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The Effects of Lead, d-Amphetamine, and Time of Day on Activity Levels in the Mouse

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DOLINSKY, Z., E. FINK, R. G. BURRIGHT AND P. J. DONOVICK. *The effects of lead, d-amphetamine, and time of day on activity levels in the mouse.* PHARMAC. BIOCHEM. BEHAV. 14(6) 877–880, 1981.—Mice were exposed to lead acetate (0.5%) pre- and postnatally, and activity levels were assessed at 21 days of age. Two measures of open field activity were employed at two different times of day across three doses of d-amphetamine. These factors influence the results observed in lead exposed mice and demonstrate that lead's effects on activity are not invariant. Implications for future research as well as the suggestion of an animal model for childhood hyperactivity are discussed.

Lead acetate d-Amphetamine Time of day Open field Activity Attention deficit disorder Hyperactivity Animal models

LEVELS of lead in our environment have been increasing, and thus the deleterious effects of this toxic heavy metal have been of particular concern [14]. Although there have been numerous reports on the behavioral effects of low-level lead exposure in rodents, it has been difficult to unequivocally determine its effects on activity. For instance, hyperactivity [3, 10, 13, 15, 16], hypoactivity [1, 4, 12], and no change in activity [8,17] following lead administration have been reported.

While Silbergeld and Goldberg [15,16] suggested that lead exposure may be an etiological factor in childhood hyperactivity, other reports fail to support their hypothesis. Experimental designs and exposure protocols used in these other studies varied considerably and may thus account for the conflicting results which have been reported by different researchers.

By now it is clear that lead's effects on activity are situation specific and thus might be expected to be altered by time of day as well as developmental stage of the animal. In particular, juvenile organisms are most sensitive to the toxic properties of lead [6]. Further, it has been shown that task specificity is an important determinant of lead's effects on behavior [8,10]. In addition, amphetamine has been reported to have therapeutic effects in hyperactive children as well as different effects on control and lead-treated mice [16,18].

Thus, the effects of lead exposure on the behavior of 21 day old male mice were examined using two measures of open field activity, at two different times of day, and across three doses of d-amphetamine. We also measured blood-lead levels to determine the effectiveness of our exposure procedure.

METHOD

The 139 male Binghamton (Fuller) HET stock mice [2] used in this study were derived over a six month period from 29 mating pairs. Three weeks prior to mating, and before any behavioral testing, group-housed male and female mice were moved from their home vivarium having an illumination cycle of white light on from 0800 hr to 2000 hr and red light on from 2000 hr to 0800 hr and were housed in a phase-shifted vivarium having a 12 hr light/12 hr dark cycle. White light onset was at 0300 hr and offset was at 1500 hr and the phase shifted vivarium was illuminated with red light during the dark phase. This phase shift was instituted to facilitate behavioral testing in the middle of the dark phase. Behavioral testing was carried out in a room adjacent to the phase-shifted vivarium. Testing in the dark-phase was under red light illumination, while light-phase testing was performed under white light illumination.

At the time of mating and three weeks after the lighting regime had been changed, two treatment groups were formed. One group of parents received a 0.5% aqueous lead acetate solution [15,16]. This solution was made by dissolving lead acetate in boiling distilled water. The other group of parents received distilled water as their sole fluid source. Both groups received Charles River Mouse Chow ad lib. Lead solutions were made afresh and solutions changed two times a week to minimize lead precipitation. Fathers were removed

from the cages 10 days after the initial mating. At the time of birth, litters were culled to a maximum of six pups, retaining as many males as possible. Typically there were from four to six males per culled litter. Data from one litter which was reduced to two pups were included. Mothers continued to drink their assigned solution through gestation and lactation and the pups had direct access to this fluid as they matured. The pups were not weaned by the experimenter. Thus, the mother and littermates remained housed together for the duration of the study, but only males were utilized in the behavioral measures of the experiment. To assess the effectiveness of the phase shifting procedure both food and fluid consumption in five control and 11 lead litters was measured twice during the experiment on arbitrary days at 1500, 2000, 0300 and 0800 hr. The pups varied in age from eight to 20 days in the litters which were so examined.

