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Intraneuronal Aluminum Accumulation in Amyotrophic Lateral Sclerosis and Parkinsonism-Dementia of Guam

Abstract. *Scanning electron microscopy with energy-dispersive x-ray spectrometry was used to analyze the elemental content of neurofibrillary tangle (NFT)–bearing and NFT-free neurons within the Sommer's sector (H_1 region) of the hippocampus in Guamanian Chamorros with amyotrophic lateral sclerosis and parkinsonism-dementia and in neurologically normal controls. Preliminary data indicate prominent accumulation of aluminum within the nuclear region and perikaryal cytoplasm of NFT-bearing hippocampal neurons, regardless of the underlying neurological diagnosis. These findings further extend the association between intraneuronal aluminum and NFT formation and support the hypothesis that environmental factors are related to the neurodegenerative changes seen in the Chamorro population.*

The indigenous (Chamorro) population of Guam represents one of three geographical foci in the western Pacific region where inordinately high incidence rates of amyotrophic lateral sclerosis (ALS) and parkinsonism in association with severe dementia (parkinsonism-dementia or PD) are found (*1*). Severe neurofibrillary tangle (NFT) formation has been demonstrated in central nervous system tissues from affected patients (*2*) and from a high proportion of neurologically intact individuals of a relatively young age (*3*). Comparatively high levels of aluminum and unusually low levels of calcium and magnesium have been found in samples of drinking water and garden soils from Guam and two other high incidence foci of ALS and PD, one in the Kii Peninsula of Japan (*4*) and the other in southern West New Guinea (*5*, *6*). Accordingly, we have, following Yase's suggestions (*7*), suspected that chronic nutritional deficiencies of calcium and magnesium and relative excesses of certain nonessential trace metals (such as aluminum) may produce aberrations in mineral metabolism, resulting in abnormal deposition of these elements in the central nervous system.

Brain tissues from eight Guamanian Chamorros (one ALS, two PD, and five neurologically normal controls), who were lifelong residents of the island, were examined. The clinical courses of the two PD cases (ages, 57 and 66 years) and the ALS case (age, 64 years) were typical and the neuropathological changes were confirmatory. The five control cases were Chamorros who died of nonneurological disorders, and, although they had not been subjected to careful neurological evaluation, they were considered to be free of any significant neurological dysfunction. Neuropathologically, none of the controls had evidence of extrapyramidal or motor neuron degeneration. On the basis of silver impregnation staining, four of the control cases (ages, 48, 48, 57, and 65 years) had no NFT-bearing hippocampal neurons, while one control case (age, 57 years) showed extensive NFT formation, despite the absence of clinically apparent neurological deficits.

Frozen sections (20 μm) of Formalin-fixed Sommer's sector (H_1 region) of the hippocampus were mounted onto pure carbon disks and were viewed by scanning electron microscopy (JEOL JSM-35). Under standardized conditions of magnification, accelerating voltage (15 kV), and beam current (0.5×10^{-10} Å), four sites (each measuring 0.5 μm in diameter) in the nuclear region and four in the perikaryal cytoplasm of each of ten neurons were selected for elemental analysis by energy-dispersive x-ray spectrometry (Kevex 7000 series). Emitted x-rays from each probe site were collected for 100 seconds, and the resultant x-ray energy spectra were evaluated for the presence of peaks related to the Kα x-ray emissions of magnesium, aluminum, silicon, calcium, manganese, and iron. In addition, the number of x-ray counts after background subtraction were recorded within a 150-eV window centered on the Kα of each of these elements.

Serial sections adjacent to the section used for x-ray analysis were stained by the Bielschowski silver impregnation method in order to verify the distribution and extent of NFT involvement. Since 95 percent or more of identifiable Sommer's sector neurons in affected cases contained NFT's, the need for direct confirmation of the presence of NFT's in individual neurons was unnecessary (Fig. 1). The secondary electron surface images of unstained sections provided sufficient cellular detail to identify individual neurons and landmarks for intracellular probing.

Prominent peaks related to the Kα of aluminum were found in 57.5 to 67.5

Table 1. Aluminum-related x-ray emissions (counts above background) from neurofibrillary tangle (NFT)–bearing and normal-appearing neurons of the Sommer's sector of the hippocampus in Guamanian Chamorros with amyotrophic lateral sclerosis (ALS) and parkinsonism-dementia (PD) and in neurologically normal controls.

