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THE STEREOTAXIC METHOD AS AN EXPERIMENTAL PROCEDURE FOR STUDYING BRAIN ACTIVITY

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Neurosurgeons and neurophysiologists have long understood that, for precise selective stimulation or destruction of the subcortical nuclei, manual surgical techniques are inadequate. This is due to the fact that sometimes such procedures have caused extensive traumatization of tissues, that profuse hemorrhages have often developed, and that serious threats of concurrent infections have been created (Bekhteryev and Mislavs'kyy, 1891 (5); Probst, 1900 (84), and others.

Therefore, some investigators have begun to use, for purposes of destroying subcortical structures, needles through which chemical substances can be introduced which destroy the tissues of the brain in the region of the needle tip, or through which a direct current of electricity can be admitted under high voltage.

The electrolytic method of selective destruction of subcortical nuclei and of stimulation of them with an electric current was first used by L. N. Simonov in 1866 and by V. M. Bekhteryev's associate, Khol'tsinger, in 1895.

In order to enhance the precision of access to appropriate regions of the brain, a number of authors began to work out the prerequisites for proper orientation. However, there was much empiricism and pure chance in many of these studies.

An important step in the development of experimental neurophysiology was the invention of the stereotaxic method of anatomical-physiological studies of the brain in 1908 by Horsely and Clark (64). This method at once substantially increased the accuracy of placement of electrodes or other instruments in the necessary areas of the brain, and the simplicity and accessibility of the method expanded the range of neurophysiologic experiments. It is important to emphasize that the placement of electrodes by the stereotaxic

method came to be performed by a practically bloodless technique, and the minimal traumatization of the brain considerably lightened the course of the postoperative period and increased the survival rate of animals subjected to operation.

Principles of the Stereotaxic Method

The stereotaxic method is based on the principles of determination of the position of the subcortical nuclei with respect to the intersection of mutually perpendicular planes which transect certain portions of the skull and brain. The method of Horsely and Clark involves such planes of departure, or as they call them, "null" planes, as the following:

(1) the basal "null" horizontal plane which passes between the centers of the external auditory canals and the inferior borders of the orbital fossae.

(2) the sagittal "null" plane, which passes through the skull between the hemispheres in the sagittal direction.

(3) the "null" frontal plane, which passes through the centers of the external auditory canals perpendicularly to the basal horizontal "null" plane.

Therefore, all planes are oriented at right angles to one another. The fact that in the majority of animals the basal "null" plane (horizontal) lies beneath the base of the brain throughout almost its entire extent has, of course, complicated the calculation of the coordinates, in connection with which this plane was subsequently raised ten mm. Some authors define the basal horizontal plane as one passing between the centers of the auditory canals and the superior borders of the orbital fossae, which, according to Whittier and Mettler, more nearly corresponds to the long axis of the brainstem.

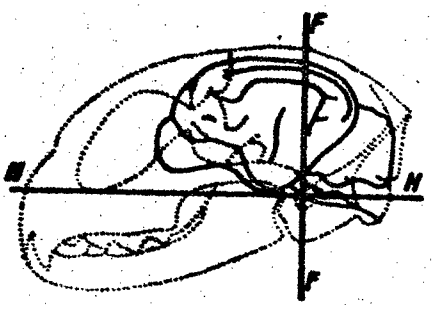

Fig. 1. The "null" planes for the cat brain

To perform an experiment with the stereotaxic method, it is necessary to place the head of the animal in a stereotaxic apparatus and to orient it carefully. For this purpose, metal rods are introduced into the external auditory canals until they rest against the bony ring of the canal. Then the head is secured by a special headgear, the uppermost projections of which fit against

the inferior edges of the orbital fossae, and curved pressure rods fit behind the teeth and, by pushing up, fix the upper jaw. Turning the apparatus brings the head into a position such that the inferior edges of the orbital fossae and the centers of the external auditory canals are in the basal horizontal plane of the apparatus. In the majority of apparatuses this process of arranging congruence is completed immediately upon fixing the head. Certain difficulties arise from the insertion of the rods into the external auditory canals, especially in dogs, due to the curvature of the cartilaginous portion. In order to facilitate this step in the procedure, it is necessary to straighten out the auditory canal by retracting the ear.

Lovenfeld and Altman propose the use of curved rods for quick and atraumatic insertion of the electrodes.

An essential condition in working with stereotaxic instruments is careful verification of the "null" position. Upon bringing the ends of the electrodes to the centers of the rods or to the control column, all scales of the apparatus should register zero.

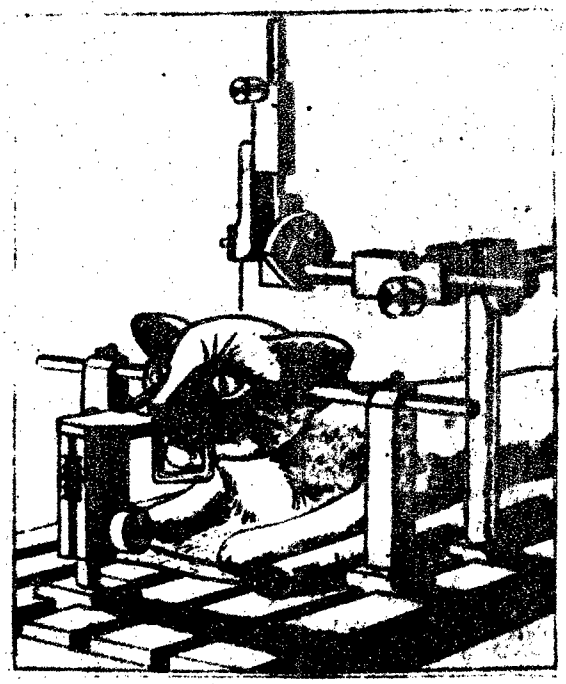

Fig. 2. Model of a stereotaxic apparatus devised by Lovenfel'd and Al'tman

After securing and orienting the head, it is necessary to ascertain the projections of the desired subcortical nuclei into which the electrodes are to be placed. For this purpose, the stereotaxic coordinates may be found in atlases or in suitable tables. These references show the area occupied by the nuclei in each frontal section, the sections being taken at one mm intervals. All sections which proceed rostrally from the frontal "null" plane are marked with a plus sign (+), along with a number indicating in millimeters the distance of the section from the "null" plane, and those proceeding caudally are similarly marked, but with a minus sign (-). In the selected section, the area is next determined for the given nuclear formation with respect to its position in relation

to the basal horizontal "null" plane. The vertical projections are designated with a plus sign (+) if they lie above the "null" horizontal plane, and with a minus sign (-) when the position is inferior to it. Likewise, the medial and lateral projections are determined. On the surface of the skull the projections of the selected nucleus are brought into congruence by adjusting the apparatus until there is agreement between the readings on the scale and the values given in the atlas for the coordinates of the nucleus.

