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THE SPONTANEOUS AND EVOKED ACTIVITY OF THE  
PRIMARY NEURONS OF STATORECEPTORS (AND OTHER  
RECEPTORS) OF THE LABYRINTH OF THE BULLFROG  
BEFORE, DURING AND AFTER AN (Pittsburgh

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FINAL SCIENTIFIC REPORT

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AND EVOKED ACTIVITY OF THE PRIMARY NEURONS  
OF STATORECEPTORS (AND OTHER RECEPTORS) OF  
THE LABYRINTH OF THE BULLFROG BEFORE,  
DURING AND AFTER AN EXTENDED PERIOD OF  
WEIGHTLESSNESS, INCLUDING ALTERNATIVE  
INTERVALS OF ARTIFICIAL GRAVITY

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## Table of Contents

Introduction	1
Progress since last report	
1.) Technical	1-2
Blockage of Nerve Conduction by Cooling	2
Experimental Work	
Test #1	3-4
Test #2	4-5
Bacterial Flora Study	5-6-7
Observation, Problems and Consequent Projected Corrective Activity	7-8-9-10-11
Progress since last report	
2.) Near Milestone: 45-60 Days Bull Frog Preparation Experiment Protocol	11-12
Explanation of Figures:	
Figure 2	13
Figure 3	13-14
Figure 4	14-15
Figure 5	15
Figure 6	15
Figures 2 thru 7	16-21

## INTRODUCTION:

The work covered by the grant had the following objectives -

- 1.) to create the laboratory facilities to support the project, "Vestibular Receptors in Space"
- 2.) to further assess a viable underwater, fully instrumented frog preparation, reaching a maximum duration of 60 days
- 3.) to develop appropriate facilities for characterizing the vestibular units, studying a) the activity at rest, b) the static and c) the dynamic properties of the same unit; different types of receptors are investigated
- 4.) to further apply and develop the automatic data acquisition and reduction; both on line and from recorded data

## A. Progress since last report

## 1.) Technical -

All the general laboratory facilities and services as described in the previous semi-annual report are installed and operational. In some specific areas work is still in progress:

- a) Aquaria - the original shielded aquarium brought in from Italy is functioning and experiments are carried out using it (see later). Two more identical aquaria have been built, tested and sent to Ames for degassing. The aquaria should return shortly.

Of the necessary equipment, a new  $PO_2$  probe (General Electric) has been tested, found satisfactory and applied to the 3 aquaria.

Three thermistors for measuring the temperature and six flow meters (1 L/min and 20 L/min) must still be procured. A master system to maintain the temperature constant has been installed. It consists of a water mixer and a sensor: chilled and hot water are mixed in the proper ratio in order to maintain the required  $62^{\circ}F$ . ( $17^{\circ}C$ ). Three new water pumps, specially built so that no metal is in contact with the water, have been acquired and are operational.

All the interfaces with the computer and the 3 aquaria (for the experiment control and data acquisition) are functional.

All amplifiers and preamplifiers for instrumenting the frogs in the additional 2 aquaria are still to be obtained.

- b) Field Mapper - the field mapper is fully operational; however, (1) the antivibratory base necessary to eliminate the external vibration is still under construction. It should be installed in the next 3-4 weeks. (2) no experiment is possible yet, as the FOEP (the frog life support system to be used with the field mapper) is still at Ames. The FOEP should be available in the next 3-4 weeks.

- c) Data acquisition - the data acquisition system is operational; however, the spike shape discriminator is, at present, operated manually and has to be integrated with the computer.
- d) Computer - the computer system is complete. The programs for the long term experiment control are finished and functioning. More work is still required for data acquisition and analysis, and for debugging.

#### Blockage of Nerve Conduction by Cooling

An experimental set up has been completed to determine the feasibility of interrupting nerve conduction by cooling. The experiment (figure 1) consists of stimulating one end of the nerve in place and recording the evoked response at the other end. In the middle a cooling element is applied to the nerve. It is a device of silver (for good heat conduction) with a hook on which the nerve lies. Through the element a coolant fluid flows. The temperature changes are recorded via microtermistors (100 $\mu$   $\emptyset$ ) on an eight channel brush recorder, together with the nerve response. The termistors are distributed as follows:

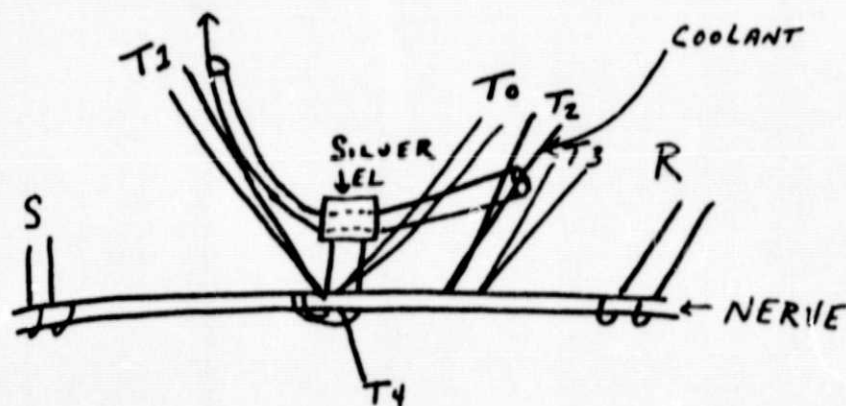


Figure 1

T1, T0, T2 and T3 are enclosed in the tip of a micropipette, while T4 is attached to the hook of the cooling element, immediately below the nerve.

T0 is on the upper surface of the nerve. T1 is affixed in the center of the nerve bundle. T2 and T3 are placed on the upper surface of the nerve at variable distances from the cooled section. The coolant element providing the nerve silver hook contact is 1 mm thick.

S = Stimulation      R = Recording

With this technique the temperature will be determined at the point at which the nerve conduction stops. The diffusion of the cooling effect, both along the nerve and in depth, will also be measured. Recovery time is assessed. The nerve is then studied histologically and ultrastructurally for possible permanent injuries.

