

## ISOLATION OF NEURONAL NUCLEI FROM RAT BRAIN CORTEX

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### INTRODUCTION

The biochemistry of "brain nuclei" has been the subject of numerous reports (1-5). Such studies are notoriously of limited significance because of the heterogeneity of the preparations employed, with

contamination (1, 2). The use of such ambiguous (5-10) criteria as size, morphological appearance, and number of nucleoli for the assessment of neuronal origin makes this nuclear preparation

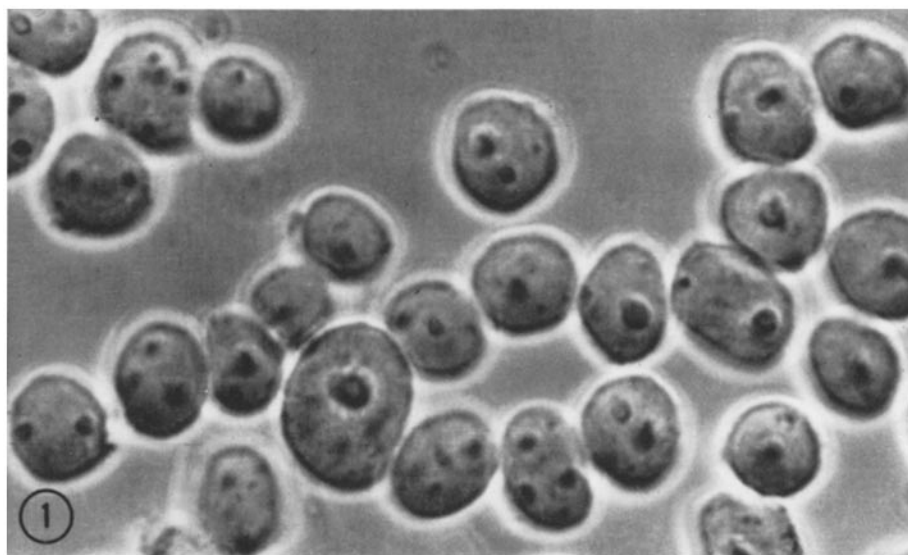


FIGURE 1 Phase-contrast micrograph of neuronal nuclei.  $\times 750$ .

neurons and glial cells both contributing to the nuclear population. The best schemes so far proposed to fractionate brain nuclei according to cellular origin yield a number of nuclear preparations, one of which is enriched in neuronal elements but nevertheless suffers from 20 to 30% glial

even less reliable. To overcome these problems we have resorted to a two stage procedure which consists of isolating easily identifiable neurons from rat brain cortex followed by gentle disruption of the cellular preparation and purification of the released nuclei. Nuclei isolated by this method can

safely be said to derive almost exclusively from neurons and to be only slightly contaminated by other cellular organelles.

#### METHODS

For each preparation 18 28-day old female albino rats (55 g body weight) of a local strain were decapitated and the brains were quickly removed in the cold.

Neuronal perikarya were prepared according to Sellinger et al. (11) with two minor modifications: (a) the dissected cerebral cortices were rinsed in an ice-cold standard medium (0.32 M sucrose, 2 mM MgCl<sub>2</sub>, 1 mM potassium phosphate, pH 6.5) before chopping in 7.5% polyvinylpyrrolidone (wt/vol), 1% bovine serum albumin (wt/vol), and 10 mM CaCl<sub>2</sub> and (b) the minced brains were filtered through four (instead

