text-free property of the individual retinal ganglion cell must occur at a later stage of development some time after the original program for spatial organization has been established at the tissue level.

R. K. HUNT
Anatomy Department and Institute of Neurological Sciences, University of Pennsylvania, Philadelphia 19104
MARCUS JACOBSON
Jenkins Department of Biophysics, Johns Hopkins University, Baltimore, Maryland 21218

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6. This is the minimal prediction for the type 1 case, in which no assumptions are made about the rules by which retinal fibers select positions in the tectum. Without knowing these rules one cannot predict, for example, whether the part of the tectum normally receiving fibers from the temporodorsal retina would be vacant in type 1, or the exact location of boundaries between shared and unshared regions of the tectum.
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Brain Aluminum Distribution in Alzheimer's Disease and Experimental Neurofibrillary Degeneration

Abstract. *Neurofibrillary degeneration is an important pathological finding in senile and presenile dementia of the Alzheimer type. Experimentally, aluminum induces neurofibrillary degeneration in neurons of higher mammals. Aluminum concentrations approaching those used experimentally have been found in some regions of the brains of patients with Alzheimer's disease.*

Aluminum is one of the most abundant elements in the earth's crust, and biological systems probably evolved in the presence of appreciable concentrations. Nevertheless, a number of observations indicate that high concentrations of aluminum may be toxic to the nervous system. The brain of an aluminum ball-mill worker, with progressive encephalopathy accompanied by dementia and convulsions, was found to contain 20 times the normal concentration of aluminum (*1*). In experimental animals, aluminum hydroxide applied to cerebral tissues induced epileptogenic foci (*2*), and subarachnoid injection of trace amounts of salts of aluminum resulted in a progressive encephalopathy characterized by a unique cellular change, that of neurofibrillary degeneration (NFD) (*3*). A similar but not identical cellular change is one of the hallmarks of Alzheimer's disease, a disease occurring after the age of 40 and producing progressive dementia (*4*, *5*).

Work in our laboratory has shown that, in the early stages of an aluminum chloride–induced encephalopathy in cats, an apparently selective impairment in short-term memory and associative learning preceded the appearance of focal neurological signs (*6*). The decline in higher nervous functions resembled in part those noted in the human conditions of presenile and senile dementia of the Alzheimer type, although the time course was shorter.

To appraise further the role of aluminum in this animal model of a human dementia, a study of the aluminum concentration in cat brain with acquisition defects was undertaken in order to establish the tissue concentration at which morphological and behavioral changes occurred. In addition, the aluminum content of the brain has been determined in humans with Alzheimer's disease. We now report the results of these studies.

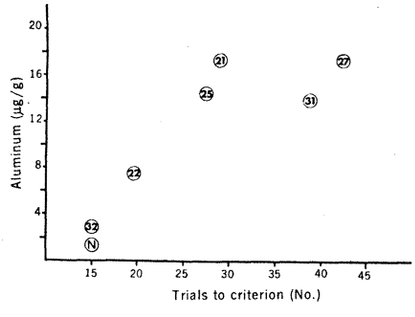

Fig. 1. Relation between acquisition of a conditioned avoidance task and brain aluminum concentrations [μg/g (dry weight)].

Eighteen cats (2.5 to 3.5 kg) were given 150 to 225 μg of aluminum (as aluminum chloride) which was injected into the ventral hippocampus, internal capsule, or cisterna cerebellomedularis (*6*). In six aluminum-treated animals behavioral testing was carried out in a one-way avoidance response acquisition task (*6*). Acquisition performance was measured on three consecutive days, beginning on day 9 after injection of aluminum chloride. These animals were killed between day 11 and day 18, and the brains were bisected in the sagittal plane. One-half of the brain was prepared for histology and stained for NFD by the method of Bielschowsky. The other half was prepared for aluminum assay. Special precautions were taken to prevent aluminum contamination of tissue during necropsy.

