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Effect of Aluminum upon Conditioned Avoidance Response Acquisition in the Absence of Neurofibrillary Degeneration

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KING, G. A., U. DE BONI AND D. R. CRAPPER. *Effect of aluminum upon conditioned avoidance response acquisition in the absence of neurofibrillary degeneration.* PHARMAC. BIOCHEM. BEHAV. 3(6) 1003–1009, 1975. – Aluminum induces neurofibrillary degeneration in cats but not rats. Cats develop a progressive encephalopathy in which an early manifestation is impaired learning-memory performance. At brain aluminum concentrations of 5 to 6 times that found in cat, rats demonstrate an initial transient weight loss and acquisition deficit immediately following intracranial injection. However, rats do not develop a progressive encephalopathy or a chronic learning deficit.

Aluminum Acquisition Conditioned avoidance response Neurofibrillary degeneration

SOLUTIONS of aluminum salts, injected intracranially in the cat, produce a progressive encephalopathy associated with an increased number of neurofilaments and loss of neurotubules in susceptible neurons [5, 11]. Deficits in retention and acquisition have been observed in the early stages of the encephalopathy [4], and are correlated both with the extent of neurofibrillary degeneration (NFD) in the neo- and entorhinal cortex [5], and with the brain aluminum concentration (B. A. C.) [6]. This laboratory previously postulated that NFD may be responsible for the impairment of learning and memory in the cat [4, 5].

To further test this hypothesis we examined the effects of intracranial injection of aluminum chloride solutions on the acquisition of a one-way avoidance response in 2 different strains of rat, in 3 experiments. The rat was chosen because previous unpublished investigations (H. Wiśniewski, D. R. Crapper) have shown that this species does not develop light microscopic evidence of NFD in response to intracranial injections of aluminum.

METHOD

Animals

Experiment 1. Thirty male hooded rats, weighing between 229–308 g, were obtained from Canadian Biological Laboratories, and were housed in individual cages with food and water available ad lib until 24 hr prior to surgery. They were kept on an alternating 12 hour light–12 hour dark cycle, and were weighed and handled each day.

Experiment 2. Twelve male wistar rats weighing between 280–324 g were obtained from Canadian Biological Laboratories. These animals were housed and treated in the same way as those in the first experiment.

Experiment 3. Thirty-two male hooded rats weighing between 216–248 g were obtained from Bio-Breeding Laboratories. These animals were handled in a manner identical to those in the first experiment except that they were not food deprived prior to surgery.

Apparatus

In all 3 experiments the test chamber was a Lehigh Valley shuttle box 20 × 45 × 19 cm, with a tilt floor constructed of 1/16 in. steel rod spaced 11 mm center-to-center. This box was divided into 2 equal compartments by a metal partition, which had a 7.5 × 12.5 cm guillotine door. Apart from the clear Plexiglas panel facing the experimenter one compartment was black and the other white. White noise at 78 dB, measured inside the chamber, was present during habituation and testing.

Solutions for Injection

Experiment 1. Aluminum, as $AlCl_3$, was injected in 2 concentrations: 0.25 M and 0.125 M at pH 3.0 in artificial C. S. F. (Ames Medium). The control solution was artificial C. S. F. adjusted to pH 3.0 by the addition of HCl. A total of 6 μl of one of these solutions was injected bilaterally into the ventral hippocampus of each animal (stereotaxic

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coordinates: P 2.8 mm from Bregma, L 4.8 mm, and 8.5 mm below the surface of the brain) [14], under pentobarbital anesthesia (50 mg/kg IP). A Hamilton microliter syringe No. 701 was used for intracranial injection. Ten animals each were injected with 1 of 3 solutions: Group 1 – C. S. F. Control, Group 2 – low [Al], Group III – high [Al].

Testing began on the ninth day postinjection for all animals. This delay between injection and testing is the same as that employed in the cat experiment (4).

Experiment 2. Six rats each were injected, as in Experiment 1, with 6 μl of an 0.25 M solution of $AlCl_3$ (group Al_W) or C. S. F. adjusted to pH 3 (group C_W). All animals were tested 9 days postoperatively.

