

A STUDY OF TOPOLOGICAL CHARACTERISTICS
IN PATTERN PERCEPTION PHYSIOLOGY
IN THE MAMMALIAN CORTEX

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0. 0 Introduction

The following is a progress report covering the first 6 months of a 12 month pilot project to study topological characteristics of the physiology of pattern perception in the mammalian cerebral cortex.

0. 1 Our specific final objective was to record simultaneously from a number of single neuronal units that were involved in mediating the perception of a stimulating sensory pattern. Our intention was to analyze this data so as to trace time fluctuating characteristics in the relationships of these units to each other. The underlying motivation was the potential use of such information in designing a new type of synthetic intelligence (automaton) machine. The schedule proposed was, in effect, a 6 month period of development of techniques, followed by a 6 month period of single unit analysis of visual cortex in the rat, to be combined with (in its terminal phase) study of multiple simultaneous single unit recordings. The present report concerns the initial, 6 month phase of developing appropriate techniques.

1. 0 Development of Experimental Methods

1. 10 Input stimuli

1. 11 Input stimuli were purposely restricted to simple, hand manipulated dark objects (3° - 5°) on a white field, normal sounds, manual touching and so on. We have not tried to develop quantitative parameters at this stage.

1. 12 Experimental psychological program.

Dr. David Ingle, an experienced experimental psychologist, joined the laboratory in order to develop appropriate behavioral tests for defining the existence of perceptual states in the experiment. The development of psychological indices has been deferred until completion of technical development. Nonetheless, his presence adds important strength to the implementation of the overall project.

1. 13 Surgical Techniques

1. 132 Anesthesia. We have developed a suitable encephale isolé preparation. For this we have used fluothane anesthesia for induction and maintenance until completion of the bulbar section. Penthrane was tried and found unsuitable owing to its prolonged anesthetic depression.
1. 131 For most of our pilot experiments we have used urethane (1.5g/kg.). Although some cortical depression occurs with urethane, anesthetic deaths are rare and the anesthesia is very stable over prolonged (8 - 12 hour) periods. We have discovered that obese rats do very poorly, the same dosage being lethal and reduced dosage unsatisfactory in terms of anesthetic depth.
1. 133 We always perform a tracheotomy and intubate with a small tracheal canula fashioned out of #14 gauge stainless steel tubing. The design is conventional except for the obtuse angle of the port. (Fig. 1) With the angled connection, it is easy to pass a thin suction tube into the trachea and major bronchi. This has proved essential since, even when using atropine (500 mgm/kgm), in prolonged experiments, severe pulmonary obstruction usually occurs and aspiration is vital.
1. 134 Body temperature was maintained by means of a thermostatically controlled, water circulating, heating pad. Bulbar sections, required artificial respiration. Owing to our inability to monitor alveolar CO₂ we were uncertain of the physiological state of the cortex and have deferred these experiments until adequate CO₂ monitoring is available.

1. 40 Stereotactic, electrode placement control and micromanipulator design.

1. 41 Design considerations included versatility in applications, ultrarigidity, precision and stability of electrode movement, maximal accessibility for sensory stimulation, reliability and reproducibility of establishing stereotactic coordinates.

1. 42 Design solutions.

The basic solution centered around the concept of establishing rigid stereotactic relationships by means of reversible magnetic mounts magnetically clamped to flat, ultra-rigid, base plane.

When precision milled for flatness, conventional reversible machinist chucks have enormous holding power on a properly ground steel base-plane. The system consists of (1) a precision X-Y carrier to which the animal is fixed in proper stereotactic head position, (2) a base plane, (3) microelectrode manipulators mounted on reversible magnets. The plane couples the animal on the X-Y carrier to the manipulator.

1. 41 Components

(1) A conventional machinist cross-slide carrier (Palmgren), when smoothed by hand rubbing of slides and dove tails, supplies precise, vibration free, X-Y motion, easily controlled, with position recoverability to 10 micron displacements. (Fig. 2)

The choice of the Palmgren cross-slide carrier was based on several considerations. (1) For a standard, commercially available X-Y platform it was far better in workmanship than other available U.S.A. products; its massivity and smoothness of action and freedom from vibration or play are excellent.

- (2) Its table is grooved with "T" slots in such a way as to provide convenient tracks for adjustable slide bars which fix the jaw and skull. Although ear bars must be used in order to provide proper skull alignment, in my experience, direct skull fixation by means of screws and epoxy cement is more secure and less traumatic to the animal. I intend to employ this mode of fixation in future experiments.

The X-Y plane is easily oriented by aligning the base of the Palmgren to a standard K & E millimeter grid and using a pointer for establishing ear bar zero.

