recording	Weight of Fish in Grams	Signal: Rest Rep. Rate	Signal: Max. Rep. Rate	Signal: Amplitude mV	Water Temp. in ^{o}C	Instrumentation
1	8/12/73	15	15	136	500	20	Amplif.: 100x Oscil. Tek. 555 Frequ. Counter for all Recordings
3	8/11/73	22	7	161	500	20	
4	8/12/73	15	15	135	500	20	

TABLE 2

Microelectrode Amplifier: Input and Output Waveforms and Results

Photo No.	Sine ⎍	Sweep cm	Gain/cm In	Gain/cm Out	Input Res.
6	1K	2 msec	1 mV	1 V	50Ω
7	10K	.5 msec	1 mV	1 V	50Ω
8	100K		1 mV	1 V	50Ω

Photo No.	Square ⎍	Sweep cm	Gain/cm In	Gain/cm Out	Input Res.
9	1K	2 msec	1 mV	1 V	50Ω
10	10K	.5 msec	1 mV	1 V	50Ω
11	100K	.05 msec	1 mV	1 V	50Ω

Photo No.	Square ⎍	Sweep cm	Gain/cm In	Gain/cm Out	Input Res.
12	1K	2 msec	1 mV	1 V	1 meghom
13	10K	.5 msec	1 mV	1 V	1 meghom
14	50K	.1 msec	1 mV	1 V	1 meghom

Photo No.	Sine ⎍	Sweep cm	Gain/cm In	Gain/cm Out	Input Res.
15	1K	2 msec	1 mV	1 V	1 meghom
16	10K	.5 msec	1 mV	1 V	1 meghom
17	50K	.1 msec	1 mV	1 V	1 meghom

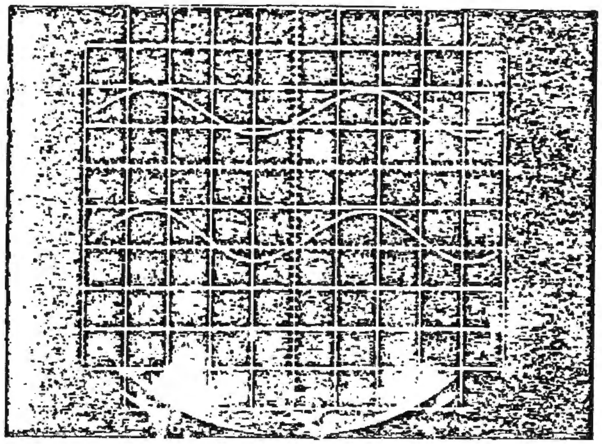

Fig. 7. Microelectrode amplifier input and output waveform and gain (see Table 2).

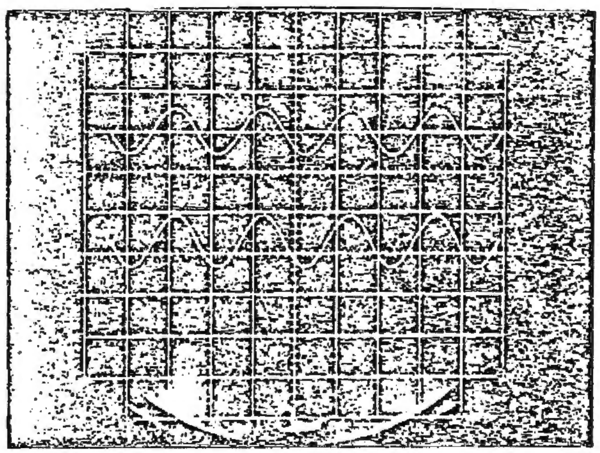

Fig. 8. Microelectrode amplifier input and output waveform and gain (see Table 2).

