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## TECHNICAL NOTE

# *A SIMPLE HISTOLOGICAL TECHNIQUE FOR LOCALIZING ELECTRODE TRACKS AND LESIONS WITHIN THE BRAIN*[1]

Histological procedures are necessary in brain stimulation or lesion work to determine the neural area which has been stimulated or damaged. Preparation of brain tissue often involves embedding and staining techniques that require specialized training, and the expense of a technician and a large assortment of special apparatus and supplies. In addition, the results of such techniques are unavailable for at least several days. A photographic method, which requires little special skill and a minimal amount of apparatus, is described here. Results can be available within minutes after the subject is sacrificed. This method has been shown to be adequate for the gross determination of lesion boundaries and electrode or cannula tip loci in brains of rats, cats, and squirrel monkeys.

## METHOD

### *Apparatus*

In addition to the standard instruments needed for perfusion, and freezing and sectioning the brain, special apparatus is required for taking photomicrographs of the sections. The photomicrographic equipment should be capable of producing a picture of a whole brain section for primary localization, as well as higher power pictures for more detailed representation. Therefore, the range of magnification should be at least from X3 to X22. The Bausch and Lomb Model L photomicrographic system is very satisfactory. Less expensive systems, such as a micro-projector-camera setup or a simple photo enlarger, have also been used. A low-power stereoscopic microscope with camera might also suffice. The camera and film may be of a common type, such as 35 mm, or Polaroid.

### *Procedure*

After perfusion and decapitation, the head is placed in the stereotaxic instrument and the dorsal half of the skull is removed. The dura covering the brain is removed and a modified knife blade placed in the stereotaxic electrode holder is used to cut out the block of tissue that contains the lesion or electrode tracks. Two cuts are made in the coronal plane, one an-

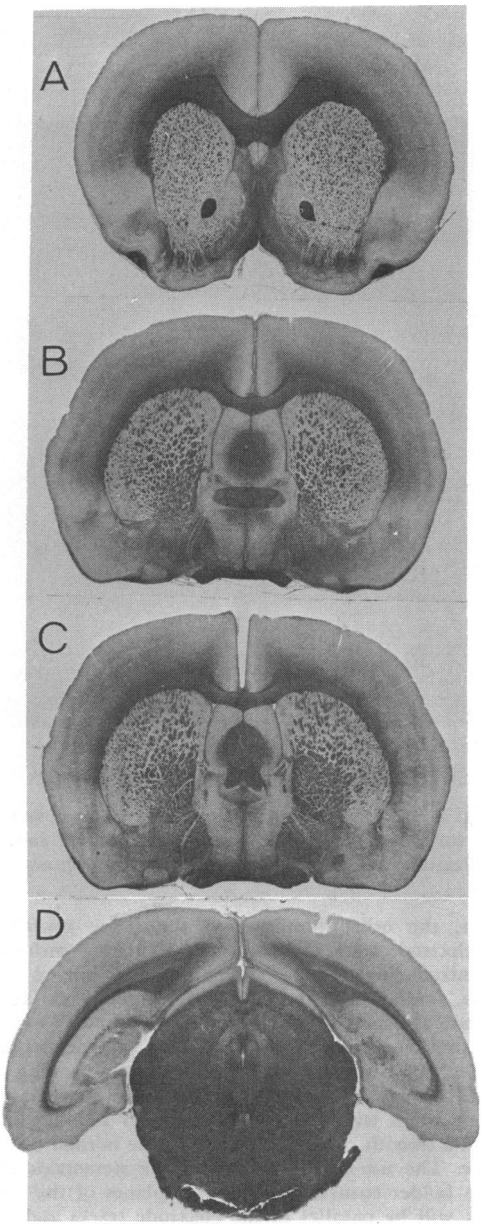


Fig. 1. Coronal sections (50 $\mu$) demonstrating several areas of the rat brain (Polaroid Type 47 film).