To fasten the jaw of the animal, I anchored the upper teeth to a bite bar, using dental acrylic. This is more secure than the conventional clamp. The acrylic is easily removed after the experiment. (Fig. 2)

- (2) The base plane is a 29" x 39" x $5\frac{1}{4}$ ", cross-ribbed, welded steel platform, ground flat by standard Blancharding machines to ± 0.0005 " flatness. No detectable torsion exists. This ultra-rigid platform provides the above mentioned connecting link between the X-Y carrier (on which the animal is fixed) and the microelectrode drive system.
- (3) The microelectrode drive system consists of (i) a rigid pillar in the form of an aluminum block (alloy, 2024-T4) $\frac{1}{2}$ " thickness walls 5" x 5" x 10", mounted on a pair of coupled magnetic chucks - the entire unit being milled for flatness after assembly (ii) a vertical locking dovetail slide for gross Z position (iii) a rigid, cast aluminum arm extending horizontally out at a 90° angle from the upright pillar, 6 $\frac{1}{2}$ " in length. Equipped with

two rotating joints, the arm has two degrees of rotatory freedom, respectively in planes parallel to and normal to the face of the pillar. (iv) The electrode is advanced by being mounted on a standard, commercial, (Bausch & Lomb) microscope body drive unit, with coarse and fine controls, the latter providing one micron displacements over a range of 2500 microns. (See Fig. 3) There is no detectable backlash and no drift in the B & L unit. The "Z" dimension is achieved by lowering the coarse control under guidance from a millimeter, vernier caliper, accurate to 20 microns. The fine control provides an additional Z axis drive of one micron per division. (v) The actual electrode carrier is machined from standard aluminum stock and fitted with a simple, phosphor-bronze, spring clip which secures the shaft of the microelectrode to the carrier. Electrical isolation is achieved by insulating the electrode carrier from the microscope drive unit with two layers of mylar and fastening with nylon screws. The guide tube assembly is similar save for a heavy screw clamp holder for the assembly. Cost of assembly is kept low by using standard stock sizes and simple design for the machining, and commercially available components for the intricate mechanisms.

This micro manipulator has several useful features:

- (i) The needle advance track is independent of the X-Y positions of the stereotactic positioner. Further, its two degrees of rotatory freedom permit any desired angulation.

- (ii) The clearance to the animal is large (9 inches in all) permitting multiple electrode arrangements without blocking access to animal or being encumbered by mutual interference.
 - (iii) The freedom of placement of each unit is enormous, not being limited by side rails or base screws. Also, other equipment may be mounted easily around the preparation.
 - (iv) Finally, individual micro-drive units can be used independently for other microelectrode work wherever one has a flat, rigid, diamagnetic surface.
- (Fig. 4)

1. 50 Micro electrode design.

1. 51 Design principles. Since I plan chiefly extracellular recording, impedance was to be relatively low. Simplicity and repeatability of construction, as well as durability and resistance to damage from penetration of tissues, were clearly desirable qualities. In particular, owing to its planned use in a multiple electrode system, smoothness and uniformity of shaft dimension were critical.

The solution adopted was to coat with corning 7740 glass a .005" diameter, straight tungsten needle, conventionally sharpened by electro-polishing the tip and cleaned at its connector end. I placed each needle inside a 2mm capillary tube and, using a Lettvin designed micro-pipette puller, melted the glass around the tungsten needle. This was done by hand pulling the capillary in two stages. I then cracked the capillary tubing from the needle near beginning of the flare, and crimp connected a fine (0.002") tungsten wire to the protruding shaft. The latter permitted easy electrical coupling

to the input stages of the amplifiers, with minimal mechanical coupling.

To improve impedance characteristics, I plate the tips with gold followed by platinum (Lettvin). The plating current cracks off approximately the terminal 5-10 microns of glass, resulting in adequate tip exposure. Occasionally, needle tips are coated with too heavy a glass layer, and must be discarded. A finished electrode (under - 550X power) may be seen in (Fig. 5).

1. 6 Implantation Technique

The aim of the system is (1) to establish closed hydraulic recording conditions. This eliminates artifacts due to relative motion of the cerebral cortex, (eg. arterial, respiratory or mechanical pulsation) and (2) to hold multiple electrodes, recording different units at different locations in the cortex.

To this end I designed a plastic ("Delrin") guide tube assembly, containing a number of #24 gauge stainless steel guide tubes friction fitted into a small plastic cylinder. The lumen of the tubes is 0.012" and permits easy passage of the glass coated tungsten electrode but allows little free play. (Fig. 6)

I embed the guide assembly over the desired cortical region in the following manner. (1) Over the desired recording area, the skull is first stripped of periosteum, cleaned, dried and the circle of the burr hole marked. (2) Using a flat bottomed dental burr (#2 inlay) I drill through the calvarium until I reach the inner bony lamella. I remove the latter by piecemeal stripping, using a micro-rongeur made from a pair of hand ground jeweler's forceps. (3) When the burr hole accepts the guide tube assembly snugly, I remove the overlying dura.