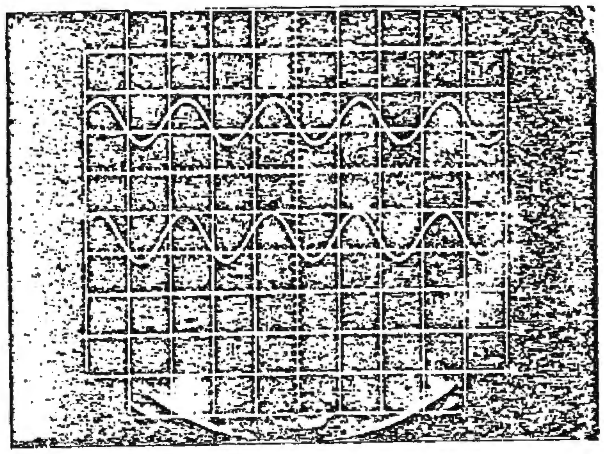

Fig. 9. Microelectrode amplifier input and output waveform and gain (see Table 2).

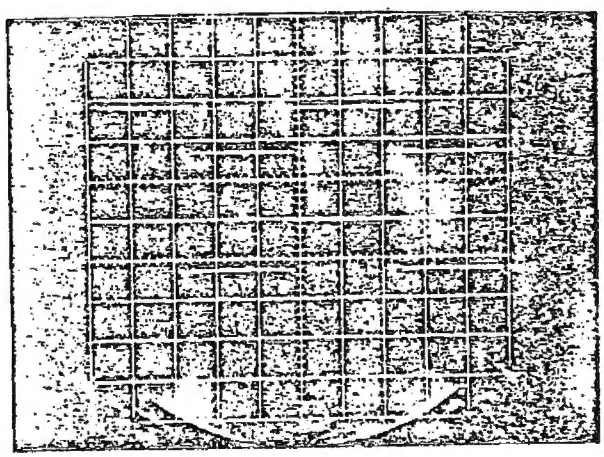

Fig. 10. Microelectrode amplifier imput and output waveform and gain (see Table 2).

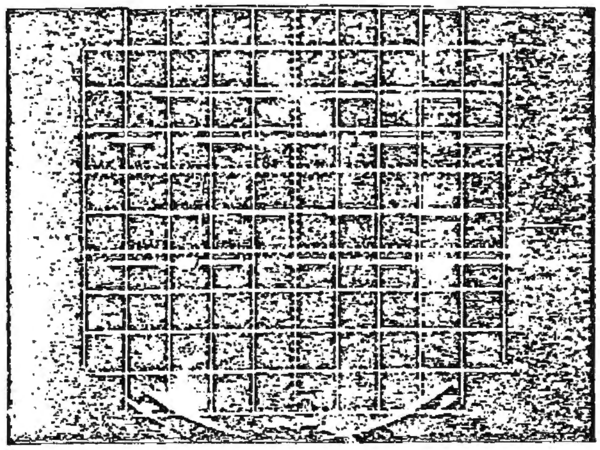

Fig. 11. Microelectrode amplifier input and output waveform and gain (see Table 2).

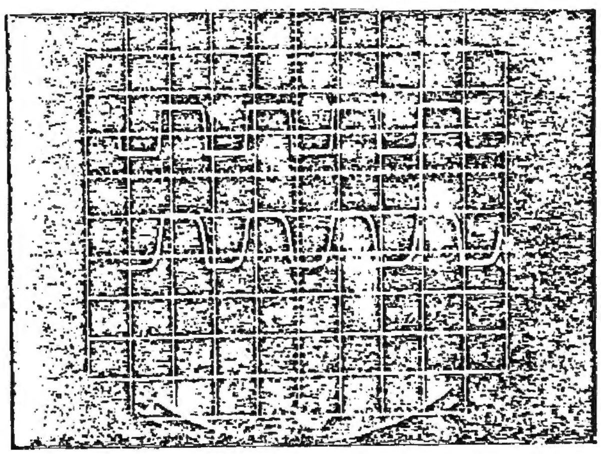

Fig. 12. Microelectrode amplifier input and output waveform and gain (see Table 2).

