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EFFECT OF SLEEP ON THE CENTRAL NERVOUS SYSTEM. (U)
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Effect of Sleep on the Central Nervous System

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The experiments reported herein were conducted according to the principles described in the "The Guide for the Care and Use of Laboratory Animals" prepared by the Committee on Care and use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council, DHEW Publication No. (NIH) 78-23, Revised 1978.

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20. ABSTRACT (Continue on reverse side if necessary and identify by block number) Neurons and glial cells form the IVth and Vth layers of frontal and occipital cortex of 24h sleep-deprived hamsters were examined under electron microscope for structural alterations. Nuclear membrane, chromatin distribution pattern, mitochondria, Golgi apparatus, and cell membrane show no changes. The partial volumes of endoplasmic reticular cisternae increased by 37% but the surface area of the endoplasmic reticulum remained constant in the neurons of sleep-deprived animals. Also, the number of neuronal		

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lysosome decreased by 36% and 18% in frontal and occipital cortex. No changes in the liver cells were detected. The astroglial cells of the sleep deprived animals were found to contain large clear vacuoles. In 80% of the sleep deprived astroglial cells the endoplasmic reticulum vesiculated with partial desolution of its membrane as compared to only 14% in the control animals. There is no change in the surface area of endoplasmic reticulum. The volumes of astroglial endoplasmic reticular cisternae increased by 162% in frontal cortex and 122% in occipital cortex of the sleep deprived animals. The fact that the surface area of the endoplasmic reticulum remains the same but the volume increases gives support to the idea that the membrane spaces seen in the astroglial cytoplasm are vesiculation of the endoplasmic reticulum rather than new formation. Incapacitating behavioral decrement associated with sleep loss may be a consequence and symptomatic expression of the type of subcellular disorganization observed in this study. These changes are reversible after 24 hrs of rest following sleep deprivation and a lysosomal stabilizer failed to prevent its occurrence.

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A. OBJECTIVE

It is generally accepted that dramatic and incapacitating behavior changes take place in human subjects due to prolonged sleep deprivation. We have assumed that the behavioral decrement due to sleep loss is a consequence and symptomological expression of cellular and subcellular level changes taking place in the brain cells during the deprivation period. Several investigators^{1,2,3,4,5,6,7} have observed morphological (light microscopic) alterations in brain cells after sleep deprivation in a number of species. The changes include chromatolysis and vacuolization of cerebral cortical cells in dogs^{3,4} and in guinea pigs.⁵ Some authors^{6,7} have reported degenerative changes in medulla and spinal cord and also in non-neural tissues.^{6,7} In a series of studies Legendre and Pieron^{8,9,10} demonstrated the presence of a "sleep hormone" in the cerebro spinal fluid of sleep deprived dogs. In these studies the cells of prefrontal cortex showed cell shrinkings, nuclear displacement, cytoplasmic vacuolization and disappearance of Nissl granules. We have reinvestigated this morphological alteration following sleep deprivation at the electron microscopic level.

- a. We have examined changes in the structure of the nuclear membrane, chromatin distribution, mitochondria, endoplasmic reticulum and the cell membrane of the cortical neurons following sleep deprivation.
- b. We have examined changes in the structure of the nuclear membrane, chromatin distribution, mitochondria, endoplasmic reticulum and the cell membrane of the cortical astroglial

cells following sleep deprivation.

- c. We have examined structural changes in oligodendrocytes following sleep deprivation.
- d. We have compared the morphological changes taking place in the frontal cortex with those taking place in the occipital cortex.
- e. We have examined if any morphological changes are taking place in the liver and kidney cells.
- f. We have examined the reversibility of structural changes in brain cells after a period of rest following sleep deprivation.
- g. We have examined the effectiveness of a lysosomal stabilizing drug in preventing structural damage during sleep deprivation.

B. RESEARCH PERFORMED

1. General Methods

Adult male golden syrian hamster (approximately 100 g) were maintained on commercial chow and water ad libitum. Animals were housed individually at constant room temperature with 12/12 light/dark cycles until the period of sleep deprivation. Total sleep deprivation was performed by gentle hand manipulation or mechanically. In the latter method, an electronic program switching device (Lyons Electronics, Trenton, NJ) alternately activated a rotating T-bar (6 cycles per minute) on the floor of a circular cage placed on a Dubnoff metabolic shaking (1 cycle per second) incubator. The efficiency of this apparatus was tested by behavioral, electro-

encephalographic and electromyographic observation. Animals were deprived of sleep for 24 hours and were compared to undisturbed cage controls sleep-waking ad libitum.

