PERMANENT IMPLANTATION OF MULTILEAD ELECTRODES IN THE BRAIN*

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Anesthesia and restraint of the animal subject are the two main factors which may modify responses evoked by stimulation of the brain. However, much of the information concerning brain physiology is of necessity based upon experiments performed under one or both of these limiting circumstances. The techniques developed thus far for the study of brain function in unanesthetized animals are described in the following paragraphs.

1. Hess,[7] one of the pioneers in the field, was the first successfully to implant electrodes in the diencephalic structures of the cat. Using the sutures of the skull as reference points, a metallic frame with a superstructure is screwed to the parietal bone. This superstructure serves to support the electrodes while they are being driven into the brain. It is then removed. Remaining are the metal frame and the electrodes. Since Hess's experiments were of short duration, aseptic precautions were not taken.

2. Variations of the remote control technique have been used by Chaffee and Light,[1] Clark and Ward,[3] Fender,[5] Loucks,[12] and Mauro.[14] In this method a small induction coil having terminals inside the brain is placed under the skin. Stimulation is accomplished by applying a magnetic field to the head of the animal and then inducing a current in the subcutaneous coil. The main advantage of this is that no leads pierce the skin. Some of the difficulties and limitations of the remote control techniques are: (a) the number of electrodes is limited to one or two; (b) it is impossible to record electrical activity from the brain; and (c) it is difficult to control the parameters of stimulation accurately.

3. Pachon and Delmas,[15] Hunter and Jasper,[9] Clark and Ward,[2,4] Hoagland,[8] Lubinska and Konorski,[13] Rheinberger and Jasper,[10] and Gastaut[6] used variations of a general procedure that consisted essentially of screwing to the skull a small plastic or stainless steel base in which were inserted the electrodes (accomplished either with or without a Horsley-Clarke apparatus). In some instances aseptic procedure was followed.

The present paper describes a technique for aseptically implanting a large number of electrodes (fourteen to forty). This makes it possible to study individually or simultaneously many points on the surface and in the

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interior of the brain for periods of as long as a year without any resulting ill effects to the animal subject. During the experiments the animal has complete freedom of movement.

METHOD

The two types of electrodes which have been employed are: (i) a "needle electrode" for study of regions in the interior of the brain; and (ii) a "plate electrode" for study of areas on the brain surface. There are slight differences in the electrodes depending on the species of animal in which they are to be used. Following are descriptions of the kinds adapted for use on the cat or monkey.

Needle electrode (Fig. 1). Enameled stainless steel wire 0.005 inch in diameter is used. Pieces of approximately 18 cm. are straightened by stretching, and the tips which are to form the needle are scraped bare of enamel for 1 mm. The opposite ends are also scraped for 15 mm., and then the perfect isolation of each electrode is tested by using an ohmmeter.

After the test one wire is held between the left thumb and forefinger and another wire is placed beside it so that the tips are 2 mm. apart (the distance may change in accordance with the needs of each experiment). By means of a small glass rod the wires are cemented with liquid plexiglas. Several coats are applied. When these are dry, a third wire is cemented in place in the same way as before; then in succession four more wires are cemented to form a single body of seven wires with each tip 2 mm. apart. These seven wires form an electrode needle that is 0.5 mm. in diameter. The opposite tips of the electrodes are soldered in some identifiable order to a miniature seven-pin socket.

The group of seven electrodes is protected by polyethylene tubing, which in the portion close to the socket is exactly adapted to the electrodes by means of a small flame and in the distal part is occluded and attached to the electrodes with liquid plexiglas.

Each wire is again tested with the ohmmeter (one pole in the socket and the other in the free tip of the wire). After washing it with soap and water, the electrode needle is immersed in Zephiran 1/1.000 for sterilization.

Plate electrode (Fig. 1). A piece of polyethylene tubing approximately 15 cm. long and 1.5 mm. in diameter is slit open at one end for 30 mm. This part is spread out and pressed with a warm iron (not too warm) to form a small plate at the end of the tubing. Using a sewing needle, seven holes are punched 2 or 3 mm. apart along the midline of this plate.

Seven pieces of enameled stainless steel wire (0.005) are cut and straightened by stretching, and one tip of each is melted to form a small ball (see Riley).[17] After assuring the isolation of all the electrodes with the ohmmeter, they are put through the individual holes prepared for them in the polyethylene plate; then all are threaded through the tubing.