At the points on the skull thus determined, the skull is trephined and the dura mater penetrated. By adjusting the micromanipulator of the apparatus, the electrodes are introduced into the required nucleus depending on the depth of its position and its relationship to the basal "null" horizontal plane. In doing this, it is necessary to keep in mind that in some atlases the horizontal plane is ten mm higher. In a number of cases the electrode is inserted carefully at an angle, especially in experiments involving the retrotentorial portion of the brainstem. The micromanipulator ensures precision of insertion and placement, as well as displacement of the microelectrode during changes of position involving distances calculated in microns and parts of a micron. The stereotaxic instrument may also be used for microelectrode recording of the potentials of individual neurons of the cerebral cortex and the spinal cord.

Stereotaxic Atlases

For insertion of electrodes into the brain by the stereotaxic method, it is necessary to know the topography of the nuclei of the brainstem at different cross-sectional levels in their spatial configurations and their relations to the "null" planes.

A general anatomical orientation in these matters is given:

(a) for monkeys, by the atlases of Monnier (81) and Olszewski (83);

(b) for rabbits, by the atlases of Warren (104), Meesen and Olszewski (79), Rose (91), Winkler and Potter (106), and others;

(c) for cats, by the atlases of Monnier (81), Ingram, Hannet and Ransom (67), Hess (60, 61, 62), Winkler and Potter (106), and Rioch (87);

(d) for dogs, by the manual of Elenberger-Baum (55), the Atlas of the Brainstem in Humans and Animals published by the Brain Institute of the Academy of Medical Sciences USSR (1), the manuals of Flatau and Jacobsohn (56, the atlas

of Hoffman (63), and the thorough works of Rioch (87, 88, 89), which are devoted to studies of the configuration of the nuclei of the optic tubercles and of the fibers which connect them.

On the basis of these antomico-histologic atlases of the brainstem of different animals, a number of experimenters and morphologists have published special stereotaxic atlases and guides.

It is pertinent to list some of these here:

(a) for monkeys, the atlas of Olszewski (83);

(b) for cats, the atlases of Jasper and Aymone-Marsan (68), Jimenez-Castellanos (69), and also of Reinoso (85);

(c) for rats, the atlas of Krieg (72);

(d) for dogs, the tables of position of some of the subcortical nuclei of the brainstem compiled by Leontovich and Mering (19) and by Mogilevs'kyy (24).

In working out the coordinates for stereotaxic atlases of the brain, use is ordinarily made of formalinized brain tissue from animals in which, prior to the study, the skull of the animal is removed and the brain dissected away in situ in the frontal and horizontal planes until the desired brain structures are exposed. Then the head is secured in the stereotaxic apparatus and the ends of the electrodes are approximated to the given nuclear formation. The positions of the nuclei are then read from the scale of the stereotaxic apparatus.

This method is used in several modifications. Thus, Loewenfeld and Altman (77) remove only half of the vault of the skull and measure the vertical coordinate. With a median sagittal section they remove half of the brain down to the level of the tentorium, which permits visualization of the cortex and brainstem to the level of the protuberances of the corpora quadrigemina. After this, at various intervals along the sagittal "null" plane, measurements are made of the coordinates of the contours of the visible formations of the brain. Since the midline ordinarily is not known precisely in the brain, measurements are made two mm from it. The structures of the brainstem are ordinarily measured in the midline, since for them the midsagittal plane is a natural plane of division.

In connection with the fact that, at a given age, condition, and sex, the measurements and configurations of the skull and brain in many animals (cats, monkeys, rats, rabbits) are relatively constant, the compilers of atlases compute arithmetic averages for the values of the coordinates of the desired nuclei. Such a method affords the possibility of representing diagrammatically the average contours of the brain formations.

There are also other methods of stereotaxic description and reconstruction of the brain in three dimensions. With one such method, fine wires are passed along the basic "null" planes which, after formalinization of the brain, are withdrawn, and serial sections are then made of the brain. With the use of these sections it is easy to compute the relationship of any nucleus to the residual defects left by the wires in the tissues along the "null" planes. This is the principle underlying the stereotaxic atlas of the brain of Jasper (68). By semischematically superimposing tracings of the nuclear formations of every tenth section of the brain, Jimenez-Castellanos (69) has been able to investigate the changes in directions of nuclei and has produced with this method a three-dimensional model of the nuclei of the optic tubercles.

In the atlas of Hess (60), the positions of the subcortical nuclei of the brainstem of the cat are calculated in angular coordinates. The original or "null" point in this technique is the point of intersection of the coronal suture with the sagittal plane. Since, in the compilation of the atlas, the electrodes were projected on the formations of the brainstem and of the cortex with respect to medial sections of the brain, the possibility is afforded of determining the proper depth for the electrodes upon insertion through any point in the skull.

Reinoso (85) in his atlas endeavors to work out the coordinates of a number of morphologic formations of the brainstem of the cat by combining in this atlas the stereotaxic method and the method of Hess, for which purpose he carefully measures the thickness of the calvarium above the projection of the formation in question and compares them with a whole series of bone orientations.

Desmedt and Franken (52) have described the retrotentorial portion of the brainstem in cats in angular stereotaxic coordinates, in connection with the fact that this part of the brainstem, as it were, is covered by the osseous cerebellar tentorium. These investigations have considerably facilitated the performance of experiments on the medulla oblongata and the pons Varolii using the stereotaxic method.

Stereotaxic Apparatus

There are several types of stereotaxic apparatus.

