

- (13) Hughes, T. R., and Klotz, I. M., "Methods of Biochemical Analysis," Vol. 3, Interscience Publishers, Inc., New York, N. Y., 1956.
- (14) Antonopoulos, C. A., Borelium, E., Gardell, S., Hamnstrom, B., and Scott, J. E., *Biochim. Biophys. Acta*, **54**, 213(1961).
- (15) Bridger, G. L., Salytsky, M. L., and Starostka, R. W., *J. Agr. Food Chem.*, **10**, 181(1962).
- (16) Smith, F., and Montgomery, R., "Chemistry of Plant Gums and Mucilage and Some Related Polysacchar-

- ides," Reinhold Publishing Corp., New York, N. Y., 1959, p. 44.
- (17) Whistler, R. L., "Industrial Gums; Polysaccharides and their Derivatives," Academic Press, New York and London, 1959, p. 652.
- (18) Hirscher, D. A., and Miller, O. H., *J. Am. Pharm. Assoc.*, **NS 2**, 105(1962).
- (19) Le Blanc, J., *Proc. Soc. Exptl. Biol. Med.*, **97**, 238 (1958).
- (20) Korst, D. R., *J. Am. Med. Assoc.*, **170**, 2076 (1959).

_____Technical Articles_____

Technique of Implanting Permanent Electrodes in Cats for Chronic Stimulation and Observation of EEG and Behavioral Effects

By ZOLA P. HOROVITZ and MAY-I CHOW

A procedure for preparing cats with permanently implanted electrodes is described. This technique, which has been used to successfully prepare over 50 of these animals, employs fixation with dental cement and the use of a direct contact connection on the top of the head. The procedure is quick, inexpensive, and very productive. This makes it quite useful in both industrial and academic pharmacological, physiological, and psychological research.

SINCE Hess' pioneer work on stimulation of the unrestrained, unanesthetized cat (1), techniques for stimulating and lesioning or for recording of electrical and behavioral phenomena in the active animal have become useful to the pharmacologist, physiologist, and psychologist. Bradley and Elkes (2), Horovitz and Chow (3), and many others, have used cats with permanently implanted electrodes (P.I.E. cat) for correlating the effects of drugs upon electrical and behavioral responses. This type of preparation has also been used for various types of chronic stimulation by Killam and Killam (4), Doty (5), and Horovitz, *et al.* (6).

The literature, unfortunately, reveals only a few complete descriptions of the techniques involved in preparing these P.I.E. animals. The techniques used by Hess, ingenious for his time, have been outdated by technical advancements. Knowles (7) in 1951 described a method of implantation that employed a base-

plate assembly. This technique requires a large craniotomy and the building of a complicated base-plate head assembly for each cat. Bradley and Elkes (8) in 1953 described an excellent procedure for implanting deep and cortical electrodes but the leads were threaded under the skin of the neck and out the animal's back. This required the animal to carry a bulky harness on its back. Delgado (9) has described a method of implanting multipolar needle electrodes; unfortunately his technique also requires a bulky harness. Delgado (10) has expertly reviewed various implantation procedures and the effects of long term stimulation and recording in animal brains.

Our laboratory has explored various aspects of many techniques for preparing the P.I.E. cat. We feel that the procedure described below is the quickest, easiest, and most productive we have tested. It requires very little laboratory preparation; every item is commercially available, and the animal requires very little maintenance care. We have successfully used this procedure to prepare over 50 P.I.E. cats within the past two years.

Received July 17, 1962, from The Squibb Institute for Medical Research, New Brunswick, N. J.

Accepted for publication August 21, 1962.
The authors wish to thank Dr. B. N. Craver for his advice and encouragement and Dr. L. J. Brannick and Mr. T. Waldron for their invaluable technical assistance.

MATERIALS

Deep Electrodes.—The deep electrodes (Fig. 1-B) are of the bipolar concentric type and consist of insulated 0.0117 in. platinum-tungsten wire inserted into 23-gauge stainless steel hypodermic tubing. The inter-polar distance varies between 0.6–0.8 mm. Each tip exposure is approximately 0.3 mm. A patch wire is soldered onto the tubing about 5 mm. from the top. The length from the tip to the patch solder joint varies, depending upon the electrode placement. Generally, the length is equal to 5 mm. plus the distance between electrode tip and surface of cortex, as determined from the cat brain atlas (11). This allows enough extra length on the electrode for cementing to the skull surface. The tubing is insulated with three or four coats of BC-341 Dolphox epoxy resin varnish and the wire with two coats of Formvar insulation.¹

Surface Corticals.—The surface cortical electrodes are 5-mm. long stainless steel rivets with the heads removed. Flexible lead wires are soldered onto the top of the rivets. The rivets are 1.5 mm. thick and insulated with Insl-X varnish,² except at the tip (Fig. 1-C).