Experiment 3. Only the 0.25 M $AlCl_3$, and C. S. F. control solutions were employed. Utilizing the same procedure, volume, and locus of injection as in the first experiment, 16 animals were injected with the aluminum solution, and 16 with the C. S. F. Group Al_1, consisting of 8 aluminum injected rats, and group C_1 consisting of 8 controls, began acquisition training on the first day postinjection. The remaining 8 aluminum injected and 8 control rats, group Al_9 and group C_9 respectively, commenced testing on the ninth day postinjection.

Test Procedure

On the first day of testing the animals were placed in the shuttle box with the guillotine door removed and allowed to explore. After 15 min the door was closed, the animal was placed in the black compartment, and mock trials commenced. Each mock trial was initiated by raising the guillotine door, which turned on the conditioned stimulus (C.S.), a white noice chopped at 10/sec. If the animal did not cross to the opposite side after 30 sec, the C. S. was terminated and the door closed. If the animal did cross to the other side the trial was automatically terminated and the door was closed. After 30 sec, the rat was returned to the black compartment. These trials were continued until the animals had made no crossings for 5 trials in succession.

Twenty-five training trials were given on the first day and 30 each on the second and third days. Each trial was initiated by the experimenter, who raised the guillotine door. If the rat did not cross to the opposite side within 10 sec, a 0.8 mA electric current was delivered through the floor rods, from a Grayson Stadler Model 700 shock scrambler, until the animal crossed and terminated both the C. S. and the shock. The door was lowered and the rat was allowed 30 sec in the safe compartment. The intertrial-interval was 15 sec. Each trial was registered as either an escape or an avoidance. Trials to criteria of 1, 5, and 10 avoidances in succession, and the number of escapes made each day were used as measures of performance.

After the last trial on the third day of testing each animal was anesthetized with pentobarbital, the skull was opened and the brain was bisected in the saggital plane. One-half was taken for histology and one-half for aluminum assay.

The above procedure was followed for all experiments with this exception: animals in Experiment 2 were tested for only 1 day (Day 9 postinjection) at the end of testing on that day they were sacrificed and B. A. C. was determined for 3 of the animals in group Al_W. The brains of the other 3 animals were taken for enzyme analysis (results not reported here).

Histological Methods

Histological examination was undertaken for animals in Experiment 1. Portions of neocortex, hippocampus, and midbrain were fixed in a 4 percent glutaraldehyde and processed for electron microscopy. The remainder of the brain was fixed in 10 percent Formalin, embedded in paraffin, and sectioned at 5 μ. Sections near the locus of injection were stained with haematoxilin and eosin (H. and E.). The Bielchowsky silver stain was employed to detect NFD.

Aluminum Assay

B. A. C. in the hemisphere anterior to the colliculi was measured by a modification of an atomic absorption method [11] employing a carbon furnace (Perkin Elmer Model 305A).

The hemispheres were divided into 3 portions. For animals in Experiments 1 and 2, the concentration of aluminum reported was taken as a weighted average of 6 assays, 2 each from the 3 portions of the cerebral hemisphere. In Experiment 3 one assay was taken from an homogenate of each portion, and the total brain concentration obtained as an unweighted average of 3 assays.

EXPERIMENT 1

RESULTS

Unlike several higher mammalian species, rats do not develop signs of a progressive encephalopathy following brain aluminum application. Doses greater than those employed in the present study also did not produce the chronic neurological signs usually seen in cat, rabbit or guinea pig. Furthermore, animals observed for 12 months postinjection failed to develop motor signs of an encephalopathy.

Brain Aluminum Content

The average normal brain aluminum concentration in 3 saline injected rats was 3.5 ± .61 μg/g dry weight. Aluminum assay in 13 injected animals revealed concentrations ranging from 2.5 to 40.8 μg/g dry weight, (Table 1). The average concentration, in Group 3, 11 days after injection was 22.3 ± 14.3 μg/g. For Group 2 the average concentration was 10.1 ± 6.9 μg/g dry weight.

Three aluminum injected rats, 1 from Group 2 and 2 from Group 3, and 1 C. S. F. injected control, all of whom were rejected from the acquisition study (see below), were allowed to survive for 12 months postinjection. These animals were then sacrificed along with 3 other noninjected rats of the same age, and all brains were analysed for B. A. C. The 3 Al injected animals had an average B. A. C. of 2.03 μg/g brain (S. D. 1.31), and the mean for the 3 normal and 1 injected controls was 0.96 μg/g (S. D. 0.46). It should be noted that the mean concentrations for the 3 aluminum injected animals sacrificed 12 months postinjection is much less than that of either of the aluminum injected groups, sacrificed on the eleventh day postinjection.

