Neuronal Soma and Whole Neuroglia of Rat Brain: A New Isolation Technique

Abstract. *Minced rat brain softened by treatment with trypsin is disrupted by filtration through nylon and steel meshes to produce a suspension of free-floating cells and debris. The cells are separated and purified by centrifugation on discontinuous sucrose gradients. Preparations of neuronal perikarya, retaining stumps of processes, so obtained are 90 percent pure and yield 33.6 × 10⁶ cells per brain (3 milligrams, dry weight). The glial cells, apparently intact with extensive branched processess, are about 70 percent pure by weight and are obtained in a yield of 6.6 × 10⁶ cells per brain (2 milligrams dry weight). The neurons are smaller and have less lipid than the glial cells.*

The mammalian central nervous system is a network of the processes and soma of neurons and glial cells. Many efforts have been made to determine the contribution of each type to the total composition and biochemistry of the brain. The most direct are those of Hyden (*1*) and Lowry (*2*) who studied single nerve cells and glial clumps dissected from nervous tissue, Only recently have investigators attempted to obtain either neurons (*3, 4*) or glia (*5*), or both (*6–8*), in bulk. We now report a new method for isolation and preparation of neurons and glial cells.

Methods reported previously use initial mechanical disruption of the tissue in various media. Of the two methods (*6, 7*) furnishing both neuronal and glial fractions, the technique of Rose (*6*) has been most frequently applied (*8, 9*). This method yields a glial-rich fraction which is poorly defined and badly contaminated (*6, 10*); the neuronal fraction, as well, may be more heterogenous than previously suspected (*11*). Our experience with mechanical disruption of brain in aqueous media has been disappointing with respect to both yield and purity of recovered fractions. The use of combinations of organic solvents, although apparently effective (*3, 7*), was rejected because of the unknown effects of these solvents on cell composition and enzymic activity. Treatment of tissue with trypsin (*12*) permits subsequent disruption to proceed more efficiently. Neuronal soma are obtained in yields 10 to 30 times higher than those reported by others (*3, 6*) and glial cells are obtained in free-floating suspension, completely apart from the tissue matrix.

Sprague-Dawley rats (*13*) are decapitated. The brains are trimmed of cerebellum and chopped fine (approximately 1 mm^3) in an ice-cold medium consisting of 5 percent glucose, 5 percent fructose, and 1 percent bovine serum albumin (*14*) in 100 m*M* KH_2PO_4–NaOH buffer (*p*H 6.0). The minced brain is added to the same medium containing 1 percent trypsin (*14*) (10 ml of medium per gram of tissue) and incubated in a shaker under oxygen for 90 minutes at 37°C. After incubation, all further steps are performed at 0° to 4°C. The incubation mixture is diluted with 0.4 volume of an ice-cold mixture of 90 percent calf serum and 10 percent phosphate buffer (*p*H 6.0), chilled in ice, and centrifugated at 140*g* for 5 minutes. The supernatant is discarded, and the softened tissue is washed twice with medium by suspension and centrifugation. The washed tissue is suspended in a small volume of cold medium and filtered with vacuum through nylon bolting cloth (Tobler, Ernst, and Traber, New York; 150 mesh) stretched over a small porcelain Hirsch funnel. Gentle stroking with a thick glass rod, while the tissue is kept moist with medium, is necessary to break up the tissue and aid the screening process. The crude suspension is then filtered at least three times through a 200-mesh stainless steel screen (74 μ aperture) to complete the tissue disruption.

Cells can be separated from the debris by centrifugation in a number of systems in which discontinuous density gradients of sucrose or Ficoll, or both, are used. The data reported here were obtained on cells fractionated on sucrose-containing gradients. All sucrose solutions are prepared in the hexose-albumin-phosphate buffer medium and adjusted to *p*H 6.0 by the addition of 0.4*N* NaOH as necessary. The cell suspension is adjusted to 10 ml per 0.5 to 1.0 g of tissue with medium, and 10-ml portions are layered onto discontinuous gradients prepared in 39-ml tubes of the Spinco SW-27 rotor. The gradient consists of 14 ml of 0.9*M* sucrose (specific gravity, 1.154), 5 ml of 1.35*M* sucrose (specific gravity, 1.204), 5 ml of 1.55*M* sucrose (specific gravity, 1.227), and 5 ml of 2.0*M* sucrose (specific gravity, 1.286). The tubes are centrifuged at 5000 rev/min (3300*g*) for 10 minutes. The layers at each interface are removed with a Pasteur pipette. Very little, if any, pellet is formed. Layer A (on 0.9*M* sucrose) and the supernatant above it have myelin and small particulate debris; layer B (on 1.35*M* sucrose) is the crude glial layer; layer C (on 1.55*M* sucrose) is a mixture of glial cells, neurons, and capillaries; and layer D (on 2.0*M* sucrose) is the neuronal fraction which is taken without further purification.