Ground and Support Screws.—The ground wire is usually three or four entwined strands of platinum wire forced into one of the temporal muscles or wound around one of the support screws. These screws are No. 4-36 \times 1/4 in. machine screws.

Electrode Connectors.—The electrode connector is an Amphenol Blue Ribbon 14 pin connector #57-40140 (Fig. 1-A). This connector was found to be the easiest to handle and solder of all the connectors tested.

Cement.—All electrodes and the connector are fixed to the skull with Caulk Grip dental cement. This cement is a quick drying powder-liquid resin that has proved to be very easy to handle. To increase the gripping effect, Caulk dental primer is painted on the skull surface prior to cementing.

METHOD

Most of our cats have been adult female cats of more than 2.0 Kg., deprived of their ovaries and uteri. We have successfully used six male cats, but in general find the spayed female a much more stable and tractable animal. At least two weeks prior to implantation, the cats were immunized against feline pneumonitis and feline infectious enteritis (distemper).

The cats are anesthetized intraperitoneally with 35 mg./Kg. of pentobarbital sodium. Sterile operating procedures have been found to be unnecessary but the electrodes are sterilized overnight under ultraviolet light and all the instruments are soaked in iodine solution³ prior to use. The head and neck are shaved and painted with antiseptic solution. The animal is placed into the stereotaxic instrument and a midline incision is made in the scalp. The incision is approximately 5 cm. in length and ends just behind the occipital protuberance. The temporal muscles are reflected to give the desired skull exposure. Small pock marks

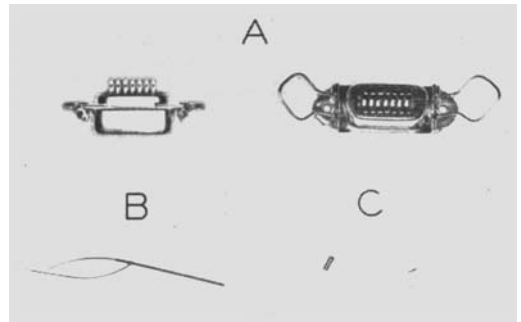


Fig. 1.—A = electrode connector; B = deep bipolar electrode; C = cortical electrode.

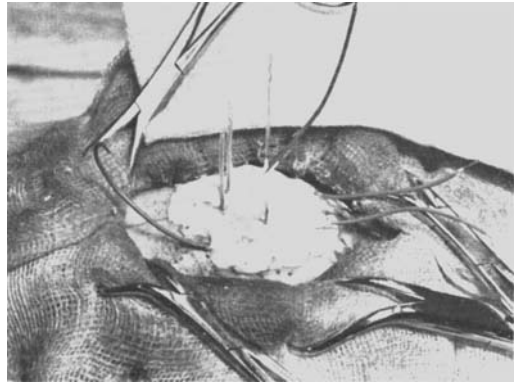


Fig. 2.—Deep and cortical electrodes cemented onto top of skull.

are made on the skull with a No. 1/2 burr on a dental drill. These marks serve as additional gripping points for dental cement and help to hold the electrodes in place. The previously zeroed electrodes⁴ are then placed into the holders and moved to the appropriate lateral and anterior-posterior positions prior to insertion. The electrodes are then lowered to a point just above the surface of the skull and the skull is marked with pencil just below the electrodes. After all the electrode skull-entry points have been marked, they are drilled with a No. 4 burr. Care is taken not to damage the dura. After drilling, any bleeding between the bone plates is stopped by plugging the hole for a few minutes with Gelfoam. After each deep electrode has been inserted into the brain, the skull surface around it is carefully dried with cotton swabs and primer and a small amount of cement applied. About 5 minutes is allowed for the cement to dry before the electrode holder is removed (Fig. 2).

The holes for the corticals are drilled with a No. 5 burr and those for the supporting screws with a No. 8 burr. After these are in place they are also cemented to the skull (Fig. 2). Using an Oryx miniature soldering iron, the previously tinned ends of the electrodes are then soldered onto the appropriate connector pins (Fig. 3). All soldered joints are tested for mechanical strength and elec-

¹ Finished electrodes available from Lehigh Valley Electronics, Fogelsville, Pa.

² Insl-X Corporation, Ossining, N. Y.

³ Weidol disinfectant, Allied Laboratories, Indianapolis, Ind.

⁴ Most of our experiments employed the Baltimore Instrument Company stereotaxic apparatus, model L, Baltimore, Md. The brochure with the instrument provides full and adequate instructions for zeroing electrodes.

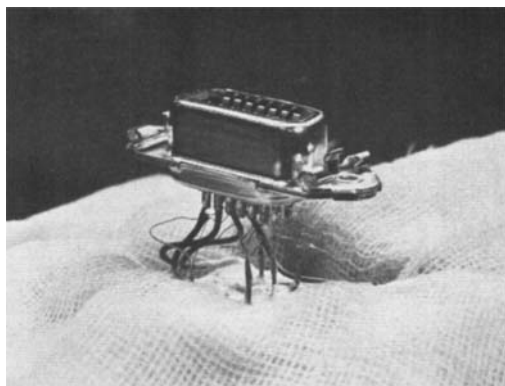


Fig. 3.—Electrode connector showing solder connections with electrodes from brain.