Histology

Detailed examination of 27 Bielchowsky and H. and E. stained slides of 7 Al injected rats and 4 C. S. F. controls failed to reveal evidence of NFD. Also, no evidence of NFD was found in tissue examined in the electron microscope.

TABLE 1

PERFORMANCE SCORES AND BRAIN ALUMINUM CONCENTRATION

N	(Al) μg/g Dry Weight	Number of Trials to: 5 Avoidances	10 Avoidances	Number of Escapes Day 1	NFD
7†	40.81	26	38	17	none
23†	38.70	30	72	20	none
45†	24.18	37	37	19	none
6*	19.29	19	31	17	
29*	19.21	19	41	10	none
9†	17.36	16	16	11	none
43†	13.97	4	4	8	none
26*	12.07	9	9	8	
22*	7.25	16	45	14	
36*	5.17	17	35	11	
42*	5.15	20	26	9	
32†	4.28	9	9	8	none
35*	2.48	7	27	7	
		r = 0.65	r = 0.49	r = 0.76	
		($p<0.02$)	($p>0.05$)	($p<0.01$)	

*Group 2 †Group 3 N = animal number

Specifically, tissue from Animal 7, which had 40.8 μg Al/g dry weight in the contralateral hemisphere, was examined in detail and exhibited no alteration in ultrastructure.

Avoidance Acquisition

Of 30 animals, 3 in Group 1, 1 in Group 2, and 4 in Group 3 were excluded from the study: 6 were aggressive and could not be handled; 1 learned to avoid shock without terminating the trial and 1 did not reach a criterion of 5 avoidances in a row. The data reported is from 22 animals.

In general, aluminum injected rats made fewer avoidances and required more trials to reach criteria than controls. However, large within group variation obscured these differences. Table 2 (upper part) gives the means and standard deviations for trials to criteria of 1, 5, and 10 avoidances in a row. Analysis of variance with unweighted means [16] revealed that the differences between groups were not significant, $F(2,19) = 1.66$, $p>0.25$. The means and standard deviations of the number of escapes made by each group on each day are given in Table 2 (lower part). Again, analysis of variance showed the intergroup differences not to be significant, $F(2,19) = 2.12$, $p>0.25$.

An examination of Table 1 indicates that the independent variable, B. A. C., was not completely controlled. There is a wide range of values, and 5 animals had concentrations less than 10 μg/g dry brain weight. To test for a correlation between performance and B. A. C. a rank order correlation was performed between B. A. C. and trials to 5 and 10 avoidances in a row, and the number of escapes on Days 1, 2 and 3 [10]. There is a significant positive correlation between B. A. C. and trials to 5 avoidances in a row, and the number of escapes on Day 1. Individual scores and correlation coefficients are given in Table 1. Correlations with the number of escapes on Days 2 and 3 were not significant ($r = 0.52$, $p>0.05$; and $r = -0.22$, $p>0.1$ respectively).

DISCUSSION

The significant correlation between B. A. C. and the number of trials to 5 avoidances in a row, and the number of escapes on Day 1, indicates that aluminum may have a deleterious effect upon acquisition of a conditioned avoid-

TABLE 2

MEAN TRIALS TO CRITERIA AND ESCAPES

	N	Trials 1	5	10	Days 1	2	3
Group 1	6	6.00 (2.83)	8.13 (3.31)	13.14 (11.29)	7.86 (2.91)	3.00 (1.91)	3.85 (2.12)
Group 2	9	4.89 (1.54)	13.44 (5.53)	25.56 (14.34)	10.33 (3.24)	5.78 (5.91)	4.55 (6.48)
Group 3	7	7.83 (4.07)	20.33 (12.87)	29.33 (25.25)	13.83 (5.49)	4.83 (3.76)	4.17 (3.19)