The crude glial layer is further purified on a second gradient. It is diluted slowly with medium to 25 ml and layered onto 5 ml of 0.9*M* sucrose over 5 ml of 1.4*M* sucrose (specific gravity, 1.211). These tubes are centrifuged at 5000 rev/min (3300*g*) for 20 minutes. The purified glial cells collect at the interface between 1.4*M* to 0.9*M* sucrose. Both cell types can be concentrated by slowly diluting them at least fivefold with medium and centrifuging them at 630*g* for 10 minutes. A portion is taken for cell counts before this final centrifugation.

Pellets are stored frozen for analysis. To determine dry weight, we washed the pellets six times by suspending them in distilled water and centrifuging them at 100,000*g* for 1 hour. The washed pellets are freeze-dried. The dry weight obtained on the freeze-dried pellets is low because water-soluble constituents are lost. For lipid analyses, the unwashed pellets are treated directly with a mixture of chloroform and methanol (*15*). The amounts of total lipid per cell do not vary appreciably, whether determined on washed or unwashed cells. Nucleic acids are extracted from the unwashed cells under conditions similar to those of Santen and Agranoff (*16*) and modified by the recommendations of Munro and Fleck (*17*), and determined spectrophotometrically.

For rats 10 to 30 days old, the yield is $33.6 \times 10^6 \pm 5.7 \times 10^6$ neurons and $6.6 \times 10^6 \pm 2.4 \times 10^6$ glial cells per brain sample (0.8 to 1.35 g, fresh weight). This quantity of cells represents a dry weight of 3.1 mg for neurons and 2.3 mg for glia, or a dry weight per cell of 93 ± 15 pg and 348 ± 112 pg, respectively. The neurons are 24.8 percent lipid ($23.1 \pm$

3.7 pg/cell) and 5.6 percent DNA (5.2 ± 0.6 pg/cell); the glia are 39.4 percent lipid (137 ± 44 pg/cell) and 3.0 percent DNA (10.3 ± 0.4 pg/cell).

The neuronal fraction is more than 90 percent pure by particle count (Fig. 1a). These cells retain only stumps of axons and dendrites. They are readily identified by their characteristic morphology, size, abundant cytoplasm, and large nuclei with a prominent nucleolus (Fig. 1, c and e). The soma diameters range from 9.5 μ to 38 μ, the median cell dimensions being 15 by 19 μ. These measurements are reasonably consistent with the observed dry weight, if one assumes that the water content is 82 percent and that the shape approximates a bipyramid.

The neuroglial fraction is less pure. About 50 percent of the particles are whole glial cells, the contamination consisting of small and fragmented neurons and processes of unidentified origin. There are no myelin vesicles or myelinated axon fragments. The observed dry weight per cell is high because only whole glial cells are included in the counts from which this figure is calculated. Based on this count, the DNA per cell of the glial fraction is twice that of the neuronal fraction. The difference in the two values for DNA can be assumed to be equivalent to the amount of cellular and nuclear contamination in the glial fraction, which is in good agreement with that estimated from particle counts. If the cellular and nuclear contamination in the glial fraction is assumed to have a maximum particle weight equivalent to the weight per neuron, the minimum purity of the glial fraction is 73 percent based on weight.

The glial cells are easily distinguished from the neurons (Fig. 1). They are apparently largely intact with highly ramified processes, small nuclei, and little perinuclear cytoplasm (Fig. 1, d and f). The processes extend 20 to 80 μ from the nucleus. It is not always possible to determine the glial type for each cell. Cells resembling all glial types (as determined in metal-impregnated sections) have been seen. Many cells seem to be intermediate between protoplasmic astrocytes, fibrous astrocytes, and oligodendrocytes.

