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EEG SPECTRAL POWER REDUCTION AND LEARNING DISABILITY IN RATS EXPOSED TO LEAD THROUGH POSTNATAL DEVELOPING AGE

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Abstract : The present study was carried out to assess electrophysiological and behavioral changes in Wistar rats caused by consuming a daily dose of lead acetate (400 µg lead/g body weight/day, given by gastric intubation, from second day of birth to 60 days of age. At 60 days of age, the lead treated rats showed in both wakeful and slow wave sleep stages, a statistically significant reduction in the delta, theta, alpha and beta band EEG spectral power in motor cortex (MC) and hippocampus (HI) with the exception of the delta and beta bands power of MC in wakeful state (WA). Lead administration was discontinued after that age for allowing rehabilitation for 40 days. Then, operant behavioral assessment was done. Results revealed that the lead treated animals took significantly more time and sessions than control normal animals in attaining criterion of learning. Hence exposure to lead in early age could result in a learning disability persisting even after discontinuation of exposure.

Key words : chronic lead toxicity, EEG power spectra, operant conditioning, learning disability, environmental effect on brain

INTRODUCTION

Lead is widely distributed in the environment and is by far the most studied of the neurotoxins. The effects of high level exposure on human neurological functioning and the deleterious behavioral effects of childhood lead encephalopathy have long been well documented (1-5). Animal behavioral studies as well as clinical observations increasingly indicate similarities between effects of childhood plumbism and syndromes of mental retardation, particularly minimal brain dysfunction (MBD) (2). Children with MBD show such impairments as poor motor coordination, hyperactivity, poor impulse or inhibitory control and a variety of learning difficulties. However, experimental investigations on the effects of lead on learning behaviour have produced contradictory conclusions (6). We have previously shown that exposure of rats to lead would cause alterations in levels of noradrenaline, dopamine and serotonin in certain brain regions (7). Power spectral EEG alterations caused by lead exposure have also not been adequately assessed in the past. In view of the above lacunae, our experimental study was extended to determine whether any impairement would be caused on operant learning and EEG power spectra in rats exposed to lead starting from postnatal age. Also, it was aimed to assess any peristent effect on learning ability even after discontinuation of lead consumption.

METHODS

Wistar male and female adult rats of about 150 days old, were mated and on confirmation of pregnancy, the females were separated and housed singly and had access to food and water *ad libitum*. The litter size was maintained to eight pups per mother. Additional stocks of rats were bred and reared under similar conditions served as a buffer colony, to make replacements for deaths or for animals sacrificed away periodically in experiments.

Experimental rats received lead at a dose of 400 µg/g body weight, per os, in the form of lead acetate daily (6 days per week) beginning from second day of age. The corresponding age matched controls received equivalent dose of sodium acetate by gastric intubation. At 60th day, the lead treatment was stopped and the rats were allowed rehabilitation for a period of 40 days. The lead exposed rats and their respective age matched controls were implanted with electrodes as described by Rajanna et al (8), epiosteally, over motor cortex and in dorsal region of the hippocampus (Stereotaxic coordinates used were 3 mm behind bregma, 1.5 mm lateral and a depth of 4.0 mm). After a day of recovery, EEG was recorded in pairs of one control and one lead treated rat, placed in a transparent perspex box (to observe behaviour), and connected to a 13

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channel EEG machine via a flexible insulated copper wire, which provided least restraint to the rats. Four of the EEG recordings, two from the normal rat and two from the lead treated rat were simultaneously processed on-line on a Nicolet (USA) computer system, to obtain power spectral printouts and plots. While watching the rat's behaviour, it was noted on the EEG record whether it remained in awake state or lapsed into spells of sleep. EEG data of awake state were separated from that of sleep periods (slow wave EEG type) in making calculations for drawing inferences.