## Experimental Work

Two partial tests have been completed on the extended bull frog preparation. The objective and the description of the tests follows:

### Test #1:

Introduction - The purpose of this test was to determine the survival capacity of the bull frog preparation within the frame of the new experimental set up, including 3 aquaria (the old one from Milan and 2 new ones built in Pittsburgh). Also, the capability of previously well fed bull frogs to withstand food deprivation and for how long, has been studied. This was carried out to assess the need of feeding the animals the artificial diet. Originally no microelectrode implant was contemplated; however, as the survival of the group of frogs in the old aquarium exceeded 31 days, after that period the frogs were instrumented with one microelectrode each and observed until death, 12 days later.

### Description:

#### 1) day: 210-217

Twelve frogs were chosen from the holding tanks, where they were kept for more than 2 months, and where they were fed living tadpoles and liver. The frogs were treated with 5 mgs of tetracycline for 3 days before surgery. The 12 frogs were paralyzed and the VIII nerves exposed. During this period and thereafter, the frogs were placed in groups of 4 in the 3 aquaria and observed day by day.

#### 2) day: 217-224

During this period all frogs in the 2 new aquaria developed red skin patches and ulcerations, and eventually died. The red patches always developed on the skin in contact with the aquaria walls. Similar observations were made in previous experiments. It was due to residual solvents in the new plexiglass material and in the glue used to assemble the aquaria. The problem was then solved by degassing the aquarium itself. In fact, the groups of frogs in the old aquarium (previously degassed) appeared to be in good health. Consequently, the two new aquaria were sent to Ames for degassing.

#### 3) day: 224-244

The group of 4 frogs in the remaining aquarium continued to be in good health, with the exception of lesions on the side of the head where the two prongs of the head holder were pressing on the skin.

#### 4) day: 244-245

As the frog preparations seem to progress well, it was decided to implant one microelectrode for each frog until the end of the experiment, plus EKG electrodes.

5) day: 245-256

During this time periodic recordings were made from the 4 frogs, until the death of the animals which took place during the last 2 days at various intervals.

The entire experiment was carried out under controlled environmental conditions (see description of a typical test later).

#### Results:

The results of this test will be discussed, together with the results of the second test.

#### Test #2:

Introduction - The purpose of this test was to experiment the effect of feeding the animal with the artificial diet, and also to record, via implanted microelectrodes, the activity of single vestibular units since the beginning of the test.

#### Description:

Two groups of 4 frogs each have been used. The first group was fed the artificial diet, slightly modified as follows:

Dosage given 3x weekly  
Daily diet/300 gm frog

1 mg each of:

L-isoleucine  
L-leucine  
L-Lysine  
L-valine  
L-methionine  
L-phenylalanine  
L-threonine  
L-tryptophane

5 mg of:

L-alanine

.48 g maltose in 6.5 ml  
distilled water

2 ml ringers  
(formula)

Gms/Liter

NaCl	6.5
K Cl	0.14
CaCl	0.12
NaHCO <sub>3</sub>	0.20
NaH <sub>2</sub> PO <sub>4</sub>	0.01

B<sub>1</sub><sup>1</sup> - .02 mg

B<sub>6</sub> - .03 mg

B<sub>12</sub> - .03 mg

B<sup>1</sup> - .2 mg

Pantothenic - .03 mg

Nicotinic - .01 mg

Calcium Chloride - 2.2 mg

Magnesium Sulfate - 1.1 mg

Vitamin C - .8 mg

Vitamin D<sup>2</sup> - .075 mg )

Vitamin K - .10 mg )

Vitamin A - .06 mg )

given monthly



This group was chosen from the frogs kept in the holding aquaria for a month and fed with living tadpoles and liver. The artificial diet feeding was started day 262, and was continued until day 297, when Test #3 began using the same group of frogs.

The second group of 4 frogs were fed the usual live diet. Then the test started according to the following routine:

1) day 260

The four frogs were paralyzed and the feeding tube and the EKG electrodes were inserted. The frogs were placed in the controlled environment aquarium (temperature 62°F., PO<sub>2</sub> 710 mm Hg water flow free 1 l/min, close circuit 30 minutes in the hr 20 l/min).

2) day 261-263

The vestibular nerve was exposed.

3) day 264

One microelectrode was implanted in one VIII nerve of the 4 frogs.

4) day 267

The periodic recording started.

5) day 289

One of the 4 frogs died. Upon inspection, a deep lesion, reaching the body cavity, was discovered at the sides of the head where the two prongs of the head holder compressed the skin. Consequently, the test was discontinued and the other frogs examined. All were affected by the same injury described above. Obviously a new way of holding the head had to be studied. Moreover, symptoms of red leg lesions had appeared in the frog since 4-5 days. This phenomenon was attributed to a large concentration of pathogenic bacteria in the water, as shown by the study of the bacterial flora (see next chapter).

#### Bacterial Flora Study

During this test periodic samplings of the water in the aquaria containing the frogs were taken and the bacteria content studied. The following results were obtained:

Date

1) 257	Sterile	no frogs
2) 262	"	frogs placed in aquaria: <u>260</u> day
3) 266	"	
4) 269	"	
5) 276	flavobacterium	2x10 <sup>3</sup> pseudomonas 10 <sup>3</sup>
6) 284	heavy flavobacterium	
7) 288	pseudomonas	10 <sup>3</sup> 2 types
8) 289	pseudomonas	10 <sup>3</sup> 2 types; enterobacterium 10 <sup>3</sup> ; gram negative rod 103. Frog died

As shown, a heavy build up of pathogenic bacteria starts after 19 days. Particularly dangerous are the 2 types of pseudomonas known as the most common cause of red leg lesions. The lesions in the frog's body facilitated the infection and the consequent death of the animal.

#### Results:

During the 2 tests 8 channels of vestibular spike data were recorded, respectively, for 11 days in the first test, and 24 days in the second test. The time limit was due to the deterioration of the frog's health, as the spike recording terminated a few hours before the death of the animal. However, in the first test, the survival time of the frog in the aquaria was 41 days (without feeding), and in the second, 29 days. The death of the animal was due to severe lesions and to the pseudomonas infection (documented in the second test only).