Standard deviation in parentheses

TABLE 3

MEANS AND SD's OF VARIABLES MEASURED FOR GROUPS IN EXPERIMENT 3

Variable	Group			
	Al_1 (n = 8)	C_1 (n = 8)	Al_9 (n = 8)	C_9 (n = 6)
B.A.C. (μg/g)	47.5 ± 18.9	4.4 ± 2.0	34.1 ± 9.3	4.1 ± 1.4
Body weight loss: Day 1 postinjection (g)	18.38 ± 7.01	4.75 ± 5.50	17.25 ± 8.29	3.86 ± 6.08
No. of trials to:				
1 avoidance	5.88 ± 2.03	3.63 ± 0.92	3.86 ± 1.25	2.50 ± 0.84
5 avoidances	17.75 ± 11.89	4.75 ± 1.75	5.50 ± 2.00	4.17 ± 1.72
10 avoidances	27.00 ± 9.61	11.63 ± 13.46	6.75 ± 3.88	5.83 ± 4.79
No. of escapes on:				
Day 1	12.50 ± 4.18	4.63 ± 1.60	4.88 ± 1.36	4.17 ± 1.72
Day 2	2.75 ± 1.83	1.63 ± 0.74	1.38 ± 0.52	1.50 ± 0.55
Day 3	1.75 ± 1.83	1.25 ± 0.46	0.88 ± 0.64	1.00 ± 0.63

ance response. Since the massive amounts of aluminum injected intracranially in these animals did not produce NFD the cause of any deficit resulting from aluminum injection must be sought elsewhere. The second experiment in this series was conceived as an investigation of the possible effects of aluminum on a number of brain enzymes. This follow-up study, however, brought into question conclusions drawn from the first experiment.

EXPERIMENT 2

The only discernable effect of the aluminum injection in the first experiment had been the correlations between B. A. C. and measures of poor performance on Day 1 of acquisition (only 3 of 22 animals failed to achieve 5 avoidances in a row on Day 1). Therefore, it was decided to test the animals in this experiment on Day 1 only, using total number of escapes as the measure of performance.

RESULTS

One aluminum injected animal was discarded from the study because of haematuria. All other Al injected animals appeared healthy and displayed no gross neurological symptoms. Group C_W, with a mean of 15 escapes (S. D. = 5.22), displayed poorer performance than group Al_W ($\bar{X}$ = 10, S. D. = 3.24). These differences are not statistically significant, $F(1,9) = 3.62$.

Three animals in group Al_W had B. A. C.'s of 71.5 μg/g, 50.4 μg/g, and 9.7 μg/g. The mean B. A. C. for 6 rats in group C_W was 2.72 μg/g ± 0.86.

The results of this experiment do not support the hypothesis that intracranial injection of aluminum interferes with acquisition of a C. A. R.

EXPERIMENT 3

To replicate and extend the findings of Experiment 1, hooded rats were tested for their ability to acquire a C. A. R. the day after intracranial injection as well as 9 days postinjection. Animals were tested 1 day after injection in order to ascertain whether deficits which do exist may not occur immediately and be ameliorated with time. Also, more subtle effects of Al injections, which might also be correlated with performance deficits, were looked for: specifically daily weight changes were recorded and closely examined for all animals.

RESULTS

As in Experiment 1, no gross neurological impairments were seen in any animal injected with $AlCl_3$. However, an effect on weight regulation which was not noticed in Experiment 1 became evident in this experiment.

B. A. C.

The B. A. C. of animals in groups Al_1 and C_1 were measured after 3 days postinjection while the B. A. C. of animals in groups Al_9 and C_9 were determined 11 days following injection. The average concentration for each group is given in Table 3. Although there is a large within group variation, the aluminum injected rats have a much

higher average concentration than the C. S. F. injected controls.

Weight Changes

On the average both the C. S. F. controls and the aluminum injected rats lost weight on the day after injection. The mean body weight loss for each group is recorded in Table 3. The difference in the amount of weight lost on the Day 1 postinjection between the aluminum injected animals and the controls was shown to be significant by an analysis of variance, $F(1,26) = 30.5$, $p<0.01$. Animals in Group Al_9 which had a mean weight loss of 18.38 g ± 7.01 on Day 1 postinjection, continued to gain weight on subsequent days at the same rate as animals in Group C_9. The correlation between B. A. C. and the amount of weight lost on Day 1 postinjection is −0.28 for Group Al_1 and −0.35 for group Al_9, neither correlation is statistically significant.

Acquisition

Two animals were excluded from Group C_9; 1 was accidently sacrificed on Day 3 postinjection, and 1 rat displayed an unconditioned crossing response to the C. S. during the mock trials which did not habituate or extinguish.

