ACCURATE PLACEMENT OF MINUTE LESIONS IN THE BRAIN OF THE ALBINO RAT[1]

WENDELL J. S. KRIEG, Ph.D.[2]

FOR accurately placing lesions in predetermined regions of the brain, the sterotaxic machine, devised by Horsley and Clarke (1908, 1920), and used for a long series of experiments by Ranson and his school (1934) has been found very useful. The instrument as designed is best applied to the brain of the monkey or cat. It has been modified by Clarke (1939) to be used for the rat by altering the clamping mechanism for the head. The instrument, however, is rather cumbersome for this purpose, and all who desire to work on smaller animals may not be provided with the Horsley-Clarke machine. Finding himself in this position, the author decided to devise a small and simple machine for making localized lesions on the rat. If one is going to do critical work on a small animal, a higher degree of accuracy is required than is usually obtained with a larger machine, and it must be possible to place smaller lesions. With the machine here described and the coordinates determined for this work, it has been found quite feasible to place lesions a cubic millimeter or a half cubic millimeter in diameter with an electrode .1 millimeter in diameter or smaller, which often leave no perceptible track. A large number of such small lesions were necessary for the problem chosen, so it was decided to determine coordinates for all points on the rat brain. These were determined by detailed measurement of a standard rat, an atlas on coordinate paper made, and coordinates taken from this as a guide to the desired lesions. The instrument has been fully tested by over 100 lesions, the location of which have been compared with that of the lesion intended. It was found that under properly controlled conditions, there was only a negligible variation between the intended and the actual lesion. It was unusual for the actual lesion to vary more than ½ mm. from the desired location, although the lesions were sometimes more or less extensive than intended. Once standardized the method proved practical, and such a large number of lesions were so easily traced, that it was decided to publish specifications for making the machine and the coordinates for the structures of the rat brain.

The original machine is available at the Institute of Neurology, Northwestern University Medical School, but those who desire to make their own machine and instruments will find the explanation and drawings adequate.

The instrument was originally built at New York University in 1935, but was not used for an extensive series of experiments until the author came to Northwestern University in 1944.

Section I. The Instruments

The instrument described and illustrated here (figs. 1 and 2) was made from a mechanical stage with a rack and pinion carrying the electrode added to take care of the third dimension. Simple arrangements have also been made for tilting the electrode carrier in any direction. The stage is mounted firmly above a heavy brass plate, which contains screw plugs to fix the external auditory meatuses with a scale on each for centering the head, and a clamp for the upper incisor teeth made from a Hofman's pinch clamp filed to accommodate the configuration of the rat's incisors, and clamped with a slotted bar to the base.

Any style of mechanical stage can be used, but the older type of Bausch & Lomb stage with a curved arm to clamp

[1]This study was aided by a grant from the John and Mary R. Markle Foundation.

[2]From the Institute of Neurology, Northwestern University Medical School. Received for publication, December 14, 1945