(1) The rectangular stereotaxic apparatus of Horsely and Clark which is composed of two parts: (a) a basic holding device for immobilization of the head and for the designation of the primary planes, and (b) a quadrilateral

rectangular calibrated frame on which the electrode device is mounted. The electrodes in this apparatus move parallel to the "null" planes. Clark and Henderson (47), and later Harris (59) added to this apparatus a mechanism for changing the angle of inclination in the sagittal plane, and Spiegel and Miller (95) brought about the possibility of sterilizing the instrument.

(2) The equatorial stereotaxic apparatus of Clark and Henderson, which consists of (a) a securing device for the head and (b) a small rotating table. This table comprises two flat, concentric rings. The outermost ring can be rotated in a circle and supports a circular arc which passes over the surface of the skull. An electrode device is mounted on the circular arc in such a fashion that it can be moved along the arc and placed at any desired angle regardless of the position of the arc. This instrument permits universal insertion of the electrodes and at the same time allows various approaches to the subcortical structures.

Brown and Henry (40), in 1935, constructed an apparatus which consists of a single piece comprising the securing device for the head and the circular arc, which can be rotated in the rostrocaudal direction about an axis passing through the centers of the external auditory canals. In this apparatus the electrodes can be insterted in various planes and at various angles. Carpenter and Whittier (45) call this apparatus a "combination of the head-holding device and of the manipulator of the goniometer".

(3) Modern stereotaxic apparatus with micromanipulators must be simple to prepare and to use, sturdy, of great precision, and must also permit insertion of the electrodes in any direction. A large number of diversified stereotaxic apparatuses are now known which have been recommended for operations on the brains of several species of animals.

Carpenter and Whittier report on the as-yet unpublished construction of the stereotaxic apparatus of Carlyle and Petri and the modification of it by King and his associates for monkeys. The stereotaxic instrument of Carpenter and Whittier (45) for monkeys, cats, and certain other laboratory animals is a U-shaped frame with devices for the ear rods and with a support for the superior or inferior borders of the orbital fossae, which together define the basal "null" horizontal plane. At the edges of the frame is a universal micromanipulator, mounted so as to be mobile, with the electrode device. With the aid of the micromanipulator the electrodes may be inserted rectilinearly in any direction and at any angle, and rotary motion may also be effected.

Stellar and Krause (98) have proposed a stereotaxic instrument for small laboratory animals constructed in accordance with the equatorial principle.

Attention is merited by the curious construction of the stereotaxic apparatus of Beattie (36) for experiments on the brains of white rats. In this, use is made of the principles of the panthograph, in which a system of levers precisely duplicates the finest movements of the receptor device in ratio of 1:4. In the apparatus the micromanipulator is closely connected with one end of the panthograph, the other end of which is moved about an enlarged contour drawing or chart of the surface of the brain of a given animal. The head of the animal is held in a strongly secured position so that the point of intersection of the bregma and the sagittal suture corresponds to the corresponding point on the chart.

Upon placing the rod of the receptor instrument of the panthograph on the picture representing the nucleus, as drawn on the chart, the electrode, which is fixed in the micromanipulator, is located precisely over the projection of the desired nuclear formation in the brain. This principle has been used by Krieg for experiments on white rats.

Of the existing stereotaxic instruments, mention should be made of the apparatus of Lister and Sherwood (75) in which a micromanipulatora and a securing device are combined. The model of Della deserves mention, also. These apparatuses belong to the class of linear stereotaxic instruments. Stereotaxic apparatuses have been described for cats by Loewenfeld and Altmann (77), for small laboratory animals by Clark (46), for monkeys and cats by Harrison (59), and also for cats, dogs, and certain other smaller laboratory animals by Cort and Harding (49). In the latter apparatus, instead of moving the electrode device in different planes, the head of the experimental animal is moved. Mention should also be made of a model for stereotaxic apparatus for dogs described by V. Traczyk (101). Hume and Canong (66) have proposed a linear rectangular type of stereotaxic instrument for dogs and monkeys.

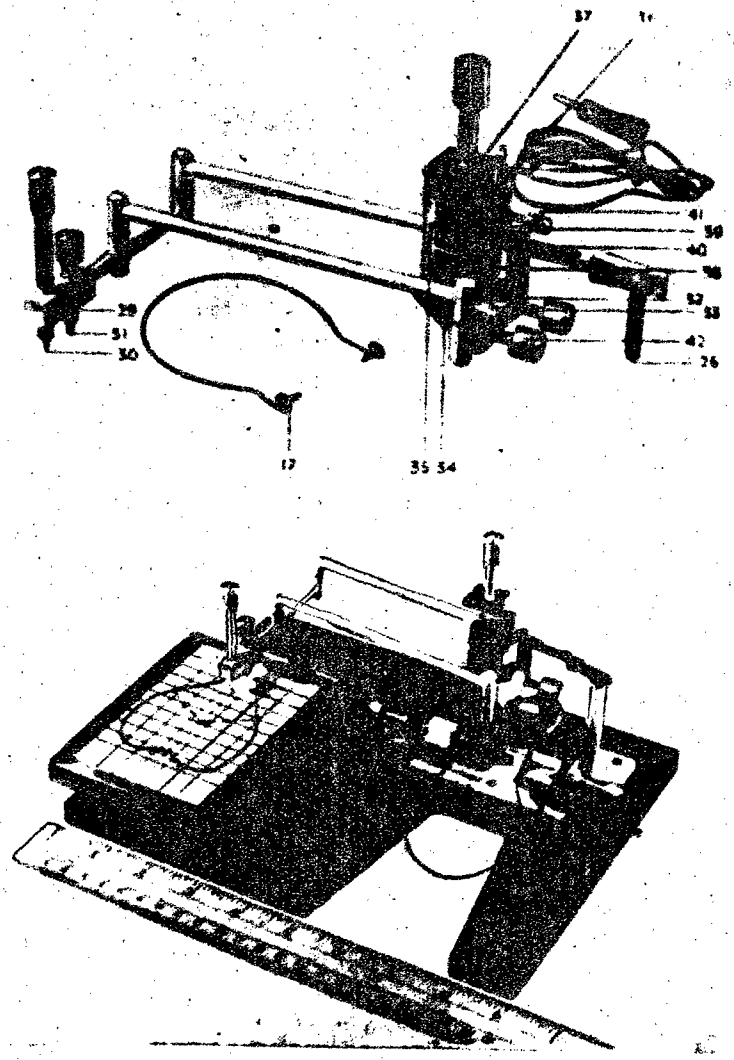

Fig. 3. Stereotaxic apparatus of Beattie for rats, with panthographic construction.

