Location of Anatomic Sites in Brains of Experimental Animals Based on Internal Landmarks[1]

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ABSTRACT The determination of the spatial positions, with respect to a head holder reference, of anatomic structures within the brains of experimental animals, based on measurements of coordinates of ventricular and internal cranial landmarks, is described. If "appropriate" landmark arrays are employed, the uncertainty in coordinate values for prechosen anatomic sites in cat brain, determined by the methods outlined in the paper, is reduced by a factor of the order of five compared to the deviations experienced when the Horsley-Clarke landmarks are employed. The instrumentation system developed for implementing the entire procedure, including precision x-ray contrast ventriculography, and the atlas and roentgenographic data used to obtain the desired coordinate information are illustrated.

As the demand for more sophistication in anatomical and functional studies of brain develops, greater precision in locating specific predetermined sites is required. And, at present, a critical need exists in experimental animal studies for increased precision in the production of lesions confined to specific anatomic structures, or specific sites therein (and for electrode placement and other purposes) without appreciable damage to (or misplacement in) other structures. With respect to lesion production, this need is particularly appaent when the ultrasonic method of producing selective changes in brain structures is employed. In this case the versatility, usefulness, and potential of the method have been severely limited by the landmark system utilized as a reference by essenitally all investigators up to the preesnt for estimating the positions of specific deep anatomic sites — that is, the axis through the "centers" of the external auditory meatuses and a "horizontal" or reference plane determined by this axis and the lowermost aspects of the infraorbital ridges.[2] This plane, employed by Horsley and Clarke in their classic ('08) publication on cranio-encephalic topography, is designated Reid's plane throughout this paper since it is determined by reference lines first introduced by Reid (1884). In this latter connection see D'Arcy Power ('36). This coordinate-landmark system has served as the basis for a tremendous amount of stereotaxic work on experimental animals and obviously its conception and use did constitute a development which provided, in conjunction with methods devised for lesion production and for electrical stimulation and recording, the basis for significant advances in knowledge of structure and function of the brain. However, the present inadequacy of this system is readily apparent (see for example Loewenfeld and Altman, '56) and can be illustrated here by a single specific example. It has been shown at this laboratory that it is completely impractical, using this coordinate-landmark system in conjunction with brain atlases, to accomplish bilateral interruption of the mammillothalamic tract, with no appreciable spreading of the lesions into surrounding structures, in a reasonable percentage of cats. Success in about one of every 25 animals can be achieved by this method. Obviously an improvement is needed and such has now been achieved by employing internal landmarks.

As the first step in improving the accuracy of localization of anatomic sites in the thalamus and hypothalamus of cats, we developed a method of employing inter-

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[2] Due to cranial asymmetry the indicated axis and sites on the infraorbital ridges may not lie in a plane. In practice the infraorbital clamps are adjusted in a lateral direction until a "stable" position is achieved.

nal cranial landmarks identified on roentgenographic projections. This method is described in a recent publication summarizing results on certain aspects of the anatomy of the mammillothalamic system (Fry et al., '63). In this reported work it was necessary to produce lesions at various sites along the intrathalamic course of the mammillothalamic tract without appreciable infringement of the lesions into surrounding structure. Although the accuracy of positioning was improved, the gain was not sufficient to justify decreasing the effort to achieve further improvement. Accordingly, the development of instrumentation and procedures for implementing precision x-ray contrast ventriculography, to provide estimates of coordinate values of anatomic sites, was pursued. The method which was developed is now in routine use in our laboratories and the increased accuracy achieved — a decrease in the uncertainty of positioning by a factor of approximately five — completely outmodes the older Horsley-Clarke procedure employing the ear bar zero and Reid's plane as landmarks. As a specific example, bilateral interruption of the mammillothalamic tract without appreciable infringement on surrounding structure can be accomplished now in three of every four cases as compared to one of 25 by the older method.

Successful achievement of precision x-ray contrast ventriculography, and the use of information so obtained to estimate the positions of anatomic sites in the brains of experimental animals on a routine basis, has required: the design and development of rigid head holders provided with cassettes incorporating markers which define precisely reproducible coordinate axes, the integration of x-ray apparatus into the system as an integral part so that the configurations of the head holder with respect to the source(s) of x radiation are accurately determined, and the development of a ventricular interface detector to indicate when a penetrating cannula pierces the ventricular wall. It has also been necessary to construct appropriate sagittal projection maps of brain and cranial structure topography and to develop, particularly when the ultrasonic method of lesion production is employed, efficient computation methods of handling the data obtained from the roentgenograms.

It is the purpose of this paper to describe the methods which the authors presently employ routinely for determining the coordinate positions of anatomic sites in the brains of experimental animals (cat, monkey).

Instrumentation

The achievement of increased precision in the localization of specific predetermined anatomic sites by the use of internal brain and cranial landmarks, as described in this paper, places more stringent requirements on the instrumentation than is the case for the Horsley-Clarke method employing the ear bar zero and Reid's plane as basic references for a landmark system. To realize the full increase in accuracy which is possible with the landmark methodology outlined here it is essential that the *geometric* uncertainty in positioning not exceed 0.1 mm. If this geometric accuracy is to be maintained in spite of the uncertainties introduced at each stage of the multi-step procedure, it is necessary that the equipment be designed and constructed with a machine tool accuracy that has not been characteristic of most of the apparatus employed in stereotaxic work on the brain up to the present time. Precision must be maintained for all components including: (1) head holder, (2) cassettes which hold the film and incorporate the indicators for the coordinate axes, (3) positioning system which supports and moves the modifying, perturbing or detecting means, e.g., electrode, cannula, etc., and (4) the structure which determines the relative positions of the x-ray source(s) and the head holder with its cassettes. The required accuracy is embodied in the instrumentation system which will be described here.