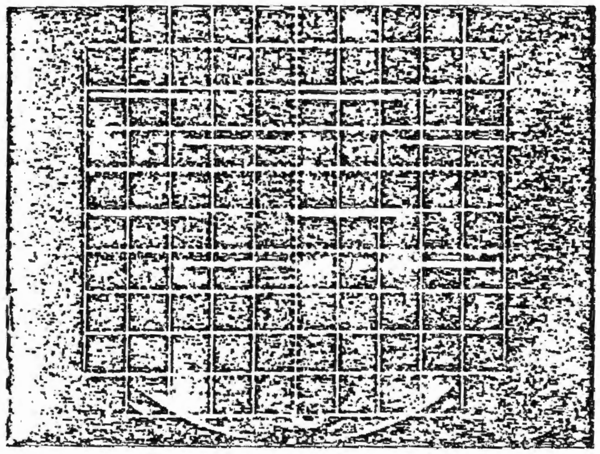

Fig. 13. Microelectrode amplifier input and output waveform and gain (see Table 2).

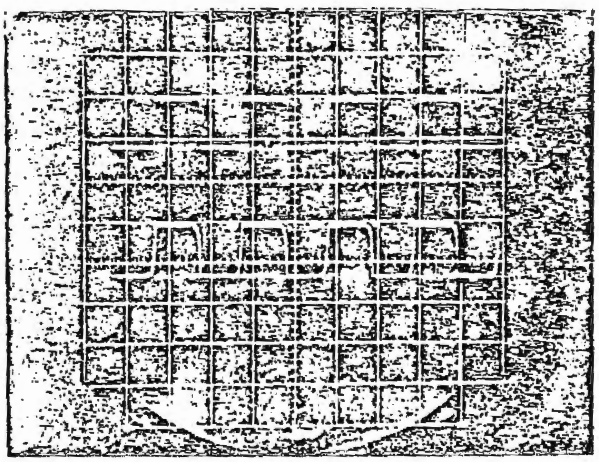

Fig. 14. Microelectrode amplifier input and output waveform and gain (see Table 2).

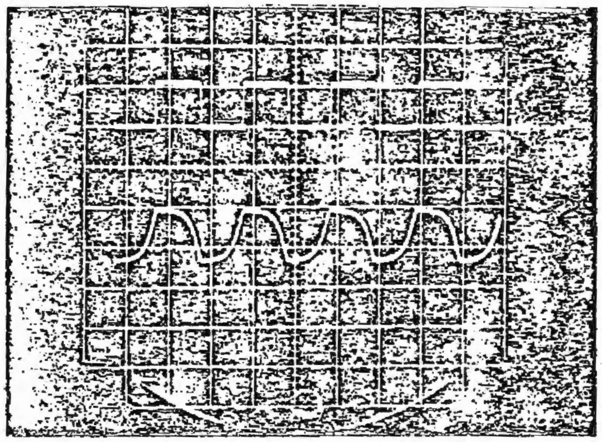

Fig. 15. Microelectrode amplifier input and output waveform and gain (see Table 2).

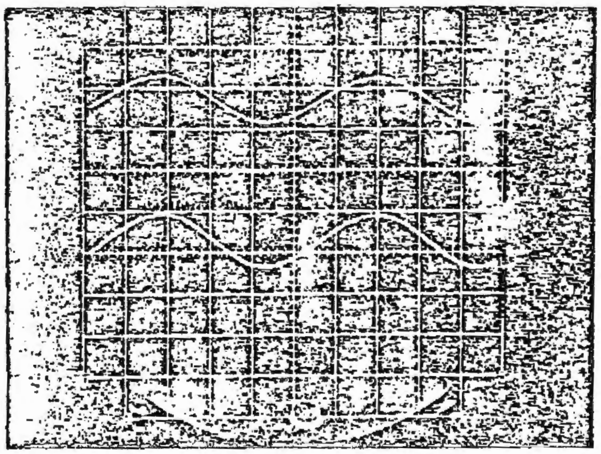

Fig. 16. Microelectrode amplifier input and output waveform and gain (see Table 2).

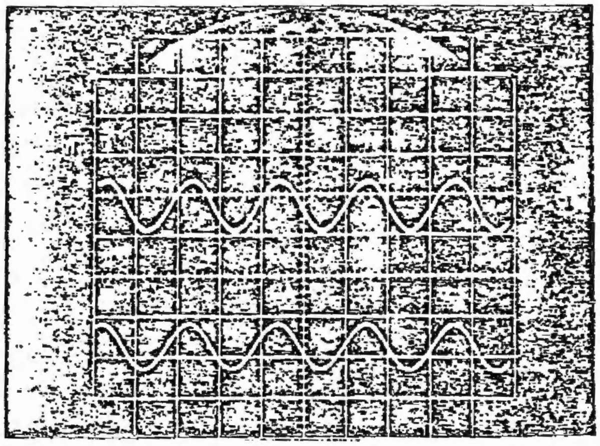

Fig. 17. Microelectrode amplifier input and output waveform and gain (see Table 2).