This is done by picking up the dura with a sharpened, hooked, tungsten needle, and dissecting it away with a fresh razor shard. One must be very careful to spare meningeal arteries or superficial cerebral cortical arteries. (4) When the dura is so removed, I immediately lower the guide assembly into the burr hole until it just overlies, but does not compress the cortex. The inner surface is usually placed just deep to the inner lamella of the cranium. The guide assembly is automatically properly positioned for later passage of electrodes because it is clamped in the same carrier that holds the microelectrodes, in a groove parallel with that holding the microelectrode. It is therefore exactly "in line". The guide assembly is fixed to the skull with sealing wax, surrounding it with a hermetically sealed, strong, support wall. The steel guide tubes of the assembly are kept clean and sealed by means of tiny wire plugs, ground flush to the bottom of the guide assembly and inserted in the guide tubes. The clamp holding the assembly is then released and the positioner racked up to accept the microelectrode carrier. This sequence may be seen in Figures 6-a-c. (5) When ready to record, the plug is removed from one of the guide tubes and the microelectrode lowered into the tube until it penetrates the cortex. The contact depth is computed beforehand. (6) After the amplifier is connected to the microelectrode, the drive lowers the microelectrode until the desired unit is being recorded. (7) To hold the electrode, I then place a tiny drop of wax at the entry point to the guide tube and a bridge of wax connecting a slender, rigid holding bar to the electrode. When both bar and guide tube points are secure, the clamp is released on the microelectrode and the manipulator racked back up for another electrode insertion.

2. 0 Data Analysis Techniques.

Recognizing the need for in-house, on-line analysis of a quantitative statistical nature I have installed a Hewlett-Packard 2116A digital computer. The general arguments for a laboratory computer in neurophysiology are clear, the main advantages being (1) steady accrual of analytic strength from hardware and programs uniquely suited to the analysis of neural data, plus (2) elimination of queing and turn around time as well as difficulty in arranging for special purpose programming at facilities not specifically designed for neurophysiological data.

The purchase of the Hewlett-Packard 2116A computer was in the following configuration:

2116A Computer with 4,096 x 16 bits of 1.6 μ s memory

ASR 33 Teletype

300 CPS Paper tape reader

Dual 8-bit D/A Card (Display Card)

Object of purchase was to establish at low cost the multichannel statistical analysis which the project required. The 2116A was chosen for its 16 bit word length, low cost, hardware priority interrupt system, availability, and manufacturer support. The machine subsequently has proven quite satisfactory on all counts.

The data analysis system plan for this project was:

1. Hardware preprocessing of nerve signals
2. Analog tape storage and retrieval of preprocessed data, using existing 8-channel recorder.
3. Online analysis visual display and hardcopy of selected statistics.

4. Special interface circuits to minimize CPU overhead so as to permit simultaneous data acquisition and analysis.
5. Fortran programming of most routines, to make machine more accessible to the experimenter.

Less manpower than anticipated was available to this project, so that the system is less sophisticated than that described above. The present capability is single-channel, assembly-language programmed, on-line analysis and display of: pulse interval statistics and pulse interval histograms and their moments, post-stimulus histograms, and pulse-rate averages. Multiple channel, interface hardware has been designed, and is being built for two channels. Simple, low cost pulse-height selectors (for preprocessing) have been designed, but we have enough units of an older design on hand to meet our immediate need.

Our object has been to keep the computation from overshadowing the experimenter or the experiment. Nothing more is expected of the system than a "super-oscilloscope", to permit the experimenter to measure what he wishes in the nervous system. Our experience with single channel analysis handled on this system has been very favorable. We expect a similarly facile multichannel system will be achievable at the present level of effort. Figures 7 a.b. illustrate the sequence of data processing from raw nerve pulse to histogram display. Figure 8 illustrates the working arrangement in the laboratory.

3. 0 Results of Physiological Recording

Three successful experiments were achieved using the system outlined above. Fig. 9 illustrated a typical recording. It was taken during an initial probing of occipital cortex in the rat. This CRO trace was recorded approximately 2 hours after initial recording while it was

held independently by its holder bar (See Fig. 6c)

4. 0 Summary

4.1 Technical barriers to a multiple simultaneous microelectric recording system have been solved.

4.2 Data processing is now complete for single channel basic statistical analysis (PIH, PSTH and conventional statistical parameters). On line display capabilities for observer-experiment interaction and graphic storage are complete.

5. 0 Future Plans (Assuming funding will be available)

5.1 Experimental. Our next immediate experiments will be a survey of occipital cortex in the rat using the closed system, probe technique. We have modified the guide insert so that it can rotate, permitting a larger number and finer mesh of sampling locations than at present. As a terminal phase of this survey we shall employ multiple simultaneous probes in animals in whom evidence of cognitive perception has been established.