In general, animals injected with $AlCl_3$ and tested 1 day postinjection made more escapes and took longer to reach criterion performance than either the C. S. F. injected controls tested 1 day postinjection or the $AlCl_3$ injected rats which were tested 9 days postinjection. The aluminum injected animals tested 9 days postinjection were indistinguishable from the corresponding C. S. F. controls in their ability to acquire the C. A. R.

The means and standard deviations of the number of trials to reach criteria for each group are given in Table 3. Due to a large inhomogeneity of variance, analysis of variance was performed on a square root transformation of the raw data. A significant 3-way interaction effect of substance injected by day postinjection by criterion level, $F(2,112) = 6.55$, $p<0.01$, necessitated comparisons of individual mean scores for each group. A Newman-Keuls procedure was used for this purpose [16], the significance level chosen for these tests was $p<0.01$. Comparisons between groups at each criteria level demonstrated that Group Al_1 required more trials to reach criteria of 5 and 10 avoidances in succession than either Group Al_9 or Group C_1. However, Group Al_1 is not significantly different from either Al_9 or C_1 in the number of trials prior to the first avoidance response. Other intergroup comparisons revealed no differences between the performance of Group Al_9 and Group C_9, or between that of Group C_1 and Group C_9 at any criterion level. Group C_9 and Group Al_9 did not require significantly more trials to reach a criterion of 10 avoidances in a row than was necessary to make the first avoidance. The number of trials prior to reaching a criterion level of 10 avoidances in a row is significantly different from the number of trials prior to the first avoidance for Group C_1. However, there is no significant difference between the number of trials to 5 avoidances in succession and either the number prior to the first avoidance or the number of trials preceeding 10 avoidances in a row. The differences between the number of trials to reach each of the 3 criteria levels for Group Al_1 are statistically significant.

The means and standard deviations of the number of escapes made by each group on each day are given in Table 3. Analysis of variance was performed on square root transformations of this data. There is a significant 3-way interaction effect of substance injected by day postinjection by test day, $F(2,112) = 9.22$, $p<0.01$. Again, a Newman-Keuls procedure was used to compare individual mean scores, a $p<0.01$ significance criterion was chosen for these tests. In intergroup comparisons Group Al_1 made significantly more escapes on Day 1 of acquisition than either Group Al_9 or Group C_1. Group Al_1 did not make significantly more escapes than either of these 2 groups on the 2 remaining days. Comparisons between the means of Group C_1 and Group C_9 and between those of Group C_9 and Group Al_9 did not reveal any significant differences on any test day. All 4 groups made significantly more escapes on Day 1 than on Day 2; however, none of the groups showed significant differences between the number of escapes made on Days 2 and 3. Thus, animals injected with $AlCl_3$ and tested on the first day postinjection were slower in acquiring a C. A. R. than either injected controls tested 1 day postinjection or aluminum injected rats tested 9 days postinjection. This latter group did not differ significantly from the controls in their ability to acquire a C. A. R. The deficit of Group Al_1 appears to be due to a failure to make avoidances on Day 1.

In order to attempt to account for the poor performance of Group Al_1 correlations were tested for between B. A. C. and the number of escapes on Day 1, and the number of trials to a criteria of 5 avoidances in a row, and 10 avoidances in a row. Correlations between weight loss on Day 1 postinjection and these measures of performance were also determined. Only the correlation between weight loss and the number of escapes on Day 1 is significant ($r = 0.77$, $n = 8$, $p<0.05$) [10].

DISCUSSION

The results of this experiment do not support the hypothesis that intracranial injection of aluminum will induce a slowly developing impairment in the ability of the rat to acquire a C. A. R., as it does in the cat. In agreement with the results of Experiment 1 and 2 there was no significant impairment of the ability of the rat injected with aluminum to acquire a C. A. R. 9 days later. However, unlike the results of Experiment 1 there was no tendency for the aluminum injected animals tested on Day 9 to perform below the level of the control group, and there were no correlations between B. A. C. and the performance measures.

The acquisition deficit seen in the aluminum injected rats, tested on Day 1, is obviously different in its time course from that observed in cats [4]. It represents an acute effect of a massive injection of $AlCl_3$ and is probably unrelated to the mechanism which produces the delayed deterioration of mnemonic function in the cat.