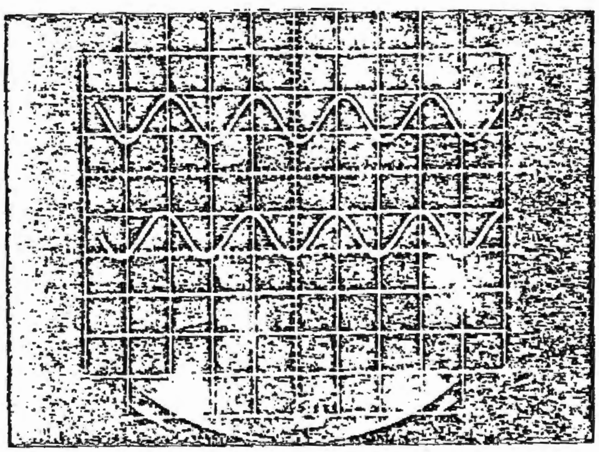

Fig. 18. Microelectrode amplifier input and output waveform and gain.

For electric receptors we found two types of mormyromasts (A and B) and one type of tuberous organ. They are confined to well defined areas of the epidermis. The epidermis of these regions has a particular structure, which is developed in the Gymnotoides in a similar way. Its essential components are columns of very thin, flat hexagonal cells 60 μm in diameter, invariable in all species and body sizes.

The height of the columns depends on location, but increases with body length. The mormyromasts are not covered by the hexagonal cells, but by small polyhedric cells which are arranged in a circular pattern.

The A-type mormyromasts possess an opening toward the surface and are evenly distributed with a relatively wide space between them. The B-type mormyromasts have no opening to the surface, and are more numerous than the A-type and are also evenly distributed.

The tuberous organs lack an open connection to the surface and form distinct patterns (Fig. 19). They can be classified according to the number of their giant sensory cells (1 to 10). All mormyromasts and tuberous organs are innervated by lateral line nerves. Only the tip of the chin with its Lorenzini ampullae is innervated by the Nerve trigeminus.

Each mormyromast is enclosed by a loop of capillaries. The common lateral line system has developed only along the trunk and the tail. In the head only deep laying canals exist, but without sensory cells.

The tuberous organs are characterized by an autorhytmic activity yielding a few mV, and with a high repetition frequency exceeding 1 kHz. The duration of the spikes are approximately 300 μsec. The transmitting electric organ of Gnathonemus petersii is located in the tailstalk, occupying 2/3's of it and represents approximate 12% of the total length of the fish.

