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Aluminum toxicity: Cognitive and cholinergic parameters

Connor, Donald Joseph, Ph.D.

The University of Alabama in Birmingham, 1988

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ALUMINUM TOXICITY:
COGNITIVE and CHOLINERGIC PARAMETERS

by

DONALD J. CONNOR

A DISSERTATION

Submitted in partial fulfillment of the requirements for
the degree of Doctor of Philosophy in the Department
of Psychology in the Graduate School,
the University of Alabama
at Birmingham

BIRMINGHAM, ALABAMA

1988

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ABSTRACT OF DISSERTATION
GRADUATE SCHOOL, UNIVERSITY OF ALABAMA AT BIRMINGHAM

Degree Ph. D. Major Subject Psychology

Name of Candidate Donald Joseph Connor

Title Aluminum Toxicity: Cognitive and Cholinergic Parameters

Administration of aluminum sulfate in the drinking water of male Sprague-Dawley rats for thirty days resulted in an inpairment of both consolidation and extinction of a passive avoidance task. The behavioral deficit was not due to nonspecific effects caused by lower fluid consumption. No impairment of performance was observed on an active avoidance task, radial arm maze or open field activity measure. Partial improvement of the deficit was produced by replacement of the aluminum sulfate solution with tap water two weeks prior to testing (p 0.05). Injection (i.p.) of the aluminum chelator desferoxamine (DFO) returned the performance of the aluminum-treated animals to control levels in a dose-dependent manner. DFO injection of control animals did not affect their activity levels or extinction on the passive avoidance task. Biochemical analysis indicated a slight (10%) but significant increase in hippocampal muscarinic receptor number after aluminum treatment as determined by tritiated quinuclidinyl benzilate (3H-QNB) binding. No changes were found in choline acetyltransferase (ChAT)

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activity, phosphoinositide hydrolysis, 3H-QNB binding in the cortex or tritiated pirenzepine (3H-PZ) binding in the hippocampus or cortex. These results indicate that the behavioral impairment is a specific, toxic effect of aluminum administration, that it can be reversed by administration of an aluminum chelator and that the impairment is not due to cholinergic degeneration.

Abstract Approved by: Committee Chairman Robert S Hope

Program Director Joan F. Loden

Date 6/9/88 Dean of Graduate School Anthony Kerwood

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DEDICATION

To my Mother and Father, this work which meant so much to me is only a small fraction of what you have meant in my life. Please take this dedication as a small token of what you have given to me.

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ACKNOWLEDGEMENTS

This dissertation, as well as my progress in science and development as a reseacher, would not have been possible without the help of certain individuals.

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I would also like to thank Michele Simonato, Michael Koenig, Tammy Casebolt and others that I have worked with for their concern and support at times when I needed it.

Finally, I must thank my parents without whose love and support I would never have been able to persevere.

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I. INTRODUCTION TO ALUMINUM TOXICITY

1. Exposure

Aluminum is a ubiquitous metal, compromising approximately 8% of the earth's crust and is present in the air as aluminosilicates associated with dust particles (Boegman & Bates, 1984). Aluminum compounds are used in many common products including deodorants, talc, cat litter, table salt, baking powders and processed cheese (Roberts 1986; Crapper-McLachlan & Farnell, 1985). The average daily North American diet has been estimated to contain between 3 and 36 mg of aluminum (Underwood, 1977; Alfrey, 1986b), however, individual exposure can greatly vary. Following the recomended dosage of some over-the-counter liquid antacids can result in ingestion of up to 2 g of elemental aluminum per day. Although long believed to be nontoxic, recent evidence has shown that aluminum can cause neurological damage. The following sections review some of the clinical and experimental evidence for aluminum neurotoxicity.

2. Aluminum Toxicity in Relation to Human Disease:

Aluminum has been implicated in the pathology of several neurological disorders associated with cognitive impairments (Wisniewski, Sturman, Shek & Iqbal, 1985; Crapper-McLachlan, 1986). The three best explored

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disorders are dialysis encephalopathy, the Parkinson-dementia of Guam and Alzheimer's disease.

A. Dialysis Encephalopathy:

Dialysis encephalopathy (also called dialysis dementia) is a progressive neurological dysfunction which is observed almost exclusively in patients who have been on hemodialysis for over 2 years (Arieff, 1985). The main symptom complex consists of speech and language disorders, dementia (personality changes, intellectual deterioration and memory loss), myoclonus and in some cases seizures (Jack, Rabin & McKinney, 1984). Initially, symptoms are present only during and shortly after dialysis. The severity and persistence of these symptoms increase with the number of treatments until the syndrome becomes constant (Alfrey, 1985). Death usually occurs within 6-8 months after the first manifestation of symptoms (Alfrey, 1986a).

The occurrence of dialysis encephalopathy has been shown to be related to ingestion of phosphate binding gels containing aluminum (Alfrey, Le Gendre & Kaehny, 1976; Wallace, 1981; Pogglitsch, Petek, Wawschinek & Holzer, 1981) and to aluminum contamination of the dialysate (Parkingson, Ward & Kerr, 1981; Rozas & Port, 1979). Contamination of the dialysate with aluminum has been associated with the use of aluminum sulfate as a coagulum in the tap water used to prepare the dialysate (Alfrey, 1984). In some cases the clinical symptoms of the encephalopathy have been successfully treated or prevented by the removal of aluminum containing gels (Wallace, 1981; Jack et al., 1984;

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Masselot, Adhemar, Jaudon, Kleinknecht & Galli, 1978), by clearing the dialysate of aluminum (Rozas & Port, 1979) or by a combination of both treatments (Platts, 1980; Poisson, Mashaly & Lebkiri, 1978; Poisson, Mashaly & Lafforgue, 1979). Readministration of the aluminum containing gels to treat elevated phosphate levels has resulted in reoccurrence of the encephalopathy (Masselot et al., 1978; Poisson, 1979).

B. Parkinson-dementia of Guam:

Natives of Guam, certain regions of the Kii Peninsula of Japan and of southern West New Guinea have demonstrated an abnormally high incidence of amyotrophic lateral sclerosis (ALS) and parkinsonism with severe dementia (PD). ALS is a progressive central nervous system disease characterized by progressive weakness and skeletal muscle atrophy stemming from degeneration of the anterior horn cells of the spinal cord, motor nuclei in the brain stem and neurons in the motor cortex (Spencer et al., 1987). PD has a much slower onset than ALS and its victims demonstrate both extrapyramidal symptoms and organic dementia. Patients with PD usually deteriorate into a vegetative state 3 to 5 years after the appearance of the disease (Chen & Yase, 1985). Occurrence of both syndromes in 1 patient is common (Elizan et al., 1966), but whether these syndromes are different manifestations of the same underlying disease is still unknown.

Accumulation of neurofibrillary tangles occurs in both ALS and PD. The tangles in the neurons of the ALS patients

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appear to be composed of neurofilaments, while the neurofibrillary tangles of the PD patients are composed of single, paired and occasionally triple helical filaments similar to the paired helical filaments (PHF) found in Alzheimer's disease (Wisniewski, Narang & Terry, 1976; Wisniewski et al., 1985; Guiroy et al., 1987). Recent studies have indicated a common subunit protein exists in both the neurofibrillary tangles in Guamanian PD and the neurofibrillary tangles of Alzheimer's disease suggesting a common pathogenesis (Guiroy et al., 1987).

The occurrence of the amyotrophic lateral sclerosis (ALS) and parkinsonism-dementia (PD) syndromes of Guam have been correlated with high environmental levels of aluminum by epidemiological studies which also excluded genetic and virological influences. The characteristic neurofibrillary tangles found in the central nervous system of patients with these disorders have been shown to contain high concentrations of aluminum (Perl, Gajdusek, Garruto, Yanagihara & Gibbs, 1982; Chen & Yase 1985; Garruto, 1985), further implicating the metal in their pathogenesis.

C. Alzheimer's Disease:

Alzheimer's disease is a progressive, degenerative, terminal neurological disorder that affects more than 2 million people in the United States, accounting for approximately 55% of all irreversible dementias (Katzman, 1986). It is estimated that over 20 billion dollars a year is spent on the general treatment and care of victims of Alzheimer's disease (Dean & Bartus, 1985). Clinically, it

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is characterized by memory loss, cognitive decline, personality change and eventual death.

The clinical diagnostic criteria for Alzheimer's disease has been greatly improved over the past few years (Ron, Toone, Garralda & Lishman, 1979), but definitive diagnosis can only be achieved postmortem. It is the presence of neurofibrillary tangles (made of paired helical filaments), neuritic plaques, granulovacular degeneration and Hirano bodies in the brains of demented patients which defines the classical pathology of Alzheimer's disease (McKann et al., 1984). The number of neuritic plaques within the cerebral cortex correlates with deficits in cognitive performance (Blessed, Tomlinson & Roth, 1968; Perry et al., 1978) and with reductions in cholinergic and somatostatinergic markers (Perry, Candy & Perry, 1983; Rossor, Emson, Mountjoy, Roth & Iversen, 1984).

Analysis of the aluminum content in brain regions from Alzheimer's patients has demonstrated elevated aluminum content when compared to age-matched controls in some studies (Crapper, Krishnan & Quittkat, 1976; Trap, Miner, Zimmerman, Mastri & Heston, 1978) but not in others (Markesbery, Ehmann, Hossain, Alauddin & Goodin, 1981; McDermott, Smith, Iqbal & Wisniewski, 1977). Recent studies have shown that both the neurofibrillary tangles (Perl & Brody 1980) and the core of the neuritic plaques (Candy et al., 1986) contain high concentrations of aluminum. Aluminum accumulation is therefore directly associated with the 2 main defining morphological characteristics

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of Alzheimer's disease (neuritic plaques and neurofibrillary tangles). Aluminum's role in the etiology of the disease, however, remains unclear.

The association between these dementing disorders and aluminum suggests that aluminum toxicity may be involved in the pathogenesis of the cognitive impairments observed in these disease states. Further evidence implicating aluminum toxicity in central nervous system (CNS) diseases is provided by experimental studies.

3. Modeling Aluminum Toxicity:

A. Morphology:

Aluminum administered orally (Greger, Bula & Gum, 1985; Arieff, Cooper, Armstrong & Lazarowitz, 1979; Slanina et al., 1986), intraperitoneally (Ebina, Okada, Hamazaki & Midorikawa, 1984), subcutaneously (Uemura, 1984a; 1984b) and by intragastric intubation (Slanina, Falkeborn, Frech & Cedergren, 1984; Slanina, Frech, Bernhardson, Cedergren & Mattsson, 1985) crosses the blood-brain barrier and accumulates in the brain. Metallic aluminum (Wisniewski, Sturman & Shek, 1982) or aluminum chloride (Wisniewski, Sturman & Shek, 1980), injected into the cisterna magna of rabbits, as well as chronic subcutaneous injection of aluminum tartrate (Uemura, 1984b), has been shown to induce the formation of unpaired neurofibrillary tangles similar to the paired neurofibrillary tangles found in the Alzheimer's patient's brain (Wisniewski, Shelk, Gruca & Sturman, 1984; Selkoe, Liem, Yen & Shelanski, 1979). The difference in these tangles may reflect a species-specific difference

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rather than different mechanisms of formation. The type of exposure (acute high level vs chronic low level) may also affect the nature of the tangles. Intracranial injection of aluminum chloride to rats failed to induce formation of neurofibrillary tangles at a concentration 5-6 times that found to induce tangle formation in rabbits or cats (King, DeBoni & Crapper, 1975).