Operant conditioning technique: Rats exposed to lead (400 µg/g body weight) for 60 days and subsequently rehabilitated for 40 days were tested under an operant conditioning paradigm, in Skinner box having a solid food dispenser. Each rat was initially exposed to the Skinner box for 30 minutes for initial orientation. In the next few sessions, each of 30 min duration, the animals were "shaped" on the continuous reinforcement (CRF) schedule, through a series of successive reinforcements given by the experimenter as follows. Every time the rat approached the pedal, a reinforcement (a food pellet) was provided. The "click" of the pedal, press and the illumination of the cue lamp (above the pedal) became a conditioned stimulus for the availability of the food pellet. Shaping was terminated as soon as the rat on it's own accord would press the pedal and look for the food pellet to drop. The time taken by each rat to associate the pedal, with the food pellet was then noted down. From then on, reinforcement was dispensed automatically. The next few sessions were each of 15 minute duration, wherein for every pedal press response, a food pellet was dispensed (CRF). After the CRF schedule (5 to 6 sessions per rat, per day), the ratio of responses required per reward was gradually increased to FR:2 and FR:5. For each of these schedules (FR:2 and FR:5), 5-6 sessions of 15 minutes each were conducted and the number of reinforcements noted.

The results were subjected to statistical analysis and the significance of difference between means was assessed by using the Student's t-test (two- tailed) (9).

RESULTS

Electroencephalographic alterations: Computerised spectral analysis of EEG of two regions of the brain (motor cortex and dorsal hippocampus) was monitored in rats with chronically implanted electrodes, during unanaesthetised free to move state (Fig. 1). The

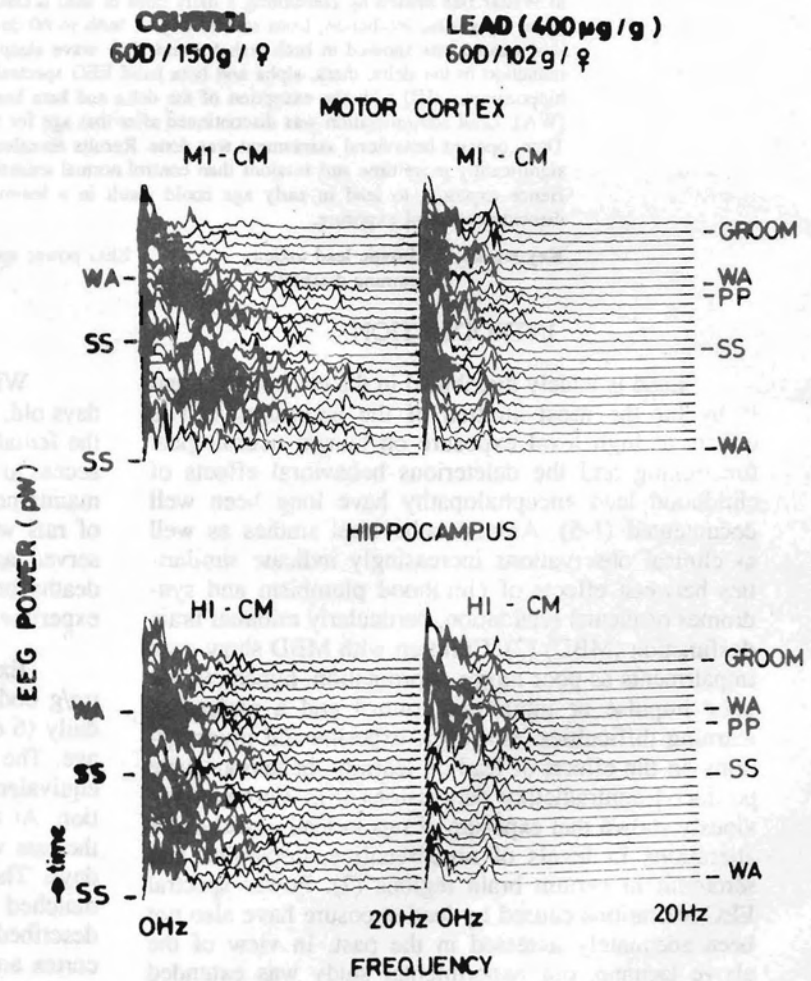

Fig. 1 : Representative examples of reduction of EEG power in spectra (0-20 Hz) of motor cortex (MI) and dorsal hippocampus (HI) in lead-consuming rat. Recording gain was similar in both control and lead-treated rat. Each trace of the spectral array represents power spectrum of EEG of 20.48 sec duration. CM: reference electrode placed over bone covering cerebellum; PP: desynchronised EEG sleep; SS: slow wave sleep; WA: wakeful periods.

data of epochs of quiet wakeful state (WA) were considered separately from that of slow sleep (SS) epochs.

