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A simple and inexpensive slicer for preparation of brain slices

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A simple and inexpensive slicer has been developed for the preparation of slices of mouse or rat brain. The instrument consists of razor blades, separated by an 0.5 mm thick polyethylene sheet (1 × 1 cm), mounted on metal screws through a hole in the center of the polyethylene sheet. Using this slicer, 6–8 uniform slices of 500 μm thickness were obtained from mouse or rat brain. These brain slices were incubated in a medium consisting of artificial cerebrospinal fluid for 1 h at 37°C under an oxygen atmosphere and the activities of various subcellular marker enzymes were assayed. The slice weights and the activities of the enzymes did not vary significantly in different batches of slices. Morphological evaluation of the slices revealed well-preserved neurons. Histochemical staining for mitochondrial enzymes revealed intense staining of neuronal cells and lighter staining of the white matter in all the regions examined. These slices could serve as a useful in vitro model for studying brain function and the effect of various toxicants on the brain.

Introduction

Brain slices are widely used for neurochemical and neurophysiological studies. Slices from various regions of the brain, maintained in a variety of chambers, have been used extensively for this purpose (Reid et al., 1988). Slices can be prepared by manual cutting, using a guide to maintain uniform thickness (McIlwain, 1961) or mechanically using a tissue chopper (McIlwain and Buddle, 1953).

Sagittal slices of whole brain are more difficult to prepare. Brain slices cut by hand using the Stadie-Riggs slicer show damage to tissue. As the brain is a very soft tissue, slices of uniform thickness and weight are difficult to obtain. We describe here a simple and inexpensive slicer which allows the preparation of uniform slices of whole brain.

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Materials and methods

Two metal screws (2 inch long) with 4 bolts fitted on them were used. One set of bolts was placed at the end of each of the screws. Razor blades were placed equidistant from each other, separated by polyethylene or perspex sheets (0.5 m thick and 1 × 1 cm square). The sheets had a hole in the center (through which they were threaded onto the screws) and were placed between razor blades (Fig. 1A and B). The other set of bolts was placed at the other end of the screws and tightened to prevent movement of the blades.

Swiss albino mice and Sprague–Dawley rats obtained from the Central Animal Research Facil-

