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Low Level Lead Effects on Activity Under Varying Stress Conditions in the Developing Rat

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BARRETT, J AND P J LIVESEY. *Low level lead effects on activity under varying stress conditions in the developing rat* PHARMACOL BIOCHEM BEHAV 22(1) 107–118, 1985 —The study was designed to determine whether lead ingestion by nursing rats would affect the way offspring reacted to the stress inducing properties of the test environment both as juveniles and mature rats Dams were exposed to diets with 0.0, 0 2, 0 4 or 1.0 percent by weight metallic lead. Mean blood-lead levels of pups at weaning were 4, 25, 36 and 55 μg/100 ml of blood respectively. The stress factor was varied by (1) changing the test apparatus, i.e , forcing rats to occupy an open field or allowing the animal to be a free agent in the start box of a T-maze; (2) testing rats under a longitudinal and a cross-sectional experimental design to vary familiarity with the apparatus, and (3) comparing behavior in the presence or absence of noise Reactivity was assessed by examining the inter- and intra-session pattern of ambulations and defecations. Analysis of data revealed that lead treated rats demonstrated the greater response to stress This response was generally dose related although recovery was dependent upon the test applied and measures taken The findings provide a conceptual framework to account for varied results across previous studies

Stress Activity Lead treated rats

A pressing need in lead research is for the assessment of possible hazardous effects of exposure to levels of toxin which do not produce obvious signs of neurotoxicity in children, namely "asymptomatic" or "subclinical" levels. An animal model (the rat) is indicated for assessment of adverse toxicologic reactions since experimental procedures and interventions can be carried out which are not possible in humans with tighter control over extraneous variables. While existing animal reports point to the possibility that early low-level lead exposure produces altered behavior in the rat that can persist into maturity, the unconfounded nature of the behavioral impairment and the duration of its effect remains to be determined [5, 6, 19].

Various attempts to assess the effect of lead on rat behavior by measuring the activity of these animals have involved a variety of devices for different time periods [6]. In the majority of such studies, means for the total test period were given thus obscuring inter and intra session patterns of activity. This diminishes test sensitivity since animals may differ in behavior at certain phases of testing [26].

There is evidence to suggest that lead affected and control rats vary in the way they react to a novel environment in studies that have provided a temporal analysis of behavior. Both decreased and increased activity in lead affected versus control animals on initial placement in the test apparatus have been reported [12, 14, 22, 29]. Lead related results were attributed to an altered reaction to novelty [14,22] and normal emotionality (control rates of grooming and defecation) but increased activity (elevated rearing and ambulation scores) [29].

It is difficult to draw conclusions from these reports. The test environments and age at testing differed, lead treated rats were raised in smaller litters than controls [29] and experimental animals showed evidence of malnutrition [12] thereby compromising results [19]. All dams drank lead acetate and the acetate component is capable of confounding lead effects [3]. Furthermore, explanations in terms of reactivity and emotionality need further exploration. A review of effects of a novel environment on rats cautions against recourse to a single emotionality construct in whatever form [1]. These findings indicate the need for measurement of changes in frequency of particular types of behavior over time to build a pattern of the overall responsiveness of the animal. This involves the recognition that responses to a novel environment will be contingent upon a number of factors. For example, the pattern of activity and defecation displayed by rats depends upon whether the rat is forced to occupy an enclosure or allowed freedom of choice of entry into the same situation [27,28]. Adaptation to novelty also depends upon the type of apparatus used [13,20], the age of rats [18], previous experience in the apparatus [27] and variation in levels of stimulus intensity [18,26].

The purpose of this paper is to show that lead treated and control rats differ in the ways they react to a test environment. To achieve this all of the above factors need to be considered in explaining the temporal differences in behav-

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ior As well, the results should not be confounded by the extraneous variables which have plagued other studies [6,19]

The procedure for lead administration was via the dam to suckling pups Elemental lead was selected in preference to a lead salt [3]. Elemental lead has been utilized in research into the effects of lead on sheep [9] and absorption of metallic lead particles from the gastrointestinal tract of the rat has been investigated using blood-lead as an index of internal lead levels [2]. The use of elemental lead is a viable means of elevating blood-lead levels and overcomes problems of attempting to control for the second radical in lead salts Although petrol containing tetraethyllead has been shown to be the main source of lead contamination in the environment, the exhaust product is inorganic lead [5]. Introducing lead via the dam is a realistic model for lead insult in human development since the mother is a likely source of lead contamination in the suckling infant Increased maternal care is an early consequence of lead treatment which means that experimental dams are no less solicitous of their pups than control rats [4]

EXPERIMENT 1

METHOD

Animals

Twenty female Wistar albino rats (from the colony maintained by the State Animal Resources Centre at Murdoch University), each with ten newborn male pups, were utilized Where dams failed to deliver ten males, litters were made up immediately following parturition, from the pool of litters born at the Resource Centre on the same day Five replications of the basic experiment were run Pups from the four litters in each replication were pooled, rank ordered by weight and distributed across dams so there were no significant weight differences between litters on PN 0 and to minimize genetic bias.

Apparatus

All litters were housed in 30×30×15 cm plastic cages in a room maintained on a 12-hr light/dark cycle and at a temperature of 23°C Food jars were fitted with an internal collar and wired to the cages to prevent spillage of powdered chow.

To measure the ambulation of offspring two open fields were utilized. For the testing of pre-weaned pups, the open field was 50×50 cm square with a 10 cm high surround White lines divided the field into 25 squares. For older rats a cylinder 100 cm in diameter with a wall 40 cm high was used. The wall and floor, like that of the first field, were painted matt black. The floor was divided by white concentric and radial lines into 21 sections.

The black wooden T-maze employed in the spontaneous alternation task had a start alley which was 84×8×9 cm deep. The first 24 cm could be isolated by a sliding metal door and formed the start box. Each goal arm was 37×8×9 cm. The final 30 cm of each arm could be closed off by a sliding metal door to constitute a goal box. The hinged tops to the start alley and goal arms were made from Plexiglas.

Procedure

Three experimental groups and one control group were used Dams assigned to the experimental groups were fed a diet of either 0.2, 0 4 or 1 0 percent by weight metallic lead in powdered rat chow. Contaminated food was prepared by weighing out ground lead (particle size 150 μm to dust) and mixing this with ground rat pellets in a blender for some 5 minutes. Dams in the control group were fed normal rat pellets in powdered form All rats drank tap water After PN 18, experimental rats received the control diet to prevent pups consuming lead directly.