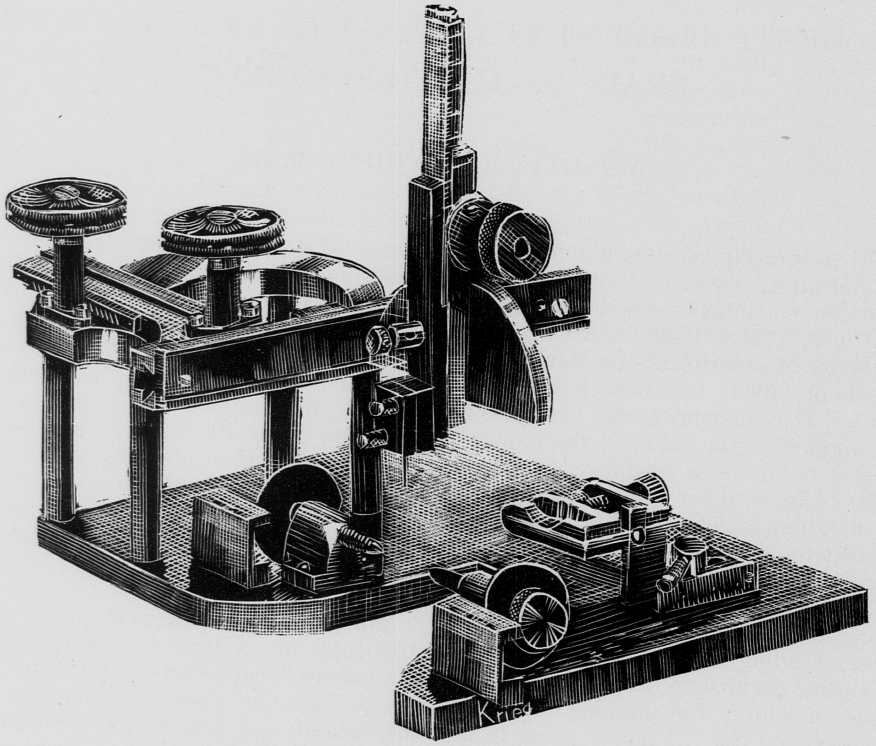

Fig. 1. Oblique view of stereotaxic machine.

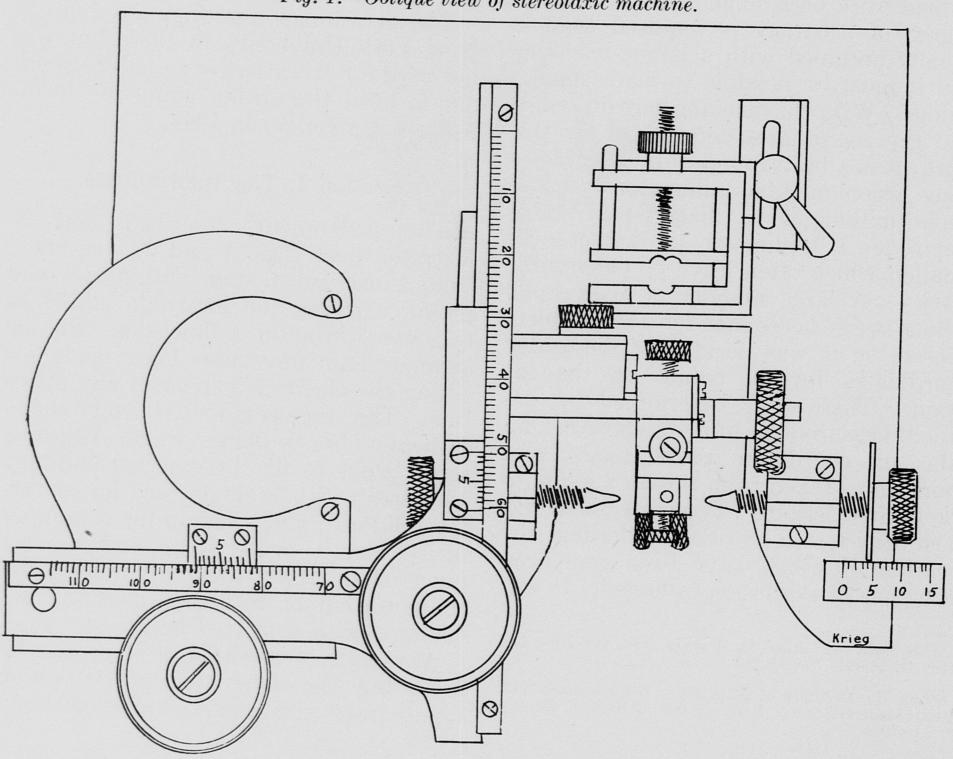

Fig. 2. Ground plan of instrument.

to the pillar of the microscope and made of brass was selected because the curved arm made a firm plate for screwing into four columns, which were in turn attached to the base.

It is quite possible to get along without the mechanism for tilting the electrode and this makes the apparatus much simpler. However, in this example the vertical arm was pivoted to a semi-circular plate divided into degrees, and held in any desired position by a screw at the back. The rod which holds the semi-circular piece to the main bar is surrounded by a collar with a screw clamp so that the vertical arm can be tilted in the sagittal plane. This is useful in making lesions of the cerebellum and brain stem to avoid the transverse sinuses. If one desires to copy this instrument exactly, in order that the coordinates given in the atlas accompanying this article may be used without translation, certain dimensions must be conformed to. These are: A, the distance of the axis of the electrode from the bar which moves in an antero-posterior plane (26 mm.); B, the distance from the center of the ear plugs to the base plate (11 mm.);and C, the distance from the upper surface of the incisor clamp to the base plate (14.5 mm.). These points establish the horizontal plane, and should be placed accurately. The actual height of the columns supporting the moveable part are not so important, since the zero point in the vertical plane can be established after the instrument is finished and by reference to the skull of the animal itself. In this instrument the distance from the top of the base plate to the bottom of the mechanical state is 40.5 mm. It is not necessary to build the sagittal and transverse scales to conform perfectly to the instrument described here, since it is possible to move or change the scales after the instrument has been made. The important point in standardization is to get the horizontal plane of the instrument to correspond to that used for the atlas of sections. It can be given a final adjustment when an actual rat skull has been measured in the instrument by altering the height of the clamp for the incisors.