Developmental Measures

Number of pups born per litter in each group was recorded. In addition, the day on which both eyelids were first separated was recorded. Body weight of 21 and 35 day old animals was recorded.

Behavioral Measures

For the open field measure half of the litters in each treatment group were randomly assigned to a light-phase testing condition, while the other half was randomly assigned to a dark-phase condition. Each animal was tested in the open field during either the middle of the light (0900–1200 hr) or dark (2100–2400 hr) phase of the light/dark cycle. Testing was carried out in the light-phase under white light illumination and in the dark-phase under red light. At 21 days post partum animals were weighed and moved to a separate testing room adjacent to the vivarium where they were injected IP with either isotonic saline (0), 1, 5, or 10 mg/kg d-amphetamine. Each of the drug×dose×lighting conditions was comprised of from 8 to 10 animals, and 6 to 8 litters were represented in each condition. Immediately after the injection the animals were placed in a small, covered, holding container (400 ml translucent plastic beaker, bottom covered with straw). At 20 minutes after the injection, the cover was removed and the cup was placed on its side in a white open field (26.7×26.7×30.5 cm) which had a smooth Plexiglas floor divided into nine, 8.9×8.9 cm squares. If the animal did not come out of the cup within 10 sec he was gently removed from the cup and placed in the open field. The number of squares crossed, nonassisted stand ups, and assisted stand ups (one or both paws on wall) was recorded for a three min period.

Blood-Lead Analysis

On Day 35 post partum animals were decapitated. Trunk blood was collected and pooled for each litter. With the help of the Department of Special Chemistry at Wilson Hospital (Johnson City, NY) the blood was analyzed for lead content using standard atomic absorption spectroscopy employing ammonium pyrolidine dithiocarbonate (APDC) chelation and methylisobutyl ketone (MIBK) extraction [5,9].

RESULTS

On the average, nine HET pups were born per litter regardless of treatment. In addition, the number of male and female pups was approximately equal and was not altered by lead treatment. One control mother died and one lead mother cannibalized three of her pups; lead and control groups had similar pup mortalities (male and female culled litters: control 8/84, lead 9/96). In addition, there was one sterile mating in the lead group and three sterile matings in the control group. Data were pooled from both males and females, and the mean day of eye opening was 13.2 for the control group and 13.5 for the lead group, $F(1,165)=6.84$, $p<0.01$.