The food intake of the four dams in each replication was measured every second day from PN 0 to PN 18 Dams were weighed every four days from parturition At PN 18 when lead was removed from the dams' diet, two pups from each litter were sacrificed for blood-lead analysis Offspring were weighed every four days from birth until being sacrificed at PN 44 To collect blood, the rat was anaesthetized and a blood sample was obtained in a heparinized syringe through a heart puncture and transferred to a heparinized tube Whole blood was treated with Triton X-100 and analyzed for its lead content using a Varian AA-575 atomic absorption spectrophotometer fitted with a CRA-90 carbon rod atomizer and an ASD-53 automatic sample dispenser [17]

To measure activity, young rats were tested every three days from PN 3 until weaning at PN 21 The ambulations of weaned rats were sampled at PN 28, 36 and 44. The same rats were tested at each age, i e , a longitudinal design was used with age variation a within groups factor Subjects were transferred to the large open field for testing on PN 28 Ambulations were scored at the end of each minute of the three minute trial and the total defecations recorded for the completed trial

The T-maze was utilized to measure spontaneous alternation. During this procedure the rat was placed in the start box, the door opened and a stop watch activated On entry to either goal arm the watch was stopped, the goal box door closed and the time recorded Each animal remained in the goal arm for 50 sec and was then removed to a bucket while the maze was cleaned Animals received five such trials in the maze and spontaneous alternation was assessed by counting the number of times an animal altered its choice from Trial n to Trial n+1

This method obviates the need for large numbers of animals and produces comparable results to those obtained if animals complete only two trials [11] Any animal that refused to make a choice within 300 sec was given no further trials at that age Rats were tested at PN 20, 24 and 40

RESULTS

All results, apart from those relating to behavior in the T-maze have been analyzed with a one between (Treatment) one within (Days) Analysis of Variance The Duncan method of multiple comparisons at the 5 percent level of significance was used to make pair-wise comparisons between groups [16]

Food Intake and Weight of Dams

There were no significant differences in food consumption or maternal weight gain between treated and control dams

Mortality of Offspring

There was no significant mortality amongst the lead exposed pups

Blood-Lead Analysis

There was a direct relationship between the dose of lead

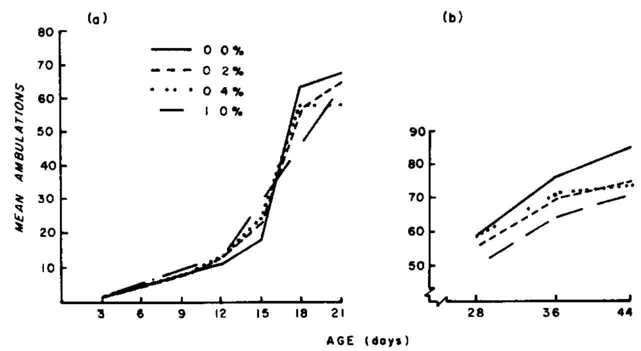

FIG 1 Mean ambulation scores in the open field in the 4 groups (a) Mean scores for juveniles at 3 day intervals to PN 21 S E. values ranged from 0 2 to 5 0 (b) Mean scores for weaned rats at 8 day intervals between PN 28 and 44 S E values ranged from 2.9 to 4 5.

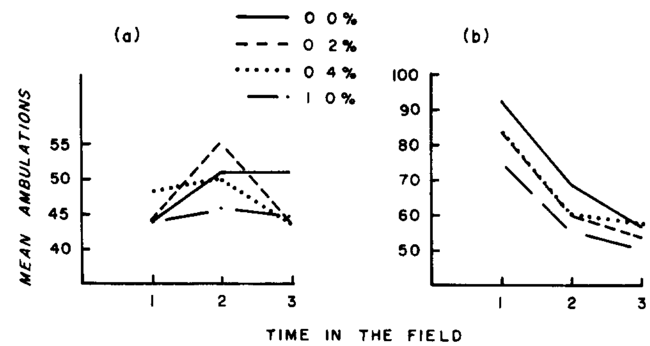

FIG 2. Mean ambulation scores by minutes in the field for the 4 groups (a) Mean scores summed over PN 15, 18 and 21. S.E. values ranged from 2.8 to 3 9 (b) Mean scores summed over PN 28, 36 and 44 S E values ranged from 3.0 to 5.0

administered to the dam and the quantity of lead present in the blood of offspring at PN 18. The mean values (±S.E.) for μg of lead per 100 ml of blood for each of the four groups were: 1.0% (87.4±11.3), 0.4% (50.4±3.0), 0.2% (31 7±3 5) and controls (1.7±0 4).

Weight of Offspring

There was a significant difference in weight between 1.0% lead treated rats and all other groups between PN 12 and PN 20 This disparity continued after weaning from lead contaminated milk at PN 21 until PN 35. After that time no significant differences between groups were evident (pre-weaning, $F(3,136)=16.4$, $p<0.01$; weaned, $F(3,136)=3.78$, $p<0.05$).

Ambulations in the Open Field

Mean ambulation scores for rats in each of the four treatment groups are shown in Fig. 1.

Ambulations of Pre-Weaned Pups (Fig 1a)

For control animals there was a striking increase in activity after PN 15 when pups had both eyes open and walking was well developed. This same pattern of locomotion was shown by the low level lead groups. However, the 1.0% group did not reach the levels of activity demonstrated by the other groups on PN 18 until PN 21. The higher activity by pups in the three lead groups on PN 15 is notable. The interaction between Days and Treatment was significant, $F(18,816)=1.66$, $p<0.05$.

Ambulations of Weaned Rats (Fig. 1b)

There was a tendency for all lead affected animals to be less active than controls except the 0.4% group on PN 28. This reduction in activity is most obvious in the 1.0% group. The analysis of results did not reveal significant differences between groups or significant interactive effects. However, differences in ambulations between groups approached significance, $F(3,136)=2.32$, $p=0.07$. The reporting of probability values close to the accepted value of significance (0.05), where this contributes to a conclusion, has been recommended [21].

Temporal Examination of Ambulation Scores

Figure 2 shows the pattern of activity in the open field for each minute of the three minute trial. In Fig. 2a the data have been summed over the three earliest test days (PN 15, 18 and 21) Figure 2b shows the data summed for the remaining ages (PN 28, 36 and 44). The figures reveal a sharply declining pattern of activity with time for the older rats which is not apparent in young rats. In the case of pre-weaned rats there was a significant Minutes effect, $F(2,272)=6.76$, $p<0.01$. No other differences were significant although the interaction between Treatment and Minutes approached significance, $p=0.08$. The analysis of data for weaned rats produced a significant Minutes effect, $F(2,272)=205$, $p<0.001$, but no significant interaction between Treatment and Minutes. The difference between groups approached significance, $p=0.08$.

The overall pattern of activity collapsed across PN 15 to 21 is not as well defined as that for older rats and could benefit from scrutiny. Therefore a further analysis of the temporal pattern of activity for young rats is provided in Fig. 3 by considering the ambulations for each of the three minutes at PN 15, 18 and 21 separately. The figure shows that experimental rats were more active than controls for each of the three minutes of the test session at PN 15 but less active on the remaining days (apart from the 0.2% group on PN 21).