Diagnosis	Age (years)	Sex	NFT's in hippocampus	Probe sites positive for aluminum (%)		Aluminum counts (mean ± S.E.)
				Nucleus	Cytoplasm	
ALS	64	M	4+	67.5	50.0	296.3 ± 26.2*†
PD	56	M	4+	62.5	55.0	236.2 ± 21.3*
PD	66	M	4+	57.5	52.5	232.1 ± 23.9*
Control (with NFT's)	57	M	3+	40.0	31.3	165.4 ± 13.2*
Control (without NFT's)	48	M	0	10.6	10.1	77.3 ± 7.2
	48	F				
	57	F				
	65	M				

*$P \leq .001$ compared to control cases without NFT's (*12*). †$P < .01$ compared to the control case with NFT's (*12*).

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percent of 120 probe sites within the nuclear region of NFT-bearing hippocampal neurons in the ALS and PD cases (Table 1 and Fig. 2, a and b). Aluminum peaks were also detected in 50 to 55 percent of probe sites within the perikaryal cytoplasm of these cells. In contrast, aluminum peaks were present in only 10.6 percent of 160 probe sites within the nuclear region of NFT-free hippocampal neurons in four control cases (Table 1 and Fig. 2c). The single control case with prominent hippocampal NFT formation had a peak for aluminum in 40 percent of 80 probe sites within the nuclear region. Aluminum-related x-ray counts above background collected from probe sites within NFT-bearing neurons of ALS and PD cases were three to four times higher than the x-ray counts emitted from probe sites within NFT-free neurons of control cases (Table 1). The NFT-bearing neurons from the control case also emitted elevated counts related to aluminum. The lower mean x-ray emission seen in this case, compared to that seen in the ALS and PD cases, may reflect the slightly lower concentration of NFT-bearing cells. Furthermore, calcium-related x-ray emissions were higher from NFT-bearing neurons than NFT-free neurons, but the differences were not as striking as those for aluminum. No significant differences were noted in the magnesium-, silicon-, manganese-, and iron-related x-ray emissions.

Mineralization of the blood vessel walls within the globus pallidus, a common finding in both neurologically normal individuals and in patients with idiopathic parkinsonism (*8*), was also noted in the PD cases (Fig. 3). Phosphorus, calcium, iron, and aluminum comprised a substantial portion of the mineral deposits within the blood vessel walls of these two cases (Fig. 4). However, the extent of aluminum deposition in the blood vessel wall was especially striking and was considerably greater than in any other neuropathological condition examined previously. In five cases of incidental vascular mineralization seen in Vermont, phosphorus, calcium, and occasionally iron were detected but aluminum peaks were not observed, except in barely detectable amounts. Nonmineralized cerebral blood vessels identified in the neurologically intact control cases were free of comparable aluminum deposition.

Intraneuronal accumulation of aluminum has been demonstrated in lumbar motor neurons in patients with ALS (*9*) and in cortical and NFT-bearing hippocampal neurons in patients with senile dementia of the Alzheimer type (*10, 11*). Our observations extend this association to include NFT-bearing hippocampal neurons in patients with ALS and PD of Guam, as well as in the neurologically intact Chamorro. We recognize that studies of the hippocampal neurons may not reflect phenomena associated with pathological changes of significance to either motor neuron or extrapyramidal dysfunction. Nevertheless, the constellation of ALS, PD, and extensive premature NFT formation encountered among the Chamorros of Guam and the Japanese living in the Kii Peninsula may well reflect shared etiological factors. The underlying environmental factors involved in the pathogenesis of ALS and

