AN ABSTRACT OF THE THESIS OF

Sara L.W. Armstrong for the Master of Science

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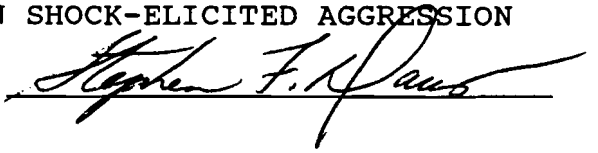
Title: THE EFFECTS OF LOW LEVEL LEAD INGESTION AND CHRONIC ETHANOL EXPOSURE ON SHOCK-ELICITED AGGRESSION

Abstract approved: 

Adult male rats were exposed to 1 of 4 conditions for a period of 74 days. Group Wat was exposed to neither lead nor ethanol, Groups Pb and Pb-ETOH were exposed to a solution of 500ppm lead and Groups ETOH and Pb-ETOH received an injection of a 30% ethanol solution. Intraperitoneal injections were administered to all subjects each day for 15 days in the amount of .25cc/100g of body weight. On the 15th day, each subject received shock-elicited aggression testing 15 minutes after the daily injection. The results indicated that subjects in Groups Pb and ETOH made significantly more and longer duration-in-aggression responses than did those in Groups Pb-ETOH and WAT.

THE EFFECTS OF LOW LEVEL LEAD INGESTION AND CHRONIC ETHANOL EXPOSURE ON SHOCK-ELICITED AGGRESSION

A Thesis

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by

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There are very few experimental studies, especially animal research, that are completed by the efforts of only one person. This study is no exception. My gratitude goes out to Ann Thompson, Celeste Thomas, and Bob Brown for their assistance.

Throughout all of my graduate career, I have had assurance and support from my family and friends. I deeply believe that I would not have made it this far without the love from my husband, Brian, the counsel of my father, and the encouragement of my mother. To you and all my friends, I dedicate this work.

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Chapter 1

Introduction

In order to fully understand any behavioral effects of the combination of lead- and alcohol-exposure, it is first necessary to examine the individual effects of these chemicals. The following sections review selected behavioral effects of lead- and alcohol-exposure, respectively.

Lead-exposure

Since the beginning of time, man has attempted to change his environment to suit his own needs. When he grew tired of carrying water from a stream, he dug wells. When he was weary from walking, he tamed horses for transportation. He invented dishes so he did not have to eat off the ground. He learned to communicate by talking and writing in order to interact with those of his own kind. He built larger and sturdier houses for protection. He even invented medicine to increase his life span. However, with all of this progress, man has paid a great price. Only now are we becoming aware of our reliance upon possibly dangerous chemicals.

In ancient Rome, lead was an extremely common metal. Pipes made out of lead carried drinking water into Roman houses. Roman wine was sweetened with lead. Goblets for

drinking water and wine, as well as dishes, were made of pewter, a mixture of lead and tin.

During the early industrial age, lead had even more uses. It was used in creating letters for early printing presses and was the actual writing element of pencils. Automobiles, run by a lead-based battery and gasoline containing lead, were created to replace the traditional horse-drawn carriage. To color these new automobiles and to brighten up one's house, lead-based paints were made.

Only recently has man realized his mistake. Lead, it seems, can build up in the body causing changes in behavior and even death. It is believed that lead contact, or exposure, may cause behavioral changes (Nation, Grover, Bratton & Salinas, 1990; Thatcher, Lester, McAlaster & Horst, 1982). So, man no longer uses lead in wines, pipes, pewter, pencils or gasoline.

Within the last few years, much research has been amassed covering the detrimental effects of lead on a variety of animals. The reaction of rodents, such as mice and rats, to toxic chemicals tend to mirror human reactions. Thus, results may tentatively be generalized from the animal model to the human population.

Schroeder and Mitchener (1971) found that lead is a highly toxic metal that leads to tumors as well as affecting the growth, survival rate, and length of life in mice and rats. In fact, Schroeder and Mitchener (1971)

were forced to discontinue their experiments with lead-exposed mice due to the decline and subsequent death of the subjects. The lead-fed rats also showed a higher rate of failures to breed, a higher rate of young deaths and more birth defects than did normal rats. By the third generation, lead-exposed rats gave birth to a significantly greater number of runts.