Systems of various configurations can be designed to implement the work, one principal difference between them residing in the arrangement of the x-ray equipment with respect to the other components. Since it is necessary to obtain at least two projections of the ventricular system and internal configuration of the skull, one can design instruments with either one or two x-ray tubes, as indicated schematically in figures 1 and 2. If a single tube is em-

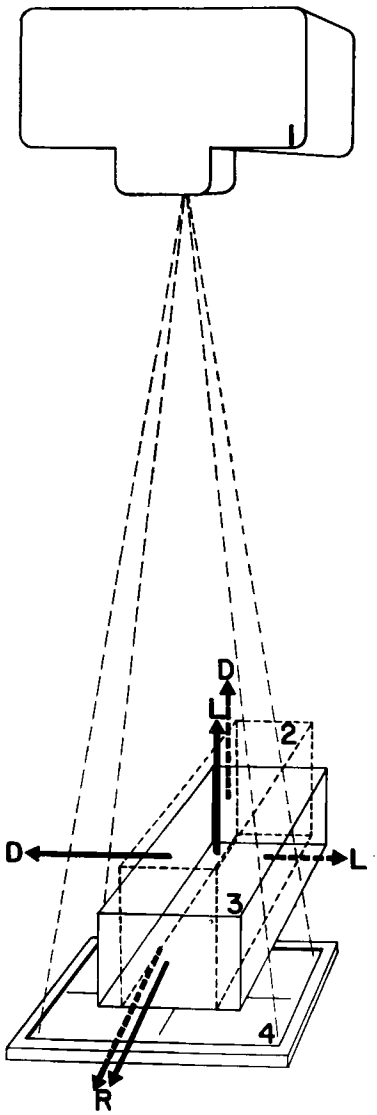

Fig. 1 Configuration including a single x-ray source (1), head of animal in two gravitational field orientations (2) and (3), and cassette (4), employed in obtaining lateral and vertical ventriculograms. D — dorsal, R — rostral, L — lateral.

ployed, it must either be reoriented between the first and second projections or the supporting structure for the animal, including the head holder must be moved (see fig. 1). In addition, if such a system is employed, it is essential that the time required for reorientation be quite short (no more than one minute) since the x-ray opaque medium, which the authors have found best for ventriculography on experimental animals, diffuses from the ventricular system in a few minutes.[3] However, no great difficulty is experienced in designing a one-tube system satisfying the criterion that reorientation require less than one minute; and the authors have employed this type of equipment, in which the head holder is moved between projections, for most of the ventriculographic procedures performed on cats and monkeys (approximately 100 animals to the present time) at this laboratory. Single-tube systems, in which the tube is fixed and the animal is moved between projections, suffer from the disadvantage that different orientations of the brain with respect to the direction of the gravitational field may be associated with different relative positions of brain structures and cranium. Relative movement is especially marked if a large fraction of the cranial vault encasing the brain has been removed. Consequently more precise results are obtained when the head of the animal is maintained in a constant gravitational field orientation for all landmark and placement procedures. For a single-tube system this requires that the tube be movable and, since the source-to-target distance should be at least a meter, the apparatus becomes bulky and heavy. Consequently a two-tube design with fixed positions of the tubes and head holder appears most practical at the present time (see fig. 2).

The authors presently employ such a two-tube system for all ventriculographic procedures, and one such design is illustrated in the photograph of figure 3. A rigid platform supports a head holder in a pin-determined position. This holder is removable from the platform although the instrument is designed so that surgical procedures, electrophysiological studies, and so forth can be accomplished with it in place. The head holder, which is illustrated in schematic detail in figure 4, is provided with ear bars and infraorbital and mouth clamps to support the heads of anesthetized animals in the usual fashion. However, these fixtures do not serve as

[3] Media presently available, which remain in the ventricles for relatively long periods of time, suffer from the disadvantage that they cause histologically identifiable changes in the surrounding tissue.

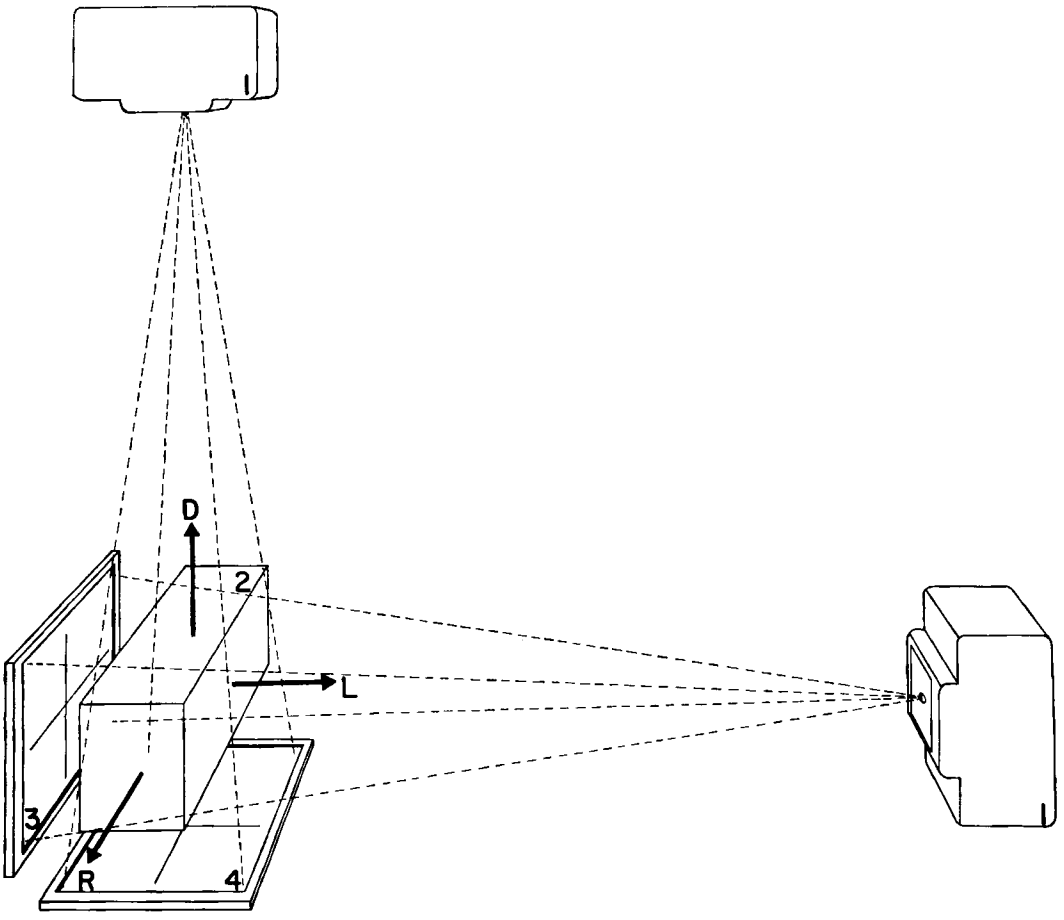

Fig. 2 Configuration including two x-ray sources (1), head of animal in a single gravitational field orientation (2), and cassettes (3) and (4) employed in obtaining lateral and vertical ventriclograms. D — dorsal, R — rostral, L — lateral.

coordinate landmarks except for determining the angular alignment about a longitudinal axis. The angular orientations of the skull about the ear bar and vertical axes do not influence the accuracy of lesion or electrode placement. This head holder also provides a second method for supporting the head of the animal, that is, by means of four pins which engage either appropriately made indentations in, or fixtures mounted on, the skull. This latter support method, which is similar to the one which the authors and collaborators have employed in work on the human (see for example Fry et al., '60), provides the advantage that procedures can be performed under local anesthesia without pain or discomfort to the animal. Of course, when the pin support method is to be employed, the animal, under general anesthesia, can be mounted in the holder first with the head supported by the ear bars and clamps and the sites for engagement of the pins can then be prepared. After the pins are in position the ear bars and clamps can be removed and a local anesthetic agent infiltrated into the tissue at the sites of the incisions to prevent discomfort when the animal is awake.