Of the 8 channels of vestibular spike train data, failure of maintaining the signal was observed in 2 cases during the 24 days test, when the electrodes became noisy. In this case, recording of spike train data lasted respectively, 6 days and 9 days.

The general feature of the spike activity was similar in all cases. (Fig 2 and Fig 3)

As shown, normally the original spike potential remained constant throughout the experiment. Occasionally an additional, possibly larger, spike might appear at a certain time during the experiment (Fig 3, 1-2), and then remain in the record until the end. More often, the activity of 2 or even 3 different units, with well recognizable spikes, were recorded in each channel (Fig 2). (Compare 23-29 with 28 where the activity of 2 different units was recorded in the same channel); Fig 3, 18; Fig 4, all records

The 3 spike train data in the channel were easily separated for analysis by means of the spike shape discriminator. Occasional noise could be easily detected, even recognized, in the record by means of the minimum interval method (Fig 4, 1). This method also demonstrated the presence of a single unit activity during analysis (Fig 2).

In the environmental condition of the experiments their activity at rest could not be recorded. Vibrations induced by walking, the operation of the water cooler and especially the close circuit water pump constituted a large stimulus for all units, with the exception of the semicircular canal units (Fig 3, 3-7-19; Fig 4-1).

On the other end interesting results were obtained because of the positive input determined by the environment. As shown in Fig 4, the close circuit water pump induces a powerful stimulation: adaptation is evident in a 30 minutes period (Fig 4-2), with a decreasing firing rate as a response to pump induced vibration. After the water pump is stopped it takes 15 to 20 minutes for the unit to be restored to the sensitivity at rest (Fig 4-3).

All statoreceptors appeared to be phase locked to the vibration profile (Fig 2-22-24-25-26-27-50-51-52). The most common result, when 2 or 3 units were recorded in the same channel, was that the units were unimodal, responding not only to the same vibration, but with very similar phase locked response. Sometimes, however, the activity of a statoreceptor responding to the vibration was recorded together with a semicircular canal not responding to the same vibration (Fig 3, Fig 4).

The so called noise is really mostly activity of distant fibers (Fig 3, compare 13 with 14). When stimulated by vibration it becomes much larger (Fig 3, compare 12 with 13).

Sometimes, apparently similar spikes actually belong to different units, as shown by the minimum interval method (Fig 2, from 8 to 14).

During the very high vibratory stimulus (30-100/sec up to 2g) no mechanical artifacts appear in the electrode pick up (Fig 2-3-4). As mentioned, the increased noise is really increased nerve activity following the high stimulation. Even in this case, however, the studied spike can be easily separated from the biological noise.

#### Observation, Problems and Consequent Projected Corrective Activity

From the above partial test the following observation can be made:

- 1) The frog preparation can withstand a long period of starvation, on the order of at least 30-40 days, without impairment of the vestibular activity. It is, however, more convenient to assure proper feeding. The tests performed so far indicate the artificial diet used is perfectly adequate in maintaining the frog's good health. Observation of the capillary circulation during surgery has shown that the blood and circulatory conditions are excellent in animals fed the diet for several months.
- 2) The close circuit water pump is the origin of a powerful stimulation. As a result, not only maximum activity is present for a large number of vestibular units, but a change of sensitivity and gain appears as a consequence of the high stimulus, lasting several minutes. It is, therefore, necessary to limit the water pump cycle to the minimum, if the condition of 0 input are to be studied, as in orbit. It has been found that 30 minutes every 24 hours of pump activity are more than enough to cleanse dead skin from the frog body. The current test (#3) intends to determine the absolute minimum of pump operation necessary for the good health of the frog. The results will determine the pump runs during the orbital experiment.
- 3) It is necessary to control the bacteria growth, and especially to eliminate the pathogenic types, especially the pseudomonas.

As suggested by the animal microbiologists consulted, in the third test, now in progress, an attempt is being made to control the bacteria growth a) by periodically adding tetracycline in the frog diet, b) by eliminating body lesions as much as possible, c) by lowering the pH of the water in the aquaria to 4 pH, adding the appropriate amount of HCl in order to destroy the pseudomonas and the flavobacterium.

- 5) The present head holder is responsible, in the long run, for injuries. It has been modified so that the frog head is suspended in the center of the holder by three stainless steel stitches in such a way that no part of the frog skin is compressed. This system is under test now, and after two weeks, no injury is present.

6) The two failures in the recording of the train spike data have shown that the technique, originally developed for the OFO-A flight experiment (to last only approximately 7 days), is not well suited for a 45-60 days preparation. Failures are mostly due to the following factors:

- a) Material: the commercially available enameled platinum wire connecting the amplifier to the microelectrode show often discontinuity in the coatings (see Fig 5A) and the isolation is not attached on the metal, but moves freely. While this fact allows a better flexibility (Fig 5C), it also occasionally breaks the sealings of the isolation coat to the microelectrode, exposing the bare metal and producing leakage.
- b) Isolation and waterproofing: the preamplifier waterproof coating develops occasional leaks, especially at the junction of the power and output wires.

(b-1) the input junction between the microelectrode and the preamplifier is, at present, waterproofed by encasing the live junction in parafin. After a period of 10-20 days in HPO water, sometimes the parafin develops cracks and water seeps in.

The microelectrode coating undergoes changes when immersed for several days in frog ringer, equivalent to the situation when implanted in the frog nerve and agar. The impedance decreases (which might explain the sudden recording of additional spike after some days of implant), indicating, perhaps the penetration of moisture across the thin layer of insulation near the tip of the electrode (Fig 6). The junction of the platinum wire with the tungsten is made through conductive epoxy. It becomes noisy occasionally (a high resistance contact). It is sought to use spot welding of the wires if possible.

In order to eliminate such failure elements, a continuous, pin hole free coating of a waterproof, highly dielectric insulator is necessary. This has to include the preamplifier-microelectrode junction.