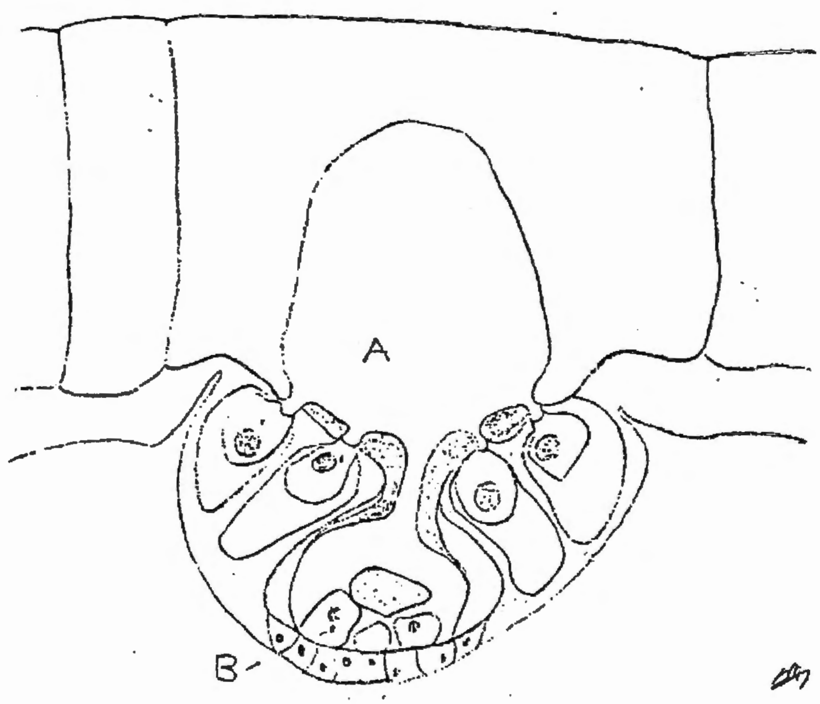

Fig. 19. Tuberous organ (electroreceptor) of Gnathonemus petersii.

The repetition rate of the impulses are influenced by light. In daylight the rest repetition rate is between 7 and 10, in the night it increases to 15-20. It will also increase considerably in the case of a stimulus affecting the fish.

The EMF of the fish with no load and out of water is between 7 and 17 volts depending on the particular specimen. The internal resistance is around a few kilo-ohms.

The electroreceptors sensory fields of Gnathonemus petersii can be clearly visualized if we put the fish in a solution of 10% buffered formaline. Figure 20 and 21 show the limits of these sensory fields.

There are between 700 and 1000 tuberous organ electroreceptors, between 800 and 1000 type A mormyromasts electroreceptors and between 2100 and 2300 type B mormyromasts electroreceptors in the skin of an adult Gnathonemus petersii. The total number of electroreceptors varies between 3600 and 4300. These are distributed on the body as follows: between 42 to 46% on the head on 41 to 44% of the electroreceptor fields; between 30 and 32% on the dorsal sides on 27 to 30% of the electroreceptor fields; and between 22 and 26% on the ventral sides on 25 to 32% of the electroreceptor fields. The total area of the electroreceptor fields may occupy between 2000 and 5000 mm^2 area for fishes between 90 and 125 mm length. Figure 22 shows the different types of mormyromast electroreceptors of Gnathonemus petersii.

With the exception of the sensory receptors of the chin which are mechanical displacement receptors and are connected to the CNS through the Nervus trigeminus, the mormyromast electroreceptors are subserved by the lateral line nerves. Figure 23 shows the main branches of the lateralis nerves system. All the mormyromasts types (tuberous, A and B) are connected to nerves forming bundles pertaining to the lateral line system and ending in the brain.

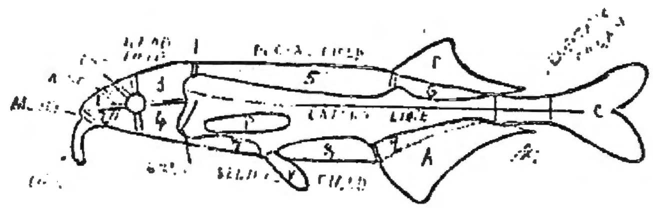

Fig. 20. The electric sensory fields of Gnathonemus petersii.

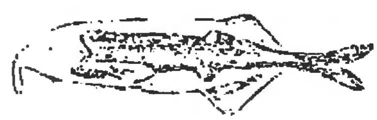

Fig. 21. Limits of the electroreceptors sensory fields of Gnathonemus petersii.

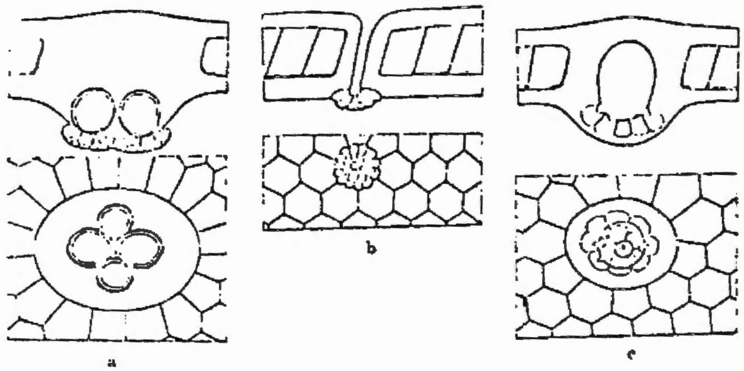

Fig. 22. Different types of mormyromasts:
a. tuberous organ
b. A-mormyromast
c. B-mormyromast
(top and cut view).

