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BEHAVIOURAL AND NEUROTOXIC EFFECTS OF CHRONIC LOW LEVEL LEAD EXPOSURE IN THE DEVELOPING RAT

A Thesis
Presented to the
School of Graduate Studies
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by
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DEDICATION

To my wife, Carol, whose generous support and kind encouragement were invaluable throughout the course of my studies.

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AIMS AND OBJECTIVES

The goal of establishing an animal model of subclinical lead poisoning in the developing organism has met with limited success. Many but not all investigators have been able to demonstrate an elevated motor activity in rodents that have received chronic lead treatment. In addition, it has been reported that lead-exposed rodents display a "paradoxical" response to CNS stimulants. Both of these observations are consistent with symptoms that have been described in children who have experienced subclinical lead poisoning. Neurochemical investigations suggest that lead induced hyperactivity in rodents may be related to alterations in noradrenergic and dopaminergic systems in the CNS. However, there is little concensus on the characteristic neurochemical changes, if any, associated with heightened locomotor activity observed in chronically lead treated animals.

There is some evidence to suggest that lead may not be equally distributed to all regions of the brain and that the metal may preferentially accumulate in some areas of the CNS (e.g. hippocampus) over others. However, the kinetics of lead distribution to different regions of the brain have not been examined. Furthermore, it remains to be determined whether or not the unequal distribution of lead in the brain will result in regional variations in the neurotoxic effects of chronic exposure to the metal.

In many of the previous studies on chronic lead toxicity, the dose of metal administered was so large that the relevance of observations made to subclinical lead poisoning is questionable. The present study was undertaken to evaluate the behavioural and neurochemical consequences of chronic lead exposure in the developing rat using truly low doses of the metal. The doses of lead selected for use in this study (0.1 and 0.025 mg lead/kg) represent 40 times and 10 times, respectively, the total daily intake of lead by the human population. It is assumed that the resulting blood and brain lead concentration in treated rats will bear some relationship to the amount of metal that is found in lead exposed

children.

A series of experiments were designed to answer the following questions:

- Does chronic exposure of rats to relatively low doses of lead result in a heightened spontaneous locomotor activity?
- Will chronic lead treatment result in a generalized increase in locomotor activity over the whole circadian cycle or will hyperactivity be restricted to specific periods of this cycle?
- Will the extent of change in locomotor activity be related to the dose of lead administered and the duration of chronic exposure?
- Will lead treated rats display an altered response to the CNS stimulant d-amphetamine and, if so, will this altered response be related to lead induced changes in basal locomotor activity?
- Do these low doses of lead result in any significant alterations in the steady state levels of noradrenaline and dopamine in various brain regions?
- Do alterations in locomotor activity of chronically lead treated rats correlate with any changes in the brain regional levels of catecholamines?
- Are there significant differences in the distribution and kinetics of lead among various regions of the brain?
- Do regional differences in lead concentration, if any, correlate with any variations among brain regions in the neurotoxic effects of chronic lead exposure?

By attempting to answer the above questions, the present study should further our understanding of the behavioural and neurochemical consequences of chronic low level lead poisoning.

1. LITERATURE REVIEW

Environmental Sources of Lead

Lead is one of the most common heavy metals present in the environment. By measuring the concentration of lead in snow strata preserved in glaciers, scientists have demonstrated that the concentration of lead in ice resulting from precipitation has risen from 1 ng/kg present in 800 B.C. to more than 200 ng/kg recorded in more recent times (Murozumi et al., 1969). This increase in the lead content of the biosphere over time has been attributed to the activities of man. In industrialized centers of the world, a primary source of lead in the environment has been the combustion of leaded gasoline by automobiles and smelter emissions (Hardy et al., 1971; Lepow et al., 1975). For urban man there are three major routes of lead exposure; the air we breathe and the food and water we consume. Therefore, some of this metal is ultimately absorbed resulting in an elevated body burden of lead (Lockeretz et al., 1975; Landrigan et al., 1975a). For example, the mean concentration of lead in the blood of a sample population from an industrialized city (Ann Arbor, Michigan) was 14.6 µg/dl. This is many times greater than 0.83 µg/dl, the mean blood level measured for a similar sized sample of natives living in a remote part of a South American jungle (Hecker et al., 1974).

The amount of lead absorbed by individuals vary greatly depending on their occupation, the geographical location of their residence and their eating habits (Lepow et al., 1975; Caprio et al., 1974; Kasowski and Kasowski, 1976; Richter et al., 1979). However, in order to establish a standard, it has been estimated by the National Research Council of Canada that the daily intake of lead by the average Canadian is about 175 µg. This is made up of 140 µg from food, 20 µg from water and 15 µg from air (NRC, 1973).

In addition to the sources of lead intake described above, children are susceptible to further lead exposure resulting from the practice of pica or by other hand to mouth activities. Up until the 1940's white lead was widely used

in the manufacture of indoor paints. Thus children with the habit of placing non-nutritional objects in their mouth and who resided in older homes with peeling plaster and paint were found to be at greater risk of experiencing an elevated lead intake (Griggs et al., 1964; Chisolm and Kaplan, 1968). McCusker (1979) in a recent paper demonstrated that there was a direct relationship between exposure to paint and plaster and a rise in blood lead levels in young children. This finding is in agreement with the observation that there is a greater incidence of pica in children with what is considered moderate (30-49 µg/dl) and high (50-67 µg/dl) blood lead levels (Johnson and Tenuta, 1979). Lead from vehicle exhaust and smelter emissions eventually fall out of the atmosphere to contaminate the dust and soil surrounding a child's home and playing area. Dust samples taken from houses in Boston were found to contain 0.1% to 0.2% lead by weight and the concentration of metal measured in Boston and Washington street dirt was greater than 0.5% (Needleman and Scanlon, 1973). This is 250 times higher than the reported concentration of this metal in the earth's crust (Merck Index). It is thought that in addition to pica, other hand to mouth activities typical of children such as sucking fingers and other unhygenic practices inadvertently result in the ingestion of contaminated dirt and dust and thus contribute to the lead intake of children (Lin-Fu, 1973; Landrigan et al., 1975a; Day et al., 1975; LePow et al., 1975). Sayre and co-workers (1974) conducted a study in which they were able to demonstrate that there was a definite relationship between the amount of lead recovered from children's hands and the household levels of metal. They argued that the lead on the children's hand originated from house dust and that as a result of the mouthing activities, it would be ingested and absorbed. This has been confirmed by Roels et al. (1980) who investigated the association between blood lead levels in children, the amount of metal on their hand and the airborne lead concentrations in the region where they lived. They determined that there was a significant relationship between lead on hands and lead in blood. They

concluded that in children the ingestion of lead from contaminated hands represented a source of intake that was of greater importance than the absorption of airborne lead via the lungs. Therefore, in addition to the daily intake of lead from contaminated food, water and air, these reports clearly indicate that children are exposed to additional amounts of lead via pica and other hand-to-mouth activities.

The greater oral intake of lead by children provides an explanation as to why their blood lead levels are elevated as compared to adults residing in the same region. Sayre and collaborators (1974) conducted a study in Rochester, N.Y. where they observed that the mean blood lead levels of a group of children (1-6 years old) was 44 µg/dl. This value was more than 2-fold greater than the mean concentration of metal in the blood 20 µg/dl of adults from the same region. Similar observations have been made by another group (Roels et al., 1980) who investigated the blood lead levels of children residing in the vicinity of a lead smelter. They found that the mean concentration of lead in the blood of children living less than 1 km from the smelter was 27.8 µg/dl in contrast to 18.0 µg/dl observed in a sample of adults taken from the same area. They also noted that the blood lead levels of children from a rural area, some distance from the smelter, was less than half (10.7 µg/dl) of that of a similar group who lived in the vicinity of the lead smelter. The observations described above emphasize that children are at a greater risk of elevated lead intake in comparison with adults.

Measures of Lead Exposure

Lead in blood exists in a dynamic state. A study of lead metabolism in humans indicated that the kinetics and distribution of the metal could be interpreted by a 3 compartment model. As a result of excretory and redistribution processes the concentration of lead in blood decreases when exposure is discontinued, the half life of lead in the blood was found to be 27 days

(Rabinowitz et al., 1973). There is evidence which indicates that the noxious effects of the metal can persist for long after the source of lead exposure is removed (Mellins and Jenkins, 1955; de la Burbé et al., 1975). Thus it is important to be able to gauge both current and past incidences of lead intake. In contrast to blood, lead is stored and retained by calcified tissues therefore the concentration of lead in teeth can be used successfully as a measure of previous lead exposure (Altshuller et al., 1962). Studies using dentine lead levels have confirmed that the amount of metal to which an individual is exposed is a function of geographical location (Needleman et al., 1972; de la Burbé and Shapiro, 1975). However, since it takes some time for lead to be incorporated in teeth, and because teeth are not readily accessible, the concentration of lead in blood is still used as the primary measure of the amount of metal absorbed by the body and in screening studies to identify high risk children.

Over the years, there has been considerable debate as to how much lead in blood should be regarded as acceptable. In 1961, a subcommittee on Accidental Poisoning (Press et al., 1961) reported that the upper limit of normal blood lead levels in children should be 60 ug/dl. This value was determined on the basis that this was the concentration associated with the onset of clinical symptoms of lead poisoning. However, epidemiological studies later demonstrated that the range of blood lead levels in the general North American population was 15-40 ug/dl (Berman, 1966; Goldwater and Hoover, 1967; Blanksma et al., 1969). Therefore the Surgeon General in 1971 (Steinfeld, 1971) recommended that 40 ug/dl instead of 60 ug/dl should be established as the maximum allowable blood lead levels. Biochemical studies have indicated that the heme biosynthetic pathway is very sensitive to the presence of lead in blood. It has been demonstrated that there is a positive correlation between blood lead levels and the concentration of protoporphyrin in blood and researchers have observed that heme biosynthesis is affected even when blood lead values were less than 40 ug/dl (Sassa et al., 1973; Tomokuni et al., 1975). In 1975 the Center for Disease

Control (1975) conducted a screening study of blood lead concentrations and erythrocyte protoporphyrin levels. A significant fraction of children with blood lead of 30-39 ug/dl were found to suffer from impaired heme biosynthesis as detected by erythrocyte protoporphyrin determination. They concluded that undue lead absorption exists when a child has confirmed blood lead levels between 30 and 79 ug/dl and/or erythrocyte protoporphyrin values of 60-189 ug/dl.

Neuropsychological Consequences of Low Lead Exposure in Children

One of the most dramatic consequences of lead intoxication is the damage done to the central nervous system (CNS) (Smith, 1964). Acute lead poisoning, usually associated with blood lead levels greater than 80 ug/dl can result in encephalopathy. This severe neurological disorder manifests itself in symptoms which include convulsions, coma and respiratory arrest (Smith, 1964; Greengard et al., 1965). In the more severe cases it can result in death (Niyogi, 1974). The persistent nature of the neurotoxic effects of the heavy metal is evident in the large percentage of children who survive acute lead encephalopathy but retain some brain damage. Mellins and Jenkins (1955) observed that 6 months following lead poisoning at least 11 out of 15 children displayed deficits in language ability and fine motor coordination.

Although it is obvious that lead in large doses is highly neurotoxic, the extent to which moderate increase in lead intake damages the CNS is somewhat less clear. Over the years, there have been a number of reports which have assessed the impact of slightly elevated body burdens of lead on children's behaviour. Many investigators have employed sensitive neuropsychological measures in order to detect subtle changes in behaviour that would normally escape clinical detection, hence the term subclinical lead poisoning is often used to describe these cases.

In 1972, de la Burbé and Choate (1972) examined a group of 4 year old children who had a history of moderate lead exposure. None had clinical

symptoms, but the mean blood lead concentration was 58.0 µg/dl. These children were compared to others from a similar socioeconomic background but who had had no unusual exposure to lead. Results indicated that there was a greater incidence of subnormal I.Q. (Stanford-Benit) in children with elevated lead exposure. In addition, the lead exposed group displayed other behavioural disorders, such as extreme distractibility, with greater frequency than did controls. A follow-up study was done 3 years later by de la Burbé and Choate on these same children (de la Burbé and Choate, 1975). The authors demonstrated that dentine lead concentrations of a sample of children from the originally defined lead exposed group (202.1 µg/g) was greater than that from the control group (111.6 µg/g). These findings confirmed that the two groups of children had indeed differed in lead intake. Psychological assessment of these 7 year olds, using the Wechler Intelligence Scale for children, indicated that the lead exposed children still had a lower I.Q. than did the controls. Other tests indicated that the lead exposed youngsters suffered from additional problems such as reduced visual and fine motor co-ordination, and these children were found to still have a shorter attention span than controls. The results of this follow up study clearly indicate that subtle behavioural disorders in lead exposed children are truly of a persistent nature.

These observations of reduced I.Q. in children with slight increases in lead body burden have been supported by other studies which point to a relationship between mental retardation and elevated lead intake (Beattie et al., 1975; Landrigan et al., 1975; Needleman et al., 1979). Landrigan and coworkers (1975b) using the Wechsler Intelligence scale test demonstrated that the I.Q. of lead exposed children (mean blood lead level of 48 µg/dl) was significantly lower than that of controls (mean blood lead level of 27 µg/dl), the mean scores being 95 versus 103. However, the association between moderate increases in lead intake and subtle behavioural deficits is not unequivical. Yotok (1972)

conducted a study in which he demonstrated that there was no difference in mental performance between controls (blood lead = 38 ug/dl) and lead exposed children (blood lead = 81 ug/dl), but he did note that both of these groups of children displayed developmental delays relative to a third group of children from a higher socioeconomic level. In another study, Lansdown and colleagues (1974) demonstrated that children living near a source of lead pollution had higher blood lead levels than those who lived further away. However, they found that measures of intelligence and behaviour were unrelated either to blood lead concentrations or to the distance of their homes from the pollution source. In both of these reports (Kotok, 1972; Lansdown et al., 1974) it was implied that social factors rather than elevated lead exposure were primarily responsible for the lower I.Q. scores and greater frequency of behaviour disturbances.