The electrode holder consists of a bar of hard rubber screwed to the vertical arm with another small block of rubber provided with a tiny groove to match the groove on the main block, and hinged with thumb screws so that it can be clamped to the main block. A small terminal in line with the electrode clamp is provided above on the block.

Considerable experimentation was devoted to developing the proper sort of electrode. Finally the simplest possible solution was adopted. The electrode consists merely of a straight piece of ready-varnished copper wire. Twenty-five gauge wire was found the most satisfactory; 29 gauge wire can be used for small deep lesions when it is desired to leave no track, and of course larger wire can be used for superficial or coarser lesions. Nichrome wire is stiffer but is undesirable because it requires straightening by tension with consequent special varnishing; a laborious and unsatisfactory process. The electrode protrudes from the tip of the clamp by a standard 12 mm.

When it is desired to place a lesion at an angle, the coordinates must of course be recalculated. The simplest way to do this is as follows: set up a standard recording lever as used for physiological experimentation which is adjustable by screws in two planes. Having determined from the atlas what the coordinates should be if the lesion were made vertically, one sets the instrument, with electrode in place, for this measurement. Approximate the tip of the recording lever to the tip of the electrode and then turn the vertical arm of the electrode holder to the desired angle, and re-approximate the two tips by means of the controls of the machine, keeping the recording lever immobile. The new readings will be the actual coordinates for the modification made by the angularity.

The instrument worked quite well without modifications from its original design except that it would have been better to provide a tilting mechanism for the whole upper portion of the instrument, so the electrode could be moved well out of the way when the animal is being fixed in or removed from the machine. To have added this feature, however, would have made necessary a recalculation of all the coordinates. With

this in mind, in designing the instrument it would only be necessary to have the mechanical stage fixed to the standards on a firm hinge.

Section II. Determination of Standard Coordinates

To obtain the desired three dimensional coordinates to contact any given structure in the brain, it was necessary to choose an ideal or standard animal, and make a series of measurements of the actual positions of the structures in the brain of that animal, and transfer these on to coordinated paper, filling in the details. This was done in the following manner: a rat was sacrificed weighing 237 grams, which was 7 months 3 weeks old, which had a nose-anus length of 220 mm. and a nose-occiput length of 53 mm. After the calvaria was exposed the animal was clamped into position in the instrument and a series of measurements was made on the exposed surface. This established the position of any points on the skull, but particularly the horizontal planes of the midline, and the craniometric points observable: the bregma, lambda, pterions, asterions. The positions of these points can be used during an operation to test whether the animal is placed properly in the instrument. The soft tissues were then pared away from the skull and the calvaria removed. A knife-like fragment broken from a razor blade was clamped in the electrode holder, and the brain sawed through by use of the controls of the instrument at millimetric intervals. Then under binocular magnification the exposed surface of the brain was examined and any points which could be identified measured while in the machine. For most millimetric planes, some 25 or more points were recorded, and their position indicated by a sketch of each exposed surface. This was continued throughout the entire brain. Then these were transferred to millimetric coordinated paper at the magnification of 10X to fill in details, and as a furtber check in identification each millimetric slice was treated as a tissue block and sectioned serially and stained. This enabled individual nuclei, small tracts and cortical areas to be added to the outlines obtained by measurement. It also enabled the atlas to be made into one with half mm. intervals by adding outlines of the section whose measurements corresponded to the mid-interval between the measured sections in front of and behind it. Then the outlines of the nuclear and fiber tract structures in the middle of the tissue block were added.

After the brain had been entirely measured the base of the skull was then measured point for point. Incidentally, this gives a reliable estimate of the normal relation between the skull and the brain, thickness of subdural space, size of cisternae, etc., in the only way that this can be properly done. It also enables lesions to be made on the cranial nerves, on the pituitary gland or on the parts of the internal ear.