B. Biochemistry:

In vitro studies of nuclear processes have demonstrated that aluminum induces the formation of DNA-aluminum complexes (Karlik, Eichhorn, Lewis & Crapper, 1980), inhibits polytene chromosome puffing in response to steriods (Sanderson, Crapper-McLachlan & DeBoni, 1982) and inhibits ADP-ribosylation in vivo and in vitro (Crapper-McLachlan, Van Dam, Farnell & Lewis, 1983). Aluminum in combination with sodium floride has been shown to interact with the G-proteins which regulate the cyclic AMP and phosphoinositide second messenger systems (Sternweis & Gilman, 1982; Katada, Bokoch, Northup, Ui & Gilman, 1984; Bellorin-Font et al., 1985; Blackmore, Bocckino, Waynick & Exton, 1985; Johnson & Jope, 1986). Aluminum also impairs glucose utilization (Johnson & Jope, 1986), inhibits the fast phase of voltage-dependent calcium influx into synaptosomes (Koenig & Jope, 1987) and inhibits glycolysis and hexokinase activity in rat brain cytosolic and mitrochondria preparations (Lai & Blass, 1984). Synaptosomal uptake of norepinephrine (NE), serotonin (5-HT) and γ-aminobutyric acid (GABA) is inhibited by aluminum while dopamine (DA) uptake shows a

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biphasic effect (Lai, Lim & Davison, 1982; Wong, Lai, Lim & Davison, 1981; Lai, Lim & Davison, 1981).

Aluminum also appears to have toxic effects on central cholinergic function. Addition of aluminum chloride to rodent isolated brain nerve ending preparations resulted in decreased choline transport (Lai, Guest, Leung, Lim & Davison, 1980). A reduction in activity of purified acetylcholinesterase (AChE) from eel electric organ and human serum was found after addition of aluminum chlorohydrate to the assay medium (Marquis & Lerrick, 1982b; Marquis, 1983), while a decrease in rat brain AChE activity followed maternal exposure to aluminum (Marquis, 1982a). Choline acetyltransferase (ChAT) activity was found to decrease after aluminum administration to rabbits in one study (Kosik, Bradley, Good, Rasool & Selkoe, 1983) but not in another (Hetnarski, Wisnienski, Iqbal, Dziedzic & Lajtha, 1980).

C. Behavior:

Few studies have assessed the effect of aluminum administration on cognitive function in animals. Intracranial aluminum injection impaired the performan : of rabbits in both a water maze (Rabe, Lee, Shek & Wisniewski, 1982) and a passive avoidance task (Petit, Biederman, Jonas & LeBoutillier, 1985) and impaired learning of a conditioned avoidance task by cats (Crapper & Dalton, 1973). Studies with rats have produced variable results. Injection of aluminum chloride into the hippocampus of rats resulted in a transient deficit in acquisition of a conditioned avoidance response (King et al., 1975), while chronic

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intubation of aluminum chloride produced no performance deficits on a shuttle task (Bowdler et al., 1979). Dietary administration of aluminum chloride produced varying effects on shuttle-box avoidance behavior depending on strain, gender and whether or not parathyroid hormone was co-administered (Commissaris et al., 1982). In rats fed 3 doses of aluminum hydroxide (1500 mg/kg, 2500 g/kg and 350 mg/kg) no significant difference between dosage groups was found on a single trial passive avoidance task and on a visual-discrimination with reversal task although a significant correlation was found between the aluminum content of some brain areas and impaired performance on these tasks (Thorne et al., 1986). Activity levels of rats have been reported to increase (Bowdler et al., 1979), decrease (Commissaris et al., 1982; Thorne et al., 1986) or not change (Bowdler et al., 1979; Commissaris et al., 1982) after aluminum administration.

The behavioral variability following aluminum administration could be secondary to differences in 1.) testing conditions between laboratories, 2.) modes of aluminum administration and 3.) susceptibilities of rats by age, gender and strain (Bowdler et al., 1979; Commissaris et al., 1982; King et al., 1975; Thorne et al., 1986). The chemical form of aluminum may also be relevant in determining the absorption (both intestinal and through the blood-brain barrier) and therefore the toxicity of the aluminum compounds. For example, daily intubation with aluminum citrate significantly increased the cortical and

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hippocampal concentrations of aluminum, but intubation with aluminum hydroxide was without effect (Slanina et al., 1984).

4. Overview of Studies

The following experiments consist of 3 main studies. The first study utilizes several classical behavioral tasks to examine a range of cognitive function in aluminum-treated rats. Modifications of some of the task paradigms were done to increase the sensitivity of the tasks to behavioral deficits. The second study examines pre- and postsynaptic cholinergic function in an effort to find a neurochemical basis for the behavioral deficit(s) found in study 1. The third and final study examines the nature of the aluminum-induced behavioral impairment(s) to determine if they can be reversed by removal of aluminum from the animal or if they are permanent.

The mode of administration and form of aluminum that was employed in the present study was chosen on the basis of previous clinical and experimental research. The chronic oral administration protocol reduces the stress and possible side-effects of other methods that have been used, such as daily administration by intubation with etherization (Bowdler et al., 1979), and is more comparable to the mode of exposure of the general populace to aluminum than acute intracranial or subcutaneous injection. The form of aluminum used (aluminum sulfate) has been shown to be leached from the soil by acid rain (Driscoll, 1985) and has been used in water purification systems as a flocculant

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(Crapper-McLachlan & Farnell, 1985). Aluminum sulfate compounds are also found in medications such as sucralfate (the aluminum salt of sucrose octasulfate) with the conventional dose of this compound providing 828 mg per day of elemental aluminum. Absorption of this compound was demonstrated by increased blood and urine levels of aluminum in control patients with normal renal function after chronic (Bannwarth, Gaucher, Burnel & Netter, 1986) and acute (Haram, Weberg & Berstad, 1987) sucralfate administration. Previous neurochemical studies have shown effects of chronic oral aluminum sulfate administration on cyclic nucleotide synthesis in the central nervous system of rats (Johnson & Jope, 1987).

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II. STUDY 1: EFFECTS OF CHRONIC ORAL ALUMINUM SULFATE ADMINISTRATION ON COGNITION

1. Introduction:

In this study, a battery of behavioral tasks was used to investigate the effect of chronic oral aluminum sulfate administration on cognitive function in rats. Behavioral testing was done utilizing passive and active avoidance tasks (aversive reinforcers) and the radial arm maze (appetitive reinforcer). These tasks allow the examination of acquisition, extinction and long- and short-term memory. Activity measures were done in order to control for non-specific effects of the treatments on behavior.

The general activity level of the treated animals was measured in an open field device. In addition to indicating the effects of treatment on activity level, this measure is necessary in order to interpret the results of the other behavioral tests correctly (e.g., latency to enter on the passive avoidance task).

The passive avoidance task measures the animal's ability to learn and remember to avoid a footshock by inhibiting a "natural" response (entering a dark chamber). It should be noted that the extinction of the avoidance response over time reflects both a component of "forgetting" the original relation (dark box = shock) and the acquisition of a new association (dark box = safe).

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The active avoidance paradigm requires the animal to take an action (step-up to a platform) in order to avoid a shock. This paradigm allows the measurment of both the acquisition and retention of the task.

An open 8-arm radial maze was used to test working memory in an appetitive reinforcement paradigm.

2. Methods:

Subjects:

Male, Sprague-Dawley rats (initially weighing 120-130g) were housed communally in a temperature (25°C) controlled room, illuminated 0600 to 1800 hours. Treated animals were maintained on drinking water containing 0.3% aluminum (as a 3.7% aluminum sulfate octadecahydrate solution; Mallinckrodt) ad libitum for 1 month. Standard rat chow was provided to control and treated animals ad libitum. During the last 3 weeks of aluminum administration, the treated animals consumed 18.0 +/- 0.8 ml of aluminum solution per day per rat (equal to 2.0 mmoles of aluminum /day/rat) and controls consumed 28.0 +/- 1.7 ml of water per day per rat.

Activity:

General motor activity was measured in an open field apparatus consisting of an 80 x 80 cm square divided into sixteen 20 x 20 cm blocks, surrounded by a 45 cm high opaque wall. At the beginning of the measurement period, the animal was placed in the middle of the field and activity was measured from 0 to 1 minute and 1 to 5 minutes. Horizontal activity was defined as the number of hindlimb

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crossings between blocks and vertical activity as the number of rearings. Groups were compared by Student's t-test.

Passive Avoidance:

A Lafayette model 12970 passive avoidance device was utilized for all passive avoidance tasks. The apparatus consisted of a runway (25 cm x 7 cm), illuminated by a 60-watt incandescent light, separated by a manually operated guillotine door from a black Plexiglas chamber (46 cm x 40 cm x 17 cm) having a grid floor through which a scrambled foot-shock (unconditioned stimulus; UCS) could be delivered.

Experiment 1: Animals were placed on the runway facing the closed guillotine door. After a 30-second delay the guillotine door was raised and the rats were allowed to enter the dark chamber and habituate in the apparatus for 3 minutes. Twenty-four hours later the procedure was repeated except the door separating the arm and the chamber was closed after entry of the rat and a 1.0 second, 0.8 mA scrambled foot-shock (UCS) was administered through the floor grid. Retention was tested 1, 2, 3, 4 and 7 days after training.

Experiment 2: In the second experiment, rats were not prehabituated to the apparatus prior to shock and the guillotine door was open at the beginning of the trial. In order to eliminate the possibility of entry as a result of a startle response from being placed on the runway, the rat was positioned facing away from the open guillotine door and was required to turn and enter the dark chamber. When

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the rat had entered the chamber, the door was closed and a 4.0 second, 0.8 mA scrambled foot shock was administered. Thirty seconds later, the subject was returned to the home cage. The shock duration was increased from Experiment 1 in order to insure that all of the control animals acquired the task and to extend the extinction period so that any difference between groups would be more easily detected. Animals were tested at 24-hour intervals until extinction criterion was achieved.

Experiment 3: In order to increase the strength of the initial association, the final passive avoidance experiment involved the same conditions as Experiment 2 except that the animals were prehabituated to the apparatus for a 3-minute period, 24 hours prior to shock. Testing was done at 24-hour intervals until extinction criterion was reached.

