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Differential Effects of Chronic Lead Intoxication on Circadian Rhythm of Ambulatory Activity and on Regional Brain Norepinephrine Levels in Rats*

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The human environment is exposed to constant contamination by high concentrations of lead which has been recognized as a principal toxicological factor to the central nervous system (CNS). The most common toxic effects of lead lead to the production of behavioral abnormalities in humans. There have been numerous investigation on psychoindustrial analysis of workers and the result suggests that lead posioning may cause memory loss in adult humans. Similar evidence has been found in adult rats experimentally exposed to lead poisoning (Danscher et al.1975). Studies with lead intoxication in rodents have shown either hyper-activity, hypo-activity, or no change in spontaneous motor-activity (Goldberg and Silbergeld 1974; Michaelson et al.1974; Sobotka and Cook 1974; Dricol and Strenger 1976; Krehbeil et al.1976; Modak and Satavinoha 1979). In our earlier studies, both hypo-and hyper-activity have been reported in lead exposed adult rats (Chandra and Shafiq-ur-Rehman 1982; Shafiq-ur-Rehman and Chandra 1983; 1984).

Changes in biochemical mechanisms and amine concentrations in the brain have been manifested in the form of varying disorders and abnormalities in behavior, including motor-activity, which has been proved with a number of psychoactive drugs (Taylor and Snyder 1971; Thornburg and Moore 1972; Benkert et al. 1973). It has been reported that increased level of cerebral norepinephrine (NE) has been shown to be associated with motor hyper-activity (Matussek and Ruther 1965), and in lead exposed rats (Goldberg and Silbergeld 1974; Michaelson et al.1974). In contrast to later reports, decreased cerebral NE level has been studied in rats exposed to lead (Wysocka-Paruszewska and Beil-Baranowska 1979). The levels of NE have been found to be elevated in the midbrain, but reduced in the straitum of lead intoxicated rats (Dubas et al.1978). Unfortunately, the later two studies did not investigate behavioral manifestations of lead intoxication. No study is available which could account for the pattern of changes in spontaneous ambulatory responses in an open field situation together with the steady state regional levels of NE in the brain of chronically lead exposed rats. Therefore, it seemed to be worthwhile to study the circadian rhythm of ambulatory activity and its association with NE levels in various brain regions of rats exposed to lead.

* I wish to dedicate this paper to the loving memory of my sister Mrs. Zaheda-Rehman.

MATERIALS AND METHODS

Young adult male albino rats of Charles Foster strain weighing between 100 and 180 grams were used. They were kept under identical diurnal conditions of 12 hour light (07-19) and dark (19-07) cycle at temperature 25-27°C and 44-48% relative humidity. The animals were provided with standard laboratory pellet food (Hindustan Lever Laboratory Feeds, India) and water ab-libitum. Six animals were taken in each group. The experimental group were given 2% lead acetate in drinking water for a period of 30 days. Separate experiments were run for behavioral, neurochemical and stress studies.

The apparatus used for open field test is illustrated in Figure 1. Briefly, it consisted of a wooden circular open arena of 82 cm diameter enclosed by a wooden wall of 38 cm height. The wooden floor was marked with three concentric circles which were divided into segments by six lines radiating from the center. The outer segments were further divided into two segments by a radiating line. Thus, 19 units of approximately equal size were used to score ambulation of animal in the arena during the test period. In the open field situation, the animal was exposed with two types of stimuli: white noise (78dB, Reference intensity 0.0002 dyn/cm) was produced by an oscillator through four speakers, and light (165 footcandle) was obtained by four photographic lamps. A transparent glass screen enclosed the arena on all the sides, the front side serving as glass door through which the subject was placed. The ambulatory score of the animal was recorded by a three channel fingeroperated electric counter. The ambulation scores were derived from the number of segments crossed by the subject. The shift of animal with its four limbs to a segment was scored as one unit of ambulation.

The ambulation scores of each animal were obtained daily at 2, 6, 10, 14, 18 and 22 hours for two minutes for 30 consecutive days. Total duration of rest and activity periods was also observed. The ambulation activity under the stress conditions of white noise and light was also recorded in another set of experiment in the open field situation on day 3, 13, 23 and 30 at the same test periods for 2 minutes.

The control animal as well as experimental groups were sacrificed by decapitation on 3,13,23 and 30 day. Brain and cervical part of the spinal cord were taken out and the former was dissected into cerebral cortex, cerebellum and brain stem. Samples of the tissues were stored in deep freeze (-40°C) before NE analyses. For the estimation of lead, the tissues were wet-ashed according to the method of Shafiq-ur-Rehman et al.(1982) and analysed by means of Atomic Absorption Spectrophotometer (PYE-UNICAM-SP-2900).