In the Institute of Normal and Pathologic Physiology of the Academy of Sciences USSR, Durinyan and Bartyzel' (12) have constructed an original stereotaxic apparatus which the authors call the "stereotaxic instrument DB-1-57". This apparatus belongs to the class of rectangular linear constructions and is designed for experiments on cats and certain small laboratory animals. Special devices in this apparatus permit placing the head of the animal at any angle of inclination, and the micromanipulator of the apparatus effects movement of the

electrodes in three mutually-perpendicular planes with a precision of up to ten microns. Particularly clever is the inclusion in the apparatus of a device for warming the animals, and also an adaptor for purposes of experiments on the spinal cord.

Meshcherskiy (23) reports on an improved stereotaxic apparatus for small laboratory animals.

Chereshnev (33) has devised a simplified apparatus, similar to a stereotaxic apparatus, for inserting electrodes into the hypothalamus in the dog. The apparatus is provided with a special head-holder and with a needle for mechanical destruction of the hypothalamic region. For insertion of electrodes into subcortical structures, use has also been made of various adaptors, holders, oriented directional sleeves, and cannulas which, ordinarily, cannot compete in precision with the stereotaxic method (Hess (60); Knowles (71); Pavlygina (27); Kogan (14); Kletskin (70); Maire (78) and others).

A number of subcortical formations can easily be demonstrated by filling the ventricular system of the brain with a substance which is radio-opaque (sodium urocon in an amount of 0.1 ml). The recording of ventriculograms permits recognition of the silhouettes of the ventricles, the hypothalamus, and the formations which border upon the third ventricle, as well as the massa intermedia and the corpora mamillaria. Using calculations derived from the ventriculograms, Hume and Canong (66) have inserted electrodes very precisely into the hypothalamus of the dog with the use of the stereotaxic apparatus. The principle of roentgen control has also been used in the experiments of Chereshnev (33). The incorporation of both stereotaxic and roentagenographic apparatus into a single instrument indisputably has great prospects. Mention may also be made of the use for this purpose of roentgenographic "grat" [?], which may facilitate considerably the simultaneous use of both of these methods.

Determination of the Localization of the Electrodes

The coordinates of a number of the subcortical nuclei cannot always be precisely located. To a considerable extent this depends on individual variations in the skull and brain of the animal being studied, on the use of different orientors and planes of section, and also on the slightest flaws in the instrumental techniques. Thus, Loewenfeld and Altman (77), in analyzing the sources of possible errors and divergencies, call attention to the mechanical instability

of the stereotaxic instrument. When the instrument was dismanteled and reassembled ten times and, following this, the end of the needle was returned every time to the same place, the error in placement averaged ± three mm; with repeated fixations of the head of the animal in the apparatus and successive releases, the error may be as great as ± 0.5 mm in all directions. Individual variations in the spatial position of the larger formations of the brainstem, according to the materials of Reinoso (85), are as great as two mm, and, according to the data of Loewenfeld and Altman, as much as five mm in a given direction.

From the studies of Bradley and Elkes (38) it can be seen that, in 32 percent of cats, considerable variations may be observed in the contours and dimensions of the skull and brain. Therefore, after concluding an experiment, it is desirable to have a morphological control in order to render precise the localization of the tip of the electrodes. This control permits definite determination of where the end of the electrode was, which structures of the brain were subjected to stimulation, and from which structures potentials were recorded. Moreover, it is always desirable to have a description of the pathomorphologic changes in the zone of the electrode. In order to determine the localization of the tip of the electrode in the brain tissue, a number of methods have been worked out. For example, Kogan (14) proposes making sections through the hardened brain with a sharp knife in the direction of the electrode, which is left in the brain in order to indicate the direction in which the sections are to be made. However, such a method provides only an approximate, rough orientation.

More suitable is the method of "designation of the vertical projection", elaborated by Hess (60). This method consists in the fact that the head of the experimental animal is placed, with the electrodes left in it, in a cold mixture, frozen, and sawed in the sagittal plane. The medial surface, thus exposed, is photographed and the brain is then thawed, after which the electrodes are withdrawn one cm. A small section of brain tissue is removed at the point where, judging by the direction of the electrodes, stimulation or destruction occurred, following which the electrodes are returned to their previous depth and photographs are again taken. The excised section of brain tissue is divided into two parts with a horizontal cut, which parts are placed at the time of photographing alongside the brain; the planes with the photofilm are then transferred to [illegible] superimposed upon the photograph of the medial section of the brain.

Suitable for marking the end of the electrode is a

method of electrolytic release of the metal from the end of the electrode and the subsequent demonstration of it in the tissues of the brain with specific chemical indicators which, in interaction with the metal, produce a staining coloration. Thus, it is possible to detect the place where the end of the electrode was by the staining of the area in the brain sections. The section containing the stained area can then be identified from the anatomic atlas.

The choice of indicator and the color of the stain depend upon which metal is used for making the electrode. Thus, for marking an electrode tip of iron or steel, use is made of a color reaction for the iron which is isolated at the anode upon electrolysis and which, following interaction with potassium ferrocyanide in the presence of hydrochloric acid, produces a blue color (the Berlin [i. e. Prussian] blue reaction) (Hess (60), A. B. Kogan (14)). For indication of the tip of a nickel electrode, the indicator used is dimethylglycine [?], which, by reacting with nickel, forms a complex salt - nickel dimethylglycinate, which has a bright orange color. This reaction is very sensitive. It is necessary only that the time between the application of the current and the staining of the section be as short as possible (Gusel'nikov (11)).