Two cassettes are provided, one for a vertical and the other for a lateral projection. The positions of these cassettes on the head holder are determined by a pin arrangement which assures a reset accu-

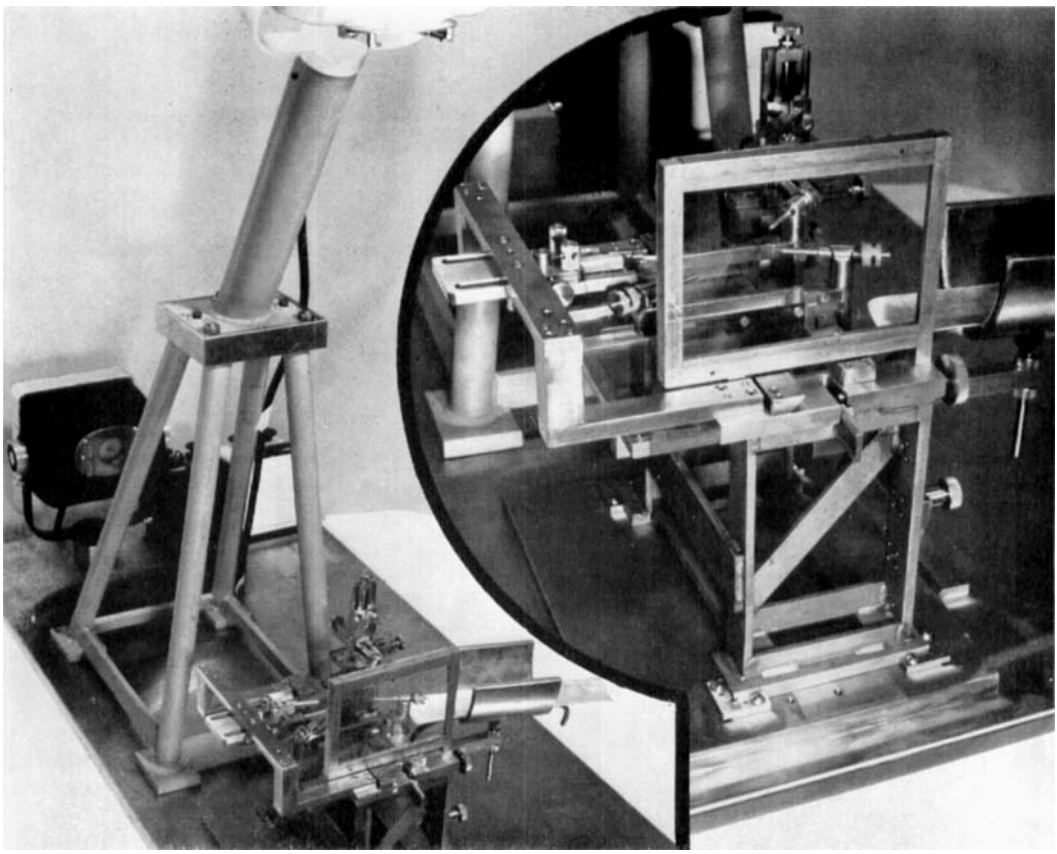

Fig. 3 Instrument for precision x-ray contrast ventriculography and stereotaxic procedures on experimental animals (cat, monkey, etc.). The equipment includes: a head holder provided with two different sets of fixtures for supporting the head of the animal (one the standard Horsley-Clarke clamps, the other a fixture to make work on unanesthetized animals feasible); cassettes mounted on the head holder and provided with markers defining references axes; a removable benchmark which coincides, when in place, with the origin of the coordinate reference system defined by the markers on the cassettes; x-ray tubes for providing two beams of x radiation; a positioning system which supports and moves electrodes, cannula, etc. The base on which the major components are mounted serves to accurately maintain the relative positions of the head holder and the sources of x radiation.

racy of ± 0.001 inch. It is essential that this degree of accuracy be provided since the cassettes not only hold the film but also support the markers which determine the axes of the coordinate system employed. These cassettes are illustrated in detail in figure 5. Lead markers mounted in a three-sixteenth inch thick lucite plate on the face of each cassette define "pinpoint" circular areas on the film to determine the coordinate axes. The system is designed so that the intersection of the rectilinear axes so determined on the lateral and on the vertical roentgenogram respectively coincides with the longitudinal and vertical, and with the longitudinal and lateral position respectively of a demountable "benchmark," which serves as a reference. This latter is provided in order to translate from the origin of the coordinate system employed on the roentgenograms to coordinate values read on the positioning system which supports and moves electrodes, cannulas, etc. The benchmark fixture is positioned on the head holder before the animal is placed in the instrument and its use, as just described, is identical to that of the midpoint of the ear bar axis as coordinate reference

in the Horsley-Clarke method employed up to the present time. Longitudinal coordinate values obtained from the lateral and vertical roentgenograms complement each other, that is, some anatomic sites seen on the lateral roentgenogram are not identifiable on the vertical, and vice versa.

The positioning system, which is mounted on the head holder, can be employed to support the cannula for the ventriculographic procedure; but it is also designed with the machine tool quality required to achieve the anatomic positioning accuracy for lesion production, and for electrical stimulation and recording, made possible by the methods described here. The axes of this positioning system are aligned accurately parallel to those determined by the lead markers on the cassettes. With this system it is possible, for example, to position the tip of an electrode in deep brain at a geometric site determined from measurements made on the roentgenograms with an accuracy of 0.1 mm. The operation of bringing the tip of an electrode (or a "dummy" probe if a site on the electrode other than the tip is to be positioned at a prescribed location) into coincidence with the benchmark is an important step in achieving this accuracy. It should be noted that this coincidence procedure can be accomplished with considerably greater precision than the placement of the tip of an electrode or probe at the midpoint of the ear bar axis as defined by the blunt tipped bars which engage the ear canals.

Of course it is essential, if the accuracy characteristic of the mechanical parts described thus far is to be maintained in the roentgenographic information, to align and maintain accurately the geometric positions of the x-ray sources with respect to the remainder of the system. This is accomplished by mounting all components on a rigid platform. In order to make the computation of coordinate values extremely simple, the x-ray tubes are positioned so that the intersections of the pairs of axes on the lateral and vertical roentgenograms are the sites, one in each plane respectively, at which the images of all structure lying on a line connecting an intersection to the corresponding "point" source of radiation are projected. Thus measurements of the positions of structural landmarks determined from the roentgenograms need be corrected only for the magnification of scale caused by the finite distance of the source. In actual practice, scales can be employed which permit direct reading of the proper values of coordinates from the roentgenograms, that is, magnification correction is automatic.