The tissues of various parts of the CNS were fluorophotometrically analysed for NE after the solvent extraction method of Welch and Walch (1969) by the help of an Aminco-Bowman Spectrofluorophotometer (American Instrument Co., Silver Springs, M.D., U.S.A) at 400/510 nm using 2 mm slits.

RESULTS AND DISCUSSION

The abmulation scores, as shown in Figure 2, were obtained every day

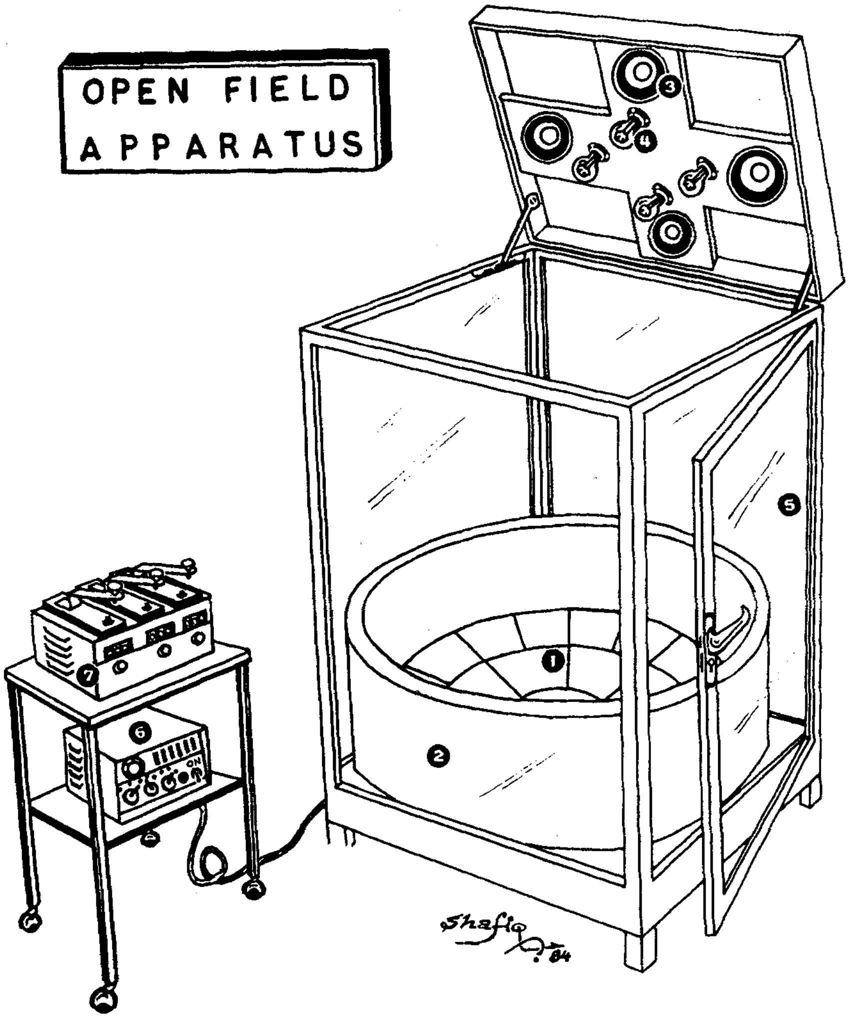

Figure 1. Schematic representation of the Open-Field Apparatus used in this work for studying and recording of rat behavior (i.e. ambulation). 1-Circular open arena, 2-Wooden wall, 3- Speaker, 4-Electric lamp, 5-Glass door, 6-Oscillator amplifier, 7-Finger operated three channel electric counter. For detail see under Materials and Methods.

as a mean of scores recorded at 2, 6, 10, 14, 18 and 22 hours individually in six rats of each group for 2 minutes. The abmulatory activity with an initial transient increase forming a parabolic response during first week of lead exposure was significant from day 2 to 6. After peak activity on day 3, the ambulation progressively declined. The hypo-ambulation response in lead exposed rats was although observed significantly from day 9 to day 17 as compared to control. A rapid increase in the ambulatory activity noticed on day 18 and then remaining significantly higher as compared to control group from then onward (Figure 2).