Following sleep deprivation the animals were given an overdose (80 mg/kg, IP) of pentobarbital. To check the effects of anesthesia on brains cell ultrastructure, some awake and asleep animals were killed by neck fracture. In the pentobarbital injected animals as soon as the anesthesia takes effect, a small volume of heparin was injected into the femoral vein to prevent clotting. The thorax was opened, a clamp was placed on descending aorta, the tip of the left ventricle was cut and a cannula was inserted into the aorta through the ventricle. The descending aorta was clamped off and a cut was made in the right atrium. The blood from the head region was washed out by perfusing with a small volume of buffered Ringer's solution which was followed by a dilute and a concentrated buffered solution of para-formaldehyde and glutataldehyde (purified) as described by Peters.¹¹ The pH and the hydrostatic pressure of the perfusion fluid was maintained close to the normal values of hamsters. To prevent distortion of the brain the head was stored overnight. Next day, the brain was removed from the skull. Desired regions of the brain was cut into small pieces (2 mm) and the pieces were thoroughly washed in hypersomolar (400 mOsm) buffer solution (pH 7.4). The pieces were then osmicated in 1% oxmic acid (same carrier buffer) for 2 hours. After osmication the tissue was thoroughly rinsed in buffer, dehydrated in alcohol changes and embedded either in Spurr low

viscosity plastic or in Epon 812. Some blocks were stained with 0.5% uranyl acetate in veronal acetate buffer before dehydration. Control and experimental animals were always worked up in pairs. One micron sections from various parts of the brain were obtained from the plastic blocks. The sections were stained in 1% Toliudine Blue in 0.5% borax solution. These sections were examined under light microscope for selecting the area to be examined under electron microscope. From cerebral cortices areas III, IV and V were chosen for electron microscopic observation. Thin sections (800Å) were stained in 5% uranyl acetate in 50% alcohol and saturated with lead citrate.

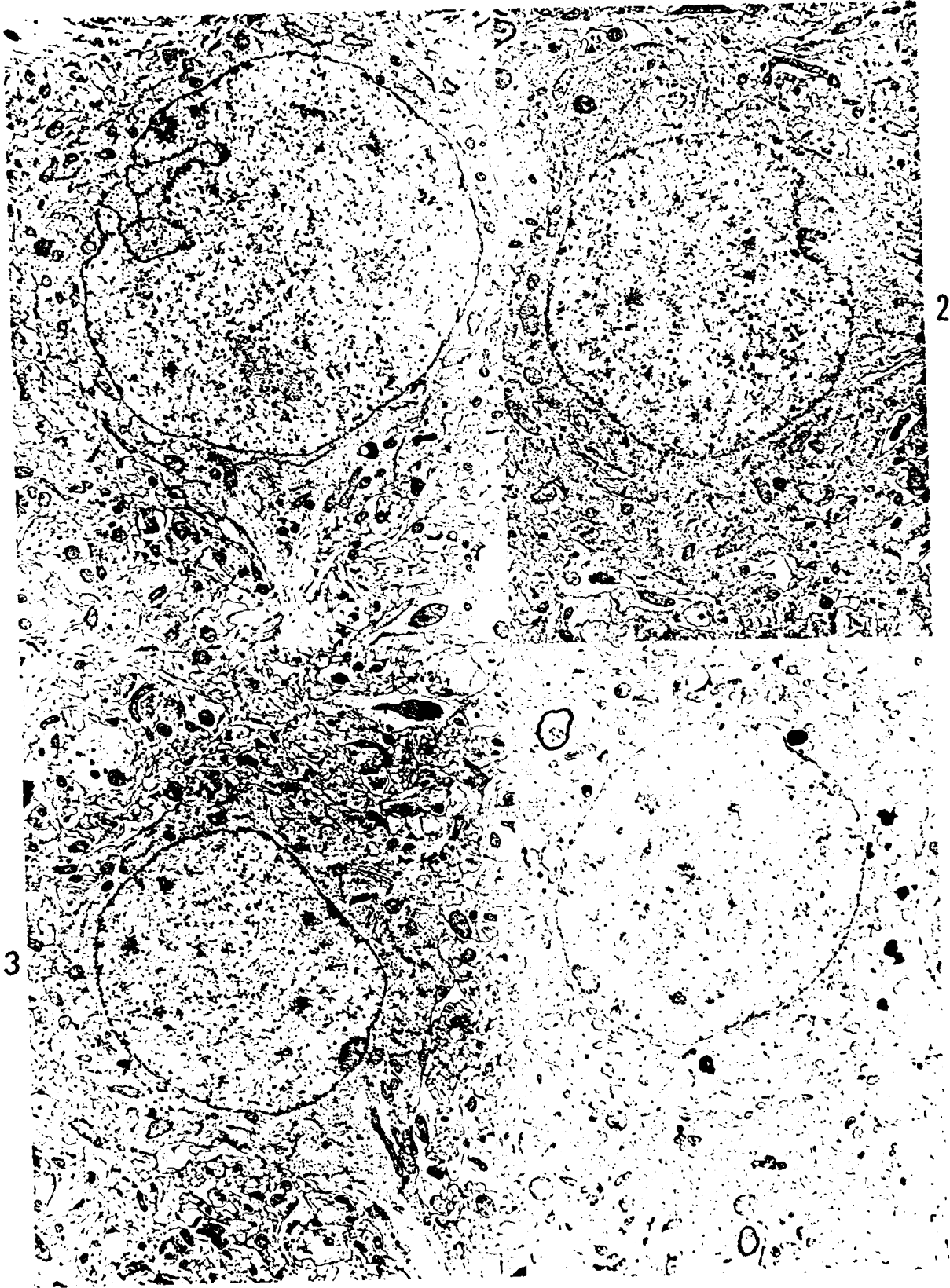
Morphometric analysis¹² was performed by taking low power (5,600x) pictures of randomly encountered neurons and astroglial cells in five different areas of the frontal and occipital cortices of each of the control and experimental animals. The pictures were projected on a standard morphometric test-grid and the number of end points of a test line falling within a given structure or the number of intersections of a given structure with the test line were counted. The partial volumes, relative surface area and relative number of a structure were determined by the use of appropriate morphometric equations¹².

