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BRIEF COMMUNICATION

A Simple and Rapid Technique for Preparing Histological Sections of Brain[1]

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SHAPIRO, R. M., J. I. BADALAMENTI AND S. D. GLICK. *A simple and rapid technique for preparing histological sections of brain.* PHARMACOL BIOCHEM BEHAV **19**(6) 1049–1050, 1983.—A rapid technique for preparing histological sections of rodent brain is described. Sectioning and staining may be completed within two hours of sacrifice.

Histological sectioning Staining

WE have developed a simple procedure for staining frozen sections of rodent brain which permits localization of lesions and electrode tracts within two hours of the death of the animal. Previously described "rapid" methods are either technically more tedious [2] or require photographing enlarged projections of unfixed tissue slices so as to obtain a permanent record [7]. Since all slices may be stained and saved, our technique does not necessitate selecting those sections thought to be most valuable at the time of processing.

METHOD

After decapitation the brain is removed and placed on a microscope slide, ventral surface down with the long axis of the brain parallel to that of the slide. The brain is quick-frozen by carefully submerging the slide with the brain on it into a small (e.g., 60 ml) beaker containing CCl_2F_2 (UCON 12, Union Carbide, NY) for 30–60 seconds. The slide (with the brain now temporarily frozen onto it) is placed onto the variable inclined plane described by Herberg and Franklin [4] and a cut is made in the desired plane. The brain is then taken off the slide and mounted on a chuck in a cryostat (−15°C). In doing so it is advantageous to prevent distortion of cortex by handling the brain only with a large forceps (Fisher No. 10-316B), the tips of which have been cooled in CCl_2F_2 immediately prior to use. Serial coronal sections (10–20 μm) are made, picking up the desired slices off the cryostat blade onto clean microscope slides; the slides are then dried on a Fisher (No. 12-594) slide warmer (40°C). The Sudan black B/neutral red stain is applied according to the following protocol adapted from Barka and Anderson [1]: (1) Rinse in 70% ethanol (10 seconds); (2) Sudan black B (7% in 70% ethanol and filtered through Whatman No. 1 filter paper; 10–15 minutes); (3) 70% ethanol (90 seconds); (4) 70% ethanol (90 seconds); (5) Distilled water (60 seconds); (6) Distilled water (60 seconds); (7) Neutral red (1% in 4% aqueous acetate buffer, pH=4.8; 3 minutes); (8) Distilled water (30 seconds); (9) Distilled water (30 seconds); (10) Distilled water (30 seconds, and until coverslipped); (11) Mount in glycerin (PUREPAC) jelly [5]; (12) When slide is dry, seal edges with clear nail polish. A typical result is shown in Fig. 1.

Some cautionary notes and comments: (1) Since coverslipping takes several minutes, no more than ten slides should be stained at one time, in order to prevent stain from coming off in Step 10. (2) As with any lipid stain, Sudan black B loses some of its potency with continued use; therefore it may become necessary to lengthen Step 2 if the stain is not freshly prepared. (3) The glycerin jelly should be stored refrigerated and warmed on a heated stir plate prior to use; if the stain is found to run on coverslipping, the glycerin jelly may be spoiled. (4) Immediately after the animal is killed the brain must be dissected, frozen, and mounted on a cryostat chuck, either in ice or in embedding fluid (e.g., M-1 Embedding Matrix, Lipshaw, Detroit). If mounting fluid surrounds the tissue, it can be stored indefinitely prior to sectioning; if, however, any space is inadvertently left unsealed, the brain may dry out. (5) Once the brain is sliced, slides can be stored at room temperature prior to staining; we have obtained excellent results from slides that were stained more than a year after the brain was sliced.