On day 3, 13, 23 and 30 marked disorders in the ambulating responses were observed, the circadian rhythm of the activity is shown in Figure 3. The increased (i.e. hyper) ambulation was found at 6 and 22 hours on day 3, and at hours 10, 14, 18 and 22 on days 23 and 30. Further, the hypo -ambulation was also recorded on the 13th day at 2, 6, 10 and 14 hours (Figure 3).

When lead-intoxicated rats exposed to simultaneous light and noise stimuli in the open field situation showed a progressive decrease in ambulatory response (Table 1).

There was a gradual but marked increase in the concentration of lead in all parts of the CNS of lead-intoxicated rats as compared to the control. The highest accumulations of lead ions were obtained in the cerebellum and spinal cord. The maximum per cent augmentation of this ion was noticed in the cerebral cortex on day 30. Surprisingly, a quick increase and retention of lead ions appeared in the cerebellum on day 13. Moreover, the cerebral cortex was the only brain region which showed a significant enhancement of lead ions on day 3 (Table 2).

The NE level of the cerebral cortex was significantly enhanced on day 3. However, on day 13, the cerebellum and spinal cord exhibited diminished contents of NE. On the day 23, the levels of NE were increased in all brain regions. Moreover, the increased concentrations of NE were discernible in the cerebellum and the brain stem on day 30 (Table 3).

Rats chronically exposed to lead showed a mixed response of ambulatory activity in an open field situation over a period of 30 days. Nevertheless, some reports have shown a lead-exposed hyper-motor-activity while others a hypo-motor-activity or no responses (for references see introduction). Neuropsychopharmacologically, it has been documented that NE plays an important role in controlling motor activity (Randrup and Scheel-Karuger 1966). Employing various experimental conditions, Matussek and Ruther (1965) suggested that the increased levels of NE were shown to be responsible for the motor-hyperactivity. We studied here the responses of ambulatory activities and the levels of NE in various brain regions on days when varying disorders in behavior were seen. Thus, it has been found that the level of NE was increased in the cerebral cortex on day 3 of hyperambulation. Earlier reports have also shown elevations in the cerebral forebrain levels of NE (Goldberg and Silbergeld 1974), and increase rate of turnover of NE (Michaelson et al.1974) in lead-intoxicated hyperactive rats. There is an initial increase