2. Results

- a. Structural changes in cortical neurons following 24 h sleep deprivation.

- i) Qualitative changes: In the neurons of areas III, IV and V of the frontal and occipital cortices of 24 h sleep deprived hamsters, we have seen no changes in the following structures: nuclear membrane, chromatin distribution within the nucleus, mitochondria, endoplasmic reticulum, and the cell membrane. With the exception of about 8% of the cells, the general appearance of the neuronal soma of the frontal and occipital cortex of 24 hr sleep deprived hamsters is quite normal (Fig. 1) (Table 1).
- ii) Quantitative changes: Although most of the cortical neurons appear normal by casual inspection, morphometric analysis reveals subtle structural changes in them. The partial volumes of mitochondria and the surface area of endoplasmic reticulum remained constant in the sleep deprived neurons (Fig. 2). After sleep deprivation, the numerical density of neuronal lysosome decreased 36% and 18% in frontal and occipital cortex. There was an approximately 35% increase in the endoplasmic reticulum cisternal volume.

Fig. 1. Low power (8,000X) electron micrographs of four neuronal soma in the frontal cortex of hamsters. Cell 2 is from a 18 hours sleep deprived hamster and cells 1,3, and 4 are from control hamsters. The ultrastructural picture of a nerve cell is unaltered after 18 hours of sleep deprivation.



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TABLE 1

Percentage of neurons judged to be normal or having the vesiculate endoplasmic reticulum in the frontal and occipital cortex of control and 24 h sleep deprived hamsters.

	Brain Regions	Neurons	
		Normal	Vesiculated
Control	Frontal	100%	0%
	Occipital	100%	0%
24 h sleep deprived	Frontal	91%	9%
	Occipital	93%	7%