Defecations in the Open Field

Figure 4 illustrates mean defecation scores for each day that more mature rats (PN 21–44) were in the open field. Defecation rates for younger rats were too low to warrant inclusion. It is notable that rats from the 0.2% and 1.0% groups showed an increase in defecation on PN 28, the first day of exposure to the large open field. The interaction between Treatment and Days was the only significant effect, $F(9,408)=2.99$, $p<0.01$.

Assessment of Spontaneous Alternation Behavior in the T-Maze

A rate of spontaneous alternation of between 60 and 70% was achieved by all groups across the three ages at which testing occurred. However, the data for the three lead groups at PN 20 and 24, when any developmental disparities were likely to be evident are equivocal because of failure to com-

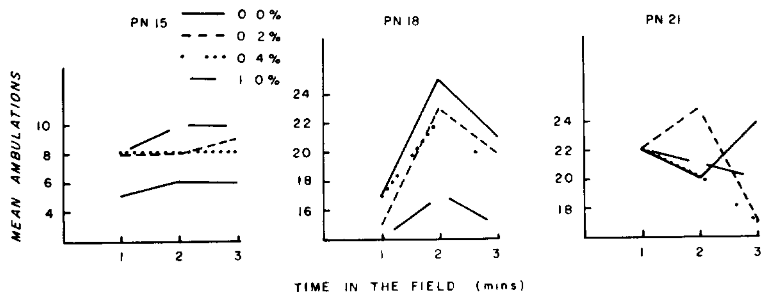

FIG 3 Mean ambulation scores in the 4 groups displayed by 1 minute intervals for 3 minutes at PN 15, PN 18 and PN 21 S E values ranged from 0 7 to 2 5

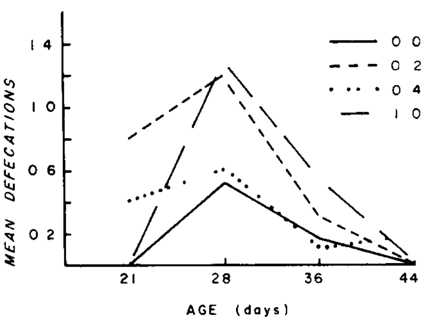

FIG 4 Mean defecation scores in the field for the 4 groups S E values ranged from 0 1 to 0 3

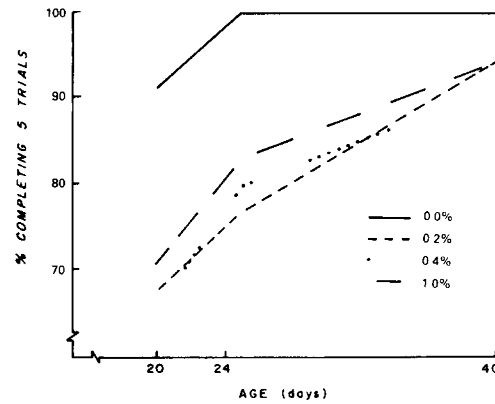

FIG 5 Percentage of rats in the 4 groups successfully completing 5 trials in the T-maze

plete trials by many of these animals This is illustrated in Fig 5 which shows percentage of rats completing five trials in the T-maze for each group at PN 20, 24, and 40. This figure shows failure on the part of experimental animals to complete successive trials in the T-maze. A Chi-square test of the number of animals which survived the five trials at each age when spontaneous alternation testing occurred revealed a significant difference between treatment groups at PN 20 (χ^2=7.85, df=3, p<0.05) and PN 24 (χ^2=8.87, df=3, p<0.05). There was no significant difference between groups with regard to the number of animals completing all trials at PN 40

Latency to Make a Choice in the T-Maze

Figure 6 shows the time taken for lead treated and control rats to make a choice in the T-maze across the five trials at each test age. When an animal failed to complete all trials, the maximum latency of 300 sec was recorded for the failed and remaining trials on that day

This figure shows that lead affected rats took more time to travel from the start box to the arm of the maze, particularly at PN 20 and 24 There was a significant Treatment effect on PN 20, F(3,136)=2.59, p<0 05, and PN 24, F(3,136)=2.97, p<0 05 There was also a significant Trials effect at both of these ages, F(4,12)=24 3, p<0 001, and F(4,12)=3.96, p<0.05. There were no significant differences between dosages at PN 40 On PN 20, the main effects occurred during Trial 1 between control and 1.0% rats and on Trial 2 between control and 0.4 and 1.0% animals At PN 24, control rats differed significantly from all lead groups on Trials 2 and 3.

Animals Which Failed to Complete Trials

Rats which failed to finish trials in the T-maze either remained in the start box or once outside the start alley remained motionless or attempted to return by gnawing at the lowered perspex door It was also observed that 50 percent of animals which failed to complete trials at PN 20 were among those rats not succeeding at PN 24

DISCUSSION

The lead dosages utilized in this experiment produced two asymptomatic groups with respect to weight gain (0 2 and 0 4%) and a third lead group with retarded growth (1 0%) Any behavioral aberrations noted in this group must therefore be interpreted in the light of this undernutrition [19]

Blood-lead values for the lower dosages at PN 18 were in accord with levels considered asymptomatic in humans, whereas internal lead levels in the 1.0% group were in the range where clinical manifestations of lead poisoning are considered likely to occur [7,10].

An examination of ambulation scores for weaned rats revealed that all animals displayed an initial burst of activity when placed into the field with attenuation of that activity

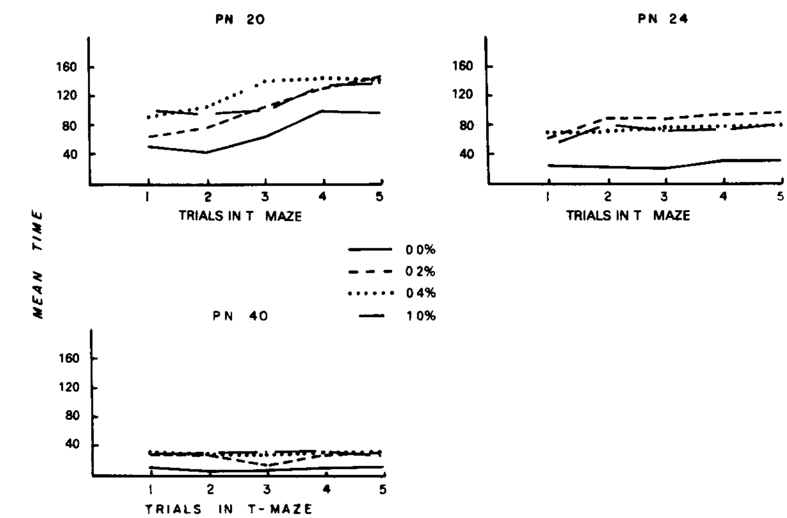

FIG 6 Mean time to make a choice on each of 5 trials in the T-maze for the 4 groups S E values ranged from 0 9 to 20 2

across the test session However, lead treated rats demonstrated ambulation scores which were consistently below control levels. Preweaned rats failed to habituate to the open field in the same way as older animals and overall, exhibited an increase in activity in the second minute followed by a levelling off or decline in the final minute. The only notable treatment effect on activity during this stage was elevated activity in lead affected rats on PN 15.