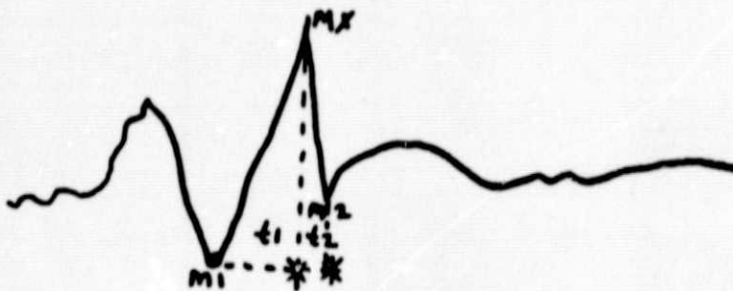
Recently, a new high performance, stable microelectrode insulator was developed and tested: Parylene<sup>1</sup>. Consequently, 10 miniaturized systems (preamplifier and microelectrode) have been ordered from Eltec Instruments, Inc. The completed preamplifier-microelectrode assembly will be coated with Parylene in a continuous layer. The 10 assemblies will be tested in about 2-4 weeks. If the test is positive the following routine will be performed:

- a) The microelectrode emitter follower system will be implanted once, and after use the preamplifier will be recovered.
- b) The recovered amplifiers will be sent back to the company to be fitted with a new microelectrode and Parylene coated.
- c) The system will be reused.

1. IEEE Transactions on Biomedical Engineering, Vol. BME-24, No. 2 March, 1977, Parylene as a Chronically Stable, Reproducible Microelectrode Insulator; Gerald E. Loeb, M.J. Bak, M. Salzman and E.M. Schmidt

- 7) Triaxial Accelerometer: The high sensitivity of the frog's vestibular receptors to all kinds of acceleratory input requires a good appraisal of the accelerations effectively reaching the frog head in any given environment. The present tests have shown how the activity, the sensitivity and the gain of the vestibular receptors are the results of the previous time history. Moreover, the impossibility of fixing the frog head with an absolute absence of any shift in order to avoid injuries induced by rigid frames compressing the skin for long periods will allow some variations of the vestibular input as a result of even limited movement relative to the applied stimulus vector. As a proper transfer function requires the knowledge of the real input to the vestibular system, it is necessary to measure accelerations directly on the head bone structure by means of a triaxial accelerometer attached to the bone itself. Tests have already shown that this can be done without difficulty by fixing a minaturized container to the head by means of screws and bolts passing through the nostrils with or without additional cementing of the container to the bone.
- 8) The large amount of data recorded, even during these 2 limited tests, makes it imperative that on line analysis of data be prepared during the tests. To do this a computerized spike discriminator is necessary. It will also solve the problem of data telemetry during the orbital flight.

The spike discriminator works on the principle that a mask of the spike to be analyzed has to be preset on the apparatus. The mask is made up by the definition of the voltage levels and time difference between minima and maximum of the spike. These values are then preset by means of potentiometers, and an output is obtained each time a signal is present where minimum to maximum amplitudes and time are within the preset range.



M1 and M2=spike minima  
 MX=spike maximum  
 t1=M1 to MX time lapse  
 t2=MX to M2 time lapse  
 $V1=(V_{MX}-V_{M1})$   $V2=(V_{MX}-V_{M2})$   
 t1, t2, V2= the respective  
 sample are set with potentiometer.

The choice of the four parameters (t1, t2, V1 and V2), however, is a time consuming job involving the play back and forth of the tape recorder a number of times, according to how many units are being discriminated, and is done just looking on the oscilloscope. This implies, besides the time involved, a certain degree of inaccuracy in borderline cases. A way to minimize this is to interface the unit with the computer and utilize the power of the computer to make a more objective choice of parameters.



Data below a certain level would be filtered from this system by the present apparatus, thus allowing the computer to respond to spike data only. The buffering allowed would permit the computer to handle multiple electrodes at a time, even when burst activity occurred. The length of the FI/FO buffer would determine the length of burst which could be handled.

The level at which data is to be considered noise should be digitally controlled - thumb wheel switches which could be replaced by the computer. This would allow the maximum amount of data for each spike to be captured at one pass. The computer in an off-line mode could then determine from which neuron a given spike was derived, without multiple playings of the analog tape.

- 9) Increasing the signal to noise ratio: At present the signal to noise ratio is limited by the input capacity, mostly due to the long enameled platinum wire (3/4 inch) necessary to avoid spring effects on the microelectrode. If a less elastic wire can be used and the length decreased (and/or the isolation coating increased) the input capacity can be minimized, and the signal to noise ratio correspondingly increased. A technical investigation into this problem is necessary. It could be performed at Ames, in house, or by the contractor making the microelectrode-emitter follower assemblies. As both solutions are possible, the determining factor could be the cost.
- 10) Glands and wires: Following the 14 frog test, at least 14 glands, wired for EKG recording, are necessary - at least 5 more spares are advisable.

Similarly, the emitter follower output and power wires are to be connected to a waterproof gland for attachment to the main amplifiers. The wired glands should be standardized to allow each frog preparation to be tested

- a) in the holding aquaria
- b) in the FOEP, for receptive field mapping
- c) in the positioning frog holder for the dynamic properties study

2.) Near Milestone: 45-60 Days Bull Frog Preparation Experiment Protocol; Including Field Mapping and the Study of the Dynamic Properties of the Vestibular Units.

(This protocol is only a sample, and in no way restricts the research work against changes, even fundamental or completely different approaches if the development of the research so requires. Moreover, it is only a fraction of the total research proposed.)