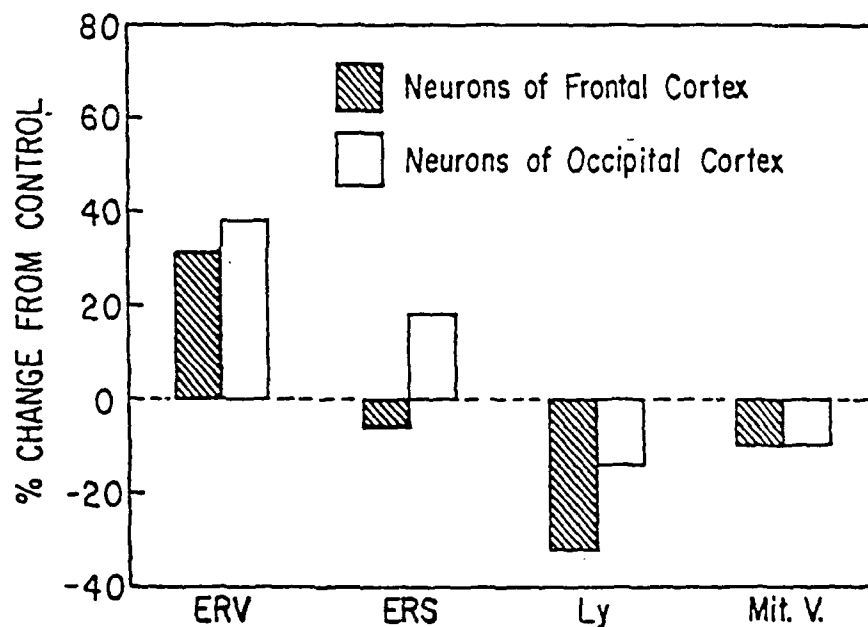


Fig. 2. Percentage changes of mitochondrial volume (Mit. V.), endoplasmic reticulum cisternal volume (ERV), endoplasmic reticular surface (ERS) area and lysosomal number (Ly) in the neurons of frontal (cross-hatched) and occipital cortex (empty bars) of 24 h sleep deprived hamster in comparison with control animals.

b. Structural Changes in cortical Astroglial Cell following
24 hr Sleep Deprivation

- i) Qualitative changes: Very striking changes in the ultrastructure of the astroglial cell has been observed (Fig.3,4) after sleep deprivation. In 67% of astrocytes in frontal cortex and 93% of astrocytes in occipital cortex the endoplasmic reticulum vesiculated to form large membrane bound vesicles scattered in the cytoplasm around the nucleus (Table 2). No changes in the nuclear membrane, chromatin distribution, mitochondria or plasma membrane have been observed in the astroglial cells.
- ii) Quantitative changes: In the astroglial cells of the occipital cortex the partial volume of mitochondria, lysosomal number and the endoplasmic surface area (Fig. 5) remained unchanged. In the frontal cortex the number of lysosomes decreases by 20% and there is no change in the surface area of endoplasmic reticulum. Consistent with our qualitative observation the volume of endoplasmic reticulum more than doubled in the experimental animals. The fact that the surface area of endoplasmic reticulum remains the same but the volume doubles gives support to the idea that the bound spaces seen in the astroglial cytoplasm are vesiculation of endoplasmic reticulum.

Fig. 3. Low power (8,000X) electron micrographs of six Astroglial cells in the frontal cortex of hamsters. Cells 3, 4, 5, and 6 are from 18 hours deprived hamsters. Cells 1 and 2 are from control animal. A narrow rim of cytoplasm surrounds the relatively large nucleus and sheets extend out among the various elements of the neuropil. Large vesicles (narrow heads) appears in the endoplasmic reticulum of the cells of the sleep deprived hamsters.