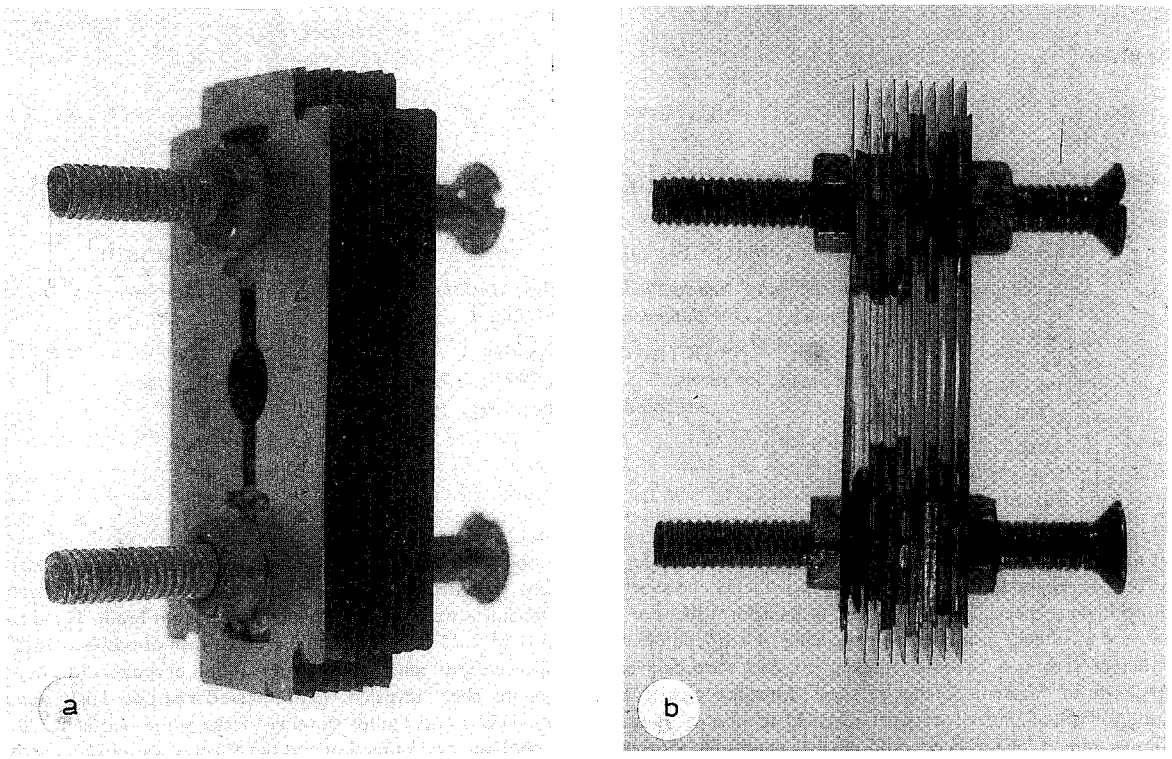

Fig. 1. Frontal (A) and lateral (B) view of slicer. Blades are placed equidistant from each other with the help of polyethylene sheets (1 × 1 cm) of uniform thickness (0.5 mm) and mounted on 2 screws. The blades are positioned by placing bolts on either side of the blades and tightening them.

ity of the Institute were decapitated and the brain was quickly removed. The brain was placed on a plastic lid (the edges of which were cut on opposite sides to facilitate movement of the slicer) as shown in Fig. 2A. The slicer and the plastic lid were pre-cooled at 4°C. The slicer was placed above the brain with an equal number of blades on either side of the median plane and gently pressed. The slicer was lifted and the slices present between the blades were carefully transferred using a fine needle into a 10 ml pre-weighed beaker containing artificial cerebrospinal fluid (aCSF) (Fig. 2B). Six to 8 slices could be prepared from each mouse or rat brain. Slices of varying thickness could also be prepared by altering the thickness of the polyethylene sheets that are placed between the blades. The time taken for preparing 6–8 slices from one mouse or rat brain was 1–2 min.

The aCSF consisted of the following (in mM): NaCl 122, KCl 3.1, $CaCl_2$ 1.3, $MgSO_4$ 1.2, glucose 10, $NaHCO_3$ 25, and KH_2PO_4 0.4 (Elliott, 1969); it was well oxygenated and the pH adjusted to 7.4 by bubbling with CO_2 prior to the addition of slices. The slices were then incubated at 37°C in an atmosphere of O_2 for 1 h. After incubation, slices were washed, blotted and homogenized in 0.32 M sucrose (5%, w/v). The homogenate was centrifuged at 1000 × *g* for 10 min to remove cell debris. Subcellular marker enzymes, namely cytosolic lactate dehydrogenase (Yoshida et al., 1975) and glyceraldehyde 3-phosphate dehydrogenase (Howland et al., 1980); mitochondrial isocitrate dehydrogenase (Cleland et al., 1969) and cytochrome *c* oxidase (Gibson and Hilf, 1983); lysosomal acid phosphatase (Cottman and Mathews, 1971), *N*-acetylglucosaminidase (Frohwein and Gatt, 1969) and β-glucuronidase (Levvy and Conchie, 1966); and membrane Na^+K^+-ATPase (Deliconstantinos et al., 1987), were estimated in the

