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Effects of Subchronic Dietary Lead Exposure on DMTP and DNMTTP Performance in Fisher 344 Rats

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EFFECTS OF SUBCHRONIC DIETARY LEAD EXPOSURE ON DMTP AND
DNMTP PERFORMANCE IN FISHER 344 RATS

by

Joshua D. Vardigan

A Thesis
Submitted to the
Faculty of The Graduate College
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Joshua D. Vardigan

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Joshua D. Vardigan, M.A.

Western Michigan University, 2007

Recent attention has focused on the possibility that even low-level Pb exposure can produce subtle neurological impairments in the absence of overt signs of toxicity. Previous behavioral assessments of learning and memory using DMTS procedures have documented learning impairments in monkeys exposed to Pb during early postnatal development, and although a variety of operant behavioral assays have documented detrimental effects of dietary Pb treatment in rats, the DMTS procedure has not been examined in rats following short-term low levels of Pb through dietary exposure. Thus, the objective of this study was to measure the degree of cognitive impairment produced by chronic dietary Pb exposure in rats and to determine the degree to which the separate aspects of this test are sensitive to that impairment. Animals exposed to Pb acetate for a 90 day period were trained on a DMTP task. Following acquisition, animals were introduced to a non-matching reversal of the DMTP design (DNMTP). Delay-dependent significant differences in response accuracy and number of sessions until criterion was met were found between treatment groups on the reversal task only. These results indicate that a sub-chronic duration of dietary Pb exposure does produce significant cognitive impairment in rats which are measureable not through the initial acquisition of the DMTP task, but in the subsequent acquisition of the reversal of that task.

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INTRODUCTION

Investigations into the neurobehavioral effects of low-level and subchronic dietary lead exposure have been motivated both by the discovery that such exposure can potentially cause significant cognitive deficits in children, and to help understand the mechanisms by which learned behaviors can be impaired. This study was undertaken as part of a series of studies in collaboration with another laboratory to examine both the behavioral impairments and immunological responses occurring in mammals as a result of exposure to environmentally relevant behavioral toxicants—specifically, those found in high quantities in the Kalamazoo River. The area into which the Kalamazoo River drains, or the Kalamazoo River watershed, stretches 162 miles and terminates in Lake Michigan. Comprising approximately 2,450 lakes and ponds and totaling 37,500 acres, the Kalamazoo watershed covers about 2,000 square miles, and it is inhabited by about 400,000 people. There is increasing recognition and concern regarding the potential for adverse human health effects that occur as a result of environmental contamination, as wildlife from this watershed have been shown to have elevated contaminant levels in blood and tissues. The primary toxicants of concern in the Kalamazoo River are PCBs, PAHs, solvents, and trace metals such as lead (Pb) and mercury (Hg).

There exists a substantial amount of evidence illustrating the rate-altering effects of responding patterns resulting from sub-chronic, low-level dietary lead exposure under fixed-interval schedules of reinforcement (Cohn, et al., 1993, Cory-Slechta, 2002). However, this effect on operant behavior has questionable relation to the observed effects

of dietary lead exposure on learning and behavior in children exposed to lead at different stages in early development. These studies have primarily investigated lead-induced correlations between fixed-interval responding and dopamine function in the nucleus accumbens--a response pattern and brain region which, though intriguingly affected by Pb exposure, are not often implicated with regard to memory function.

One of the primary cellular actions of Pb is the blocking of voltage-gated calcium channels resulting in the reduction of evoked transmitter (primarily glutamate) release (Evans *et al.*, 1991, and Minnema *et al.*, 1988), even at low concentrations (Büßelberg *et al.*, 1993). Dose-dependent reductions in glutamate release in Pb-exposed animals have previously been demonstrated using in vivo microdialysis in the hippocampus (Lasley & Gilbert, 1996), which is considered to be the major site of the action of Pb on the brain (Petit *et al.*, 1983). Furthermore, the phenomenon known as Long-Term Potentiation (LTP), a key process of hippocampal synaptic plasticity, is studied as an important component of the cellular basis of learning and memory (Roman *et al.*, 1999). The effects of lead on certain glutamate pathways in the hippocampus, and specifically on glutamate NMDA receptor function, the most extensively studied synapse type involved in the generation and maintenance of LTP, have also been well-established (Gilbert and Lasley, 2007, Zhu *et al.*, 2005, Wang, 2007). LTP is widely accepted as a primary phenomenon in the study of memory formation, and reductions in LTP generation have furthermore been shown to affect performance in memory assays such as the Morris water maze, a spatial memory task frequently used to reveal Alzheimer's-related memory impairments

(Jeon, *et al.*, 2007). Other research has suggested that exposure to environmentally relevant levels of Pb during early life produces deficits in hippocampal synaptic plasticity in the form of LTP and spatial learning in young adult rats (Nihei, 2000), and it has even been demonstrated that LTP deficits may occur at least in part due to lead-induced decreases in neuronal nitric oxide synthase (nNOS) activity (Zhu, *et al.*, 2005), and subsequent nitric oxide (NO) production (Chetty, *et al.*, 2001). Given that the effects of Pb on the central nervous system lie in interactions with the hippocampus and processes underlying this major component of memory (LTP), investigations into the behavioral effects of lead are warranted such that the relationship between Pb, LTP, and behavioral impairment can be tested.

