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SHORT COMMUNICATIONS

Standardized excision of small areas of rat and mouse brain with topographical control

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WHEN neurochemical studies are performed on brains of small animals, it is difficult to excise well defined areas with good reproducibility by free-hand dissection. The topographical reproducibility is considerably improved if brain slices with defined frontal planes are first prepared and cylindric tissue pieces are then punched out of these slices according to given coordinates. Equipment designed for this procedure is described in the following. So far, it has been used in studies on monoamines carried out with a miniaturized fluorometric method.[1,2]

DISSECTION EQUIPMENT AND PROCEDURE

The equipment consists of a microtome and a punching apparatus.

Microtome. Brain slices are cut with a razor blade (part of Gillette five blade strips) that is fixed on to a movable hold which allows vertical and horizontal movements (4 cm) in one plane (Fig. 1). Downward movements are retarded by springs. The table (6 × 7 cm, coated with araldite) is displaced at a right angle to the blade by a micrometer screw (precision 0·05 mm; one circle of the screw yields 1 mm of axial displacement). The table can be inclined (0–30°) so that frontal planes can be cut at various angles. In order to permit work with frozen brain, the table is equipped with a water-cooled Pelletier element that allows a temperature of −25° to be reached at 20 A.

Punching apparatus. The punching apparatus consists of a stereomicroscope (stereomicroscope III Zeiss; magnification usually 20×) and a punching device. Both are fastened to the same support (Fig. 2). Cylindric tissue pieces are punched out by a glasstube (0·5–5 mm i.d., usually 1 mm) that is moving in the vertical direction. The punched tissue pieces are pushed out of the tube by a steel pin which enters the tube when it is again raised. The punching device can be displaced by 90° in the horizontal plane in order to free the observation field of the microscope. When the punching device is caught in the zero position, the glass tube is concentric with the cross lines of an ocular of the stereomicroscope. The stage (4 × 4 cm; coated with araldite) is driven by two micrometer screws along the x- and y-axes of the horizontal plane (precision 0·01 mm; one circle of the screw yields 0·5 mm of axial displacement). The stage is again cooled to −25° (at 7 A) by a built-in Pelletier element. Both Pelletier elements are connected to an appropriate power supply constructed in the Department.

Tissue preparation. When unfixed brains had to be dissected, good results were obtained only when the tissue was frozen. In this case, the brain was rapidly removed after decapitation and immediately frozen on to a glass slide cooled by dry ice, with additional cooling of the surface by carbon dioxide gas. The brain was then stored at −25° for no longer than 1–2 days. Subsequently, the brain was divided into slices (usually about 1 mm thick) by frontal cuts on the microtome at predetermined antero-posterior levels. For this purpose, the ventral surface of the brain was frozen onto the surface of the microtome table cooled to −20°, with the sagittal fissure perpendicular to the plane of the razor blade. The dorsal rim of the *fissura prima* (posterior circumference of the cerebral cortex) served to fix the frontal zero plane. We found that in the rat it was possible to define certain frontal planes according to their antero-posterior distance from the frontal zero plane. Because of its smaller size, the mouse brain was preferably cut at intervals of 0·1 mm under continuous visual control until the desired frontal plane was reached. The thickness of a given frontal slice (e.g., anterior midbrain) was then calculated according to the ratio of the longitudinal extensions of rat vs mouse brain. The brain slices were wrapped in plastic foil in order to avoid desiccation, and were again briefly stored at −25° (1–2 days). In a second step, tissue cylinders (usually 1 mm in dia) were punched out of the slices. The temperature of the stage of the punching apparatus was set at −20°. For various frontal planes of mouse and rat brain, a suitable point of origin of x-, y-coordinates has been chosen. The cross lines of the ocular of the stereomicroscope were first centered on this point. The stage was then moved to the coorindates of the center of the area to be punched out. The position was controlled in the microscope so that minor variations could be corrected. The procedure was the same for mouse and rat brain. The tissue cylinders were immediately transferred to precooled microhomogenizers kept on dry ice. These were closed by ground glass stoppers in order to prevent any changes in water content.