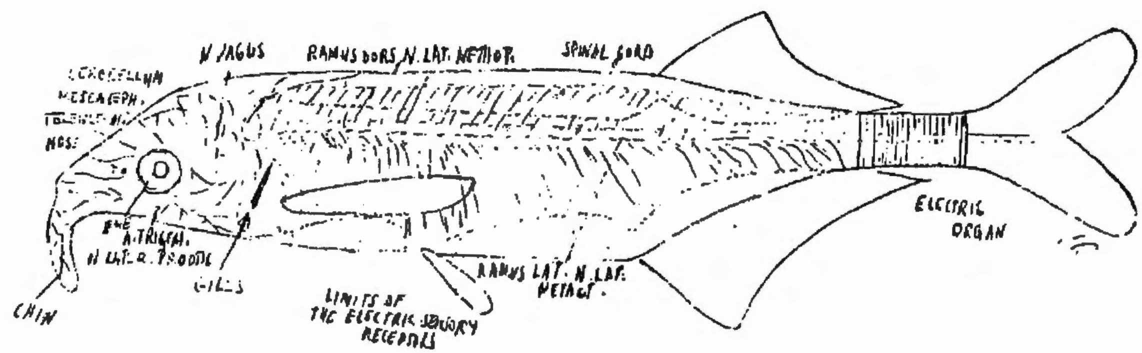

Fig. 23. The lateral line nerves of the electric fish Gnathonemus petersii.

The tuberous organ electroreceptors are autorythmic and the EMF may reach a few millivolts. The repetition rate varies from 550 to 3900 with the most often encountered repetition rate between 0.95 and 1.95 kHz.

Figure 24 shows a comparison between sensitivity and density of the electroreceptors in the epidermis of Gnathonemus petersii and Fig. 25 shows the autorhytmic activity of the electroreceptors near the chin and near the eye.

Figure 26 shows the autorhytmic activity of the mechanical displacement sensory organs as an effect of bending the proboscis of the chin.

Experiments in this direction would be continued to record waveform and changes in repetition rate as a result of different stimuli.

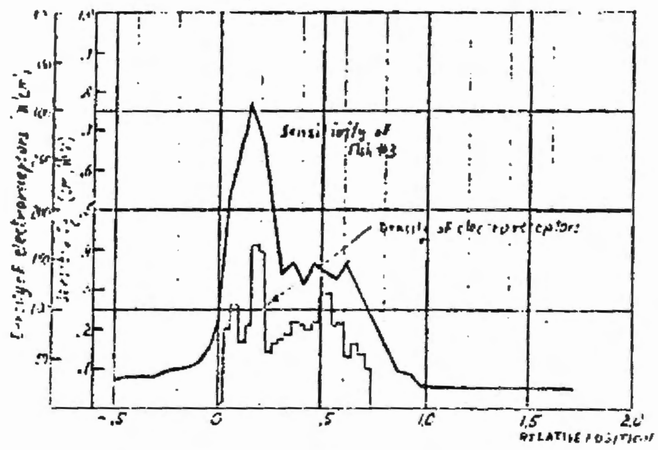

Fig. 24. Comparison between sensitivity and density of the electroreceptors of Gnathonemus petersii in the epidermis.

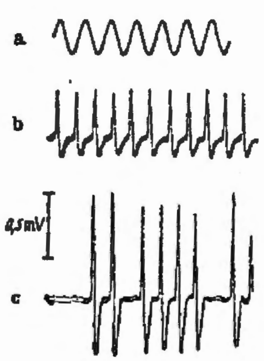

Fig. 25. Autorhytmic activity of the electroreceptors of Gnathonemus petersii:

a. 500 Hz calibration signal
b. electroreceptors near the chin
c. electroreceptors near the eye