Taken together the results show that lead treated and control rats differ in their reaction to the novelty of the open field. The 15 day old rat is particularly aroused by a novel environment [8]. In the present experiment this was the first age of exposure to the field when rats had at least one eye open. Thus the event would be novel with the appearance of visual information. Lead affected pups were more active than controls across the three minute trial. Campbell and Raskin [8] have explained the arousal in 15 day old rats as reflecting stress to an unfamiliar environment rather than curiosity or exploratory behavior. Stress is often denoted by physiologists as all forces or stimuli in the environment capable of inducing changes and adaptations in the organism. The magnitude of environmental stress can only be measured indirectly through the response of the animal [24]. In this case lead treatment enhanced the stress effect.

Weaned rats forced to occupy a novel space exhibit high initial activity which declines throughout the session [18,27]. The motivating agent for the first burst of activity has been interpreted as escape oriented behavior [27]. Lead treated rats failed to demonstrate this activity to the same extent as controls and were less active overall. In the present experimental situation animals had been regularly exposed to an open field since PN 3 thus having the opportunity to habituate to the environment. Under these conditions lead treated rats become less reactive than controls.

While these lead effects were more pronounced at the highest level where results were confounded with undernutrition, asymptomatic levels produced a similar trend in behavior pointing to a dose dependent lead effect.

It has been concluded that a rat placed in the open field first seeks an escape from the field with high activity and low defecation. When this is not successful activity declines and, with increasing stress, defecation increases [18]. On PN 28 in this study, defecation scores for rats in the 0.2 and 1.0% groups were significantly above control levels. This was the first day of exposure to the large open field. It is not possible to determine whether this lead related increase in defecation occurred towards the end of the trial, since boli were counted after the three minute trial ended. Experiment 2 will present a temporal analysis of defecation which shows that lead treated rats demonstrate a greater stress effect than controls.

In the T-maze, where animals were free to leave a start box, a lead effect was again evident. At PN 20 and 24 control rats revealed more rapid running on the initial trials than lead affected rats, to the extent that a significant number of experimental subjects failed to complete trials. All rats revealed short trial latencies at PN 40 and no lead effect was observed at this age. Longer trial latencies in the T-maze on the part of experimental rats may also reflect enhanced susceptibility to stress. Where the rat is a free agent, the initial stress response is immobility [20,26].

Like the results from other lead studies this experiment has demonstrated that, in terms of activity, lead treated rats can be hyperactive, hypoactive or no different from controls. By recognizing that factors such as previous experience, age and test apparatus determine how an animal reacts to the stress of novelty, these seemingly varied results become more meaningful. Experiment 1 has suggested that lead treated rats are likely to exhibit an enhanced response to stress in a novel situation compared with controls. When the situation is familiar and therefore less stressful the response of experimental rats is likely to fall below control levels. The aim in the second experiment was to maintain the stress of a novel situation by testing rats in the open field at one age only. As well, the added stress of loud noise was introduced for some groups. Response measures were taken at frequent intervals with both ambulations and defecations recorded every 30 seconds.

EXPERIMENT 2

METHOD

Animals

Thirty-six female Wistar albino rats, each with eight newborn male pups, were utilized in this study. Four replications of the basic experiment were carried out in the no noise condition and five in the noise condition Pups were cross-fostered as previously described.

Apparatus

The housing of animals and the open fields employed were described in Experiment 1

Noise was generated through a speaker mounted 20 cm above the wall of the open field Noise intensity was measured at 85 db at the center of the field floor

Procedure

The placement of animals into groups, diet preparation, measurement of food intake and weight of dams parallels procedures in the first experiment Pups were weighed every four days to PN 16 and on PN 18 and PN 20 Litters were reduced to six pups on PN 4, principally to ensure viability in the highest lead group, by sacrificing the smallest pups in the 1 0% group and matching color-coded animals in remaining groups.

To measure blood-lead levels, blood was collected from the dams at PN 21 and offspring on PN 21, 28, 38, 50 and 74 after behavioral testing was completed for the particular animals to be sampled. Samples of blood were taken from the rats of selected replications at each of the above PN days and five out of the nine sets of animals were utilized for this purpose. It was assumed that these blood-lead values would be typical of the wider population. The remaining four sets of animals were allowed to survive and regularly weighed to determine whether normal weight levels were achieved in young affected by lead in the preweaning period.

Ambulations and defecations were measured under two conditions, that of noise and no noise at PN 15, 18 and 24 in the smaller of the two open fields and at PN 35, 47 and 70 in the larger field. Thus each of the six rats in a litter was tested at one of these ages and then removed from this aspect of the experiment To examine rats at the desired range of ages between PN 15 and 70, a necessary condition given that adaptation to novelty changes with age [18], all comparisons were made between the six age groups within each noise condition rather than between the noise and no noise condition, the developmental sequence being the main concern This experiment was therefore cross-sectional in design with age variation a between groups factor Each rat was observed for three minutes at the same time on three consecutive days from the commencement age. Ambulations and defecations were scored every 30 seconds. The test room was at 23°C and the amount of incident light was 44 lux.

RESULTS

The results for both the no noise and noise condition are presented side-by-side. Food intake and weight of dams, blood-lead levels of pups and pup weights have been analyzed with a one between (Treatment), one within (Days) Analysis of Variance Ambulation and defecation scores in the open field were analyzed in two configurations. To examine ambulation and defecation differences between groups by half-minutes over days and at each test age, scores were analyzed with a two between (Treatment, Age), two within (Half-minutes, Days) Analysis of Variance Pairwise comparisons were made as in Experiment 1

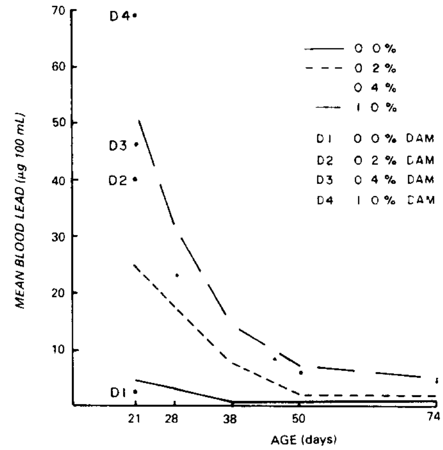

FIG 7 Mean blood-lead levels for dams and pups in the 4 groups taken over 5 ages between PN 21 and PN 74 S E values ranged from 0 2 to 5 6

Food Intake and Weight of Animals

There was no significant difference between the food intake of dams in the four treatment groups in either the no noise or noise condition. Dams in the 1 0% and to a lesser extent in the 0 4% group, exhibited an initial weight drop between parturition and PN 4 The analysis of data failed to produce a significant Treatment effect in rats used for either noise condition but did result in a significant Treatment by Days interaction in both conditions, F(15,60)=2 58, *p*<0 01, F(15,80)=1 98, *p*<0.05

