A REPORT ON THE TECHNIQUES DEVELOPED FOR CHRONIC IMPLANTATION OF ELECTRODES IN THE ALBINO RAT BRAIN[1]

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INTRODUCTION

In 1954, Olds and Milner published the results of a study on the effects of electrical stimulation of the septal region of the albino rat. It was shown that there were "rewarding" properties associated with this electrical stimulation (Olds and Milner, 1954). The rats would, in effect, stimulate themselves by pressing a lever. It was further shown that the rats would not respond at any high rate unless stimulation of the septal area was associated with the lever presses.

The purpose of this paper is to describe the electrode implantation techniques developed for the inexpensive replication of such studies as that of Olds and Milner. All too often small institutions or unproven investigators are without funds and thus are unable to conduct desired research. It is felt that application of the techniques described in this paper can result in relatively inexpensive implantation of intracranial electrodes.

ELECTRODE CONSTRUCTION

In view of the high cost of implantation electrodes, it is often imperative that the experimenter be capable of the design and construction of functional electrodes. The following procedure can result in the production of satisfactory electrodes. The required materials are as follows (see appendix for source of materials):

1. Small phono plugs of Jacks
2. Stainless Steel Suture Wire (32 gauge)
3. Epoxylite Solution

The phono jack has a continuous rod running from the top of the phono jack to the base. The jack was placed against a grinding wheel and filed down so that the bradded end was sheared off enabling the phono jack to be disassembled (see Fig. 1).

A three inch piece of stainless steel suture wire was wound around the filed end of the center pole and was carefully soldered (see Fig. 2). Often the solder would not adhere to the stainless steel

[1]This report reflects work carried out while the author was at Baylor University, and was done in collaboration with a colleague, William Mark Reid.

Fig. 1. Phono Jack Disassembled

wire and it was necessary to file a small groove in the center pole. A three and one-half inch piece of stainless steel suture wire was then wound firmly within the threaded grooves of the jack base and soldered into place (see Fig. 2).

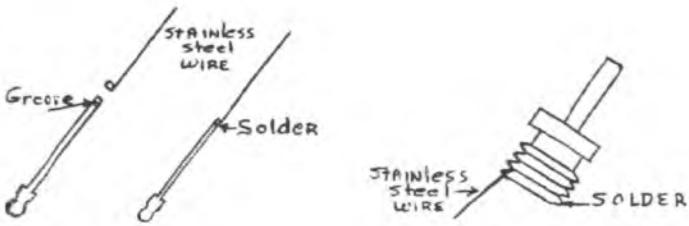

Fig. 2. Soldering of Stainless Steel Wire to Phono Jack

The next step was to dip the center pole with its wire into the epoxylite solution up to the rounded knob and bake for approximately 5 minutes at 225°F. This process was repeated until a total of three coats had been applied. The same procedure was applied to the jack base and its wire. The base portion of the electrode was dipped only up to the point of solder. Both parts were then baked for 20 minutes at 225°F. The phonojack (electrode) was reassembled and the two wires twisted together as near to the base as possible (see Fig. 3).

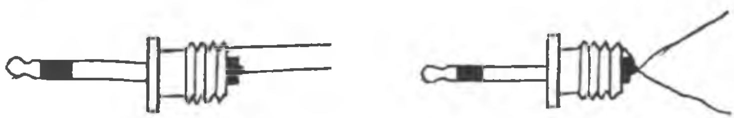

Fig. 3. Phono Jack Reassembled and Twisting of Stainless Steel Wire

With the two wires held between the thumb and index finger, the electrode was spun with the other hand. The wires twist easily and with practice, a professional job results. Approximately one inch of twisting was sufficient for most purposes (see Fig. 4).

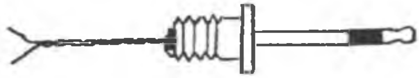

Fig. 4. Completion of Electrode Wire Twisting

The electrode wires were then dipped in the epoxylite solution and allowed to bake for 5 minutes at 225°F. The electrode was redipped and allowed to bake for 20 minutes at 225°F. The electrode was removed from the oven and cut to the desired length (For septal placement, the length was 6.5 mm from the bottom washer). The twisted electrode wires were then bent until they were in line with the center pole and twirled lightly against a fine grinding wheel until the convolutions of the twisted wires were no longer present. With the aid of a magnifying glass, the tips of the two wires were separated and bent apart at an angle of about 80 degrees (see Fig. 5).