The yield of cells recovered from the total number in the brain sample is difficult to determine since the calculations of the number of cells per gram of rat brain vary so widely (*18*), as do the estimates of the ratio of glia cells to neurons. However, our purified cell fractions together account for 14 percent of the total DNA in the sample. It is probable that we recover 20 to 30 percent of the neurons and 5 to 10 percent of the glial cells.

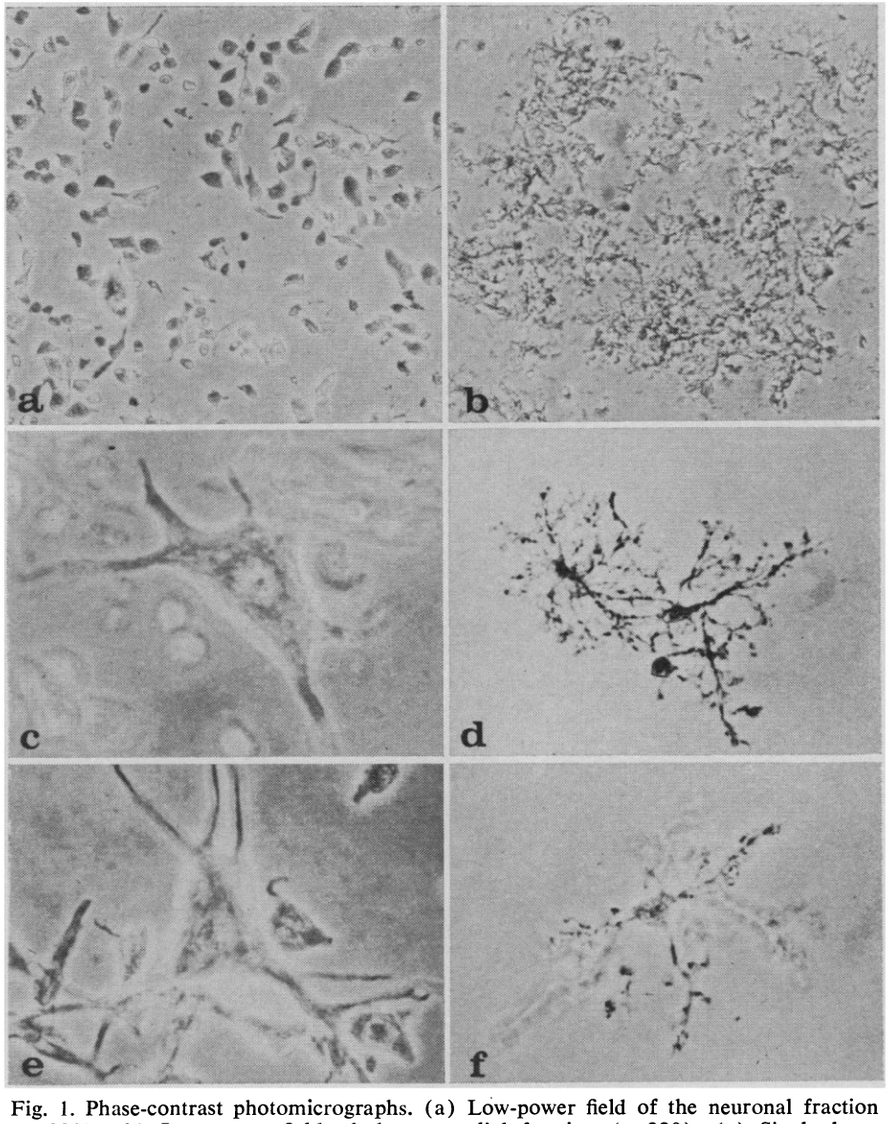

Fig. 1. Phase-contrast photomicrographs. (a) Low-power field of the neuronal fraction (× 220). (b) Low-power field of the neuroglial fraction (× 220). (c) Single large neuron (× 670); length of perikaryon is about 45 μ. Some smaller cells are out of the focal plane. (d) Three typical glial cells (× 490); longest process about 60 μ. (e) Large neuron and several smaller ones (× 600). (f) Single glial cell (× 680). Nucleus and three processes in focus; other processes out of the focal plane.