The results showed that in both the MC (Fig. 2) and HI (Fig.2), there was a significant reduction in the delta, theta, alpha and beta band powers. The EEG power change was low in both states of behaviour. Only the delta and beta band power of MC in wakeful state was not significantly altered in the lead treated animals.

Alterations of conditioned behaviour: Rats aged 100 days (having received lead from second day to 60th day and subsequently no lead for 40 days) were tested for their performance in operant conditioning paradigm with food reward, so as to assess whether there was any residual effect of lead toxicity on learning ability. By this time (100 days), the animals recovered from their body weights to about 85% of controls, this difference was statistically significant. The results showed that lead treated animals took significantly more time and sessions than the control animals in learning the operant behaviour (Fig. 4). Even after having accomplished learning in all the reinforcement schedules, (CRF, FR:2, FR:5), the operant pedal press rates of the lead treated animals were significantly lower than that of controls. Thus the results showed a residual effect on learning behaviour in lead treated animals, even after cessation of lead exposure for 40 days.

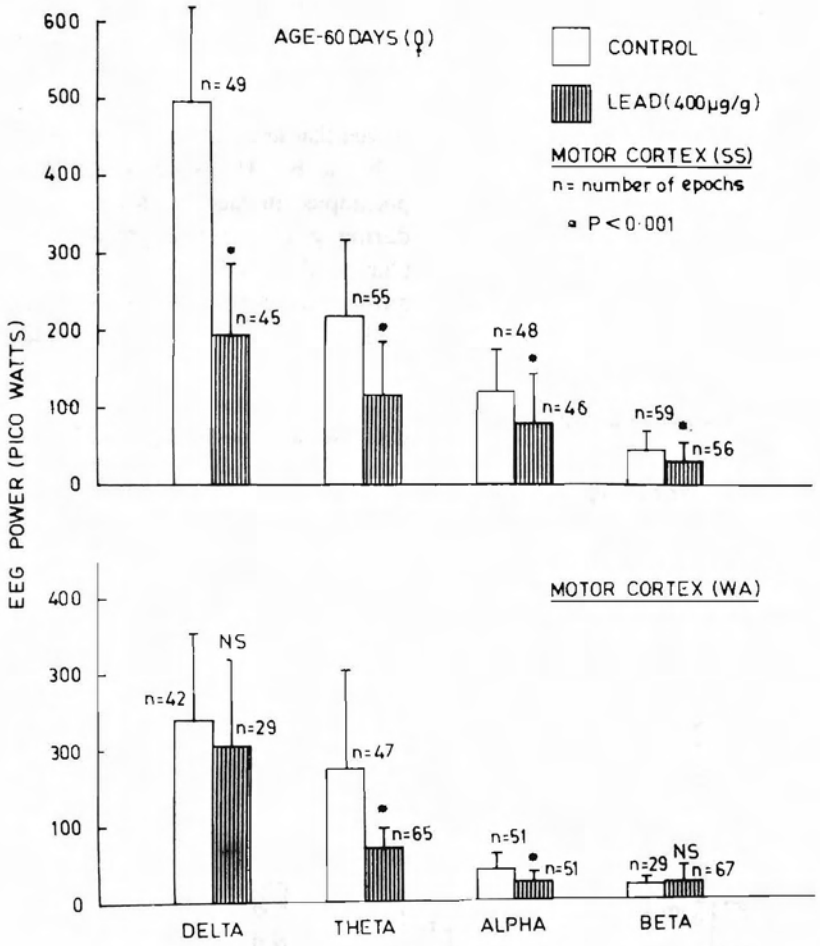

Fig. 2 : EEG spectral power of motor cortical area in lead exposed rats and control rats at 60 days of age, during states of slow wave sleep (SS) and quiet wakefulness (WA) Rats. were given 400 µg/g lead acetate or sodium acetate (for controls) by gastric intubation, starting from 2nd postnatal day, 6 days/week for 60 days. Values are mean ± SD of 4 rats in each group. Number of epochs used for each bar of the histogram is indicated above it. *(Student's 't' - test; two-tailed).