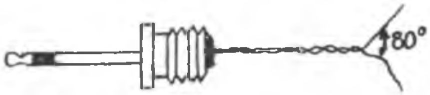

Fig. 5. Separation of Electrode Tip

The next step was to test the resistance of the electrode with an ohmmeter. If the electrode exhibits no resistance, it is not a functional electrode. Infinite resistance had to be present. A lack of infinite resistance was often corrected by unraveling the wires for a short portion of their length. The electrode wires were dipped and baked 5 minutes at 225°F for a total of eight coats. The electrode was then baked for one-half hour at 225°F and for an additional half hour at 275°F.

The next step was to immerse the electrode up to the bottom washer in a super-saturated saline solution and to check the resistance. If the insulation formed by the exopylite solution was porous, a lack of resistance or a fluctuation of resistance resulted. Additional coatings of the epoxylite solution applied as previously described usually corrected this defect. Often dipping the tip of the electrode in candlewax aided in eliminating a lack of resistance.

The final filing was done when an infinite resistance was attained. The electrode was held at a 60 degree angle from the grinding wheel and spun lightly until the ends of the wires were down to within the diameter of the rest of the electrode wires. The further back the electrode wires were filed, the closer together were the contact points of the electrode (see Fig. 6).

If carefully executed the preceding procedure should lead to adequate electrodes. Once familiarization with the principles involved is attained, innovations and adaptations are possible.

OPERATIVE TECHNIQUES

The following is a brief review of surgical procedures involved in the chronic implantation of intracranial electrodes. These proce-

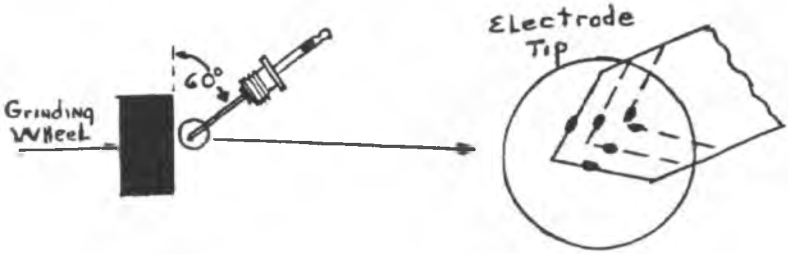

Fig. 6. Filing of Electrode Tip

dures were developed to fit the needs and abilities of a small laboratory with limited funds and personnel and may be revised or improved to fit the needs and characteristics of individual laboratory situations.

1. *Anesthesis:* It was found that the use of 300 to 400 gram rats was desirable because of the thickness of the skulls of these animals and because less difficulty was encountered in getting the dental acrylic to adhere to the skull. The rats received intraperitoneal injections of 60 mg/cc Diabutal at .001 times the body weight in grams. With healthy animals a 15 minute waiting period was required before initiation of the surgery. If the animal was found to be infected with respiratory diseases (ex. pneumonitis), a 45 to 60 minute waiting period was used. The length of this waiting period with diseased animals often resulted in the animal recovering from anesthesis before or during the operation. In these cases, regular anesthesis ether was used to re-anesthesize the animal. Occasionally, pulmonary edema developed in the anesthesized animals and it was necessary to administer small amounts of atropine to counter these symptoms.

In general, this anesthesis procedure should render the animal unconscious until the surgical procedure is completed. Anesthesis ether may be used in small quantities if the effects of the Diabutal are not of sufficient length.

2. *Surgical Procedure:* It is realized that no description of surgical procedure is an adequate substitute for actual experience. The purpose of any technical procedure is to accomplish a goal. The goal of healthy animals with inexpensive but accurately placed permanent electrodes in the septal area was accomplished through the use of the following techniques. A partial list of the supplies and materials essential to the success of the surgical procedure is found in the appendix.

Following the waiting period after anesthesis, the stereotaxic earplugs were placed in the external auditory meatus of the rat. The rat was then placed in the stereotaxic instrument (Modified Johnson

Stereotaxic Device). This procedure involved the placement of the earmounts equidistant from both sides of the stereotaxic instrument so that the head of the animal is centered in the instrument. The nose of the animal was placed in the upper incisor bar of the instrument and lowered 5mm. This point was exactly 6 mm rostral from instrument zero, which is the center of the earplugs. (The procedure would vary as a function of the type of stereotaxic instrument utilized.)

After the nose was secured and positioned in the upper incisor bar, a longitudinal incision was made with the surgical scissors extending from between the eyes to the back of the skull. This incision involved only the top layer of the skin and was made after all hair had been removed from the surgical region of the rat. The incision was spread open and the underlying layers of skin and tissue exposed. These underlying layers of skin and tissue were then spread open with pointed scissors until the skull region was exposed. The skull was usually covered with a thin film of tissue that had to be scraped clean with a sharp, sterile scalpel. Care was exercised in scraping the skull since rupturing of capillaries within the bone often resulted in killing the animal, increasing the complexity and length of the surgery, or causing the dental acrylic to fail to adhere to the bone surface.