Pups from the 1.0% group failed to gain weight at the same rate as offspring in the remaining groups. The analyses produced a significant Treatment and Days effect and a Treatment by Days interaction for rats used in both noise conditions, F(3,92)=18 3, *p*<0 001, F(6,552)=2698, *p*<0.001; F(18,552)=17.0, *p*<0 001, and F(3,116)=9 0, *p*<0.001, F(6,696)=3111, *p*<0.001, F(18,696)=5 4, *p*<0.001 The 1.0% group were lighter than all other pups from PN 4 to PN 20 These results are in accord with those of Experiment 1. Weaned rats from 1.0% dams continued to demonstrate below normal body weights after removal from lead The analysis produced a significant difference between groups and a significant Days effect, F(3,95)=4 1, *p*<0 01, F(6,552)=2100.0, *p*<0 001 The 1.0% rats failed to reach normal weight by PN 70 whereas in the previous experiment, rats in this group had shown a weight "catch-up" by PN 40

Blood-Lead Analysis

Figure 7 illustrates the mean blood-lead levels in dams at weaning and offspring at five ages between PN 21 and PN 74 There was a dose related elevation of blood-lead levels in pups although these levels were below those of corresponding dams Lead levels declined after pups were removed from contaminated milk Data analysis on the lead levels in pups produced a significant Treatment and Days effect and a

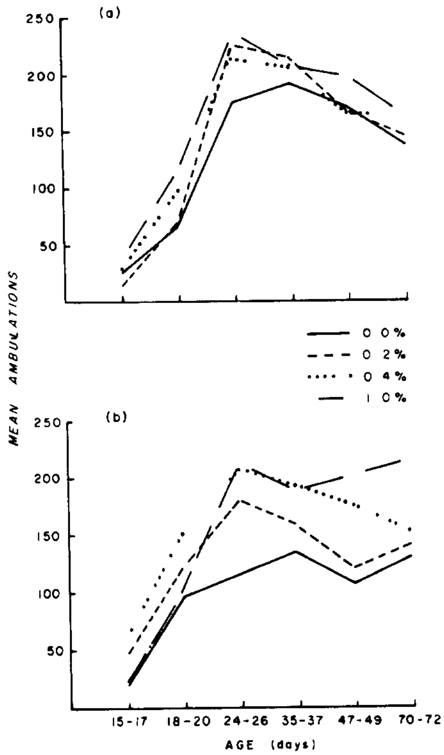

FIG 8 (a) Mean ambulation scores in the 4 groups for each 3 day block over 6 ages in the no noise condition S E values ranged from 7 3 to 33 9 (b) Mean ambulation scores in the 4 groups for each 3 day block over 6 ages in the noise condition S.E values ranged from 5 9 to 27 8

significant Treatment by Days interaction, $F(3,16)=45.5$, $p<0.001$; $F(4,64)=74.63$, $p<0.001$; $F(12,63)=8.6$, $p<0.001$

If the mean blood-lead levels from Experiment 1, which were taken on PN 18 (1.0%, 87.4±11.3; 0.4%, 50.4±3.0; 0.2%, 31.7±3.5, 0.0%, 1.7±0.4) are compared with the values from this experiment on PN 21 (1.0%, 55.0±5 1; 0.4%, 36.0±4.7; 0.2%, 25.0±5.6, 0.0%, 4.0±1.5) it is apparent that lead levels begin to fall as soon as the dam is removed from the contaminated diet on PN 18

Ambulations in the Open Field

Mean ambulations for each age group by lead levels are shown separately for the no noise and noise condition in Fig. 8 As set out above, comparisons can only be made within each noise condition From this figure lead affected rats tend to be more active than controls. In the no noise condition (Fig. 8a), this elevated activity is most notable just after weaning with little variation between groups after this stage. Hyperactivity on the part of lead treated rats is more obvious in the noise condition (Fig 8b), with only the 0.2% rats exhibiting recovery in the immediate post-weaning period.

In the no noise condition the analysis failed to produce a significant Treatment effect There was a significant Age effect, $F(5,72)=18.4$, $p<0.001$ but no significant interaction between Treatment and Age. The analysis produced further significant effects, namely a Half-minute effect and interactions between Half-minutes and Treatment and between Age and Half-minutes in the field, $F(5,360)=44.3$, $p<0.001$; $F(15,360)=2.5$, $p<0.01$; $F(25,360)=4.3$, $p<0.001$. These results will be examined when the activity of young and older rats is compared.

The analysis of scores in the noise condition did reveal a significant Treatment effect, $F(3,96)=8.82$, $p<0.001$. There was, as in the case of the no noise condition, a significant Age result, $F(5,96)=20.7$, $p<0.001$ but no significant interaction between Treatment and Age. There was a Days effect, $F(2,192)=13.1$, $p<0.001$, and Half-minutes effect, $F(5,48)=34.9$, $p<0.001$, as well as significant interactions between Days and Age, $F(10,192)=5.6$, $p<0.001$, Age and Half-minutes, $F(25,480)=3.5$, $p<0.001$, Days, Treatment and Age, $F(30,192)=1.4$, $p<0.05$ and Age, Days and Half-minutes, $F(50,960)=1.61$, $p<0.01$. These effects will be examined below.

Scores were then summed for young pre- and immediately post-weaned rats (PN 15, 18 and 24) and for older rats (PN 35, 47 and 70), to examine the course of activity at each 30 sec interval over each 3 minute period.

Figure 9 displays the results for juveniles in both the no noise and noise conditions. It is evident that without noise (Fig. 9a), young lead treated rats, particularly from the 0.4 and 1.0% groups, tended to be more active on initial placement in the field, although by Day 3 this applied only to animals in the highest lead group. In the noise condition (Fig. 9b), all lead affected rats were more active initially and tended to maintain this elevated activity beyond the initial minute in the field. The 1.0% juveniles, particularly on Day 1, were less active than might be expected from the results in Experiment 1. The 1.0% pups in this experiment appeared to have more nutritional problems than those in Experiment 1 since they failed to achieve control weights after weaning from lead contaminated milk. This may account for a lag in the lead-related arousal effect

A one between (Treatment), two within (Half-minutes, Days) Analysis of Variance in the no noise condition produced one significant effect, a Half-minute effect, $F(5,60)=6$ 8, $p<0.001$ This supports the observation that activity declined with time in the field but the trend towards higher activity by lead treated young was not significant. In the noise condition, there was a significant Days effect, $F(2,32)=11$ 1, $p<0$ 001, and Half-minutes effect, $F(5,80)=5.9$, $p<0$ 001. Activity declined with days and once again this behavior changed with time, generally decreasing between the first and second half-minute in the field but then tending to increase again. As in the no noise condition, the activity of experimental rats was not significantly above control levels.