For indication of capillary microelectrodes, Leman (73) recommends filling the capillary with electrolyte containing silver nitrate. After finishing the study, heating the proximal end of the electrode will cause the electrolyte to move from the capillary into the tissues of the brain, where the area can be recognized on histologic sections by the usual reactions for silver. Silver nitrate may be replaced as the electrolyte by a solution of lead chloride containing ammonium sulfide; which produces the blackish-brown stain of lead sulfide.

These methods may be used only in acute studies, since, when the capillary electrode is left in the tissues for more than 20 minutes, there is progressive diffusion of the electrolyte into the surrounding tissues. For studying the localization of the end of silver electrodes in chronic studies, use may be made of a similar method of demonstrating silver deposited in the tissues by the electrolytic method. It is also possible to locate the tips of microelectrodes or of macroelectrodes in nerve tissue with the aid of a silver impregnation method developed by Andersen (34).

A quick method of detecting the electrode tip in brain tissue by silver impregnation is reported also by M. Scheibel and A. Scheibel (93). This method produces a very pronounced microscopic picture in the zone of the electrode tip.

A very nice method for localization of the electrode involves the demonstration in the brain tissue of a zone of electrocoagulation. For microelectrodes this method has been worked out by Mollica, Rossi, and Venturelli (80). The authors destroy the tissue with the ends of bipolar electrodes, through which they pass a direct electric current from a 90-volt battery, through a potentiometer with condensers arranged in parallel. Destruction of the tissue is effectuated under the control of a microammeter. The authors present a scale for the relationship between the strength of the current, the diameter of the tip of the microelectrode, the exposure time, and the area of tissue destroyed. For example, with electrodes of 37 and 12 microns in diameter, a current of 100 microamps, and an exposure time of five seconds, an oval area of necrosis is created in the tissue measuring 200 by 250 microns. With a current of 20 microamps and an exposure time of 20 seconds, the area of necrosis measures 120 by 180 microns. The sections in which the necrosis is most pronounced are then stained and their identity determined with the use of the atlas.

Electrodes

The development and progress of the stereotaxic method has been closely connected with improving electrode technology, especially that of microelectrodes. Electrodes have been developed for the cortex and for the subcortex. The electrodes for the subcortex are also called deep electrodes. Deep electrodes of minimum diameter must be sturdy and must not be deformed upon being inserted through the brain substance. Since these electrodes must serve not only for the recording of potentials but also for stimulation of the various structures of the brain, they must not have a large internal supporting structure. Reduction in the diameter of the electrode leads to reduction in the amount of damage done to the brain and increases the precision of localization of recording. Of particular significance is a strong insulating cover for the deep electrodes, which must also permit sterilization of the electrodes. Originally the electrodes were placed in fine glass insulating tubes (Bekhterev (4), Horsley and Clark (64)). Enamel coating was soon recognized as being more suitable, with the tip left uncoated. Gum lacquer, plastic substances, glyptal, and bakelite have also been used as insulating substances. A particularly good method of covering the electrodes is the application of plexiglass, which consists in repeated dippings of the electrodes in a chloroform solution of plexiglass.

The material dries quickly and creates a uniform, durable film. Immersion of the electrodes in a solution of photographic film in acetone also produces a durable and elastic insulation. These substances do not incite any untoward reactions in the brain tissues contiguous to the electrodes.

Hess (60) deserves credit for the clever method of checking the insulating properties of electrodes by observing the process of gas formation during application of a weak direct current to the electrode while it is immersed in salt solution. In the presence of disruptions in the insulating material, the release of small gas bubbles above the discontinuity is observed.

Two types of deep electrodes are in use: the needle type and the capillary type. At first, a platinum-iridium alloy was used as the material for making electrode needles. However, Hess did not believe that there were special advantages of platinum electrodes over steel. In 1934, Ranson constructed electrodes out of nichrome wire. Kogan used fine wires, to the tips of which he fastened platinum or silver slivers.

An effective method is that of the electrolytic covering of the tips of the electrodes with gold, nickel, or silver. The necessity of processing the electrode stems in the same way can be circumvented by the use of chemically inert metals (nickel, nichrome, constantan, silver, stainless steel, molybdenum, tungsten). These metals do not exert a deleterious influence on the nervous tissue and are not themselves subject to corrosion.

In 1928, Bronk (39) reported on the use of deep electrodes made in the form of hollow needles with a core. Such concentric deep electrodes of the needle type are now used in one modification or another in the majority of investigations. The most extensively used are the electrodes developed by Delgado (51). The material for their manufacture consists of wires of stainless steel 0.05 mm in diameter, covered with four wrappings of teflon. From this wire, segments of different lengths are made. The ends of the separate wires are left bare for a distance of one mm. Then six such electrode wires, taken together, are immersed in dilute plexiglass in such a way that distances of three mm are left between the ends of the separate wires (see Fig. 4). The electrode bundle is then coated with a thin coating of polyethylene. To the free ends of the electrodes are attached recording leads. These electrodes may be left implanted for very long periods of time (one to two years).

Fig. 4. The Delgado electrode

The Burlo (42) electrodes are composed of polyethylene tubes or polyvinyl tubes perforated at different levels for fine wires. Each recording electrode is composed of wires 0.004 mm in diameter covered throughout their lengths with insulation, with the exception of one mm at the tip. Each wire is led out through an opening in the polyethylene tube and bent. For greater sturdiness of electrode construction, steel stylets 0.015 mm in diameter are inserted in the tubing. Such an electrode affords the possibility of recording bioelectrical potentials along the course of the electrode channel at different depths in the brain.

Bradley and Elkes (30) used electrodes made of two segments of stainless steel 0.02 mm in diameter with fine electrolytic tips 20 microns in diameter. The electrodes were covered with a lacquer insulation, with subsequent thermal processing.

These same steel needles have been used in the investigations of Roytbak (29). In a number of other experiments he used constantan wires 80 microns in diameter.

Brun and Emerson (41) report on a simplified non-irritating electrode for chronic insertion in the subcortical regions of the dog brain. The needles of the electrodes are made of nichrome covered with a thermoplastic enamel with an interelectrode distance of two to three mm. The insulation is nine layers thick. Cadilhac and Passouant-Fontaine (43) used deep electrodes in the form of hollow steel casings with pointed ends covered with lacquer insulation. The diameter of such a casing is 0.6 mm. Within the casing is inserted an insulated steel or nichrome wire which protrudes 0.5 mm beyond the end of the casing.