To identify appropriate internal landmarks roentgenographically, it is necessary to employ x-ray contrast ventriculography in order to reveal boundaries of ventricular structure with sufficient definition. However, the implementation of ventriculography on experimental animals such as the cat and monkey is not so readily accomplished as it is on the human. In these experimental animals the ventricular system is of considerably smaller size than in the human so that the maintenance of the tip of a cannula at a specific location within it must be achieved by a mechanical supporting system. In addition, since the cannula must be of small diameter (No. 22), the criteria which are employed to indicate penetration in the human are practically useless. Therefore it was necessary to develop an instrument for determining unambiguously when ventricular penetration occurs.

The device which was developed utilizes a cannula provided with a readily remov-

Fig. 4 Diagram of head holder and attachments. The Horsley-Clarke fixtures, 8, are shown in position on the holder frame, 9. The fixture, 2, shown at the top of the figure, can be employed for supporting the head of unanesthetized animals by engaging the four pins, 1, with appropriately prepared sites on the skull. Both fixtures can be attached to the frame simultaneously and either can be mounted or dismounted from its position with an animal's head in the holder and supported by the other. The benchmark, 5, which serves as the reference for electrodes or other devices supported on the positioning system, is provided on a fixture, 4, which fastens into position in place of one of the ear bars. The reference position of the benchmark coincides with the origin of the coordinate system defined by the markers, 7, on the cassettes, 6 — the lateral cassette is shown in position on the holder but the horizontal cassette is shown displaced to the lower left. The cassettes are provided with pins which insure high repositioning accuracy on the holder frame. The positioning system, 3, shown mounted on the frame, is machine tool quality and thus provides for accurate placement of electrodes, cannula, etc., at prescribed positions.