The dissection technique was tested mainly with regard to its possible usefulness in the determination of monoamines within circumscribed brain areas.[1,2] Some more general features may be discussed briefly in the present context. The reproducibility of the method was investigated by histological techniques as well as by weight determinations.

Histological control of topographical reproducibility. Formalin-fixed frozen brains were cut at various levels on the microtome and cylinders containing certain areas were punched out of the slices. Serial sections through the frontal slices were subsequently prepared according to current histological techniques. The position of the two frontal planes of a given slice as well as the emplacement of the cylindric hole were controlled in the sections. Formalin-fixed brains were used because tissue structure was much better preserved than in brain frozen on dry ice before fixation. It had been found that formalin fixation alone did not detectably change the antero-posterior extension of rat or mouse brain (retraction occurred during the subsequent steps of the histological procedure). The histological controls revealed that the levels of the various frontal planes as well as the positions of the excised cylinders could be reproduced with sufficient precision. Thus, in five rat brains the anterior and posterior borders of, e.g., the substantia nigra, were determined by histological control as being situated at 4·5 mm (3 rats) or 4·0 mm (2 rats) and 2·5 mm (5 rats) anterior of the frontal zero plane. The borders of nuc. raphes medialis + dorsalis were found (positive values = anterior of zero plane) at 0 mm and + 1·5 mm (4 rats)/2 mm (1 rat), those of nuc. locus coeruleus at 0 mm and −0·95 mm (3 rats)/−1·4 mm (2 rats), those of nuc. arcuatus hypothalami at +4·5 mm (3 rats)/+5·0 mm (2 rats) and +6·5 mm (4 rats) or +6·0 mm (1 rat). Subsequently, the following two antero-posterior levels were chosen for the cuts in the rat: "substantia nigra" +3 and +4 mm, "raphe" 0 and +1·5 mm, "locus coeruleus" 0 and −1·4 mm, "nuc. arcuatus" +5 and +6·5 mm (for further topographical details, cf. ref. 1). An example of a typical cylinder hole comprising the substantia nigra of a rat brain is shown in Fig. 3.

Weight determination. In order to obtain information on the variability of the size of the tissue cylinders, such cylinders (slightly less than 1 mm thick and 1 mm in dia) were prepared from several areas of formalin-fixed frozen mouse brains. In contrast to the procedure described later, the weight was then determined on a microbalance (Mettler) at room temperature. The determinations showed that the variations between individual cylinders from a given brain region were relatively small. They were nevertheless such as to require a weight determination for every piece of tissue [examples: 1. midbrain raphe, weights of single cylinders (mg): 0·490, 0·540, 0·460, 0·425, 0·495, 0·290, 0·415; mean ± S.D. 0·445 ± 0·080. 2. substantia nigra, single values (mg): 0·580, 0·550, 0·560, 0·680, 0·770; mean ± S.D. 0·628 ± 0·095. 3. amygdala, single

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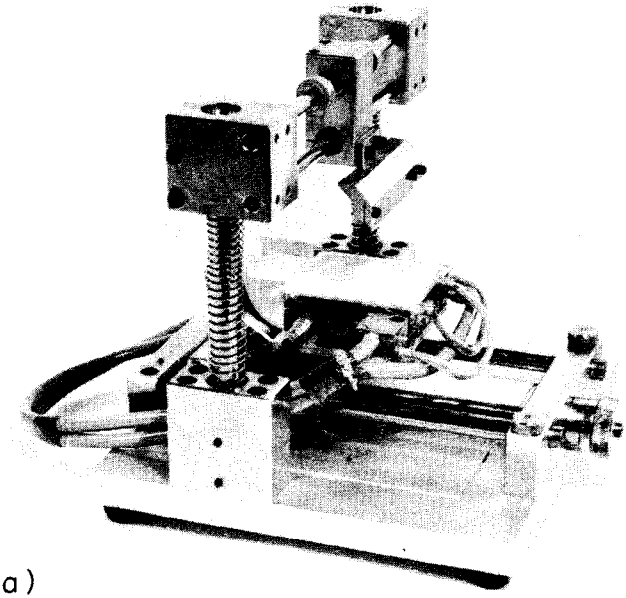
FIG. 1. View (a) and construction drawing (b) of the microtome. (1) Razor blade, (2) hold of the blade carried on the horizontal support (3) which in turn is moved along the vertical support (4). Both connections are provided with ball bearings. Springs sustain the horizontal support. (5) Table with Pelletier element. The table can be inclined from 0 to 30° around the pivot (6). (7) Power supply of the Pelletier element. (8) Micrometer drive.