Introduction: The test, typically is carried out on a group of 10 frogs. While it is obvious that to achieve statistically valid results the test should include the largest possible number of biological preparations, it is felt that the 10 frog group is sufficient for the purpose. The limit to 10 animals is due to the available equipment. In effect, each frog provides a maximum of 6 channels of information; namely:

1. Vestibular unit 1
2. Vestibular unit 2
3. EKG
4. X axis acceleration
5. Y axis acceleration
6. Z axis acceleration

The recording of the signals is made through a 64 input multiplexer (Fig 7). Ten frogs will provide 60 channels of information to be connected to 60 multiplexer inputs. The remaining 4 are used for house-keeping data ( $PO_2$ , temperature, free fresh water flow, close circuit water flow). The test is performed in stages, as follows:

**Preliminary Stage:** From 50 to 100 bull frogs are kept in the receiving aquarium at all times, fed with living tadpoles and liver. After receiving, each frog is treated for 5 days with aureomycin (tetracyclin). The frogs are checked for possible disease, especially for development of skin redness or ulcers.

**Stage 1:** After at least 2 weeks (or 1 month) 25 frogs are transferred to 2 separate aquaria and fed with the artificial diet for 2 weeks (or 1 month). Weight increase and general health conditions of the frogs are evaluated during this period.

**Stage 2:** Out of the 25 frogs, 14 are chosen and paralyzed following the OFO-A technique. They are instrumented with the EKG electrodes and the feeding catheters in the ventral lymphatic sac. On ten of the 14 frogs, the 3 axial accelerometers will be fixed on the skull, when available. The 14 animals are distributed in the 3 specialized aquaria (with close circuit water flow,  $HPO$ ,  $PO_2$ , and temperature recording - for detail see OFO-A conclusive report, Contract NAS2505/27699), and the EKG and head accelerations monitored continuously. The frogs are fed regularly, 3 times a week, with the artificial diet.

**Stage 3:** After variable periods of 2-10 days, the main surgery is performed on all frogs, exposing the vestibular nerves.

**Stage 4:** After a second variable period of 2-10 days the 10 frogs with accelerometers are fully instrumented with microelectrodes in the VIII nerves, either on both sides or on one side only, and replaced in the aquaria. The remaining 4 frogs are kept in reserve, as controls.

**Stage 5:** After a minimum of 10 hours the periodic recording of all the signals as described in the introduction begins through the multiplexer, one frog at a time for a minimum of ten minutes. The recording is normally repeated 12 times in the 24 hours. The analog data are recorded on a 14 channel tape recorder and also subjected to various on-line quick look analysis (for more details on the analysis program see the proposal submitted for SSM3). This stage lasts for 45-60 days, max. During this period each frog is submitted to various tests according to the kind of unit under investigation. The statoreceptors are tested for receptor field in the field mapper and for dynamic responses using Dr. O'Leary's special technique (see SSM3 proposal). The semicircular canal and vibrosensors are tested for dynamic properties only. The tests might be repeated several times to determine the variability of the responses. In the intervals the background activity is recorded.

After 60 days the test is terminated. It might be continued if necessary for better understanding of the results.



## Fig 2 Test #1 Frog #1:

3 units appear in this recording: a large one (A upper row, 1-7) and two smaller ones, B1 and B2 mixed (second row, 8-11) and are separated via the spike shape discriminator (B1 - 14 and B2 - 13).

In 5 and 12 response to vibration respectively of Unit A (5) and B1 (12): note the phase lock in the envelope of the vibration stimulus on the descending branch of the quasi sine acceleration (X) and in the ascending branch (Y). Unit B2 also responds in the same way



(19). The responses remain the same throughout the experiment: 22-24-25-26-27-30-31-32.

Records 15-18 and 20-21: spike analysis according to the diagram of Fig 2: on the abscissa M1-Mx; on the ordinate Mx-M2; the different spikes appear as a cluster of white dots, the dimension equal to the noise area.

Records 23-29: a minimum interval is evident, equal to approximately 20 msec for Unit A (23) and 30 msec for Unit B1 (29).

Record 28: corresponding to Unit B1 + B2 (8 through 11) does not show a minimum interval, although the firing is closely modulated by the water pump induced vibration.

Calibrations in the figure. Days are numbered according to the day of the year, on top of each record (245 = Sept. 2, 1977)

## Fig 3 Test #2 Frog #3:

2 units appear: one (b) since the beginning (3-7), and a 2nd one (A), larger, from day in the year 285 to 287.

As shown, the characteristics of the spike remain consistent (see also, analysis via spike shape discriminator, performed as in Fig 2, 8-14; 16-17; 20).

Unit b. does not respond to vibration: (19) the white dots corresponding to the spikes are randomly distributed along the envelope of the vibratory curve: Compare with 21 (Unit A) which respond to the vibration and it is phase locked to the top of the vibratory curve. 18 shows the minimum interval of Unit B, before (bp), during (dp) and after (ap) the close circuit water pump is activated. The minimum interval appear to be approximately 60 msec.

12: starting of the pump (pump 1). 16 (pump 2): 10 minutes after the pump started. 20 (pump 3): 25 minutes after pump started. 13 (after pump 1): immediately after the pump stopped. 17 (after pump 2): 20 minutes after the pump stopped.

As shown, the very high vibration (up to 2g, 60/sec) induced by the water pump strongly stimulate Unit A (cluster of white dots on top of record 12) and not at all Unit b (cluster of white dots in the middle of record 12). Also, the noise (dots on lower left corner of record 12) increases remarkably. As the stimulation continues, the area of the cluster on top and bottom of records 12-16-20 decreases, indicating adaptation of the Unit A and most of the units in the noise, while the dots corresponding to Unit b (middle cluster) remain the same. At the end of the pump run (13) the upper cluster does not appear, indicating blocking of firing of unit A, whereas Unit b (middle cluster) is still present. Noise, too, is reduced, indicating inhibition or depression of most of the activity of the nerve. However, several units seem to behave as Unit b. In fact, the basic noise still contains more nerve activity. When the nerve is dead (same conditions) the noise is reduced much further (record 14: in this case electric noise only is present). A better illustration of the adaptive behavior of the response of Unit A, and lack of response and no adaptive behavior of Unit b is shown in Fig 4.

Calibrations in the Figures.

Fig 4 Test #2 Frog #3:

Sequential minimum interval: the first spike trigger horizontally the time base, while the tracing is moved vertically downward. Thus, the 2nd run of the timebase is shifted downward. Each spike corresponds to one white dot.