For all paradigms, retention and extinction were assessed by the latency to enter (LTE) the dark chamber. Entry was defined as all four paws of the animal being on the grid floor of the dark chamber. The maximum latency was set at 5 minutes. Acquisition criterion was achieved when the rat withheld entry from the dark chamber for 5 minutes. Extinction criterion was achieved when the rat entered the dark chamber in less than 1 minute on 3 out of 4 consecutive days. The acquisition of the task and the number of days to reach extinction criterion were compared between groups using Fisher's exact test and by ANOVA with unequal n.

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Active Avoidance:

A Lafayette model 85150 step-up active avoidance device was used for all active avoidance tasks. The device consisted of a lower (shock) chamber with a grill floor and an upper (safe) chamber with a retractable back wall. The conditioned stimulus (CS) consisted of a 5-watt light and the unconditioned stimulus (UCS) was a scrambled foot shock. Concurrent with the presentation of the CS, the back wall of the upper chamber retracted to allow access to the safe chamber. To make an avoidance response, the animal had to climb into the upper chamber within the 10-second CS interval. If an avoidance response was not made, the UCS was administered for 10 seconds or until an escape response was made by the animal climbing into the upper chamber. The rat was then given a 10-second rest period. Upon conclusion of the trial, the back wall of the upper chamber was moved forward and the animal returned to the lower chamber. After a 10-second intertrial interval, the procedure was repeated.

To determine the effect of aluminum on the acquisition of the task, animals were treated with aluminum (0.3% for 1 month) prior to training. The acquisition schedule consisted of 10 trials per session with 1 session every other day and a UCS shock level of 0.3 mA. Acquisition criterion was defined as 9 avoidance responses in 10 trials (with the first 5 responses being avoidances) on 2 out of 3 consecutive days. In order to assess the strength of acquisition, 20 trials without shock were given 24 hours after acquisi-

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tion criterion was reached. Data were analyzed by ANOVA with unequal n and by Student's t-test.

To test the effect of aluminum on the retention of an active avoidance task, rats were trained on the active avoidance task prior to aluminum administration. Training sessions consisted of 20 trials per day with a UCS shock level of 0.8 mA. Training criterion was achieved when the rat established 18 avoidance responses in 20 trials with the first 5 responses being avoidances. Beginning 48 hours after reaching criterion, aluminum was administered for 30 days. To assess retention without new learning or recall being primed by the UCS, the first test session consisted of 20 trials without shock. No-shock trials continued until a stable extinction criteria was achieved (5 or fewer avoidance responses per session with none of the first 5 responses being an avoidance). Following this, shock trials were resumed until training criterion was reacquired. Data were analyzed by Student's t-test.

Radial Arm Maze:

A standard, wooden open 8-arm radial maze was used to assess working memory in an appetitive task paradigm (Olton & Samuelson, 1976). The apparatus consisted of a black wooden 8-arm maze elevated 100 cm above the floor. The center platform was octagonal and 30 cm in diameter with 8 arms, 75 cm long and 7 cm wide, spaced at equal distances. At the outer end of each arm a 2 cm wide and 1 cm deep hole served as the reward cup.

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Animals were deprived of food for a 24-hour period prior to the beginning of initial training. The pre-aluminum training consisted of a habituation, a shaping, and an acquisition session. In the habituation session 4 baits (dry Fruit Loops) were placed equidistant along each of the 8 arms and the rats were allowed access to the baits for a 5-minute period. This procedure was repeated 24 hours later. The shaping sessions were then begun by reducing the number of baits per arm over the next 4 days until only the bait in the reward cup was presented.

In acquisition training, water deprivation was used as the motivating variable with water-soaked Fruit Loops as the reward. Food was provided ad libitum, but access to water was restricted to 1 hour after each session. During the acquisition sessions, rats were placed in the center of the maze and allowed to enter the arms and eat the bait. Arm entries and reentries were recorded until the rat had obtained all baits or a 5-minute period had elapsed. Acquisition criterion was reached when all 8 baits were obtained within 5 minutes with less than 2 arm reentries (errors) per trial on 4 out of 5 consecutive days. Aluminum administration began 48 hours after acquisition criterion was reached.

After 30 days of aluminum administration, animals were retested. Procedures were the same as described in acquisition training. Rats were allowed access to water or to the aluminum sulfate solution for 1 hour and 2 hours, respectively, after each daily session. The number of days

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to reacquire the task and the number of arm reentries (errors) per day were analyzed by Student's t-test and ANOVA.

3. Results:

Activity:

No significant differences were observed between control and aluminum-treated rats in horizontal or vertical activity at 0-1-minute, 1-5-minute, or 0-5-minute time intervals ($p > 0.05$).

Passive Avoidance:

Initial (pre-shock) latency to enter the dark chamber was not different between the control and treated groups for any of the 3 passive avoidance experiments.

Experiment 1: Aluminum sulfate administration did not produce a deficit in acquisition or retention of the avoidance response in Experiment 1. All rats in both the control and treated groups withheld entry for the maximum time allowed (5 minutes) at 1, 2, 3, 4, and 7 days.

Experiment 2: All control animals demonstrated a LTE of 5 minutes on the first day after training while only 3 of the 9 aluminum-treated animals reached criterion on day 1. The remaining 6 animals reached criterion on days 2 - 4 ($p < 0.05$; Fisher's exact test). The learned response extinguished significantly faster in the aluminum-treated group than controls ($p < 0.01$; Fig. 1). Rank analysis showed that the number of days to reach the initial 5-minute LTE acquisition criteria was inversely correlated to

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Figure 1: Effect of aluminum sulfate administration (30 days) on extinction of a learned passive avoidance response (Experiment 2). Animals were trained without prehabituation to the apparatus as described in Methods. Bars represent percent of each group to reach extinction criteria. Open bar represent control rats (n = 4) and shaded bars represent aluminum-treated rat (n = 9).

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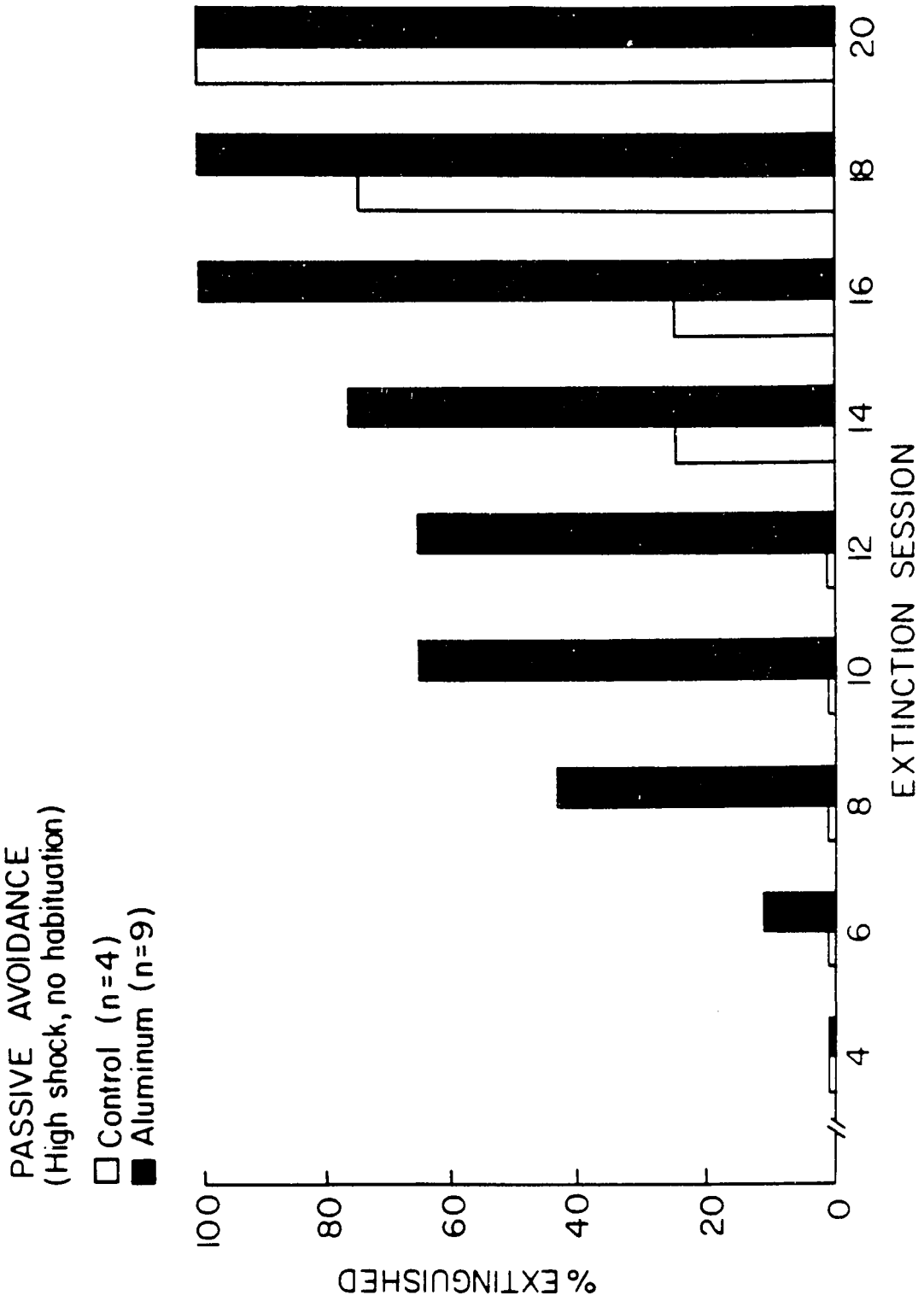

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the number of days to extinction criteria (Spearman's r = -0.78; $p < .05$).

Experiment 3: The addition of a 3-minute prehabituation period 24 hours prior to shock resulted in all of the control animals and 7 out of the 8 aluminum-treated animals acquiring the 5-minute LTE criteria on day 1. The remaining aluminum-treated animal reached criterion on day 2. As in Experiment 2, administration of aluminum caused a significantly faster extinction of the passive avoidance response compared to control animals ($p < 0.001$; Fig. 2).

Prehabituation to the apparatus 24 hours prior to shock significantly increased the mean number of days to extinction for both the aluminum-treated ($p < 0.01$) and control ($p < 0.001$) animals (Experiment 2 vs. Experiment 3). Prehabituation increased the mean number of days to extinction by 84% for control animals (from 17 to 32 days) and by 70% for aluminum-treated animals (from 10 to 17 days).

Active Avoidance:

Pretreatment with aluminum sulfate did not affect the number of sessions required to reach acquisition criteria (control = 5.72 +/- 1.05, aluminum = 6.0 +/- 0.73). No significant differences were found between groups in the number of avoidance responses or on the interaction of group x days ($p > 0.5$ and $p > 0.1$, respectively; Fig. 3). A significant days-effect was found, reflecting learning of the task ($p < 0.05$). The treated and control groups also

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Figure 2: Effect of aluminum sulfate administration (30 days) on extinction of a learned passive avoidance response (Experiment 3). Rats were trained with prehabituation to the apparatus as described in Methods. Bars represent percent of each group to reach extinction criteria. Open bars represent control rats (n = 5) and shaded bars represent aluminum-treated rats (n = 8).