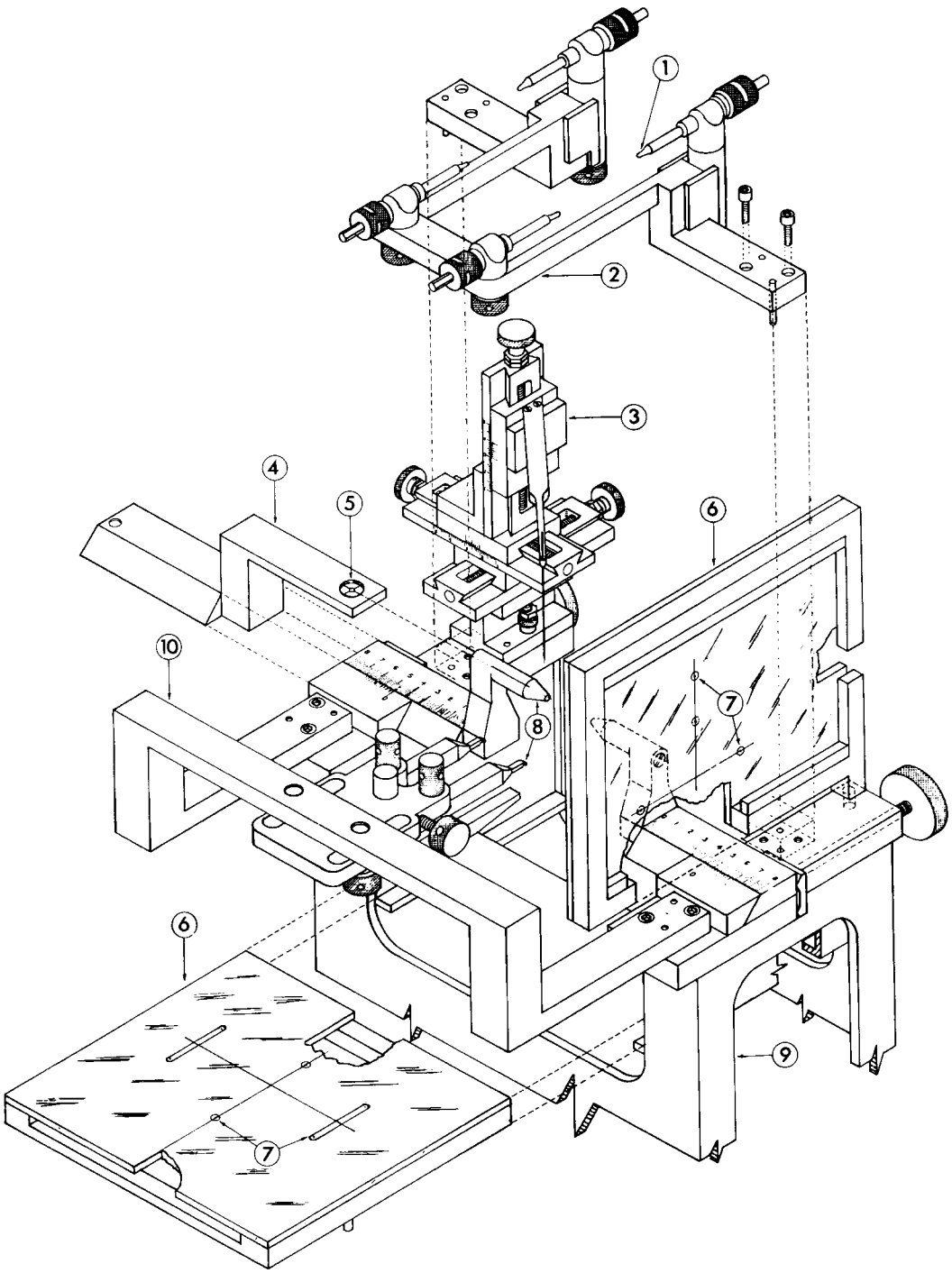

Figure 4

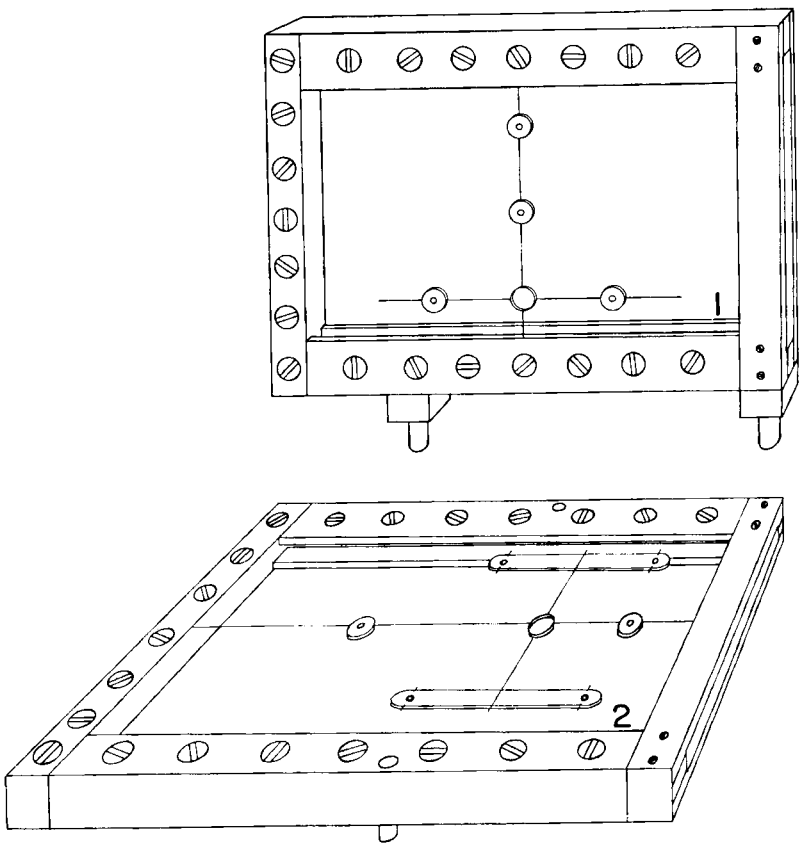

Fig. 5 Diagram of the precision cassettes which provide the rectilinear reference axes to achieve high accuracy in the coordinate values of brain and internal cranial landmarks. The lead inserts containing the "pinholes" which define these axes are shown mounted in the lucite faces of the cassettes. (Vertical, 1, and horizontal, 2.)

able center electrode (diameter 0.010 inch), insulated with Teflon except for a ¾ mm length at the tip, which projects 1¼ mm beyond the end of the cannula (Fry et al., '62). Passage through the ventricular wall is identified by detecting the change in electrical impedance of the medium surrounding the tip and adjacent portion of the metal cannula when the end of the assembly, illustrated in figure 6, passes from tissue into cerebrospinal fluid. The assembly, which is supported and moved by the positioning system, is designed so that a quick-disconnect fitting provides for separation of the stylet-inner-electrode and attached connecting concentric cable from the cannula. In use the outer conductor of the cable is electrically continuous with the cannula and instrument casing, thus making the detector insensitive to electrical pickup. The result of the impedance measurement is presented on a meter with the deflection from a preset value proportional to the magnitude of the impedance change. The deflection of the meter pointer, which occurs when the tip of the cannula-electrode assembly moves from subcortical white matter, corpus callosum, or other similarly dense fiber tract region into the cerebrospinal fluid of the ventricular system, is a decrease of 30 to 40% for an operating frequency of 2,000 c/sec and for the size and configuration of cannula-electrode assembly currently employed in routine ventriculography procedures on cat and monkey at our laboratories.

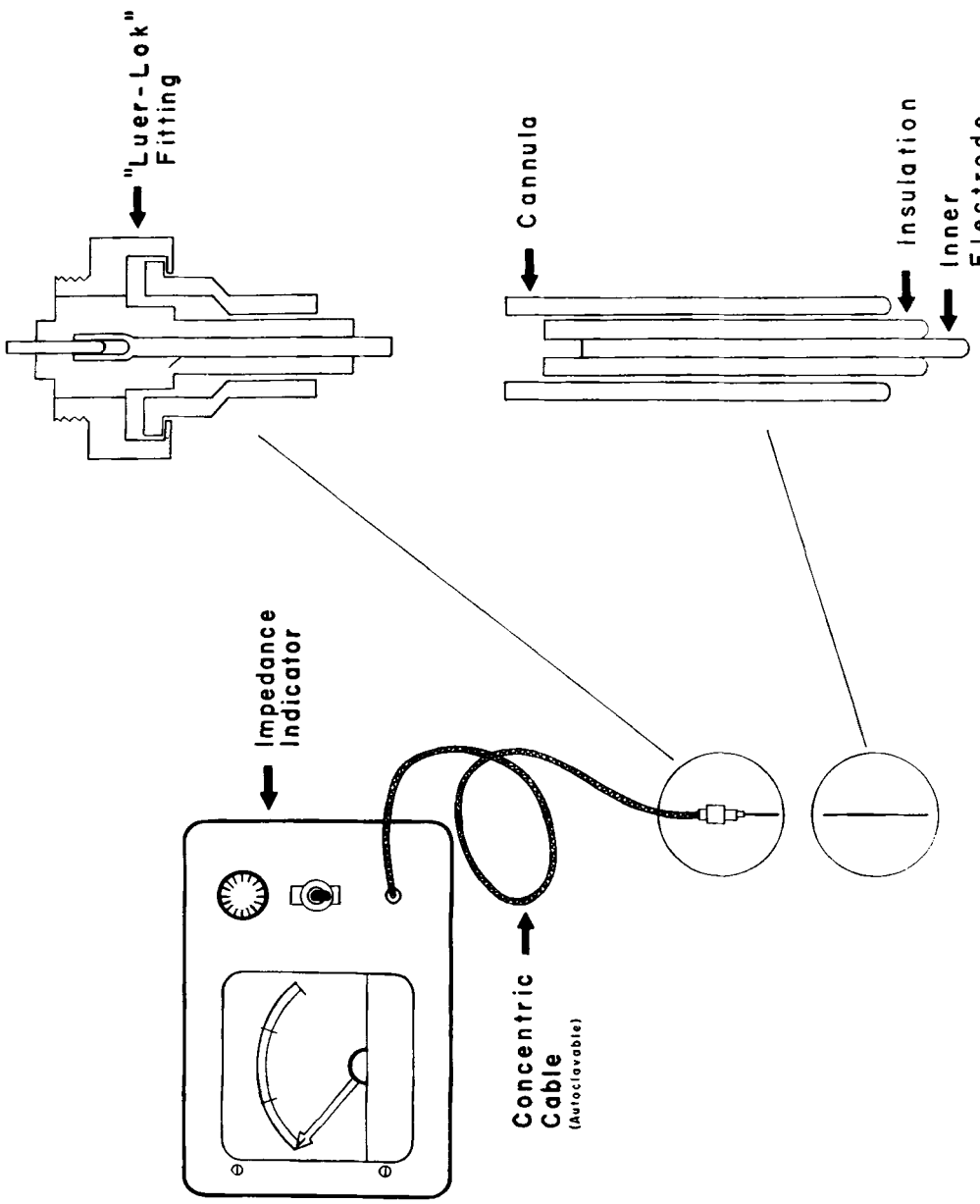

Fig. 6 Diagram of electrode-cannula assembly, connecting cable and electronic impedance indicator used in implementing ventriculography on experimental animals.