Record 1, Unit b: A 60 msec minimum interval appears continually, except at the arrow. That single event is explored in Record #4 immediately below. The lower tracing (arrow) show occasional electric noise responsible for the artifact and lack of a minimum interval at that time. The upper tracing corresponds to usual unit activity. Unit A, first on left, and Unit b (on right) are shown.

Record 1 also shows that Unit b is not affected by the close circuit water pump. The dots appear at random following the minimum interval with no correlation with the pump cycle. On the contrary, Record 2 and 3 (continuous) show that Unit A respond to the pump cycle. A minimum interval is present at approximately 40 msec. The following activity is completely modulated by the pump cycle (see also Fig 3 Record 21). However, the response becomes increasingly less as the pump stimulation continues (from top to bottom minutes of pump run are indicated on left of 2 and on right of 3).

C - corresponds to additional stimulation provided by the short burst of vibration due to the periodic working of the water cooler (the vibration is transmitted through the floor). As the bursts are nearly equally spaced in time, and very similar in intensity, they provide a good test of the unit sensitivity and gain. This is shown in (3). Immediately at the end of the water pump run, not only is there no spontaneous activity, but nearly no response to the cooler induced vibration (first C on top). With time the response to C becomes increasingly larger. The last two C evoke equal responses. When the water pump starts again a massive response follows.

From the record it appears that approximately 15 minutes are required, both for adaptation to high stimulation (Record 2) 0 to 15 minutes, decrease of gain, and for readaptation to no stimulation (Record 3) 35 to 50 minutes, gain return to normal.

Fig 5: Scanning electromicroscope photograph of the enameled 25m platinum wire. In A a large discontinuity of the coating is shown. In B the irregularity of the coating is evident. In C sharp bending of the wire does not break the insulation.

Fig 6: Scanning electromicroscope photograph of the tip of a typical microelectrode. As shown, a clear area appears at the very tip of the electrode. However, a very thin film of glue is still present. After immersion in frog ringer for 10-12 days the film on the tip becomes permeated by the ringer solution which diffuse above the boundary line increasing the recording area (lower record, higher gain). The glue film appears swollen. Correspondingly, the impedance decreases to 1/5. This is due to the fact that the varnish used (insulex) is not a high enough water repellent. The proposed parylene has a moist diffusion coefficient several order of magnitudes smaller than insulex.