Clearly, there are multiple mechanisms by which Pb may affect the production and maintenance of LTP. Given what is known of the properties of Pb and the requirements for LTP, it is possible that the nature of this effect and its relationship to behavior may not be more easily elucidated than through the testing of an array of possible rescue-treatments and regimens. The interaction of the cholinergic and glutamatergic systems in the generation of LTP was recently expanded upon by Wang (2007), whereby peripheral administration of the cholinergic agonist Carbachol (CCh) was shown to attenuate Pb-induced LTP reductions in the dentate gyrus of the adult rat hippocampus. CCh is a compound that does not normally cross the blood-brain barrier, so its effects when administered peripherally are surprising, and suggest that Pb exposure may even compromise the blood-brain barrier to some degree. Lead is also known to

disrupt the binding of MK-801, a non-competitive NMDA receptor antagonist, while causing disruptions in performance on discriminative operant tasks (repeated acquisition). Cohn and Cory-Slechta (1993) demonstrated a curious subsensitivity of Pb-exposed rats to both the accuracy-impairing and response rate-altering properties of MK-801 by showing that declines in accuracy produced by this glutamate antagonist were attenuated by Pb exposure. Zhu *et al.*, (2005) found that developmental low-level lead exposure could in fact raise the level of NMDA receptor type 2A (NR2A) mRNA expression in the hippocampus of rats, which could suggest that, if MK-801 exhibits a higher affinity instead for the NR2B subunit, this account of mRNA production alteration may account in part for lead's disruption of MK-801 action. This suggests that, although Pb interferes with the actions of MK-801, MK-801 is not likely to affect the actions of Pb after the exposure period.

An alternative account of the physiological effects of Pb apart from LTP production is offered by Verina, *et al.* (2007), who noted detrimental effects of Pb on hippocampal granule cell neurogenesis and morphology. This observation is expounded by Jadhav (2000), who demonstrated that glutamate co-treatment functioned to augment Pb-induced cytotoxicity, while NMDA receptor antagonism partially blocked cell death induced by extracellular Pb exposure—effects that are modulated by intracellular calcium (Ca). Specifically, generation of reactive oxygen species (ROS), shown to correlate with cytotoxicity, was observed in response to Pb exposure in a dose-dependent manner, and was both dependent upon and exacerbated by the presence of glutamate. This Pb-induced

cell death was shown to be partially blocked by addition of MK-801, the binding of which has already been shown to be disrupted by Pb-exposure (Gilbert and Lasley, 2007), suggesting that MK-801 may in fact work in opposition to the detrimental effects of Pb, but only when co-administered, and not as a rescue agent. Additionally, an *in-vitro* study by Oberto, *et al.*, (1996) revealed that a low dose of Pb (1 microM) is sufficient to induce cellular apoptosis in cultured newborn rat cerebellar cells, characterized by cell shrinkage and DNA fragmentation. This dose is well within the range of doses known to induce cognitive impairment in children, and its effects were also found to be attenuated by treatment with a voltage-sensitive calcium agonist known as Bay K8644 (Oberto, *et al.*, 1996). These data suggest that the mechanism by which Pb affects learning and memory may be due at least in part to its promotion of apoptosis *prior* to the acquisition new behavior, and its role in increasing glutamate function (or interacting with increased glutamate function), resulting in the overproduction of ROS and subsequent excitotoxic states, and perhaps not, in this instance, a direct action on receptor functioning or LTP production *during* the task acquisition and discrimination behavior.

Interestingly, although NMDA-mediated LTP in the hippocampus has shown to be a determiner of performance accuracy in the Morris Water Maze, (Leggio, *et al.*, 2006, Yaka, *et al.*, 2007) Pb exposure has been shown not to disrupt spatial learning (Gilbert, 2005). This observation is consistent with the findings of a neuropsychological examination of Mexican children performed in 2001, in which children with a history of chronic exposure to lead and arsenic were examined according to the parameters of the

Wechsler Intelligence Scale for Children. Indeed, Pb exposure was shown not to have an effect on the particular factor of the assessment which purports to measure spatial memory. It was shown, however, to be associated with performance detriments on the sequential factor, which is said to measure short-term memory and attention process, (Calderon, *et al.*, 2001, Vance and Singer, 1979) a finding which has been reported elsewhere in primate studies using a Delayed-Matching-To-Sample, or DMTS, procedure (Rice, 1984). This procedure is a common tool for measuring short-term memory, typically at increasing intervals, by requiring the subject or participant to match a choice sample presented among a number of alternative choice samples to a singular previously presented sample. Sometimes referred to as working memory, or the ability to temporarily retain task-relevant information across a delay, short-term memory performance tends to decrease with increasing delay duration, indicating that this type of memory fades across a delay (Kalenscher, *et al.*, 2005).