Each electrode is soldered in some specific order to the pins of a miniature radio tube socket, and with liquid plexiglas the electrodes are fixed to the posterior part of the plate. Employing suction, a small amount of liquid plexiglas is passed through the tubing and dried in place with a current of air. The tubing is closed by applying a flame to the end close to the socket and liquid plexiglas to the distal extremity.

The electrode may be used with the small balls protruding from the plastic plate, but it is better to abrade the balls with a fine emery wheel to form minute flat buttons.

Surgery for Implantation

Needle electrodes. The animal is operated upon under general anesthesia (e.g. Nembutal), and aseptic precautions are taken which include the sterilization of the stereotaxic instrument. When the head and the posterior part of the neck have been shaved, the animal is placed in the Horsley-Clarke apparatus.

An incision, 4 or 5 cm. long, is made exactly in the midline of the head ending at the occipital protuberance. Another very small incision is made in the posterior part of the neck through which a rubber tubing is passed beneath the skin in the direction of the principal incision. By means of a strong needle the base of the occipital protuberance is perforated and strong sutures are then passed through.

The coordinates of the stereotaxic instrument are adjusted so that the exact point may be marked on the skull. Using a trephine 1.5 mm. in diameter this point is opened. The previously placed rubber tubing is used to pass the needle electrode beneath the skin of the neck so that it may be attached to the micromanipulator of the Horsley-Clarke instrument. The dura is punctured and the electrodes are inserted to the desired depth. The small hole in the skull is closed with dental cement, which holds the electrodes firmly in position. Only a minute quantity should be used in order to avoid the formation of an excessive protuberance on the skull.

When the cement is dry (in about five minutes), the needle electrode is removed from the micromanipulator and is curved 90 degrees for application to the surface of the skull. Then the electrodes are fixed to the occipital protuberance by means of the sutures that were placed earlier (Fig. 2).

The implantation is repeated for each group of electrodes. The muscle, subcutaneous tissue, and skin (intradermic) are sutured, and the operation is finished.

Plate electrodes. The surgical technique for the implantation of plate electrodes in order to study the surface of the brain is similar to that described above with the following differences. The Horsley-Clarke instrument is not needed. The trephining of the skull must be about 1 cm. in diameter. The dura is opened with a fine knife and the plate electrode is slipped between the brain and the dura to the desired position. (It is also possible to make the craniotomy larger and to place the electrodes under visual control.) The electrodes are fixed in position by means of sutures passed through the bone at the edge of the orifice of trephining. The electrodes are rigid enough to stay placed, supported by the sutures, and at the same time flexible enough to remain in position in spite of cerebral pulsatile movements.

Adaptation of the technique to different circumstances. In a small animal such as the mouse there is no occipital protuberance for fixing the electrodes and it is necessary to perforate the occipital bone without piercing

the dura in order to pass the sutures through. In this animal it is necessary to reduce the size of the burr hole, thus a trephine of only 0.5 mm. is used. Also, the distance between the electrodes which make up the needle or the plate must be reduced to 1 mm. (instead of 2 mm.), but of course this distance depends upon the type of experiment.

If one wishes to study only a few points in several different regions of the brain (for example, eight zones situated at different coordinates), needles or plates with only two, three, or four electrodes may be used. In such a case it is possible to solder the electrodes to the miniature radio tube socket after the implantation. During the soldering, the electrodes must be cooled with ice in order to avoid conduction of heat to the brain. If the needle electrode is made up of only three fine wires, it is convenient to increase the size of the first one to give rigidity to the needle. Therefore, the first electrode will have a diameter of .010 inches instead of .005 inches.

The sockets of the electrodes must be protected by a plastic collar for support and to avoid interference with the movements of the animal (Fig. 3). Cats and dogs accept the collar without difficulty, but mice and monkeys try to remove it during the first days. For this reason either of these species must become accustomed to a collar for two weeks before the operation. In experiments of long duration the normal head motility of an animal may cause breakage of the electrodes because of the continuous bending at the point of suturing to the occipital protuberance. To avoid this, electrodes must be protected by a spring made by winding a fine stainless steel wire around the plastic tubing. The spiral should start 2 to 3 mm. before the suture and end 10 mm. behind it. This flexible spiral buffers the movements and, furthermore, may be used as an indifferent electrode for monopolar stimulation or recording.