5.2 Data Analysis. (1) We shall expand our data intake capacity so that it can accomodate simultaneous 8-channel input (2) We wish to add multi-channel, analog-to-digital capacity in order to analyse slower wave form responses (the "slow potentials", 0.1 to 100 cps bandwidth) (3) We plan to add computer-controlled, visual display, as well as monitoring, for synchronizing data analysis with stimulus presentation (4) Finally, we wish to develop programs (and arithmetic capacity) for more complicated analysis such as study of cross-correlations of rates of change of statistical functions of activity in multiple brain loci.

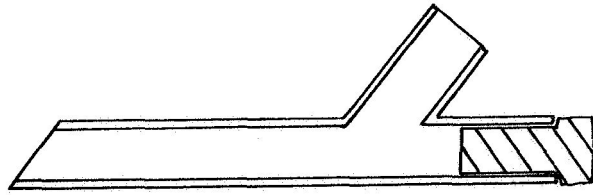


Figure 1. Tracheal cannula for rat. Made of thin wall #14 gauge stainless steel tubing. Slant of entry port facilitates passage of suction tube for clearing trachea and bronchi. Hatched area is insert plug to seal off cannula from upper trachea.

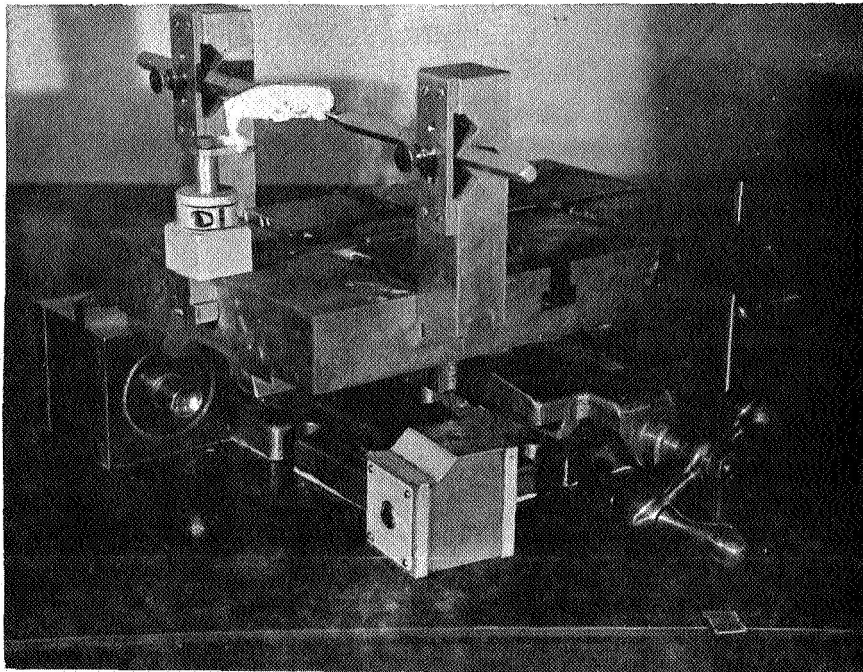


Figure 2. Palmgren cross-slide carrier adapted for "X-Y" stereotactic electrode implantation. Note "T" shaped slots in floor of carrier which accept slides holding jaw and ear bar fixation jigs. Set-screws in slides permit adjustment from rodent to large primate skulls. Magnetic chucks are used to lock carrier into position. Vertical rod at right rear establishes ear bar zero with reference to grid on base plane.