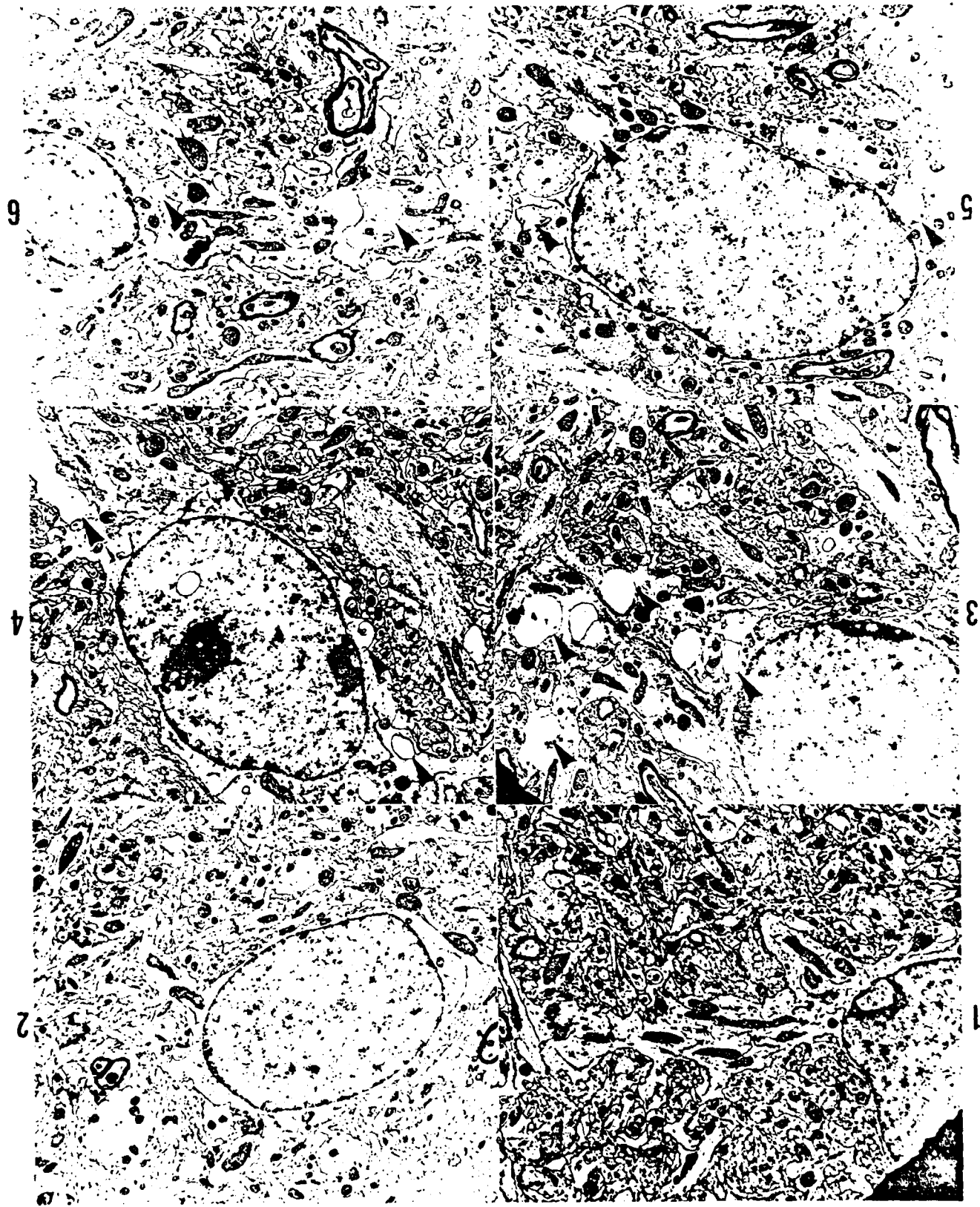


Fig. 4. Shows, on the top, a higher magnifications of an astrocyte from a control animal (cell #1 in Fig. 3). In the cytoplasm of this second cell, seven large membrane bound vacuoles are visible (marked by X). These spaces appear to be dialated spaces of endoplasmic reticulum. The vacuole in the upper right-hand corner suggests its continuity with endoplasmic reticulum (marked by an arrow).



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TABLE 2

Percentage of glial cells judged to be normal or having the vesiculate endoplasmic reticulum in the frontal and occipital cortex of control and 24 h sleep deprived hamsters.

	<u>Brain Regions</u>	<u>Astrocytes</u>		<u>Oligodendrocytes</u>	
		<u>Normal</u>	<u>Vesiculated</u>	<u>Normal</u>	<u>Vesiculated</u>
Control	Frontal	90%	10%	100%	0%
	Occipital	83%	17%	--	--
24 h sleep deprived	Frontal	33%	67%	100%	0%
	Occipital	7%	93%	--	--