The absence of a correlation between B. A. C. and the degree of acquisition impairment, in Group Al_1 animals, does not rule out the possibility that the slow learning exhibited by these animals is due to a transient disruption of normal brain function produced by aluminum. In vitro, the replacement of calcium by aluminum, at concentrations as low as 10^{-5} M, in the external medium causes a large increase in axonal membrane resistance, which severely impairs the axon's ability to conduct action potentials [1].

Furthermore, 10^{-4} M aluminum has been found to double the activity of bovine erythrocyte acetylcholinesterase in vitro [12]. Either of these effects, if present in vivo, could disrupt normal brain function.

The weight loss suffered by all aluminum injected animals is difficult to explain. However, a disruption of brain function, as postulated above, might result in the alteration of food intake and/or metabolism. Certainly, a large loss of body weight would prove stressful and could have a deleterious effect on performance. Although this hypothesis is supported by the positive correlation between the amount of weight lost and the number of escapes on Day 1 for Group Al_1, the evidence is not conclusive.

GENERAL DISCUSSION

At this time there is no evidence to support the hypothesis that the impairment of learning and memory induced by intracranial aluminum injection can occur in the absence of neurofibrillary degeneration. Previous studies employing cats have shown that the degree of impairment following aluminum injection is correlated with the extent of NFD, occurring in the neo- and entorhinal cortex [5]. In the present experiments no conclusive evidence of a chronic learning deficit following aluminum injection in the rat has been found. Even at brain concentrations of 5 to 6 times that found in the cat [6], aluminum injected rats did not display evidence of NFD or of more than a transient depression in their ability to acquire a C. A. R.

The functional basis of this species difference is unknown at the present time. The rat appears to lose intracranial injected aluminum at a much faster rate than cat. In Experiment 3, Group Al_1, sacrificed 3 days postinjection, has a somewhat higher B. A. C. than Group Al_9, sacrificed 11 days postinjection (Table 3). Also, aluminum injected rats allowed to survive for 12 months after injection had a mean B. A. C. which was only 2 times that of controls, and much less than animals sacrificed 11 days postinjection. In comparison, a cat which survived aluminum injection for 3 years had a mean concentration of 4.7 μg/g $\pm$ 1.7 (mean of 15 samples). This is more than 3 times the normal value of 1.4 μg/g $\pm$ 0.5 (mean of 23 samples from 4 cats). While 2 cats injected with the same concentration, 10 μM/brain, and sacrificed 14 days postinjection had mean B. A. C.'s of 6.9 μg/g $\pm$ 4.0 (mean of 32 samples) and 8.4 μg/g $\pm$ 6.3 (mean of 31 samples) [7]. Although the last 2 values have very large S.D.'s compared to that for the chronic cat, this only reflects the patchy distribution of injected aluminum [7]. The metal may become more evenly distributed with time, and in any case, probably less than half the injected aluminum was lost from the cat brain between 14 days and 3 years postinjection. Nevertheless, the rapid loss of brain aluminum in rat does not explain the resistance of this species to large amounts of the metal.

Recent histochemical investigations have revealed that in a variety of plant and animal cells aluminum binds to chromatin preferentially [8]. Chromatin bound aluminum has been observed in the brains of cats and rabbits which were injected with aluminum, and which demonstrated histological evidence of NFD [3,8]. Attempts made so far to study the distribution of aluminum in the rat brain following injection have not been successful due to the anomalous properties of this tissue in response to the morin stain. At present it is not known whether differences in the subcellular binding of aluminum may account for species differences in response to aluminum application. Studies on subcellular localization are now in progress in our laboratory.

Neurofibrillary degeneration may well be an epiphenomenon of aluminum neurotoxicity, however, only animals which develop NFD display alterations in acquisition and retention. Electrophysiological evidence support the hypothesis that neurons with NFD loose postsynaptic potential activity and become either non-functional or disfunctional [2, 7].

The species variation in aluminum neurotoxicity may be important in interpreting the elevated aluminum content found in some forms of human senile and presenile dementia in which deterioration in short term memory is an early symptom: i.e. Alzheimer disease [15]. In this condition brain aluminum concentrations approaching those neurotoxic to cat are found in regions with extensive neurofibrillary degeneration [6, 9]. Precise definition of the intracellular binding sites for aluminum in cat and rat may aid in the interpretation of the pathogenic significance of the elevated aluminum content found in human disease.

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