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BRIEF COMMUNICATION

Lead-Produced Changes in the Relative Rate of Open Field Activity of Laboratory Rats[1]

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DRISCOLL, J. W. AND S. E. STEGNER. *Lead-produced changes in the relative rate of open field activity of laboratory rats.* PHARMAC. BIOCHEM. BEHAV. 8(6) 743–747, 1978. – Four groups of rats, continuously exposed to one of two lead acetate solutions, ad lib water or a limited amount of water, were tested for three daily 5 min periods on the open field. The effects of treatment on activity, relative to animals drinking ad lib water, depended upon the concentration of the lead acetate solution. Animals exposed to a 10^{-4} M lead acetate solution showed increased overall activity while animals exposed to a 10^{-2} M lead acetate solution showed changes in the relative rate of activity. Activity was not affected by limiting the amount of water consumed. These findings illustrate the importance of recording activity in a manner which allows assessment of changes in activity as well as absolute level.

Lead acetate Lead and activity Lead and behavior Open field

A NUMBER of investigators have reported increases in the motor activity of laboratory rodents exposed to lead compounds during early development [7, 9, 10, 11, 13]. In addition, it has been suggested that this phenomenon provides a non-human model of lead-produced hyperactivity in children [12]. There are, however, a number of findings which are not consistent with this conclusion. For example, Sobotka and Cook [15] exposed rat pups to lead acetate and reported no differences in the activity of 24–28 day old lead-exposed and control animals tested for 30 min on a photoactometer. Sobotka, Brodie and Cook [14] reported similar results for both the initial 15 min and the final 30 min of a 60 min test period in a photoactometer for one month old lead-exposed and control rats. Brown [3] tested 7 week old lead-exposed rats in a photoactometer for 15 min and obtained no differences in activity when compared with controls. In the same study, 7 week old lead-exposed rats showed levels of activity, rearing and defecation during a 5 min test on the open field which were similar to those of control animals. Driscoll and Stegner [5] tested 30 day old rats for 2 min on the open field and reported lower activity scores for rats exposed to a 10^{-2} M lead acetate solution while no difference in activity was found for rats exposed to water or a 10^{-4} M lead acetate solution.

The major reasons for the lack of consistency in these findings appear to be differences in (a) techniques used to record activity, (b) methods of administration and dosage levels of lead, and (c) length and detail of activity recording. The present study addressed itself to the third of these concerns by examining the behavior of rats on the open field for three daily 5 min periods. Activity scores were recorded each minute to allow analysis of change in activity as well as total activity levels.

METHOD

Experiment 1

Nine adult albino female rats, bred from stock originally obtained from Simonsen Laboratories (Sprague-Dawley derived), were paired with nine adult males of the same strain and provided with ad lib Purina chow. These animals were not experienced breeders. Three pairs were randomly assigned to each of three lead exposure conditions in which one of the following drinking solutions was provided: 10^{-2} M (2070 μg Pb/ml) lead acetate (high lead); 10^{-4} M (20.7 μg Pb/ml) lead acetate (low lead); or water (control). Once assigned to a lead exposure condition, all animals were maintained on the same drinking solution throughout the experiment.

When visual inspection showed females to be pregnant, males were removed. Seven litters with at least 10 pups occurred; litters larger than 10 were immediately culled to 10. One litter in the low lead condition contained 5 pups and one litter in the high lead condition contained 6 pups. Pups were weighed every third day until Day 10 and on Days 14, 21 and 28.

At 30 days of age, 30 control (21 males, 9 females), 25

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low lead (13 males, 12 females) and 26 high lead (16 males, 10 females) animals were tested individually on a square open field which measured 1.3 meters on a side and was divided into 16 equal squares. Testing was conducted in normal fluorescent lighting (approximately 270 lx) at or near the beginning of the light phase of a 12/12 light–dark cycle. Each animal was tested for a period of 5 min on each of three consecutive days. The number of squares that an animal entered during each minute of the test was recorded. Daily defecation scores were also recorded.

Experiment 2

One litter of 11 high lead animals and one litter of 10 low lead animals were reared using the procedures of Experiment 1. In addition, 8 animals from one litter were assigned to a limited water condition in which the mother and her pups were given access to the average amount of water consumed by high lead animals in previous experiments. This amount was adjusted for litter size. At 30 days, the limited water group (5 females, 3 males) was tested on the open field using the procedures previously described.

On Day 33, animals were killed with CO_2. Blood, liver and kidneys were removed and preserved by freezing. Lead content of tissues was determined by anodic stripping voltammetry using an internal cadmium standard [6].