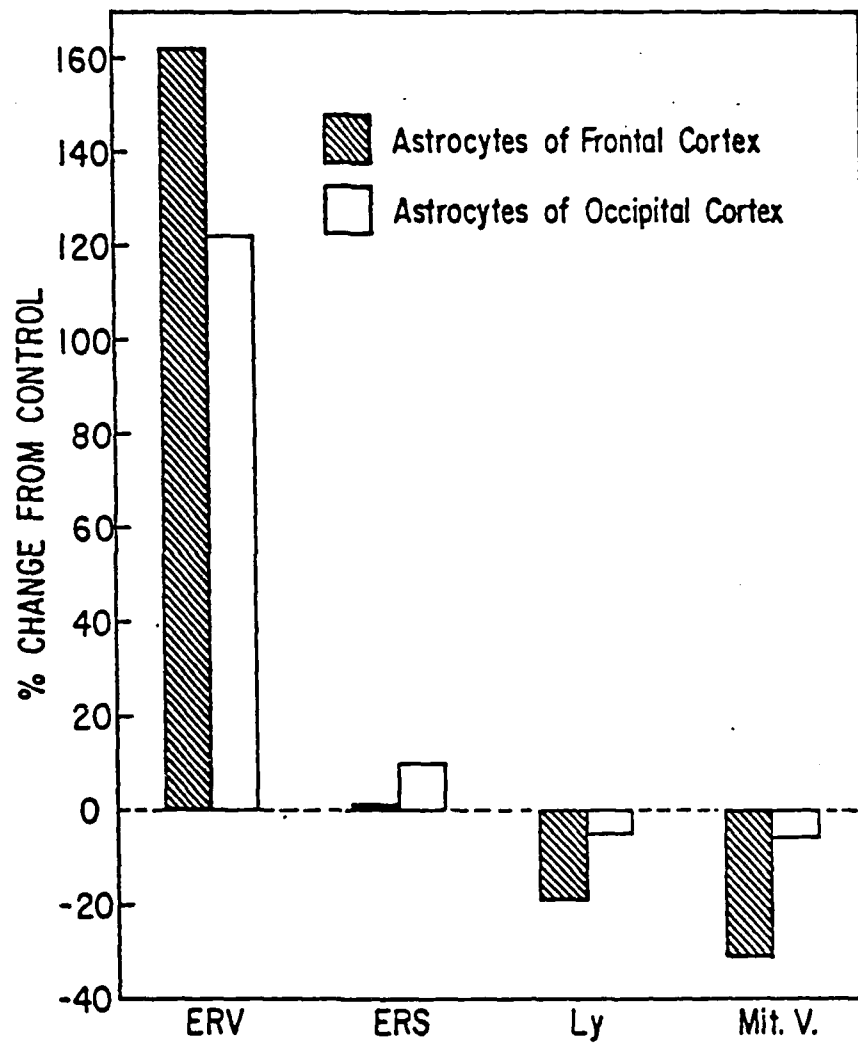


Fig. 5. Percentage changes of mitochondrial volume (Mit. V) endoplasmic reticular cisternal volume (ERV), endoplasmic reticular surface (ERS) area and lysosomal number (Ly), in the astrocytes of frontal (cross hatched) and occipital cortex (empty bars) of 24 h sleep deprived hamster in comparison with control animals.

c) Structural Changes in Cortical Oligodendrocytes following 24 h Sleep Deprivation.

We have not detected any structural changes in the oligodendrocytes, following sleep deprivation (Table 2).

d) Comparison of Structural Changes in the Frontal Cortex versus Occipital Cortex.

The overall pattern of structural changes are very similar between these two areas (Fig. 2, 5; Table 1,2). The decrease in lysosomal number both in the neurons and in the astroglial cells are slightly more pronounced in the frontal cortex than in the occipital cortex.

e) Non-neural Tissue.

To check if the ultrastructural changes observed in the brain cells of sleep deprived animals is a generalized phenomenon occurrence also in non-neural tissues, we have examined the ultrastructure of the liver and kidney cells. The overall appearance of these cells is the same as in control and experimental animals. Specifically, we saw no changes in nuclear membrane, chromatin distribution within the nucleus, ribosomal distribution, mitochondria, endoplasmic reticulum and the cell membrane.

f) Reversibility of Structural Changes.

When the animals are allowed to sleep for 24 hours following sleep deprivation, the vesiculation of the endoplasmic reticular cisternae in the astrocytes is no longer detectable. This length of recuperative sleep is adequate for reversing the morphological change.

g) Lysosomal Stabilizing Drug.

Chloroquine administered intraperitoneally (every 6 hrs) during the deprivation period does not protect astrocytic vesiculation

3. Significance of the Observed Changes

A variety of stressful situations are known to stimulate neuroprotease (lysosomal activity)¹³. A number of conditions like exercise¹⁴, infection¹⁵, hypoxia and chemicals like progesterone¹⁶, are known to cause simultaneous increase in neuroprotease activity and sleeping time. We have proposed that prolonged wakefulness or sleep loss increases neuroprotease activity, which causes subcellular disorientation in brain cells and is behaviorally manifested in sleepiness and associated functional decrement.