DISCUSSION

EEG changes : Significant alterations of EEG spectral power were revealed in this study. Needleman (5) reported that children having > 20 PPM dentine lead were found to be significantly impaired on IQ, auditory processing and reaction time

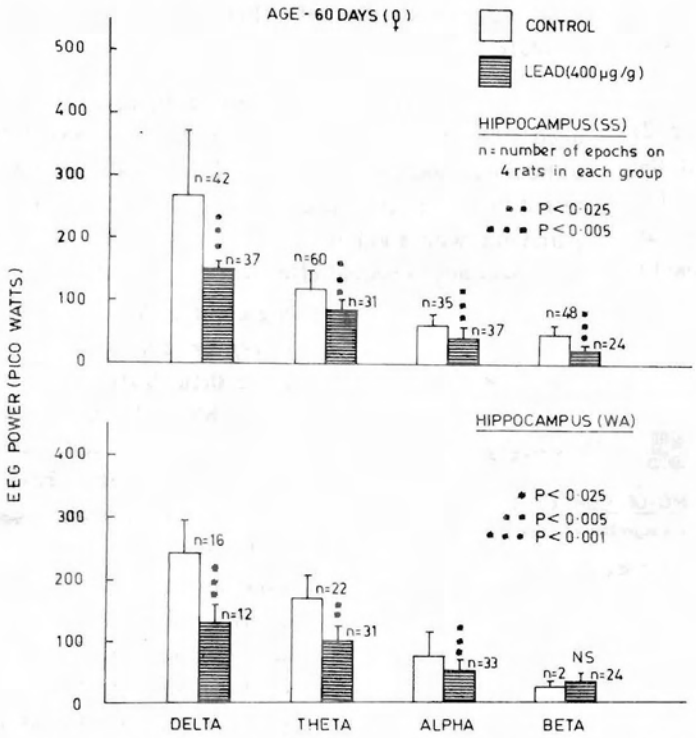

Fig. 3 : EEG spectral power of dorsal hippocampus of control group and lead exposed rats. Other legends are as in Fig. 2.

under varying intervals of delay. EEG of these children had higher proportion of delta in central, parietal and occipital regions, bilaterally. They had also less alpha in occipital and midline central, and parietal regions. McCarren (10) reported that lead administered to rat pups during nursing period resulted in adult having a shift of the modal frequency of hippocampal theta (4-12 Hz), from 7 Hz to 5 Hz, leaving 8-12 Hz activity unaffected. Burdette and Goldstein (11) have observed that lead selectively depressed only the 6-7 Hz power in the hippocampus, in rats exposed to lead during gestation and pre-weaning phases. They did not study the rats during the period of exposure to lead, but only about 55 days after discontinuation of exposure to lead, whereas the present study provides data on EEG power spectral altera-