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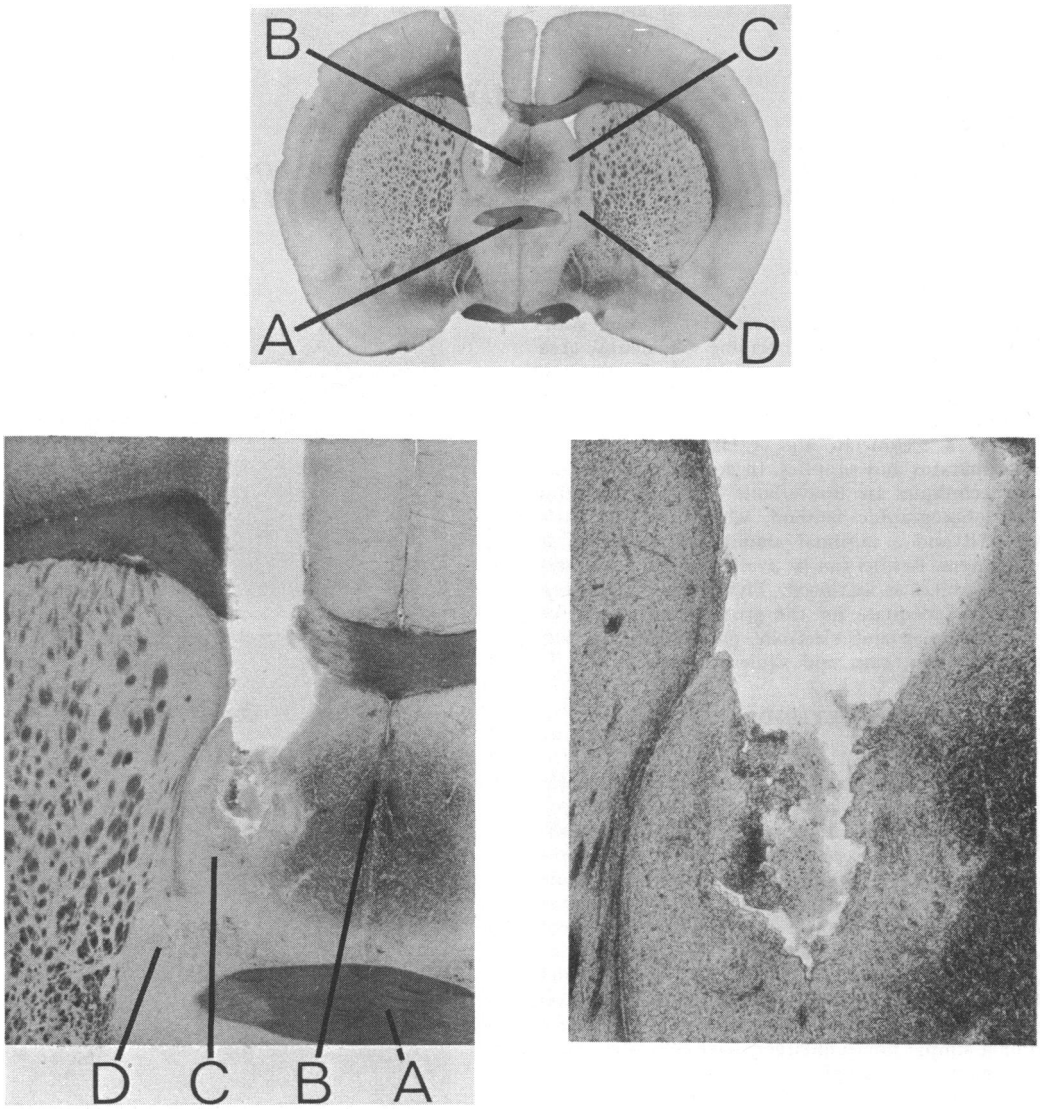


Fig. 2. Different magnifications of a coronal section (50 $\mu$) of a rat brain. The section is at the level of the anterior commissure and displays a 21-gauge cannula track. The lower magnifications provide information for localization and the highest magnification shows damage around the cannula tip (Polaroid Type 55 P/N film).

terior, the other posterior to the tissue containing the electrode track or lesion. These cuts are performed by introducing the 2-in. long, slender, convex knife blade, similar in shape to a #23 scalpel blade, at the far lateral aspect of the brain. By alternately extending and retracting the knife by use of the electrode carrier depth-control knob, while slowly moving the knife through the midline to the near lateral aspect of the brain with the electrode carrier lateral-control knob, a smooth cut will be made in the normal coronal plane. The use of the knife and the stereotaxic electrode holder ensures that the end planes of the tissue block will be parallel to the electrode tracks and also, typically, will correspond to the brain atlas plane. Cranial nerves are then severed and the tissue block is removed from the skull with the aid of a spatula, washed in tap water, and placed on the stage of a freezing microtome. Unstained sections are then taken (50 $\mu$ for rats; 75 $\mu$ for cats and monkeys) and selected for mounting on microscope slides. Localization can then be done immediately with the aid of an atlas or after a permanent record of the sections is obtained.

Although localization may be obtained merely by inspecting the unstained section, it is usually desirable to have some permanent record of the appearance of the section, or sections, from which the localization was made. The traditional procedure is to prepare these sections for mounting upon microscopic slides. Frozen sections may be placed in an alcohol bath, then mounted on a slide. It is then necessary, however, to

prepare this material in order to demonstrate structures that can no longer be differentiated optically. Therefore, the tissue is traditionally put through a series of solutions to accomplish differential chemical uptake in order to accentuate various structures. This stained tissue is permanently kept on a slide for microscopic observation. The procedure proposed here is to obtain a photographic representation of a wet unstained section, in which fluids are still present and where structure remains intact and discriminable. This can be done employing a photomicrographic camera, preferably one that allows both gross and more detailed representations of the sections to be obtained. The photos can be taken on a regular-type black and white film and the sections saved in formalin until the film is developed and the results noted or, preferably, Polaroid film can be used to produce rapid photographic prints.