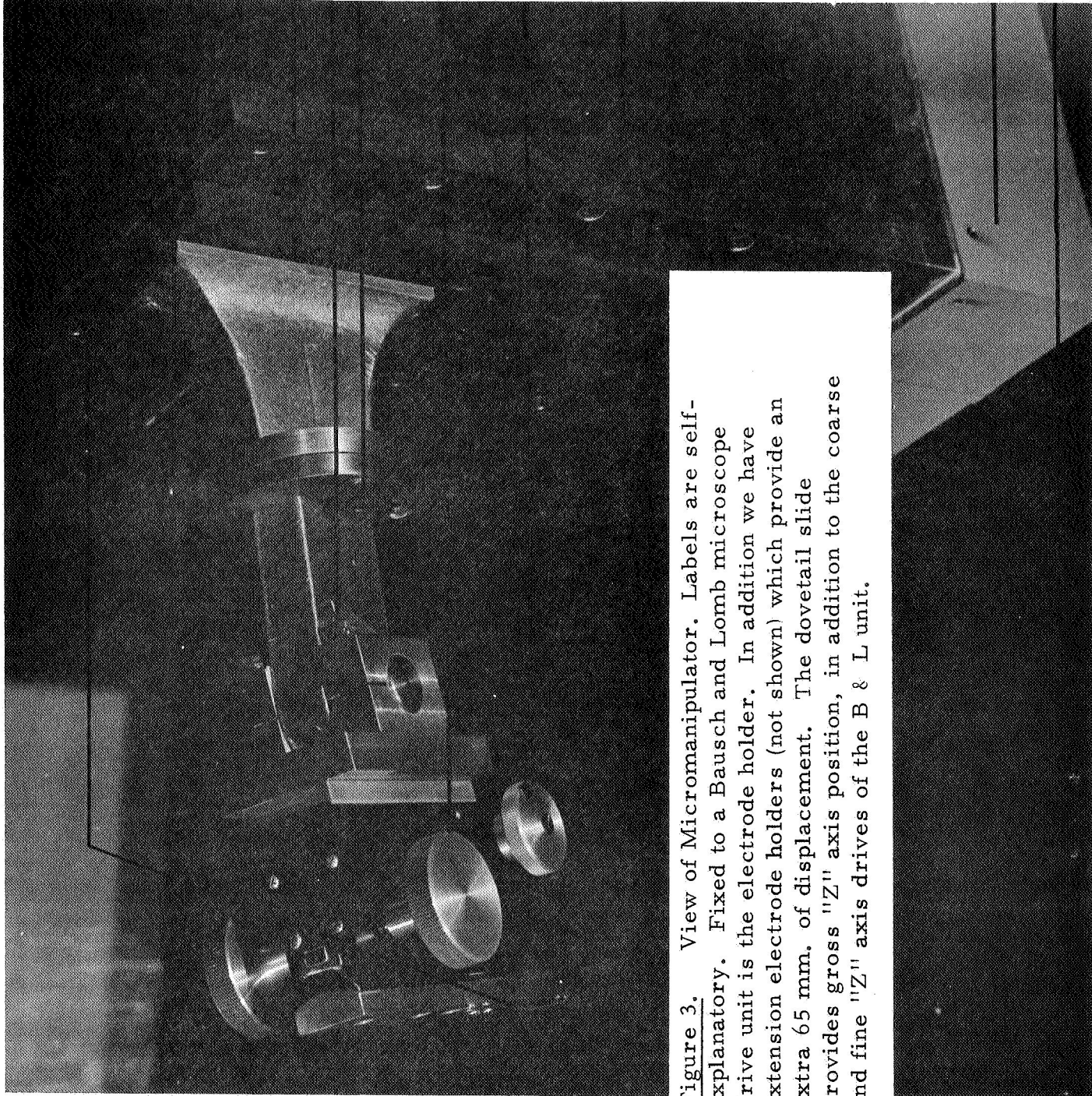


Figure 3. View of Micromanipulator. Labels are self-explanatory. Fixed to a Bausch and Lomb microscope drive unit is the electrode holder. In addition we have extension electrode holders (not shown) which provide an extra 65 mm. of displacement. The dovetail slide provides gross "Z" axis position, in addition to the coarse and fine "Z" axis drives of the B & L unit.

Electrode clamp

Vertical gross position
lock (locks dovetail)

Dovetail

Lock of first angle
Lock of second angle

Coarse Z drive

Calibrated Z drive

Aluminum support pillar

Magnetic chuck

Steel base plate

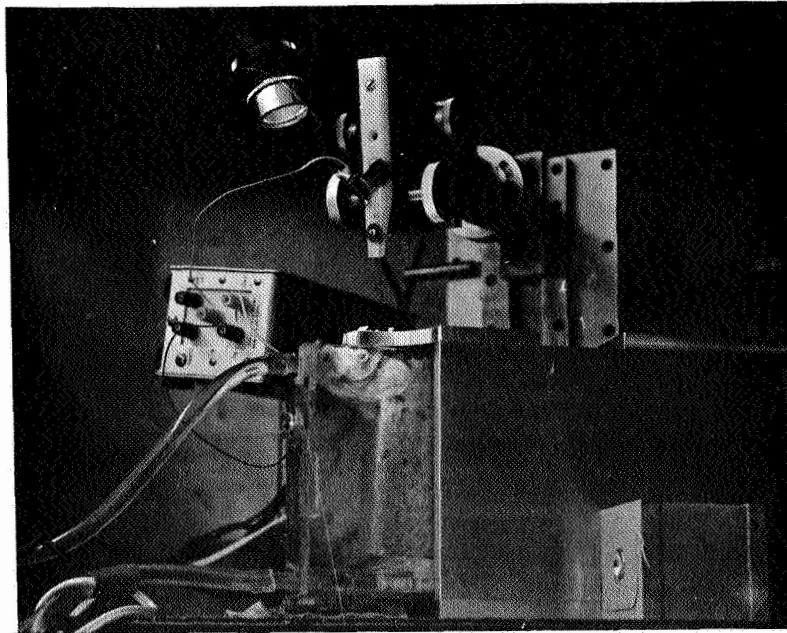


Figure 4. Example of independent use of micromanipulator holder, here being employed in the study of the optic tectum of the fish. Again, note use of magnetic chucks. Not shown is a micrometer drive X-Y positioner for motion of the skull with respect to the microdrive.