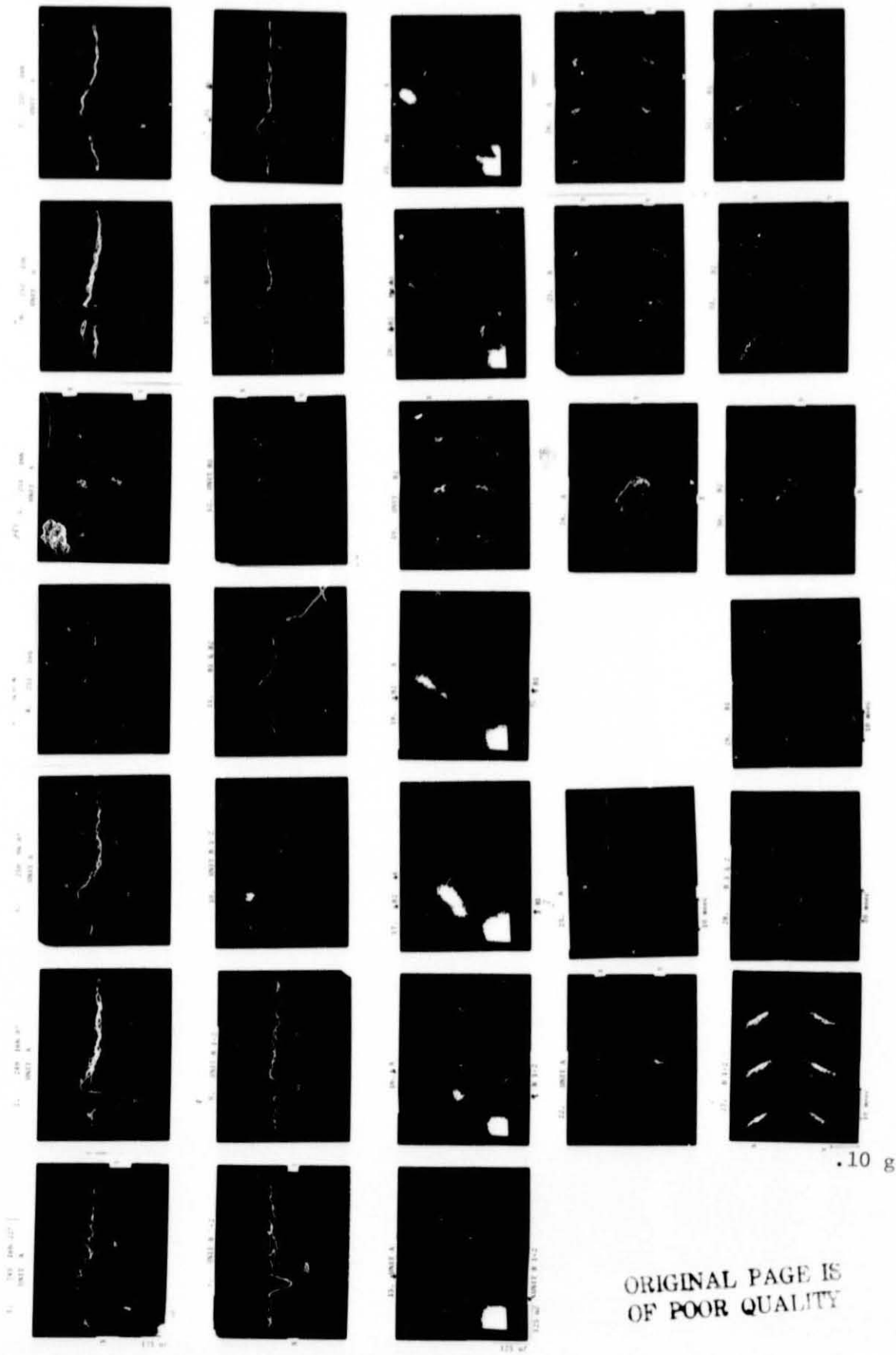
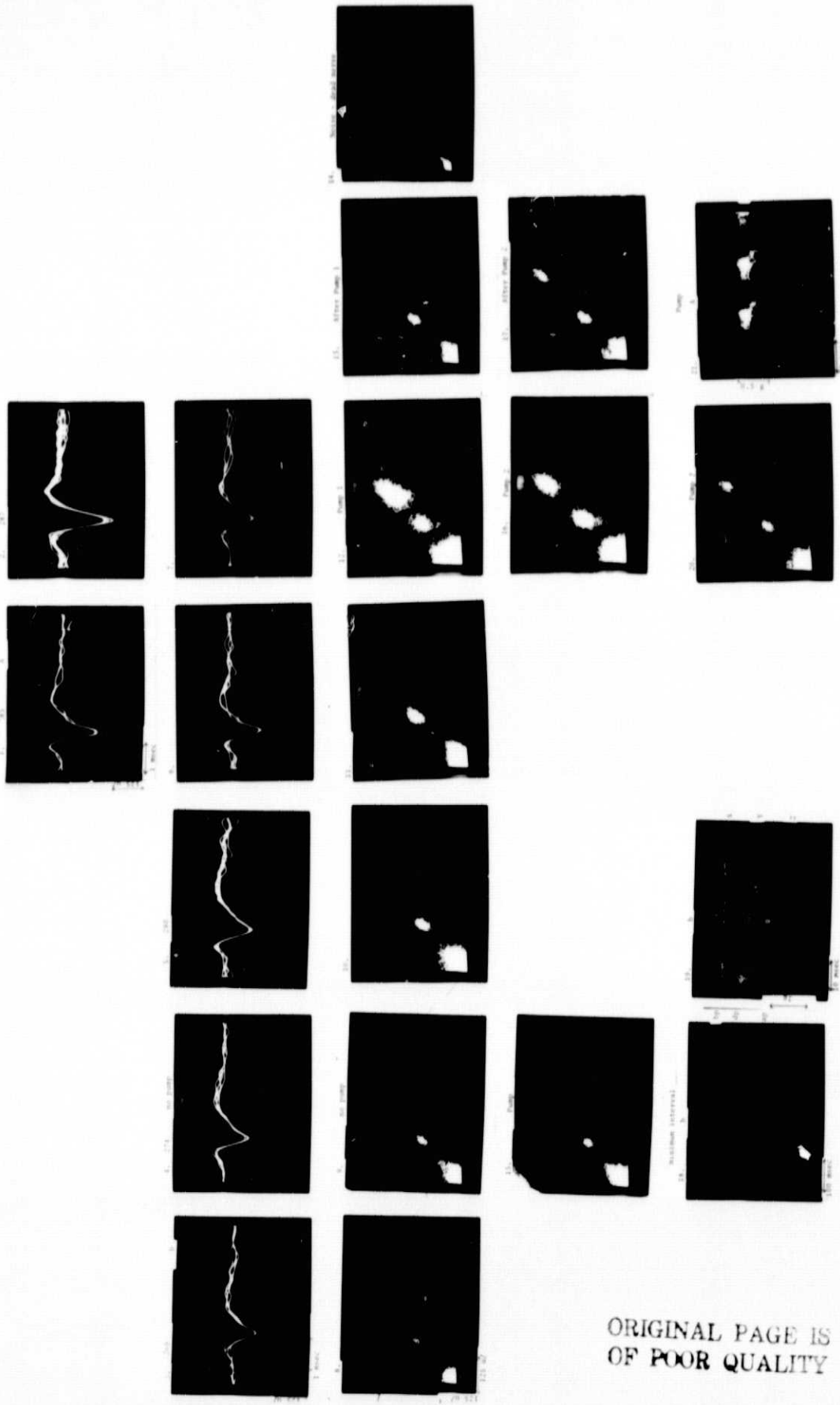
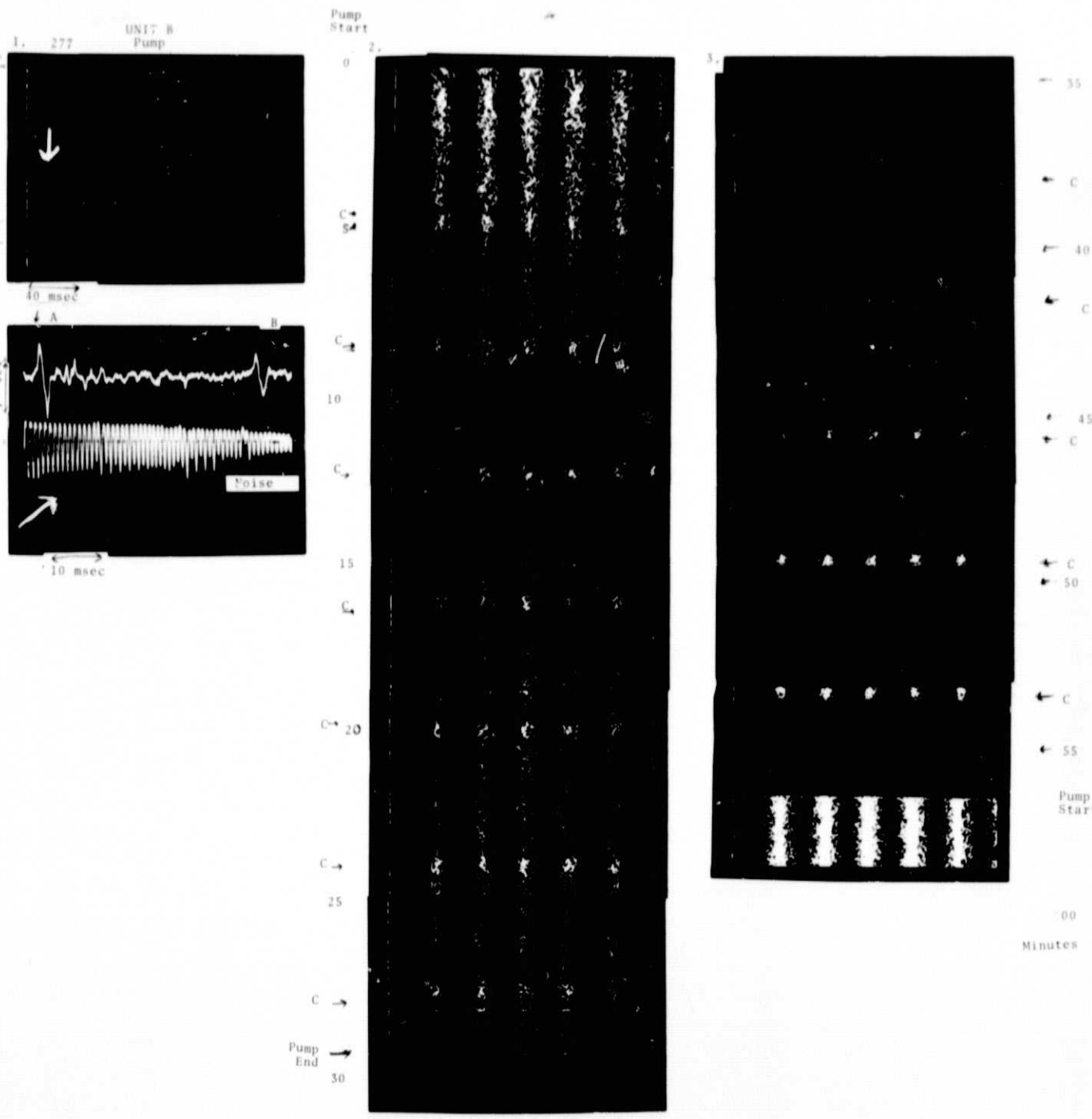