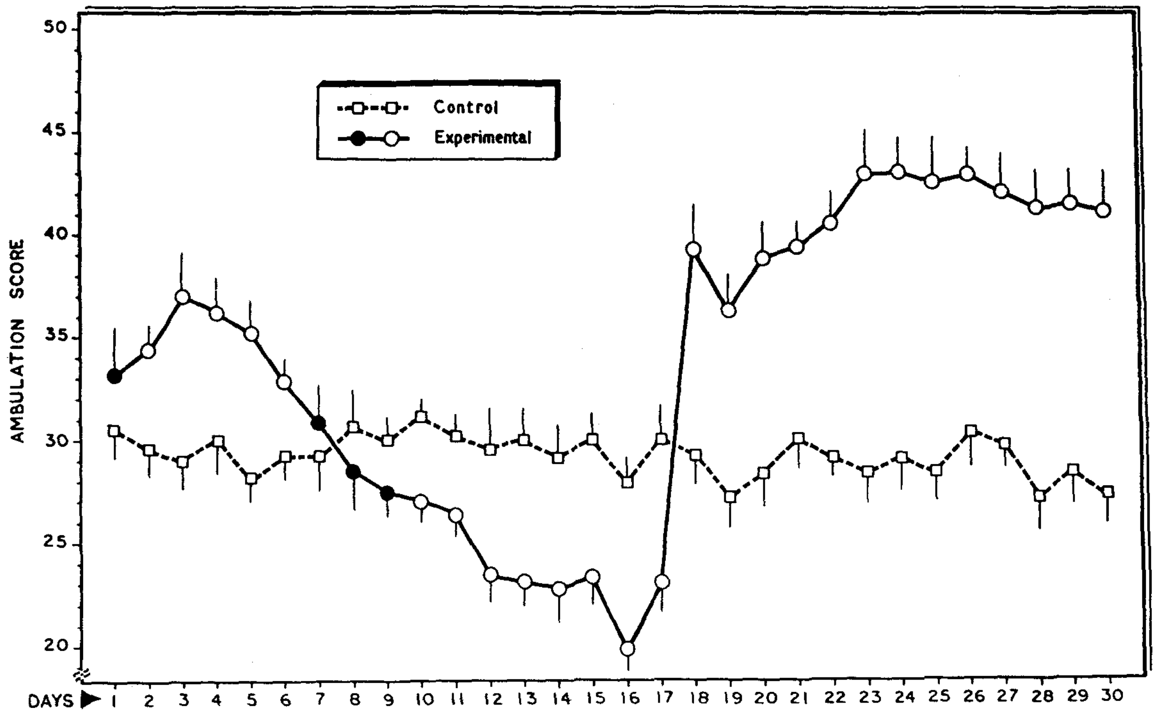

Figure 2. Time course of ambulatory behavior of rats ingesting tap water, or 2% lead acetate in drinking water, for a period of 30 consecutive days. Shown are Mean ± SE of six rats. Open circles indicate significant differences compared with the corresponding control; P at least $\angle 0{\cdot}05$; Student's *t*-test. Filled symbols stand for non-significant differences.

Table 1. The effect of light and sound stress on the ambulation of rats following lead intoxication (2% lead acetate in drinking water for 30 days).

	Group	Day 3	Day 13	Day 23	Day 30
Simultaneous Light & Sound-Stress effects on Ambulation	C	35.00 ±3.60	36.86 ±3.22	34.20 ±2.46	37.00 ±3.10
	E	33.20 ±3.42	28.75^{*} ±2.21	18.70^{**} ±1.62	8.40^{***} ±0.68
	% Change	(-5)	(-22)	(-45)	(-77)

*,**,and *** indicate, respectively, P∠0.05, P∠0.01, and P∠0.001 (Student's t-test). C represents for control, while E stands for experimental (i.e. lead-exposed).

in the ambulation reaching a maximum on day 3 and thereafter decreasing steadily; however, these authors described their animals as showning a state of spontaneous hyperactivity. According to Sobotka and Cook (1974) that the possibility of their (Goldberg and Silbergeld 1974; Michaelson et al.1974) finding of spontaneous hyper-motoractivity may be related to their method of exposing the pups to lead (4 per cent) through feeding the lactating mothers, altered quality and quantity of milk supply or abnormal maternal behavior that may, in turn, influence offspring behavior.

In this present study, although the hyper-ambulation was altered after the first week of lead exposure, the hypo-ambulation gradually fell until the 17th day. Developed hypo-ambulation observed here could be explained by suppression of circadian rhythm of ambulatory activity at 2, 6, 10 and 14 hours. Thus markedly increased contents of lead ions resulted in diminution of the levels of NE in the cerebellum and spinal cord during the period of hypo-ambulation an observed on day 13. In turn, it appeared that reduced levels of NE might have been responsible for decreased ambulation. A similar effect on cerebral NE levels has been observed by others (Wysocka-Paruszewska and Beil-Baranowska 1979). The hyper-ambulatory activity was further developed in lead exposed rats on the 4th day of third week which then continued upto the 30th day. It appears that the increased levels of NE have been implicated in modifying the ambulatory behavior and at present a reasonable explanation of such effects may be through modification in circadian rhythm of ambulatory activities observed at 10, 14, 18 and 22 hours. This may support the role of this amine (i.e.NE) in the evaluation of both the hypo- and hyper-ambulatory activity.

Control animals when exposed to simultaneous sound and light stress showed increased ambulatory response. On exposing the rats to lead this increased ambulation was blocked. This might be due either to a change in the amine concentrations in the brain or some sort of accumulation of lead ions in selective brain regions.

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Table 2. Levels of lead in different regions of the rat C. N. S. following lead intoxication (2% lead acetate in drinking water for a period of 30 days).

Region	Group	Day 3	Day 13	Day 23	Day 30
Cerebral cortex	C	0.95 ±0.08	0.92 ±0.12	0.98 ±0.08	0.91 ±0.09
	E	3.26* ±0.28 (+243)	4.28** ±0.31 (+365)	6.80*** ±0.48 (+594)	7.66*** ±0.61 (+742)
Cerebellum	C	1.23 ±0.03	1.24 ±0.03	1.23 ±0.02	1.25 ±0.09
	E	1.42 ±0.04 (+15)	7.69*** ±0.52 (+520)	10.32*** ±1.02 (+739)	11.12*** ±1.02 (+790)
Brain stem	C	0.94 ±0.10	0.97 ±0.08	1.02 ±0.09	0.99 ±0.07
	E	1.04 ±0.11 (+11)	2.23* ±0.18 (+130)	3.42** ±0.21 (+235)	4.89*** ±0.38 (+394)
Spinal cord	C	3.44 ±0.42	3.47 ±0.30	3.60 ±0.30	3.92 ±0.28
	E	4.22 ±0.53 (+23)	9.74** ±0.64 (+181)	11.62*** ±0.90 (+223)	15.22*** ±1.21 (+288)

Values expressed as μg/g of fresh tissue, Mean ± S. E.