RESULTS

Experiment 1

Figure 1 shows the mean number of squares entered on the open field during each minute of the 5 min test period over the three days of testing and the summed daily activity for each day of testing. An initial analysis of variance revealed that sex differences did not occur and the data were pooled for both sexes. A repeated measures, un-

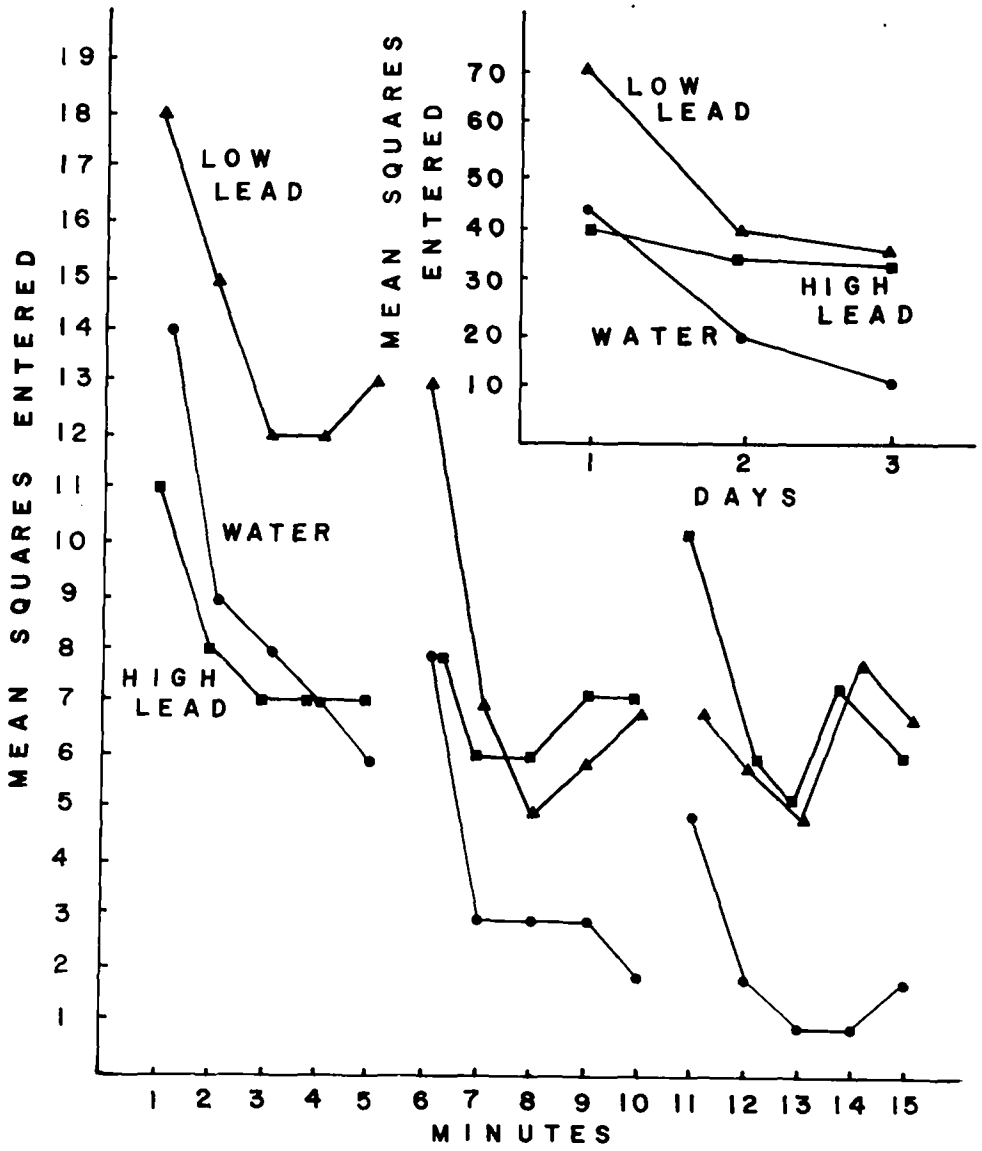

FIG. 1. Number of open field squares entered by 30 day old rats ingesting 10^{-2} M lead acetate (high lead, N = 26), 10^{-4} M lead acetate (low lead, N = 25) or water (N = 30) in three daily 5 min tests.