This alleged Pb-induced short-term memory impairment observed in primates on an MTP task has yet to be replicated in a rodent model, which is the animal whose tissue is most commonly used in the examination of the physiological characterization of the effects of Pb on the nervous system at the cellular and molecular level. Additionally, solid data of Pb-induced performance impairment on a matching task that is reducible to rodent-based operant chamber manipulations offer a product more readily comparable to results from other similar rodent studies. Important relevant literature contributing to the goal of systematic, functional evaluation and categorization of learning and memory

operations is most often a product of research using basic operant modes of assessment in rodents, and much of this research involves invasive techniques much more widely used in such rodent models, including genetic knockout, brain lesions, and microinjection techniques. The demonstration of a specific type of operant performance impairment and the potential rescue of that impairment ought to be a good step towards determining the molecular mechanisms by which Pb exposure induces cognitive disruption--specifically whether either LTP function or cytotoxicity are primarily involved in the short-term memory processes involved in DMTP performance. To that end, our laboratory previously undertook an investigation into the effects of 30 day dietary lead exposure on the acquisition of a rat adaptation of the DMTS procedure, notated as Delayed-Matching-To-Position, or DMTP. This assay proved to be insensitive to any lead-induced behavioral changes, thus provoking the present investigation to include the addition of a post-acquisition reversal of the task, or Delayed-Non-Matching-To-Position (DNMTP), and a moderate lengthening of the exposure period from 30 to 90 days. Hilson and Strupp (1997) demonstrated that subchronic dietary lead exposure could induce behavioral impairment on the reversal of an olfactory discrimination task, and that such impairments did not occur or were not detected on the acquisition of the original discrimination task.

Hypotheses: Two hypotheses were proposed in this investigation. The first hypothesis was that lead-exposed animals would not differ from controls in the number of sessions required to meet the criterion level of performance on the DMTP task, nor would they

differ in accuracy of performance at any of the delay values once criterion is reached. The second hypothesis was that lead-exposed animals would exhibit both a dose-dependent and a delay-dependent impairment of performance relative to control animals on the reversal (DNMTP) task. That is, lead-exposed animals would show a greater number of sessions to meet the criterion level of performance on the DNMTP task, and the accuracy of responses should differ when compared across individual sessions, with the most notable differences occurring at the largest delay interval between the highest exposure group and the control group.

Accuracy on Hilson and Strupp's olfactory discrimination task, however, was measured as Response vs. Non-Response method, illustrating stimulus control inasmuch as the subjects' ability to discriminate the stimuli in the presence of which a response is followed by reinforcement, and the stimuli in the presence of which that same response is not reinforced. The present investigation expands upon this evidence in an attempt to show the extent to which dietary lead exposure may affect stimulus control over a Correct-Response vs. Incorrect-Response choice paradigm. The Response vs. Response type memory assay is more similar to the style of tasks used in the assessment of cognitive impairment in humans than is the Response vs. Non-Response type, particularly those used for the early detection of Alzheimer's Disease onset, nearly all of which utilize response choices.

METHODS

Subjects: Twenty-four male Fisher 344 rats were used in this study, and were obtained at 21 days of age from Charles River Laboratories (Portage, MI). Animals were individually housed in metabolic cages upon arrival to animal facilities, and they were acclimated to the colony for one week prior to beginning treatment. For the duration of the study, that colony was maintained on a 12 hour light/12 hour dark cycle under constant temperature (20 C +/- 2 C) and humidity (50% +/- 5%) in accordance with the general principles of animal husbandry stated by the U.S. Department of Health, Education, and Welfare (National Research Council, 1996). Following completion of the lead-exposure period, animals were transferred to standard rectangular polypropylene housing with *ad-libidum* access to regular drinking water. Food was restricted to maintained animals at approximately 85% of their free-feeding weights. Behavioral Data were obtained from only 23 of the rats because one animal died prior to the collection of behavioral data.

Apparatus: Behavioral testing was conducted in eight operant conditioning chambers (MED Associates Inc., Georgia, VT), measuring 28 cm x 21 cm x 21 cm. Each chamber contained two retractable levers, one 28V house light for entire chamber illumination, a fan to provide white noise and ventilation, and one stimulus light each in the panel above each lever (panel lights). Programming of experimental events was conducted with MED-PC software installed on an IBM-compatible computer.

Lead Exposure Procedures: Each of the 24 animals was randomly assigned to one of three groups, denoted as X, Y, and Z ($n = 8$). Lead-acetate was then administered in the drinking water of groups X, Y, and Z, at concentrations of 0, 50, and 500 ppm respectively, and continued for a period of 90 days. One subject in the Y treatment group (50 ppm) was found dead on three days following cessation of Pb treatment but prior to behavioral testing; thus no behavioral data were collected from this animal, and group Y was reduced to $n = 7$. Bodyweights, as well as feces and urine samples, were collected approximately every two days during the exposure period.

Behavioral Procedures: The 23 surviving rats were assigned to three different testing squads with roughly an equal number of animals from each treatment group assigned to each squad. The experimenters were blind regarding the lead treatment of each rat until after the completion of behavioral testing. Behavioral training began approximately one week following the concurrent cessation of lead exposure and implementation of food restriction.