Whittier and Kettler (105) used concentric electrodes with cores of tungsten insulated with glass threads and covered with a sheath of fine nickel tubing. This sheath was in turn covered with four layers of insulation.

Microelectrodes may be capillary or metallic. Capillary microelectrodes have a covering of insulation and a contact the diameter of which is measured in microns or parts of a micron. They are used for recording potentials of the brain in acute studies and permit recording the electrical activity of single nerve cells and even of parts of a nerve cell. Capillary microelectrodes are produced from fine glass tubing by drawing apart the ends of the tubing. If a large tube is filled with tubes of smaller diameter and this aggregate is then drawn in the same manner, it is possible to produce a multichannel capillary microelectrode (Vis (103)). The capillaries are filled with an electrolytic solution of potassium chloride or an easily-fused alloy of indium and lead (Vis (103); Caldwell and Downing (44)). However, capillary microelectrodes have a very high internal resistance (up to 40 megohms), and hence, for the recording of biocurrents, a strong preliminary amplification is needed. Moreover, with prolonged retention of the microelectrodes in the nervous tissue, there is inevitably a diffusion of the electrolyte from the capillary. In connection with this, for experiments involving prolonged recording, such types of microelectrodes cannot be used. The needle electrodes are better for this purpose. The advantages of the needle microelectrodes consist in an extraordinarily small amount of tissue traumatization, considerable elasticity, and the possibility of using them for recording both the respiratory and the pulsatile waves in the brain.

In the literature, descriptions are given of needle microelectrodes made of tungsten (Kogan (13), Hubel (65)), of stainless steel (Li-Chon-Lon and Jasper (74)), and of nickel or nichrome (Gusel'nikov (11)). In order that the microelectrode may have the smallest possible diameter, it is processed in a mixture of nitric and hydrochloric acids or it is sharpened by an electrolytic method. By this means, the tip of the electrode may be given a highly-varied configuration and any degree of sharpness. Thus, Kogan produced microelectrodes with diameters of three to five microns and Hubel produced some with a diamter of one micron and even less. The microelectrodes may be used for recording potentials from different areas of the cortex as well as from the subcortex. Depending upon this, only their arrangement and degree of reinforcement need be varied. A detailed description of the micromanipulator and the head-holding device used in microelectrode experiments is given in the works of Kogan (13) and Baumgarten (35) and others.

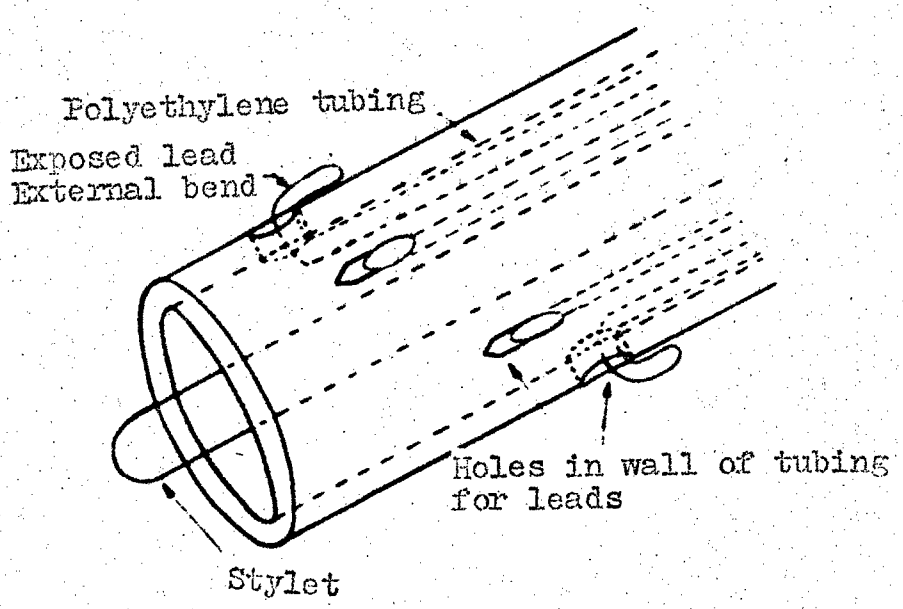

Fig. 5. The Durlo electrode

The recording of bioelectric potentials from various cortical zones, especially in chronic experiments, requries maximum approximation of the recording electrode to the surface of the brain. Attempts to record potentials through the imperforate skin inevitably lead to the production of artifacts in connection with the recording of muscle currents, which in animals it is extremely important to eliminate. Even secure fixation of the heads of animals does not eliminate these currents. A method has been described of putting a needle into the skull bone before each experiment. This needle is insulated throughout its entire length with the exception of the tip. Such needles serve as cortical recording electrodes (Sakhiulina, (30)). A number of investigators have proposed special couplings which can be screwed into the trephine hole in the skull. Into these couplings are inserted sleeves, at the ends of which there are flat, elongated plates which are applied directly to the dura mater of the brain.

Use may be made of thin, pliable surface electrodes which transmit the pulsatile waves of the brain. It is possible also, by a twist of the sleeve, to move the flat, eccentrically situated contacts of the electrode around the restricted area of the cortex within the limits of the diameter of the trephine hole (Hogan). A cortical electrode in the form of a special cell with pins for contacts

has been described by Gurevich (9, 10) and Laptev (17, 18). An electrode may also be made with a screw inserted through the skull above the zone of projection of the cortical analyzer which is to be studied (Khvoles (30, 32)).

For recording potentials in different spheres of the cortex, use has been made of electrodes of the needle type which are inserted into the cortex to the necessary depth. The principle of their construction does not differ from that of the deep electrodes used for the subcortex. The length of the electrodes is, of course, different.