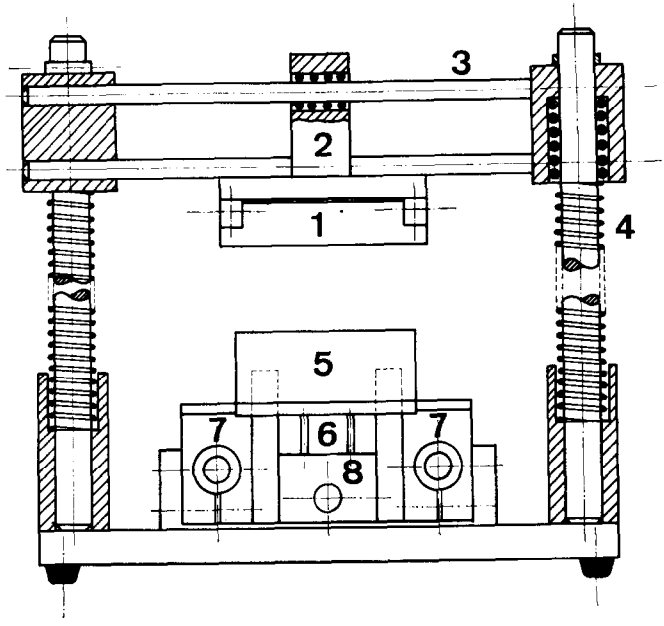
FIG. 2. View (a) and construction drawing (b) of the punching apparatus. (1) Common support for Zeiss stereomicroscope III (2) and horizontal arm (3) of the punching device, which is shown in zero position. (4) Hollow cylinder (chromium-plated brass) connected with the horizontal arm by two bars (5) that allow for horizontal displacement. (6) Micrometer drive serving to center the glass tube to the crosslines of the ocular. A piston (7) which carries the glass tube (8) is fixed by two screws through vertical slits of the cylinder to two lateral bars (not shown). The piston is sustained by springs placed on the inside of the cylinder and is moved in the vertical direction by pressing the bars. A metal pin (not shown) fixed to the top of the cylinder, runs through a hole in the center of the piston. It reaches as far as the lower end of the glass tube when the piston occupies its resting position (as on the drawing). (9) Stage containing a Pelletier element. The micrometer screws for displacement along the *x*- and *y*-axes are shown on the photograph.

FIG. 3(a). Frontal slice of a rat brain at the level of the anterior midbrain. Two tissue cylinders (1 mm in diameter) were punched out at the position of the substantia nigra.

FIG. 3(b). Microphotograph of frontal section through one side of the anterior midbrain of a rat, with circular hole punched out at the emplacement of the substantia nigra. At this level, the tissue cylinder contained most of the zona compacta and the major part of the zona reticularis of the substantia nigra, together with parts of the medial lemniscus, and impinged somwhat on the border of the reticular formation. ml = medial lemniscus, c = crus cerebri, vt = area ventralis tegmenti, rf = reticular formation, cg = corpus geniculatum, mb = mammillary body.