The increase in neuronal and glial lysosomal number following sleep deprivation is understandable in light of our working hypothesis and is consistent with our biochemical data. In a related study we have examined the possibility that during the stress of sleep deprivation neuronal lysosomes are labilized and hydrolases leak out

of them. We measured the total activity of three hydrolytic lysosomal enzymes (Cathepsin D, Acid phosphatase and N-acetylglucosaminidase) before and after sleep deprivation. In general there was a slight decrease¹⁸ in the total activity of these enzymes in several brain areas after 18 hrs of sleep deprivation (Fig. 6, 7, and 8). The loss of these enzyme activity may be an indication of macromolecular degradation during sleep deprivation.

The extensive damage observed in the astroglial cells may be the cause of the functional decrement observed in sleep deprivation. It is not clear how the central nervous system dysfunction is related to the astrocytic changes. Recent studies have indicated that the glial cells may function as a K^+ and neurotransmitter sink. A damaged astroglial cell may have a sufficiently altered function to be unable to maintain the ionic environment of the neurons. It is interesting to note that similar vesiculation of endoplasmic reticulum has been observed in rat astrocytes in experimental cerebral encephalopathy following liver bypass¹⁹.

Our attempts to prevent astrocytic vesiculation with chloroquine did not succeed. Perhaps other lysosomal stabilizers would be able to do that. Also, it would be worthwhile to determine if lysosomal stabilizers are able to prevent functional decrement following sleep deprivation.

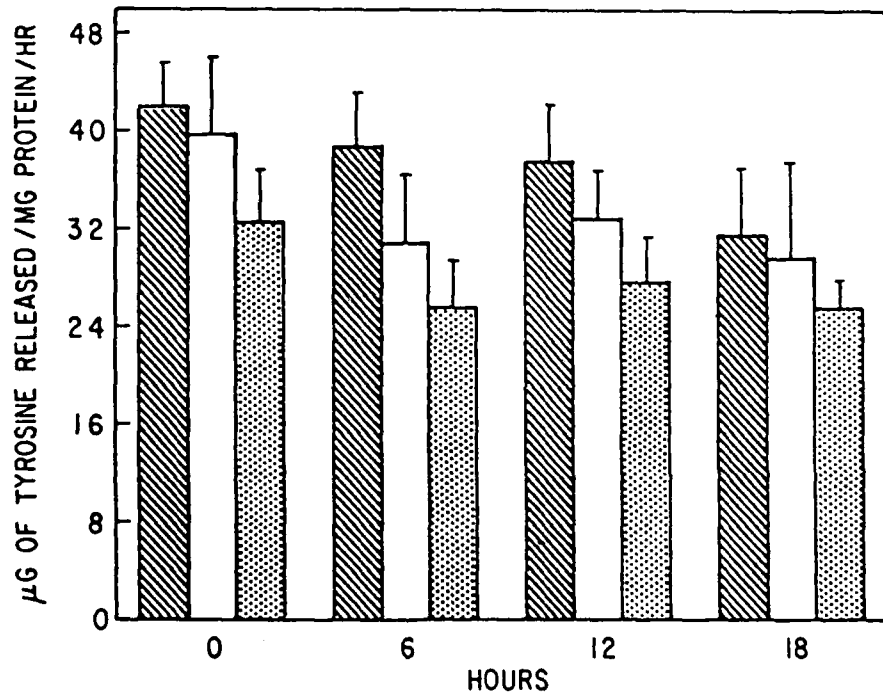


Fig. 6. Cathepsin D total (triton activated) activity of three brain regions as a function of hours of sleep deprivation values are mean \pm SEM of 6-14 animals. Cerebral cortex = hatched bars; upper brain stem - clear bars; lower brain stem = dotted bars.

