After ethanol administration, the changes observed in the steady-state concentrations of metabolites may significantly influence regulatory processes in brain. Such alterations may be the cause of the acute pharmacological action of alcohol on the central nervous system and may be associated with the neural disorders observed during chronic alcohol consumption.

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Age Changes in the Neuronal Microenvironment

Abstract. *The extracellular space of the rat brain was visualized by electron microscopy in sections of cerebral cortex fixed by freeze-substitution. The volume of tissue occupied by the extracellular space was estimated stereometrically, and was found to decrease from 20.8 percent in 3-month-old rats to 9.6 percent in senescent animals, 26 months of age. This decrease in extracellular space indicates an age-associated change in the microenvironment of nerve cells.*

The extracellular tissue compartment of the brain and spinal cord (extracellular space) is composed primarily of submicroscopic channels between cellular elements (*1*). It is believed to be the site of physiologically important reactions, involving metabolites and ions, upon which neuronal metabolism depends; it thus constitutes the microenvironment of neurons (*2*). Its submicroscopic distribution appears to be more adequately preserved by freeze-substitution than by more traditional methods of chemical fixation (*1*, *3*), and its volume is readily estimated by the stereometric assessment of electron micrographs of brain specimens preserved by freeze-substitution (*1*). In the normal adult rat the extracellular space occupies about 22 percent of the volume of superficial cerebral cortical tissue (*1*).

It has been demonstrated that the volume of the extracellular space in mammalian brain changes gradually during the course of early postnatal development (*1*). In the cerebral cortex of the rat, the volume of the extracellular space changes from about 40 percent during the first week after birth, to about 35 percent during the second week (*1*). During the third week, when electroencephalographic activity acquires adult-like characteristics (*4*), the volume of extracellular space was estimated to be about 26 percent, which closely approaches the volume in the adult (*1*). Under normal conditions, the volume of the extracellular space in the brain of the rat appears to remain relatively unchanged until 22 to 24 months, when senescent characteristics become evident (*5*).

The extracellular space of the brain appears to contain certain anionic substances that may be glycoproteins or mucopolysaccharides (*2*). In this regard, it resembles the ground substance of connective tissues (*6*). Because the ground substance of connective tissues condenses, becomes less hydrated, and is replaced by collagen as animals age (*7*), we asked if the extracellular space of brain also changed in the latter part of the life span. The apparent role of the extracellular space in neuronal nutrition further impelled our question.

Eleven male Sprague-Dawley rats were obtained commercially at 1 month of age, housed in the animal care facility of Northwestern University, and fed a standard chow diet with freely available water. At the time of this study, eight animals were 26 months old and three were 3 months old. They were killed by decapitation after which the calvaria and dural membranes were rapidly removed, and the brain was exposed. A thin slice of cerebral cortex, from the parieto-occipital region near the midline, was excised and frozen within 30 seconds in Freon-22 that had been previously chilled with liquid ni-

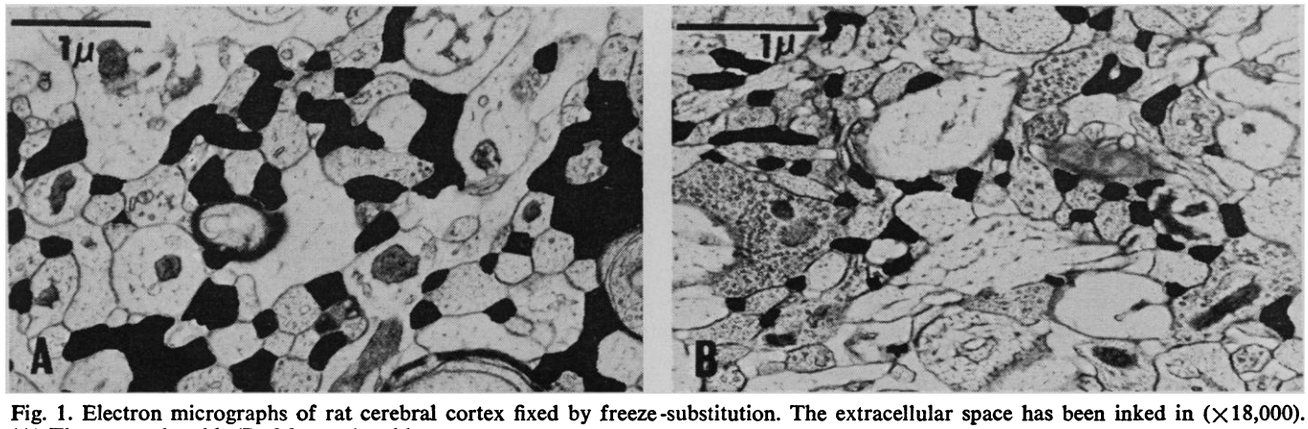

Fig. 1. Electron micrographs of rat cerebral cortex fixed by freeze-substitution. The extracellular space has been inked in (×18,000). (A) Three months old; (B) 26 months old.