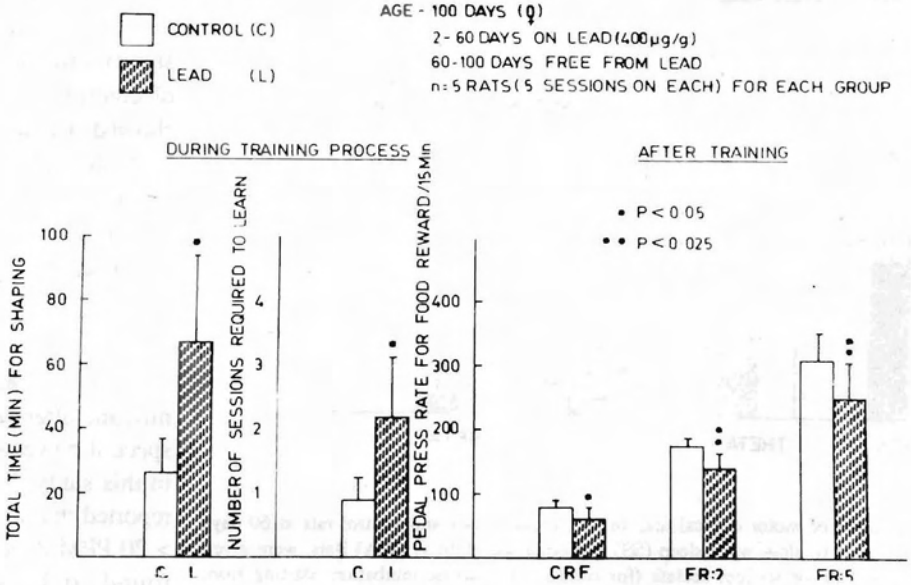

Fig. 4 : Operant learning acquisition and performance tested at 100 days of age in rehabilitated rats that previously had lead exposure till 60 days of age. Testing involved pedal pressing for food reward in Skinner box in continuous reinforcement (CRF) and fixed ratio (FR:2, FR:5) schedules. n=5 rats (5-6 sessions in each) for control (C) and lead (L) group. Other legends are as in Fig. 2.

tions occurring during exposure to lead at 60 days of age. Our study revealed a significant reduction in EEG spectral power in all bands.

The basis for the changes observed in our study could be due to altered development of dendrites, synaptogenesis (12,13), and possibly of reduced mitosis of glial cells and microneurons, and altered cell metabolism due to lead toxicity. Alterations of cell metabolism could cause changes of membrane composition in the neuronal elements on either side of the synapse (14). The reduction of EEG power in all the bands could imply a reduction in synaptic currents of dendrites and also a change in the manner of synchronization in the synaptic activities.

Following lead consumption in rats, there would be a selective accumulation of lead in hippocampus (15). Collins *et al* (16) have not only confirmed it but also showed that significant amounts of lead will persist in brain tissue for as long as 2 to 4 weeks after withdrawal of the lead treatment. Our lead exposure regimen was similar in doses and route to that of Toews *et al.* (17) who observed that with 400 µg/gm dose, lead levels in brain reached a peak at 30 days of age and termination of the lead treatment from that age resulted in a gradual fall of brain lead levels to about 30% of that of prior-peak level by 120 days of age (i.e. after 90 days rehabilitation). In our present study the lead exposed rats were rehabilitated for a period of 40 days following last dose of lead, hence the lead levels in brain would be higher than that observed in Toews's study (17). A previous study on neurotransmitters (noradrenaline, dopamine, serotonin) (7) using the same dose, route and time period of exposure as was followed in the present study, indicated that major amine pathways in the HI would be significantly affected by lead.

Learning disability : The present study showed a learning disability in lead treated group. Brown (18) reported that learning handicap can be produced in rat by doses of lead which alter neither body growth nor indices of developement if the rat is exposed to lead for the first 10 days after birth, suggesting that early postnatal brain may be especially susceptible to lead toxicity. The prolonged period of synaptogensis in hippocampus (19) may offer a vulnerability to the substantial lead induced pathology as is observed in this area relative to other brain structures, like in reduction in the width of cell layers (20) and in complexity of dendrites and synaptic profiles (13, 20-27). The present study showed that hippocampal EEG power is significantly reduced under lead toxicity. Harley (23) found that animals with hippocampal lesions exhibited random arm selection in a sunburst (radial arm) maze, in contrast to non-operated controls or animals with cortical lesions who selected maze arms oriented towards the goal. That deficit in a spatial test and the present findings of decreased operant performance, may be due to motor cortical as well as hippocampal aberrant development (due to lead consumption) as reavealed by the present data of EEG power reduction, and by a previous study on neurotransmitters (7). Levin *et al* (24) exposed Rhesus monkeys to lead (0.7 mg/kg, body weight/day) from soon after birth to about the end of first year, and later tested them between 7 and 9 years of age on delayed spatial alternation task (DSA). They found deficits in performance on the test. The results of our study, like the other types of studies mentioned above, establish that lead exposure in early stages of brain development can lead to significant handicap in learning, persisting even at a much later age.

