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Isolated removal of hypothalamic or other brain nuclei of the rat

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Due to recent developments in biochemical and endocrinological micromethods we are now able to assay small brain particles, such as brain nuclei and subdivisions of nuclei. The following technique enables quick and simple removal of separate portions of the unfixed brain of the rat. The technique is naturally adaptable to any other species.

The procedure consists of following steps.

(1) After decapitation remove carefully the whole brain and bisect it by making a section perpendicular to the cortical surface at the caudal end of the hemisphere.

(2) Position the part of interest upon the specimen holder of the microtome and freeze it on with carbon dioxide or dry ice for 45–90 sec.

(3) Place the holder in a cryostat chamber at a temperature of —10 °C for 10–15 min.

(4) Cut 200–300 μm thick frozen sections and mount them on conventional glass slides. Store the slides in the cryostat.

(5) Place one slide upon the surface of a metal or glass container (petri dish) filled with dry ice. Paint the background black or place a metal plate between the petri dish and the slide.

(6) Remove the required brain particles by punching them out with a special needle under a stereomicroscope. The needles should be of stainless steel and of 5 cm overall length. The inside diameter of the lumen should narrow at the tip to 200, 300, 500, or 1000 μm, depending upon the size of the nucleus to be removed. The thickness of the wall at the tip should be about 0.1 mm. (Polish the point of the needle plane perpendicular to the long axis.)

The needles have to be kept cold on dry ice before use. Select the needle necessary to match the size of the given area, punch it out and remove the pellet from the lumen by blowing it out directly into the required chemical or dish.

The pellets of the 300 μm section weigh approximately 10, 20, 60 or 230 μg according to the inside diameter of the needle used.

In the unstained sections, the shape of the brain, the ventricles and the major tracts serve as landmarks for localization during the process of removal. Staining of the sections with cresyl violet for 45–60 sec after removal aids control and docu-

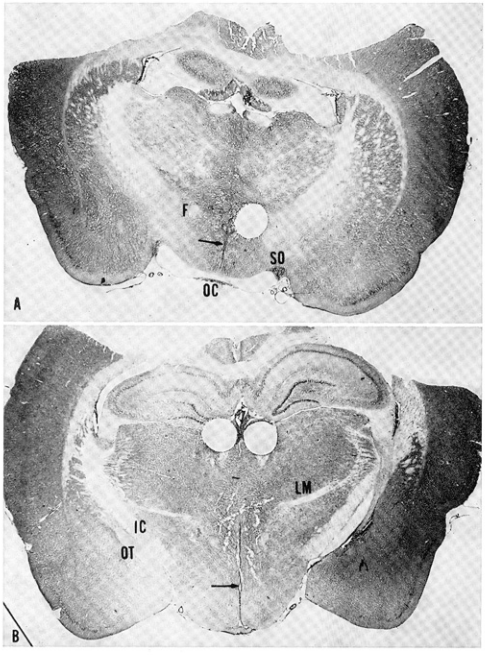

Fig. 1. Sections of the rat diencephalon. × 7. A: the hole represents the removed paraventricular nucleus at the right side using a needle of 1000 μm inside diameter. SO, supraoptic nucleus; OC, optic chiasma; F, fornix. The third ventricle is indicated by arrow. B: the holes represent the removed habenular nuclei using a needle of 1000 μm inside diameter. LM, lemniscus medialis; IC, internal capsule; OT, optic tract. The third ventricle is indicated by arrow.

mentation (Fig. 1). The process is quick: 15 different hypothalamic nuclei or subdivisions can be removed from a series of sections in about 10 min.

Separate reports are in progress about the results of different biochemical determinations of the removal hypothalamic nuclei.