Figure 10 illustrates the activity pattern of older rats in both noise conditions. Once again the most striking effects were the elevated initial activity by lead treated rats in the no noise condition (Fig. 10a) and initial elevation in the noise condition (Fig. 10b) which was maintained across time

The analysis of ambulation scores in the no noise condition produced a significant Half-minute effect, $F(5,60)=46.3$, $p<0.001$, as well as a significant Treatment by Half-minutes interaction, $F(15,60)=2.4$, $p<0.01$. The interaction between Days and Half-minutes was also significant, $F(10,120)=2.2$, $p<0.05$. The interaction between Treatment and Half-minutes in the field shows that groups exhibited different patterns of activity within trials This would support the observation that lead affected rats, particularly subjects from the 0.4 and 1.0% groups, were more active during initial stages in the field.

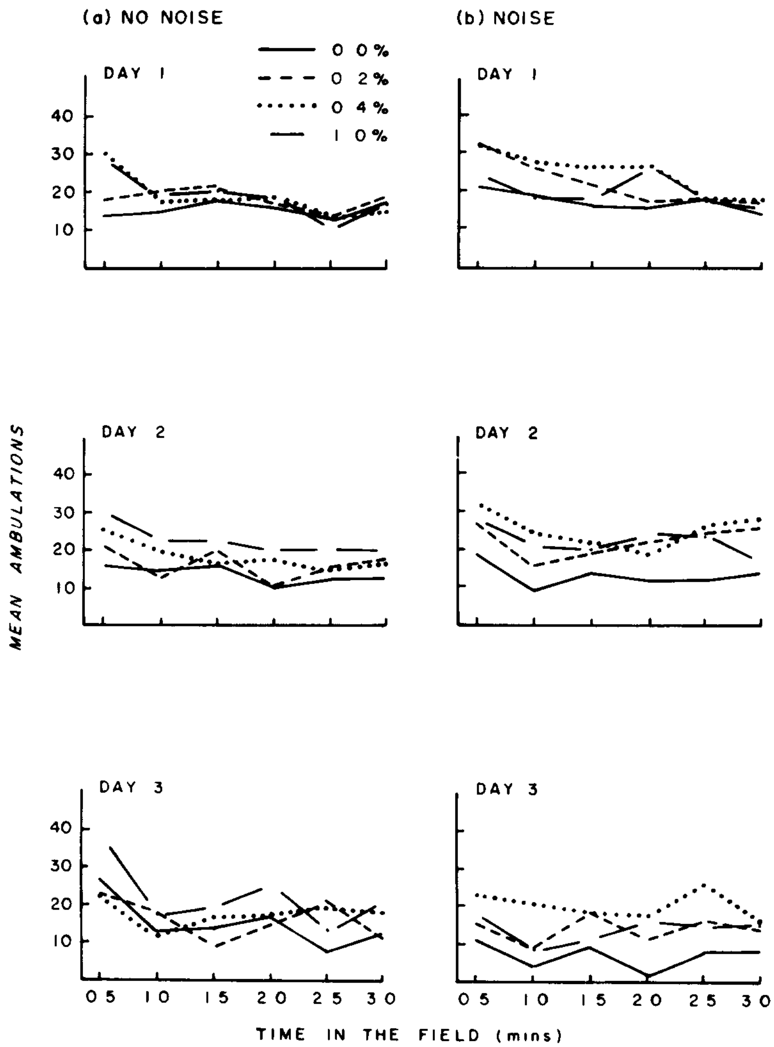

FIG 9 (a) Mean ambulation scores in the no noise condition for juvenile rats in the 4 groups Scores are displayed by half-minutes over 3 days of testing S E values ranged from 1 5 to 5 9 (b) Mean ambulation scores in the noise condition for juvenile rats in the 4 groups Scores are displayed by half-minutes over 3 days of testing S E values ranged from 1 6 to 5 6

In the noise condition, a one between, two within Analysis of Variance produced a significant Treatment effect as well as a significant Half-minute effect and a Days by Half-minutes interaction, F(3,16)=3 6, p<0 05, F(5,80)=36.9, p<0 001; F(10,160)=1 9, p<0 05 On Day 1 rats in the 0.4 and 1 0% groups were more active than controls at the 1.0 minute mark, with subjects in the highest dose remaining more active at the 1.5 and 3 minute marks On Day 2, 0.4 and 1.0% rats were more active during the first half-minute and after one and a half-minutes. The 1.0% group remained more active at the 2.5 minute mark By Day 3 only the 1.0% subjects exhibited elevated activity and this occurred at the 2 0 and 2.5 minute segments.

The overall Analysis of Variance for the no noise and noise conditions produced a significant Age by Half-minutes interaction. Figures 9 and 10 show that the intrasession decline in activity from the first to the last half-minute was more characteristic of the older animals A significant Treatment by Age by Days interaction was achieved with noise and is also reflected in these figures Rats in the highest lead groups were more active as mature animals on all three days in the field, whereas control rats revealed this trend on Day 3 only

Defecations in the Open-Field

Defecation scores were analyzed in a similar fashion to ambulation results Under the no noise condition defecation levels were low and did not produce any significant effects

Figure 11 plots the defecation scores for each group in the noise condition and shows generally increased rates of defecation with age and higher scores by lead treated rats, particularly at the 0 4 and 1 0% levels. A two between two within Analysis of Variance in this condition resulted in a