In addition to disrupting development, lead-exposure also affects behavior. Nation et al. (1990) found that certain behavioral changes were attendant on lead ingestion. Their animals were trained to bar press in order to receive food. The lead exposed rats showed "an increase [in] movement, decreased rest time and increased amount of vertical activity, relative to a control condition" (Nation et al., 1990, p. 102). Similarly, heightened levels of bar pressing in lead-exposed rats were noted by Nation, Frye, Von Stultz and Bratton (1989). Their lead-exposed rats also bar pressed significantly more than did animals on a lead-free diet.

One possible reason for the behavioral changes may be found on the neurochemical level. Lead has been shown to have an effect on the production of the neurotransmitter serotonin, which is thought to influence the sleep/wake cycle. Cupo and Donaldson (1988) discovered that chicks who had been exposed to lead had significantly higher levels of 5-hydroxyindoleacetic acid [5-HIAA], the

precursor of serotonin, in their brains than did chicks who had not received lead. Perhaps the increase in 5-HIAA, and the subsequent increase in serotonin, results in a change of sleeping patterns and consequently, a change in behavior.

Physical development and behavior changes are not the only detrimental effects of lead-exposure. Thatcher et al. (1982) found a strong negative relationship between scores on the Wechsler Intelligence Scale for Children - Revised (WISC-R) and the amount of lead found in the children's hair, i.e., the lower the scores, the higher the lead content. They discovered that children who had been exposed to lead scored significantly lower on overall I.Q., performance I.Q., and verbal I.Q. than did non-lead-exposed children. Additionally, there was a higher frequency of learning disabilities, hyperactivity and general behavior disorders in those children with the higher amounts of lead. Thus, it appears that exposure to this toxin may affect cognitive functioning as well as physical development and behavior.

Although the adverse effects of lead are well documented, lead still remains in our environment. Numerous slums and underdeveloped foreign countries continue to use older lead pipes and lead-based paint. Many cars still continue to run on leaded gasoline. Factories release lead into the atmosphere.

Alcohol Effects

Another chemical that man has used and abused, is alcohol. Neolithic man discovered alcohol (Ray & Ksir, 1987). Berries fermented resulting in alcohol; perhaps cavemen enjoyed the feelings of relaxation that such fruit instilled. Cavemen may have given the fermented fruit some religious value, as did the Catholic Church (Ray & Ksir, 1987). In the time that Jesus of Nazareth lived, wine was used for celebrating weddings and births. Later, the Catholic Church declared that wine was the sacred blood of the Savior.

In the early nineteenth century, alcohol was found to have medicinal purposes. Doctors used alcohol to relax a patient before surgery, so alcohol became known as a type of anesthesia (Ray & Ksir, 1987). Doctors also discovered that infection would not spread if alcohol had been used as a disinfectant (Ray & Ksir, 1987).

Relevant to the present project, it is noteworthy that research relating ethanol exposure and aggressive responding has been reported. Tramill, Wesley and Davis (1981) demonstrated an increase in hostility or aggressiveness due to low level (.25 cc per 100 grams of body weight) ethanol exposure. Likewise, Tramill, Gustavson, Weaver, Moore and Davis (1983) found that rats injected with an ethanol-saline solution containing 30% ethanol made more and longer aggressive responses when

given a mild electric shock, than did animals injected with isotonic saline. Thus, the relationship appears to be rather straight forward: chronic ethanol challenges result in higher levels of aggression.

Rationale for the Present Study

Researchers at Texas A & M University have found that the effects of ethanol may be negated by lead exposure. For example, Nation, Grover, Burkey, McClure and Bratton (1992) showed that rats receiving a lead-diet and ethanol injections displayed better performance on bar pressing tasks than animals receiving only ethanol. Lead-exposed rats were less sensitive to the suppressive effects of ethanol, so it would take more ethanol in a lead-exposed animal to produce the same level of intoxication as an animal given only ethanol.

Nation, Grover, and Bratton (1991) tested to see if rats that were deprived of liquid for 24 hours would continue to lick a solution of sucrose and water even if the animal's tongue was given a mild electric shock after every few licks. Rats given an injection of ethanol prior to testing made the most licks and received the most shocks. Animals receiving a lead-adulterated diet received the same amount of shocks as animals receiving neither lead nor ethanol. The group given ethanol and the lead diet received more shocks than either the lead only or the lead and ethanol free group. However, they received fewer

tongue shocks than the ethanol only group. It seems that lead-exposed animals need more ethanol than non-exposed animals to produce a similar state.