FIGURE 2



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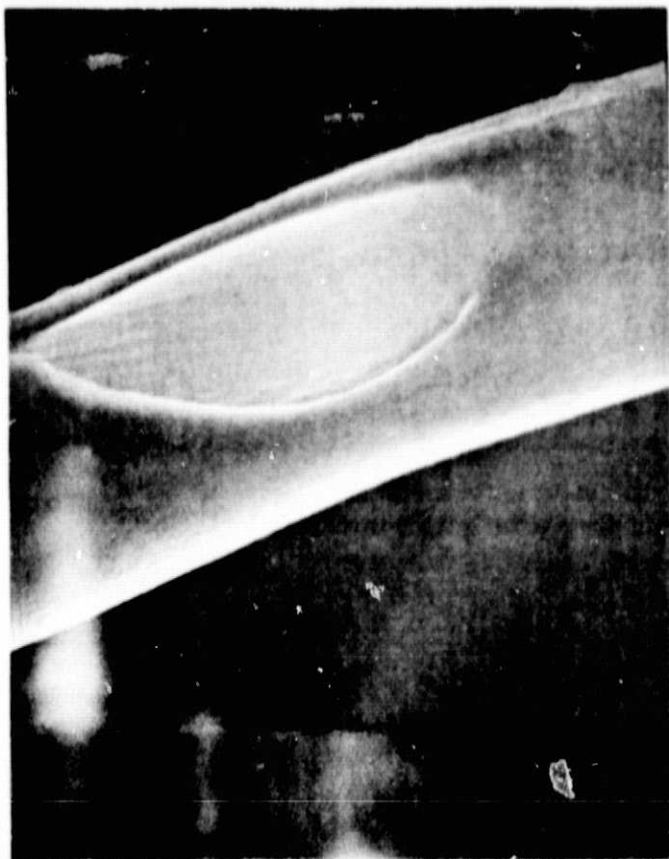
FIGURE 3



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FIGURE 4

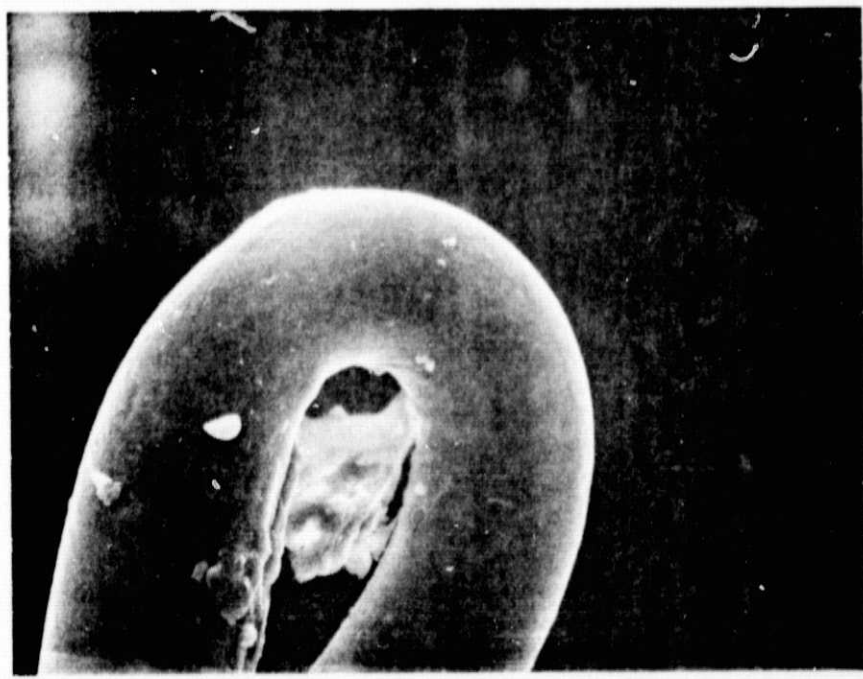
A



B



C



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FIGURE 6

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S1-4 = Spare Frogs Control

1-10 = Instrumented Frogs

