A Technique for Making Thin Brain Slices for Stereotaxic Measurements

Technical Note

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An instrument has been designed which makes accurate 5 mm sequential sections of fixed brain tissue. Measurements of macroscopically identifiable cerebral structures can be made from these sections and stereotaxic charts constructed for use in surgery. The method initially has been used to define the spatial relationships of the dentate nucleus of the cerebellum to the fourth ventricle in order to carry out stereotaxic operations in the posterior fossa. The technique has minimized the technical artifacts often associated with embedding, sectioning, and staining of brain tissue. It utilizes a technique of radiographic alignment of the brain specimen to obtain an exact plane for sectioning.

Method

The technique for preparing the cerebellum and brain stem for sectioning is carried out in three steps: 1) the preparation of the brain and its radiographic alignment; 2) the sequential sectioning of the brain into exact 5 mm slices and 3) the direct measurement of cerebellar structures on the unstained or stained sections of the fixed brain.

Step 1: Preparation

The brain is suspended from the basilar artery in 15% neutral formalin for a period of 3 to 5 weeks. The brain stem and cerebellum are then removed en bloc by sectioning through the midbrain at the level of the colliculus. A liquid radio-opaque medium is injected through the foramen of Magendie into the fourth ventricle and aqueduct of Sylvius. The material, which contains 5% agar and 1% barium sulfate in 60% Methyl glucamine, rapidly solidifies when it cools. The injected specimen is placed in a lucite box with detachable sides 4½ in. square (Fig. 1), on a base of jelled 5% agar. The two opposing sides of the lucite box are divided into squares by leaded grid lines which are used for radiographic alignment of the midsagittal plane of the cerebellum and brain stem.

The midline of the specimen is first visually aligned with the grid lines. The box is then placed 40 in. from the focal point of the x-ray tube. The central beam of the x-ray is so aimed that it will pass through the center of the two opposing grid lines. The precise midsagittal plane is then determined by superimposing the grid lines over the midsagittal plane of the radio-opaque outline of the aqueduct and fourth ventricle (Fig. 2). A final fine adjustment of the specimen in relationship to the grid line is done by using 1 × 2 in. lucite plates which can alter the position of the specimen in either the horizontal or vertical direction. Once the midsagittal...
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FIG. 2. An enlargement of a roentgenogram of the fourth ventricle injected with radio-opaque material. Left: The fourth ventricle viewed from a dorsoventral direction. The single cross represents the perfect superimposition of the two crossed lead lines on the opposite faces of the lucite box. The vertical line in the center of the image of the fourth ventricle represents the true midsagittal plane. This line is used for aligning the central blade in the knife carriage for sequential midsagittal sections. 1) aqueduct, 2) superior lateral recess, 3) foramen of Luschka, 4) vallecula cerebelli. Right: The same image of the fourth ventricle viewed in a rostrocaudal direction. The vertical line divides the floor of the fourth ventricle at its midpoint assuring further accuracy for the true midsagittal cut. 5) fastigium, 6) superior lateral recess, 7) foramen of Luschka, 8) sulcus limitans in the floor of the medulla.

plane has been determined the specimen is prepared for sectioning.

The lucite box, with its radiographically aligned specimen, is filled with a solution of 5% cooled agar, which is allowed to gel. Two silk sutures are inserted through the agar block at right angles to the midsagittal plane. When the block is sectioned, the cut ends of the suture material embedded in the block can be used for additional points of orientation. The specimen in the agar block is now ready for sectioning and is attached to the base of the machine. The true midsagittal plane determined by the radiographic alignment is centered beneath the central knife base of the machine.

Step 2: Brain Cutting

The brain cutter is constructed of stainless steel and consists of a supporting frame for a carriage containing multiple stainless steel blades (Fig. 3). There are 11 fine ribbon knife blades of stainless steel (.007 in. thick, 7½ in. in length and ½ in. in width) mounted parallel and separated by a distance of 5.0 mm. A tension of 25 lbs is maintained by the separation bars. The blades held in the carriage are driven by an electric motor which oscillates at a frequency of 5 cps. A hand screw mechanism advances the oscillating carriage down through the specimen. The lucite box is placed on the base of the machine. The true midsagittal plane determined by the radiographic alignment is centered beneath the central knife base of the machine. The true midsagittal plane determined by the radiographic alignment is centered beneath the central knife base of the machine.

FIG. 3. The cutting machine with multiple blades below A. The lucite box contains the cerebellar specimen aligned for sectioning.