There are many confounding variables that can result in a misinterpretation of data in studies of this nature. Elevated lead intake occurs more frequently in inner-city area which are often associated with economic disadvantages. Consequently there are a number of other factors such as poor educational facilities, unstimulating home environment and inadequate nutrition that could handicap a childs mental development. These situations could distort the effect that lead may have on a child's behaviour. Another source of controversy has been the sensitivity of behavioural tests employed, obviously if the appropriate measures are not used then deficits will not be observed. Interestingly, both Kotok (1972) and Lansdown and colleagues (1974) acknowledged the possibly that the psychological tests they utilized may not have been sensitive enough to detect subtle behavioural alterations that could result from subclinical lead poisoning.

In 1979, Needleman and coworkers (1979) published on a comprehensive study in which they attempted to analyze some of the shortcomings of many of the earlier papers. Using dentine lead levels so that they could more accurately gauge a child's history of lead exposure, they identified two groups of school

children within a population of first and second graders. The "elevated lead group" were children with dentine metal levels greater than 20 ppm, whereas the controls, or the "low lead group" as they were referred to, had concentrations of less than 10 ppm in their teeth. After controlling for 39 possible confounding variables including medical history, socioeconomic status and parents' I.Q., members of the "high lead group" were found to have a significantly lower I.Q. than those in the "low lead group". In addition, the children that had an elevated lead intake also scored less well than controls on other tests of verbal ability and were found to be less attentive and more impulsive. Based on their results the authors concluded that subclinical lead poisoning appears to be associated with neuropsychological disorders that may interfer with a child's classroom performance.

It has also been suggested (David et al., 1972; Baloh et al., 1975; Beattie et al., 1975) that some incidences of hyperactivity in children may be the result of subclinical lead poisoning. Hyperactivity is a multifaceted behavioural disorder and in accordance with this the American Psychiatric Association Diagnostic and Statistical Manual (DSM III, 1979) has suggested that it be renamed as "attention disorder deficit with hyperactivity". The diagnosis of this behavioural disorder is not made solely on the basis of exaggerated body movement. The following criteria have been established by the Diagnostic Statistical Manual:

a) Exaggerated body movements, excessive crawling, climbing, walking or fidgeting are characteristic. The quality of the activity as well as the quantity differs from the norm.

b) Poor attention span, difficulty in maintaining attention for a long enough period to complete a task that has been started.

c) Impulsive behaviour, chronic impatience and low tolerance threshold.

d) The above abnormalities persist for a duration of at least one year.

When counselling and behaviour modification techniques fail to improve a child's condition, medication, in particular d-amphetamine and methylphenidate, are often prescribed. This seemingly paradoxical response to CNS stimulants is another feature of the hyperactive syndrome. Clinical studies with hyperactive children have demonstrated that following treatment with these agents, originally pointless activity becomes more goal oriented (Conners et al., 1967), in addition, impulsive behaviour (Campbell et al., 1971) and aggression is notably reduced (Winsberg et al., 1972). There have also been reports that treatment with CNS stimulants results in a longer attention span (Sykes et al., 1972), improved learning abilities and better performance of fine motor tasks (Knights and Hinton, 1969).

In a study by David et al. (1972) the body burdens of lead in hyperactive and non-hyperactive groups of children were compared. The diagnosis of hyperactivity was determined by the observations of physicians, teachers and parents. The physician's rating scale was based on the incidence of increased motor activity, poor impulse control, short attention span, hyperexcitability and low frustration tolerance. Children diagnosed as being hyperactive were subsequently found to have significantly higher blood lead levels and the post-penicillamine urine lead concentration of these children was also greater than that of controls. Penicillamine is a metal chelating agent which mobilizes much of the lead stores in the body. In 60% of the hyperactive group versus only 20% of controls, the urine lead levels were in the toxic range. These findings thus indicate that hyperactive children have an elevated body lead burden relative to controls. This observation by David et al. (1972) has been confirmed by other reports of elevated locomotor activity in children with moderately increased lead levels (David, 1974; Baloh et al., 1975; Rummo et al., 1979). It is interesting to note that while not all the studies of children with elevated lead burdens have reported a greater incidence of exaggerated body movement, for the most part authors have noted other symptoms of the hyperactive

syndrome in lead exposed children such as greater distractibility (Mellins and Jenkins, 1955; de la Burbé et al., 1972), fine motor dysfunction (Pueschel et al., 1972; de la Burbé et al., 1975; Landrigan et al., 1975b) and heightened irritability (Pueschel et al., 1972). In the study by Needleman and colleagues (1979) hyperactivity per se was not reported in children from the high lead group. However, it is interesting to note that these children did display with greater frequency than did the low-lead group, other behavioural patterns that are typical of the hyperactive syndrome such as greater distractibility, lack of persistance, inability to follow directions and lack of organization. The authors explain this discrepancy by suggesting that teachers were relunctant to label a child as being "hyperactive". This observation by Needleman and coworkers (1979) emphasizes how examiner bias can interfer with the accurate assessment of neuropsychological function when subjective measures are utilized and underlines the need for more objective studies of the relationship between elevated lead intake and hyperactivity.

Animal Models of Chronic Lead Poisoning

In an attempt to better understand the behavioural consequences of chronic increased lead intake many researchers have undertaken animal studies of lead poisoning. Since locomotor activity of small animals can be measured with little or no bias on the part of the researcher and with relative ease by using locomotor activity measuring devices, animal studies have for the most part focused on the question as to whether or not subclinical lead poisoning results in hyperactivity of developing animals. At the same time, numerous investigators have examined various neurochemical parameters in brains in an attempt to elucidate the mechanism by which lead may alter behaviour.

Pentschew and Garro (1966) demonstrated that a diet of 4% lead carbonate administered to lactating rats can cause encephalopathy in their suckling offspring. Whereas the mother rats were able to tolerate the 4% lead diet, their litters developed symptoms of acute lead poisoning. Between 23 and 29

days of age, 90% of the lead exposed offspring developed transient paraplegia which lasted no longer than 2 weeks and about 85-90% of these animals died during this period. Examination of the brains from the poisoned litters indicated that much of the damage was confined to the cerebellum and was attributed to an increased permeability of the intracerebral capillaries. Neuropathological changes in the brain have also been reported in immature mice that have encountered an increased level of lead intake via mother's milk (Rosenblum and Johnson, 1968). In another study of lead encephalopathy in rats Goldstein and coworkers (1974) also observed hindlimb paralysis between 26 and 28 days of age in the offspring of mothers that were treated with lead in a similar manner as were those in the study by Pentschew and Garro (1966). The brains of these animals revealed diffuse hemmorages and severe edema which was more prominent in the cerebellum than in other brain areas. The authors proposed that the observed damage was the result of increased capillary permeability and further suggested that cerebellar capillaries are more vulnerable to lead than those in other regions of the brain. These injuries observed in rats exposed to large doses of lead are in accordance with hemorrhagic and edematous encephalopathy observed in children suffering from acute lead poisoning (Lin-Fu, 1973 and Barltrop, 1969). The introduction of this animal model of lead poisoning initiated an extensive study on the effects of lead on the behaviour and associated neurochemical systems in developing rodents.

Kinetics of Lead

In view of the greater vulnerability of newborn animals to the toxic effects of lead, many studies have compared the kinetics of the metal in suckling rodents and in adults. Experiments have indicated that age is an important variable which influences the extent of lead absorption from the gastrointestinal tract (GIT). In 1971, Kostial and colleagues (1971) demonstrated that whereas 55% of an oral dose of radioactive lead was absorbed and retained over a 3 day period by 1 week old pups, only 1% was taken up from the GIT in older animals

(4 months old). Forbes and Reina (1972) have also observed that age influences the intestinal absorption of the metal. These authors noted that prior to weaning about 90% of the lead dose is absorbed but within two weeks after weaning the fraction of metal taken up from the GIT drops to 15%. Findings of elevated absorption of lead in immature rodents have been confirmed in monkeys (Willes et al., 1977; Pounds et al., 1978).

The intestinal uptake of lead has been shown to be influenced by diet. Quarterman and Morrison (1975) demonstrated that a reduced intake of dietary calcium and phosphate in adult rats resulted in an elevated absorption of lead. These results were later confirmed and extended by Conrad and Barton (1978) who reported that in the GIT lead is absorbed primarily in the duodenum. They also found that calcium, iron and zinc decrease the intestinal absorption of the metal. In addition, Conrad and Barton (1978) reported that substances which complex with lead and increase its solubility enhance absorption of the metal. Milk is the principal dietary intake of suckling pups and it has been shown that the presence of milk in a diet significantly increases the intestinal uptake of lead in both adult and immature animals (Kello and Kostial, 1973). Moreover, it was demonstrated that the milk sugar, lactose, is capable of enhancing the absorption of lead from the GIT in weaning rats. Bushnell and DeLuca (1981) reported that lactose in physiological quantities administered by gastric intubation, significantly increased the intestinal uptake into blood of a single dose of radiolabelled lead that was also administered by gavage. Based on the findings of the above studies, it was concluded that milk, the principal food of suckling animals, influences the intestinal absorption of lead and consequently may play a complex role in the increased sensitivity to the metal observed in developing animals.

In addition to the differences in intestinal absorption of lead between developing and adult animals, differences in the kinetics of lead distribution and excretion have also been described. Momčilović and Kostial (1974) noted

that the rate of elimination of lead from the whole body was lower in suckling pups (15 days old) as compared to adult (150 days old) rats. This observation of prolonged retention of lead in immature rats have been confirmed in mice by Keller and Doherty (1980) who demonstrated that the rate of lead elimination from the whole body of 10 day old mice was less rapid than elimination of the metal from mice that were 13 to 16 weeks old. Thus it appears that the greater vulnerability of immature rodents to lead poisoning could be due to an elevated uptake and prolonged retention of the metal by these younger animals.

Both clinical (Barry and Mossman, 1970; Barry, 1975) and animal studies (Mykkänen et al., 1979; Mykkänen et al., 1980) on the distribution of lead to the various organs of the body indicate that the lowest concentrations of the metal can be found in the brain suggesting that the CNS is better protected against lead exposure. However, there is evidence to suggest that in immature animals the brain is more accessible to the metal. Following a single dose of radiolabelled lead administered intraperitoneally, Momčilović and Kostial (1974) observed that a greater fraction of the dose had accumulated and was retained in the brain of suckling rat pups when compared to the adults. Furthermore, a study on the effect of age on tissue distribution of lead (Mykkänen et al., 1979) has demonstrated that whereas liver and kidney lead levels parallel the decline in blood lead, with increasing age and duration of treatment the metal content of the brain remains consistently elevated for up to 7 weeks of age. Therefore the increased deposition of lead in the brain incurred by an animal during its early stages of development can persist into adulthood and continue to exert its toxic effects on the CNS.

In addition to interorgan differences in lead distribution there have been reports that lead is not deposited equally to the different areas of the brain. One of the first reports of regional variation in lead distribution came from Stowe and colleagues (1973) who reported that in dogs receiving chronic lead

treatment the concentration of lead in the occipital gray region of the brain (2.347 ppm) was significantly greater than the lead content of seven other brain areas examined. Fjerdingstad and others (1974) reported that in untreated rats the concentration of lead in the hippocampus was much greater than that in half-brains and it was later demonstrated that there is also a selective accumulation of the metal in the amygdaloid regions of the rat brain (Danscher et al., 1975). The findings of preferential deposition of lead in the hippocampus has been confirmed in chronically lead treated rats in a recent study by Fox and colleagues (1982). It is interesting to note that the heavy-metal selectively accumulates in the hippocampus, an area of the brain thought to be involved in the process of habituation to a novel stimulus and the central regulation of goal directed behaviour (Sanwald et al., 1970; Powell and Hines; 1974).

Effect of Chronic Lead Treatment on Locomotor Activity

Silbergeld and Goldberg (1973) were among the first to report on lead induced hyperactivity in rodents. Newborn mice were exposed to lead via its transfer from the mother's drinking water to milk on which the suckling animals fed. The dams were exposed to three levels of metal; 2, 5 or 10 mg lead/ml of drinking water. At all doses the locomotor activity of lead treated offspring was significantly elevated relative to age matched controls. The lead poisoned mice were also found to be more aggressive than the untreated groups as demonstrated by a greater frequency of fighting among littermates. However, the doses of metal used in this study were so large that some of the animals developed symptoms of severe neurological damage such as ataxia of the hindlimbs, the largest level of lead exposure even produced convulsions and deaths. In addition, growth and physical development was retarded in pups at all levels of treatment. This observation is of particular significance in view of the recent studies which have demonstrated that undernutrition or food deprivation alone can cause hyperactivity in rodents that have not been treated with lead (Loch

et al., 1978; Yamamoto and Kutscher, 1981).

Michaelson and coworkers (Sauerhoff and Michaelson, 1973; Michaelson and Sauerhoff, 1974) were first to report on lead induced hyperactivity in the rat. Their experimental design was considered an improvement over that of Silbergeld and Goldberg (1973) in two respects. First the dose of lead administered was reduced to a level such that symptoms of severe neurological damage were not evident. Secondly, the control pups were raised by pair fed mothers. Pair feeding is the process by which control mothers were given a restricted quantity of regular rat chow that was equivalent in weight to the amount of lead contaminated food consumed by dams in the treatment group. In so doing, the discrepancy in nutritional intake between untreated and lead exposed mothers was minimized. This was a necessary control because Michaelson and Sauerhoff (1974) had demonstrated that lactating rats raised on a lead contaminated diet (5% lead acetate) consumed 30% less food than controls which would result in undernutrition of the pups, thus introducing a confounding variable. No significant differences in physical development was observed between the offspring of lead treated and pair fed control mothers. However, examination of behaviour revealed an elevated locomotor activity and heightened aggressiveness in the lead exposed group as compared to the offspring of pair-fed controls. These observations by Michaelson and Sauerhoff (1974) clearly demonstrated that lead induced hyperactivity in the rat is not necessarily associated with retarded body growth.