The technique described here has been used in this laboratory to investigate a relationship between brain morphology and behavior [3]. It has also been used, with different

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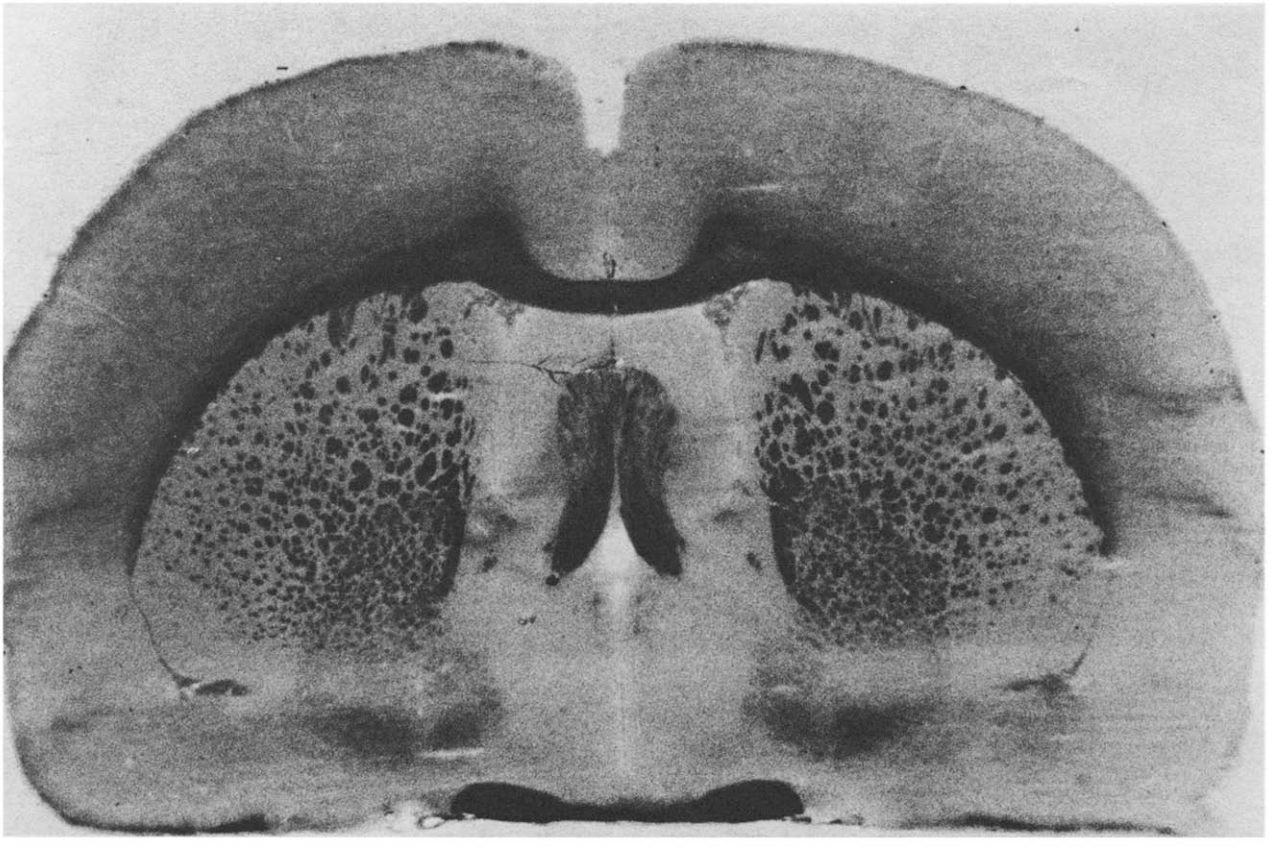

FIG. 1. Photomicrograph of coronal section (20 μm) through rat brain at 1.2 mm anterior to bregma [6]. Note that globus pallidus can be distinguished from caudate-putamen.

stains (toluidine blue and Astrablau), in quantitative studies of mast cells in rat brain (Goldschmidt *et al.*, in preparation). The method should prove valuable for neuroscience laboratories which routinely require visualization of brain tissue.

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