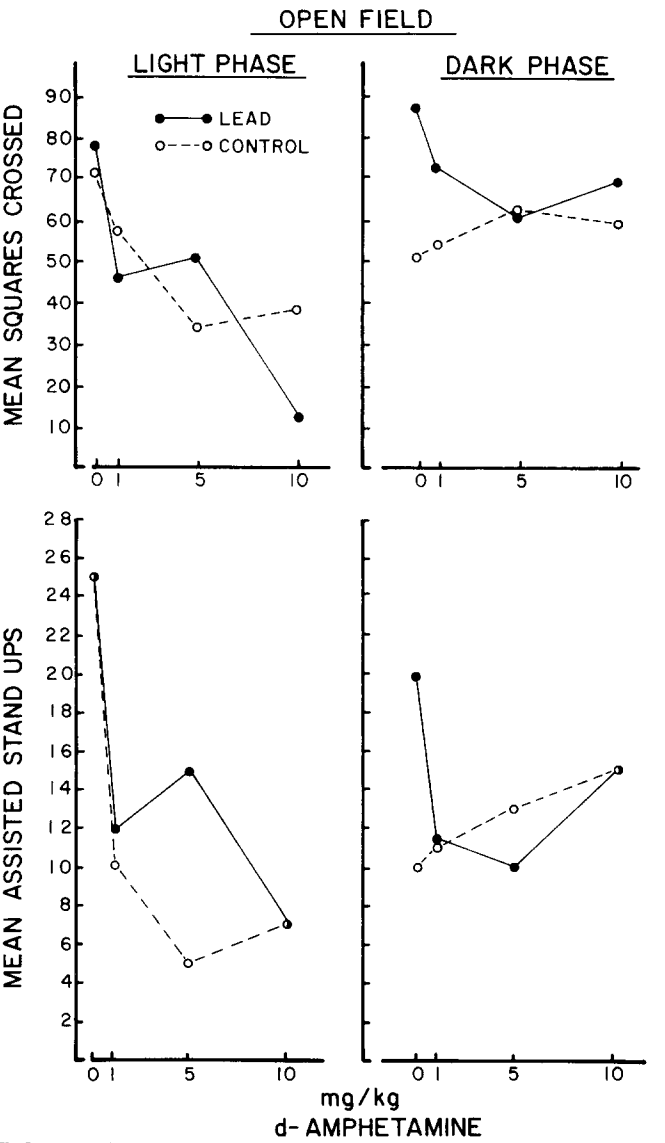

FIG. 1. Activity levels in control and lead treated animals receiving d-amphetamine during open field testing in light- and dark-phase. Note: Each point represents independent groups.

Both control and lead groups consumed approximately two times as much water and food during the dark phase of the shifted cycle than during the period of time corresponding to the dark-phase of the pre-shifted cycle. Thus, a normal diurnal pattern of consummatory behavior was established by our phase-shift procedure. Although, particularly in the dark, there were some indications of depressed food and fluid intake in lead treated mice, comparisons between groups were not performed because the litters were not equated with respect to the number or age of pups.

Body Weight

Lead treated mice weighed less than controls at both 21 (9.3 vs 11.3 g) and 35 (18. 6 vs 21.6 g) days of age (Treatment effect: $F(1,131)=86.64$, $p<0.0001$). In addition, the control group gained an average of 10.3 grams from age 21 to 35 while the lead group gained only 9.3 grams (Treatment×age: $F(1,131)=11.17$, $p<0.001$).

Blood-Lead Levels

Lead treated mice had a mean blood-lead level of 102.8 μg/% while the control group's blood-lead level was only 0.84 μg/%, $F(1,16)=70.23$, $p<0.0001$. These results indicate that our exposure regime was successful in elevating blood-lead levels.

Open Field Activity

Within the context of this experimental design several *a priori* questions are relevant with respect to lead treatment, activity levels, and the effects of d-amphetamine. Thus, the results observed in the open field were considered within the framework of an unweighted means analysis of variance and planned comparisons. In lieu of an overall analysis of variance, main effects and interactions of the dose and treatment factors within each level (light, dark) of the phase factor were performed. In addition, activity levels between lead and control groups were compared at each of the 0 dose conditions [7]. One animal was dropped from the analysis because the number of squares crossed by this subject was 8 SD's above the mean of the other animals in its group. In addition, stand up data from several animals were inadvertently not recorded. Frequency of non-assisted stand ups was low in all groups, thus only squares crossed and assisted stand up data were analyzed. It should be noted that all points in Fig. 1 represent data from independent groups.

Squares Crossed

The response to amphetamine was more apparent in both the control and lead treated groups in light- as compared to dark-phase testing. At 0 mg/kg (saline injections) during dark-phase testing lead treated animals crossed more squares than controls, mean=87 vs mean=51; $F(1,17)=5.84$, $p<0.05$ under red light, but both groups were similar in showing relative unresponsiveness to amphetamine at 1, 5 or 10 mg/kg.

In contrast, when animals were tested in the light-phase under white illumination, amphetamine decreased activity in both groups, Dose main effect: $F(3,61)=13.41$, $p<0.001$. However, a differential response to amphetamine was noted between the groups, Dose×Treatment: $F(3,61)=2.84$, $p<0.05$, and can be attributed primarily to the fact that lead treated animals crossed fewer squares than controls at the 10 mg/kg dose, $F(1,61)=5.28$, $p<0.05$. Further, in contrast to dark-phase testing, saline (0 mg/kg) injections did not differentiate the groups with respect to the number of squares crossed.