On the first day of training, subjects were exposed to a one-hour session of fixed time schedule of food delivery in which one food pellet was delivered into the food receptacle every 60 seconds (FT 60s). Subjects were placed individually into one of the eight operant chambers one group at a time, and all levers were retracted so as not to be present in the chamber during the FT 60s period. This was essentially a respondent conditioning procedure to pair the sound of the pellet drop with the availability of food. Food delivery was not contingent upon any response during this session. Upon

completion of this procedure, subjects were exposed to five 40 minute lever-press-training sessions in which one of the two levers was inserted into the chamber until pressed. Delivery of a food pellet was contingent upon the animal pressing the inserted lever, immediately after which the lights above each sample lever were illuminated, the lever was retracted back out of the chamber and, following a short delay, one of the two levers was again randomly selected and presented.

Following completion of the preliminary lever-press training, the Delayed-Matching-To Position (DMTP) task procedure was implemented similar to those described by Reading and Dunnett (1991). Initial training sessions were conducted for 40 minutes with a 0 s delay and were essentially match-to-position tasks. At the beginning of each trial, either lever (sample) was randomly inserted into the chamber. Any nose entry into the food delivery hopper resulted in the offset of the panel lights and the simultaneous insertion of both levers (choice) into the chamber. A response on the choice lever matching the sample lever for that trial constituted a matching (correct) response, whereas a response on the choice lever not matching the sample lever for that trial constituted a non-matching (incorrect) response. All correct responses resulted in the onset of the panel lights and the delivery of one food pellet into the food delivery tray. The panel lights were turned off and both levers retracted at the first nosepoke into to food hopper following the correct response. Conversely, each incorrect response resulted in the immediate retraction of both choice levers, no reinforcement, and the offset of the house light for a period of 5 s (timeout). Following timeout, the house light was turned back on, signifying the onset of the next trial. As a correction procedure, incorrect trials were repeated.

Once animals met the criterion of at least 90% accuracy for three consecutive sessions under the 0 s delay condition, where accuracy was calculated as percent correct trials per session, a three second delay between the response on the sample lever and the presentation of the choice levers was implemented randomly into half of the trials per session. Progressively higher delay intervals (10s, 30s) were implemented as criterion was met at each preceding delay value (i.e., at least 90% of the total responses were correct responses). Each delay value occurred an equal number of times per session, and all sessions lasted for 40 minutes.

Data Analysis: Accuracy scores underwent arcsine data transformation for statistical comparison (Howell, 1997, Reading & Dunnett, 1991, Winters & Dunnett, 2004). This was due to the fact that the calculation of variance for proportions such as these necessarily depends upon the particular value of each datum in the set, such that the homogeneity of variance assumption is violated. Since the proportion (or percentage) value for each datum also represents a probability value, the calculation of group variance becomes calculated as a probability of a probability, and is thus skewed. The arcsine transformation converts the proportions into their decimal angular components, and in doing so allows the data sets to meet the assumptions of homogeneity of variance. Pairwise comparisons, where appropriate, were made using Fisher's Least Significant Difference (LSD). Graphs displaying percent accuracy data and sessions to criteria were generated using Microsoft XL 2003, and arcsine transformations for analysis purposes were performed on the accuracy data using MINITAB 14 for Windows (MINITAB, Inc., State College, PA). One-way Analysis of Variance tests and Fisher's LSD post-hoc

analyses were performed using SPSS 14.0 for Windows (SPSS, Inc., Chicago, IL).

RESULTS

Delayed-Matching-To-Position (DMTP): Subjects exposed to Pb at either level did not differ from controls in their acquisition of the matching task, nor in their performance accuracy. All subjects acquired the matching task at approximately the same rate. Each group exhibited nearly 100 percent accuracy at the 0s delay condition, with accuracy declining at essentially equal rates between groups as delay values increased, indicated by the downward slope in Figure 1. Total percent accuracy scores for the 0, 50, and 500 ppm exposure groups did not differ at all ($85.48\% \pm 2.55\%$, $82.62\% \pm 1.83\%$, and $84.54\% \pm 1.90\%$, respectively.) Concordantly, no group reached total accuracy criterion for the task in significantly fewer trials than any other. The 0ppm subjects required $6.5 \pm 1.59(\text{SEM})$ sessions to meet criterion, the 50ppm subjects required $7.29 \pm 1.96(\text{SEM})$ sessions, and the 500ppm subjects required $4.75 \pm 0.73(\text{SEM})$ sessions (Figure 2).

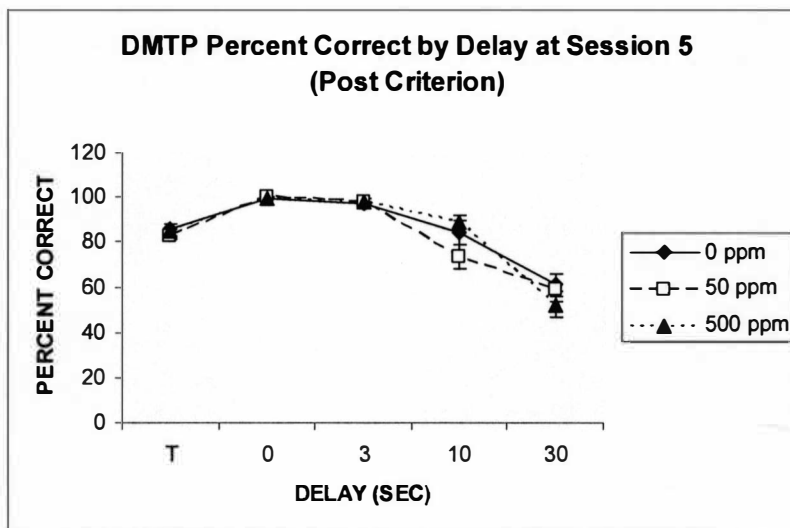


Figure 1. No significant group differences were observed at any delay value following acquisition of the DMTP task. 'T' represents average accuracy across delay values.