As discussed earlier one of the characteristics of the hyperactive syndrome in some children, is their paradoxical response to CNS stimulants (Sykes et al., 1972; Winsberg et al., 1972). This clinical observation too has been duplicated in animal models of lead induced hyperactivity. Silbergeld and Goldberg (1974) reported that lead exposed hyperactive mice displayed an unusual response to centrally active drugs. As expected injection of amphetamine and methyl phenidate increased the locomotor activity of controls. However,

in lead treated hyperactive mice these drugs significantly suppressed their elevated motor activity. Furthermore, a CNS depressant drug, phenobarbital, produced as expected a decrease in the motor activity of control animals but caused a further increase in the already heightened locomotion in lead poisoned mice. A more extensive pharmacological investigation of chronic lead poisoning (Silbergeld and Goldberg, 1975) revealed that lead treated hyperactive mice displayed altered locomotor responses to a number of other centrally active drugs including apomorphine, atropine, benzotropine, L-DOPA, fenfluramine and physostigmine. Lead exposed mice were found to respond in a similar manner as did the controls to other drugs; for example, chlorpromazine caused sedation in both groups of animals. Neostigmine a quaternary anticholinesterase which in contrast to physostigmine poorly penetrates the blood-brain barrier and consequently had minimal central activity had no effect on lead induced hyperactivity.

The phenomenon of lead induced hyperactivity in rodents is not without controversy. In contrast to previous studies, Sobotka and colleagues (1974 and 1975) failed to observe an increase in basal locomotor activity of lead poisoned rats although they were able to duplicate the earlier findings of a "paradoxical" response to d-amphetamine by animals that had received chronic lead treatment (Sobotka and Cook, 1974). Unlike the earlier experiments, in these studies by Sobotka and coworkers (1974 and 1975) lead was administered daily directly to the pups via gastric intubation and doses of 9, 27 and 81 mg lead acetate/kg were used. However, Overmann (1977) observed a significantly elevated motor activity in rats that were also treated directly (by gavage) with similar doses of lead (10, 30 and 90 mg lead acetate/kg; daily). Therefore it does not seem that the difference in the method of lead administration could account for the varied results. Other workers (Krehbriel et al., 1976; Cutler et al., 1977; Grant et al., 1980; Rafales et al., 1981) have reported an unaltered motor activity in animals exposed to lead indirectly via the mother's

milk in a similar manner as had been used in earlier experiments which reported incidences of hyperactivity (Sauerhoff and Michaelson, 1973; Silbergeld and Goldberg, 1973). It would therefore appear that the method of lead administration to the pup (be it either directly via gastric intubation or indirectly via mother's milk) is not the confounding variable which could explain the inconsistent findings. The controversy surrounding the effects of lead on motor activity is further fueled by observations of reduced locomotor activity in lead poisoned rodents. Driscoll and Stegner in 1976 reported a significantly lower level of open field motor activity in rats that were exposed to lead via their mother's milk. The metal was administered to the dams via their drinking water using a similar concentration (4 mg lead acetate/ml) as was given by Silbergeld and Goldberg (1975). Furthermore, the running wheel activity had been found to be significantly reduced in the offspring of rats treated with 0.1 mg lead chloride/ml (Verlangieri et al., 1979) and 1 mg lead acetate/ml (Hastings et al., 1977) in their drinking waters. The offspring of mice exposed to 0.025, 0.05 and 0.1 mg lead acetate/ml have also been found to be hypoactive relative to controls as measured by magnetic locomotor activity measuring devices (Modak and Stavinoha, 1979).

Of thirty papers on the effect of sublethal lead exposure on locomotor activity, about 50% have demonstrated lead induced hyperactivity, 33% found that the metal had no significant effect and 17% have reported a reduced locomotor activity in lead exposed animals. Because of the varied methods of lead administration employed it is difficult to establish a dose-response relationship between lead and locomotion. However, as has been discussed earlier, there have been a few cases where different experiments that were using similar doses of metal and the same route of administration have produced conflicting results, suggesting that variables other than the dose of lead and method of administration may be responsible for at least some of the conflicting

results.

Strain dependent variation in the circadian pattern of locomotor activity has been observed in rats (Lemmer et al., 1981) and there have been reports of differences in locomotor response to centrally active agents between various strains of mice have also been reported (Davis et al., 1974; Anisman et al., 1975). In addition, Mykkanen and coworkers (1980) demonstrated that there is a varied sensitivity to lead poisoning between different strains of rats. With regard to physical and neurological development of newborns they observed that the Hooded strain was less susceptible than the Wistar strain to lead intoxication. Over the years, there have been at least 13 reports on lead and locomotor activity which have utilized the Sprague-Dawley strain of rat, 8 (62%) have found hyperactivity in the lead treated animals. Of 5 studies that used the Long-Evans strain 3 (60%) noted an elevated motor activity in the poisoned animals. Moreover, 48% (n=21) of those investigators that used rats reported significant increases in locomotor activity whilst a similar proportion of the mice 57% (n=7) were also found to be hyperactive. Since the proportion of different strains and species which have reportedly displayed hyperactivity following lead treatment is similar, it would appear that the lead induced hyperactivity is not strain or species dependent.

It has been demonstrated that hyperactivity in lead poisoned rats is of a transient nature (Reiter, 1977; Dubas and Hrdina, 1978). Dubas and Hrdina (1978) reported that the motor activity of lead treated rats was significantly elevated between 6 and 10 weeks of age but had returned to within the range of control activity levels at the age of 12 weeks. This would suggest that age at the time of activity measurement may be a confounding variable. However, upon examination of many experimental reports on postnatal lead exposure and locomotor activity we found that the age range of those rats reported to be hyperactive (13-84 days) was similar to that of the non-hyperactive lead treated cases (21-90 days). From this we can conclude that age alone

is not likely to be the variable responsible for the inconsistent locomotor activity findings in lead treated rodents.

When measuring locomotor activity some authors (Sauerhoff and Michaelson, 1973; Krehbiel et al., 1976; Dubas and Hrdina, 1978) have recorded the total body movements of groups of animals collectively whilst others (Silbergeld and Goldberg, 1973; Sobotka and Cook, 1974) have assessed the locomotion of each animal individually. A distinction should be made between these two methods of measurement, since group activity in addition to recording the spontaneous locomotor activity of individual animals, also reflects the degree of social interaction between members of the group. Upon examination of the results from previous experiments on lead and behaviour we noted that 60% of researchers that measured group activity reported an elevated locomotor activity in poisoned animals. Of the remainder who recorded the motor activity of individual animals a similar fraction (61%) reported incidences of lead induced hyperactivity. This comparison indicates that although the two methods of measurement record different types of body movements, it does not appear to make any difference to the detection of lead induced hyperkinesis.

The locomotor activity of rodents is characterized by its relationship to the dark/light illumination cycle in a circadian rhythm. Rodents are nocturnal animals and as such are always markedly more active during the dark period as compared to the light period of a circadian cycle (Holmquest et al., 1966). Lemmer and Berger (1978) demonstrated that a dopamine β-hydroxylase inhibitor FLA 63 elevated motor activity during the light phase, but in contrast caused a reduction in activity during darkness. This phase dependent variation in locomotor response has also been observed in lead treated rodents. In a study by Dolinsky and coworkers (1981) the locomotor activity of mice treated with lead pre- and postnatally was examined for 3 hours, at different times during the circadian cycle. The authors observed that whereas the locomotor activity of lead exposed mice was significantly greater than that

of untreated animals during the dark phase no difference in locomotion was noted between the animals that had received lead treatment and control during the light period. It is interesting to note that in instances where lead induced hypoactivity has been observed and where the locomotor activity of lead exposed rodents was compared with that of controls during both the dark and light cycle, some investigators (Reiter and coworkers, 1975; Modak and Stavinohoa, 1979) have reported a significant reduction in both the nocturnal and diurnal activity of chronically lead treated rodents as compared to controls.

On the basis of the comparisons made earlier between the controversial studies on the effects of lead on locomotor activity, it appears that the method of lead administration, species and strain differences, age at time of testing, or group versus individual activity measurements do not singularly explain the conflicting findings of previous experiments. However, we cannot rule out the possibility that an interaction between some or all of the above factors may be responsible for some of the inconsistent results observed from the different laboratories.

Central Regulation of Locomotion

Central regulation of locomotor activity is thought to be mediated at least in part by dopaminergic and noradrenergic systems in the brain (Benkert et al., 1973; Pijnenburg and Van Rossum, 1973). Dopamine containing neurons in the rat brain are condensed systems with highly specific and organized pathways that are located primarily in the upper mesencephalon and diencephalon regions (Moore and Bloom, 1978). There are three major components to the system: 1) the nigro-striatal pathway which originates from the substantia nigra and ventral tegmental area; the axons of cells in this pathway terminate principally in the neostriatum and globus pallidus; 2) the mesocortical pathway; the neurons of this pathway have their cell bodies in the ventral tegmental area and terminate in the frontal and cingulate cortex as well as the limbic system. The constituent of the

mesocortical pathway that innervates the nucleus accumbens and amygdala is sometimes distinguished and identified as the mesolimbic system; 3) the tuberoinfundibular system; dopamine containing neurons originating in the arcuate nucleus project their axons to the pituitary stalk. This system is important for central neuroendocrine regulation.

In contrast to the dopaminergic pathways, the noradrenergic neuronal system in the rat brain is a diffuse network innervating many regions of the CNS (Moore and Bloom, 1979). Noradrenaline containing neurons originate from cell bodies located in the region of the pons and medulla. The locus coeruleus is made up of A_6 and A_7 cell bodies and contains the most abundant noradrenergic neuron population. Axons from this group of cell bodies project rostrally via the dorsal catecholamine bundle and the median forebrain bundle to innervate the cortex, thalamus, hypothalamus and other structures of the limbic system. A second grouping of cell bodies A_1, A_2 and A_5 are also located in the medulla. These neurons collectively form the ventral catecholamine bundle and via the median forebrain bundle thay innervate the septal area, the periventricular region and the substantia nigra among other structures of the brain.

The role that catecholamines play in the regulation of locomotor activity has been extensively investigated. Benkert and coworkers (1973) pharmacologically manipulated catecholamine metabolism in rats to cause an increase in endogenous noradrenaline and dopamine levels in brain. Associated with the rise in whole brain concentrations of catecholamines the authors observed a significant elevation in the locomotor activity of rats, they also noted an increased incidence of fighting among these animals. Similar observations were made by Pijnenburg and van Rossum (1973). In their study exogenous dopamine injected directly into the nucleus accumbens of nialamide-pretreated rats caused an increase in motor activity of the animals. In addition, the authors noted that dopamine produced a greater stimulation of locomotion than did noradrenaline. It was later demonstrated (Pijnenburg et al., 1975) that in the nucleus

accumbens the dopaminergic system is the principal pathway via which catecholamines exert their effects.

Amphetamine is a sympathomimetic agent which enhances the activity of both noradrenergic and dopaminergic systems in the CNS (Glowinski and Baldessarini, 1966; McKenzie and Szerb, 1968; Moore, 1969; von Voigtlander and Moore, 1973; Horn et al., 1974). The behavioural effects of d-amphetamine are dose dependent. Low doses (1.5 mg/kg) elevate locomotor activity while high doses (5 mg/kg) produce stereotypic behaviour. Using this varied response to amphetamine Kelly and colleagues (1975) demonstrated that locomotor activity and stereotypic behaviour in rats are probably controlled by separate dopaminergic systems in the brain. They observed that 6-hydroxydopamine induced lesions of the dopamine pathways in the caudate nucleus caused a suppression of the stereotypic behaviours normally associated with high doses of amphetamine but did not alter the elevated locomotor activity response to low doses of the drug. On the other hand, similarly induced lesions of the dopaminergic systems in the nucleus accumbens had no effect on the stereotypic response to high doses of amphetamine and dramatically suppressed the elevated locomotor activity normally caused by low doses of the CNS stimulant. Kelly and colleagues concluded that the dopamine pathways in the caudate are essential for amphetamine induced stereotypy while the dopaminergic system in the nucleus accumbens probably play an important role in locomotor activity responses to the drug.

The importance of noradrenergic mechanisms in the regulation of locomotor activity has also been demonstrated. The administration of an α adrenergic receptor blocking agent (phenoxybenzamine) to rats was found to inhibit the spontaneous locomotor activity of these animals indicating that the proper functioning of the central noradrenergic systems is necessary for the regulation of basal motor activity in these animals (Maj et al., 1972a). It is also thought that amphetamine induced hyperactivity in rats is mediated partly via the stimulation of central α-adrenergic receptors located in the brain (Maj

et al., 1972b). Andén and coworkers (1977) examined the role of noradrenergic systems in L-dopa induced hyperactivity, and concluded that noradrenaline receptor activation was probably involved in mediating the elevated locomotor activity response to L-dopa. Lemmer and Berger (1978) proposed that whereas dopamine may be involved in regulation of nocturnal locomotion, noradrenaline is probably associated with the control of diurnal locomotor activity.

Neurochemical Consequences of Chronic Lead Poisoning

The possible involvement of brain catecholamines in lead induced hyperactivity was first reported on by Sauerhoff and Michaelson (1973) who observed a significant decrease in whole brain dopamine levels of lead exposed hyperactive rats. Since this report there have been several investigations into the concentrations of noradrenaline and dopamine in the brains of lead exposed rodents. The findings of reduced dopamine content in the whole brain of poisoned animals (Sauerhoff and Michaelson, 1973) has been confirmed and extended by further reports of significantly lower levels of this catecholamine in the cortex (Dubas and Hrdina, 1978), striatum (Jason and Kellogg, 1981) and hypothalamus (Dubas et al., 1978). However, other studies have found no changes in the dopamine concentrations in the whole brain (Golter and Michaelson, 1975; Schumann et al., 1977) or brain regions such as the cortex (Grant et al., 1976; Sobotka et al., 1975), striatum (Grant et al., 1976; Dubas and Hrdina, 1978) and hypothalamus (Grant et al., 1976) in rodents that have received chronic lead treatment during postnatal development.