As a check on the accuracy of the measurements and to visualize operative approaches, a wax plate reconstruction was made from the faired outline of the slices as finally determined for the atlas of coordinates. Thus we have a model of the fresh rat brain at a magnification of 10X linear. Needless to say, this method is far preferable to the use of measurement on fixed and stained specimens, since it gives the true measurements of the fresh brain in the machine, and is presumably the only Born wax plate reconstruction made from slices of fresh material. Figure 3 is a projection onto the horizontal plane of the outline of cortical areas and other significant structures.

This procedure of course consumed a great deal of time, and was made with the greatest accuracy that could be obtained. Hence it would be a considerable saving of time if any future instrument could be made so that its measurements can conform to those given here, and the atlas of coordinates be used directly. The atlas of transverse sections as published here (fig. 4-6) is made at a magnification of 4x to save space. If an atlas with a magnification of 10 is desired, it is an easy matter to photograph sections and enlarge them. The atlas perhaps needs no explanation; attempt has been made to outline all significant structures including the cortical areas.

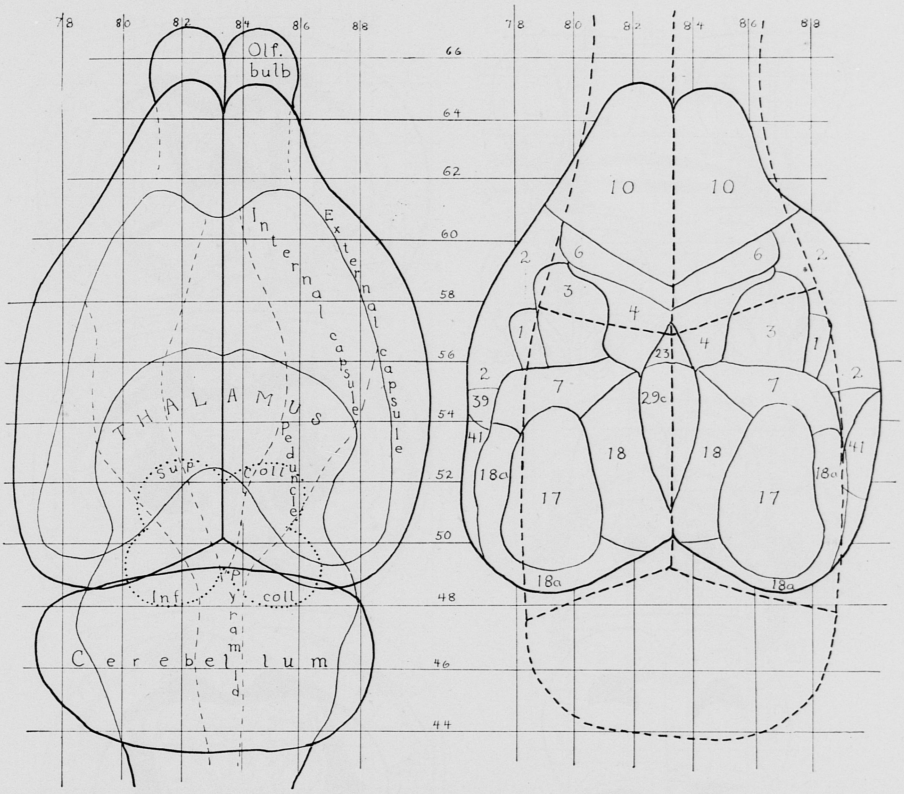

Fig. 3. External outline, principal internal structures, and positions of some of the cortical areas shown on a dorsal reconstruction made directly from measurements of the fresh brain. The outline has been faired only enough to give smooth lines. The heavy dotted lines indicate the positions of the principal dorsal skull markings.

Section III. The Operation

Anyone who makes use of the atlas will also have to develop an operative technique, and much time can be wasted in discovering for one's self the many minor changes that are necessary for success. For this reason the operative technique as finally used will be described in some detail.