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Facilitation of Spindle-Burst Sleep by Conditioning of Electroencephalographic Activity While Awake

Abstract. *A slow-wave electroencephalographic rhythm recorded from the sensorimotor cortex of the waking cat has been correlated behaviorally with the suppression of movement. Facilitation of this rhythm through conditioning selectively enhances a similar pattern recorded during sleep, the familiar spindle burst. The training also produced longer epochs of undisturbed sleep. The specific neural mechanism manipulated during wakefulness appears to function also in sleep and to be involved with the regulation of phasic motor behavior.*

Localized electroencephalographic (EEG) recordings from sensorimotor cortex in the cat show a very distinctive slow-wave pattern (12 to 14 count/sec), which occurs in brief trains periodically during the waking state. This activity has been termed the sensorimotor rhythm (SMR) (*1*). It is observed in the quiet, alert animal and has been related specifically to the suppression of previously trained motor responses (*1*, *2*). When the SMR in a naive hungry cat is reinforced with food, a conditioned EEG response associated with stereotyped motionless postures develops (*3*). Such training makes it possible to increase significantly both the occurrence of the SMR and the related suppression of movement.

The waking SMR is similar to EEG spindle-burst activity recorded during slow-wave or quiet sleep (*4*) both with respect to frequency-power spectrum and to cortical topography (*1*, *5*). This similarity (Fig. 1) suggested that the two phenomena are related. The objective of the present study was to test this hypothesis. We attempted to determine whether enhancement of the SMR in the waking animal, achieved through the use of EEG conditioning procedures, causes changes in the pattern of quiet sleep. Quantitative effects were sought in the amount of spindle-burst activity and in the duration of sustained quiet sleep epochs.

Eight adult cats were prepared surgically for localized EEG recording. The frontal sinus was opened bilaterally, and pairs of jeweler's screws were threaded into the frontal bone over lateral pericruciate cortex on both sides (Fig. 1). Leads from these, a pair of posterior cortical electrodes, and standard eye and neck muscle electrodes were attached to a connector which was fixed to the skull with dental cement. Later the animals were placed in a recording chamber, which was equipped with an automatic feeding device, and connected through a counterweighted cable system and slip-ring assembly to an electroencephalograph. After adaptation to this chamber, three independent records of sleep were obtained as controls in advance of experimental training. These consisted of continuous recordings of EEG, electrooculograph, and data from neck muscles through several sleep cycles. With localized EEG recordings in the cat, quiet sleep is easily identified by the simultaneous occurrence of recurrent spindle bursts from sensorimotor cortex and slow waves from posterior cortex (Fig. 1). This pattern is punctuated frequently by motor adjustments or spontaneous shifts in the EEG back to the patterns of the waking or drowsy states. These interruptions are usually brief and give way again to a sustained pattern of quiet sleep. The result is a series of quiet-sleep epochs which are terminated eventually by a period of active sleep. This sequence defines the sleep cycle in the cat and at least two such cycles were obtained from each animal during the three tests before training. Subsequently, the animals were assigned randomly to two groups of four each. Both groups were then trained to receive food by producing specific patterns of EEG activity from sensorimotor cortex. Group 1 was reinforced during daily sessions for producing SMR activity, while group 2 was reinforced for producing low voltage, fast (LVF) activity. A training session consisted of 60 such reinforcements. Most animals reached maximum performance after 2 to 4 weeks of daily training. On the final or test session, they were allowed to obtain unlimited reinforcement and remained in the chamber until several complete sleep cycles were registered. Training was then reversed for the two groups and a second sleep recording was eventually collected in a similar manner. A final sleep recording was obtained again 1 month after the termination of all training.

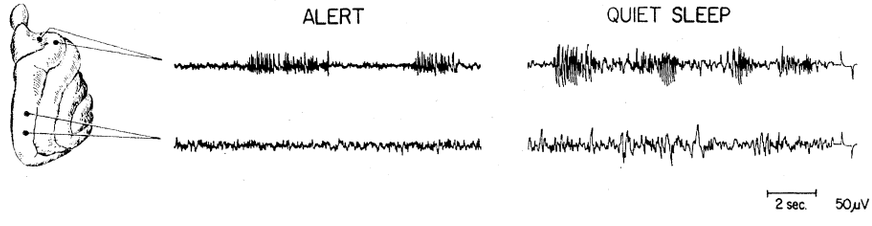

Fig. 1. Localized EEG recordings obtained from the cat during alert wakefulness and quiet sleep. Conditioned sensorimotor rhythm activity, shown as recorded in the waking animal, can be compared with the spindle-burst pattern which develops over this same cortical area during quiet sleep.

Instrumental EEG responses were established by previously described