The results of investigations into brain regional noradrenaline levels of lead exposed animals have also been controversial. In their 1973 study, Sauerhoff and Michaelson reported no significant changes in the whole brain concentration of noradrenaline in treated rats relative to controls. In a reappraisal, Golter and Michaelson (1975) found that noradrenaline levels were elevated in the brains of lead exposed rats. Analysis of the noradrenaline content of the brain by regions has failed to resolve this controversy. In

rodents receiving chronic lead treatment during postnatal development researchers have observed that the noradrenaline levels in the cortex may be reduced (Dubas and Hrdina, 1978) or unchanged (Grant et al., 1976) and in the striatum, the level of this catecholamine has been observed to be lower (Dubas et al., 1978; Dubas and Hrdina, 1978) or unaltered (Grant et al., 1976).

In addition to investigating the steady state levels of noradrenaline and dopamine in the CNS of lead poisoned animals, researchers have also examined other measures of catecholamine function both at the presynaptic and post-synaptic level. Much of the research has concentrated on dopamine and relatively little work has been done on the noradrenergic systems.

The effect of chronic lead treatment on the turnover rate of dopamine in various regions of the rat brain has been investigated extensively by Govoni and others (Govoni et al., 1978; Govoni et al., 1979; Memo et al., 1979; Govoni et al., 1980; Memo et al., 1980). These researchers have consistently observed that in lead poisoned rats dopamine turnover is elevated in the nucleus accumbens, reduced in the striatum but remains unaltered in the substantia nigra. These findings have been confirmed in part by a study from another laboratory which also demonstrated a significant reduction in the rate of dopamine turnover in the striatum of lead poisoned rats (Jason and Kellogg, 1981). In contrast to the experiments conducted by Govoni and colleagues (1978-1980) in which they measured the accumulation of dopamine metabolites following the inhibition of synthesis with α-MPT, Jason and Kellogg (1981) estimated the rate of turnover by measuring the rate of decline in the levels of monoamine itself following the administration of the tyrosine hydroxylase inhibitor.

Experiments which examined the effects of lead on ^{3}H spiroperidol binding to dopaminergic receptors (Govoni et al., 1979) demonstrated no significant alterations in the number (B_{max}) or affinity (K_D) of dopamine receptors in the striatum of rats following chronic lead treatment. Lucchi and colleagues (1981) investigated the effects of lead on the two subclasses of dopamine receptors, D_1

and D_2. The authors reported that while chronic treatment with the metal had no significant effect on the D_1 receptors in various brain regions they noted an increase in the number of D_2 receptors (B_{max}) in the striatum and a reduced number (B_{max}) of the same receptors in the nucleus accumbens in lead exposed rats relative to control. The observation that in vivo lead exposure has no effect on the B_{max} or K_D of dopamine receptors linked to adenylate cyclase is in agreement with earlier studies which found that dopamine stimulated adenylate cyclase and cAMP levels were unaltered in various brain regions of chronically lead treated rats (Govoni et al., 1979; Govoni et al., 1980). There have been reports of reduced adenyl cyclase activity in the CNS following in vivo and vitro lead exposure (Nathanson and Bloom, 1975; Ewers and Erbe, 1980). However, in contrast to Govoni et al. (1979) the above researchers (Nathanson and Bloom, 1975; Ewers and Erbe, 1980) did not specifically measure the activity of dopamine-sensitive adenylate cyclase. Therefore the reduced basal adenylate cyclase activity that they observed following lead exposuremay be a reflection of changes in neurotransmitter mechanisms that are not specific to the dopaminergic pathways in the brain.

Numerous studies have attempted to correlate neurochemical alterations in the CNS with hyperactive behaviour resulting from lead exposure. Researchers have reported that noradrenergic mechanisms may be enhanced (Golter and Michaelson, 1975; Silbergeld and Goldberg, 1975; Dubas and Hrdina, 1978) or suppressed (Dubas and Hrdina, 1978) in various brain regions of lead treated hyperactive animals. On the other hand, there are a number of papers which suggest that there is a correlation between depressed function of dopaminergic pathways in the brain and hyperactivity in rodents treated with lead. The high affinity uptake of dopamine by forebrain synaptosomal preparations from lead treated hyperactive mice have been reported to be lower than that of controls (Silbergeld and Goldberg, 1975). In addition, Dubas and Hrdina (1978) observed that the transient elevation in motor activity of lead exposed rats was in

temporal phase with transient reductions in dopamine content of the cortex, hypothalamus and midbrain. Further investigations have reported a significantly slower rate of dopamine turnover in the striatum (Govoni et al., 1980) and a decrease in the K^+ induced release of this catecholamine from forebrain slices as well as a reduction in the dopamine stimulated adenylate cyclase activity of the neostriatum (Wince et al., 1980) in hyperactive rats that were poisoned with the metal. Hence while it is apparent that hyperactivity in lead poisoned rodents may be the result of metal induced changes in catecholamine neuronal systems of the brain, researchers are yet to characterize the exact neurochemical alterations associated with this behavioural disorder.

II. MATERIALS AND METHODS

Experimental Design

Lactating dams of the Sprague-Dawley strain (Canadian Breeding Farm Ltd.) with one-day old litters were housed individually in plastic cages and were given Purina Laboratory Chow and tap water ad libitum. Lead measurement by atomic absorption spectrophotometry revealed that the concentration of lead in a random sample of lab chow was 0.211 µg/g. At two days of age the pups were pooled and randomly cross-fostered, 10-12 pups were returned to each dam and an attempt was made to have equal numbers of both sexes in each litter. The pups were weaned at 22 days of age on the same diet fed to their mothers and at 29 days they were separated on the basis of sex. Control and lead-treated rats were housed in separate cages.

Lead was administered to the pups by gastric intubation, a polyethylene tube was passed down the oesophagus and into the stomach via which an appropriate volume of lead acetate solution was dispensed. Controls received an equal amount of sodium acetate. All solutions were made up in distilled water. During the first two weeks of treatment the concentrations of the lead acetate and sodium acetate solutions were such that a volume of 5 µl/g body weight was administered to each pup. As the animals grew, the concentrations were adjusted regularly so that the volume given to each rat did not exceed 0.3 mls. Treatment was carried out at the same time each day.

The body weight as well as the onset of developmental landmarks such as incisor eruption, hair appearance, eye opening and ability to walk were monitored daily. At the end of the exposure regimen, the animals were sacrificed by decapitation and blood was collected from the trunk. The blood was stored in heparinized polypropylene tubes at -4°C until used for blood lead measurement. The brain was rapidly removed then placed on an ice-cold aluminum block and carefully dissected into cerebral cortex, striatum, hippocampus and midbrain as defined by Glowinski and Iversen (1966). Brain regions were placed in poly-

propylene tubes and frozen immediately in liquid nitrogen then stored at -40°C until analyzed. Regions dissected from one half of the brain (sagittal plane) were used for the assay of noradrenaline and dopamine concentrations or for the estimation of the turnover rate of noradrenaline while samples from the other half were used for the determination of lead content.

The data presented in this thesis are the results of six series of experiments (see Table 1). Series A was designed to be a reference study which would enable us to compare our results with regard to the effects of lead on the circadian pattern of locomotor activity, with observations made by other researchers who had used similar doses of the metal. Beginning at 3 days post partum pups received daily 10.0 mg lead/kg, via gastric intubation until 4 weeks of age (Series A). The diurnal locomotor activity patterns of these animals were measured at 3 and 4 weeks of age. In addition, at ~ weeks we compared the locomotor responses of control and treated animals to the CNS stimulant d-amphetamine.

In order to investigate the consequences of moderate lead exposure, groups of rats were treated via the oral route with 0.1 mg lead/kg daily from 3 days until 4, 6 or 8 weeks of age (Series B, C and D). The basal circadian motor activity as well as the locomotor response to d-amphetamine were recorded when the animals were 4, 6 and 8 weeks old. At these ages we also examined the steady state levels of noradrenaline in the cerebral cortex and hippocampus, as well as the concentration of dopamine in the striatum and hippocampus The lead content of four brain regions, the cerebral cortex, striatum, hippocampus and midbrain were also determined. A group of control and lead exposed rats were withdrawn following 8 weeks of treatment and kept for an additional 2 weeks (Series D). In these animals we examined the effects of withdrawal from elevated lead exposure on locomotor activity, on brain regional catecholamine levels and on the concentration of lead in blood and various areas of the CNS.

TABLE 1

Schedule of Lead Treatment

Series	Dose; Duration of Pb Exposure*	Age at SLA Measurement‡ Basal	d-Amp. Response	Age at CA and Pb measurement§
A	10.0 mg/kg; 8 wks	3,4	7	-
B	0.1 mg/kg; 4 wks	4	4	4
C	0.1 mg/kg; 6 wks	6	6	6
D	0.1 mg/kg; 8 wks	8,10	8	8,10
F	0.025 mg/kg; 8 wks	3,5,6,~		8

*Beginning at 3 days and continuing until 4, 6 or 8 weeks of age lead (Pb) was administered to rat pups daily by gastric intubation as a lead acetate solution. Controls received an equal amount of sodium acetate. In Series D, a group of animals that had been exposed to 0.1 mg lead/kg for 8 weeks were withdrawn from treatment and kept for 2 weeks at which time (i.e., 10 weeks of age) behavioural and neurochemical measurements were conducted.

‡At indicated ages, the individual spontaneous locomotor activity (SLA) of equal numbers of control and lead exposed rats was simultaneously recorded. In addition, the locomotor response of control and treated animals to 3.0 mg/kg d-amphetamine (d-Amp.) was compared.

§At the end of the exposure regimen the steady state levels of catecholamines (CA), noradrenaline and dopamine, were measured in various regions of the brain. In Series D, the rate of turnover of noradrenaline in the hippocampus was also determined. Following chronic lead treatment the concentration of metal was measured in blood and various brain regions.

In an attempt to establish a "no effect" level of lead exposure, we further reduced the dose of lead administered. Groups of newborn rats were treated in a similar manner as described above with 0.025 mg lead/kg until 8 weeks of age (Series E). The circadian pattern of locomotor activity of these animals was recorded at various times during postnatal development. The steady state levels of catecholamines in the striatum, hippocampus and cortex, as well as the concentration of lead in blood and brain regions were also measured.

Experimental Procedures

Measurement of locomotor activity

At appropriate ages, the individual locomotor activity of equal numbers of sex-matched (both sexes) control and lead treated rats was simultaneously recorded over a 23 hour period. Locomotor activity was recorded in an isolated room, with a 12 hour dark/light cycle (6:00 a.m. to 6:00 p.m.) and with controlled temperature (24°C) and humidity (60%). Food and water were available ad libitum for the duration of locomotor activity measurement.

The apparatus used to measure locomotor activity consisted of a low intensity oscillator, the frequency of which is determined by the capacitance between two insulated aluminum plates (20" x 10"). A standard polycarbonate cage (19" x 10" x 8") containing a rat was placed between these plates. Movements of the animal caused a change in capacitance between the two aluminum plates which in turn altered the frequency of the oscillator. The number of cycles of the oscillator was monitored during successive 90 msec intervals, any movement by the animal which caused a change in frequency by a predetermined magnitude was detected and recorded as one count by a computerized counter. Activity is defined as the number of 90 msec periods during which body movement was registered in a one-hour interval, therefore it is expressed as counts per hour. There were 8 such electronic activity measuring devices connected to a Commodore PET 2001 Series computer. However, because there were more animals than there were

recording devices, and since one session lasted 23 hours, it was necessary to subdivide the sample population of control and treated rats into groups and conduct the activity measurements on successive days.

Response to d-amphetamine

The locomotor response to d-amphetamine was investigated in rats that had received 10.0 mg lead/kg for 7 weeks or 0.1 mg lead/kg for 4, 6 and 8 weeks. A d-amphetamine sulfate solution was made up in saline, to a concentration of 1 mg/ml. At selected ages, equal numbers of control and treated animals were injected intraperitoneally with 3.0 mg d-amphetamine sulfate/kg body weight in an alternating fashion, i.e., a control rat then a lead exposed followed by another control, or visa versa. All animals were in their cages and activity measurements began within 10 minutes following the time when the first rat was injected.

For animals treated with the 10.0 mg/kg dose of lead the response to d-amphetamine by control and lead treated rats was compared in a manner similar to that outlined by Sobotka and Cook (1974). The animals were placed in the locomotor activity measuring device for two hours to allow them to become accustomed to the novel environment. The SLA during the third hour of recording was used as the baseline activity. Following the measurement of basal locomotion, the rats were injected with amphetamine sulfate and motor activity was recorded. The response to d-amphetamine during the subsequent 3 hours was expressed as the mean percent increase in SLA counts above the baseline activity levels.