Materials needed: medium size scalpel, kept sharp; very small pointed bistoury; a small sharp spade-ended instrument, edge about 3 mm. broad, for scraping muscle from bone; rat-toothed forceps, small forceps, medium sized pointed scissors; small pointed scissors; curved mosquito forceps; two spring hook retractors made from safety-pins; skin clip remover; dental drill, with angle head attachment; square dental burs of medium size with stops soldered on, cotton mounted on tooth picks; apparatus for making lesions by direct current; hollow ear plugs connected as spring tongs; sterotaxic machine. On the table, but not sterilized, should be medium size pointed scissors, medium size forceps, automatic hair clippers (desirable but not indispensable, only the fine head should be used); magnifying loupe of medium power with attached lamp.

The operation itself is best done without help. (1) Anesthetize the animal with nembutal injected into the abdomen. It is very difficult to determine the exact amount of nembutal to use. The weight range of animals utilizable is limited between about 230 grams and 300 grams if the lesions are to be accurately placed. Animals of 250 grams require .22 cc. of nembutal. Heavier rats of the same age group require proportionately more, lighter rats require

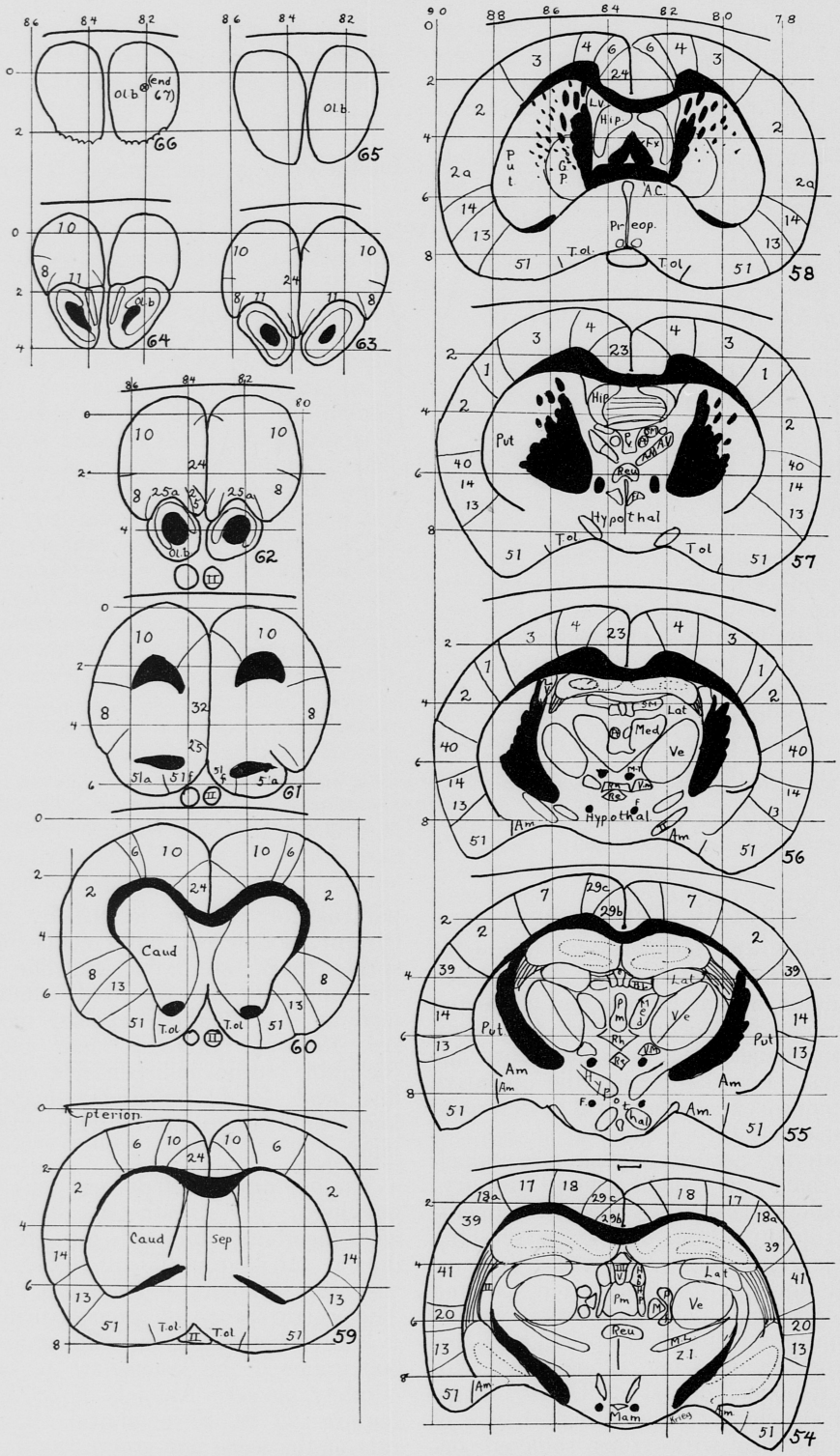

Fig. 4. Series of outlines of cross sections of rat brain at millimetric intervals, showing location of significant structures. For legend see fig. 6.