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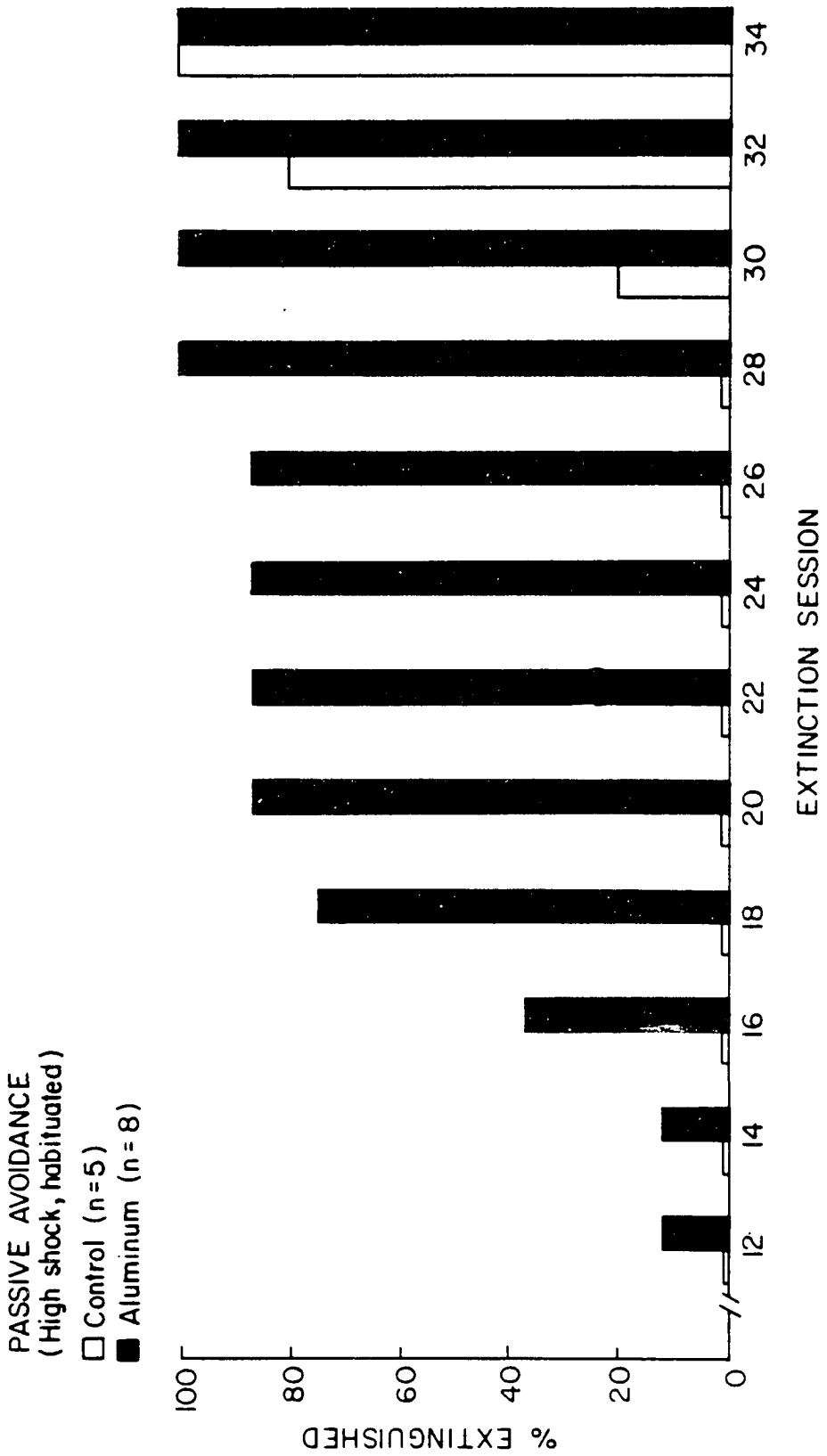

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Figure 3: Effect of aluminum sulfate administration (30 days) on acquisition of the active avoidance task. Data are expressed as the mean number of avoidance responses per session +/- S.E.M. Open bars represent control rats (n = 4), and shaded bars represent aluminum-treated rats (n = 6).

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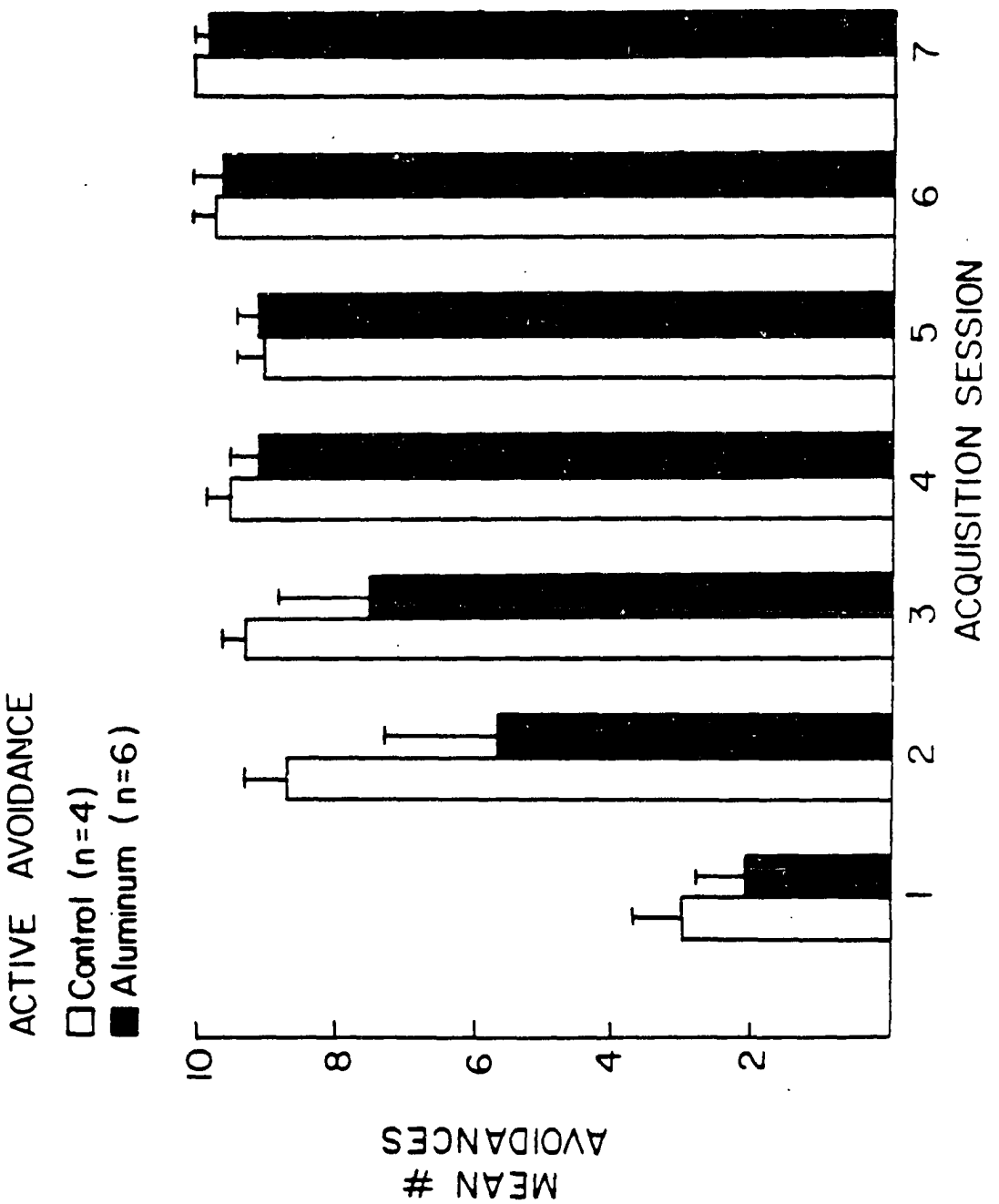

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behaved similarly in the no-shock condition (number of active avoidance responses: control = 14.5 +/- 3.6, aluminum = 16.0 +/- 2.6; n = 4 - 6).

In the active avoidance retention paradigm, the mean number of days to initial acquisition criteria (pre-aluminum sulfate administration) did not differ between the group to be treated with aluminum and control animals. Aluminum treatment did not significantly alter the number of trials (without shock) to reach extinction criteria nor alter the reacquisition of the task. The number of avoidance responses during the first session of extinction trials and the first session of reacquisition trials were also similar between aluminum-treated and control animals (Table 1).

Radial Arm Maze:

The mean number of days to reach initial acquisition criteria was not significantly different between the control animals and the animals to be treated with aluminum (5.8 +/- 1.5, and 4.2 +/- 0.13, respectively; n = 4 - 10, $p > 0.1$). After 1 month of aluminum sulfate treatment, the mean number of days to reacquire task criteria was not significantly different between the 2 groups (control = 7.0 +/- 1.08; aluminum = 5.2 +/- 0.42, $p > 0.07$). Analysis of arm reentries also showed no significant interaction effect of group x sessions or any significant difference between groups.

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Table 1: Effect of aluminum administration on retention of the active avoidance response. Animals were trained to criteria on the active avoidance response. After thirty days of treatment with aluminum sulfate, the animals were tested for retention of the avoidance response in the absence of the UCS (shock). After the avoidance response was extinguished, the UCS was reinstated and reacquisition trials began. Data is expressed as means +/- S.E.M.

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PRE-TREATMENT		
	(n = 6)	(n = 4)
DAYS TO ACQUISITION:	3.1 ± 0.1	3.3 ± 0.3
30 DAY TREATMENT PERIOD	ALUMINUM	CONTROL
POST-TREATMENT		
DAYS TO EXTINCTION (no shock):	2.4 ± 0.6	2.5 ± 0.3
DAYS TO REACQUISITION (with shock):	1.8 ± 0.2	2.5 ± 0.3
AVOIDANCES ON EXTINCTION TRIAL ONE (no shock):	13.5 ± 4.0	15.3 ± 3.5
AVOIDANCES ON REACQUISITION TRIAL ONE (with shock):	15.2 ± 1.0	15.3 ± 1.5

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4. Discussion:

A series of behavioral tests were employed to determine the neurotoxic effects of chronic oral aluminum sulfate administration in the male Sprague-Dawley rat. The results indicate that this administration paradigm produced specific impairments of cognitive function, manifest as deficits in acquisition/consolidation and retention of the learned response in a passive avoidance task. The observation of these impairments was found to be dependent upon the experimental constraints under which the task was performed. Moreover, the cognitive impairments appear specific to this task because no deficits were observed in acquisition or retention of an active avoidance task or in performance on a radial arm maze task.