After the surface of the skull was bared, 2 hemostats were placed approximately ¼ inch from each end of the incision. The underlying layers of skin, rather than the uppermost layers, were grasped with the hemostats and then drawn to the sides of the skull. The stereotaxic instrument was then set at the coordinates dictated by the stereotaxic atlas. The electrode carrier holding the dental drill was then lowered carefully until it just touched the skull. Extreme care was taken to avoid puncturing the skull with drill pressure. The drill was activated and lowered until the dura mater was reached. Again, extreme care was taken to avoid rupturing the dura mater. Four small holes were then drilled approximately ½ inch rostrally around the electrode hole. These four holes were slanted at an angle of approximately 45 degrees away from the electrode hole. The drill was then removed. Jeweler's screws (.08 stainless steel, ⅛ inch long) were screwed into the skull, again taking care to avoid puncturing the dura mater. The electrode holder was mounted in the electrode carrier of the stertotaxic instrument. The electrode was "zeroed in" to the instrument zero position prior to anesthesis. The electrode was then positioned via the stereotaxic instrument at the desired location dictated by the stereotaxic atlas.

The skull was thoroughly dried. This step was most essential. Dental acrylic was then placed around the electrode base while the electrode holder was still in place. Care was taken to cover the screws with acrylic so that the electrode was securely anchored into place. The acrylic was then allowed to dry. When the acrylic was dry, the electrode holder was carefully removed and the incision wiped clean to facilitate further drying. Flowers of sulfur was sprinkled within the wound to aid in the prevention of infection, and the wound was sutured. Any excess tissue or skin was trimmed from the area adjacent to the electrode to avoid shorting the electrode. The rat was then removed from the stereotaxic instrument. Careful observation of the rat was practiced in order to avoid death after surgery. Often, artificial respiration and/or atropine was administered in order to restore proper breathing. Following each operation, pencillin was administered to avoid infection.

When the animal was sufficiently recovered, a super-saturated sugar solution was administered orally. The rat was then placed in a small, cylindrical cage lined with small mesh wire to avoid damage to the electrode. Vitamin drops were added to the animal's water, and a warm environment was maintained for at least 24 hours to aid in post-operative recovery.

Again, it should be emphasized that as the experimenter becomes familiar with the surgical procedures, the preceding techniques may be altered to fit the individual needs of the experimenter.

POST-OPERATIVE TECHNIQUES

1. *Testing Procedure:* The response box consisted of a large levered Skinner Box, eleven inches long, five inches wide, and twelve inches high. The top of the box was open to allow passage of the electrode leads. The lever activated a microswitch in the stimulation circuit so that when depressed, the animal received electrical stimulation. This response box was similar to the response box used by Olds and Milner (1954).

The electrode harness, which enables free movement by the rats, consisted of electrical wires soldered to the female portion of the phonojack. This arrangment was suspended above the response box with the aid of expansion springs.

In the initial training of the rats, a 150 microamps (ua) alternating current with a duration of 0.3 seconds was used. As the rats developed the ability to perform the task, the current was lowered until optimal performance was obtained at as low an amperage as was possible. It was found that there was individual variation as to the optimal amount of current with a mean optimal current of approximately 50 ua.

The testing procedure was initiated four days following surgery unless complications developed. The actual decision as to whether the animals were "septal" animals was based on a procedure desccribed by Olds and Milner (1954). On the fourth day following surgery, the rats were given a pre-testing session of about one hour in the response box. Each animal was placed on the response lever by the experimenter with the stimulation amperage set at 150 ua. During this first session, the amperage was varied to determine the threshold of a "just noticeable" effect upon the rats' behavior. If the animals did not respond regularly from the start, they were placed on the lever periodically.

2. *Training Procedure:* Two approaches to the training of the septal animals were used. The first consisted of the use of operant procedures, i.e., the animal's behavior was shaped in the Skinnerian tradition. The second approach was to place the animals in the response box and allow them to "discover" the relation between intracranial stimulation and lever pressing. This second approach was utilized only after success of the operational procedure was assured.

3. *Performance Procedure:* On subsequent days, the rats were placed in the box for about 3½ hours each day; these trials consisted of 3 hours of acquisition (stimulation available) and ½ hour extinction (stimulation unavailable). During the acquisition period, the rats were allowed to stimulate themselves with an amperage which was just high enough to produce some noticeable response in the resting animals. Since this threshold amperage fluctuated with the passage of time, the experimenter reevaluated the threshold at ½ hour intervals, unless the animal was responding regularly. At the beginning of each acquisition session and after each amperage threshold test, the experimenter placed the rats on the lever once.