Further evidence of an increase in tolerance due to lead exposure was found in a study by Nation, Dugger, Dwyer, Bratton and Grover (1991). They discovered that when given a choice between water and ethanol, lead-exposed rats consumed greater amounts of ethanol. This effect was not observed in non-lead-exposed animals. This "pattern of increased ethanol drinking among lead-treated animals may derive from diminished sensitivity to 10% ethanol, which is normally considered aversive to rats" (Nation, Dugger, Dwyer, Bratton & Grover, 1991, p. 478). Thus, lead may attenuate, or dilute, the normal effects of ethanol.

Since studies have shown that lead causes behavior changes (Nation et al., 1990; Nation et al., 1989) and since aggressive states have been reported when the animals are exposed to low levels of ethanol (Tramill et al., 1983; Tramill et al., 1981), one would expect that rats who had been exposed to a combination of both toxins would be extremely aggressive. However, if lead exposure counteracts the effects of ethanol (Nation, Grover, & Bratton, 1991; Nation, Dugger, Dwyer, Bratton & Grover, 1991; Nation et al., 1992), then animals exposed to both lead and ethanol should have lower levels of aggressive responding than animals exposed either to lead

or ethanol alone.

In the present investigation four groups of rats were studied to see if there were any differences in the level of aggression following exposure to a different combination of lead- and/or ethanol- exposure. The first (control) group was exposed to neither lead nor ethanol. This group was used to determine what the "normal" level of aggressive responding would be. The second group of rats was only exposed to ethanol. The third group was exposed only to lead. These groups were utilized to discover whether each toxin independently increased aggressive responses. In order to ascertain the combined efforts of the two toxins, the fourth group was exposed to both lead and ethanol.

Based upon the previous data, the following predictions might be entertained. The ethanol and lead only animals would be expected to display nondifferential, but high, levels of aggression. Lower, and perhaps indistinguishable, levels of aggression should be shown by the control and the lead-and-ethanol-exposed groups.

Chapter 2

Method

Subjects

Twenty-seven male albino Holtzman rats (Sprague-Dawley Company, Madison, WI) served as subjects. The animals were approximately 38 days old at the beginning of the experiment and 113 days old at the time of testing. The animals were housed in the Emporia State University animal vivarium in Visser Hall. They were maintained in individual wire-mesh cages with both food and water freely available throughout the experiment.

Apparatus

Testing took place in a shock-elicited aggression apparatus consisting of a restraint tube, a shock source (Stoelting, Model 26170), a target rod (Lafayette, Model 80111, omnidirectional lever), an impulse counter (Lafayette, Model 58022), and an electronic digital timer (Lafayette, Model 80200). The opaque plastic restraint tube measured 21.5 cm in length and 7.5 cm in diameter.

The subject was placed into the restraint tube with the tail extended through a 1.5 cm hole in the enclosed end. Then the tube and subject were placed on a wooden platform containing the target rod. The rod was parallel with the floor and extended across the middle portion of the open end of the tube. Attack upon the rod resulted in

activation of the counter and timer.

A wooden dowel was attached with adhesive tape to the subject's tail. Two copper wires, spaced 7 cm apart, were fixed to the dowel and served as electrodes. In turn, the wires were connected to the shock source.

Procedure

Upon arrival from the supplier, the animals were randomly placed into cages and randomly assigned to one of four groups: Group WAT (water, n = 6), Group ETOH (ethanol, n = 7), Group Pb-ETOH (lead, ethanol; n = 7), Group Pb (lead, n = 7). Groups WAT and ETOH had free access to water for the 74 days preceding testing. Groups Pb-ETOH and Pb had free access to a solution of 500ppm lead during this period. The lead solution was prepared by mixing .92 grams of lead acetate per liter of water. Bottles for all groups were weighed and filled every day. All bottles also were thoroughly scrubbed every 5 days to remove any precipitate that had formed.

Groups WAT and Pb were given an injection of a .09% saline solution each day for the 15 days prior to and including testing. Groups ETOH and PB-ETOH received a daily injection of an ethanol-saline mixture consisting of 30% ethanol on these days. All injections were administered intraperitoneally in the amount of .25cc/100g of body weight.