Control and aluminum-treated animals behaved similarly when a standard passive avoidance paradigm was employed (Experiment 1). In order to assess more subtle influences of aluminum on behavior, the sensitivity of the task was increased by decreasing the saliency of the events (cues) through (i) omitting prehabituation to the apparatus, (ii) eliminating the 30-second door delay and (iii) continuing trials until extinction criterion was reached by each animal. Utilizing this protocol, deficits in both the acquisition/consolidation and retention of the tasks were evident (Experiment 2).

The longer period required for most of the aluminum-treated animals to acquire the passive avoidance behavior when compared to controls (Experiment 2), demonstrated one

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of the potential problems of measuring learning at only the 24-hour retention interval. That is, entry into the dark box in less than 5 minutes at the 24-hour test period may be an indication of an impaired acquisition/consolidation process rather than of a retention deficit. In order to test retention of the task in the absence of a deficit in acquisition/consolidation, the initial strength of association between the CS and the UCS was increased by prehabituating the animals to the apparatus 24 hours before the training trial (Experiment 3). In this situation, all of the controls and all but one of the aluminum-treated animals reached acquisition criterion on day 1 and the remaining animal achieved criterion on day 2. In the absence of a significant impairment of acquisition/consolidation, the aluminum-treated animals still extinguished significantly faster than controls, indicating that retention of the task was impaired independently of its initial acquisition.

An increase in general motor activity could potentially confound performance on passive avoidance and many other behavioral tasks. Aluminum administered by other methods has been reported to increase (Bowdler et al., 1979), decrease (Commissaris et al., 1982; Thorne et al., 1986) or produce no change (Bowdler et al., 1979; Commissaris et al., 1982) in activity levels. In this study, activity measurements did not reveal any significant difference between groups when analyzed in 0-1-, 1-5- or 0-5-minute intervals. The 0-1-minute interval was analyzed separately

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to exclude the possibility that an initial activity difference would be masked by the longer measurement period (0-5 minutes). That is, since extinction criterion in the passive avoidance task was set at an LTE of 1 minute or less, even a brief increase in the initial activity, which would be insignificant over the total interval (0-5 minutes), could effect the measurement of the extinction of the passive avoidance response.

Sensitivity to foot-shock could also influence the performance of the rats on the behavioral tasks. Since the acquisition of the active avoidance task and of the passive avoidance task in Experiments 1 and 3 was similar between the aluminum-treated and control animals, altered sensitivity does not appear to account for the differences between the 2 groups in performance of the passive avoidance task. No obvious signs of dehydration (skin turgor, ungroomed fur, etc.) were present in the aluminum-treated animals at the time of testing.

The lack of significant differences between the treated and control groups on radial arm performance, active avoidance acquisition or retention, and acquisition or extinction in passive avoidance Experiment 1 has several possible interpretations. There are indications that different neuronal subsystems are associated with the performance of different behavioral tasks. Unlike tasks such as the radial arm maze which appear to be largely dependent upon intact cholinergic hippocampal function (Eckerman, Gordon, Edwards, MacPhail & Gage, 1979; Stevens, 1981),

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performance of the passive avoidance task seems to be disrupted by lesions of several CNS regions. Hippocampal (Winocur, 1985), striatal (Prado-Alcala, Fernandez-Samblancat & Solodkin-Herrera, 1985) and basal forebrain (Altman, Crosland, Jenden & Berman, 1985) lesions have all been shown to effect passive avoidance performance. It is possible that the neuronal systems subserving passive avoidance learning are more accessible or susceptible to aluminum toxicity than the systems underlying the learning and retention of the other tasks.

The memory requirement of the task may also be important in considering the effects of aluminum. The radial arm maze is considered to be a measure of working memory and is dependent upon an appetitive reinforcer (Olton, Becker & Handelmann, 1979) while the passive avoidance task measures reference memory and utilizes a negative reinforcer. Also, in the passive avoidance paradigm, aluminum was administered prior to training while in several of the other tasks (active avoidance retention, radial arm maze), animals were trained on the task before exposure to aluminum. Therefore, aluminum may interfere with the consolidation and retention of new memory rather than alter the retrieval of established memories acquired previous to aluminum exposure. It should be noted however, that aluminum did not effect active avoidance acquisition which may indicate that the different characteristics of each task (multi vs. single exposure to the UCS, go vs. no-go response, etc.) may draw on different systems.

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In summary, administration of aluminum in a paradigm that is relevant to *in situ* human exposure produces a specific behavioral impairment in the rat. The next study examines possible neurochemical substrates for this cognitive change.

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III. STUDY 2: EFFECTS OF CHRONIC ORAL ALUMINUM SULFATE ADMINISTRATION ON CHOLINERGIC FUNCTION

1. Introduction:

A close association has been shown to exist between cholinergic activity and cognitive function (Drachman, 1977; Spencer & Lai, 1983). In clinical studies, choline acetyltransferase (ChAT) activity was found to be reduced in the frontal and occipital cortex of hemodialysis patients manifesting the symptoms of dialysis encephalopathy (Perry et al., 1985). Neurochemical studies of patients with the PD complex of Guam have revealed a reduction in ChAT and AChE activity in the neocortex and limbic system. The degree of loss of cholinergic markers in these areas correlated well with the presence of neurofibrillary tangles (Chen & Yase, 1985). In Alzheimer's disease, degeneration of the cholinergic system is the most pronounced neurochemical abnormality (Davies & Maloney, 1976; Bowen, Benton, Spillane, Smith & Allen, 1982) and is highly correlated with the degree of dementia (Perry et al., 1978). In experimental studies, memory loss similar to that observed in normal aging has been induced in man by an injection of scopolamine (a muscarinic antagonist) (Drachman & Leavitt, 1974). Rats and monkeys injected with muscarinic antagonists have shown similar deficits in performance on memory tasks (Bartus & Johnson, 1976; Drew & Miller, 1973;

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Swonger & Rech, 1972; see Spencer & Lai, 1983 for review). The specific involvement of the cholinergic system in the induction of these impairments has been demonstrated by their reversal with physostigmine (an anticholinesterase) but not with general CNS stimulants such as methylphenidate (Bartus, 1978) or amphetamine (Drachman, 1977).

In order to determine if the cognitive deficits observed after aluminum sulfate treatment were related to impairment of the cholinergic system, biochemical markers of presynaptic (choline acetyltransferase activity; ChAT) and postsynaptic (muscarinic receptor binding) cholinergic integrity were measured. Receptor-coupled inositol phospholipid hydrolysis after *in vivo* aluminum administration was also measured because this second messenger system has been shown to be inhibited by aluminum added *in vitro* (Johnson & Jope, 1986).

2. Methods:

Subjects:

Male, Sprague-Dawley rats (initially weighing 120-130 grams) were housed communally in a temperature (25°C) controlled room, illuminated 0600 to 1800 hours. Treated animals were maintained on drinking water containing 0.3% aluminum (as a 3.7% aluminum sulfate octadecahydrate solution; Mallinckrodt) ad libitum for 1 month. Standard rat chow was provided to control and treated animals ad libitum. During the last 3 weeks of aluminum administration, the treated animals consumed 18.0 +/- 0.8 ml of aluminum

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solution per day per rat (2.0 mmoles of aluminum/day/rat) and controls consumed 28.0 +/- 1.7 ml of water per day per rat.

Choline Acetyltransferase:

ChAT activity was measured using the method of Fonnum (1975). Homogenates of brain regions were incubated in triplicate for 20 minutes at 37°C in buffer containing [14C] acetyl-CoA (0.2 mM; ICN Radiochemicals), choline (10 mM) and eserine (1 mM). The reaction was terminated by extraction of the synthesized [14C]-acetylcholine into an organic phase containing scintillation fluid.

Muscarinic Receptors:

The method of Vickroy et al. (1986) was employed to measure muscarinic receptor binding in homogenates of brain regions. Triplicate samples of homogenate were incubated for 1 or 2 hours at 25°C in 2 ml of 10 mM phosphate buffer containing 2.5 nM [3H] pirenzepine (3H-PZ; New England Nuclear) or 0.60 nM [3H] quinuclidinyl benzilate (3H-QNB; New England Nuclear), respectively. Incubations were terminated by rapid filtration through Whatman GF/B filters followed by a wash with 8 ml of ice cold buffer. Blanks contained 1 mM atropine sulfate. Protein concentration in the incubation tubes were 0.06 mg/ml and 0.03 mg/ml for 3H-PZ and 3H-QNB binding, respectively.

Inositol Phospholipid Hydrolysis:

Phosphoinositide metabolism was determined by measurement of [3H]myo-inositol-1-phosphate (M1P) accumulation using a modification of the method of Berridge, Downes, and

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Hanley (1982). Cortices or hippocampi were sliced (0.3 mm) in 2 perpendicular directions using a McIlwain tissue slicer. The slices were washed several times and preincubated for 60 minutes at 37°C in media (NaCl, 122 mM; KCl, 4.9 mM; MgSO4, 1.2 mM; NaHCO3, 3.6 mM; dextrose, 11 mM; HEPES, 30 mM; bubbled with 95% CO2 for 20 minutes, pH adjusted to 7.3 with NaOH) to restore energy balance. Media was renewed after 30 minutes of preincubation. Slices were then washed several times and incubated in 500 ml of media containing 0.53 uM myo-[2-3H]inositol (American Radiolabelled Chemicals) and 10 mM LiCl for 60 minutes at 37°C. Carbachol (5 mM), norepinephrine (200 uM), K+ (25 mM), or media (basal) and CaCl2 (1.3 mM) was then added to the prelabeled slices and incubated for 60 minutes. The reaction was stopped by rapidly washing the slices twice with 6 volumes of ice-cold media, and addition of 1.5 ml of CHCl3/MeOH/12N HCl (1:2:0.01). After a 20-minute extraction period, 1.0 ml of chloroform and 0.5 ml of H2O were added and the lipid and aqueous phases were separated by centrifugation. The lipid phase was dried overnight at room temperature and counted in 5 ml of scintillation fluid.

The aqueous phase was mixed with 0.5 ml of a 50% AG1x8 slurry (formate form, BioRad) and 1.0 ml H2O. The mixture was vortexed and added to a 10 ml plastic column. The resin was washed with 8 ml of 5 mM Na-tetraborate/60 mM Na-formate and 6.0 ml of 200 mM NH4-formate in 0.1 M formic acid (to elute inositol monophosphate; MIP). Ten ml of

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scintillation fluid was added to the final eluate and the radioactivity was determined.

Protein determination:

Protein concentration was determined by the method of Lowry, Roseborough, Farr, and Randall (1951).

3. Results:

There were no significant differences of ChAT activity between the aluminum-treated animals and controls in either the cortex or the hippocampus (Table 2). Muscarinic receptor number as determined by [3H]-PZ and [3H]-QNB binding was similar in the cortex of control and aluminum-treated rats. Although the Bmax of 3H-PZ binding in the hippocampus was not different between groups, there was a small (10%), but significant ($p < 0.05$) increase in the Bmax of 3H-QNB binding in the hippocampus of the aluminum-treated rats compared with controls.