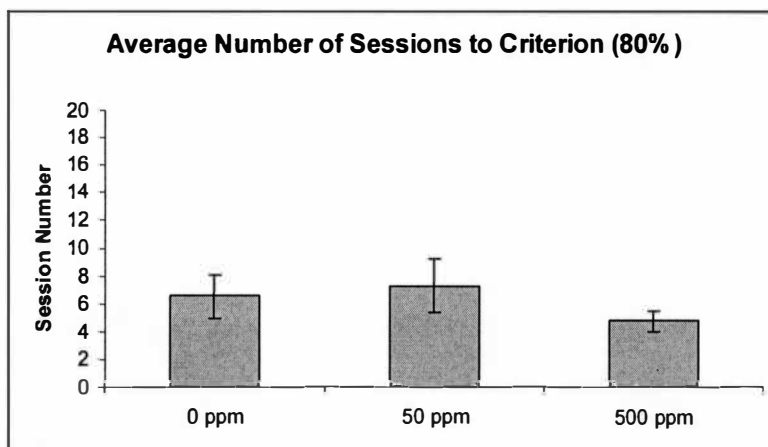


Figure 2. The average number of sessions needed to meet 80% overall accuracy for three consecutive days following the introduction of the 30s delay was not significantly different among groups.

Delayed-Non-Matching-To-Position (DNMTP): A sharp difference between control (group X) and Pb-treated animals (groups Y and Z as represented by 50 ppm and 500 ppm exposure levels, respectively) is evident at the 10s delay at session 10 of the reversal assessment (overall, $p < 0.05$; X vs Y, $p < 0.05$; X vs Z, $p < 0.01$; Y vs Z = 0.87) and only a marginal difference at the 30s delay ($p = 0.078$), whereas by session 20, control subjects exhibited significantly higher performance accuracy at both the 10s (overall, $p < 0.05$; X vs Y, $p = 0.093$; X vs Z, $p < 0.01$; Y vs Z, $p = 0.311$) and 30s delays (overall, $p < 0.05$; X vs Y, $p = 0.132$; X vs Z, $p < 0.01$; Y vs Z, $p = 0.147$) than the Pb-exposed animals. Curiously, the significant differences between high-exposure and control treatment groups also appearing at the 10s delay of session 10 are not evident again until session 20 (Figure 3).