On the day of testing, each subject was tested

individually 15 minutes after receiving its injection. Following placement in the test apparatus, the subject was given a 3-minute period to habituate to the apparatus. Following habituation, each subject received 3 minutes of tail-shock administration consisting of 60, 2.50-mA, 300-msec shocks. The intershock interval was 3 seconds. The number of aggressive responses and the length of time spent in responding was recorded for each subject.

Chapter 3

Results

A separate unweighted means analysis of variance (ANOVA) was performed on the response and duration data. In all instances an alpha level of 0.05 was set as the criterion for determining significance.

Prior to analysis the response rates were converted to $\log_{10}(x_i + 1)$ scores in order to insure normality of distribution. Group mean responses are shown in Figure 1. The analysis of variance detected a significant difference for the groups factor, $F(3, 23) = 6.089$, $p < .003$. Subsequent Newman-Keuls tests showed no significant difference in the rate of aggressive responses between Groups Pb and ETOH or was there a difference between Groups WAT and Pb-ETOH. However, subjects in Groups Pb and ETOH made significantly ($p < .01$) more aggressive responses than did those in Groups Pb-ETOH and WAT.

The second analysis of variance looked for differences in the duration of the aggressive responses. Group mean duration of aggression (seconds) is shown in Figure 2. Significance was found between the groups, $F(3, 23) = 7.149$, $p < .001$. Subsequent Newman-Keuls tests showed that Groups WAT and Pb-ETOH did not differ significantly. Group ETOH spent significantly ($p < .01$) longer in contact with the target rod than did either Group

WAT or Group Pb-ETOH. Group Pb had significantly ($p < .01$) longer duration-in-aggression scores than any of the other groups.

Figure Caption

Figure 1. Mean $\log_{10}(X_i + 1)$ aggressive responses for Groups Pb, ETOH, Pb-ETOH, WAT.