weighted means analysis of variance [8] revealed a significant main effect of Minutes, $F(14,1092) = 28.58$, $p<0.001$; a significant main effect of Lead $F(2,78) = 12.76$, $p<0.001$; and a significant interaction, $F(28,1092) = 3.75$, $p<0.001$. To clarify the nature of the interaction, activity scores were analyzed for the 5 min of each day of testing. An analysis of the first 5 min showed a significant main effect of Minutes, $F(4,312) = 23.93$, $p<0.001$, and a significant main effect of Lead, $F(2,78) = 20.91$, $p<0.001$. The interaction was not significant. A similar analysis of variance performed on the second 5 min of testing revealed a significant main effect of Minutes, $F(4,312) = 15.77$, $p<0.001$, a significant main effect of Lead, $F(2,78) = 4.80$, $p<0.05$, and a significant interaction, $F(8,312) = 2.78$, $p<0.01$. The interaction was further examined using F tests for simple effects [4]. These tests showed that significant interactions occurred between the high lead group and the water group, $F(4,312) = 2.93$, $p<0.025$, and between the high lead group and the low lead group, $F(4,312) = 4.23$, $p<0.005$. Activity scores of the water and low lead groups did not interact. Analysis of the third 5 min test period produced results similar to those of the first 5 min with a significant main effect of Minutes, $F(4,312) = 8.79$, $p<0.001$, a significant main effect of Lead, $F(2,78) = 9.65$, $p<0.001$, but no interaction.

The summed daily activity scores were also subjected to analysis of variance. This analysis produced results similar to the previous analysis of activity by minutes with a significant main effect of Days, $F(2,156) = 51.42$, $p<0.001$, a significant main effect of Lead, $F(2,78) = 13.29$, $p<0.01$, and a significant interaction, $F(4,156) = 7.23$, $p<0.001$. These data were further examined using F tests for simple effects. Analysis of the Lead x Days interaction showed that the high lead group interacted significantly with both the low lead group, $F(2,156) = 12.44$, $p<0.001$ and the water group, $F(2,156) = 9.04$, $p<0.001$, but these two groups did not interact with one another.

Summed daily activity scores were also analyzed separately for each day of testing. Analysis of Day 1 activity revealed a significant effect of Lead, $F(2,78) = 21.60$, $p<0.001$. A Newman-Keuls' multiple range test [4] showed that on Day 1, the low lead group ($\bar{X} = 70.2$ squares) was significantly more active than the high lead group ($\bar{X} = 40.0$ squares, $p<0.01$) and the water group ($\bar{X} = 44.0$ squares, $p<0.01$) but these two groups were not different from one another. A significant Lead effect also occurred on Day 2, $F(2,78) = 4.81$, $p<0.025$, but a different pattern emerged with the water group, ($\bar{X} = 19.6$ squares) significantly less active than the low lead group ($\bar{X} = 37.8$ squares, $p<0.05$) and the high lead group ($\bar{X} = 33.7$ squares, $p<0.05$). These two groups were not significantly different. The same pattern was evident on Day 3 with a significant main effect of Lead, $F(2,78) = 9.56$, $p<0.001$. The water group ($\bar{X} = 11.0$ squares) was once again less active than the low lead group ($\bar{X} = 34.9$ squares, $p<0.01$) and the high lead group ($\bar{X} = 33.4$ squares, $p<0.01$) while these two groups were not significantly different. Defecation scores were also subjected to analysis of variance but did not differ signficantly for the 3 groups.

Means and standard deviations of the body weights of the three groups of Experiment 1 and the limited water group of Experiment 2 are presented in Table 1. Analysis of variance of the weights of the water, low lead and high lead animals showed these groups to be significantly different ($p<0.05$) on all days except Day 7 when the differences approached significance, $F(2,78) = 2.94$, $0.10>p>0.05$. Newman-Keuls' multiple range tests showed that the high lead group was significantly lighter than both the water and low lead groups on all days except Day 7 ($p<0.05$). Low lead and water groups differed significantly only on Days 1 and 21 ($p<0.05$).

Experiment 2

Activity scores for the ad lib water group of Experiment 1 were compared to those of the limited water group using an unweighted means analysis of variance. While there was a significant main effect of Minutes, $F(14,504) = 21.92$,

TABLE 1

BODY WEIGHTS IN GRAMS OF RATS INGESTING AD LIB WATER, 10^{-4}M LEAD ACETATE (LOW LEAD), 10^{-2}M LEAD ACETATE (HIGH LEAD) OR A LIMITED AMOUNT OF WATER

		Days of Age						
		1	4	7	10	14	21	28
Ad lib water	$\bar{X}$	7.10	9.25	11.66	17.02	23.66	42.04	72.63
N = 30	SD	0.61	0.59	1.32	2.27	3.05	2.79	8.29
Low lead	$\bar{X}$	6.78	9.01	11.97	17.40	24.37	34.69	72.57
N = 25	SD	0.55	0.83	1.59	2.60	2.98	5.62	9.67
High lead	$\bar{X}$	5.29	8.08	10.61	15.11	20.23	27.42	51.65
N = 26	SD	0.43	1.44	3.25	3.04	3.79	5.14	8.55
Limited water	$\bar{X}$	7.02	9.11	12.14	17.75	21.25	31.80	54.30
N = 8	SD	0.33	0.62	0.98	1.05	0.73	1.22	2.47

$p<0.001$, reflecting a reduction in activity over time for both groups, there was no effect of drinking condition and no interaction. The mean body weights of the limited water group, shown in Table 1, were compared to those of the high lead group of Experiment 1 using the t^* statistic which does not assume homogeneity of variance or equal group sizes [16]. The limited water group was significantly heavier than the high lead group on Days 1, 4, 10 and 21 ($p<0.05$). These groups were not significantly different on body weight on Days 7, 14 and 28.