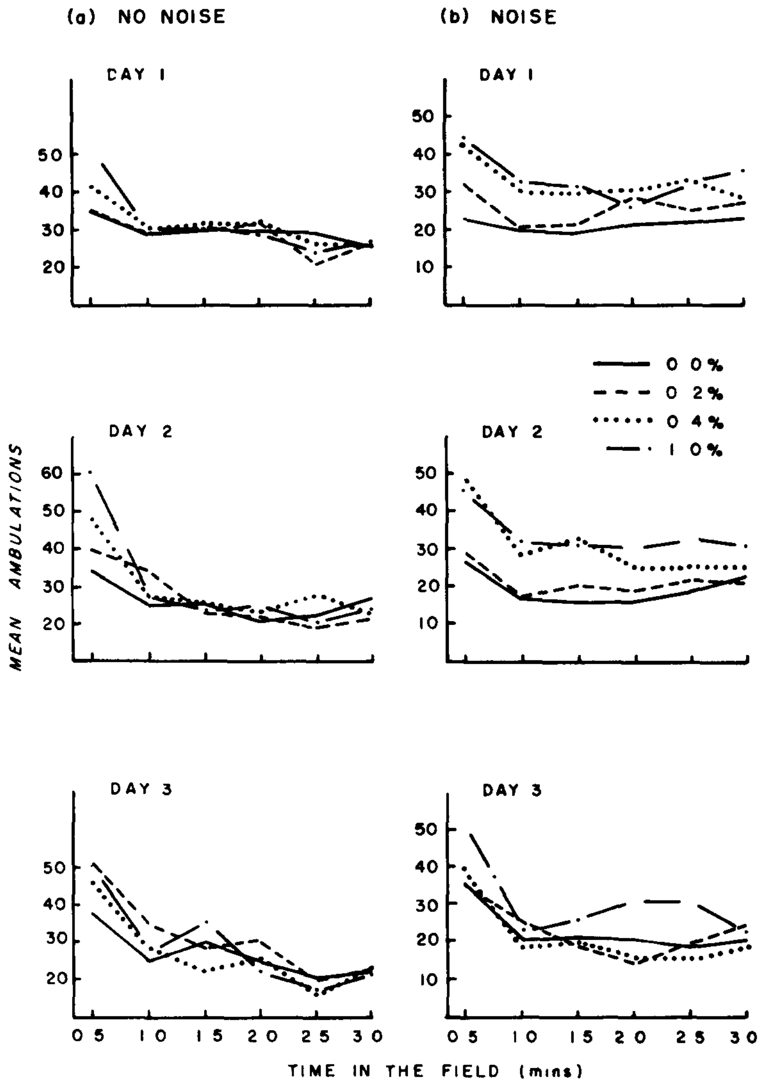

FIG 10 (a) Mean ambulation scores in the no noise condition for older rats in the 4 groups. Scores are displayed by half-minutes over 3 days of testing. S.E values ranged from 1 3 to 6 5. (b) Mean ambulation scores in the noise condition for older rats in the 4 groups Scores are displayed by half-minutes over 3 days of testing S E values ranged from 0 9 to 5 2

significant Treatment and Age effect, F(3,96)=2.8, $p<0$ 05; F(5,96)=4.1, $p<0$ 01. There was also a significant Days and Half-minutes result as well as a significant interaction between Age and Half-minutes in the field, F(2,192)=5.7, $p<0.01$; F(5,480)=21 4, $p<0.01$; F(25,480)=2.8, $p<0.001$. These results will be examined below.

Figure 12 reveals defecation levels at each 30 sec interval for the three days of testing for rats in the noise condition as juveniles (Fig. 12a) and more mature animals (Fig. 12b). Figure 12a shows that as juveniles (PN 15–24) there were no obvious treatment differences apart from an elevation in defecation scores by 0 4% rats on Day 2 There was an increase in defecation across days and most defecation occurred in the last minutes in the field. The analysis produced two significant effects which support the above observations, namely Days and Half-minutes, F(2,32)=3 55, $p<0.05$; F(5,80)=10.1, $p<0.001$.

Figure 12b illustrates mean defecation rates across the three days of testing for older rats (PN 35 to 70). This figure shows that defecation for older rats is also more likely towards the end of a trial and is highest in the 0 4 and 1.0% groups A one between two within Analysis of Variance produced a significant Treatment effect, F(3,16)=4.65, $p<0.05$, and an interaction between Treatment and Half-minutes that approached significance, $p=0.06$. There was a significant Days and Half-minute effect, F(2,32)=5.0, $p<0.05$; F(5,80)=9.6, $p<0.0001$. Pair-wise comparisons revealed that treatment effects were due to the 1.0% group. Animals at this dosage level defecated more than controls at the 2.5 minute interval on each of the three test days.

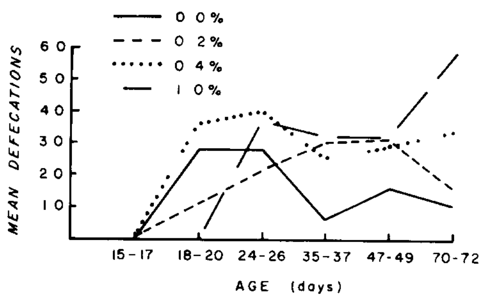

FIG 11 Mean defecation scores in the 4 groups for each 3 day block over 6 ages in the noise condition S E values ranged from 0 5 to 1 2

The significant interaction between Age and Half-minutes achieved in the overall analysis can also be illustrated by Fig 12 If the data over the three days for both juvenile and mature rats is considered, older animals exhibited more defecation across all minutes in the field than younger conspecifics

DISCUSSION

In keeping with the results in the previous experiment this study has produced two lead groups demonstrating normal weight gains (0.4 and 0.2%) and a low weight lead group (1 0%)

Lead loaded rats revealed a dose related elevation in blood-lead levels at the time of weaning from lead contaminated milk at PN 21 After this age, blood-lead levels fell rapidly to be near control levels by PN 50 The maximum

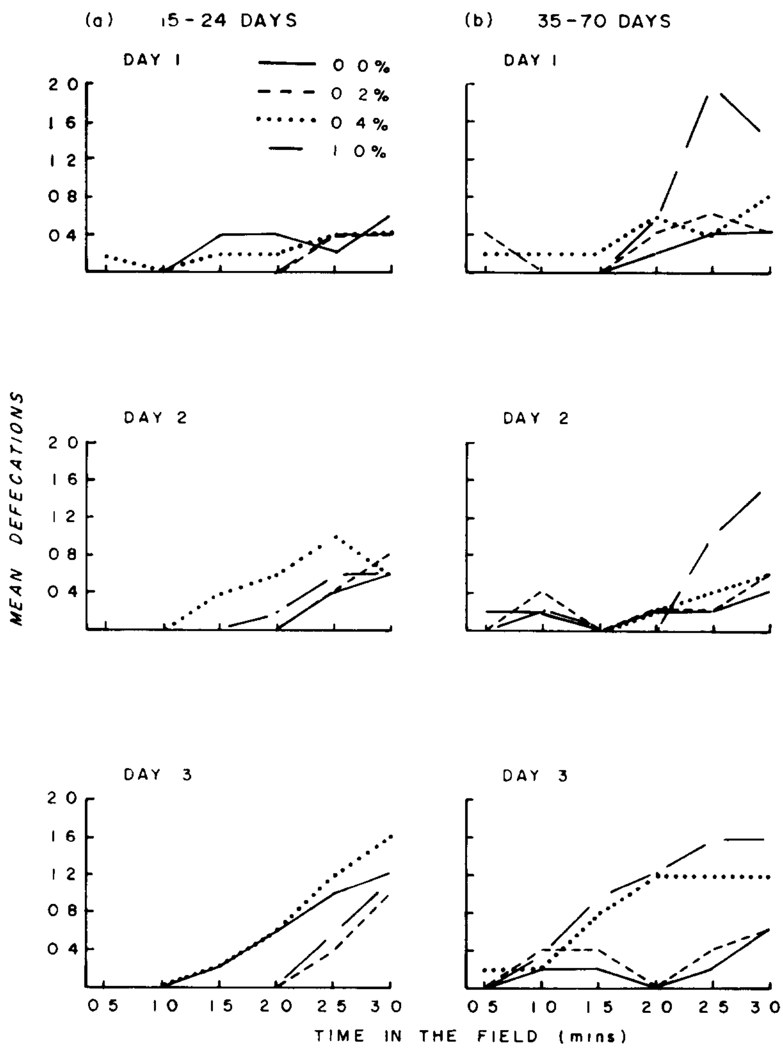

FIG 12 (a) Mean defecation scores in the noise condition for juvenile rats in the 4 groups Scores are displayed by half-minutes over 3 days of testing S E values ranged from 0 1 to 0 3 (b) Mean defecation scores in the noise condition for older rats in the 4 groups Scores are displayed by half-minutes over 3 days of testing S E values ranged from 0 1 to 0 3 blood-lead levels were found in 1.0% offspring at PN 21 (mean, 55 μg/100 ml) although levels would be higher immediately after weaning at PN 18 In keeping with the findings from the first experiment, lead levels in 0.2 and 0.4% pups were in the range considered asymptomatic in the human population.