Inositol phospholipid hydrolysis demonstrated a similar resistance to aluminum treatment. Basal, carbachol (5 mM), norepinephrine (200 uM) and K+ (25 mM) stimulated production of [3M]M1P were not significantly different in cortical or hippocampal slices from the aluminum-treated rats compared with controls (Fig. 4).

4. Discussion:

Although no general effects of aluminum on cholinergic function were found, a small increase in hippocampal muscarinic receptor number was observed. This may reflect a slight decline in hippocampal cholinergic presynaptic activity. However, the results indicate that the aluminum

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Table 2: Effects of chronic oral aluminum sulfate treatment on cholinergic markers. ChAT activity and radioligand binding assays were carried out as described in Methods. Values are expressed as mean +/- S.E.M. in nanomoles/minute/mg protein for ChAT and femtomoles/mg protein for radioligand binding assays. *$p < 0.05$.

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Brain Region	ChAT (n = 4 - 6)	[^{3}H]-QNB (n = 8 - 9)	[^{3}H]-PZ (n = 5)
Cortex			
Control	0.73 +/- 0.08	796 +/- 29	292 +/- 27
Aluminum-treated	0.73 +/- 0.03	804 +/- 20	288 +/- 20
Hippocampus			
Control	1.13 +/- 0.03	591 +/- 10	243 +/- 14
Aluminum-treated	1.28 +/- 0.06	642 +/- 14*	246 +/- 14

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Figure 4: Effect of aluminum sulfate administration (30 days) on phosphoinositide hydrolysis. Rat cortical or hippocampal slices were prelabeled with [3H]-inositol and the release of [3H]-M1P was measured as described in Methods. Data are expressed as the percent [3H]-M1P/[3H]-lipids +/- S.E.M. Open bars represent control rats (n = 4), and shaded bars represent aluminum-treated rats (n = 4).

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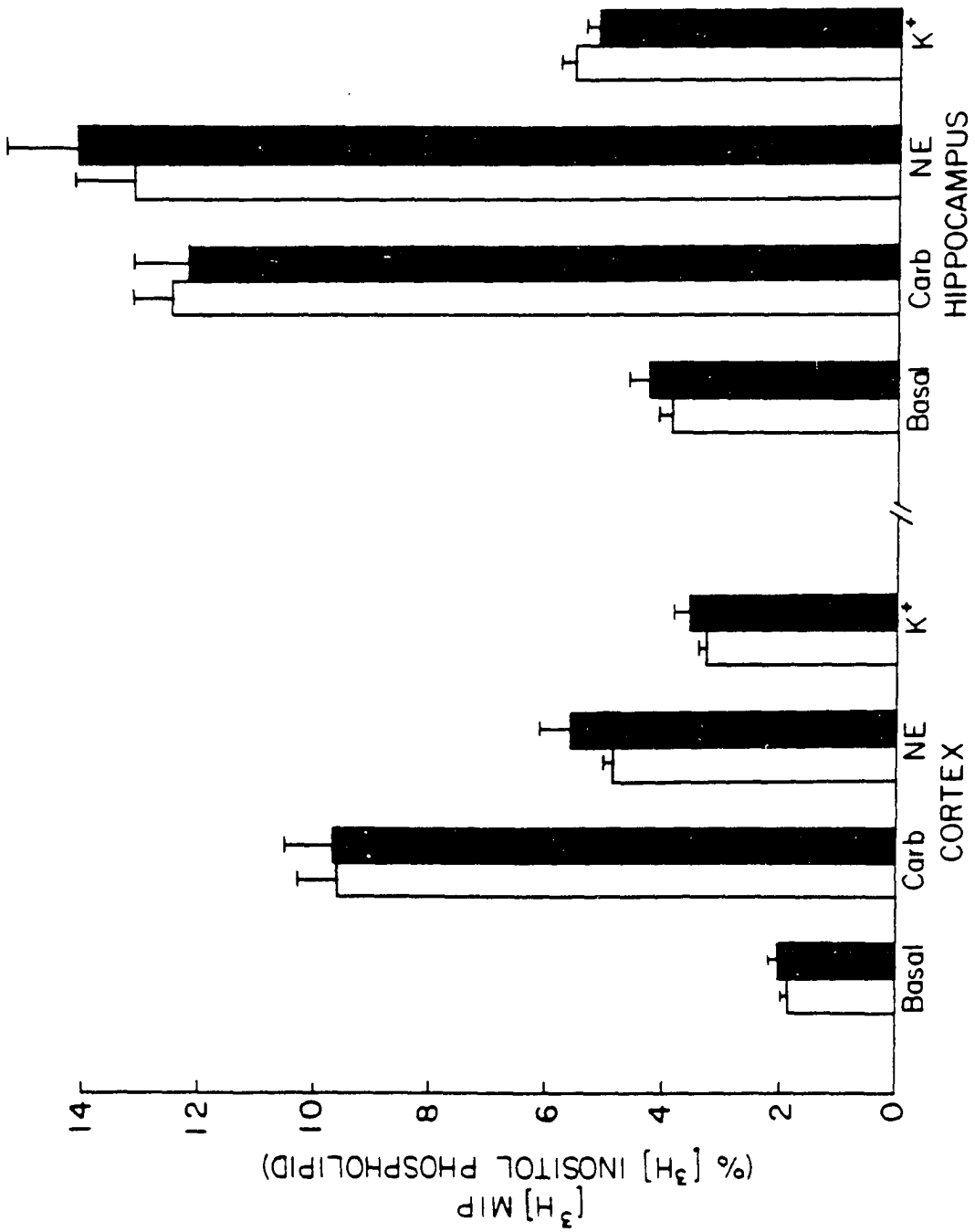

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treatment did not lead to cholinergic degeneration since ChAT activity, carbachol-stimulated phosphoinositide hydrolysis and binding of another ligand (3H-PZ) were unaffected. The inability of aluminum to effect other cholinergic parameters in the hippocampus is consistent with the normal performance by the aluminum-treated animals on the radial arm maze (Study 1) as this task has been shown to be dependent upon normal hippocampal cholinergic function (Eckerman et al., 1979; Olton et al., 1979; Stevens, 1981).

Although *in vitro* aluminum inhibits carbachol-induced hydrolysis of inositol phospholipids in brain slices (Johnson & Jope, 1986), no inhibition was observed following chronic *in vivo* administration of aluminum. This indicates that an irreversible alteration of this receptor-coupled system did not occur with this treatment. Preparation and washing of the brain slices during the assay may have removed the aluminum from the slices, masking a direct effect. Alternately, a longer treatment period may be required to expose the CNS to sufficient aluminum to induce an irreversible effect on this system. An initial shock or trauma to the tissue prior to aluminum exposure (such as changes in membrane fluidity or cell metabolic activity) as may occur in the preparation of brain slices for the phosphoinositide assay and in some clinical situations (e.g., Alzheimer's disease, dialysis encephalopathy) could also be necessary to make the neurons more susceptable to aluminum toxicity.

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IV. STUDY 3: REVERSAL OF A BEHAVIORAL DEFICIT INDUCED BY CHRONIC ORAL ALUMINUM SULFATE

1. Introduction:

The neurological lesions of Alzheimer's disease and the Parkinson-dementia of Guam are generally considered irreversible with our current level of knowledge. However, several methods for reversing the pathology of dialysis encephalopathy have been tried with varying success. Reduction in the exposure of dialysis patients to aluminum through discontinuation of the oral aluminum hydroxide gels and removal of aluminum from the dialysis fluid by the use of a deionizer has reduced the number of encephalopathy cases and in some instances induced partial remission of an already present encephalopathy (Poisson et al., 1978; Masselot et al., 1978; Poisson et al., 1979; Rozas & Port, 1979; Wallace, 1981; Platts, 1980). Similarly, in aluminum-loaded animals with normal renal function, spontaneous elimination of aluminum from tissues can occur but does so very slowly (Verbeelen, Smeyers-Verbeke, Van Hooff, Van Der Straeten & De Roy, 1986).

A far more efficent way to treat the dialysis encephalopathy syndrome is by using chelators to facilitate aluminum removal. The most clinically effective chelator at present is deferoxamine (DFO) (Ackrill & Day, 1985; Swartz, 1985). DFO is a byproduct of the mold Streptomyces and was

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originally used to treat iron overload in dialysis patients (see Pippard & Callender 1983 for review). It was subsequently shown that DFO is also effective in removing aluminum and reducing the symptoms of encephalopathy in a dialysis patient (Ackrill, Ralston, Day & Hodge, 1980). Recent studies have confirmed and expanded this finding (Ciancioni et al., 1984; Payton, Junor & Fell, 1984; Hercz, Salusky, Norris Fine & Corburn, 1986; Warady et al., 1986). In cases where hemofiltration alone did not improve dialysis encephalopathy or increase aluminum removal, co-treatment with DFO significantly reduced the patient's symptoms after only 4 infusions (Pogglitsch et al., 1981). The inability of hemofiltration alone to remove aluminum is probably due to the metal's tendency to bind to serum proteins such as albumin and transferrin and to sequester in bone and tissue. DFO can detach aluminum from these proteins and allow removal of the metal by dialysis (Leung, Hodsman, Muirhead & Henderson, 1985). Infusion of DFO also increased plasma aluminum levels in patients removed from aluminum exposure, indicating that DFO is able to mobilize aluminum from body tissue stores (Ackrill, Ralston & Day, 1986). Intravenous (i.v.), intramuscular (i.m.) or intraperitoneal (i.p.) injection of DFO were all seen to be effective in producing aluminum mobilization and removal (Molitoris et al., 1987).

In experimental studies, DFO increased the survival and aluminum excretion in the urine and feces of animals injected i.p. with aluminum nitrate (Domingo, Llobet, Gomez & Corbella, 1986). DFO infusion was also effective in

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removing aluminum from the tissues of rabbits given sub-chronic subcutaneous injections of aluminum (Melograna & Yokel, 1983; 1984). The average reduction in aluminum levels from the organs examined in this study was 26% compaired to a theoretical maximum of 39% if all the DFO infused had chelated with aluminum. This demonstrates that DFO can be quite efficient as well as effective in reducing aluminum tissue burden.

In study 1, impaired performance of rats on a passive avoidance task was found after adminstration of a 3.7% aluminum sulfate solution in the drinking water for 1 month. The final study tests the reversibility of this behavioral impairment by (i) discontinuing aluminum administration for a 2-week period prior to and throughout testing and (ii) injection of DFO in addition to the discontinuation of aluminum administration.

2. Methods:

Subjects:

Male, Sprague-Dawley rats (initially weighing 120-140 grams) were housed communally in a temperature (25°C) and light (12 hour light/dark cycle) controlled room. Standard rat chow was provided to control and treated animals ad libitum throughout the study.