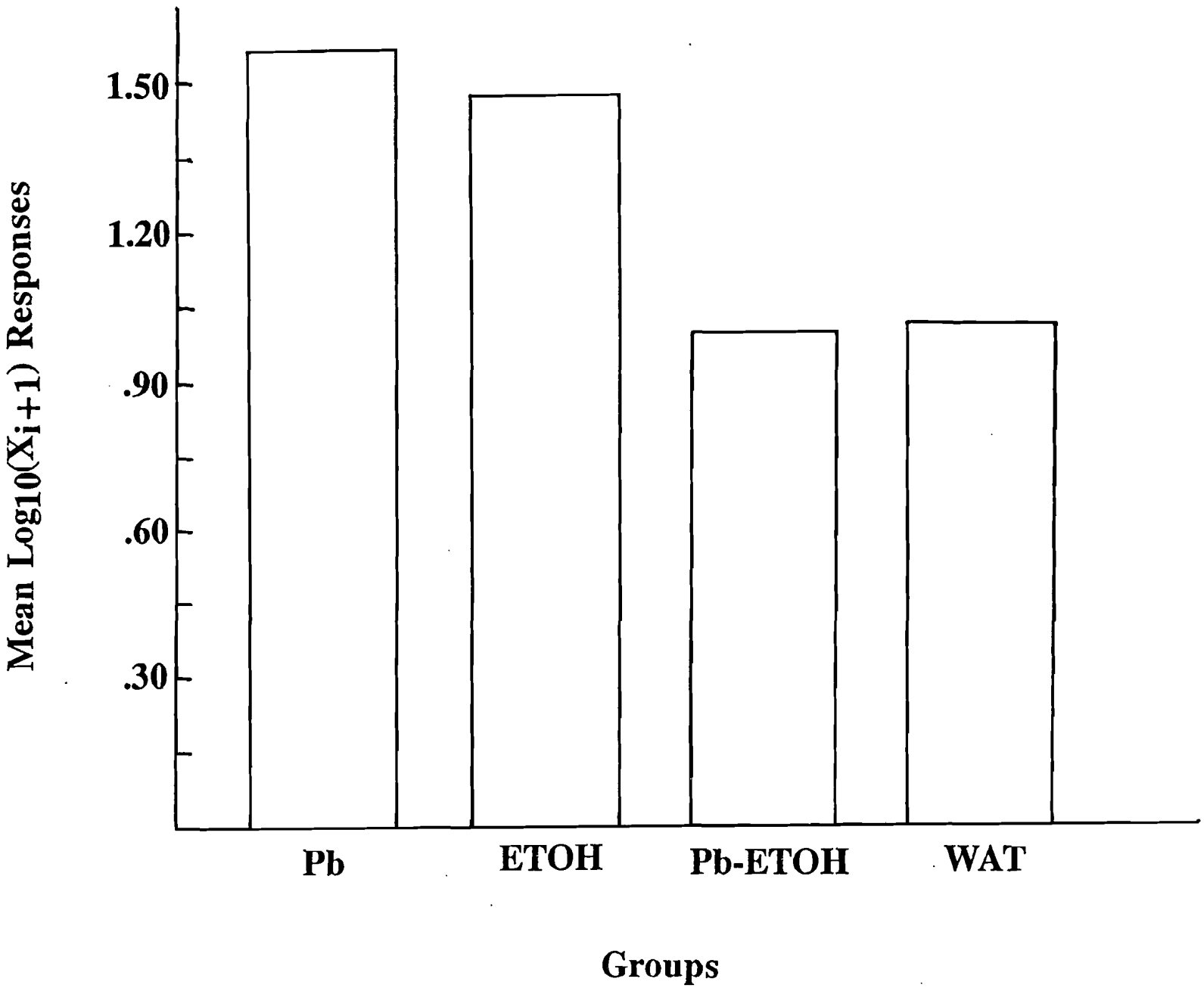
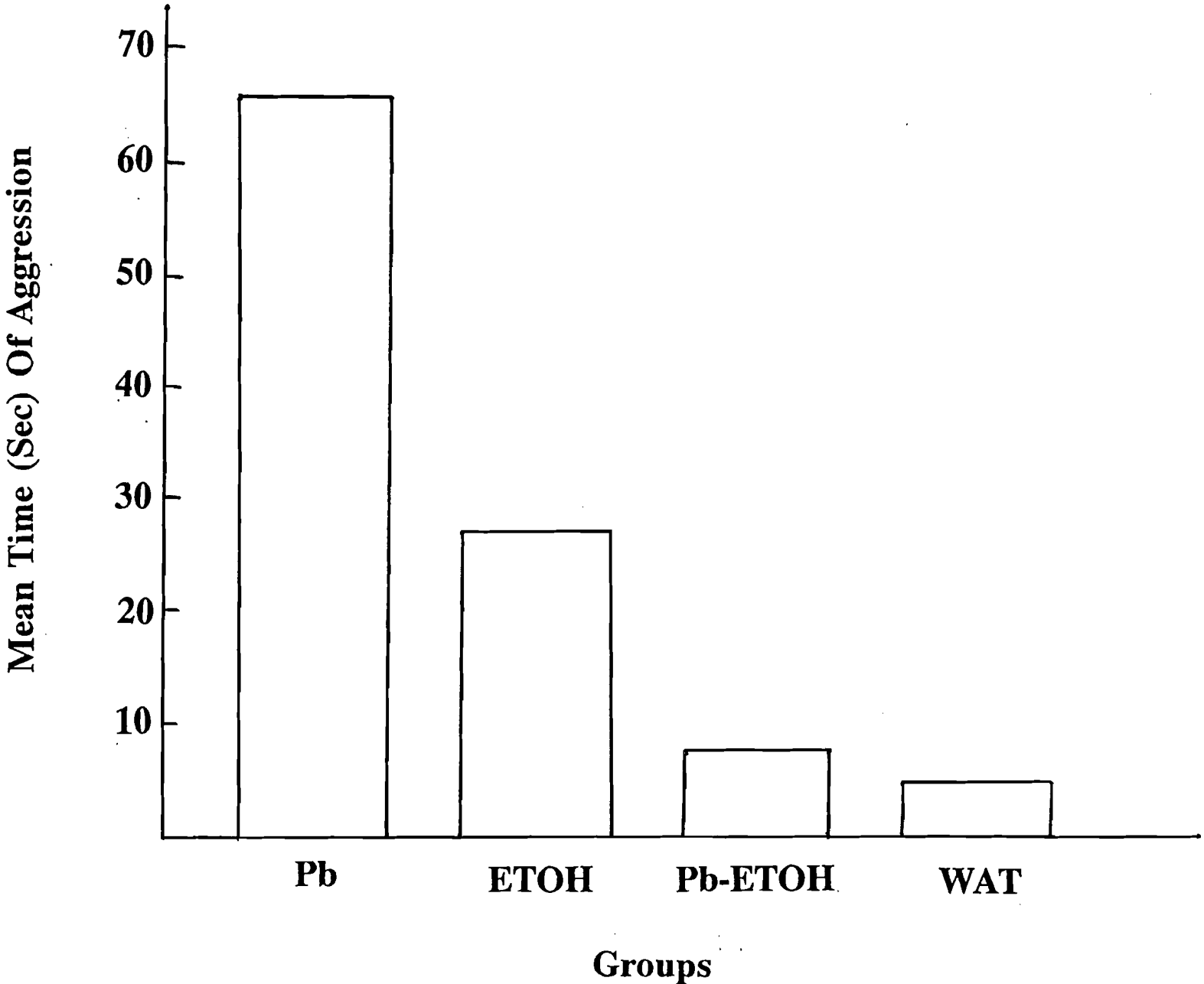