As in Experiment 1, it is evident that age and level of stress (noise in this case) need to be taken into account to explain activity and defecation differences between lead treated and control rats.

The frequency of defecation increases with age and in response to the stress of continuing confinement to an open field or stressors such as shock and noise [15,18] An examination of defecation rates in the open field under the no noise condition showed low levels of defecation which did not discriminate between groups. With the added stress of noise, defecation rates were more substantial At the juvenile stage (PN 15 to 24), all rats demonstrated an inter- and intra-session increase in defecation. Between PN 35 and 70 the within session increase in defecation was marked for rats from the 1 0% group on all days and 0.4% rats on the third day.

As set out in Experiment 1, limiting a rat to an open field produces high initial activity which declines with time. If the temporal pattern of activity is examined for young rats in the no noise condition, there was a non-significant early elevation in activity for 0.4 and 1.0% rats. The ambulation results for rats between PN 35 and 70 revealed a significant interaction between treatment and half-minutes in the field. This was due to initial elevation in activity by lead treated rats However, this elevation occurred in the first 0 5 minutes and in contrast to results from the noise experiment (see below), activity was not kept above control levels beyond this time

An assessment of ambulation scores for young rats under the stress of a noise revealed that all groups exhibited a similar intra- and inter-session pattern of activity and although lead treated rats were more active than controls, the differences were not significant. Older lead loaded rats were significantly more active than controls, particularly subjects from the 0.4 and 1 0% groups. For rats from the 0.4% group this elevated activity occurred during the initial minute in the field and was maintained for the first half of the trial on Test Days 1 and 2. Subjects in the highest lead group were also most active at this time and remained active across the whole session. They were still significantly more active by the third test day.

It is notable that the pattern of an intrasession decline in activity was more characteristic of older rats in all groups and the establishment of this pattern of activity made more apparent the resultant significantly elevated activity by experimental subjects Furthermore, lead affected rats showed a significant increase in their activity with age characterized by an elevated initial burst of activity upon introduction to the field and the maintenance of this behavior over half-minutes in a dose related manner

In this cross-sectional study where rats were tested at one age only, lead affected rats responded to the stress inducing properties of the open field by showing greater initial activity and higher defecation rates toward the end of trials than controls This effect was most apparent in older rats and in the presence of a noise but was also evident without the added stressor In the previous longitudinal experiment, rats had been removed from the home cage and placed in the open feld on 10 occasions before the completion of testing at PN 44 and were, therefore, becoming increasingly familiar with test situations and less likely to find the experience stressful Elevated activity and defecation in lead treated rats were achieved on only two occasions At PN 15, the first day of testing when rats would have visual awareness of the environment, experimental animals exhibited elevated activity On PN 28, the age at which rats were first placed in the larger open feld, lead treated rats demonstrated higher defecation scores

Once again the effect of lead on behavior is varied, ranging from small or non-existent differences between groups to substantial and significant disparities in activity and defecation However, by recognizing that response to novelty changes with age and level of stress (noise, previous experience) it becomes apparent that lead loading exacerbates the typical stress response for a rat under prevailing conditions of testing

GENERAL DISCUSSION

Much of research into the effects of lead treatment on behavior has been concerned with simply producing a difference between groups This has led to a number of different results under varying test parameters. The present studies have shown that when the findings are viewed within a particular conceptual framework a coherent picture emerges. By recognizing that the reaction of rats to the stress of a novel environment is dependent upon a number of factors, it becomes evident that lead loaded rats exhibit an enhanced response to stress. The nature of this response depends on the test apparatus used (open field or T-maze), age of subjects (pre and immediately post weaned versus adults), previous experience (continuous placement in the apparatus versus experience at one age) and level of stimuli (no noise and noise conditions). Thus it has been demonstrated that lead effects can be seen as consistent, if the subtleties of animal behavior are taken into account rather than searching for gross differences per se.

The testing of animals across a number of ages permits conclusion to be made in respect to the duration of behavioral aberrations after the cessation of lead treatment. The analysis of blood-lead levels in offspring at regular intervals between PN 21 and 74 revealed a dose related decline in lead levels after weaning from the lead loaded dam and there was evidence of behavioral recovery as levels fell. Ambulation scores in the open field under the noise condition produced well defined disparities between groups. Under these conditions there was a dose related recovery in behavior. Offspring from the 0.2% group reduced their activity to control levels by PN 35 and subjects in the 0.4% group approached control levels of activity by PN 70 However, rats from 1 0% dams continued to be more active after this age, and at a time when blood-lead levels were quite small (mean, 10 μg/100 ml) All experimental groups evidenced a behavioral recovery in the T-maze by PN 40 Rehabilitation appears to be dose related and occurs as blood lead levels fall However, recovery was not always apparent and it is likely that the type of test applied to the animal and variables measured determine whether lead effects are ameliorated. For example, lead treated rats were still hypoactive with respect to controls at PN 44 after being tested in the open field from PN 3 and although testing may not have proceeded to an age when recovery would be observed, the behavior of experimental rats appeared to be increasingly divergent from control levels as the rats matured. The display of increased defecation by 0 4 and 1.0% rats in the open field under the noise condition was no less apparent by PN 70 than at earlier ages

For results to be unequivocal behavioral aberrations can only be ascribed to lead in the absence of an accompanying weight loss. While behavioral effects in this study were generally more apparent in rats from 1.0% dams, significant alterations in behavior were also discovered in 0.2 and 0 4% rats Recovery was more likely in rats with low lead levels but was also evident in 1.0% rats in the T-maze and no experimental subjects exhibited recovery when repeatedly tested in the open field. These parallels between behavioral deficits in all experimental rats suggests that results are likely to be a product of lead toxicity rather than nutritional deficiencies An alternative possibility that cannot be ruled out is that lead at varying dosage levels results in poorer quality milk so that all lead affected rats are suffering nutritional deficiencies of varying degrees However, the most significant and dramatic effects in this study occurred after animals were weaned and when nutritional deficits, if any, would be declining

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