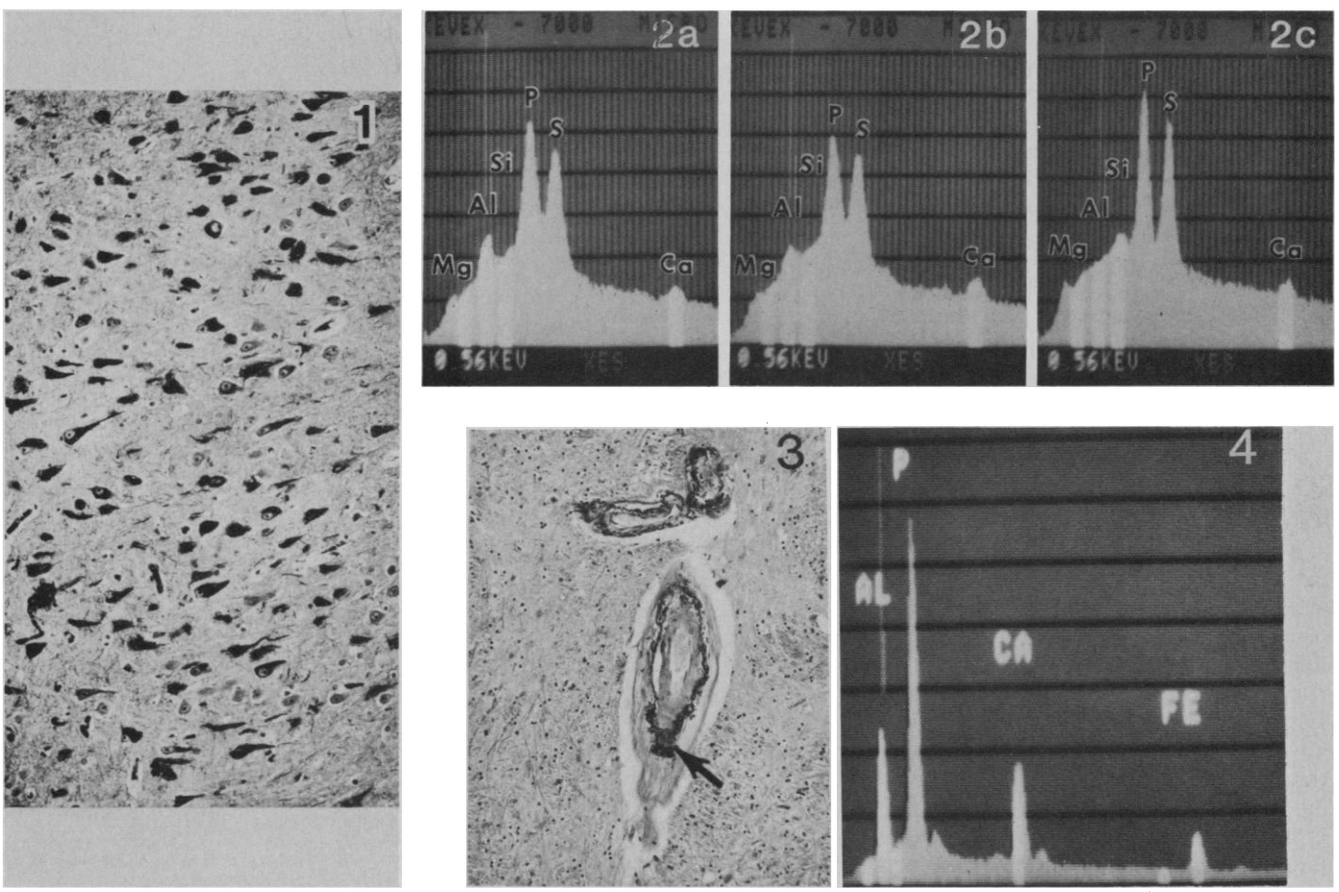

Fig. 1. Bielschowski silver impregnation stain of Sommer's sector (hippocampus) of Guam ALS case showing extensive NFT formation (×200). Fig. 2. Partial energy x-ray spectra of probe sites of NFT-bearing neurons of (a) ALS, (b) PD, and (c) NFT-free control. Note the prominent peaks for aluminum present in (a) and (b) but not in (c). Fig. 3. Hematoxylin-eosin stain of blood vessels of the globus pallidus of a PD case showing (see arrow) vascular mineralization (×200). Fig. 4. Partial energy x-ray spectrum obtained from probe site of mineralized blood vessel of PD case. Note prominent peaks for aluminum, phosphorus, calcium, and iron.

PD in the three genetically and geographically isolated foci remain to be determined. Preliminary analyses of environmental samples indicate that each of these regions have soils and local water supplies that are rich in aluminum but are virtually devoid of calcium and magnesium (*5–7*).