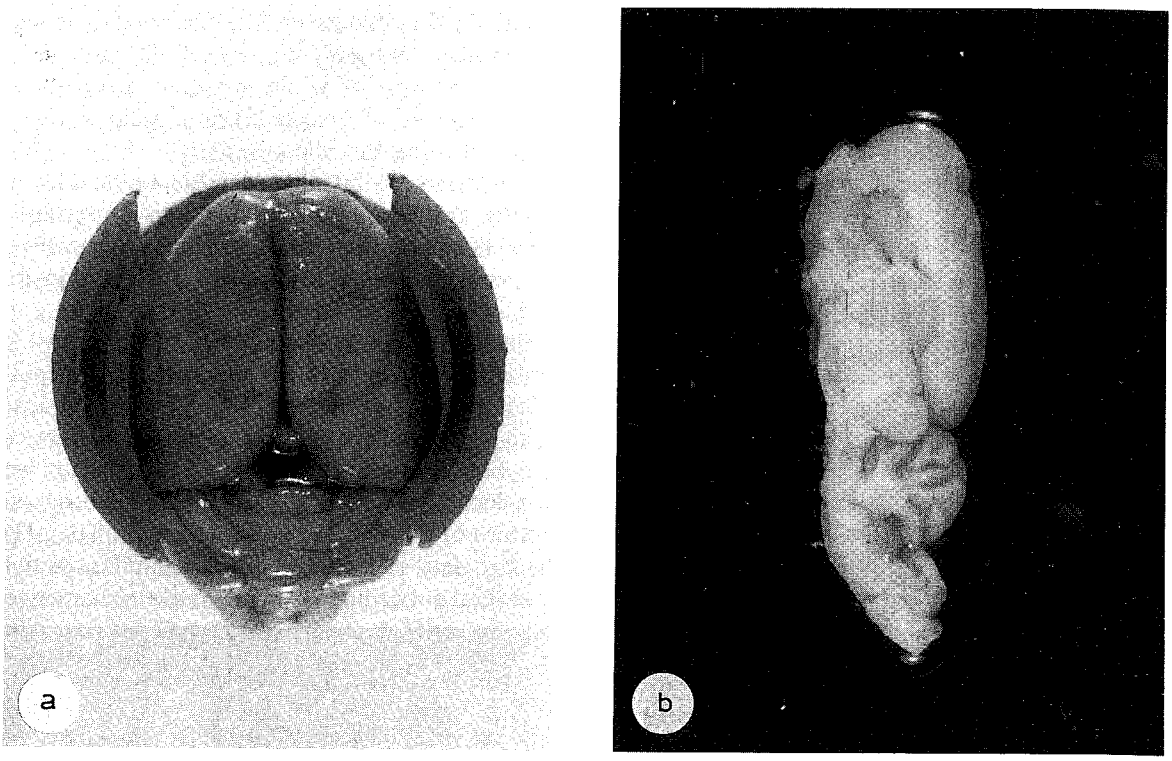

Fig. 2. A: Freshly isolated rat brain mounted on a plastic lid suitably modified by cutting on both edges. This prevents the movement of the brain during the slicing procedure. The slicer was placed above the brain and gently pressed. B: A sagittal slice of rat brain prepared using the above slicer.

TABLE I

ENZYME ACTIVITIES IN RAT BRAIN SLICES BEFORE AND AFTER INCUBATION IN ARTIFICIAL CEREBROSPINAL FLUID

	0 h	1 h
(1) Slice weight (mg)	98.9 ±15.5	
(2) Lactate dehydrogenase	128.0 ± 3.1	124.8 ±3.5
(3) Acid phosphatase	2.56 ± 0.11	2.35 ±0.09
(4) *N*-Acetyl glucosaminidase	1.13 ± 0.08	0.87 ±0.04
(5) β-Glucuronidase	0.022± 0.003	0.015±0.001
(6) Cytochrome *c* oxidase	ND	38.52 ±1.3
(7) Isocitrate dehydrogenase	ND	7.8 ±0.3
(8) Na^+, K^+-ATPase	5.1 ± 0.4	5.34 ±0.23
(9) Glyceraldehyde 3-phosphate dehydrogenase	78.4 ± 3.5	67.32 ±2.14

Rat brain slices were incubated in aCSF for 1 h at 37 °C under O_2. Enzyme activities were determined in the homogenates of brain slices before and after 1 h of incubation. Activities are expressed as μmol product formed/h/mg protein and are mean ± SEM (n = 15–40). ND = not determined.

homogenates prepared from the brain slices. Leakage of the cytosolic enzyme lactate dehydrogenase from the slices into the aCSF was monitored as a measure of cellular damage during incubation. Protein was determined by a dye-binding method (Bradford, 1976).

In order to determine the relative preservation of various areas in the slices, sagittal slices from mouse brain were collected in aCSF and immediately fixed in paraformaldehyde/lysine/periodate. Six μm thick paraffin sections were cut along the whole plane of the slices at different depths and stained with hematoxylin–eosin and Luxol fast blue/PAS for myelin. Different areas of the brain in the same section and corresponding areas in sections cut at different depths from the outer edge were examined for morphological evidence of cell preservation. In addition, 15 μm thick frozen sections of sagittal slice were incubated in aCSF with 10% glycerol (for cryopre-

servation) for 30–60 min and histochemically stained for NADH-tetrazolium reductase, succinate dehydrogenase and Na^+,K^+-ATPase (Pearse, 1972a, b).

Sections were examined microscopically for regional variation in the intensity of staining.