The mean and standard deviation of the lead content of blood, liver and kidneys for high lead, low lead and limited water groups are shown in Table 2. The lead content of each tissue type was examined separately using analysis of variance. The differences in blood lead were significant, $F(2,26) = 3.44$, $p<0.01$, as were the differences in liver lead, $F(2,26) = 6.65$, $p<0.005$, and kidney lead, $F(2,26) = 8.93$, $p<0.005$. Newman-Keuls' multiple range tests further showed that for all three tissue types, the high lead condition produced lead concentrations significantly greater than those produced by the low lead or limited water conditions ($p<0.01$ for all comparisons). It should also be noted that the lead content of the tissues of animals exposed to the high lead condition was extremely variable. The low lead condition and limited water condition did not produce significantly different concentrations of lead in the tissues analyzed.

TABLE 2

LEAD CONTENT OF BLOOD, LIVER AND KIDNEYS OF RATS INGESTING 10^{-2}M LEAD ACETATE (HIGH LEAD), 10^{-4}M LEAD ACETATE (LOW LEAD) OR WATER

		Tissue		
		Blood (ppm)	Liver (ppm)	Kidneys (ppm)
High lead	$\bar{X}$	1.06	0.72	3.84
N = 11	SD	1.36	0.71	3.47
Low lead	$\bar{X}$	0.21	0.11	0.61
N = 10	SD	0.13	0.06	0.25
Limited water	$\bar{X}$	0.14	0.06	0.17
N = 8	SD	0.08	0.06	0.09

DISCUSSION

Two major effects of lead ingestion, both presumably dependent upon the level of exposure, are apparent from these data. First, exposure to the low lead solution increased overall activity relative to controls but the change in activity over time was similar for these groups. On the other hand, exposure to the high lead solution produced mainly a difference in the rate of change in activity with high lead animals exhibiting a slower reduction in activity over time than did control or low lead animals. It is interesting to note that exposure to the low lead solution produced effects on open field activity even though the tissues of low lead animals did not show significant lead concentrations. Apparently, exposures to low lead concentrations is sufficient to produce behavioral effects when such exposure occurs during early development. In addition, examination of Table 2 shows that the mean lead concentration of low lead tissues is higher in all three tissues than the mean lead concentration found in control tissues, although these differences were not statistically significant. Further refinement and reduction of error in the measurement technique may allow detection of these differences.

The reduction in body weight for animals exposed to high concentrations of lead in food or water has been confirmed a number of times [5, 7, 9, 11] and is apparently due to reduced intake of the lead-containing commodity [7,9]. Differences in activity in this experiment do not seem to depend completely upon weight differences since limited water animals at the end of the experiment were similar to high lead animals in weight but were similar to ad lib water animals in activity. In addition, studies of undernourished rats have shown that the body weight of these animals must be at least 50% less than that of controls for differences in open field activity to be observed [1,2]. However, since limited water animals were significantly heavier than high lead animals on some days, particularly during the critical suckling period, the effects of undernutrition on activity cannot be completely ruled out.

The findings of the present study on open field activity illustrate the complexity of measurement when activity levels are changing at different rates for different experimental groups. Most studies of the effects of lead on activity have investigated total activity over a block of time, a procedure which may mask differential rates of change. It is possible that such differential rates of change may be responsible for some of the discrepancies in the findings. To illustrate this possibility, let us examine some of the conclusions that could have been drawn if the present study had terminated at different points. Examining total activity scores after the first 5 min, the conclusion drawn would be that low lead exposure increased activity relative to controls but that high lead exposure had no effect on activity. If only high levels of exposure were used, it would be concluded that lead did not affect activity at all. If the high lead animals in this experiment did not further reduce their activity with experience as Fig. 1 suggests, a continuation of the experiment for a longer period of time might have produced results indicative of higher activity levels for both high and low lead animals. These possible interpretations illustrate that both lead exposure and measurement procedure are important variables in the study of lead-induced changes in the open field activity of rodents. Further, these findings demonstrate the importance of considering changes in relative activity over time, as well as absolute activity levels.

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