Assisted Stand Ups

The assisted stand up data tend to parallel those seen for the squares crossed data. Once again the response to amphetamine was more apparent in both the control and lead treated groups during light- as compared to dark-phase testing. Furthermore, in the dark phase, lead treated animals displayed more assisted stand ups than controls at 0 mg/kg, mean=20 vs mean=10; $F(1,11)=8.77$, $p<0.025$; but, as in the squares-crossed data, both groups were relatively unresponsive to the administration of amphetamine.

As was noted in the squares-crossed data with animals tested in the light phase, amphetamine decreased assisted stand ups in both groups, Dose main effect: $F(3,53)=14.22$, $p<0.001$. However, in contrast to the squares-crossed data, there was no statistically significant interaction with respect to amphetamine responsiveness. Further, as was noted in the squares-crossed data, saline injections did not differentiate the groups with respect to assisted stand ups during light-phase testing.

It is of interest that lead treatment may alter light/dark activity patterns in the open field relative to controls. That is, saline injected control animals tested in the light phase showed greater levels of activity than comparable controls tested in the dark phase. This pattern of light/dark activity was statistically reliable in the assisted stand up data, mean=25 vs mean=10; $F(1,11)=8.77$, $p<0.025$, with a similar trend suggested by the squares-crossed data, mean=70 vs mean=50; $F(1,15)=3.37$, $p<0.1$. However, lead-treated animals injected with saline did not show any significant differences in these activity measures during light- vs dark-phase testing.

DISCUSSION

This study emphasized that the behavioral effects of lead exposure are not invariant and are influenced by the parameters employed in an experimental design. Although mice were tested only once during the light and once during the dark, it is evident that such factors are important when evaluating both the effects of lead and amphetamine on behavior. In addition, dosage level of amphetamine as well as the dependent measure chosen to assess activity can be critical factors in differentiating control and lead-treated animals. Thus, caution should be exercised in generalizing results obtained from any set of conditions for the purpose of presenting typical or expected behavior elicited by lead exposure.

The importance of such points is apparent when considering the suggestion that lead exposure in animals provides a model for hyperactivity in children. One group of researchers [11] have shown that normal and hyperactive children do not differ in the behavioral and cognitive effects of a single dose of amphetamine. Both groups showed decreased activity and increased ability to direct attention. These findings argue against a "paradoxical" effect of amphetamine as a factor which differentiates normal and hyperactive children. In the present study, amphetamine decreased activity in both control and lead treated mice, however only during light phase testing. This finding also suggests the absence of a "paradoxical" effect of amphetamine in lead treated versus control mice, and provides some evidence for the utility of a lead based model for childhood hyperactivity. However, such a model will demand continued discovery and investigation of the parameters important in influencing lead's effect on behavior. Although the literature on lead exposure has reported the use of a wide variety of experimental protocols, we do not yet have a good understanding of the factors which may interact with and alter the behavioral effects of lead ingestion. In addition, evaluation of such a model cannot be simply accomplished since the important behavioral components of childhood hyperactivity as well as factors affecting them are poorly understood and constantly being reassessed. This is demonstrated by the fact that childhood hyperactivity has

recently been renamed "Attention Deficit Disorder" to emphasize that attentional deficits, rather than hyperactivity *per se*, are perhaps the important components of the disorder [18]. It thus becomes difficult to employ animal research to directly characterize a human disorder whose definitional components are not firmly established.

Future research should be directed towards a more systematic and rigorous examination of how various experimental parameters influence the behavioral effects observed with lead exposure. Such research, both animal and human, in conjunction with clinically derived insights should provide a much improved understanding of the nature of human behavior disorders which may be associated with and/or effectively modeled by exposure of the organism to lead.

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