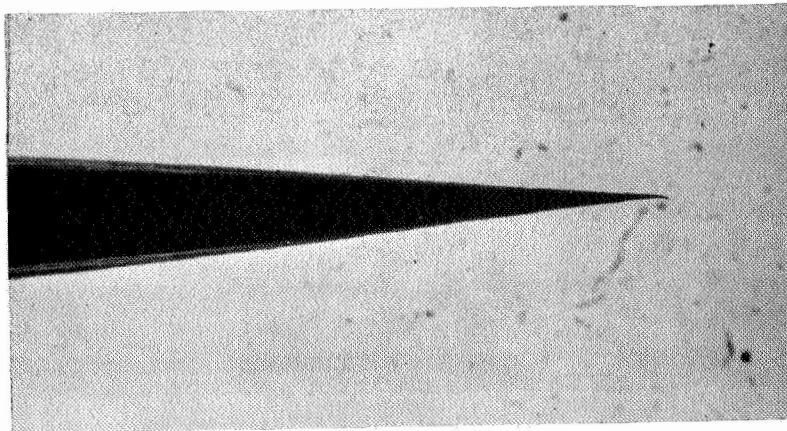
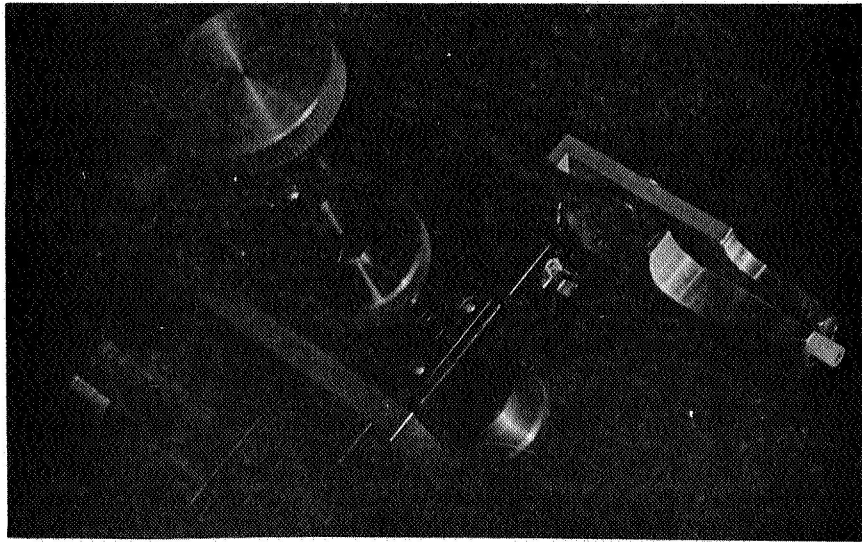
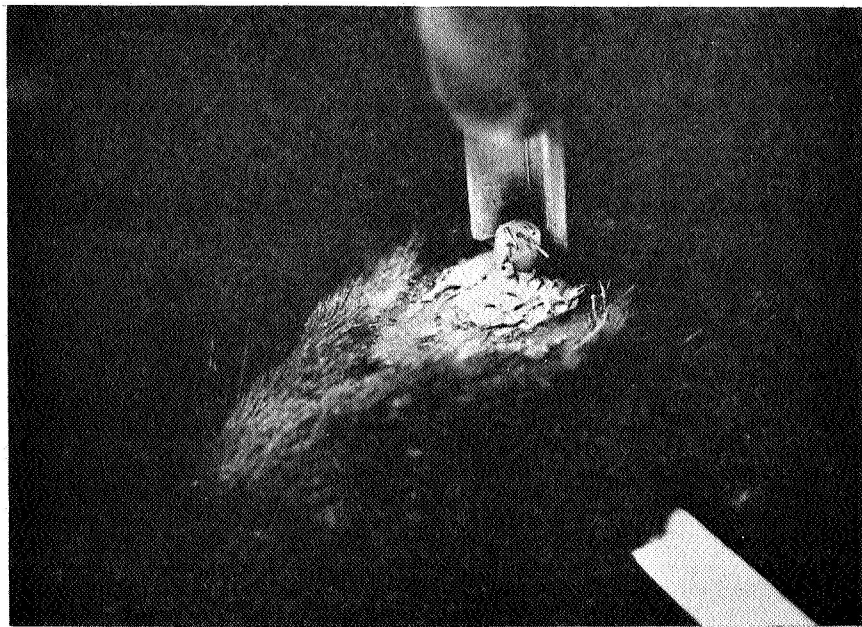


Figure 5. Light microscope view of tip of microelectrode (550 power). Lighter margin is the sheath of glass. Tip exposure is around 7 microns.