For those rats that were treated with 0.1 mg lead/kg for 4, 6 and 8 weeks, the procedure was altered slightly in order that we might investigate the locomotor response to d-amphetamine throughout the circadian cycle. In these cases control and treated animals were injected with the stimulant then placed into the locomotor recording cages. No acclimatization period was allowed prior to administration of d-amphetamine and no baseline activity levels were measured. The response to the drug is expressed as the mean hourly SLA counts for the

Neurochemical Assays

Concentration of catecholamines in brain

The steady state levels of noradrenaline and dopamine in the various brain regions were measured using a fluorometric technique according to the methods of Laverty and Taylor (1968). The brain tissue samples were homogenized in 3 ml of acidified butanol (0.1% v/v conc. HCl in n-butanol). The homogenate was then centrifuged at 1400 g for 10 minutes and the supernatant which contains the catecholamines extracted from the brain tissue was transferred to a capped test tube together with 5 ml of n-heptane and 1.0 ml of 0.1 N HCl. This mixture was shaken for 10 minutes then centrifuged for 5 minutes in order to separate the aqueous from the organic layer. For the analysis of dopamine a 0.2 ml aliquot of the 0.1 N HCl extract was mixed with 1 ml of 0.1 M potassium phosphate buffer (pH 8). Then 0.1 ml of iodine solution (0.25 g iodine and 5.0 g potassium iodide dissolved in 100 mls of distilled water) was added. Exactly 4 minutes later 0.25 ml of alkaline sulfite (2 g sodium hydroxide plus 0.5 g sodium sulfite and 0.2 g disodium ethylenediamine-tetraacetate (EDTA) in 20 ml of distilled water) was mixed in, followed exactly 5 minutes later with 0.1 ml of glacial acetic acid. The mixture was shaken vigourously and the test tubes were incubated in a 60°C water bath, in the dark, for 40 minutes. The samples were then cooled on ice and the fluorescence read within one hour after the end of incubation.

The procedures used for the oxidation of noradrenaline were similar to that outlined above for dopamine with only minor modifications. A 0.2 ml aliquot of the 0.1 N HCl extract was mixed with 0.6 ml of 1.0 M phosphate buffer (pH 7). The same amount (0.1 ml) of iodine solution as used in the dopamine assay was added. However, the volume of alkaline sulfite and glacial acetic acid was increased to 0.5 ml and 0.2 ml respectively. The time interval between the addition of reagents was the same as those used for the oxidation of dopamine. Samples were not incubated in a 60°C water bath, instead the fluorescence was

read after allowing the samples to stand at room temperature for 20 minutes.

For the measurement of dopamine fluorescence an excitation wavelength of 330 nm and an emission wavelength of 390 nm was used. Noradrenaline fluorescence was read with excitation = 380 nm/emission = 480 nm. An Aminco Bowman spectrophotofluorometer (American Instrument Company, Maryland, U.S.A.) was utilized for the fluorescence determinations. The concentration of catecholamine was determined from a standard curve. The standards were prepared by making up (0, 0.05, 0.10, 0.20 and 0.50 µg/ml) concentrations of dopamine and noradrenaline in 0.1 N HCl then subjecting them to the same oxidation reactions as described above for the catecholamines extracted from brain samples. In order to produce zero standards (blanks) the order in which iodine and alkaline sulfite are added is reversed so that the oxidation of contaminants into fluorophores is prevented.

Catecholamine turnover in the brain

The rate of catecholamine turnover can be estimated from the decline in monoamine levels after the inhibition of synthesis. Tyrosine hydroxylase is the rate limiting enzyme in the synthesis of catecholamines, by blocking the activity of this enzyme in vivo with α-methyl para-tyrosine (αMPT) the production of noradrenaline and dopamine are effectively halted. Inhibition of tyrosine hydroxylase in an intact animal will result in a decline in the steady state levels of catecholamines in brain, due to physiological processes such as neurotransmitter release and metabolism at the level of the synapse. By measuring the concentration of catecholamine at varying times after the injection of αMPT the rate of decline can be determined. In the absence of a tyrosine hydroxylase inhibitor and at steady state conditions the loss of catecholamines in the CNS is replaced by synthesis of new monoamines and at equilibrium the rate of noradrenaline synthesis is equal to the rate of efflux. Therefore the rate of depletion of catecholamines following the administration of αMPT is an estimate of the rate of monoamine synthesis and is usually refered to as the rate of

On the basis of results obtained from the measurement of noradrenaline and dopamine concentration in various brain regions the turnover rate of noradrenaline in the hippocampus was examined. The procedure used was a slight modification of that described by Brodie and colleagues (1966). Following chronic treatment with 0.1 mg lead/kg for 6 weeks, control and lead exposed rats were injected with 250 mg/kg αMPT intraperitoneally then sacrificed 0.5, 1 or 2 hours later. A group of animals were not given αMPT, measurement of the levels of noradrenaline in the hippocampus of these animals indicated the initial concentration of the monoamine, i.e., the steady state levels that were present at time zero after the administration of αMPT. The log of noradrenaline concentration versus time was plotted. From the slope of the straight line and the steady state levels of noradrenaline at time zero, the fractional rate constant (K) and the turnover rate of noradrenaline in the hippocampus were calculated.

The concentrations of noradrenaline in the hippocampus was measured according to the method of Laverty and Taylor (1968) as described earlier. A 2% solution of αMPT was prepared by dissolving 1.0 g of DL-α methyl-tyrosine methyl ester hydrochloride in about 5 ml of 4 N NaOH. The pH was adjusted to 9 with 10 N HCl then the volume was brought up to 50 ml with distilled water.

Lead determinations in blood and brain regions

The concentration of lead in blood and brain regions was measured by flameless atomic absorption spectrophotometry. Whole blood was prepared for analysis using a modification of the method described by Richter and colleagues (1979). A 100 µl aliquot of blood was placed in a polypropylene test tube and diluted with 400 µl of 0.5% (w/v) Triton X-100 (0.05 N HNO_3). The diluted blood samples were then allowed to stand at room temperature for one hour after which a 10 µl aliquot was injected into the graphite furnace of the spectrophotometer. Each sample was done in duplicate.

Preliminary studies in our laboratory confirmed the findings of Richter and coworkers (1979) that dilution of blood samples with Triton X-100 prepared

with acid rather than water enhances the recovery of lead. Our investigations revealed that by increasing the concentration of HNO_3 in which 0.5% (w/v) Triton X-100 was made up from 0.01 N to 0.05 N we were able to increase the recovery of lead from blood samples by 64% (Table 2). We therefore chose to use 0.5% (w/v) Triton X-100 prepared with 0.05 N HNO_3. Furthermore, we observed that by allowing the diluted blood sample to stand for 1 hour before proceeding with analysis, the recovery of lead from prepared blood samples increased about 7 fold (Table 3) relative to that noted if no incubation time was allowed. This finding is in agreement with the methodology described by Nise and coworkers (1978) who allowed their diluted samples to stand for 30 minutes before measuring the lead levels.

Brain samples were prepared for lead measurement using a modification of the procedure outlined by Gross and Parkinson (1974). The tissues were weighed then placed into polypropylene test tubes, to which was added 2 µl/mg tissue of tetramethyl ammonium hydroxide (TMAH) (25% in methanol). The samples and added base were then incubated in a water bath at 70°C for 2 hours, by which time the brain tissues had been digested. To the thick syrupy liquid in the test tubes 8 µl/mg tissue of 1 N HNO_3 was added and mixed vigourously on a vortex agitator, then allowed to stand at room temperature for 1 hour before proceeding with lead measurement. A 10 µl aliquot of the solubilized tissue was then injected into the graphite tube of the furnace. Each sample was assayed in duplicate.

As was the case with blood, we observed that dilution of the solubilized brain samples with 1 N HNO_3 rather than water resulted in a greater recovery of lead from tissue. In addition, we observed that incubation of the sample in the presence of HNO_3 for one hour improved recovery of the metal by 77% as compared to when no incubation period was allowed (Table 3). Using the same procedure described above for brain, we were able to measure the lead content

TABLE 2

Effect of nitric acid concentration on the measurement of lead from blood samples

Tissue	Solvent	
	0.5% Triton X-100 in 0.01 N HNO_3	0.5% Triton X-100 in 0.05 N HNO_3
	Absorbance*	
Blood†	0.368	0.603

†Samples of blood from lead treated rats (100.0 mg lead/kg; p.o., daily for 5 days) were diluted (1:19) with Triton X-100 prepared either in 0.01 N HNO_3 or 0.05 N HNO_3.

*Lead was measured as the mean absorbance resulting from the injection of 10 µl aliquots of prepared sample into a flameless AAS.

Each sample was analysed in duplicate.

TABLE 3

Effect of incubation time on the amount of lead detected in blood and brain samples

Tissue		Duration of incubation (min.) 0	30	60
		Absorbance		
Blood†	Control	0.023*	0.040	0.030
	Lead	0.044	0.368	0.346
Brain‡	Control	0.122	0.133	0.134
	Lead	0.134	0.250	0.237

†Blood samples from a lead exposed (100.0 mg lead/kg; p.o., daily for 5 days) and control rats were diluted (1:19) with 0.5% Triton X-100 (0.01 N HNO_3).

‡The whole brain was digested with 2 µl/mg tissue of TMAH and then diluted (8 µl/mg tissue) with 1 N HNO_3.

The samples were incubated for 0, 30 or 60 minutes at room temperature before proceeding with analysis.

*Lead was measured as the mean absorbance resulting from the injection of 10 µl aliquots of prepared sample into a flameless AAS.

Each sample was analysed in duplicate.

in a random sample of Purina Laboratory Chow, as well as the concentration of metal in the stomach contents from a one week old untreated pup.

The concentration of lead in a sample was determined from simultaneously run calibration curves. These were prepared by adding known amounts of the metal (12.5 ppb - 50.0 ppb) to blood or brain samples (cerebral cortex) from control animals.

A Jarrell-Ash Model 850 (Division of Fisher Scientific Company) atomic absorption spectrophotometer (AAS) with a background corrector equipped with a Jarrell-Ash FLA-100 graphite furnace attachment for the flameless method of analysis was used. Absorbance was measured at an analytical wavelength of 283.3 nm and with a spectral bandpass of 0.4 nm. The operating conditions of the graphite furnace were as follows: DRY, 30 sec at 100°C; ASH, 45 secs at

425-450°C; ATOMIZE, 5 secs at 2500°C. Argon gas flow rate was 1.5 L/min.

The temperature and duration of the ash cycle is critical to the proper measurement of lead. If the temperature is too high or the cycle is too long, lead will vaporize and escape from the furnace resulting in an artificially low reading. On the other hand, if the temperature is too low or if the duration of the cycle is not long enough, residual material in the furnace may result in excess background absorption which can cause an artificially high absorbance reading, particularly if there are relatively low concentrations of lead in the sample. Background absorption occurs when the vapor of sample residue (usually organic material) absorbs light at the analytical wavelength either by molecular absorption or by scattering the light beam. Within certain limits this problem can be corrected by the background integrator of the AAS. However, it is still essential to minimize the background absorbance of a sample by optimizing the conditions of the ash cycle.

In order to verify that we were using proper furnace conditions we compared the calibration curves of tissue lead standards with that of reagent standards. Reagent standards consist of known amounts of lead (12.5 ppb - 50.0 ppb) added to the solvents used in the preparation of blood and brain samples for lead analysis. They are therefore similar to tissue lead standards except for the absence of blood or brain tissue. As can be seen in Figures 1 and 2, the tissue standard curve is above and parallel to that derived from the reagent standards indicating that the recovery of lead from brain and blood tissue blanks (zero standards) was greater than that from their respective reagent blanks and thus demonstrating that our methodology was sensitive enough to detect and measure the small amounts of endogenous lead present in the blood and brains of control animals.

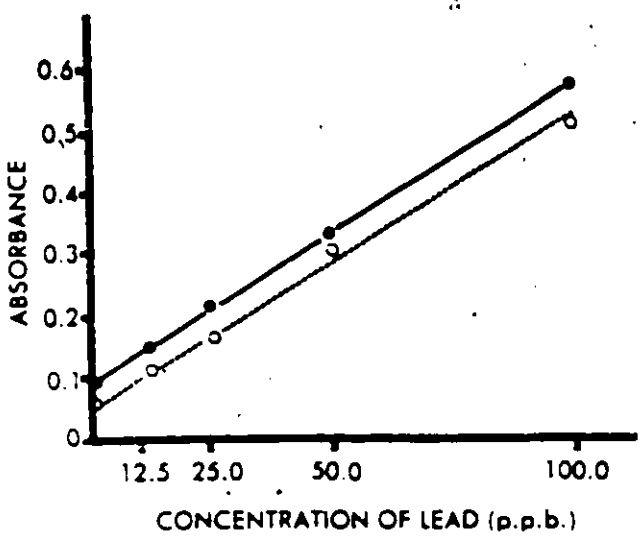


FIG. 1: Brain and reagent standard curves of lead determined by flameless atomic absorption spectrophotometry. Lead standards in brain (●——●) were made up by adding known amounts of lead (final concentration equal 12.5, 25.0, 50.0 and 100.0 ppb) to brain samples digested with 10 µl/mg of 20% Tetramethyl ammonium hydroxide (TMAH) in 1 N HNO_3. Reagent lead standards (o---o) were prepared by adding known amounts of lead (final concentration equal 12.5, 25.0, 50.0 and 100.0 ppb) to a solution of 20% TMAH in 1 N HNO_3. Each point is the mean of two determinations which differed by less than 15%.

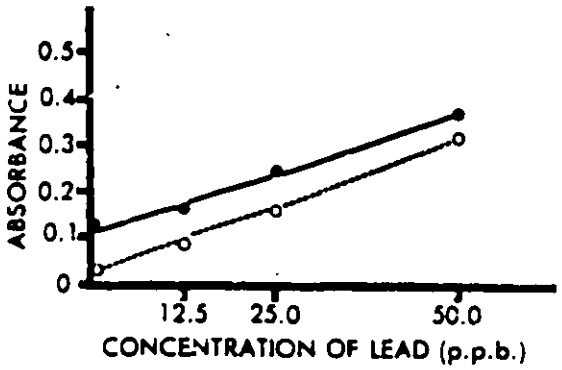


FIG. 2: Blood and reagent standard curves of lead determined by flameless atomic absorption spectrophotometry. Lead standards in blood (●——●) were made by adding known amounts of lead (final concentration equal 12.5, 25.0 and 50.0 ppb) to blood samples diluted (1:4) with 0.5% (w/v) Triton X-100 in 0.05 N HNO_3. Reagent lead standards (o---o) were prepared by adding known amounts of lead (final concentration equal 12.5, 25.0 and 50.0 ppb) to 0.5% (w/v) Triton X-100 in 0.05 H HNO_3. Each point is the mean of two determinations which differed by less than 15%.