The extinction procedure consisted of allowing the animal to remain in the response box for ½ hour with no stimulation current available. The behavior exhibited during the acquisition and extinction sessions are thus available for comparison. At no time during the preceding procedure were the animals deprived of either food or water and no reinforcement was used except the electrical stimulation.

CONCLUSION

It is felt that the validity of the experimental procedures described in this paper is adequate. The goal was the successful implantation of intracranial electrodes for purposes of electrical stimulation at the lowest possible cost. To some degree, this goal was attained. It should be pointed out that histological procedures are usually necessary to verify location of the electrodes following sacrifice of the animals.

APPENDIX

1. Stereotaxic Instrument
   C. H. Stoelting Company
   424 N. Homan Avenue
   Chicago 24, Illinos
2. Diamond Cutting Disc—2T Not Perm. Mt.
   For Straight H. P.
   Densco, Incorporated
   Denver, Colorado
3. Electrode Insulator—Epoxylite 6001-M
   The Epoxylite Corporation
   S. El Monte, California
4. Dental Acrylic—Nu Weld Improved
   L. D. Caulk Company
   Milford, Delaware
5. Depilatory—Nair
   Carter Products, Inc.
   New York, New York
6. Round Head Machine Screws—Stainless Steel, 0-80, ⅜ in. Type 303
   Metal Products Company, Inc.
   Castleton-on-Hudson, New York
7. Rat Ear Plugs for the Stereotaxic Instrument—Cat. No. SA-150
   C. H. Stoelting Company
   424 North Homan Avenue
   Chicago, Illinois
8. Dental Burrs No. 2
   Ransom and Randolph Company
   Toledo, Ohio
9. Miscellaneous Supplies—Availalable at most medical supply firms

Gauze pads	Scalpel Blades—#10
Swabs	Scalpel Handle
Hemostats	Dental picks
Suture Material	Tweezers
Suture Needles—#20—⅜ Circle	Syringes (2cc, 22 to 25g, ⅝ inch)

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Krieg, W., 'Accurate Placement of Minute Lesions in the Brain of the Albino Rat." *Quarterly Bulletin, Northwestern University Medical School,* Chicago, Illinois, 1946, 20(2), 199.

Olds, J., and Milner, P., "Positive Reinforcement Produced by Electrical Stimulation of the Septal Area and Other Regions of the Rat Brain." *Journal of Comparative and Physiological Psychology,* 1954, 47, 419-427.

Pellegrino, L., and Cushman, Anna, *A Stereotaxic Atlas of the Rat Brain.* New York: Appleton Century Crofts, 1967.

Sheer, E. E. *Electrical Stimulation of the Brain.* Austin, Texas: University of Texas Press, 1961.

Stereotaxic Atlas of the Albino Rat, C. H. Stoelting Company, 424 North Homan Avenue, Chicago 24, Illinois.

ABSTRACT

The purpose of this paper is to describe the technical procedures involved in the surgical implantation of electrodes in the brain of the albino rat. These procedures are designed to be performed in a small laboratory with limited funds and with relatively unsophisticated personnel. Discussions of the pre-operative, operative, and post-operative techniques are included. A discussion of suggested methods for the design and construction of the implantation electrodes is presented, and a partial list of supplies and their sources follows.

RESUMEN

El propósito de este trabajo es el de describir los procesos técnicos involucrados en la implantación cirúrgica de electrodos en el cerebro de una rata albina. Estos procesos están diseñados para ser llevados a cabo en un pequeño laboratorio con fondos limitados y con un personal relativamente no sofisticado. Se incluyen discusiones de técnicas pre-operativas, operativas, y post-operativas. Se presenta una discusión de los métodos sugeridos para el diseño y la construcción de los electrodos de implantación, y le sigue una lista parcial de materiales así como de su procedencia.

RESUMO

O propósito dêste trabalho é de descrever os procedimentos técnicos relacionados com a implantação cirúrgica de eletrodos no cérebro do rato albino. Estes procedimentos são desenhados para permitir a realização dêstes experimentos num laboratório pequeno, com uma verba limitada e com pessoal de formação mínima. Descrições das técnicas pre-operativa, operativa e pos-operativa são incluidas. Uma discussão de métodos sugeridos para o desenho e construção do eletrodo de implantação é apresentada e inclue-se uma lista parcial de materiais, bem como os nomes e endereços dos fornecedores.