Human tissue was collected from deceased patients in which the clinical history, physical examination, and pneumoencephalogram were compatible with the diagnosis of Alzheimer's disease. Pathological examination of a portion of brain revealed brain atrophy, extensive neurofibrillary degeneration, senile plaques, and hippocampal pyramidal cell granulovacular degeneration. Removal of tissue was supervised to minimize possible aluminum contamination.

Aluminum was assayed by the atomic absorption method (*7*). By this technique as little as 0.1 μg/ml can be detected. The only measurements excluded from this report were from samples in which (i) all of residue could not be redissolved (three cat samples and one human sample) and (ii) the signal-to-noise ratio was less than 2 in the spectrophotometer (one human sample).

In our initial experiments we found that aluminum was not homogeneously distributed in the tissue. In order to establish that the variation was of biological origin and not due to lack of precision of the analytical technique, repeated assays were made for aluminum in homogenized brain and liver. The aluminum content was found to be reproducible in different samples, within 10 percent, even at the level of 1 μg/g (dry weight).

The aluminum concentration in several regions of the feline brain is listed in Table 1. The samples denoted frontal and occipital poles and were largely cor-

Table 1. Aluminum concentrations in regions of brains from normal and aluminum-treated cats.

Cat No.	Days after Al	Al injected (μg)	Al concentration [μg/g (dry weight)] in tissue				Region
			Frontal	Occipital	Central white	Other	
			Normal				
36			0.7	0.8	2.6		
38			2.7	0.6	1.5	0.6	Cortex
39			1.9	2.6		2.4	Cortex
						1.5	Cerebellum
40			0.8	1.4	1.0	1.5	Cortex
			1.5	2.1			
Mean			1.5	1.5	1.7	1.5	
S.D.			.8	.8	.8	.7	
			Aluminum injected				
2	4	225	13.7				
11	6	225	4.6	11.2	11.6	13.6	Hippocampus
12	6	225	6.8	8.0		38.7	Brainstem
15	32	150	32.2	42.3		11.7	Cerebellum
21	18	180	17.6			14.6	Spinal cord
22	18	180	9.1	5.9		43.5*	Hippocampus
25	15	210	20.4	8.5		39.7*	Hippocampus
26	14	210	14.1	13.4	22.2		
27	11	210	17.7	17.4	38.0*		
28	10	210	9.0	12.5	12.6	67.9*	Central (gray and white)
31	12	210	26.1	1.2	62.8*		
32	11	210	3.2	1.7	140.1*	4.8	Hippocampus
1160	1	210	13.2	24.2			
Mean			14.4	13.2			

* Sample site in region of injection.

Table 2. Aluminum concentration in histologically verified Alzheimer's disease in patients H.M., L.T., and C.H. Patients N.N., R.A., and L.M. are normal age controls.

Patient	Age	Source	Al [μg/g (dry weight)]
		Biopsy Alzheimer's disease	
H.M.	67	Cortical biopsy	11.5
L.T.	64	Cortical biopsy	9.9
		Cerebrospinal fluid	0.12*
		Perfusion media	0.06*
		Necropsy Alzheimer's disease	
L.T.	64	Mesial frontal and temporal cortex	9.4, 9.1, 6.8, 6.8, 6.6, 6.3, 6.3, 5.4, 5.4, 4.8, 4.2, 4.1
		Other cortical regions	3.7, 3.2, 3.1, 3.1, 3.0, 3.0, 2.6, 2.6, 2.2, 1.8, 1.7, 1.4, 1.3, 1.1, 1.1, 1.1, 1.0, 0.9
		Optic nerve	1.8
		Brachium conjunctivum	0.8, 0.7, 0.9
C.H.	88	Mesial temporal and frontal cortex	9.7, 9.7, 7.2, 6.1, 5.9
		Lateral frontal, temporal, and parietal cortex	4.8, 4.6, 4.2, 4.2, 3.6, 3.3, 2.1, 2.1, 1.9, 1.9, 1.5, 1.3, 1.3, 0.63, 0.54
		Occipital cortex	1.4, 1.4
		Thalamus	0.62, 2.1
		Central white	3.6
		Medulla: central gray	5.8
A.S.	85	Parahippocampal gyrus	8.4
		Medulla: central gray	4.0, 3.8
		Other cortical regions	2.8, 2.3, 1.7, 1.6
		Necropsy normal brain	
N.N.	64	Cortex	2.7, 2.3, 1.2, 0.5, 0.23, 0.46
		Subcortical white	1.1, 0.6
		Mean	1.1
R.A.	45	Mesial temporal and frontal cortex	2.5, 2.4, 2.3
L.M.	54	Mesial temporal cortex	0.53, 0.62, 1.1
		Mesial and orbital frontal cortex	0.43, 1.0, 1.1