Statistical Analysis

All results are expressed as means ± S.E.M. Differences in circadian locomotor activity rhythm (both basal and following d-amphetamine) between control and lead exposed rats were analysed by two-way ANOVA with repeated measures over one factor (time), followed by Scheffe's post hoc test of significance. The two-tail Student's t-test was used for the comparison of cumulative locomotor activity between control and treated rats as well as for analysis of the data on d-amphetamine response that were expressed as percent of preinjection activity. The significance of differences in the concentration of noradrenaline and dopamine was also tested by the two-tail Student's t. A one-tail Student's t-test was also used for the comparison of blood and brain lead levels between control and treated rats. However, for analysis of inter-regional variations in lead content within the brain, two-way ANOVA together with Scheffe's post hoc test was used and the two-tail Student's t-test was employed to examine the time course of change in tissue lead levels. The significance of difference in the slope of noradrenaline depletion following αMPT (hence the turnover rate constant) between control and lead exposed rats was determined by using the test of parallelism (Tallarida and Murray, 1981). Differences between means were considered significant when the calculated p values were less than 0.05.

Drugs and Chemicals

d-Amphetamine sulfate was a donation from Smith, Kline and French Laboratories (Montreal). All other chemicals were obtained commercially in the highest purity available. For lead analysis "virgin" polypropylene test tubes were used.

Growth and Physical Development

Daily exposure of newborn rats to 10.0 mg lead/kg was found to have no significant effect on growth, as measured by body weight gain (Fig. 1). In addition, the time of appearance of certain characteristics of physical development such as hair growth, incisor eruption, eye opening and ability to walk were also unaffected in rats exposed to this dose of lead (Table 1). The onset of these developmental landmarks in rats treated with the 0.1 mg/kg dose of lead was also similar to that observed in controls (Table 2) and until 3 weeks of age there were no significant differences in growth between untreated and lead exposed animals (Fig. 2). However, slight but significant decreases in body weights were noted at 4 and 5 weeks of age in both sexes of treated rats and at 4 weeks only the females were found to weigh significantly less than their respective controls and by 7 weeks of age the body weights of both sexes of lead exposed rats had returned to within control values. It was also observed that chronic exposure of rats to the lowest dose of lead (0.025 mg/kg) during postnatal development did not significantly alter their body weight gain (Fig. 3

Circadian Spontaneous Locomotor Activity

By expressing the SLA of the rats on an hourly basis over a period of 24 hours we were able to establish and compare the circadian patterns of locomotor activity in control and lead treated rats. As can be seen in Figures 4-8 the SLA of these animals is usually dramatically elevated during the initial two to three hours following the onset of measurement. This heightened activity represents the initially exploratory behaviour that is exhibited by an animal when it is placed into a novel environment, in this case the new surroundings of the isolated room and the locomotor activity measuring device. Once the animals have settled down, their locomotor activity during the remainder of the light phase is always markedly lesser than that during the dark phase of the 12 hour dark light lighting cycle. This observation is in accordance with the fact

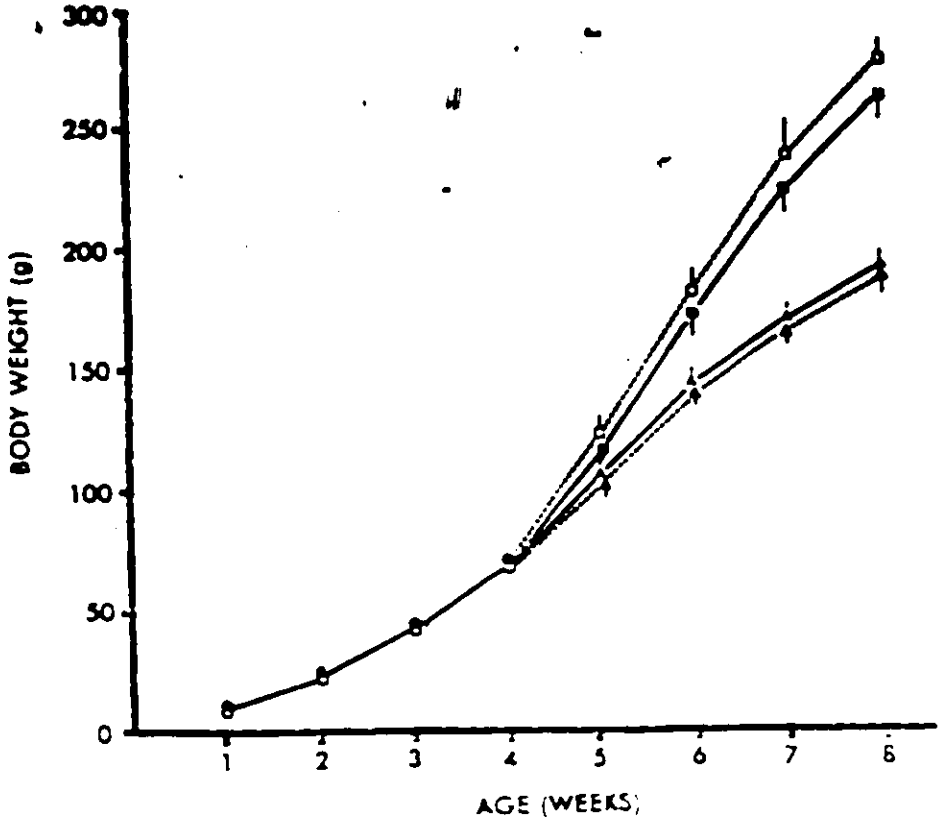


FIG. 1 The body weight gain of rats treated with 10 mg lead/kg during postnatal development (open symbols). Pups (circles) were given 10.0 mg lead/kg daily by gastric intubation from 3 days until 8 weeks of age. Controls (closed symbols) received an equal amount of sodium acetate. At 29 days of age the animals were divided by sex ▲,— males, ■,— females. Each point is the mean ± S.E.M.

TABLE 1

Effect of chronic exposure to 10.0 mg lead/kg on the appearance of landmarks of physical development

Treatment*	Hair Appearance	Incisor Eruption	Eye Opening	Walking‡
	Age§ (Days)			
Control	9	10	16	16
Lead	9	11	15	16

*Beginning at 3 days of age pups were given 10.0 mg lead/kg daily by gastric intubation. Controls received an equal amount of sodium acetate.

‡The developing pups were monitored daily for the appearance of body hair, the eruption of incisor teeth, open eyes and for the ability to walk.

§Each value is the mean age of the litter at the appearance of the various markers of physical development.

TABLE 2

Effect of chronic exposure to 0.1 mg lead/kg on the appearance of landmarks of physical development

Treatment*	Hair Appearance	Incisor Eruption	Eye Opening	Walking‡
	Ages (Days)			
Control	11	12	16	17
Lead	12	13	16	17

*Beginning at 3 days of age pups were given 0.1 mg lead/kg daily by gastric intubation. Controls received an equal amount of sodium acetate.

‡The developing pups were monitored daily for the appearance of body hair, the eruption of incisor teeth, open eyes and for the ability to walk.

Each value is the mean age of the litter at the appearance of the various markers of physical development.

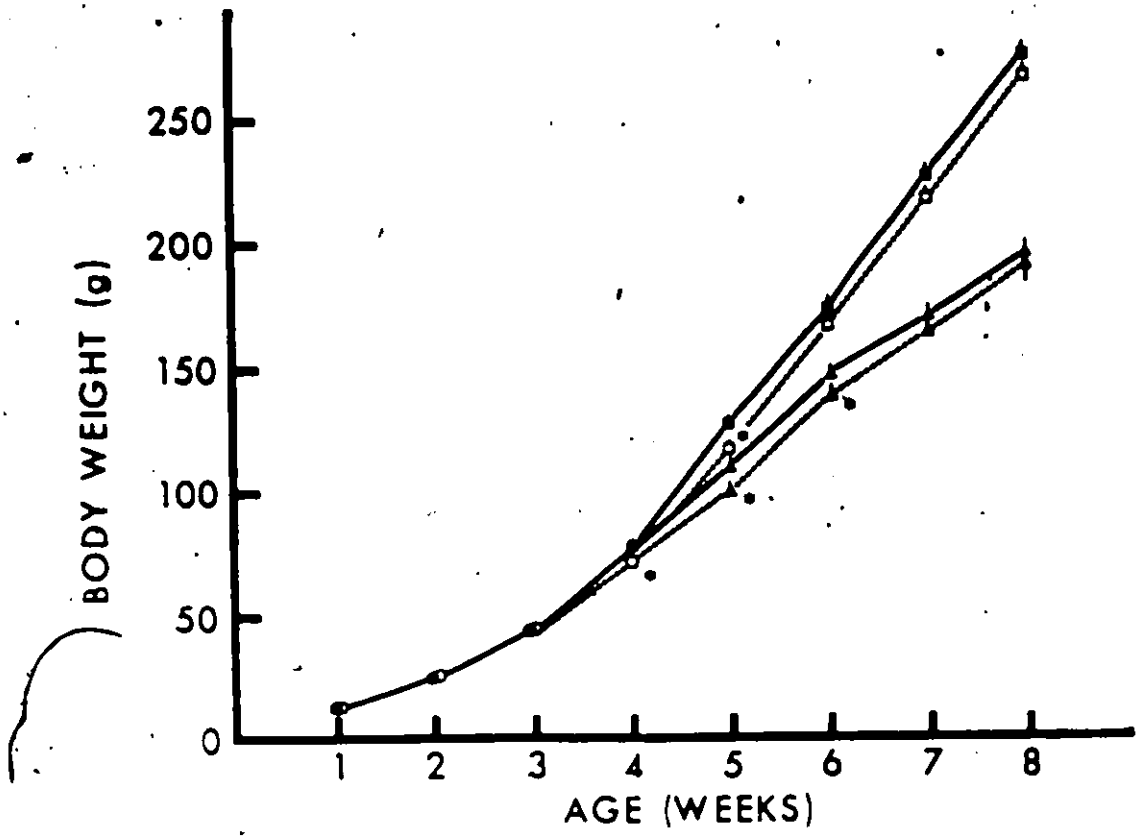


FIG. 2: The body weight gain of rats treated with 0.1 mg lead/kg during postnatal development (open symbols). Pups (circles) were given 0.1 mg lead/kg daily by gastric intubation from 3 days until 8 weeks of age. Controls (closed symbols) received an equal amount of sodium acetate. At 29 days of age the animals were divided by sex (■,□: males; ▲, △: females). Each point is the mean ± S.E.M.

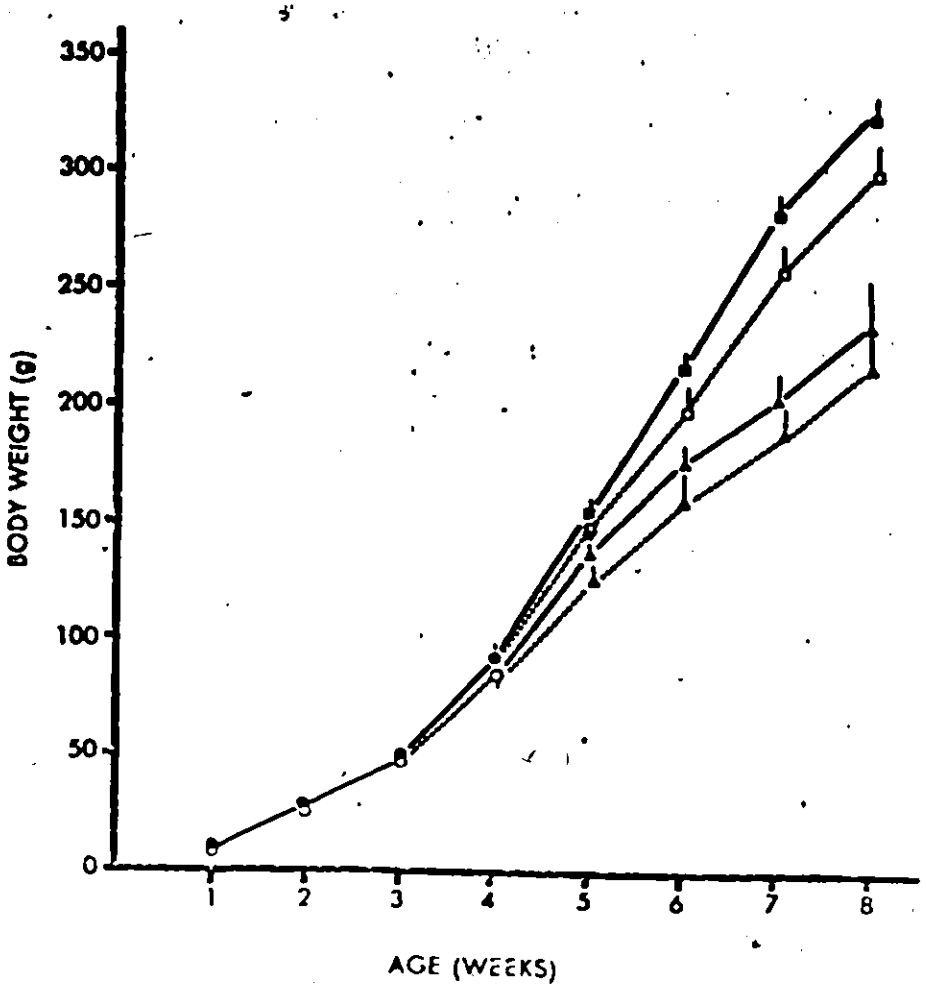


FIG. 5: The body weight gain of rats treated with 0.025 mg lead/kg during postnatal development (open symbols). Pups (circles) were given 0.025 mg lead/kg daily by gastric intubation from 5 days until 8 weeks of age. Controls (closed symbols) received an equal amount of sodium acetate. At 20 days of age the animals were divided by sex (■, □: males; ▲, △: females). Each point is the mean ± S.E.M.

that rats are nocturnal animals and therefore are more active during the dark period. Furthermore, as can be seen in Figure 6 the circadian pattern of 4 week old rats is flatter than that of the more mature animals, i.e., the nocturnal activity of 4 week old rats lack the typical peaks of activity observed at 8 and 10 weeks. This observation suggests that the regulation of locomotor activity in rats is also influenced during ontogenic development.