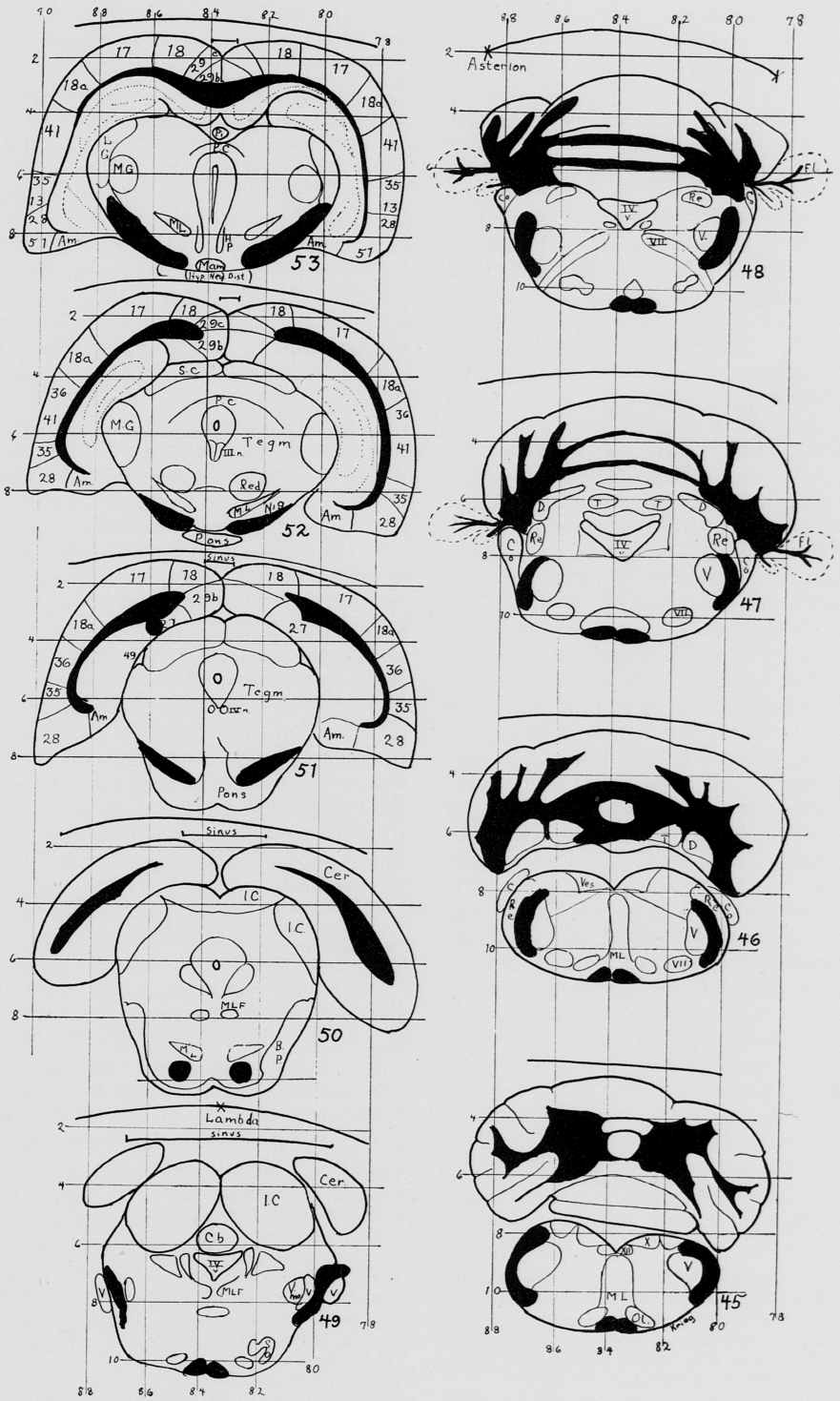

Fig. 5. Continuation of series of sectional outlines of rat's brain. For legend see fig. 6.