* Wet weight (μg/g).

tical gray matter; but some white matter could not be completely excluded. The aluminum values in four normal cats range from 0.6 to 2.7 μg/g (dry weight) with a mean of 1.5 μg/g. In 13 brains, after aluminum injection, the concentration in frontal pole gray matter ranged from 3.2 to 32.2 μg/g (dry weight) with an average of 14.4 μg/g. In the occipital pole a mean value of 13.2 μg/g was obtained.

The concentration of aluminum in the stereotactic target regions of injection are labeled by asterisks. Only in two animals, cat 31 and cat 32, was aluminum chloride infused into the internal capsule. Animal 32 had 140 μg/g in the region of injection, normal concentrations in the occipital region, and slightly elevated concentrations in the frontal cortex. Animal 31 had high concentrations in the frontal cortex and normal concentrations in the occipital cortex. Injection into central white matter appears to retard the widespread diffusion of aluminum. As noted in Table 1, there was no apparent relation between the concentration of aluminum in the tissue and the time after injection in the interval between 1 and 32 days.

Six treated cats performed in a one-way conditioned avoidance response acquisition task. In the graph of Fig. 1, the average concentration of aluminum in the cerebral cortex is plotted in relation to the number of trials required to reach the criterion of 15 consecutive avoidances. The numbers within the circles refer to the experimental number assigned to the cat for which aluminum levels and trials to criterion were obtained. The mean aluminum concentration in the cortex in four normal animals was 1.5 μg and the mean number of trials to criterion for these four animals was 15.0. (*N* in Fig. 1). The average number of trials to criterion for 16 other normal animals was 16.6. Animal 32 had normal performance and normal aluminum concentrations in the cortex. Animal 22 required 20 trials to reach criterion, and the mean concentration of aluminum in the cortex was 7.5 μg/g. Animals 21, 25, 27, and 31 required 28 to 42 trials to reach criterion and the average concentration of aluminum in the cortex exceeded 12 μg/g. It is evident that, for the short-term experimental aluminum encephalopathy, aluminum concentrations in excess of ten times normal are associated with slow acquisition of the avoidance task.

We have shown that a relation also exists between the number and distribution of neurons with NFD and performance in the acquisition task (*6*). Histological examination for the regional distribution of NFD in animals whose cortical aluminum concentrations are shown in Fig. 1 suggests a relation between the concentration of aluminum and the density of NFD. Animal 32 (Table 1 and Fig. 1) had extensive NFD only in regions adjacent to the site of injection, and no NFD was noted in the cerebral cortex. Animal 21 had extensive NFD in the entorhinal cortex, the hippocampus, and scattered lesions in neocortex. Animal 27 had extensive NFD in all neocortical regions, entorhinal cortex, hippocampus, and brainstem. This animal had an average frontal and cortical concentration of aluminum of 17.5 μg and the slowest acquisition rate of 42 trials to criterion. Animal 31 had no NFD in the cortex of the occipital pole, but large numbers of lesions in cortex of the frontal pole and entorhinal region. Aluminum concentrations were 26.1 and 1.2 μg/g in frontal and occipital poles, respectively. Although effects of aluminum on brain structures other than cerebral cortex may alter acquisition performance, these data suggest that cortical tissue concentrations above 12 μg/g (dry weight) in the cat are associated with extensive NFD and profound alterations in higher nervous functions.