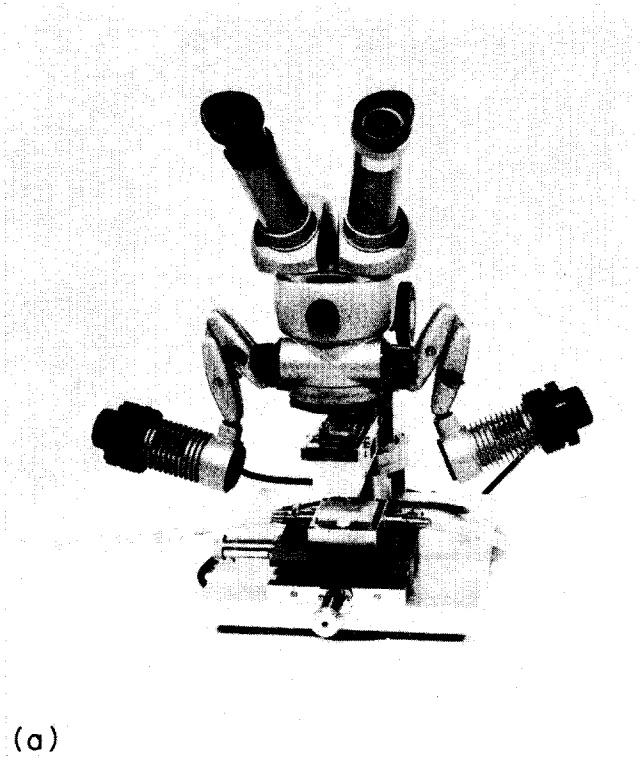


(a)

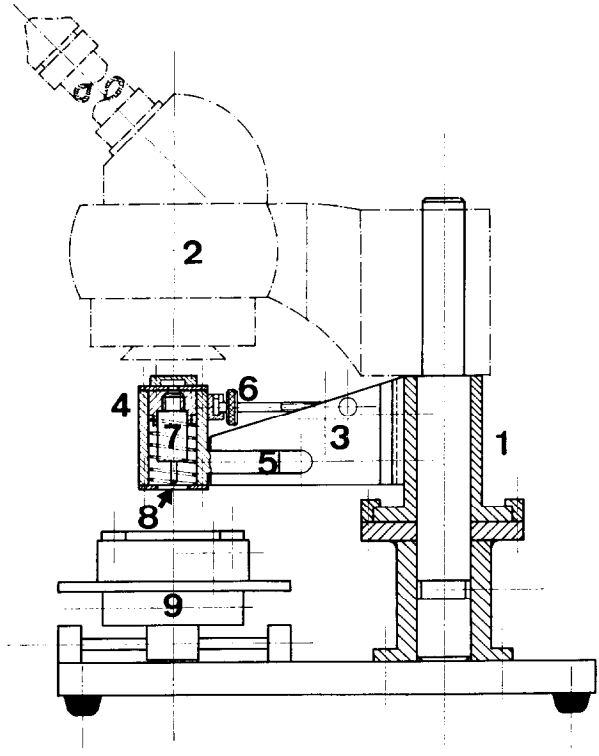


(b)

FIG. 1



(a)



(b)