Presented in Figure 4 is the circadian locomotor activity of rats exposed to 10.0 mg lead/kg for 3 weeks. We observed that during the initial hour of measurement (10:00 - 11:00 hrs) the SLA of treated rats was significantly greater (by 191%) than that of controls. In addition, during the last quarter of the dark period (4:00 - 7:00 hrs) there was a tendency for lead exposed animals to be hyperactive relative to controls (ANOVA: $F(1,11) = 5.42$, $p<0.05$) and there were two time intervals (5:00 - 6:00 hrs and 6:00 - 7:00 hrs) during which the activity of treated rats was significantly ($p<0.05$, Student's t-test) greater than that of untreated animals. Moreover, a comparison of the total locomotor activity counts (i.e. the sum of the recorded body movements) during the dark period (Table 3) revealed that indeed the nocturnal activity of poisoned rats was significantly greater (by 64%) than that of controls. The hyperactivity observed in 3 week old lead treated rats was a transient phenomenon. Measurement of the locomotor activity of animals exposed to the 10.0 mg/kg dose of metal for an additional week revealed no significant alterations in the diurnal SLA rhythm of 4 week old lead poisoned rats when compared to age matched controls (Fig. 5).

Summarized in Figure 6 is the circadian SLA cycle of rats treated with 0.1 mg lead/kg daily for varying periods during postnatal development. Whereas 4 and 6 weeks of exposure to this dose of lead had no significant effect on locomotor activity, treatment with the metal for 8 weeks did result in hyperactivity. Two-way ANOVA indicated that there was a significant interaction between treatment and time ($F(7,84) = 4.48$, $p<0.05$) during the initial light

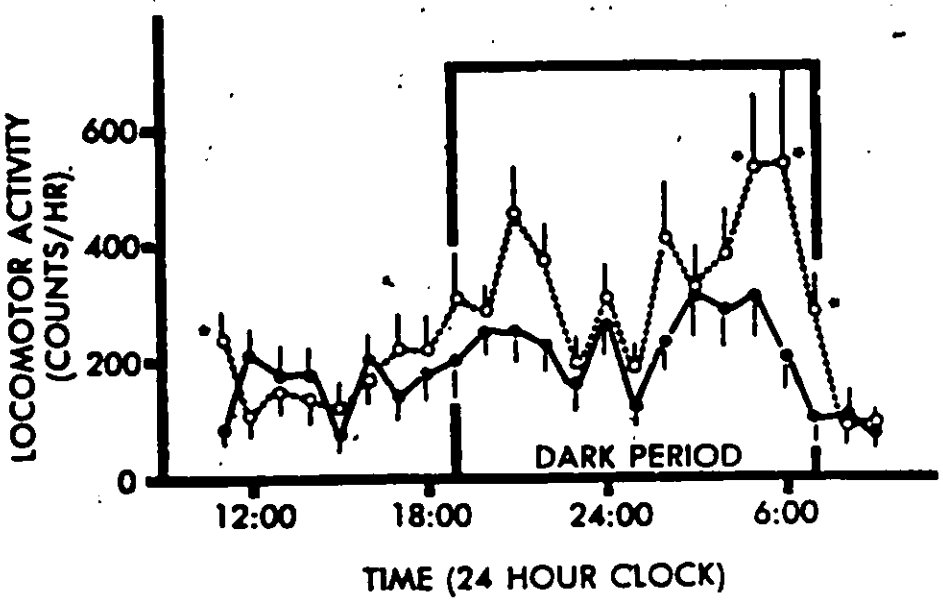


FIG. 4: The circadian spontaneous locomotor activity (SLA) of rats treated with 10.0 mg lead/kg for 3 weeks (o---o). Pups were given 10.0 mg lead/kg daily by gastric intubation from 3 days until 3 weeks of age. Controls (●——●) received an equal amount of sodium acetate. The SLA was individually recorded over an 11 hour light/12 hour dark illumination cycle, by an electronic activity meter. Each point is the mean ± S.E.M. of 8 rats. *Significantly different from control; $p<0.05$.

TABLE 3

Cumulative nocturnal locomotor activity of rats treated daily with 10.0 mg lead/kg from 3 days until 3 weeks of age

Time Interval	Treatment†	Total Locomotor Activity (counts)
19:00-7:00 hrs	Control	2922±301‡ (100)
	Lead	4779±827* (164)

†Pups were given 10.0 mg lead/kg daily by gastric intubation from 3 days until 3 weeks of age. Control received an equal amount of sodium acetate.

‡Values are the means ± S.E.M. of 6 rats. Data are also given as percentages (in parentheses) taking the value of controls as 100 percent.

*Significantly different from control values ($p<0.05$).

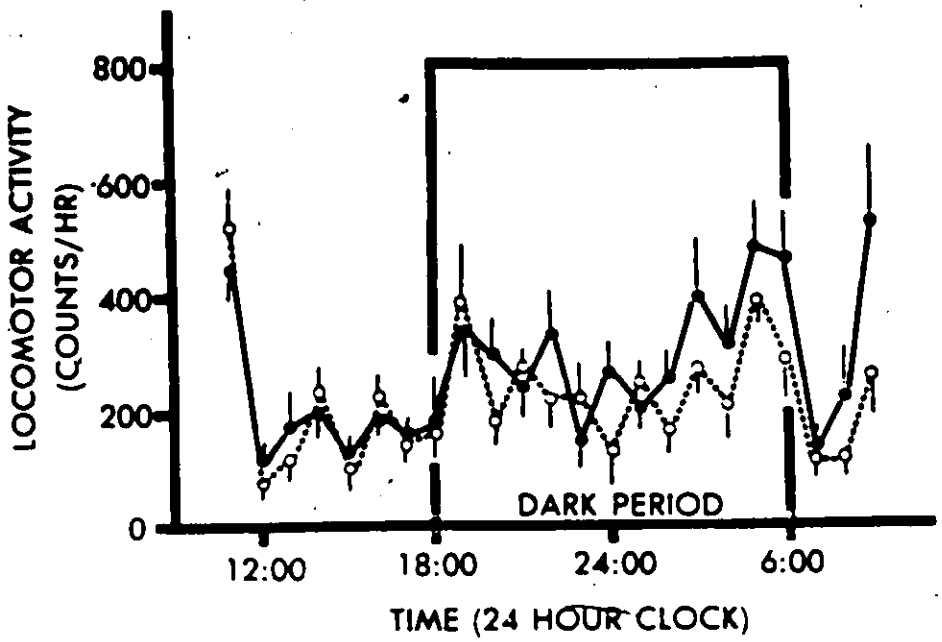


FIG. 5: The circadian spontaneous locomotor activity (SLA) of rats treated with 10.0 mg lead/kg for 4 weeks (o---o). Pups were given 10.0 mg lead/kg daily by gastric intubation from 3 days until 4 weeks of age. Controls (●——●) received an equal amount of sodium acetate. The SLA was individually recorded over an 11 hour light 12 hour dark illumination cycle, by an electronic activity meter. Each point is the mean ± S.E.M. of ~ rats.

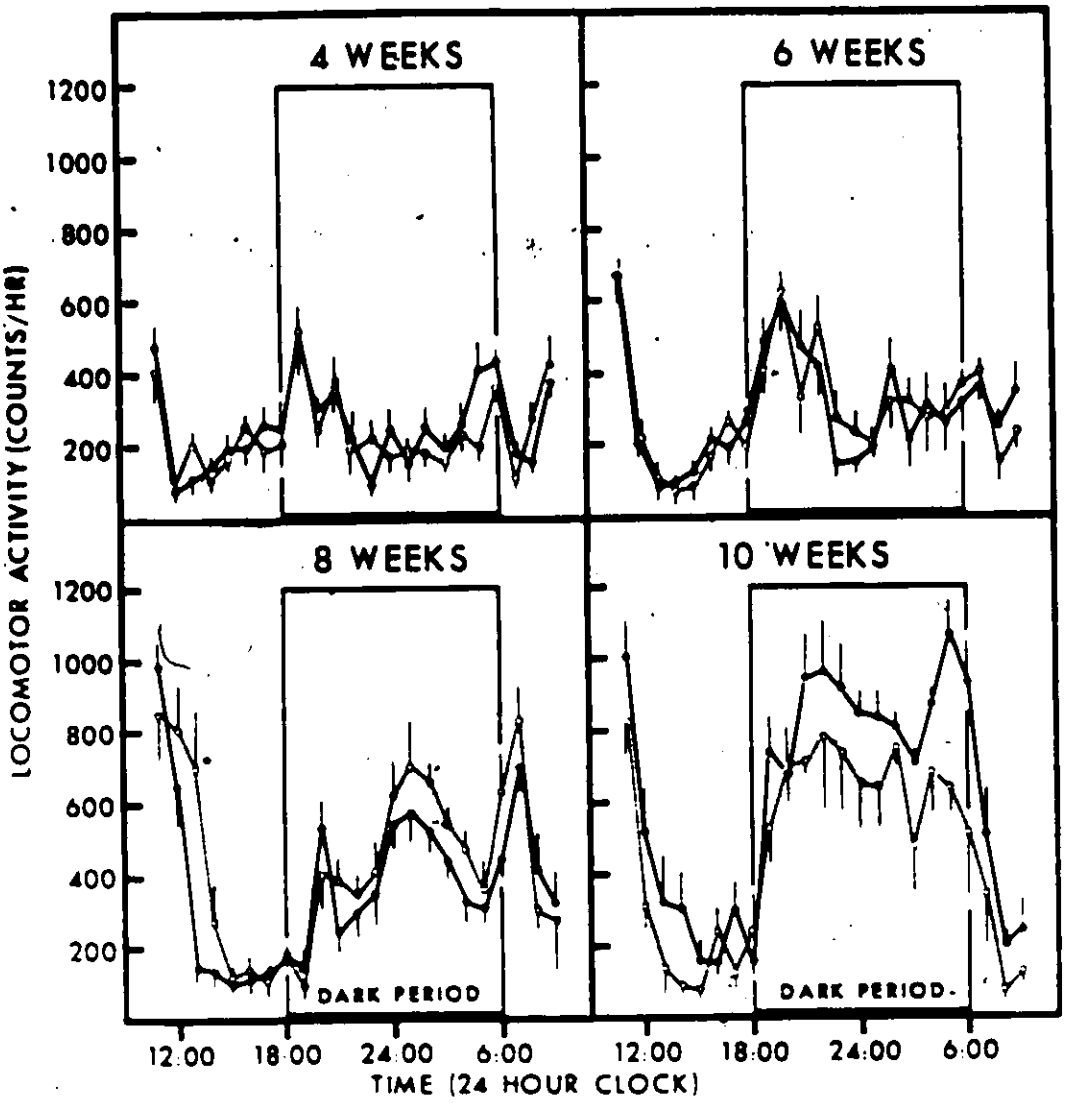


FIG. 6: The circadian spontaneous locomotor activity (SLA) of rats that were exposed to 0.1 mg lead/kg for various periods of time during postnatal development (o---o). Pups were given 0.1 mg lead/kg daily by gastric intubation from 3 days until 4, 6 or 8 weeks of age. Controls (●——●) received an equal amount of sodium acetate. Treatment was discontinued at 8 weeks and a group of animals were kept until 10 weeks of age. The SLA was individually recorded over an 11 hour light/12 hour dark illumination cycle, by an electronic activity meter. Each point is the mean ± S.E.M. of 6-7 animals at 4, 6 and 8 weeks and of 4 rats at 10 weeks. *Significantly different from the control value; $p<0.05$.

phase (10:00 - 18:00 hrs). Post hoc analysis of these data revealed that between 12:00 and 13:00 hrs the SLA of lead treated rats was significantly elevated as compared to that of controls. However, during the dark period (18:00 - 6:00 hrs) the locomotor activity of rats receiving the metal for 8 weeks was not significantly altered relative to that of untreated animals ($F(1,12) = 2.66$, $p>0.05$).

When motor activity was measured two weeks after lead was withdrawn (i.e. at 10 weeks of age) the reverse trend was apparent. The animals appeared to be hypoactive relative to controls over much of the 23 hour period, including the initial hours of measurement. Two-way ANOVA indicated that there was a significant effect of withdrawal on SLA ($F(1,6) = 6.15$, $p<0.05$) during the light period (10:00 - 18:00 hrs). However, no points of significantly reduced locomotor activity were indicated by Scheffe's post hoc test. Analysis of the locomotor activity of these animals, during the dark phase (18:00 - 6:00 hrs) revealed that the SLA of previously lead treated rats was not statistically significantly reduced relative to controls ($F(1,6) = 3.70$, $p>0.05$).

We further analysed the data obtained at 8 and 10 weeks of age by comparing the total locomotor activity counts of treated rats with that of controls during the period from 10:00 hrs to 14:00 hrs (Table 4). At 8 weeks, the cumulative SLA of rats receiving the 0.1 mg/kg dose of lead was significantly elevated (by 69%) relative to controls. Following the withdrawal of treatment for 2 weeks a rebound phenomenon was apparent; the cumulative locomotor activity of previously lead exposed rats was found to be significantly lower (by 35%) than in controls.