6a

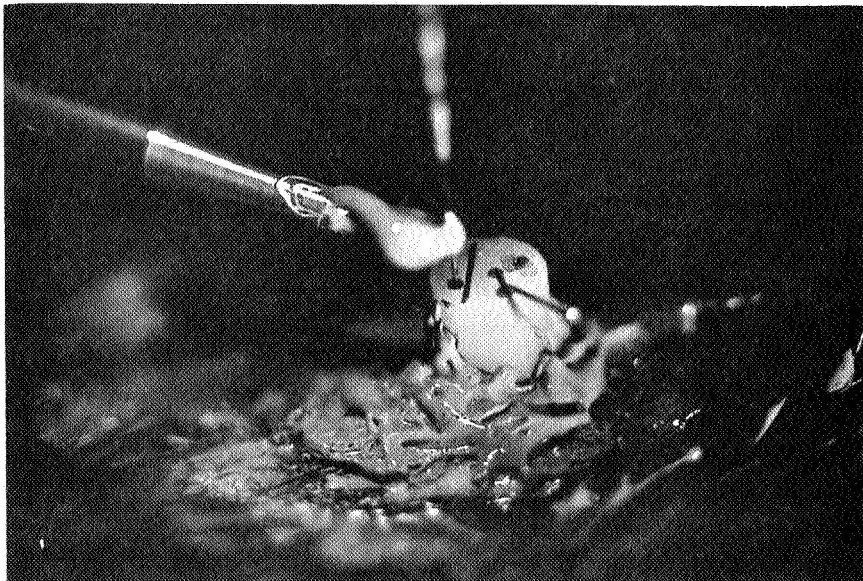


6b

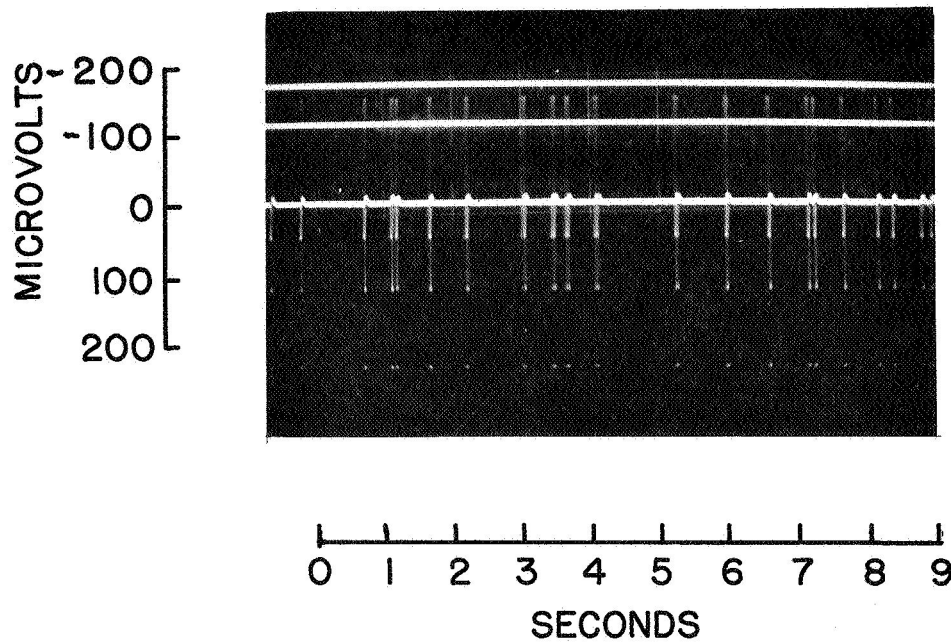
Figure 6 (a-d) Implantation of guide tube assembly and microelectrode. (a) guide assembly clamped in holder on B & L drive unit. (b) guide assembly in place in burr hole in skull of rat. Note wall of sealing wax, and release mode of guide assembly holder. (c) microelectrode inside guide tube, recording units in occipital cortex of rat. Note that electrode is still attached to microelectrode holder on B & L drive. (d) B & L drive and microelectrode holder are now removed. Electrode is independently supported by holder bar.