Discussion

The implanted electrodes are well tolerated. No infection occurs despite the omission of antibiotics, and the animals remain in such excellent health that they jump and play with their companions without displaying any ill effects whatsoever.

Each needle of seven electrodes cemented and protected by plexiglas has a diameter of 0.5 mm. The remaining length of the electrode, which is protected with polyethylene tubing, has a diameter of 1.5 mm. This small size, aseptic technique, and the use of materials that cause minimum tissue reaction[10, 11] allow long-term placement of the electrodes within the brain. Cerebral damage is limited to that resulting from the insertion of the needle which causes a small gliosis (Fig. 4), and the electrodes may stay in for several months or even a year without detriment to the animal. In the case of plate electrodes there is no damage at all.

The stability of the position of the electrodes—both surface and depth—makes it possible to carry on an investigation for as long a time as is neces-

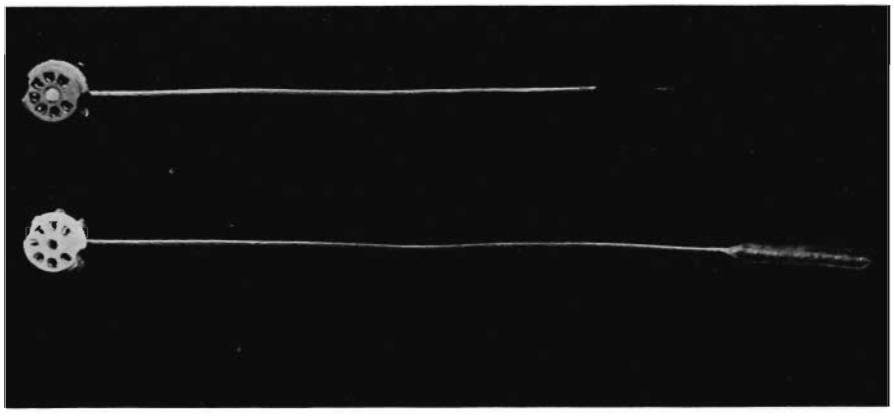

FIG. 1. Needle electrode (up) for studying the inside of the brain. It is formed by seven isolated wires with each tip 2 mm. apart from the others. The "needle" has a diameter of only 0.5 mm.

Plate electrode (down) for studying the surface of the brain. The "plate" holds the tips of the seven electrodes in place on the cerebral cortex.

FIG. 2. Two groups of needle electrodes implanted in the nuclei of the amygdala of a squirrel monkey. Placed with the use of a Horsley-Clarke instrument, they are cemented to the bone and tied to the occipital protuberance.