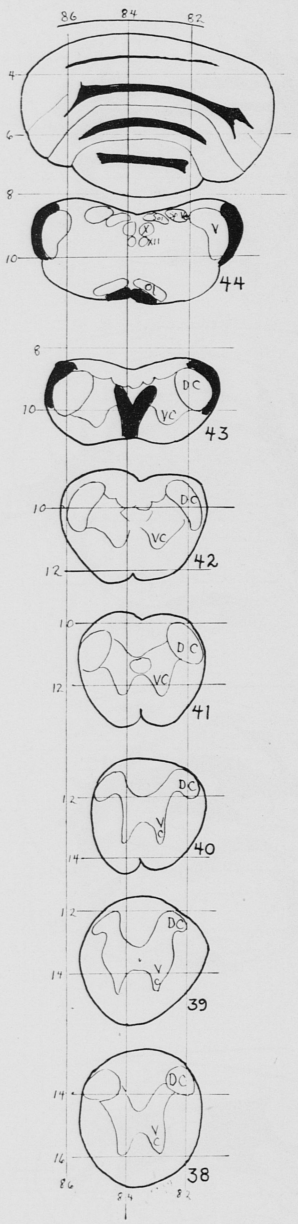

Fig. 6. Conclusion of series of sectional outlines of rats' brain. Represented in solid black are, anterior commissure, medullary centers of cerebrum and cerebellum and cortical projection pathway. The solid line at the top of each section marks the outside of the skull.

Abbreviations:
A.C., *anterior commissure;*
Am., *amygdala;*
A.M., *anterior medial nucleus;*
A.V., *anterior ventral nucleus;*
B.P., *brachium pontis;*
Caud., *caudate nucleus;*
Cb., *cerebellum;*
Cer., *cerebellum;*
Co., *cochlear nuclei;*
D., *dentate nucleus;*
D.C., *dorsal cell column;*
F., *fornix;*
Fi., *filiform nucleus;*
Fl., *flocculus;*
G.P., *globus pallidus;*
Hab., *habenula;*
Hip., *hippocampus;*
H.L., *lateral habenular nucleus;*
H.P., *habenulopeduncular tract;*
Hyp., neu., dist., *neural and distal parts of hypophysis;*
Lat., *lateral nucleus;*
L.G., *lateral geniculate;*
L.V., *lateral ventricle;*
M., *medial nucleus;*
Mam., *mammillary body;*
Med., *medial nucleus;*
M.G., *medial geniculate;*
M.L., *medial lemniscus;*
M.L.F., *medial longitudinal fasciculus;*
M.T., *mammillothalamic tract;*
Nig., *nigra;*
Ol., *inferior olive;*
Ol.b., *olfactory bulb;*
P.C., *posterior commissure;*
Pf., *parafascicular nucleus;*
Pi., *pineal;*
Pm., *paramedian nucleus;*
Preop., *preoptic nucleus;*
Pt., *paratenial nucleus;*
Put., *putamen;*
Pv., *paraventricular nucleus;*
Re., *restiform body;*
Red., *red nucleus;*
Reu., *reuniens nucleus;*
Rh., *rhomboid nucleus;*
S.C., *superior colliculus;*
Sept., *septum;*
S.M., *stria medullaris;*
S.O., *superior olive;*
Sol., *solitary tract;*
Sp. ve., *spinal vestibular nucleus;*
T., *tectal nuclei;*
Tegm., *tegmentum;*
T. ol., *olfactory tubercle;*
V.mo., *masticator nucleus;*
V.C., *ventral cell column;*
Ve., *ventral nucleus;*
Ves., *vestibular nucleus;*
Vm., *ventralis medialis nucleus;*
Z.I., *zona incerta;*
II-XII, *cranial nerves or nuclei II-XII;*
III v., *third ventricle;*
IV v., *fourth ventricle.*