Procedure for determining coordinates of landmarks and internal brain sites

The production of lesions, placement of electrodes for stimulation or recording, etc. can proceed immediately after the completion of the ventriculographic procedure which provides the landmark information underlying the determination of the coordinates of chosen brain sites; that is, the animal need not be removed from the apparatus after ventriculography and before instituting subsequent procedures. However, in some cases it is desirable or necessary to interpose an extended time interval between ventriculography and stereotaxic procedures to follow. This is often the situation when multiple procedures are planned and it is currently the method followed when ultrasonic lesion arrays are placed in the brain. In such cases where brain modification or electrode placement follows ventriculography after a lapse of time, it is not necessary to duplicate precisely the position of the animal's head in the holder. This is a consequence of the fact that the landmark information evident on the roentgenograms at the time of ventriculography can be transferred to skull roentgenograms taken any time the animal's head is repositioned in the holder. This is accomplished by superimposing corresponding roentgenograms to align the skull images, and then marking the useful information on the skull roentgenograms taken at the time of the planned stereotaxic procedure.

For ventriculography the animal's head is fixed in the instrument and, under sterile conditions, the site of penetration of the cannula-electrode assembly is prepared by incising the scalp and making a small burr hole (for ultrasonic irradiation work on the cat, the ventriculographic procedure is preceded by a craniectomy two weeks or more earlier and consequently one need only remove the plastic replacement in this case). The position of the site of penetration of the cannula can be decided with sufficient accuracy from external landmarks, for example, a chosen distance from an externally judged midsagittal plane and a chosen anterior-posterior coordinate position measured with respect to the external acoustic meatuses. After the tip of the electrode-cannula assembly (supported by the positioning system) penetrates the cortex, the deflection of the meter pointer is adjusted to some convenient position and the assembly is moved into the brain. When the change in pointer deflection indicates ventricular penetration, the stylet-electrode is removed.[4] With some experience, failure to identify penetration occurs not more frequently than once in about 100 procedures on the cat. After one of the cassettes is placed in position on the head holder, a syringe containing an appropriate amount of x-ray opaque medium (Conray[5]) is slipped into the fitting on the end of the cannula and the medium is injected at a rate to cause mixing with the cerebrospinal fluid. It should be noted that the authors consider "Conray" the medium of choice for such work. It is a water soluble iodinated compound and is rapidly excreted, so that within ten minutes after injection practically no evidence of its presence can be detected on roentgenograms. Its use in the required quantity is accompanied by negligible to slight transient neurological signs in practically all cases, and histological preparations of tissue sections present no evidence of changes induced in the structures bordering the ventricular system. Excellent x-ray contrast is obtained on injection of 0.6 cm^3 of the medium into a lateral ventricle of the cat as can be seen from the typical lateral and vertical roentgenograms illustrated in figure 7 (a,b). The axes employed in the measurement of the coordinate values of the landmarks are shown scribed on the roentgenograms; the projected boundary configurations of the two lateral, third, and fourth ventricles are

[4] It should be noted that, in general, with the small cannula size employed, no cerebrospinal fluid can be aspirated.

[5] "Conray" is the Mallinckrodt Chemical Works' aqueous solution of methylglucamine methalamate, 28% by weight of iodine.

Fig. 7 Typical ventriculograms for cat. The projected ventricular boundaries and internal cranial configuration provide a variety of internal landmarks. (a) Vertical ventriculogram exhibiting the third ventricle and the aqueduct of Sylvius. Accurate coordinate values for the midsagittal position of deep brain at specific longitudinal sites can be determined from this roentgenographic projection. (b) Lateral ventriculogram exhibiting projections of the lateral and third ventricles, the aqueduct of Sylvius and the fourth ventricle.