trogen to −154°C. The frozen tissues were transferred to a 1 percent solution of osmium tetroxide in absolute acetone at −78°C for 3 days, after which time they were brought to room temperature, rinsed in absolute acetone, and embedded in Araldite-502. Sections, 0.5 μm thick, were cut perpendicular to the pial surface and examined with a light microscope. By this means, areas of the molecular layer of the cerebral cortex were identified in which damage due to ice crystal formation appeared minimal. These selected areas of tissue were isolated, sectioned at 500 to 700 Å with an ultramicrotome (Sorvall MT-2), and examined in an electron microscope (Hitachi HU-12). Five electron micrographs were prepared from each selected tissue area at an electron optical magnification of ×10,000, and were uniformly enlarged photographically. A transparent regular point lattice, composed of small holes 0.9 cm apart, was superimposed on each electron micrograph, and the relative volume of extracellular space was estimated stereometrically by counting the holes overlying the images of extracellular space (*8*).

In all sections selected and examined with the light microscope at a magnification of × 1000, the ice-crystal artifact was minimal for a depth of about 20 μm beneath the pial surface. The cytoplasm of neuronal, glial, and vascular elements appeared relatively homogeneous, and it was not possible to distinguish between tissues from the old and young animals. With the electron microscope, tissue preservation was seen to be more variable, but cytoplasmic organelles were identifiable, and plasma membranes were essentially intact. The extracellular space appeared as lakes of variable dimensions, which were noticeably smaller in tissues from the old animals. The mean volume of cerebral cortex occupied by extracellular space was 9.6 percent (range; 4.3 to 15.0 percent) in animals 26 months old (Fig. 1). In 3-month-old animals, the mean volume of cerebral cortex occupied by extracellular space was 20.8 percent (range, 18.7 to 22.3 percent) (Fig. 1), which agrees closely with our previously reported findings of a 21.7 percent mean volume of extracellular space in the cerebral cortices of four young adult female Sprague-Dawley rats (250 g each), prepared similarly by freeze-substitution (*1*). The mean volumes of extracellular space for 3-month-old and 26-month-old rats were compared with a Mann-Whitney U test (*9*); a significant difference was suggested by a result of $P < .001$. These data are summarized in Table 1.

The function of the extracellular space in normal brain is not known, but

Table 1. Estimated volume of extracellular space in electron micrographs of frozen-substituted cerebral cortex of rats, 26 and 3 months of age. In each tissue specimen, the percentage of tissue volume occupied by extracellular space was estimated in five different electron microscopic fields. The average volumes are expressed as means ± standard deviation; EM, electron microscopy.

Rat (No.)	Volume per EM field (%)	Volume per rat (%)
	26 months old	
1	12.1	13.1 ± 2.2
	11.7	
	11.0	
	14.7	
	16.1	
2	4.2	4.3 ± 0.0
	4.0	
	4.0	
	4.5	
	4.6	
3	12.6	15.0 ± 2.0
	14.5	
	16.5	
	13.8	
	17.6	
4	9.2	11.1 ± 2.2
	12.5	
	13.8	
	8.7	
	11.3	
5	6.9	6.5 ± 0.1
	7.0	
	6.2	
	6.0	
	6.3	
6	6.7	6.9 ± 0.4
	6.9	
	7.5	
	6.8	
	6.6	
7	11.0	11.0 ± 0.9
	10.8	
	10.5	
	12.5	
	10.3	
8	10.3	9.3 ± 0.9
	8.1	
	9.1	
	10.0	
	9.0	
Average volume (%) per age group: 9.6 ± 3.4		
	3 months old	
9	18.5	18.7 ± 1.5
	20.5	
	19.8	
	17.0	
	17.6	
10	20.5	22.3 ± 1.7
	24.5	
	21.8	
	23.6	
	21.1	
11	20.4	20.6 ± 1.1
	20.9	
	22.4	
	19.8	
	19.5	
Average volume (%) per age group: 20.8 ± 1.5		

can be anticipated from studies of nervous tissues in leech and amphibia, which indicate the free diffusion of ions and small metabolites through it (*10*). Since the brain contains essentially no connective tissue, the extracellular space appears to perform a histophysiological role comparable to that performed by the ground substance of connective tissues in nonnervous organs.

We have shown here that the volume occupied by the extracellular space in cerebral cortex of senescent Sprague-Dawley rats (that is, older than 24 months) is approximately half of that which it occupies in the normal adult. This complements our earlier finding that the transport of catecholamines through brain tissues is reduced greatly in the senescent animal (*11*). Exogenous norepinephrine and dopamine were introduced into the caudate nuclei of Sprague-Dawley rats at ages of 1, 3, 9, and 26 months. In the senescent animals, the spread of catecholamines through striatal tissue was about half of that found in younger animals (*11*). Since exogenous catecholamines are transported through brain tissue, at least in part, by extracellular pathways (*12*), these data suggest a generalized decrease in transport of materials through the extracellular space, and indicate an age-related alteration in the relation of the neuron to its microenvironment during senescence.

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