In the literature there are descriptions of cortical plate electrodes which are small polychlorvinyl sheets through which fine wires of stainless steel with rounded tips are passed. After trephine holes are made in the skull, the electrodes are inserted into the space between the dura mater and the skull and are secured with sutures to the edge of the trephine hole (Delgado (50). Bradley and Elkes (38) have constructed electrodes for the cortex from small silver balls which are secured at the required place with the aid of screws made of stainless steel and screwed into the skull bone. Down the centers of the screws are holes through which insulated conductors can be passed and joined to the silver balls. The screw may be used, if necessary, as an indifferent electrode.

The electrodes are secured with the above-mentioned sleeves or other devices, and cemented with dental cement or with plastic acrylic material. Our medical industry produces a self-hardening plastic of the AST-2 type. For fixation of the electrode, it is necessary in advance to dry the channel of the trephine hole and to place in it a thick mixture of monomer and polymer. For quick hardening, it is desirable to apply to the hardening mixture a cotton tampon moistened with sterile hot water. After five to eight minutes the trephine hole is closed with a very strong plexiglass filling through which electrodes can be inserted into the brain.

The free ends of the electrodes must be left outside for joining to the recording apparatus or to the stimulatory apparatus. Pulling the free ends out through the operative incision and leaving them free causes great difficulties and exposes them to damage.

Rather clever is a method described by Delgado (50, 51) and Bradley and Elkes (38). This method consists in the fact that all the recording leads are led out in pairs through separate incisions in the interscapular area. The incisions are then sutured around the leads. Miniature sockets are then affixed to the free ends of the leads.

Through the vertebral column of the animal an apparatus is then secured, onto which the socket is fastened and fixed. During the experiment the contact is fitted into the socket, thereby joining the electrodes to the recording apparatus.

Cadilhac and Passouant-Fontaine (43) have attempted to suture to the skin a miniature panel of radio tubes to which the recording leads might be attached. However, one can scarcely count on the reliability and strength of such a method of securing the commutation apparatus.

More reliable is a method of securing the commutation apparatus to the surface of the skull, described by Sukachev (31); Lur'ye and Trofimov (21), and Lyubimov and Trofimov (22). This method consists in the fact that, to the surface of the skull, a device of soldered conductors leading to the electrodes is screwed with stainless steel screws. After suturing the operative incision, part of the device with the contacts is left free above the skin, which must be protected against damage by being covered. The recording apparatus is attached to this device during the experiment.

A report has also been published on a remote-control method of transmitting stimuli and of recording bioelectrical processes in the brain. Especially great prospects are opened up in connection with recent advances in physics and radioelectronics. If fine, insulated, metal electrodes are inserted into a nucleus of the brain of a cat without being brought out to the surface of the skull, and the animal is then placed within the field of force of a radar transmitter, an electric current will be generated in the electrodes which will stimulate the appropriate parts of the brain (mentioned by D'yerno (30)).

Nikolai (82) uses a crystalline germanium device of small dimensions, the leads from which are attached to nerves, muscles, intestines, and other organs. Upon placing the animal within the field of force of a high-frequency generator, a pulsating current develops which creates a stimulatory effect. He correctly points out that the development and perfecting of this method holds great promise for stimulation and for recording potentials of the brain at a distance, especially in studying the functions of the nervous system in animals which move about freely.

Greer and Riggle (57), using the stereotaxic method, inserted into the brains of mice electrodes with a semiconductor receiving apparatus mounted on them. Placing these mice in the field of certain radiofrequencies, it was possible to elicit stimulation of certain areas of the brain and, accordingly, to influence the behavior of the animals.

Verzeano and French (102) worked out a scheme for

radio reception of the impulses for stimulation of the brain. The reduction in the clearance gauge of the apparatus mounted on miniature semiconductors makes it possible to utilize a receiving apparatus under the skin and permits stimulation of animals at a distance of 25 meters from the transmitter.

Experimental Brain Injuries with the Use of the Stereotaxic Method

The insertion of electrodes with the aid of the stereotaxic apparatus into certain areas of the brain is carried out not only for purposes of stimulation or recording bioelectrical activity, but also for damaging these structures.

Selye and Verzhi produced lesions in the optic tubercles of dogs by application of an electric current to bipolar electrodes, with resultant clearly defined and sharply localized lesions. Horsley and Clark (64), in their classic experiments with stereotaxic apparatus, also produced localized injury with the aid of electrolysis at the cathode. It has been observed that lesions produced at the cathode are ordinarily more marked than at the anode, and have an irregular shape because of the gas which is formed upon electrolysis. The amount of brain tissue destroyed is proportional to the magnitude of the current passed through the electrodes. This is characteristic of the first seconds of exposure to the current, but later, with prolongation of the exposure, the extent of injury does not increase in proportion to the magnitude of the current nor to the time of its application. Lesions at the cathode, thus, are irregular in shape and should be computed in advance.

Whittier and Mettler (105) created electrolytic lesions at the cathode in nuclei of the brainstem in 80 monkeys and illustrated how variable are the lesions which are obtained. These investigators stated that it is impossible to anticipate the shape and size of the lesions. A current of high voltage may have no advantages over direct current, and lesions created by such a current are not confined to the tip of the electrode, due, among other things, to the fact that there is intense gas formation in the zone of electrolysis.

Leontovich (20) proposed using monopolar electrolysis, with the active electrode at the anode, in order to coagulate nuclear formations in the brainstem. In this, she observed well defined and clearly delimited foci of dry coagulation necrosis due to dehydration of the tissues upon passage of the current. Gas formation with this method is negligible, and the dimensions of the foci of necrosis

produced at the anode depend on the magnitude and duration of the current and may be calculated in advance.

Sharply defined necrosis of brain tissue is caused by the implantation of glass capillaries filled with radon granules. According to the findings of Edwards and Bagg (54), with a dose of one millicurie of radioactivity, there are foci of necrosis four mm in diameter. Thermal necrosis may be elicited with the use of electrodes of the thermocautery type or by heat induction in implanted steel magnets.

In the literature there are reports on a method of producing local lesions with a narrow, directed ultrasonic beam (Lynn, Zwemer, Chick and Miller (76), and others), and on a method of producing radionecrosis by irradiating certain areas of the brain with X-rays in a dose of 2850 r (Russell, Willson, Tansley, (92)). Of considerable promise is the use, for destruction of sub-cortical nuclei, of focused ultrasonic beams oriented transversly with the stereotaxic apparatus.