REFERENCES

1. Byers RK, Lord E. Late effects of lead poisoning on mental development. *Amer J Dis Child* 1943; 66: 471-494.
2. David O, Clark J, Voeller K. Lead and hyperactivity. *Lancet* 1972; 1: 900-903.
3. Chisolm JJ. The use of chelating agents in the treatment of acute and chronic lead intoxication in childhood. *J Pediat* 1968; 73: 1-5.
4. Lin-Fu JS. Undue absorption of lead among children. A new look at an old problem. *New Engl J Med* 1972; 286: 702-710.
5. Needleman HL. Lead at low dose and behaviour of children. *Acta Psychiat Scand* 1983; 67 (Suppl. 303) : 26-37.
6. Christopher W, Ian K. Lead Neurotoxicity: A review of the biochemical, neurochemical and drug induced behavioral evidence. *Prog Neurobiol* 1984; 22:59-87.

7. Shailesh Kumar MV, Desiraju T. Regional alterations of brain biogenic amines and GABA/glutamate levels in rats following lead exposure during neonatal development. *Arch Toxicol* 1990; 64:305-314.

8. Rajanna B, Mascerenhas C, Desiraju T. Deviations in brain development due to calorie undernutrition and scope of their prevention by rehabilitation: alterations in the power spectra of the EEG of areas of the neocortex and limbic system. *Dev Brain Res* 1987; 37:97-113.

9. Snedecor GW and Cochran WG. *Statistical methods* 1967. (Oxford and IBH Publ), New Delhi.

10. McCarren M. Short and long term effects of neonatal lead exposure in rats: behaviour and neurophysiology. *Diss Abstr* 1982 (cited in Ref. 11).

11. Burdette LJ, Goldstein R. Long term behavioral and electrophysiological changes associated with lead exposure at different stages of brain development in the rat. *Dev Brain Res* 1986; 29: 101-110.

12. Manalis RS, Cooper GP. Presynaptic and postsynaptic effects of lead at the frog neuromuscular junction. *Nature (London)* 1973; 243:354-356.

13. Alfano DP, Petit TL. Neonatal lead exposure alters dendritic development of hippocampal dentate granule cells. *Exp Neurol* 1982; 75: 275-288.

14. Niklowitz WJ. Subcellular mechanisms in lead toxicity. Significance in childhood encephalopathy, neurological sequelae and late dementias. In : Roizin L, Shiraki H eds. *Neurotoxicology*. New York, Raven Press, 1977; 289-298.

15. Fjerdingstad EJ, Danscher G, Fjerdingstad E. Hippocampus: selective concentration of lead in normal rat brain. *Brain Res* 1974; 80: 350-354.

16. Collins MF, Hrdina PD, Whittle E, Singhal RL. Lead in blood and brain regions of rats chronically exposed to low doses of the metal. *Toxic Appl Pharmacol* 1982; 65 : 314-322.

17. Toews AD, Blaker WD, Thomas DJ, Gaynor JJ, Krigman MR, Mushak P, Morell MP. Myelin deficits produced by early postnatal exposure to inorganic lead or triethyltin are persistent. *J Neurochem* 1983; 41: 816-822.

18. Brown DR. Neonatal lead exposure in rat : decreased learning as a function of age and blood lead concentration. *Toxicol Appl Pharmacol* 1975; 32 : 628-637.

19. Murthy KD, Desiraju T. Synapses in developing cingulate and hippocampal cortices in undernourished rats. *Neuro Report* 1991; 2: 433-436.

20. Petit TL, Alfano DP, LeBoutillier JC. Early lead exposure and the hippocampus: A review and recent advances. *Neurotoxicology* 1983; 4 : 79-94.

21. Alfano DP, LeBoutillier JC, Petit TL. Hippocampal mossy fibre pathway development in normal and postnatally lead-exposed rats. *Exptl Neurol* 1982; 75: 308-319.

22. Campbell JJ, Williams Er, Balltrop D. Motor neurone disease and exposure to lead. *J Neurol Neurosurg Psychiat* 1970; 73 : 1-6.

23. Harley CW. Arm choices in a sunburst maze: effects of hippocampactomy in the rat. *Physiol Behav* 1979; 23: 283-290.

24. Levin ED, Bowman RE, Wegert S Vuchetich J. Psychopharmacological investigations of lead-induced long term cognitive deficit in monkeys. *Psychopharmacology* 1987; 91 : 334- 381.