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General Anesthetics Hyperpolarize Neurons in the Vertebrate Central Nervous System

Abstract. *The effect of general anesthetics on frog motoneurons and rat hippocampal pyramidal cells was examined with sucrose gap and intracellular recording, respectively. A number of volatile and intravenous anesthetics directly hyperpolarized the motoneurons. The potency of these agents in hyperpolarizing motoneurons was strongly correlated with their anesthetic potency. While the responses to barbiturates and α-chloralose were blocked by γ-aminobutyric acid antagonists and were dependent on the chloride gradient, the responses to all the other anesthetics tested were generated by a separate mechanism. Intracellular recording from hippocampal pyramidal cells suggested that an increase in potassium conductance accounts for these responses. Such a nonsynaptic action would contribute to the decreased neuronal responsiveness observed for these compounds and thus to their anesthetic action.*

General anesthetics have been reported to have numerous synaptic effects in the central nervous system (CNS). In general, it has been found that synaptic excitation is depressed while synaptic inhibition is preserved or augmented (*1–3*). While physiological studies have focused primarily on synaptic transmission, there is some evidence that anesthetics can have nonsynaptic effects on the postsynaptic membrane. Barbiturates act directly on vertebrate neurons in a manner similar to that of the inhibitory transmitter γ-aminobutyric acid (GABA) (*2–5*). Evidence from studies of invertebrates (*6*), myelinated nerves (*7*), and artificial membranes (*8*) suggests that general anesthetics may increase the permeability of membranes to potassium. However, except for a recent report on ethanol (*9*), such an effect has not been demonstrated for vertebrate CNS neurons. We report here that a number of general anesthetics hyperpolarize central neurons and that this response appears to be due to an increase in potassium permeability.

Thirty-three experiments were performed on isolated frog spinal cords (*10*). Frogs (*Rana pipiens*) were chilled on ice and their spinal cords were removed, hemisected, and placed in a sucrose gap chamber. The activity of motoneurons or primary afferents was recorded by placing the ventral or dorsal root across the sucrose gap, and the potential difference across the gap was monitored with two calomel electrodes. Intracellular recording experiments were also done on CA1 pyramidal cells from rat hippocampal slices (*11*).

Ether caused a slow, dose-dependent hyperpolarization at concentrations similar to that required to induce anesthesia (Fig 1A). The maximum hyperpolarization was modest, rarely exceeding 2 mV. In the same preparations GABA caused large hyperpolarizations (up to 8 mV) and the maximum responses for pentobarbital and α-chloralose approached the size of the GABA response (*4*). Several anesthetics were tested on the frog motoneurons; all were found to cause a hyperpolarization. In Fig. 1B the minimum effective concentration of these anesthetics is graphed against the anesthetic concentration. There is a strong correlation (r = .90) between the hyperpolarizing action and the clinical effect of these agents; furthermore, the concentrations producing these actions are similar to the anesthetic concentrations (*12*). In four preparations the local anesthetic procaine, in concentrations up to 5 mM, did not elicit a hyperpolarization. Xylocaine (5 mM), another local anesthetic, caused a small hyperpolarization (0.7 mV) in two of three preparations.

It has been reported that both barbiturates and α-chloralose have a GABA-like effect on frog motoneurons (*4*). However, neither picrotoxin nor bicuculline, which block the action of GABA, affected the response to ether (Fig. 2A). The responses to the other anesthetics were also insensitive to GABA antagonists. Changing the chloride gradient across the motoneuron either with ammonium chloride, which blocks chloride extrusion (Fig. 2A) (*13*), or by reducing extracellular chloride (Fig. 2A) did not reduce the response to ether but did reduce or abolish the hyperpolarizing response to GABA. These results suggest that ether and the other anesthetics tested do not hyperpolarize motoneurons by increasing chloride conductance. This conclusion is supported by sucrose gap recordings from frog primary afferent fibers. At this site, GABA had a depolarizing action, presumably because of a reversed (depolarizing) chloride gradient (*14*). While pentobarbital (*15*) and GABA depolarized primary afferents, ether had a hyperpolarizing action (Fig. 2B). All the other anesthetics included in Fig. 1B also hyperpolarized primary afferents except for α-chloralose and phenobarbital, which produced depolarizations.

We studied the effects of ether and

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