In brains of humans with Alzheimer's disease (Table 2), aluminum concentrations approach 12 μg/g in some regions. Biopsy material from the brains of patients H.M. and L.T. contained 11.5 and 9.9 μg/g (dry weight) of aluminum, respectively. The usual light and electron microscopic configuration of human NFD (*4*) was present in tissue from cortical regions near the assay site. The aluminum content of the surgical perfusion media to which the biopsy material might have been exposed in patient L.T. contained 0.06 μg/ml. The cerebral spinal fluid of this patient contained 0.12 μg/ml. When the patient (L.T.) died several months later, postmortem examination of the brain confirmed increased aluminum concentration, and 9.4 and 6.3 μg/g were found in several frontal and temporal cortical regions. A wide range of concentrations were encountered; several samples had values between 3.0 and 5.4 μg/g and some were in the normal range of 0.7 to 2.6 μg/g. Patient C.H., aged 88, had a particularly high density of NFD in one parahippocampal gyrus. In the contralateral parahippocampal gyrus, 9.7 μg/g of aluminum was present. In an atrophic gyrus of frontal lobe a value of 4.8 μg/g was noted, and in some regions of the occipital cortex only 1.4 μg/g. Patient A.S., aged 85, had low concentrations of aluminum in most cortical regions, but the parahippocampal gyrus contained 8.4 μg/g.

Concentrations of aluminum in normal human brains are also shown in Table 2. Patient N.N., aged 64, died suddenly from myocardial infarction and had no evidence of intellectual impairment. The mean value of aluminum in that brain was 1.1 μg/g, with a range of 0.23 to 2.7 μg/g (dry weight). Patients R.A. and L.M. had a range of 2.5 to 0.4 μg/g (dry weight). These normal values correspond to those reported by McLaughlin *et al.* (*1*) in which spectrographic analysis of eight normal brains revealed less than 3 μg/g (dry weight).

A characteristic pathological finding in Alzheimer's disease is the patchy distribution of NFD. A similar distribution for the pathogenic agent might be expected, and the variation in aluminum concentration in cortical regions is noteworthy. The density of NFD in the experimental model is often very much higher than that encountered in Alzheimer's disease. Nevertheless, the surprisingly high tissue concentration of 9 to 11 μg/g in some regions of brain in Alzheimer's disease, compared to the average values of 14 μg/g in cat brain with experimental NFD, suggests that aluminum may be a neurotoxic factor in the human disease. However, the atomic absorption method measures the total amount of aluminum, and the possibility that this element is bound in a nontoxic form cannot be excluded at this time. It will be necessary to identify the tissue binding sites in both experimental NFD and Alzheimer's disease to further establish the role of aluminum in the pathogenesis of the disease. In addition, not all of the pathological changes in Alzheimer's disease are readily explained by these findings and our study does not exclude the possibility of other etiological factors.

D. R. CRAPPER
Departments of Physiology and *Medicine, University of Toronto, Toronto 5, Ontario, Canada*
S. S. KRISHNAN
Departments of Physiology and *Medicine, University of Toronto*, and *Center for Forensic Sciences, Toronto*
A. J. DALTON
Department of Psychology, Mental Retardation Center, Toronto

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Heart Activity and High-Pressure Circulation in Cirripedia

Abstract. *Pulsating hemolymph pressures of remarkable magnitude for invertebrates are prevalent in the Pacific gooseneck barnacle. Mean pressures of 250 centimeters of water are common with pulse pressures up to 70 centimeters of water. The pulsations are distinctly rhythmical and the pulsation rate is highly temperature-dependent. The results strongly suggest that in cirripeds hemolymph is circulated by muscular contractions of a functional heart.*

The most familiar members of the crustacean order Cirripedia are the acorn barnacles and the gooseneck barnacles. Hemolymph circulation in Cirripedia is not well understood and ideas concerning its occurrence are controversial. Present views include the notions that a circulatory system is completely lacking (*1*), that a unidirectional flow of hemolymph is set up by contractions of the body musculature (*2*, *3*), and that an organized