A method has been reported for inducing necrosis by mechanical means, using a cannula with a trochar. Upon rotating the trochar there is removal of nervous tissue, which depends on the direction of the cannula and the angle of rotation of the cutting edge of the trochar.

It is possible to achieve experimental destruction or stimulation of certain nuclei of the brain by injecting into them chemical substances which cause neurolysis. As the neurolytic substance, one can use zinc chloride, chromic acid, silver nitrate, mercuric chloride, aluminum hydroxide, alcohol, solutions of novocaine, and so forth. A number of authors inject these substances through needles or cannulas oriented with the stereotaxic apparatus toward the appropriate nucleus of the brain.

Mention should also be made of a method for destroying the strio-pallidary system by the intraperitoneal injection of manganese chloride. In addition, along with destruction of these regions of the subcortex, lesions have been noted in the pons Varolii, cerebellum and spinal cord, and also in the liver (Carpenter and Whittier (45)).

According to the findings of Richter (86), carbon disulfide vapors cause destruction preponderantly in the basal ganglia, the most constant lesions appearing in the striatum and the pallidum.

By using the stereotaxic apparatus, Traczyk (101) implants a polyethylene cannula in the lateral ventricles of dogs. This method ensures precise and predictable insertion of the tip of the cannula into certain areas of the lateral ventricles of the brain, and is deserving of attention in connection with the fact that methods of perfusion of the

ventricles of the brain and of the introduction into them of fluid substances are being used more and more in physiology and pharmacology.

From the findings in the literature, it is apparent that physiologists now have at their disposal sufficiently reliable methods of selective stimulation and destruction of certain regions of the brain. Improvements in the construction of electrodes and in the stereotaxic method have provided the technical basis for recording bioelectric processes in nerve centers, individual nerve cells, and even parts of nerve cells, thereby facilitating a precise neurophysiological analysis of their activity. These methods, in combination with the method of conditioned reflexes, afford the opportunity to discover the significance of different nuclear complexes of the brainstem and their position in the system of cortical analyzers.

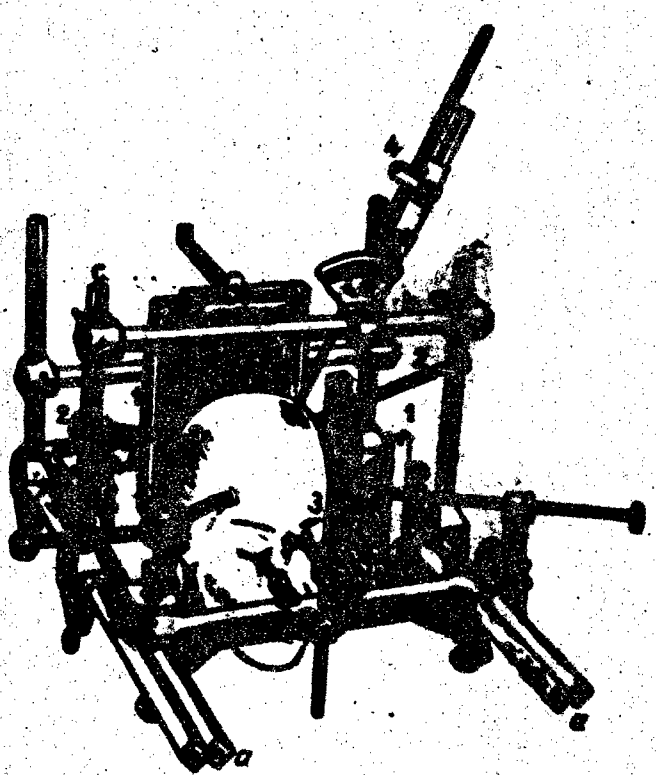

Fig. 6. Stereoencephalotome, with a device for ventriculography, constructed by Becker, Founds, and Peacock: 1 - movable cassette for taking roentgenograms; 2 and 3 - apparatus for fixation of the skull; 4 - electrode holder; a, b, and c - indicators of the basic planes of the instrument.

The achievements of the stereotaxic technique in experiments on animals, improvements of the apparatus, and increasing precision in the insertion of electrodes into the desired zones, have led clinicians to the idea of using the stereotaxic principle, and of constructing stereotaxic apparatus, for performing operations on the subcortical structures of people.

In some countries, models of stereotaxic apparatuses have already been developed for use on people and are called "stereoencephalotomes." Reports on such instruments have been made by Spiegel, Wycis, and Marks (97), Hayne and Meyers (58), Becker, Founds, and Peacock (37), Richter (86) and others.

Orientation in these apparatuses is effected not on the bones of the skull but on the "null" planes of Horsely and Clark, and on intracerebral structures, the horizontal "null" plane being established with the aid of roentgen projections along lines passing through the anterior and posterior commissures of the brain. Some authors orient themselves on the massa intermedia or on the center of the foramen of Monroe. The positions of the nuclei are worked out from a stereotaxic atlas for the human brain. Richter has for a long time used the phantom principle for determining the positions of the desired subcortical structures. Especially good analyses of the methods of stereoencephalotomy and of stereotaxic anatomy, as adapted to people, are given in the monographs of Spiegel and Wycis (96) and Talairach and associates (100).

For treatment of cancer of the pituitary and of certain other regions of the brain, neurosurgeons use the stereotaxic method to insert radioactive isotopes into the brain, and to establish prolonged drainage from the posterior part of the third ventricle into the lateral cistern, or from its anterior portion into the basal cistern, in hydrocephalus.

At the First International Congress of Neurological Sciences in Brussels, in 1957, particular attention was given to the use of the stereotaxic method for the surgical treatment of a number of disorders, including Parkinson's disease (6).

The destruction of the nuclei of the striopallidary system by electrocoagulation or by chemical neurolysis (alcohol, novocaine) for the most part ensures a stable reduction in the rigidity, tremors, and other symptoms of this disease. The addition to the neurolytic substance of a contrast medium permits demonstration of the silhouette of the zone of nuclear destruction and regulation of the degree of lysis. At present, this method of treatment is being used extensively in the Soviet Union as well as abroad.

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