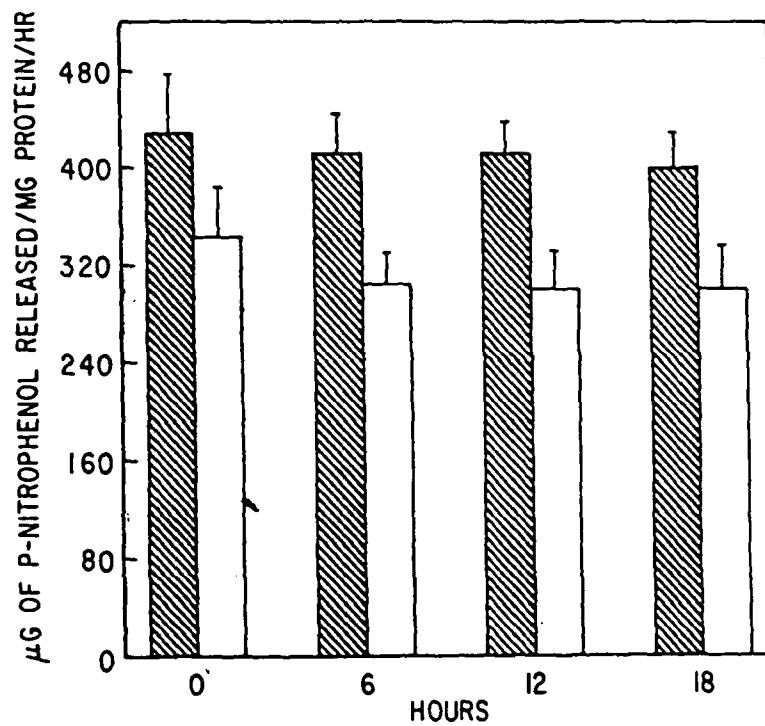


Fig. 7. β -N-acetyl-glucosaminidase total (triton activated) activity (ordinate) of three brain regions as a function of hours of sleep deprivation (abscissa). Values are mean \pm SEM of 6-14 animals. Cerebral cortex = hatched bars; upper brain stem = clear bars; lower brain stem - striped bars.

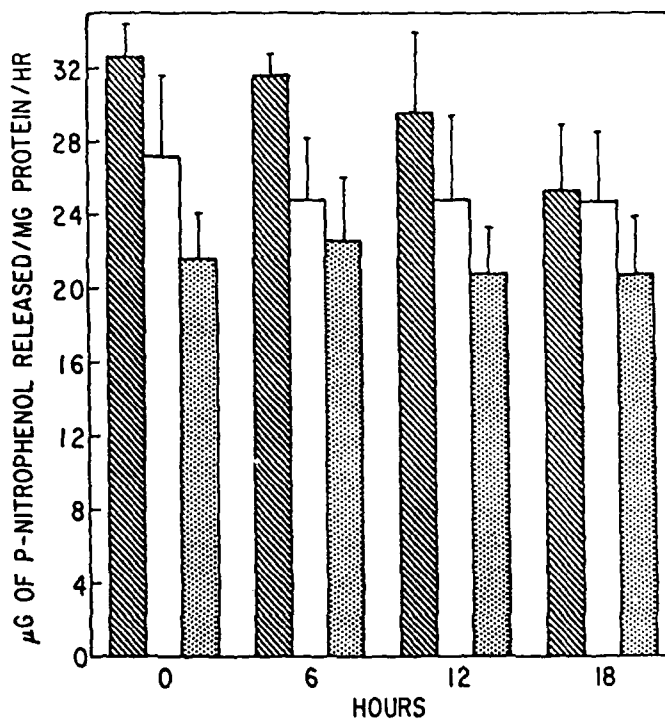


Fig. 8. Acid Phosphatase total (triton activated) activity (ordinate) of two brain regions as a function of hours of sleep deprivation (abscissa) values are mean \pm SEM of 6-14 animals. Cerebral cortex = hatched bars; upper brain stem = clear bars.

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d. Publications

1. Sinha, A. K. C. S. Lee and B. K. Ghosh. Vesiculation of astroglial cells in hamster following 24 h sleep deprivation. *Soc. Neurosc. Abs.* 5: 699, 1979.
2. Sinha, A. K. and B. K. Ghosh. Effects of sleep deprivation on brain cell morphology (being prepared for submission to the "Brain Research").

e. Professional Personnel Associated with the Research Effort

1. Dr. B. K. Ghosh. Prof. of Physiology & Biophysics. CMDNJ-Rutgers Medical School.
2. Dr. M. K. Poddar
3. Dr. G. Chakraborty
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f. Interaction

Parts of this work was presented in the following seminars and scientific meetings:

1. Air Force Review of Basic Research - 1978.
2. Rutgers Medical School - Physiology Department Faculty Seminar - 1979.
3. Society for Neuroscience Annual Meetings - 1979.
4. Air Force Review of Basic Research - 1979.
5. Air Force Review of Basic Research - 1980.

g. Patent

In my judgement, no patentable observations were made during the course of this study.

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