Aluminum and DFO Treatment:

All aluminum-treated animals were maintained on water containing 0.3% aluminum (as a 3.7% aluminum sulfate octadecahydrate solution; Mallinckrodt) for an initial 4-week period.

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Animals were randomly divided into 7 groups as follows (see Table 3):

Group 1: (C) Controls were maintained on tap water ad libitum for the initial 4-week treatment period and throughout the study. Behavioral testing was begun at the end of the 4-week period.

Group 2: (DEP) Water-deprived controls were maintained on tap water throughout the study but were yoked by fluid consumption to the aluminum treated animals (see Table 4). Testing was begun at the end of the 4-week period.

Group 3: (C+D) Control animals were allowed access to tap water as per group 1. After the initial 4-week period animals were injected i.p. with DFO (75mg/kg) every other day during the fifth week (4 injections). Animals were then allowed a further week (week 6) prior to behavioral testing to match the treatment schedules of groups 4, 5, and 6.

Group 4: (DH) Aluminum-treated animals injected with a high dose of DFO were maintained on drinking water containing 3.7% aluminum sulfate for a 4-week period. The drinking solution was then changed to tap water and the rats were injected with DFO (75mg/kg) as described for group 3. Animals were continued on tap water for a sixth week prior to testing and throughout the testing period.

Group 5: (DL) Aluminum-treated animals injected with a low dose of DFO were treated as described for group 4 except a lower dose of DFO was injected (30 mg/kg).

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Table 3. Treatment paradigm for study 3. C=controls, DEP=water deprived controls, C+D=control animals given DFO (75mg/kg), DH=aluminum-treated animals injected with DFO (75mg/kg), DL=aluminum-treated animals injected with DFO (30mg/kg), ALW=aluminum-treated animals withdrawn from aluminum treatment 2 weeks prior to testing, AL=animals continuously maintained on aluminum.

* animals were tested after the initial 4 week treatment period.

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Drinking Solutions and Drug Administration

GROUP	WEEK 1	2	3	4	5	6	TESTING PERIOD
C	<---- H2O -----				*	*	H2O
DEP	<---- H2O -----				*	*	H2O
C+D	<---- H2O -----				<--- H2O ---> (HDFO)		H2O
DH	<----- AL -----				<--- H2O ---> (HDFO)		H2O
DL	<----- AL -----				<--- H2O ---> (LDFO)		H2O
ALW	<----- AL -----				<--- H2O ---> (saline)		H2O
AL	AL				*	*	AL

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Table 4. Effects of aluminum administration on fluid consumption and body weight gain. Data expressed as (ml) / (grams) per day per rat. Groups are as described in methods (see also Table 1). Data expressed as means +/- std. dev..

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GROUP	WEEK			
	1	2	3	4
C	26.6 ± 0.5/5.32 ± 0.06	25.2 ± 1.9/4.00 ± 0.4	25.2 ± 3.8/4.95 ± 1.2	29.4 ± 2.2/4.80 ± 1.1
DEP	8.5 ± 5.9/0.56 ± 0.3	15.8 ± 1.3/2.47 ± 1.7	16.8 ± 1.7/4.02 ± 1.4	17.1 ± 2.7/5.60 ± 0.5
C + D	25.8 ± 0.7/4.78 ± 0.8	27.8 ± 1.4/6.30 ± 0.9	28.7 ± 1.2/5.14 ± 1.0	28.4 ± 1.5/3.93 ± 0.7
DH	9.7 ± 3.7/1.18 ± 0.6	15.7 ± 1.1/4.05 ± 0.8	16.0 ± 1.5/4.52 ± 0.7	18.7 ± 0.9/3.75 ± 0.6
DL	10.5 ± 3.5/1.47 ± 2.3	16.1 ± 1.3/4.76 ± 1.3	16.7 ± 1.4/4.40 ± .07	15.5 ± 1.1/3.60 ± 0.7
ALW	10.8 ± 4.2/0.79 ± 1.5	16.2 ± 0.8/4.62 ± 1.1	15.4 ± 1.8/4.14 ± .06	18.8 ± 0.2/4.45 ± 1.0
AL	9.9 ± 5.1/0.80 ± 0.7	15.3 ± 2.0/4.57 ± 0.7	15.7 ± 1.2/4.34 ± 1.1	19.1 ± 0.8/5.32 ± 0.4

Group 6: (ALW) Aluminum-treated animals with a "wash-out" period were maintained on the aluminum sulfate solution for 4 weeks as above and were then switched to, and continued on, tap water at the end of the 4-week treatment period. During the fifth week this group was injected with saline i.p. in a volume and schedule to match group 4. Animals were allowed a further week on tap water (week 6) prior to behavioral testing.

Group 7: (AL) Aluminum-treated animals were administered the aluminum-sulfate solution throughout the treatment and testing periods. Behavioral testing was begun immediatly after the initial 4-week treatment period.

Open Field Activity:

General motor activity was measured prior to passive avoidance training. An open field apparatus was utilized, consisting of an 80 x 80 cm square base divided into sixteen 20 x 20 cm blocks, surrounded by a 45 cm high opaque wall.

At the begining of the measurment period, the animal was placed in the middle of the field and activity was measured from 0 to 1 minute and 1 to 5 minutes. Horizontal activity was measured as the number of hindlimb crossings between blocks and vertical activity as the number of rearings.

Passive Avoidance:

A Lafayette model 12970 passive avoidance device was utilized. The apparatus consisted of a runway (25 x 7 cm), illuminated by a 60-watt incandescent light, separated by a

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manually operated guillotine door from a black plexiglass box (46 x 40 x 17 cm) having a grid floor through which a scrambled foot-shock (unconditioned stimulus; UCS) could be delivered.

Animals were placed on the runway facing away from the open guillotine door and allowed to turn and enter the dark chamber. The guillotine door was then closed and the rat allowed to habituate in the apparatus for 3 minutes. Twenty-four hours later the procedure was repeated except a 4-second, 0.8 mA scrambled foot-shock was administered through the floor grid 40 seconds after closing the guillotine door. Thirty seconds after UCS administration, the subject was returned to the home cage. Animals were tested at 24-hour intervals until extinction criterion was achieved.

Acquisition and extinction were assessed by the latency to enter (LTE) the dark box. The maximum latency was set at 5 minutes. Acquisition crtierion was reached when the rat withheld entry for the maximum latency period. Extinction criterion was achieved when the rat entered the dark box in less than 1 minute on 3 out of 4 consecutive days. The number of days to acquire the task and the number of days to reach extinction criterion were compared among groups.

Data Analysis:

One-way analysis of variance (ANOVA) was employed to assess treatment effects on activity levels (open field) and on the number of days to reach acquisition and

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extinction criteria (passive avoidance). Post-hoc analysis of significant ANOVA was preformed utilizing Duncan's multiple range test (significance set at 95% confidence level).

3. Results:

Fluid consumption and body weight:

Animals maintained on the aluminum sulfate solutions during the 4-week treatment period showed an initial suppression of fluid consumption and weight gain which improved over time (Table 4). When the drinking solution was changed from the aluminum solution to tap water, all aluminum-treated animals demonstrated increased fluid consumption up to, but not beyond, control levels. Fluid intake-yoked control animals (DEP) showed weight gains similar to the aluminum-treated group (AL).

Open field activity:

No significant difference between groups was seen in the 0-1-minute or 0-5-minute time periods for horizontal ($F=1.8$, $p>.1$ and $F=1.0$, $p>.4$ respectively) or vertical ($F=.83$, $p>.5$ and $F=.28$, $p>.9$ respectively) activity (Table 5).

Passive avoidance:

All but 1 of the 49 animals achieved acquisition criterion on day 1. The remaining rat (from the DL group) did not reach criterion by day 4 and was dropped from the study.

The number of days to reach extinction criteria was profoundly influenced by the various treatments (Fig. 5).

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Table 5. Effects of aluminum administration on open field activity. Number of crossings (horizontal) and rearings (vertical) in a 1-minute and 5-minute period. Groups are as described in methods (see also Table 1). Data expressed as means +/- std. error.

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	Horizontal		Vertical	
Group (n)	0 - 1'	0 - 5'	0 - 1'	0 - 5'
C (7)	27.0 ± 1.8	100 ± 8.2	6.1 ± .9	23.8 ± 3.0
DEP (6)	26.8 ± 2.2	89.0 ± 8.9	6.1 ± 1.0	20.3 ± 3.2
C + D (6)	28.3 ± 2.8	105 ± 8.9	8.0 ± 1.0	23.1 ± 3.0
DH (4)	21.7 ± 2.4	86.0 ± 10.7	4.7 ± 1.9	18.2 ± 3.2
DL (4)	29.7 ± 2.4	103 ± 10	7.0 ± .8	21.7 ± 4.0
ALW (6)	23.7 ± 2.0	80.6 ± 8.9	6.2 ± .8	21.3 ± 3.2
AL (7)	23.3 ± 1.8	91.0 ± 8.2	5.8 ± .5	21.3 ± 3.0

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Figure 5: Reversal of the effect of aluminum sulfate administration on extinction of a learned passive avoidance response. Animals were treated as described in methods (see also Table 1). Treatment groups were as follows: C = control, DEP = water-deprived controls, C+D = controls given DFO, DH = aluminum-treated animals injected with a high dose of DFO, DL = aluminum-treated animals injected with a low dose of DFO, ALW = aluminum-treated animals wihtdrawn from the aluminum sulfate solution prior to testing, AL = animals maintained on aluminum throughout the study. Extinction criterion was achieved when an animal entered the dark chamber in under one minute (LTE < 1 minute) on 3 out of 4 consecutive days. (ANOVA analysis: $p<0.0001$).