FIG. 2

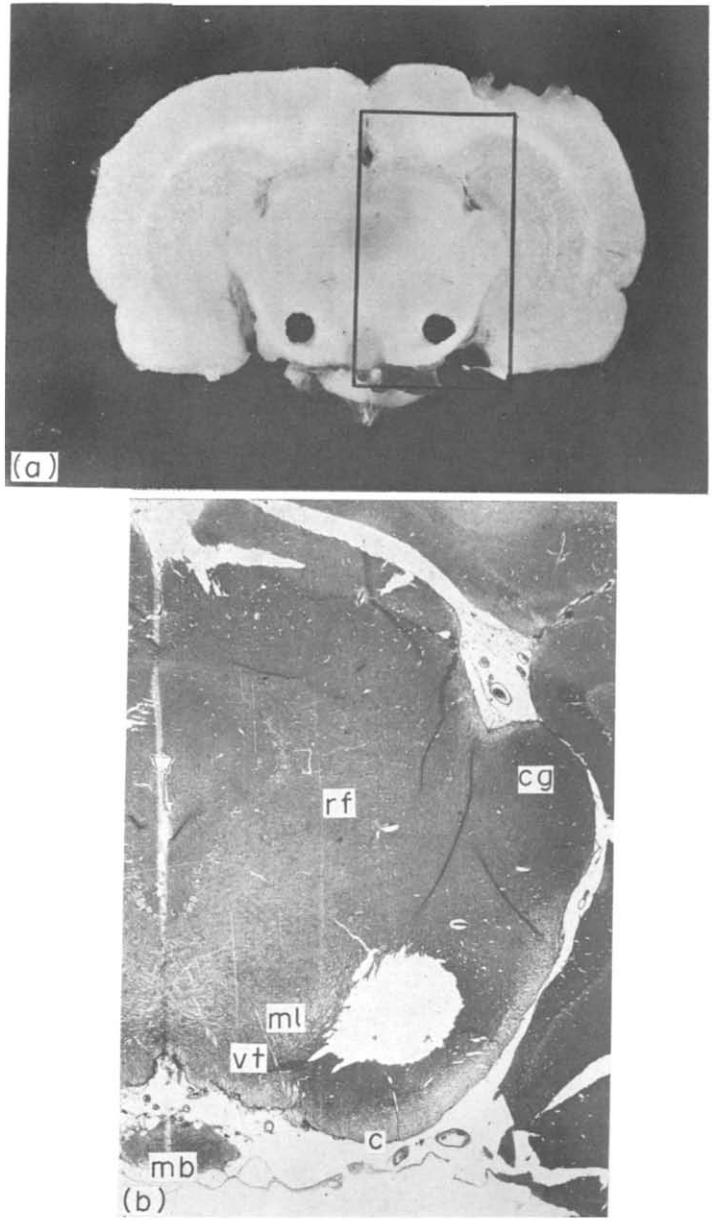


FIG. 3

values (mg): 0·580, 0·700, 0·670, 0·460, 0·610, 0·600; mean ± S.D. 0·603 ± 0·083]. The variations are probably due mainly to differences in the thickness of the frontal slices which may be caused, for example, by temperature variations of the frozen brain during the cutting procedure.

Special problems arise when weight determinations are to be performed on *unfixed frozen tissue* that should not reach room temperature. It is then necessary to follow a strictly standardized procedure because condensation of water vapour as well as evaporation may considerably influence the weight of tissue pieces of this size. As a rule, all weight determinations were carried out on closed microhomogenizers in order to keep the total water content of the homogenizer chamber constant. The pre-cooled homogenizer was weighed without complete temperature equilibration according to the following schedule: the homogenizer was taken from dry ice and wiped for 30 sec, then exposed to ambient temperature of 24° for 60 sec. The homogenizer was subsequently wiped for another 60 sec in order to remove remaining moisture and was then weighed. Thirty seconds later, a second weight determination was carried out, which should yield the same value. In this way, variations in the weight of a given homogenizer with tissue were reduced considerably. For three different homogenizers stored on dry ice prior to weight determination, the following mean values were obtained from six determinations on a microbalance (Mettler): no. 1: mean ± S.D. 16·958766 ± 0·00021 g ($n = 6$), no. 2: 17·012005 ± 0·00017 g ($n = 6$), no. 3: 17·198992 ± 0·000081 g ($n = 6$). When the weight of the tissue contained in the homogenizer is small (2 mg), the variations are still compartively high (5–10 per cent). Nevertheless, weight determination appears to be more reliable than reference to the individual tissue cylinder as a unit.

In conclusion, the dissection technique presented herein appears to be a practical one. It permits the excision of small pieces (0·5–1 mg) of any desired area of a diameter between 0·5 and 5 mm from rat or mouse brain with sufficient precision. The main difficulties actually arise from weight determinations, especially if frozen tissue is to be weighed. It may then be useful to employ additional reference systems. According to our recent experience with monoamine determinations in these small tissue samples,[2] protein content represents a more convenient reference system.

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REFERENCES

1. M. SCHLUMPF, Thesis, Eidgenössische Technische Hochschule, Zürich (1973).
2. M. SCHLUMPF, W. LICHTENSTEIGER, H. LANGEMANN, P. G. WASER and F. HEFTI, *Biochem. Pharmac.* **23,** 2437 (1974).

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Effects of methadone on steroid biosynthesis in rat adrenocortical cells

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CLINICAL studies of the effect of methadone upon hypophyseal-adrenal axis have shown that 17-hydroxysteroids are within the normal range. Most[1] but not all[2] patients on methadone maintenance respond normally to insulin hypoglycemia and exogenous adrenocorticotropic hormone (ACTH), whereas diurnal rhythm[1] and cold stress response are impaired.[3] Neither of these studies incorporated a direct measure