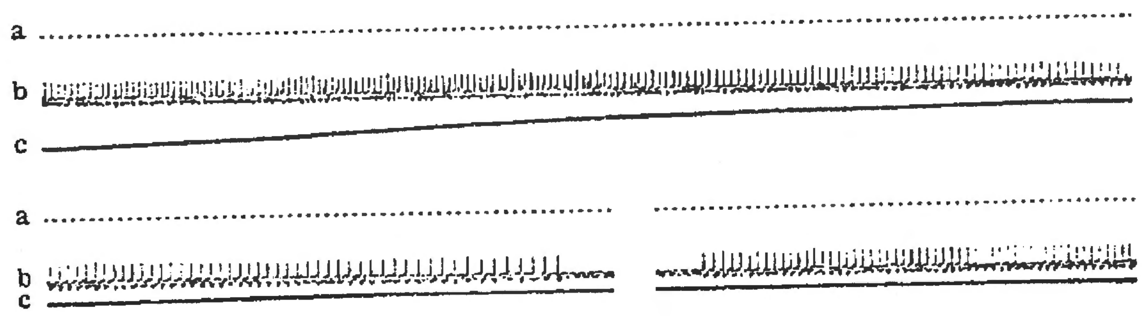

Fig. 26. Electric activity from the nervus lateral anterior innervating receptor near the proboscis of a mechanical displacement on the chin of <u>Gnathonemus petersii</u> when the proboscis has been moved upwards.

a. time marks = 50 Hz

b. electric activity in the nerve

c. movement of the chin proboscis

4. LARGE WATER TANK PREPARATION FOR EXPERIMENTS

The 12 ft. diameter, 4 ft high water tank has been prepared for the other experiments that will follow for the Phase II investigation (Fig. 26).

Heating the water is done with 2 x 1000 watts heaters controlled by an "YSI" temperature controller to ± .1°C and is normally held at 25°C. The heaters are in a separate 30 gallon tank and are connected to a relay switching them on and off and controlled by the temperature controller. Two 9 gallon per minute pumps are pumping in and out the water from the 30 gallon tank from and into the large tank (Fig. 27).

Rails with nylon strings are provided for the silver-silver chloride-platinized-silver-chlorized electrodes which can be moved from one end to the other end of the tank (Fig. 28). The electrodes are connected to a remote controlled differential amplifier (ampl. fact. x 4200) suspended over the tank and from the amplifier to the differential oscilloscope Tektronix type 555 (Figs. 29, 30 and 31).

An electric fish can be suspended in one of the restraining devices shown in Fig. 32. The fish restraining devices are provided with stainless steel end electrodes which are connected to an audio-amplifier (ampl. fact. = 300) and to the oscilloscope and displayed on a second beam.

Our preliminary experiments show good promise for recording the changes in the field of electric fishes produced by them as a result of stimuli they are presented with.

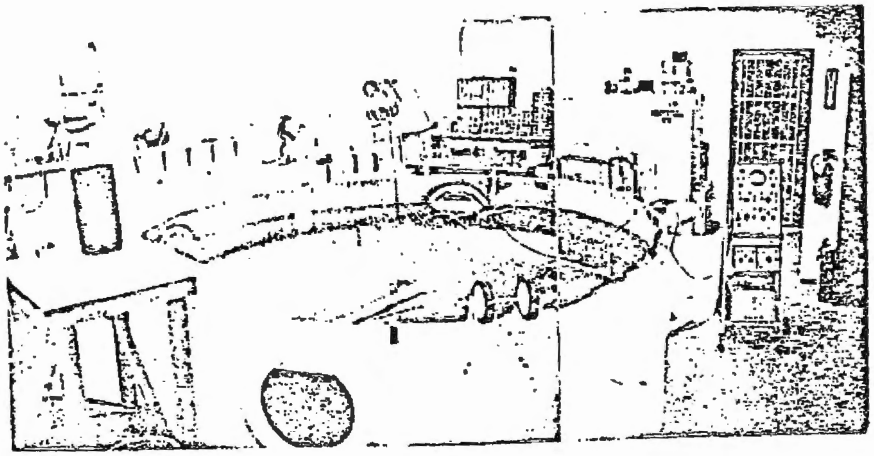

Fig. 27. 12 ft. diameter fiberglass tank provided with heating, filtering, countercurrent aeration and double rails for electrode support.