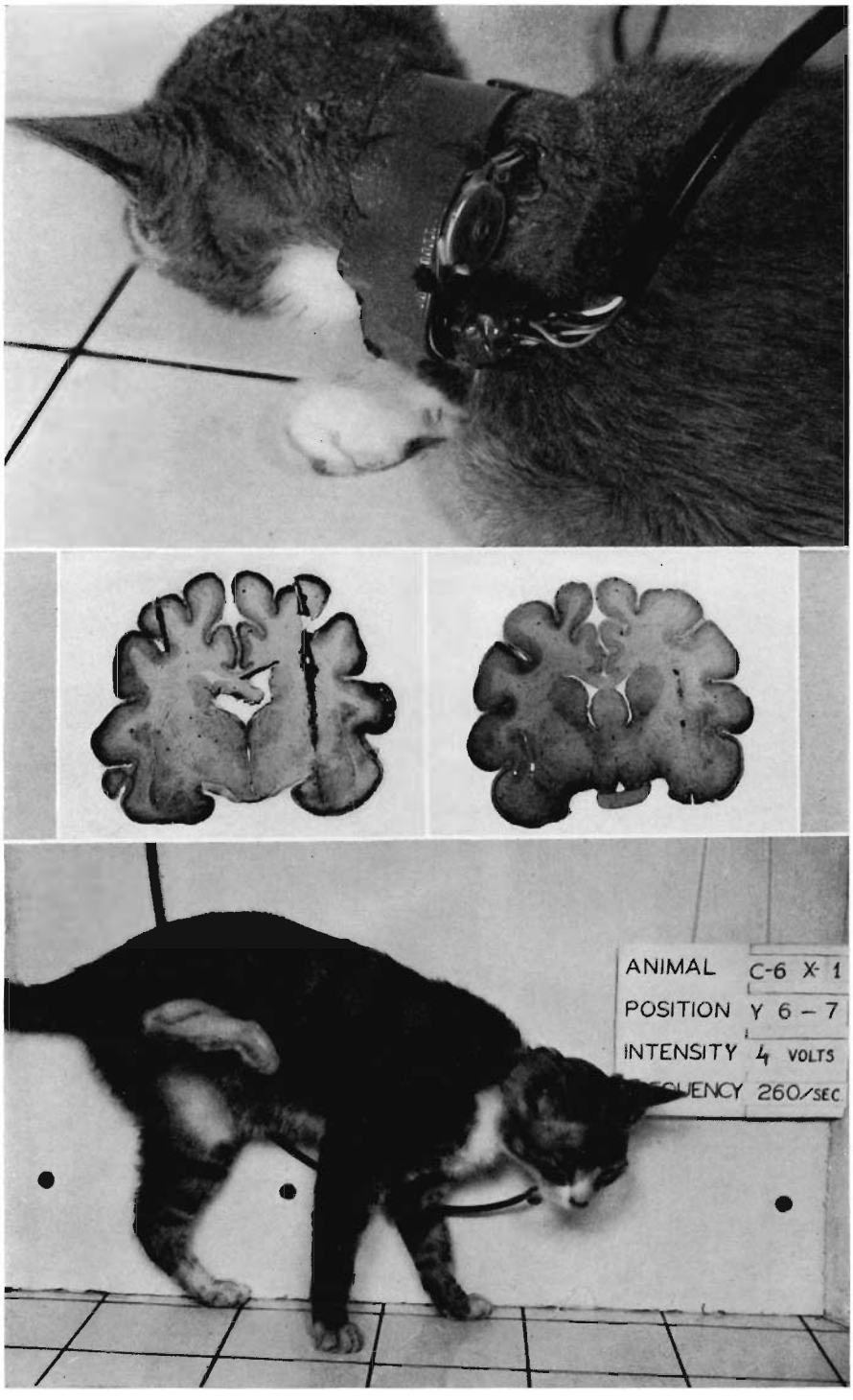

FIG. 3. Electrode slides beneath the skin of the neck and ends in a small female socket. Connection for stimulation, recording, or destruction is made by plugging in the special male socket.

FIG. 4. Path of the needle electrodes inside the brain. After months of implantation they are well tolerated. Only a small gliosis appears in the path of the electrodes.

FIG. 5. Stimulation of cortex buried inside the sulcus cruciatus in the conscious cat. A motor effect is evoked without emotional disturbance of the animal.

sary to make repeated observations of the functions of specific areas of the brain under varied conditions. The durability of the preparation permits its use in problems which require training, such as those encountered in psychology.

The avoidance of anesthesia has great value because drugs modify the excitability of the brain, and because during anesthesia sensitivity and normal activity are absent. Implanted electrodes make possible studies of behaviour, emotions, etc.

One of the greatest advantages of the method is the multiplicity of the electrodes. In general fourteen electrodes (two groups) or twenty-eight electrodes (four groups) have been used, but if necessary it is easy to increase the number (in some experiments I have used forty electrodes). The multiplicity of electrodes is especially useful for making correlations between different regions of the central nervous system.

By means of implanted electrodes the following operations may be performed: (a) stimulation, (b) recording, (c) making lesions by electrocoagulation, (d) recording of evoked potentials, and (e) all combinations of the preceding operations such as simultaneous or delayed stimulations of cerebral or cerebellar points, chronic stimulation (for example, one hour daily) of particular regions, etc.

The animal is connected to the recording or stimulating instruments by using a plastic-shielded, seven-wire cable ending in a male socket whose female equivalent is situated on the neck of the animal. Connection is made by merely plugging the sockets together. The system provides a good contact and is very convenient. The simplicity of the plugs overcomes the complexity of earlier techniques, one of which required the soldering and unsoldering of each wire for every experiment.