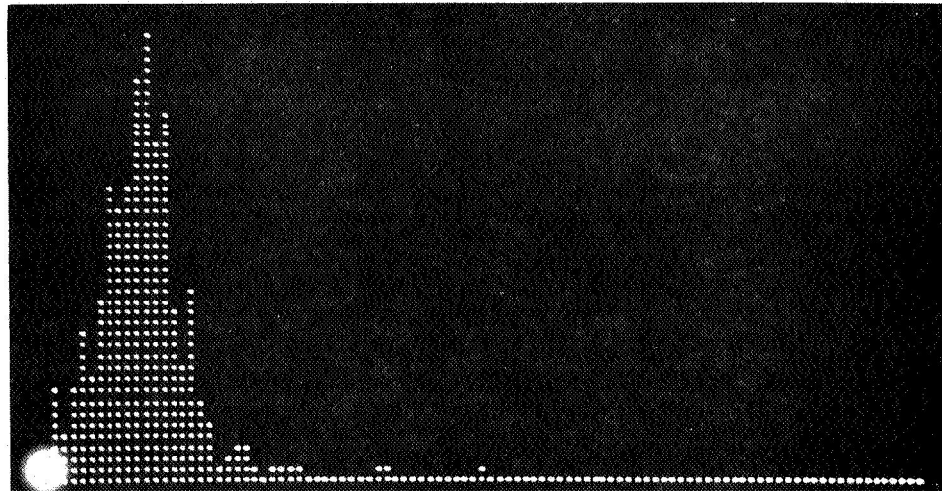
6c



6d



7a



7b

Figure 7 (a-b) Data sequence. (a) Pulse height selection of desired pulse (from photically driven cell). Pulses are raw data. Horizontal lines represent upper and lower voltage levels of acceptance window. Dots are marks of shaped pulses fed to computer for interval analysis. (b) histogram compiled from intervals between nerve pulses. 40 seconds of recording.

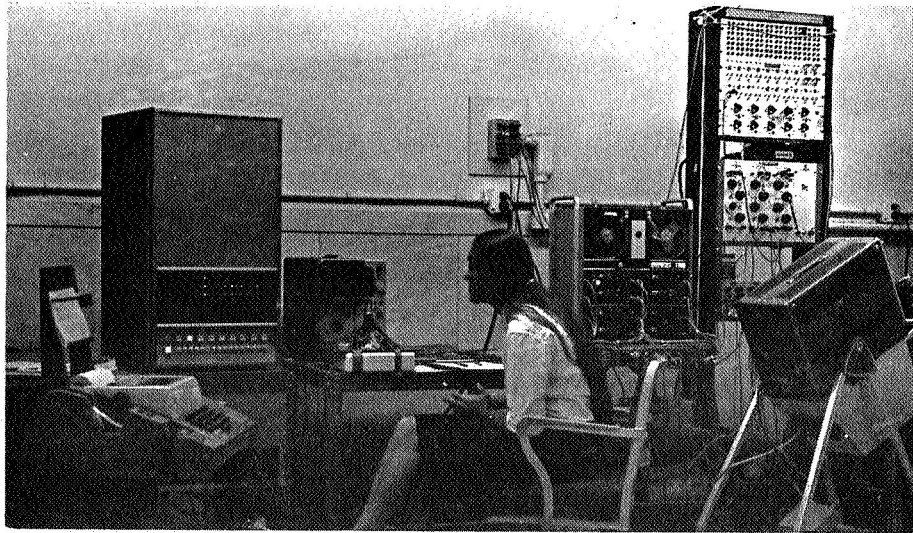


Figure 8. Illustration of current arrangement of data analysis equipment. Tripod mounting is for 35 mm. film recording camera for permanent storage of visual display. Tall rack at right is analog computer.

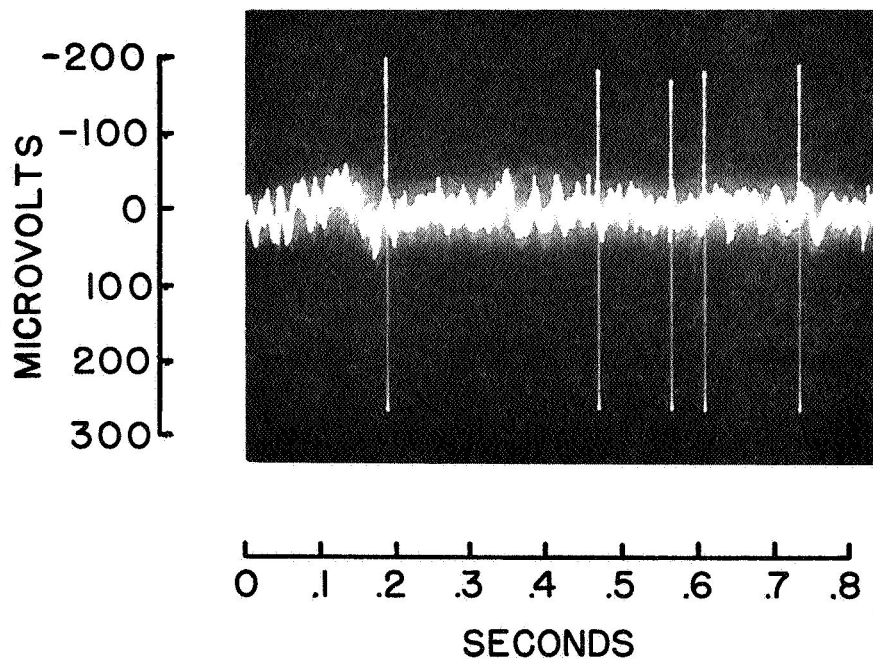


Figure 9. Single sweep of CRO trace of unit recorded in occipital cortex of rat. Unit is being held independently by holder bar.