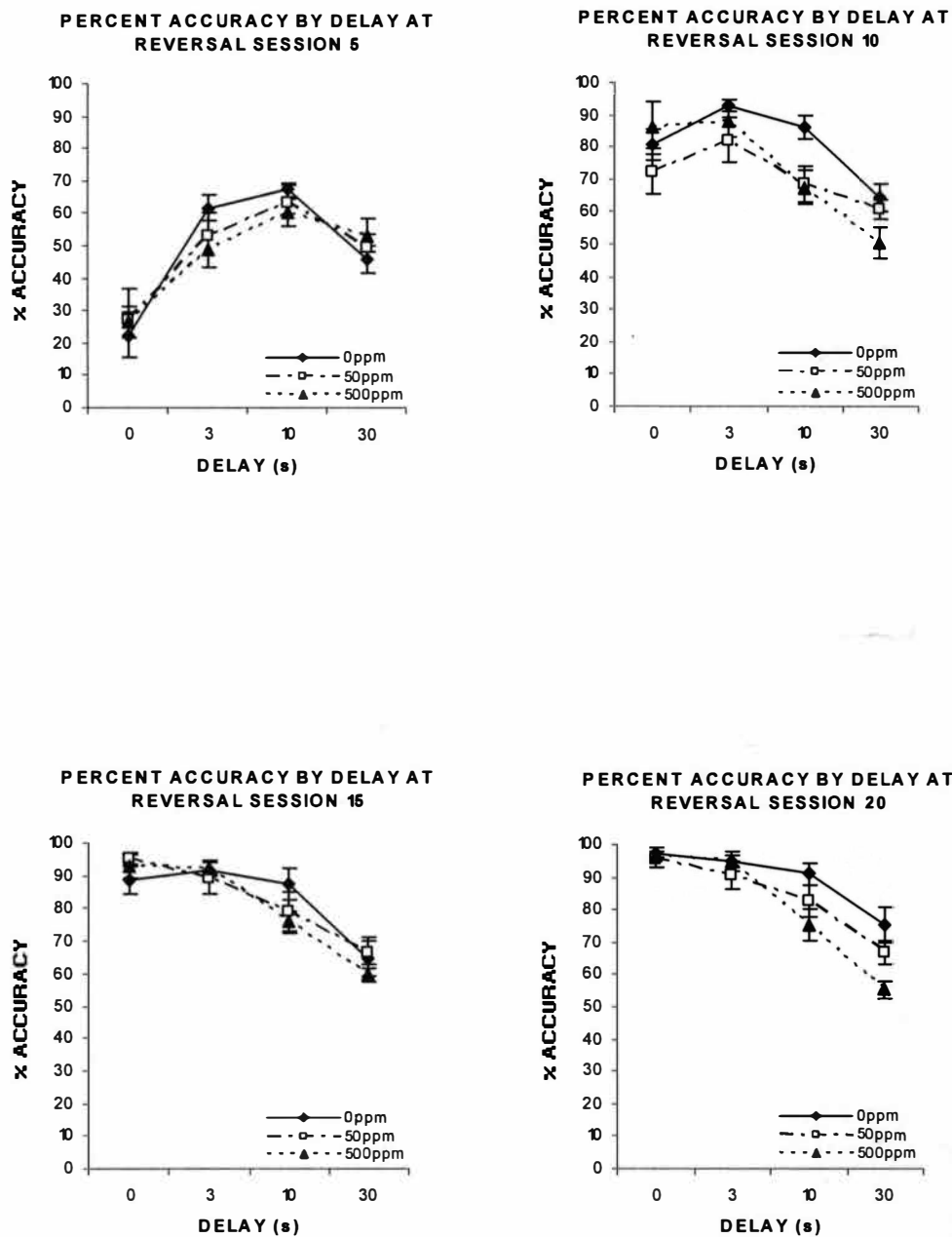


Figure 3. Clear significant differences in group accuracy of performance are notable at both the 10s and 30s delay by the 20th session following acquisition of the reversal (DNMTP) task. Non-Pb-treated animals performed with much higher accuracy than Pb-treated groups ($p=0.028$ and 0.016 , respectively).

Overall performance across all delay values at session 20 was also significantly higher for control animals ($p < 0.05$), with a significant pairwise difference evident between control and high-exposure animals ($p < 0.01$) and a marginal difference between control and low-exposure animals ($p = 0.069$). No difference was detected between Pb groups ($p = 0.381$, Figure 4). Significant differences were also evident when comparing the number of sessions required to reach 80% criteria (overall, $p < 0.05$; X vs Y, $p = 0.08$; X vs Z, $p < 0.01$, Y vs Z, $p = 0.329$). The 0 ppm group (X) reached criterion following reversal in 13.25 ± 1.00 sessions, whereas the 50 and 500 ppm groups (Y and Z) reached this criterion in 17.14 ± 1.64 sessions and 19.25 ± 1.71 sessions, respectively (Figure 5).

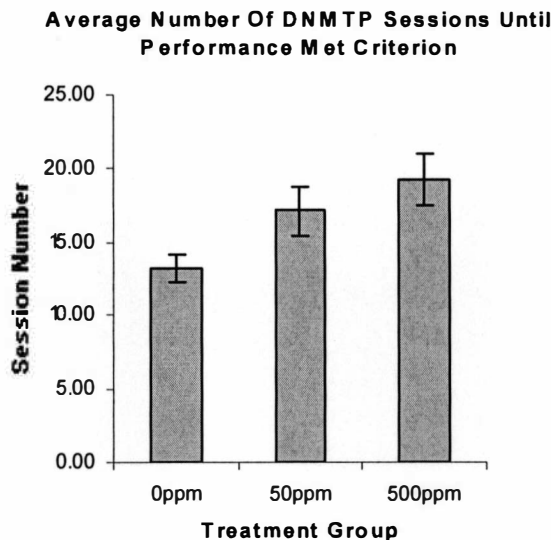


Figure 4. The average number of sessions needed to meet 80% overall accuracy for three consecutive days following the introduction of the 30s delay was significantly higher in both Pb-treated groups on the reversal (DNMT) task ($p = 0.025$), illustrating a slower acquisition, or rate of learning, in those groups.

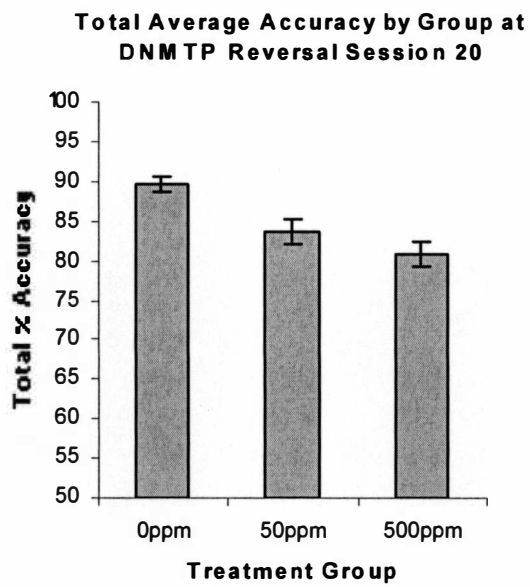


Figure 5. The control group exhibited significantly higher overall accuracy across all delay values by session 20 ($p = 0.026$).