An animal accustomed to a collar and a plastic leash is unable to differentiate between his leash and the plastic cable and therefore does not know he is the object of attention. Thus inhibitory factors, so important in behaviour studies, are avoided. Freedom of movement during an experiment depends on the length of the plastic cable which, especially for stimulation, may be very long allowing unconstrained activity.

Usually experiments are conducted on a specially constructed stage (Fig. 5) that has good illumination, transparent windows, and a clearly legible adjustable sign on which can be indicated the day, hour, animal number, position of electrodes, and the parameters of stimulation. The stage arrangement is particularly useful for taking still and moving pictures and for making sound recordings. For stimulation the only necessary equipment is the stimulator, therefore it is easy to do experiments in any location, outside or inside the stage—an advantage since it prevents the animal from becoming conditioned to its surroundings. This is especially the case when an experiment involves repeated stimulations of areas dealing with emo-

tional behaviour (thalamus, hypothalamus), but it is not so important in the stimulation of the motor regions.

A well-known stereo technique is used for implantation of the needle electrode, exact placement at a predetermined point being easily accomplished. Histological study of the brain is necessary in order to check the exactitude of the position of the electrodes. In the plate electrodes a still picture and visual inspection will give the position of the electrodes. To know their precise location a continuous current is passed between electrodes 1 and 7 for five seconds to produce a small electrolytic lesion which can be found when the animal is sacrificed.

Two arguments may be presented against the technique. First, the presence of a foreign body inside the brain may disturb normal function. The importance of this factor is probably very small in the case of plate electrodes which produce no damage, but it is greater in the case of needle electrodes which produce a small but destructive lesion of brain tissue. After several months of implantation some gliosis appears around the needle electrodes. However, the extent of this gliosis is limited to about 0.5 mm. around the path of the needle. The histological aspect of the rest of the surrounding brain tissue is entirely normal.

Second, since the animals have spontaneous activity (motor and electrical), one must differentiate between normal activity and that artificially evoked by the experiment. Fortunately, since the experiments may be repeated as many times as necessary, it is possible to ascertain the difference. The spontaneous movements of the animal very appreciably complicate the electrical recording of the brain activity because of the artefacts produced by these movements. Differential amplification reduces artefacts, and in some experiments one must wait until the animal becomes inactive or sleepy. Since recording does not disturb the animal, it is easy to obtain good results. This technique has been tried successfully on various kinds of animals. Mice, cats, dogs, and monkeys with electrodes implanted in the frontal lobes (superficial and hidden cortex), lateral hypothalamus, amygdaloid nuclei, cingulate gyrus, cerebellum, etc., have been studied for periods of months. The presence of the electrodes did not disturb the health of the animals, and after regrowth of the hair shaved off for the operation it was impossible to differentiate between normal animals and those that had been operated upon.

Good biological tolerance of implanted electrodes and their successful use for local stimulation, recording, or destruction suggest the possibility of applying this technique to humans for diagnosis and therapy of cerebral disorders. This problem is at present under consideration.

Summary

A technique for implanting electrodes for long periods of time inside the brains of mice, cats, dogs, and monkeys has been described. Two kinds of

electrodes were presented: plate electrodes for the surface and needle electrodes for the interior of the brain. The first is placed under visual control, and the second by means of the Horsley-Clarke instrument. The particular advantages of this technique are as follows:

1. Large numbers of electrodes can be employed (14 to 40).

2. Several discrete points of the brain (surface and interior) can be studied individually or simultaneously.

3. Because of the shape and small size of the electrodes, there is no brain trauma with the use of the plate electrode, and a very limited amount of trauma in the case of the needle electrode (the diameter of the needle formed by seven electrodes is only 0.5 mm.).

4. It has been established that by employing biologically inert materials in the construction of the electrodes no tissue reaction is evoked by the plate electrode and only a very limited gliosis by the needle electrode.

5. Virtually all regions of the brain are made accessible for study.

6. The electrodes may remain implanted for long periods of time without causing any observable adverse effect upon the health or behaviour of the animal subjects. (Maximum period of observation one year.)

7. The electrodes may be employed for stimulation, recording, and production of lesions (electrocoagulation).

8. During experiments the animals have complete freedom of movement.

9. Connecting the animal to the electrical instruments is accomplished merely by plugging two sockets together at the neck of the animal.

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