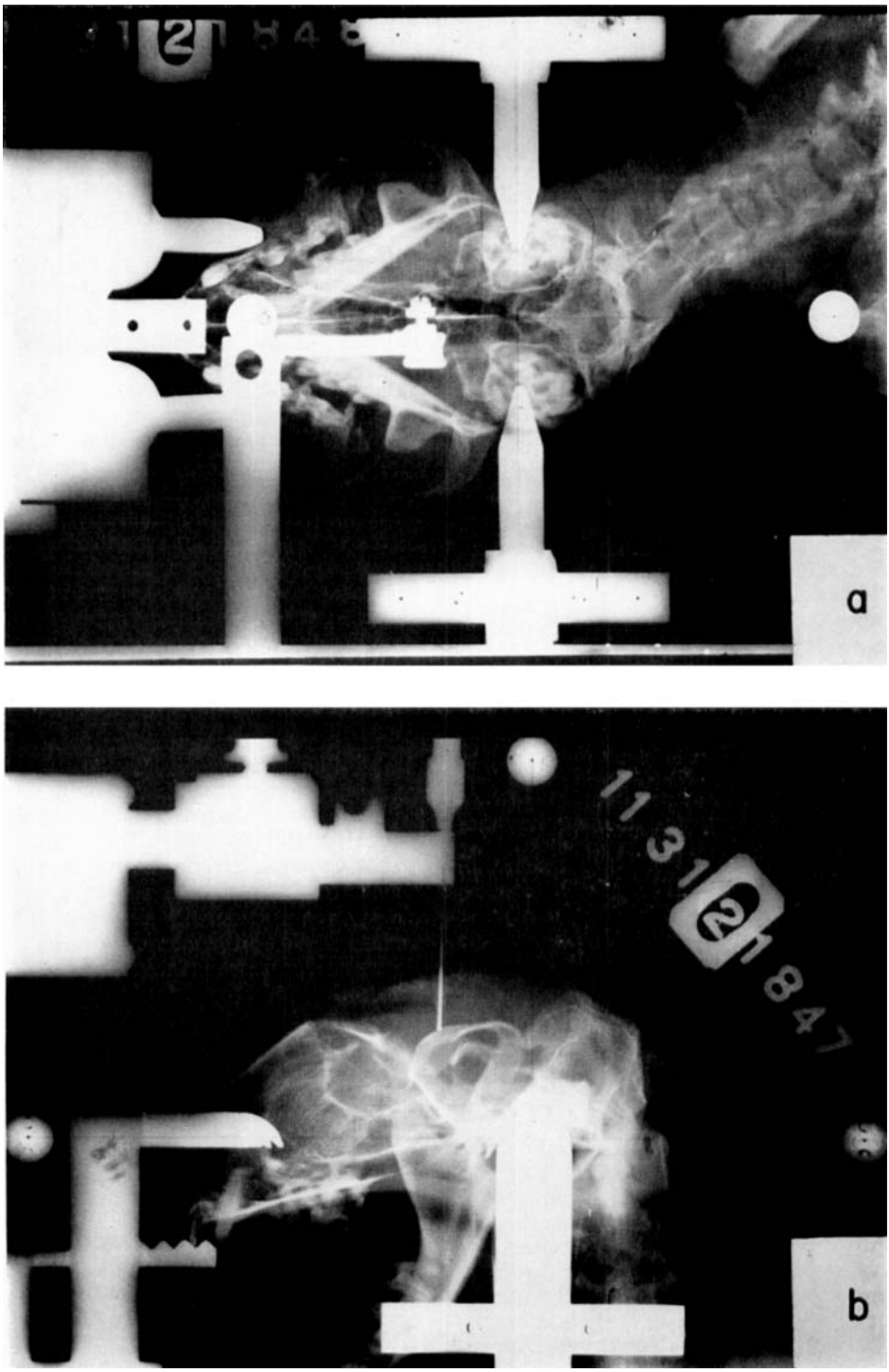

Figure 7

clearly illustrated, and the aqueduct of Sylvius is well delineated. From such roentgenographic detail it is obviously possible to derive a considerable amount of landmark information.

The landmarks, which serve as a basis for computing the coordinates of any specific anatomic structure, are decided on the basis of the experience gained from employing a number of landmark configurations in order to choose one to provide "optimum" values for the coordinates of the anatomic sites or structures of interest. Specific examples will be indicated subsequently. At this point it should be noted that the lateral roentgenographic projection exhibits a number of extremely useful brain landmarks, e.g., the ventricular boundary contours of the anterior and posterior commissures and adjacent structures, etc. Internal bony landmarks of considerable utility are the contour of the base of the brain case and the posterior clinoid process.[6]

Since interanatomic distances (landmark to landmark, landmark to chosen anatomic site) are not constant from one brain to another, optimum estimates of coordinate values for chosen sites are not realized without a method of scaling to take into account the different relative configurations characteristic of individual brains. The authors currently employ linear scaling methods in calculating values for the coordinates of anatomic sites of interest from measurements of the coordinate values of landmarks. These methods can be illustrated best by specific examples; however, it is first necessary to discuss sagittal anatomic projection maps.

The sagittal projection map for cat, figure 8, exhibits all the landmarks employed in the calculation of coordinate values for the structures discussed in the examples and also the midsagittal planar projections of boundaries of specific anatomic structures. The map shown in the figure was constructed from: Jasper and Ajmone-Marsan's ('54) atlas for the diencephalon, a series of slides prepared in the authors' laboratories for the brain stem, and brain cutdowns in situ for information on the relative positions of bony landmarks and adjacent brain structures. Appropriate scaling was used to match the interanatomic distances of the cat brain employed for the brain stem reconstruction with those of the Jasper atlas in the region of overlapping. It should be noted here that any one brain or appropriately scaled combination can be employed to construct this type of sagittal map.

At the present time two different methods of data handling are employed to determine for any specific subject animal the coordinates (longitudinal and vertical) of chosen anatomic sites from the information summarized on the sagittal map and the data obtained from the lateral ventriculogram. In the first method the roentgenogram is optically projected at adjustable magnification onto the sagittal map. By adjustment of the magnification it is possible to bring into coincidence chosen pairs of landmarks and to simultaneously satisfy certain specific criteria with respect to others, as will be illustrated below. When the criteria are satisfied a mark, determined in position by projection of a chosen anatomic site appearing on the sagittal map, is placed on the lateral roentgenogram to designate the estimated projected position of the anatomic site in the subject animal. If electrode placement work is to be implemented immediately after ventriculography (i.e., without removing the animal from the holder), then the coordinate values, longitudinal and vertical, of the position of this mark on the lateral ventriculogram are measured with respect to the rectilinear axes scribed on the roentgenogram. If electrode or other placement work is to be done later, when the animal is returned to the holder, then the position of the mark is transferred to the lateral skull roentgenogram taken at that time and the coordinate measurements are made. Simple arithmetic computation then yields the values to be set on the positioning system to place the penetrating electrode, other instrument or means at the desired site.

A second method of employing the sagittal map and lateral roentgenographic information to obtain the longitudinal and vertical coordinates of specific anatomic sites involves the use of a computer. This

[6] In the cat the posterior clinoid is not readily observed on lateral roentgenograms in about 40% of the animals, but its longitudinal position can practically always be determined from a vertical roentgenogram.