DISCUSSION

The results of this study indicate short term dietary Pb exposure does have detrimental cognitive effects in Fisher 344 rats, as measured by the reversal of the DMTP procedure once it has been acquired. Previous reports concur with the finding that Pb-induced behavioral impairments are exhibited not through the initial acquisition of a task, but in the subsequent acquisition of the reversal of that task (Hilson and Strupp, 1997). It may be arguable that the DNMTTP task itself is simply more difficult than DMTP, perhaps by lending itself less to spatial-mediating strategy. Pache, Sewell, and Spencer (1998) present an opposing view, however, demonstrating that the known robust anti-cholinergic amnestic agent scopolamine produced delay-dependent effects only on the DMTP portion of a combined matching and non-matching protocol while producing delay-independent effects on the DNMTTP trials. They suggest that this delay-independent effect may reflect a non-specific sensorimotor impairment or perhaps an effect on reference memory rather than short-term memory function. Given that behavior was examined on a combined schedule, however, any general motor impairment ought to be necessarily evident in all aspects of the trial, so it is unlikely that non-specific effects are indicative of general sensori-motor impairment in this instance. Curiously, the rest of the results reported by Pache *et al.* (1998) conflict with those of the present study, in which delay-dependent differences were evident in the non-matching (reversal) sessions and were not observed in the matching (DMTP) sessions as might be expected if the latter protocol were indeed a better indicator of short-term memory function, or if Pb exposure

functions to affect performance in any way similar to scopolamine. Given scopolamine's anticholinergic effects, it is worth noting that Popke (2003) observed a particularly remarkable relationship between the cholinergic and NMDA receptor systems involved the anticholinesterase toxicity typical to Alzheimer's Disease, and that cholinergic (specifically nicotinic) agonists have been shown to offset learning and memory impairments induced by MK-801 in rats—a phenomenon which may prove to be functionally similar to Pb effects on MK-801 actions (Levin, et al., 1998). Taken in conjunction with the effects of the cholinergic agonist CCh demonstrated by Wang (2007), this evidence warrants behavioral testing following co-administration with cholinergic agonists during the Pb exposure period.

Non-specific, or delay-independent performance decrements as observed by Pache, *et al.* (1988) may be effects specific to the anti-cholinergic actions of scopolamine; furthermore, scopolamine-induced delay-dependent effects on DNMTTP trials like those observed in the present study could reasonably be expected to be observed to a greater extent than on the DMTP trials (rather than not at all) if in fact the DNMTTP task were generally a more difficult one. The question as to whether the DNMTTP task specifically is the more reliable method of gathering discernible data on Pb-induced cognitive impairments in rats, or if in fact the reversal of any acquired task is sufficient, could be raised, given the results of the present study. Given that the reversal challenge reveals performance error due to a perseveration to the previously correct response, in this way it is similar to the non-matching procedure itself, which essentially

requires the animal to respond on the operandum opposite that which was previously required. It might, therefore, be useful to undertake a similar investigation in which the order of the behavioral assessments is reversed.

The obvious ideal objective would be to gain a more specific notion of the nature of error produced by Pb exposure and, if discernible, derive from it clues as to which of the previously discussed physiological mechanisms are responsible for the observed behavioral alterations. It is, of course, possible that Pb could produce detrimental physiological effects somewhere far enough upstream to cause several different observable physiological changes which, all things considered, could produce more than one type of behavioral impairment. Alternatively, several such physiological changes could result in one major behavioral impairment, which the present research may bring us little closer to categorizing. It is also conceivable that there may exist some physiological effects of lead exposure that are altogether unrelated to behavioral effects, or at least those that have yet been measured.

The significant disparities in performance accuracy exclusively at the 10s delay appearing by the 10th post-reversal session and disappearing by the 15th session, only to reappear again by session 20, may merely be indicative of the gradual learning occurring in all groups during the process of acquiring of the reversal task. The control group acquired accuracy at the 10s delay much earlier than the treatment groups, but *no* animals had yet performed with any notable level of success at the 30s delay. By session 15, treatment groups had begun to perform at relatively the same accuracy as control animals

on the 10s second delay, suggesting that, given enough history of exposure to an operant task, lead-exposed animals are able to retain short-term working discriminative memory across significant intervals to at least some similar degree to that of controls. By session 20, however, control animals were again performing with significantly higher accuracy at the 10s delay, and had also reached a level of marginal accuracy ($75.27\% \pm 5.19\%$) at the 30s delay, while the 50 ppm and 500 ppm exposure groups had only discriminated with $66.76\% (\pm 3.61\%)$ and $55.15\% (\pm 2.92\%)$ accuracy, respectively.

Due to the multiple observable effects of Pb on behavior as well as on neurophysiological function, it is indeed difficult to reconcile the present study with past research in such a way that decisive mechanisms can be extracted and confronted as true culprits. However, it could perhaps be said with some amount of confidence that, as chronic co-treatment with CCh has previously been shown to attenuate Pb-induced LTP reductions, the improvement of DNMTTP (reversal) performance following co-administration of CCh during the Pb exposure period would be a strong indicator in favor of LTP-reduction as the mechanism for impairment (Wang, 2007). Furthermore, being that the reversible LTP detriments recorded by Wang (2007) were not specifically the result of cell death, it would be especially important to investigate whether CCh post-treatment could improve performance on the reversal task, in an attempt to indicate a non-permanent nature of Pb toxicity. Alternatively, glutamate post-treatment could be tested as a rescue measure, as Pb-induced reduction in glutamate release is a possible component of LTP reduction.