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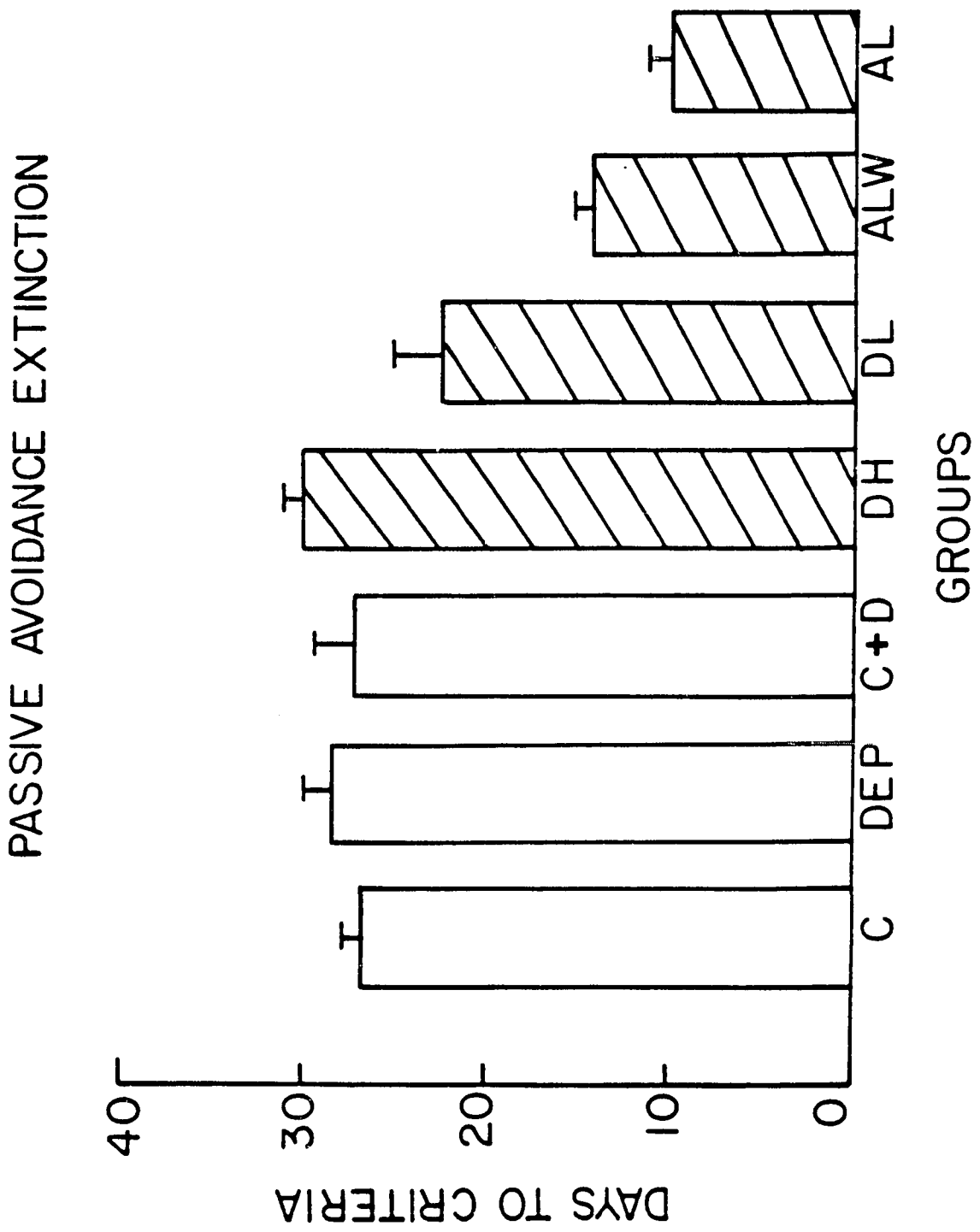

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Statistical analysis revealed a marked overall group effect ($F= 22.7$, $p<0.0001$). Animals treated continuously with aluminum (Al) extinguished significantly faster than all other treatment groups ($p<0.05$). This effect was not secondary to a decrease in fluid intake since the number of days to extinction was similar between the CON and the DEP groups.

The reduction in the number of days to extinction criterion caused by aluminum administration was completely reversed by withdrawl of the aluminum treatment when combined with injection of the high dose of DFO (DH). Although the DH group performed slightly better than controls neither group was statistically different from the controls given DFO (C+D) or the DEP group ($p>0.05$). The lower dose of DFO (DL) significantly improved performance of aluminum-treated animals compared to the Al group, but did not return the behavior to control levels ($P<0.05$). Insertion of the 2-week washout period alone (AlW), also resulted in improved performance when compared to the Al group, but not to the extent demonstrated by either of the drug treatment groups ($p<0.05$).

4. Discussion:

The present study confirms the findings of Study 1 that chronic oral aluminum sulfate administration leads to a faster extinction of the learned response in this passive avoidance paradigm. Developmental effects induced by the lower fluid intake of the aluminum-treated animals did not appear to account for the behavioral deficit since the

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water-yoked controls (DEP) performed at normal levels. Also, the improved performance of animals withdrawn from the aluminum sulfate solution (AlW) provides further evidence that the impairment is not a developmental phenomenon. The possibility that the behavioral impairment was due to a simple dehydration/rehydration effect was discounted since returning aluminum-treated animals to control levels of performance required a high dose of DFO and was not seen by simply removing the aluminum sulfate solution during the 2-week "wash-out" period. The performance of the DEP group also showed that any dehydration due to the reduction in fluid intake did not impair performance on the passive avoidance task. Finally, no signs of dehydration (skin turgor, lack of grooming, etc.) were detectable in any of the animals at the time of testing.

The lack of any significant differences in open field activity at any of the time periods measured supports the conclusion that the toxic effects of aluminum are not due to nonspecific mechanisms (Table 5). Although not measured in this study, the results from Study 1 indicate that there is no apparent difference in sensitivity to footshock between the control and aluminum-treated groups. Similarly, a DFO induced change in shock-sensitivity was not apparent since DFO had no effect on the extinction of control animals (CON vs C+D).

Injection with DFO reversed the behavioral impairment caused by aluminum treatment in a dose dependent manner (Fig. 5). The large variance in the DL group may indicate

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individual animal differences in susceptibility to aluminum or to the drug treatment. The administration of a high dose of DFO to the aluminum exposed animals improved their mean performance slightly above control levels (CON vs DH), but did not have any significant effect on rats not exposed to aluminum (CON vs C+D). When the DFO injected control and aluminum-treated high dose DFO groups were compared (C+D vs DH), no statistical difference was found. It is apparent, therefore, that DFO exerts its effect by removing aluminum from the animal's system and not through non-specific mechanisms. This view is strenghtened by the inclusion of a 1-week period between the last DFO injection and the beginning of the passive avoidance task since DFO has been shown to have a short metabolic half life in the dog ($t_{1/2}$= 75 minutes) and is rapidly excreted in the rat (Peters, Keberle, Schmid & Brunner 1966). Human hemodialysis patients demonstrated a greater than 90% reduction in plasma DFO levels within the first hour after DFO infusion (Ono et al., 1986). The presence of DFO in the animal after the sixth week is therefore very doubtful.

In summary, the behavioral deficits reported in Study 1 have been confirmed and can be reversed by injection of an aluminum chelator. Some improvement in retention was produced by removing aluminum from the animal's water during a "wash-out" period but injection with a chelator was required to return the behavior of the aluminum-treated animals to control levels. The behavioral deficits and their reversal do not appear to be due to developmental or

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nutritional influences but rather to a reversable toxic effect of the aluminum administration.

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V. CONCLUSIONS:

1. Summary:

The results of this dissertation can be summarized as follows:

1.) Chronic oral aluminum sulfate administration induced a specific deficit in the acquisition/consolidation and retention of a passive avoidance task.

2.) No behavioral impairments were noted on open field activity, active avoidance or on the radial arm maze task.

3.) Chronic oral aluminum sulfate administration did not lead to cholinergic degeneration or effect phosphoinositide hydrolysis.

4.) The behavioral impairment caused by this treatment was reversible by administration of an aluminum chelator.

These results demonstrate the potential for neurotoxic effects of aluminum utilizing a treatment paradigm that is relevant to *in situ* human exposure. The reversibility of this model suggests that it may be relevant to the study of dialysis encephalopathy or the early stages of AD and PD. In the final section of this dissertation, these results will be discussed with regard to the aluminum related clinical disorders and implications for future research.

2. Implications for Future Research:

As discussed in the general introduction, aluminum toxicity has been related to dialysis encephalopathy, the

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Parkinson-dementia (PD) complex of Guam and Alzheimer's disease. In all of these disorders, elevated levels of aluminum have been found in the whole brain or in CNS morphological abnormalities associated with the disease. The characteristic neurofibrillary tangle formation seen in Alzheimer's disease and the PD syndrome is generally not observed in dialysis encephalopathy. Recent work however has shown that excess tangle formation can also occur in some cases of dialysis encephalopathy (Scholtz, Swash, Gray, Kogeorgos & Marsh, 1987). Although the nature of the tangle formation (unpaired, paired, etc.) and characteristics of these tangles (staining, antibody binding, etc.) may vary, there is evidence that the tangles from PD and Alzheimer's disease patients may result from a common precusor (Guiroy et al., 1987). The differences between the tangles in these three diseases may result from different rates of, levels of, and/or routes of exposure to aluminum. Other disease states that may be co-present with the aluminum exposure may also influence the development and nature of these disorders. For example, in dialysis patients, onset of dialysis encephalopathy is often correlated with sudden hospitalization (immobilization stress), surgery, or other physiological stressors (Arieff, 1985). In the case of the PD of Guam, other neurotoxins present in the diet (B-N-methylamino-L-alanine, BMAA) as well as other causative factors, may interact with aluminum toxicity to influence the course of the degenerative process (Spencer et al., 1987). However, for all the variations in clinical

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and morphological presentation, aluminum seems to be a common denominator amoung these diseases.

Support for the common aluminum based etiology of these disorders is found in experimental studies. Neurofibrillary tangles and an encephalopathy-like syndrome has been induced in rabbits after intracisternal injection of aluminum salts. These tangles are morphologically different than those manifest in AD but similar to those in ALS. This may be due to species-specific differences in response to aluminum or related to the exposure factors mentioned above. Rodents are generally believed to be resistant to this effect, but a recent study has shown development of encephalopathy-like symptoms after i.c.v. injection of aluminum tartrate to rats (Lipman, Colowick, Lawrence & Abumrad, 1988). Although neurofibrillary tangles have still not been investigated in detail in rodents, it should be noted that the presence of tangles in dialysis encephalopathy is also quite variable and again may be related to the type of exposure (acute, high dose) of both populations compared to that associated with PD and Alzheimer's disease (chronic).

The interrelationship of the three clinical diseases as discussed above, suggests future directions for utilization of this model in the investigation of human cognitive disorders. Co-administration of a neurotoxin (BMAA), lesion of a particular brain region (e.g., nucleus basalis magnocellularis), disruption of synaptic activity (eg. electroconvulsive shock) or other stressors may increase

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the vunerability of the associated neuronal system to aluminum toxicity and produce manifestation of a symptomatology (including morphological changes) similar to that seen in humans. In this view, the lack of effect of aluminum on some of the behavioral tasks in Study 1 can be quite useful. For example, AF64A (a cholinergic-specific neurotoxin) has been shown to be able to induce a dose dependent decrease in ChAT activity (Johnson et al., in preperation) and performance on an active avoidance task (Connor, unpublished observations). Utilization of this neurotoxin in a low dose may allow us to target the neuronal population associated with performance on the active avoidance task and make them more susceptable to aluminum's toxic effects. This enhanced toxicity could be monitored by both biochemical (ChAT) and behavioral (active avoidance) measures. The chronic, oral aluminum sulfate administration paradigm, which mimics 1 type of human in situ aluminum exposure, in combination with a variety of neurotoxins and/or lesioning techniques, may provide us with even more viable model systems for studying the pathology of the aluminum-associated clinical disorders as well as enlightening us as to the relations between these disease states.

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