Results and discussion

Brain slices are used extensively for metabolic, electrophysiological and neurotoxicological studies (Lynch and Schubert, 1980; Hollinder et al., 1989). Slices of a particular region have also been used for these purposes (Seren et al., 1989). We describe here a simple, inexpensive slicer for the preparation of whole brain slices of uniform thickness and weight (Table I). By varying the dimensions of the spacer between the blades, slices of any desired thickness can be obtained. The slicer is compact and easy to use. Both coronal and sagittal sections of whole rat or mouse brain as well as slices of selected brain regions can be prepared using this slicer.

The brain exhibits considerable regional heterogeneity and for certain studies it is important to have slice preparations which are representative of the whole brain. Sagittal slices of rat brain may offer this.

Sagittal slices of rat brain prepared as described above were incubated in well oxygenated aCSF maintained at pH 7.4. Since the brain is richly supplied with oxygen, incubations were carried out in an O_2 atmosphere. Brain slices were also incubated in an atmosphere consisting of a mixture of oxygen and carbon dioxide. The pH in the two sets of incubations were similar. Activity of several subcellular enzyme markers, namely cy-

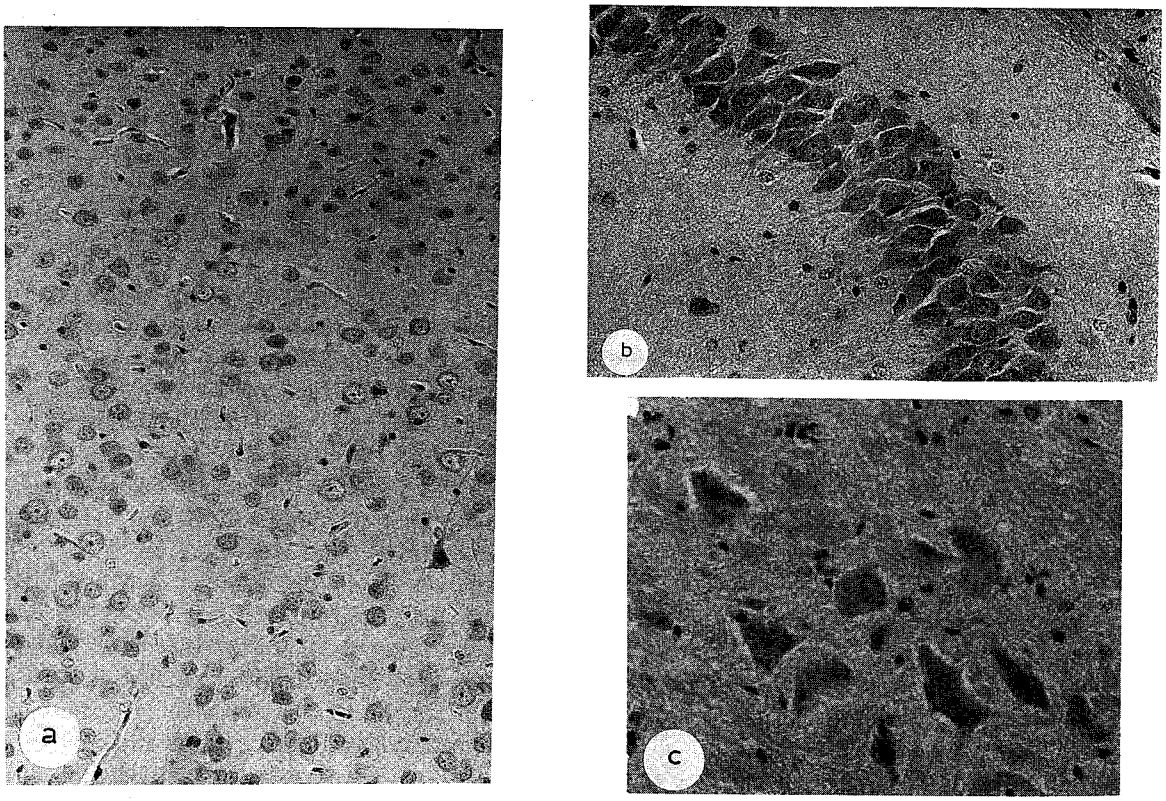

Fig. 3. Light microscopy of sections of mouse brain slice, stained with hematoxylin–eosin. A: Cerebral cortex showing well preserved neurons with lamellar architecture along the superficial section cut from the outer aspect of a sagittal slice from mouse brain (×40). B: Well preserved hippocampal neurons of the CA1 area of a deeper section of mouse brain slice (×160). C: large neurons of the brain stem containing the normal complement of Nissl substance of a section from mouse brain slice (×160).