Figures in parentheses indicate % increase.

C = Control.

E = Lead-exposed.

* = P ∠ 0.05, Student's *t*-test.

** = P ∠ 0.01, Student's *t*-test.

*** = P ∠ 0.001, Student's *t*-test.

Table 3. Effect of lead (2% lead acetate in drinking water for a period of 30 days) ingestion on the regional brain levels of NE at various test periods.

Region	Group	Day 3	Day 13	Day 23	Day 30
Cerebral cortex	C	1.624 ±0.082	1.622 ±0.098	1.620 ±0.074	1.625 ±0.088
	E	2.098* ±0.084 (+29)	1.716 ±0.096 (+6)	2.128** ±0.098 (+31)	1.786 ±0.100 (+10)
Cerebellum	C	1.268 ±0.084	1.265 ±0.064	1.269 ±0.064	1.266 ±0.071
	E	1.244 ±0.098 (−2)	1.044* ±0.062 (−17)	1.496* ±0.076 (+18)	1.726** ±0.090 (+36)
Brain stem	C	1.024 ±0.069	1.022 ±0.082	1.021 ±0.066	1.020 ±0.070
	E	1.032 ±0.071 (+1)	1.046 ±0.074 (+2)	1.286* ±0.072 (+26)	1.438** ±0.083 (+41)
Spinal cord	C	1.001 ±0.072	1.012 ±0.069	1.010 ±0.086	1.014 ±0.084
	E	1.012 ±0.069 (+1)	0.774* ±0.042 (−24)	1.004 ±0.072 (−)	1.018 ±0.010 (−)

Values expressed as μg/g of fresh tissue, Mean ± S. E.

Figures in parentheses stand for % increase (+) or decrease (—).

C = Control.

E = Experimental (lead-exposed).

* = P ∠ 0.05, Student's *t*-test.

** = P ∠ 0.01, Student's *t*-test.

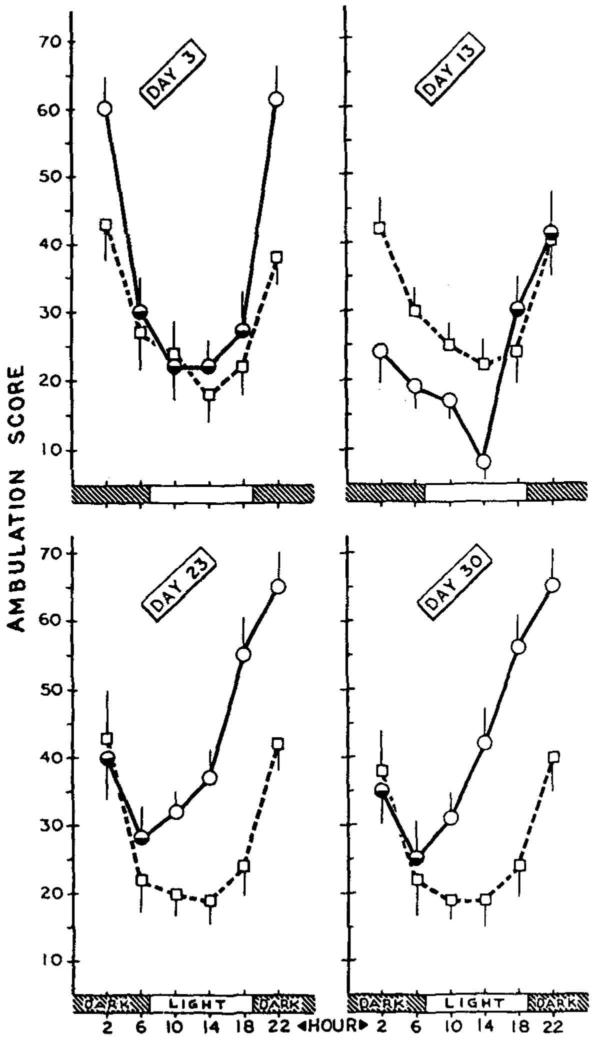

Figure 3. Circadian rhythm of ambulatory activity of rats ingesting tap water-□-,or 2% lead acetate in drinking water -○-, for a period of 30 consecutive days. Shown are Mean ± SE of six rats. Open circle indicate significant differences compared with the corresponding control; P at least ∠0·05; Student's t-test. Half filled circles indicate non-significant differences.

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