Although MK-801 exerts effects on LTP in opposition to those of Pb, post-treatment with MK-801 administered throughout behavioral training and testing should not be sufficient to reverse this performance deficit in the way that it has been shown to reverse Pb-induced apoptosis, as this type of cell destruction is irreversible. Stephens and Cole (1996) recorded a significant MK-801-induced decrease in performance accuracy on a DMTP task in rats, and it has been established that both MK-801 and Pb exposure in isolation each impair induction and maintenance of LTP. It is indeed curious, then, that NMDA antagonists such as MK-801 work in opposition to the effects of Pb exposure when introduced together, and that Pb would attenuate MK-801-induced impairments in a similar fashion. Indeed, Gilbert and Lasley (2007) recently reported that Pb exposure blocks the ability of MK-801 to reduce LTP generation in the dentate gyrus of the hippocampal formation. As the magnified post-synaptic potential observed in the LTP phenomenon is NMDA-dependent, it is suggested that developmental Pb exposure disrupts the binding of MK-801 in such a way as to profoundly attenuate its effects in the presence of LTP-induced upregulation of NMDA receptors. Oddly, Pb exposure is also known to reduce presynaptic glutamate release (Lasley & Gilbert, 1996), which would seem to be somewhat in opposition to its post-synaptic effects on NMDA antagonist binding. If this effect is in fact related to the tendency for Pb exposure to impair LTP induction on its own, then it is a factor not present in Gilbert and Lasley's investigation, as LTP was induced by generating artificial stimulus trains in that instance. It follows, then, that despite its opposing effects on LTP

to those of Pb, MK-801 should only be expected to improve performance if the impairment is apoptotic in nature, and only if it is administered prior to behavioral testing.

CONCLUSIONS

The current study has demonstrated that a relatively subchronic exposure period (90 days) of lead acetate at levels as low as 500ppm induce delay-dependant performance deficits on the non-matching reversal task (DNMTP) of the DMTP procedure. While performance accuracy in all groups declined in a delay-dependent manner, accuracy scores for the high-exposure animals were lower at the 10s and 30s delays by session 20, and the number of sessions to criteria was lower for the control group, confirming our second hypothesis. Most deficits observed in the low exposure group were marginal, with the only real significant difference appearing at the 10s interval of the 10th session, indicating that either 50ppm is too low a dose to induce cognitive impairments in rats, 90 days is too short an exposure period, or this assay is yet too sensitive to any impairments produced at in this way. In accordance with our first hypothesis, no differences were detected on the DMTP procedure, suggesting that the DNMTP reversal is a sensitive and reliable rodent assay for detecting Pb-induced cognitive impairment with considerable choice vs. choice face validity.

This assay provides an operant behavioral means of examining the effects of an array of agents on rescuing or altering Pb-induced impairments as a means of discovering the mechanisms at work, specifically whether the effects are primarily related to cellular processes occurring during learning, such as LTP, or permanent effects occurring prior to learning, as in the case of cytotoxicity. Flora, *et al.*, (2007) recently reported that a combination treatment with multiple Pb-chelating agents, which can bind with Pb to reduce the impact of its actions and allow it to be excreted safely, was sufficient to reverse Pb-induced apoptosis in adult rat brain preparations. Future studies should then

investigate the degree to which the observed Pb-induced behavioral impairments can be prevented by concurrent administration of such Pb-chelators, as well as NMDA receptor antagonists (namely MK-801), and calcium modulation. Increases in performance accuracy following any of these treatments would likely be an indicator that Pb is influencing short-term memory primarily through irreversible cytotoxic mechanisms, and not by hindering neuroplasticity. Co-treatment with CCh throughout Pb exposure could be expected to attenuate our observed performance impairment if in fact the impairment detected on this assay is a result of Pb-induced alterations in LTP processes, but effective treatment may not be limited to the exposure period, and this effect on LTP may be found to be reversible. As CCh co-treatment increased LTP amplitudes in Pb-exposed animals only through co-treatment and not through post-treatment, post-treatment with CCh should be investigated as a possible rescue agent (Wang, 2007). However, if LTP generation were being reduced due to decreases in transmitter release, glutamate treatment or introduction of Pb-chelating agents following exposure during the training period could also be expected to attenuate behavioral impairments somewhat. If, however, the mechanism by which Pb produces cognitive impairments is indeed through cytotoxicity, the unfortunate indication of such a discovery would be that the cognitive impairments suffered by exposed children are likely permanent. Together with the previous research involving the cellular and molecular effects of Pb on the central nervous system and behavior, the present study provides a reliable mode of further elucidating the function and rescuability or preventability of Pb-induced cognitive impairments that should be expandable to human populations.

APPENDIX

Institutional Animal Care and Use Committee Approval Form



Date: December 16, 2005

To: Lisa Baker, Principal Investigator
Jay Means, Co-Principal Investigator

From: Robert Eversole, Chair

Re: IACUC Protocol No. 05-11-03

Your protocol entitled "Neurobehavioral Effects of Subchronic Dietary Lead Exposure in Fisher 344 Rats" has received approval from the Institutional Animal Care and Use Committee. The conditions and duration of this approval are specified in the Policies of Western Michigan University. You may now begin to implement the research as described in the application.

The Board wishes you success in the pursuit of your research goals.

Approval Termination: December 16, 2006

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