tosolic lactate dehydrogenase and glyceraldehyde 3-phosphate dehydrogenase; mitochondrial isocitrate dehydrogenase and cytochrome *c* oxidase; lysosomal acid phosphatase, *N*-acetylglucosaminidase and β-glucuronidase, and membrane, Na^+,K^+-ATPase, did not differ significantly in successive slice preparations as shown in Table I. Enzyme assays were carried out at the beginning of the experiment and after 1 h incubation, and only a small decrease in the specific activities of certain enzymes was observed (Table I). Cellular damage was monitored by measuring the leakage of the cytosolic enzyme lactate dehydrogenase into the medium consisting of artificial cerebrospinal fluid. Only 2.5% of lactate dehydrogenase activity leaked out from the slice into the medium after 1 h of incubation (data not shown).

Neurons in different areas of the brain slices were fairly well preserved morphologically with no evidence of swelling, disruption or nuclear pyknosis. Sparsely dispersed, occasional dark neurons were noted in the cerebral cortex, cerebellum and brain stem in sections which were cut deeper from the edge of the slice. Similar morphology has been observed earlier in hand-cut and chopped slices (Garthwaite et al., 1979). Occasional vacuoles were also seen in the white matter. No myelin pallor or breakdown was evident (Fig. 3A–C). Histochemical staining for mitochondrial enzymes, i.e., NADH-tetrazolium reductase (Fig. 4A, B) and succinate dehydrogenase (not shown), revealed a staining pattern corresponding to neuronal density and size. White matter fiber tracts were negative. Similarly, the vascular pattern was equally delineated in all areas, on staining for Na^+, K^+-ATPase (not shown). This indicates that the slices cut by the method described above were quite well preserved and no damage to any particular area was discernible. Morphological evaluation was also carried out in slices incubated for 1 h at 37 °C in aCSF. These slices were well preserved and the morphological evaluation using enzyme histochemistry and hematoxylin-eosin staining revealed that these slices were not significantly different from freshly cut slices (data not shown). Similar results have also been obtained by Garthwaite et al. (1979).

The use of sagittal slices of whole brain could provide an effective model for studying the alterations in brain following exposure to neurotoxic chemicals (Pai and Ravindranath, 1990; Ravindranath and Pai, 1990). The slicer described in this report could also be used to prepare coronal sections of the brain. There is an increasing need for the development of in vitro assays for neurochemical evaluation. Brain slices may prove to be an effective in vitro model for such studies, since it takes the regional heterogeneity of the brain into account.

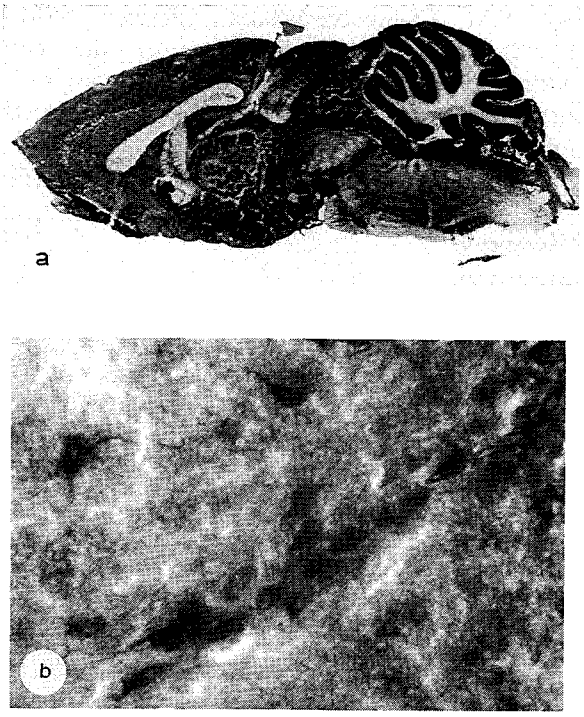

Fig. 4. A: Whole mount of 15 μM thick frozen section of mouse brain sagittal slice, histochemically stained for NADH-tetrazolium reductase. The intensity is proportional to the neuronal density. The white matter is stained very light (×8). B: Brain stem neurons showing strong reaction for NADH-tetrazolium reductase (×120).

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