Mean Log10(Xi+1) Responses
1.50
1.20
.90
.60
.30
Pb
ETOH
Pb-ETOH
WAT
Groups

Figure Caption

Figure 2. Group mean duration of aggression (seconds) for Groups Pb, ETOH, Pb-ETOH, and WAT.


Mean Time (Sec) Of Aggression
70
60
50
40
30
20
10
Pb
ETOH
Pb-ETOH
WAT
Groups

Chapter 4

Discussion

Consistent with previous findings (Nation et al., 1990; Nation et al., 1989), the animals exposed to only lead were more active than the animals in the non-exposed group. In the aforementioned studies by Nation et al. (1990) and Nation et al. (1989), the increased activity-level of their animals was observed via an increase in bar pressing. In the present experiment, the animals directed activity towards the target rod. More responses and longer-duration responses were recorded for lead-exposed animals than non-exposed animals.

The performance of the group only exposed to ethanol corroborated the results of previous research (Tramill et al., 1983; Tramill et al., 1981). By showing an increase in number of responses and the duration of these responses over that of the non-exposed group, this ethanol-exposed only group clearly supports the theory that chronic ethanol challenges result in higher levels of aggression (Tramill et al., 1981).

The most interesting result of this study dealt with the group of animals exposed to both lead and ethanol. These rats made fewer aggressive responses and spent less time in contact with the target rod than animals exposed to either toxin alone. In fact, there was no significant difference reported between the lead- and ethanol-exposed

group and the control group, which was exposed to neither toxin. This "seemingly unaffected" behavior lends support to the theory put forward by Nation, Dugger, Dwyer, Bratton & Grover (1991) which states that lead-exposure may attenuate, or dilute, the normal effects of ethanol-exposure.

Although the present study showed that together lead- and ethanol-exposure will not result in behavior different from that of exposure to neither toxin, there are still many questions left unanswered. One limitation of this study, as with previous studies (Nation et al., 1989; Nation et al., 1990; Nation, Grover, & Bratton, 1991; Nation, Dugger, Dwyer, Bratton & Grover, 1991; Nation et al., 1992; Tramill et al., 1981; Tramill et al., 1983), is that of the sample population. All of the subjects were male albino rats from the Sprague-Dawley Company (Madison, WI). One wonders if the same results would be obtained if the subjects were female rats or a different breed of rat.

Although the effects of toxic chemicals on rats tend to mirror those of humans, it is difficult to generalize with great confidence from studies having data from only one small subgroup within the entire rat species. Further research should be directed towards finding a complete understanding of the behavioral effects of the combination of lead and ethanol in the overall rodent population. Additional research should be directed towards

comprehending what is happening on the neurochemical level when both toxins are ingested, as well as finding other chemicals that when administered concurrently will attenuate any negative behavioral effects, such as aggression.

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May 15, 1993

THE EFFECTS OF LOW LEVEL LEAD INGESTION AND CHRONIC ETHANOL EXPOSURE ON SHOCK-ELICITED AGGRESSION

Doug Cooper

Signature of Graduate Office Staff Member

May 4, 1993

Date Received