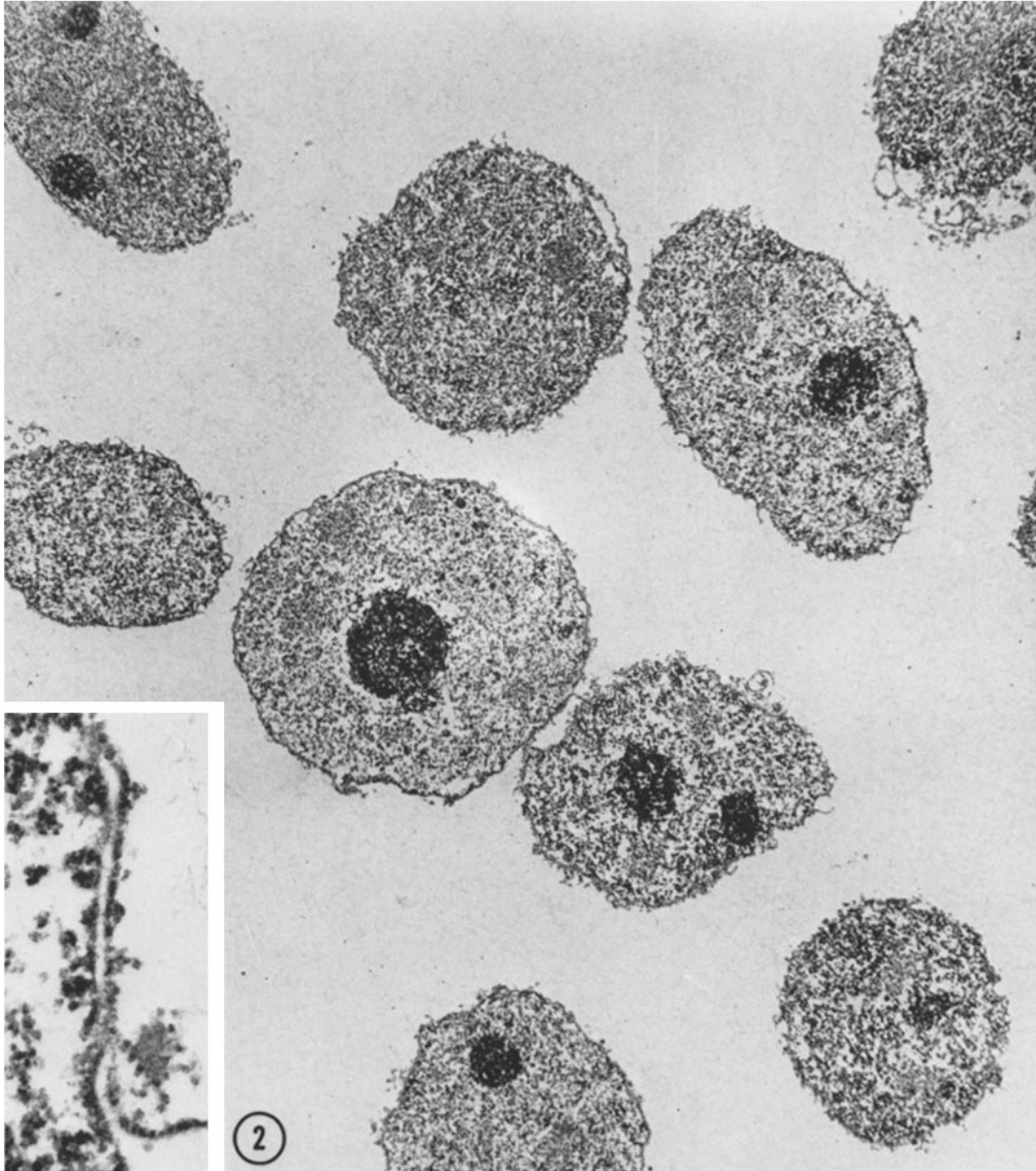


FIGURE 2 Electron micrograph of neuronal nuclei ( $\times 5,500$ ). The section shown in the *inset* ( $\times 44,000$ ) is representative of the structural preservation of the nuclear membranes. Note small cytoplasmic fragment attached.

of three) mesh sizes of nylon bolting cloth (670, 335, 115, 75  $\mu\text{m}$ ). The isolated perikarya were suspended in 50 ml standard medium, the purity of the preparation was checked by phase-contrast microscopy, and one-fifth volume was put aside for later biochemical analysis. The remaining portion was washed three times in 0.02 M Tris-maleate (pH 6.5), centrifuged at 5,000  $g$  5 min, and homogenized with 25 strokes in 5 ml of the same buffer using a tight-fitting glass Dounce homogenizer (clearance 0.06 to 0.02 mm). A low speed centrifugation (750  $g$  10 min) yielded a crude nuclear preparation, which was suspended in 12 ml standard medium and rehomogenized (three strokes). The homogenate was layered over six discontinuous sucrose gradients consisting of 1.5 ml 2.2 M sucrose and 1.5 ml 2.3 M sucrose in 2 mM  $\text{MgCl}_2$ -potassium phosphate (pH 6.5). Centrifugation at 30,000  $g$  30 min in a Beckman SW 50.1 rotor (Beckman Instruments, Inc., Spinco Div., Palo Alto, Calif.) resulted in a final nuclear preparation in the form of a pellet.

## RESULTS AND DISCUSSION

The purity of the nuclear preparation with respect to its neuronal origin is expected to closely parallel

the composition of the parent perikaryal fraction. Sellinger et al. (11) estimate that the latter contains 90% neurons. Our own differential cell counts were in agreement with this value. We therefore conclude that 90% of our nuclei derived from neurons and by this virtue were superior to any other preparation of neuronal nuclei so far reported (1, 2).