proportionately less. Thus the resistance to anesthesia in animals seems to depend upon the rate of development or general physical state. A 300 gram rat from a litter whose litter mates are approximately 250 grams may require as much as .33 cc. while .21 cc. may kill a 230 gram member of such a litter. In the rat there is a very narrow margin between ineffective anesthesia and death. All deaths at operations are due to too much anesthetic, unless one of the large sinuses is opened. The best place for injection is in the abdominal cavity in the region of the groin and care should be taken to see that the needle enters the abdominal cavity, or the anesthesia will not be effective. Anesthesia may be regarded as sufficiently deep when the animal responds to pinching or clipping the ears by only a wince or a moderate scratching movement of the hind legs. (2) The top and sides of the head should be closely clipped from the middle of the nose to the end of the neck including also the area under the ears. The vibrissae should be clipped off. (3) Scrub the top of the head and ears with a sponge soaked in 70 per cent alcohol and held in the forceps, holding the animal's nose upwards to avoid getting alcohol in the eyes. The ear tongs are next to be inserted. This is rather difficult to do properly until a little skill is acquired. It is necessary to make a cut in the lower edge of the rim of the meatus, about 3 mm. long so that one can look directly into the meatus. Spread the tongs with the fingers and place one and then the other in the meatus. Be sure to check that the plug is actually in the meatus not in the false passage just above it. When properly placed a crackling sound can usually be heard and the eyelid is closed in reflex response. (4) Mark the animal by nicking the ears. If the response is only slight to this manipulation, the animal is ready for operation. (5) With the loupe in place, grasp the skin over the occiput with the skin forceps, and make a midline cut through the scalp as far forward as the line between the eyes. Cut the strands of connective tissue connecting the scalp with the bone. (6) Open the incision by placing the spring retractors. One goes near the front end of the incision, with the points on either side stuck into the deep aspect of the skin, the other is placed behind in the same manner. (7) With a sharp scalpel cut along the edge of the exposed calvaria, up to the edge of the temporal muscles. Connect the two cuts by a transverse one at the anterior edge of the incision, and with the scalpel scrape back the galea and periosteum leaving them attached occipitally. Thus the skull is bared. (8) Place the animal in the stereotaxic machine. With the ear clips in place, this is simplified. Place the left plug in the left screw and then the right in the right, and tighten the right screw until there is no more play. Clamp the upper incisor teeth, pushing down on the muzzle to make sure that the clamp catches. If the front teeth are not properly clamped measurements at the anterior part of the skull will be off the standard. (9) Swab the surface of the skull and center the head in the machine. Set the electrode for the midline and then move the skull to the right or left by means of the ear screws to make the sagittal suture correspond with the position of the electrode. The position of the head in all three planes may be checked by a three dimensional reading of the bregma, where the frontal and parietal bones meet in the midline. This can be referred to a standard. It is apparently subject to some variation, however. (10) Set the instrument for the sagittal and transverse planes of the desired lesion, and drop the electrode down to the surface of the skull. Having already placed the anal or indifferent electrode, turn on the current momentarily. This will make a tiny spot which can serve as a guide in placing the dental drill. (11) Move the electrode well out of the way, and drill the hole for the lesion. If there is a slight amount of hemorrhage it will generally stop if swabbed; if the hemorrhage is more abundant one must wait. Usually there is none, and the dura is not severed by the drill. (12) Replace the electrode and move it down to the desired depth. Turn on the current for the proper time. Using 2 millamperes of direct current, a duration of fifteen seconds will make a cortical lesion about 1½ mm. in diameter.

Five seconds is best for deep lesions in small structures, as the thalamic nuclei. Too long a period in deep lesions causes the electrode to heat up and make a lesion in the track. Remove the electrode. (13) Place any other lesions that may be desired. (14) If a lesion is to be placed through the side of the skull, it is necessary to cut down and scrape free of the bone a small portion of the temporalis muscle. If left attached at the base it will heal very nicely. (15) Remove the animal from the instrument by releasing the incisor clamp and screwing out the right ear screw. Remove the ear plugs. (16) Arrange reflected muscle and periosteum in place as well as is convenient, and close the wound with skin clips.

If the Marchi method is used the time should be shortened in the rat to from eight to ten days. The modification of Swank and Davenport has given excellent results. The brain need be divided into only four pieces; three slices passing through the cerebrum, the middle slice being the thinnest, and the fourth including brain stem and cerebellum. Counterstaining with thionin at pH 4.18 is quite feasible and aids greatly in localizing the lesions.

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