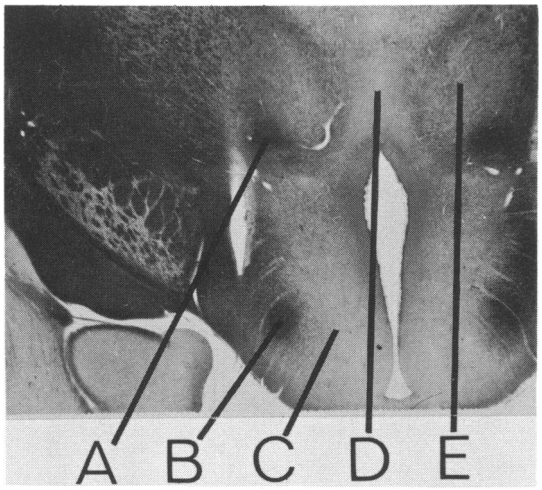


Fig. 3. Mid-hypothalamic coronal section (75 $\mu$) of a cat brain. The electrode track displayed is in the left lateral hypothalamus (Polaroid Type 47 film).

Aperature setting, focus, lens power, and shutter speed of the photomicrographic apparatus can be initially calibrated and the settings recorded for assorted tissue specimens so that little trial and error is necessary on subsequent occasions. In this way, usually only fine focusing of the image is then required for each section.

Since it is desirable to get as large a print as possible of the relevant area of tissue, a Polaroid camera back has been used in conjunction with Polaroid sheet film pack (Type 55 P/N) to give a 4 by 5 print as well as a negative for making further prints.

Figure 1 shows sections of rat brain cut in the DeGroot (1959) coronal plane. These are at the level of: A, the anterior septal area; B, the anterior commissure; C, the columns of the fornix; and D, the pons. Figure 2 contains a series of photos of a rat brain section from the level of the anterior commissure containing a 21-gauge cannula track. The lowest magnification is adequate for localization in this case, but the medium shot also gives helpful information concerning penetration depth. The highest magnification, obtained with a low-power lens on a microscope, demonstrates more adequately the damage present around the cannula tip. In each of the three photos fiber tracks are clear (*e.g.*, A. anterior commissure; B. columns of fornix) and nuclear masses can be seen (*e.g.*, C. lateral septal nucleus; D. bed nucleus of stria terminalis). In Fig. 2, the visualization of both types of structure in one section (rather than for fibers or nuclei separately as is often more conventional) aids interpretation of all the structures affected by the procedure. Also, of course, the use of unstained, unembedded tissue results in a preparation that is less distorted or shrunken, and thus is a more accurate representation of the experimental state.

Figure 3 demonstrates an electrode track in a cat brain sectioned at the level of the dorsal and ventromedial hypothalamic nuclei (Jasper and Ajmone-Marsan, 1954). Fiber tracks such as the mamillo-thalamic tract (A) and the fornix (B) are easily visible, and the outline of nuclear groups, such as the ventro-medial nucleus of the hypothalamus (C), the reuniens nucleus (D) and the medial part of the ventral nucleus (E) of the thalamus can also be seen.

Modifications of the method described here are possible, depending upon the relative importance of speed of results, equipment costs, *etc.* Guzmán, Alcaraz, and Fernández (1958) have described a procedure for acute preparations where the section is used as a negative in a simple photo enlarger. Another procedure (Powell, 1964) employs an inexpensive micro-projector in conjunction with a 35-mm camera, using roll film or a Polaroid back and film. This procedure, however, requires that the room be darkened during the actual photographic process.

The present method yields complete results quickly and at low cost. Complicated and expensive staining procedures are eliminated and the resulting representation of brain tissue is less distorted or shrunken and contains excellent delineation of brain fibers and a good demonstration of nuclear outlines in the same unstained section. In addition, use of positive/negative Polaroid film provides an immediate permanent record, a negative for making enlargements or prints for publication, and a representation similar to a cell stain process.

The efficacy of the present method derives from the ability of the microscope and camera to differentiate structure successfully on the basis of differences in refractivity, a phenomenon only present to any degree in more normally shaped, fluid-filled cells. The accuracy limits of the present method are unknown and can be determined only by further investigation with more sophisticated optical techniques such as phase contrast procedures. Because of this, it is only reasonable that more traditional procedures be employed when studies of microstructure are undertaken. As is clear from the data presented, the method is adequate to determine electrode and/or lesion location with sufficient accuracy to answer a broad range of questions often asked by many researchers.

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