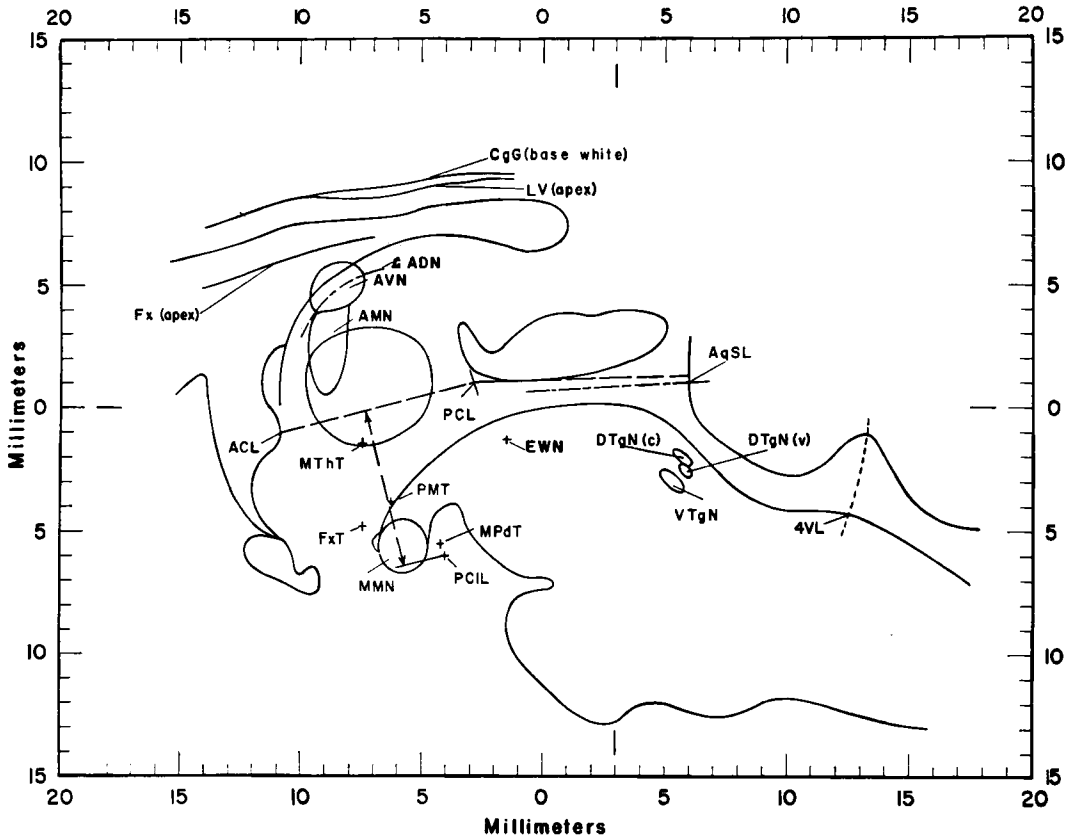

Fig. 8 Sagittal projection map of portion of cat brain exhibiting landmarks and some anatomic structures of specific current interest for the authors. The information available from this map, together with various scaling methods, are employed to provide values of the longitudinal and vertical coordinates of specific anatomic structures, or sites therein. The two pairs of short line segments indicate the vertical and longitudinal positions of the origin of coordinates employed in the Jasper and Ajmone-Marsan atlas with respect to the coordinate system of the projection map. Meaning of abbreviations: (right hand letter of symbols, L — landmark, N — nucleus, T — tract) ACL — anterior commissure landmark; PCL — posterior commissure landmark; AqSL — aqueduct of Sylvius landmark; PClL — posterior clinoid landmark; AVN, AVM — anterior ventral and medial nuclei of thalamus; ADN — center (dorsoventral) of anterior dorsal nucleus of thalamus; MMN — medial mammillary nucleus; VTgN — ventral tegmental nucleus; DTgN (c), DTgN (v) — subdivisions of dorsal tegmental nucleus; MThT — mammillothalamic tract; PMT — principal mammillary tract; MPdT — mammillary peduncle; FxT — fornix; Fx (apex) — apex of subcallosal fornix; CgG (base white) — base of white matter in medial aspect of cingulate gyrus; LV (apex) — apex of lateral ventricle.

latter procedure is particularly useful when the coordinate values of a large number of anatomic sites are to be determined. In this case the coordinates of specific point landmarks are measured from the lateral roentgenogram and these values, together with the values of the sagittal map coordinates for the same anatomic landmarks and for the anatomic sites of interest, are introduced into the computer. The computer code is formulated to employ linear scaling to take into account differences in landmark-landmark distances between sagittal map and brain of subject animal. A disadvantage of the present computer method is that entire contours of landmarks are not utilized. However, future plans include the formulation of more sophisticated coding to permit the use of landmark contours.

It should be noted that the positions of the sites indicated in anatomic structures on the sagittal map are not simply the result of plotting atlas data; instead they represent the accumulation of experience resulting from the placement of lesions in the indicated anatomic structures in a number of brains by the method described here. In this sense the sagittal map undergoes continuing modification with improvement in the choice of site coordinates with respect to old or new landmark configurations. The particular sites indicated on the map illustrated in figure 8 are those that have received our attention.

The vertical roentgenogram provides the information on machine coordinates of the midsagittal position of the deep brain at the longitudinal coordinate values corresponding to the anatomic structures of interest. At the present time, lateral values with respect to the midsagittal position are obtained directly from published atlases or from transverse tissue sections available at the laboratory — that is, no lateral scaling is employed. In some cases the vertical roentgenogram also provides longitudinal coordinate values for certain landmarks.

The following specific examples illustrate the optical projection method for determining machine coordinates for anatomic sites. Since the *present* computer method utilizes much less of the available landmark information it will not be illustrated here.

The machine coordinate values for specific anatomic sites in the medial and lateral mammillary nuclei, principal mammillary tract, mammillary peduncle, and neighboring structures are obtained as follows: (1) project the lateral roentgenogram onto the sagittal map, (2) superimpose ventricular contours of the mammillary complex and adjust the relative angular configuration so that the angular deviations of the directions of both the posterior commissure landmark and the contour of the optic chiasm from the mammillary bodies are approximately equal, (3) adjust the magnification to equate the distance between mammillary complex and base of massa intermedia, (4) place a mark on the roentgenogram at the position corresponding to the projected image of the anatomic site of interest on the sagittal map and measure the longitudinal and vertical coordinate values of the mark with respect to the axes determined by the markers on the cassette. Values for the machine coordinates then follow by simple arithmetic computation. The lateral machine coordinate value for the anatomic site is obtained by combining the value of the lateral coordinate of the site as obtained from an atlas of transverse sections with the deviation (measured on vertical roentgenogram) from the midline of the machine of the midsagittal position of the third ventricle at the longitudinal location corresponding to the anatomic structure.

The optical projection method of landmark data handling is also currently employed for placing lesions in the dorsal and ventral tegmental nuclei of von Gudden and in neighboring structures. In this case it is convenient to employ, in addition to the "point" landmark determined by the intersection of the axis of the aqueduct of Sylvius with the posterior boundary of its cisternal expansion at the head of the fourth ventricle (see fig. 7b), the "ventral" boundary of the lateral projection of the aqueduct and anterior part of the fourth ventricle. The image of the roentgenogram is projected onto the sagittal map and magnification and translation adjustments are made to (1) maintain parallelism of the axes of the aqueduct, (2) superimpose the longitudinal positions of the point landmark, (3) equate the distances between the point landmark and the ventricular boundary contour of the posterior commissure region, and (4) superimpose the "ventral" boundary of the aqueduct and fourth ventricle in the longitudinal position at which the anatomic structures of interest lie. Marks determined by the positions on the sagittal map of the tegmental structures of interest are then placed on the roentgenogram to indicate the estimated positions of the anatomic sites in the subject animal. Longitudinal and vertical coordinate values for each site are then measured with respect to the axes determined by the markers on the cassette. Lateral coordinate information is obtained from an atlas of transverse sections and from measurements, made on the vertical roentgenogram, of the deviation of the

axis of the aqueduct from the midline of the machine at the longitudinal location of the anatomic structures.

A third example illustrating the optical projection method involves the interruption of the mamillothalamic tract. Reference should be made to the sagittal map where a marker indicates the location of this tract at a position corresponding to its midthalamic course, that is a site lying approximately halfway between the posterior position where it arises from bifurcation of the principal mammillary tract and the anterior position where it begins to sweep dorsally toward the anterior nuclei of the thalamus. The landmark criterion employed is as follows: (1) superimpose the lateral roentgenogram onto the sagittal map and adjust the magnification to equate the lengths of the intercommissural line, (2) maintain parallelism between the intercommissural lines, while superimposing the ventralmost aspect of the bases of the massa intermedia. A mark is then placed on the roentgenogram at the site of projection of the specific location along the mammillothalamic tract and its longitudinal and vertical coordinate values are measured. From the vertical roentgenogram determine the midsagittal position of the third ventricle at the longitudinal location corresponding to the site indicated.

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