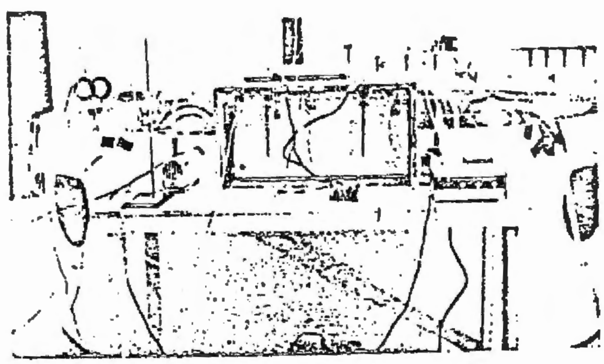

Fig. 28. Heating tank provided with automatic control of temperature to 0.01^{o}C.

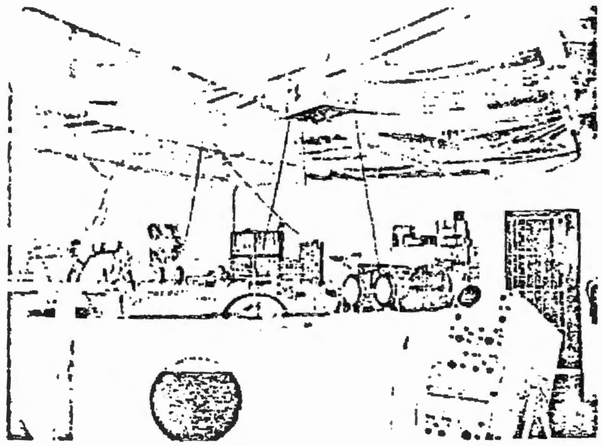

Fig. 29. Differential amplifier hanging over the water tank.

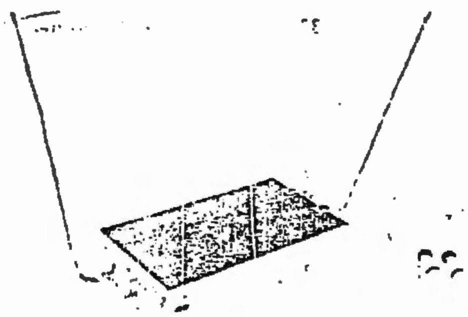

Fig. 30. Close look at the differential amplifier used in conjunction with the electrodes in the water tank to record electric activity of electric fishes.

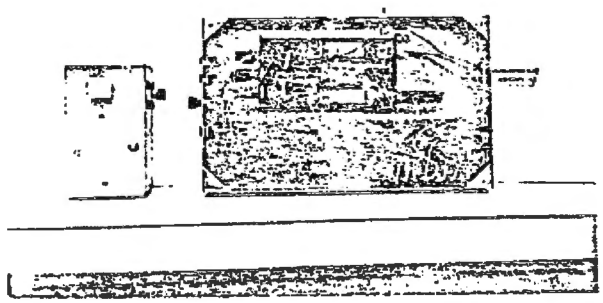

Fig. 31. Differential amplifier with remote control.
Ampl. factor = x 4000, noise = 1 microvolt.

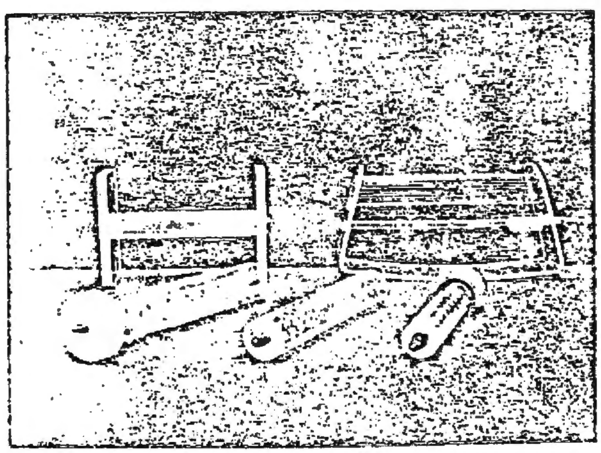

Fig. 32. Devices for restraining electric fishes in the water tank.