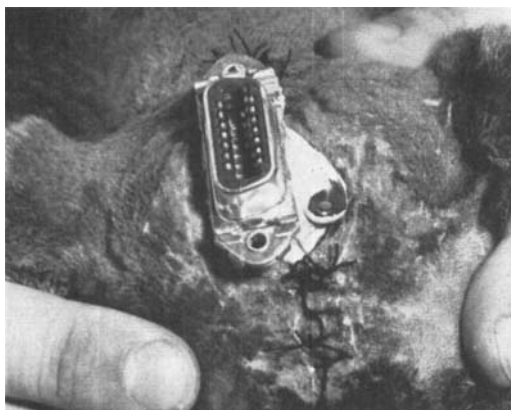


Fig. 4.—Finished preparation with venous cannula.

trical conductivity. The wires are then carefully coiled to avoid shorting and the connector is placed as close to the skull surface as possible. The connector and wires are then cemented to the skull. Care must be taken at all times not to abrade the insulation on the lead wires. The incision is then closed with No. 2-0 surgical silk thread and the animal removed from the stereotaxic instrument. The cat is usually placed in a warm cage overnight and is, in most cases, alert and hungry the following morning. Figure 4 illustrates a completed preparation. The rubber diaphragm on the right is cemented to the skull and attached on the inside to polyethylene tubing which is brought down under the skin and inserted into the jugular vein for subsequent intravenous injections. Figure 5 is an X-ray of another P.I.E. cat.

After a few preliminary scratchings, the cats do not disturb the plug. Any infection that develops under the skin surrounding the connector can be kept under control by cleaning and washing with iodine solution. More severe infections have been successfully treated with a triamcinolone-antibiotic combination ointment.⁵

To record or stimulate, an amphenol male connector No. 57-30140, attached by cable to the stimulator and electroencephalograph apparatus, is connected to the female connector on the animal's head. The connectors are durable, and since the contact is a wiping one there is always a clean contact surface.

It has been our experience that this technique of fixation with dental cement and the use of a direct contact connection on the top of the head is more practical and less bulky, has a longer duration and is better tolerated than any other procedure presently described in the literature.

⁵ Mycolog ointment, E. R. Squibb and Sons, Div. of Olin Mathieson Chemical Corp., New York, N. Y.

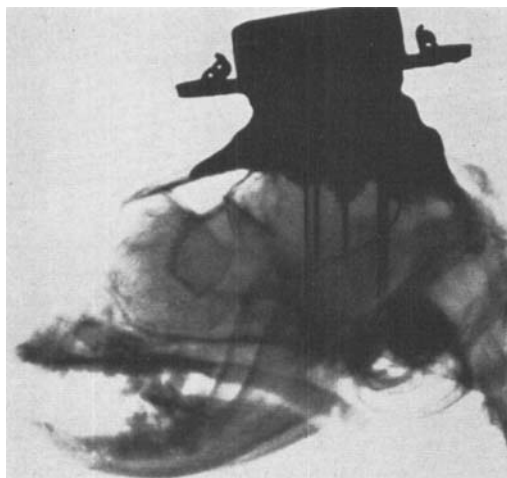


Fig. 5.—Sagittal X-ray of finished preparation.

REFERENCES

- (1) Hess, W. R., "Functional Organization of the Diencephalon," Grune & Stratton, New York, N. Y., 1949.
- (2) Bradley, P. B., and Elkes, J., *Brain*, **80**, 77 (1957).
- (3) Horovitz, Z. P., and Chow, M., *J. Pharmacol. Exptl. Therap.*, **137**, 127 (1962).
- (4) Killam, E. K., and Killam, K. F., "The Effects of Pharmacologic Agents on the Nervous System," Williams and Wilkins, Baltimore, Md., 1959, p. 245.
- (5) Doty, R. W., *Ann. N. Y. Acad. Sci.*, **92**, 943 (1961).
- (6) Horovitz, Z. P., Chow, M., and Carlton, P., *Psychopharmacologia*, **3**, 455 (1962).
- (7) Knowles, W. B., *Proc. Soc. Exptl. Biol. Med.*, **76**, 315 (1951).
- (8) Bradley, P. B., and Elkes, J., *E. E. G. Clin. Neurophysiol.*, **5**, 451 (1953).
- (9) Delgado, J. M. R., *Yale J. Biol. Med.*, **24**, 351 (1952).
- (10) Delgado, J. M. R., in "Electrical Stimulation of the Brain," University of Texas Press, Austin, 1961, p. 25.
- (11) Jasper, H., and Ajmone-Marsan, C. A., "Stereotaxic Atlas of the Diencephalon of the Cat," National Research Council of Canada, Ottawa, 1956.