Phase-contrast and electron microscopy of the final nuclear preparation revealed a high degree of purity. There was negligible contamination by capillaries (one small fragment per 500 nuclei), and some preparations also contained a few clumped mitochondria. The nuclei were of various sizes and had between one and three distinct nucleoli (Fig. 1). Both the inner and outer nuclear membranes were well preserved over most of the circumference with occasional small cytoplasmic remnants attached (Fig. 2).

Biochemical analyses of the nuclei and comparison to the parent perikarya gave evidence of an enrichment of nuclear markers, with a concurrent reduction of labels associated with other subcel-

TABLE I  
*Biochemical Parameters of Neuronal Perikarya and Nuclei*

Biochemical parameter	Assay method	Marker (ref. 19) for	Perikarya	Nuclei	Purification factor
Lactate dehydrogenase (EC 1.1.1.27)	Ref. 12	Cytoplasmic sap	3.0 $\pm$ 1.8	0.081 $\pm$ 0.079	37
Total ATPase (EC 3.6.1.3)	Ref. 12	Various types of membranes	95.0 $\pm$ 23.5	10.0 $\pm$ 6.6	9.5
NADH diaphorase (EC 1.6.99.3)	Ref. 12	Endoplasmic reticulum	9.1 $\pm$ 3.7	0.34 $\pm$ 0.15	27
Acid phosphatase (EC 3.1.-3.2)	Ref. 13	Lysosomes	0.32 $\pm$ 0.11	0.005 $\pm$ 0.008	63
Succinate dehydrogenase (EC 1.3.99.1)	Ref. 14	Mitochondria	0.38 $\pm$ 0.10	0.0027 $\pm$ 0.0045	140
Cytochrome oxidase (EC 1.0.3.1)	Ref. 15	Mitochondria	2.1 $\pm$ 0.6	0.067 $\pm$ 0.053	31
NMN adenylyltransferase (EC 2.7.7.1)	Ref. 16	Nuclei	0.075 $\pm$ 0.016	0.20 $\pm$ 0.03	2.6
Cell count (in millions)			260 $\pm$ 120	120 $\pm$ 60	
DNA (mg)	Ref. 17		1.65 $\pm$ 0.79	0.76 $\pm$ 0.34	
RNA (mg)	Ref. 18		3.91 $\pm$ 1.27	0.58 $\pm$ 0.16	
Protein (mg)	Biuret		36.2 $\pm$ 10.5	6.38 $\pm$ 2.00	
Protein/DNA ratio			21.9 $\pm$ 3.8	8.4 $\pm$ 2.0	2.6
RNA/DNA ratio			2.49 $\pm$ 0.35	0.80 $\pm$ 0.14	3.1

Perikarya and nuclei were sonicated in saline before biochemical analysis. Enzymic activities ( $\pm$  SD) were expressed as micromoles substrate converted per milligram DNA per minute, except for adenylyltransferase whose activity was expressed as micromoles per milligram protein per hour. Yields of DNA and cell counts refer to a standard preparation from 18 rats.

lular components (Table I). Succinate dehydrogenase and cytochrome oxidase gave evidence of a small but definite contamination of the nuclei with mitochondria. Enzymes primarily associated with cytoplasmic sap, endoplasmic reticulum, various types of membranes, and lysosomes indicated a degree of purification better than 27- to 63-fold, except for ATPase, whose high nuclear activity might be interpreted as resulting from its possible localization in the nuclear membrane. The enrichment of nicotinamide mononucleotide (NMN) adenylyltransferase, an absolute nuclear marker, paralleled the observed decrease of the protein/DNA ratio, and this enzyme activity reflected the functional integrity of the purified nuclei.

The yield of purified nuclei suffices for most biochemical purposes. Variations in yield were primarily due to somewhat fluctuating recoveries of the parent perikarya. In contrast, the recovery of nuclei in the second step of the preparation was less variable and consistently amounted to  $46 \pm 9\%$ . The loss of nuclear elements resulted from trapping of impure nuclei on the sucrose gradient and was not due to destruction of the nuclei during homogenization. This was substantiated by our repeated failure to detect DNA in the low speed supernate after homogenization (our unpublished data). Apart from bearing substantial amounts of cytoplasmic remnants the retained nuclei had the same morphology as those recovered in the pellet and consequently gave no indication of differential losses resulting, for example, in an accumulation of non-neuronal elements in the final preparation.

In conclusion, the reported method is simple and rapid. It affords, by way of the intermediate perikarya, neuronal nuclei of high purity and in good yield.

Received for publication 20 June 1973, and in revised form 5 September 1973.

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