

SERIAL FROZEN SECTION OF WHOLE BRAIN¹

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During the past decade, reconstructions of monkey brains lesioned or implanted for behavioral studies have been time consuming. The imbedding process especially has taken precious months that delay completion of an otherwise finished study. To overcome this difficulty the present method, developed for rapid location of electrode implant sites, was modified to allow serial analysis of whole monkey brains.

At the time Ss are sacrificed their brains are perfused with 10% formalin. The brain is then fixed for a week in 10% formalin (a rat brain requires only 48 hr.), neutralized with chalk or CaCl₂ after which a small unwanted portion, caudally or rostrally, is cut away and a small metal pin is inserted to form a guide hole through the entire length of brain. The brain is then placed in 20% alcohol for 36 hr. If a Technicon is available or a Lipshaw Tissue Plunger and Rotator, the time can be shortened to 12 hr. When ready to section, the metal pin is removed and the brain is frozen, using a freezing block in which dry ice is evaporated in 70% alcohol. For most work, a modification of the type furnished by the American Optical Co. is advantageous. The well is enlarged to permit greater circulation of the alcohol and better temperature control. The mounting base for the brain in the well is made removable and interchangeable so that other mounting bases of varying diameters can be used for different size brains or tissue blocks.

Sections are floated in 20% alcohol and mounted onto prepared glass slides with the aid of a sable brush. Sections adhere to the slides better if Mayer's fixative is applied the day before needed, and the slides incubated at 37° C overnight. The slides can be numbered at this time, using Labink, which after it has dried overnight does not come off during any staining process.

An entire monkey brain can be sectioned, requiring 5 to 7 hr. of fairly constant temperature control. In this instance, a single, large, thick piece of dry ice is used. If it is of such diameter and height that, as it melts, it fills the entire freezing well around the mounted brain and yet retains the same height as the brain, a more even temperature is obtained. Thus, as one sections, the ice melts just fast enough to clear the edge of the knife as it is drawn across. Intermittent cutting, as well as uneven freezing, results in poor sections. This is especially true of larger mounts where not only the temperature but the sectioning should be fairly constant.

¹We sincerely wish to thank Dr. Ross Adey under whose guidance many of these modifications of technique were accomplished.

For serial reconstructions of lesions, sections are cut at 50 μ ; every tenth section is saved; every twentieth stained with aniline Thionin. When the site of electrode implantation is sought, sections are cut at 80 μ and every other one saved in the approximate vicinity of the implant. For this purpose, Alum Carmine or Neutral Red may be used in addition to or instead of Thionin.

Warming plates at two temperatures, 35° and 42° C approximately, are used for drying sections. Usually an hour is sufficient, moving the slides from the warmer temperature to the cooler one after a few minutes. Additional drying is necessary in an incubator at 37° C overnight.

Ian Hine at Melbourne University, Melbourne, Australia, has devised an ingenious method for sectioning brains that are poorly fixed, necrotic, or have large lesions. A raw potato is peeled and a hollow scooped out sufficiently to hold the portion of brain to be cut. The potato is attached to the aluminum mount with water, and water is poured around the brain, inside the potato cup, to facilitate freezing. The entire mass freezes evenly, and one can section a very narrow slice of brain in this fashion if approximately 5 cm of potato is left under the brain as a base. One should not align the knife with the base of the brain as is routine; instead, the first section made should be parallel with the knife blade and if necessary the block itself rotated on the microtome to achieve this.⁶ It is often difficult to position the brain inside the potato cup evenly, hence the reversal of alignment. In cutting, the potato can easily be brushed away as one mounts each section. This method has proven invaluable where there are extreme temperature changes in the laboratory. The potato casing covers all vulnerable spots that tend to thaw rapidly, and maintains an even frozen condition for hours. The potato does not interfere with Thionin staining, but starch granules are apparent when one uses Neutral Red stain.

Summary.—A technique for making serial sections of whole monkey brains is reported. This technique allows a saving of months over the usual celloidin imbedding procedure when reconstructions of lesions or electrode implantations are needed. The technique speeds the reporting of neurobehavioral experiments.

Accepted July 19, 1962.