Presented in Figure 7 are the circadian patterns of locomotor activity of rats that were treated with 0.025 mg lead/kg for varying periods of time during postnatal development. We observed no changes in the locomotor activity of 3 week old lead treated rats as compared to controls. Animals that had received this dose of metal for 5 weeks were found to be hyperactive during the first

TABLE 4

Cumulative locomotor activity of rats treated daily with 0.1 mg lead/kg from 3 days until 8 weeks of age

Time Interval	Treatment	Age	
		8 Weeks	10 Weeks†
10:00-14:00 hrs	Control	1927±113 (100)	2139±219 (100)
	Lead	3256±445* (169)	1379±164* (65)

†Pups were given 0.1 mg lead/kg daily by gastric intubation from 3 days until 8 weeks of age. Controls received an equal amount of sodium acetate. Treatment was discontinued at 8 weeks and a group of control and lead treated rats were tested 2 weeks later (i.e. at 10 weeks of age).

‡Values are the means ± S.E.M. of 4-7 rats. The cumulative spontaneous locomotor activity is expressed as the total counts between 10:00 hrs and 14:00 hrs. Data are also given as percentages (in parentheses) taking the value of controls as 100 percent.

*Significantly different from the control values ($p<0.05$).

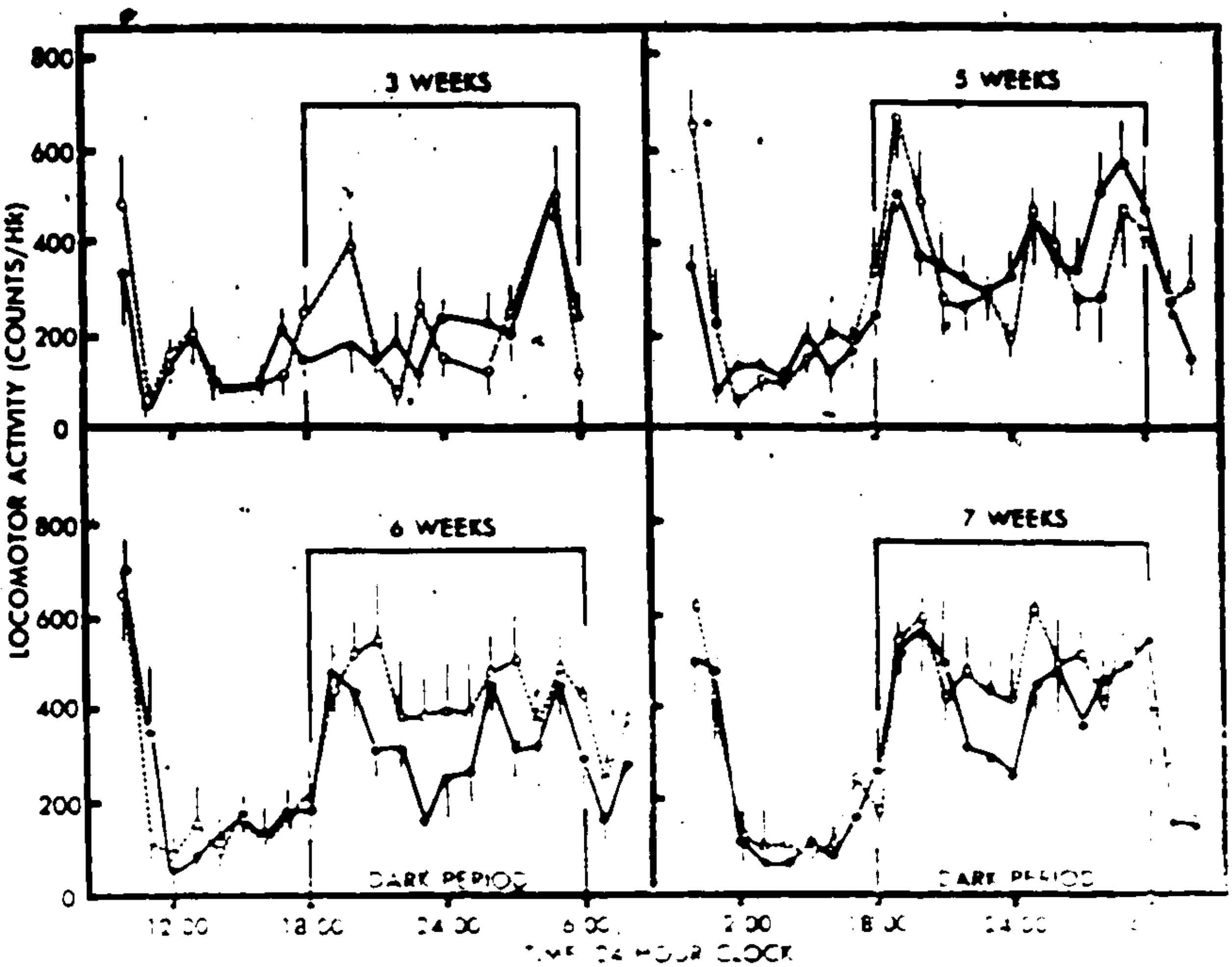


FIG. [illegible] The circadian spontaneous lo[illegible]motor activity [illegible] rats that were exposed to [illegible] mg lead/kg for various periods of time during postnatal [illegible] lopment (o----o). Pups were given [illegible] mg lead/kg daily by gastric [illegible] from [illegible] days until [illegible] weeks of age. Controls (●——●) received [illegible] amount of sodium acetate. The [illegible] was individually recorded over an [illegible] light/12 hour dark illumination [illegible] by an electronic activity meter. [illegible] point is the mean [illegible] S.E.M. [illegible] animals. [illegible] significantly different from the control value, p [illegible]

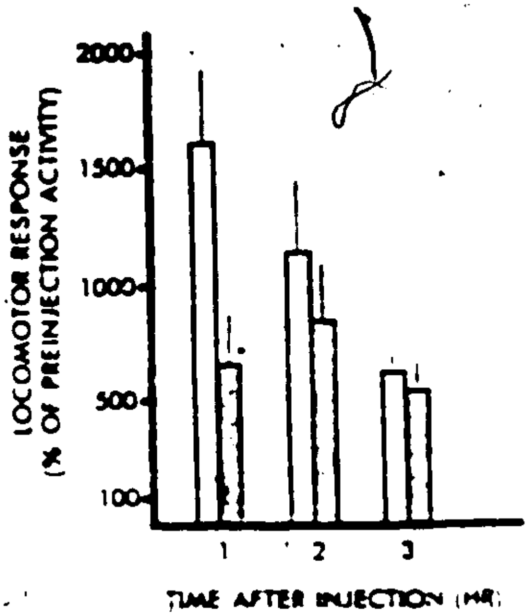
hour of measurement (10:00 - 11:00 hrs) but displayed no other significant alterations relative to controls for the remainder of the circadian cycle. Treatment with 0.025 mg lead/kg for 6 and 7 weeks did not have a significant effect on the circadian locomotor activity of rats, indicating that the lead induced hyperactivity observed in 5 week old animals was a transitory occurence.

Locomotor Response to d-Amphetamine

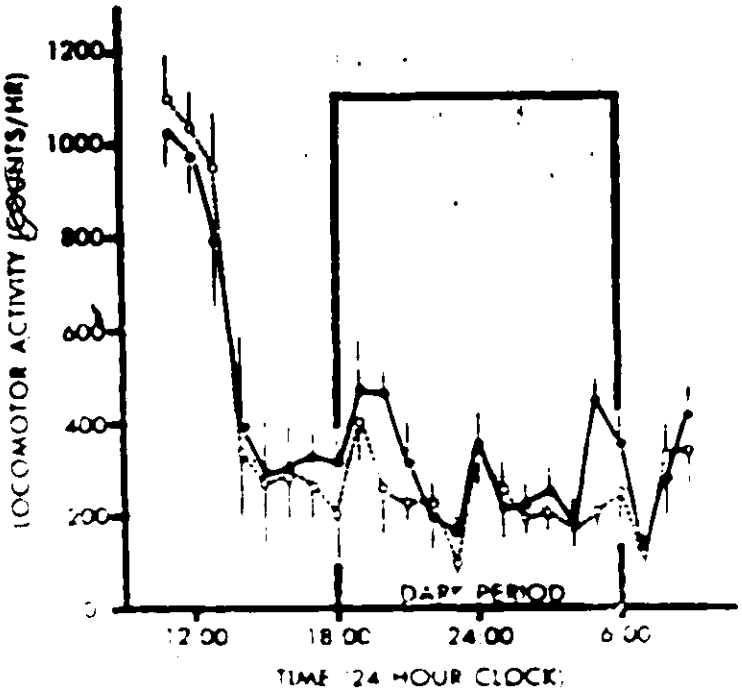
In addition to assessing the effects of chronic lead exposure on basal locomotor activity we also compared the motor responses of lead treated and control animals to d-amphetamine sulfate.

For the one hour period prior to the injection of d-amphetamine, the SLA of control animals (72 counts/hr) was similar to that of the lead exposed rats (103 counts/hr) demonstrating that there was no difference in basal locomotor activity between the two groups. During the one hour interval following the injection of [illegible] mg/kg d-amphetamine sulfate, the SLA of control rats increased to a level that was about 16X greater than the basal locomotor activity recorded one hour before the administration of the CNS stimulant (Fig. 8). In contrast to this, rats treated with 10.0 mg lead/kg for 7 weeks during postnatal development responded to the same dose of drug with only a 7-fold elevation in locomotion above basal activity levels indicating that chronic lead exposure had caused a reduced motor response to the given dose of d-amphetamine. Within the second hour following the administration of d-amphetamine, we noted that there was no significant difference in locomotor response between control and lead exposed rats.

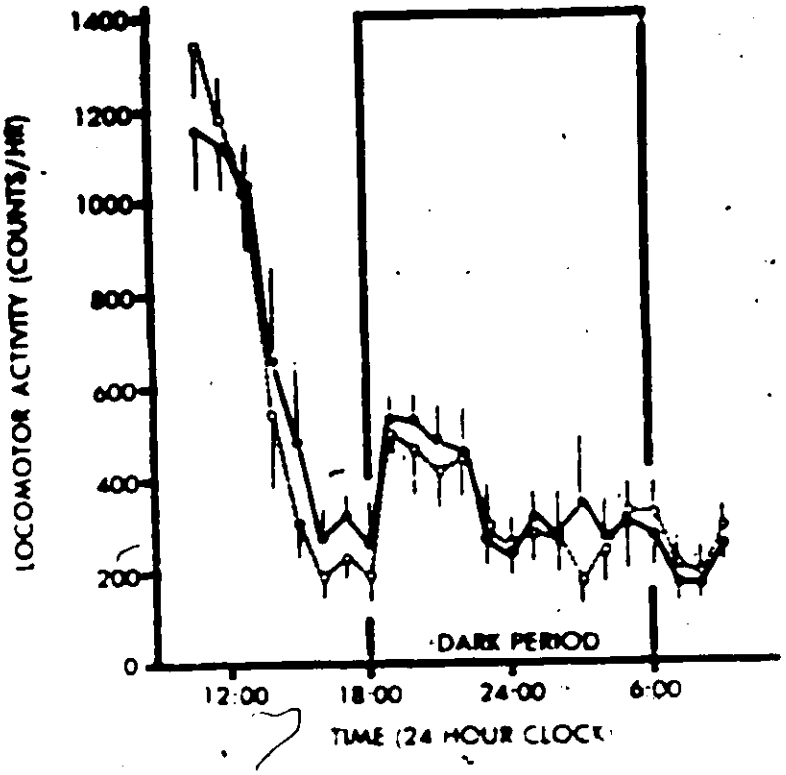
We also investigated the effects of d-amphetamine sulfate on the locomotor activity of rats that were chronically exposed to a lower dose of lead (0.1 mg/kg) for 6 and 7 weeks during postnatal development as expressed in figures 9 [illegible] amphetamine injection at [illegible] weeks of age. However, at



[illegible] The effect of d-amphetamine on spontaneous locomotor [illegible] (SLA) of rats exposed to 10.0 mg lead/kg for [illegible] weeks. [illegible] were given [illegible] mg lead/kg daily by gastric intubation [illegible] until [illegible] weeks of age. Controls (□) received an equal [illegible] of sodium acetate. Following one hour recording of [illegible] activity, lead treated and control animals were [illegible] intraperitoneally with [illegible] mg/kg of d-amphetamine and monitored [illegible] an additional [illegible] hours. The resulting elevation in SLA [illegible] exposed and control rats is expressed as a percent of their [illegible] preinjection activity levels which did not differ [illegible] between control [illegible] and lead treated [illegible] [illegible] activity was individually [illegible] activity meter. Values given are the mean [illegible]



[illegible] The effect of d-amphetamine on the circadian spontaneous locomotor activity [illegible] rats exposed to [illegible] mg lead kg for 4 weeks [illegible] were given [illegible] mg lead kg dai[illegible] gastric intubation from [illegible] days until 4 weeks of age [illegible] ●—● received an equal amount [illegible] d-amphetamine [illegible] mg kg was administered intraperitoneally [illegible] and lead treated rat then their [illegible] activity was recorded [illegible]



The effect of d-amphetamine on the circadian spontaneous locomotor activity of rats exposed to 0.1 mg lead/kg for 6 weeks. o---o Pups were given 0.1 mg lead/kg by gastric intubation from 3 days until 4 weeks of age. Controls o——o received an equal amount of sodium acetate. d-Amphetamine 2 mg/kg was administered intraperitoneally to control and lead treated rats then their motor activity was recorded over an 11 hour [illegible] dark illumination [illegible] activity before [illegible] rats.

8 weeks (Fig. 11) lead treated rats were found to be less active than controls during the first 3 hours following the injection of d-amphetamine (ANOVA: ($F(1,8)=7.39$, $p<0.05$). No marked difference in the overall SLA pattern were noted between lead exposed and untreated animals during the remainder of the circadian cycle.

Brain Regional Catecholamine Levels and Noradrenaline Turnover Rate

Summarized in Table 5 is the steady state levels of noradrenaline in the hippocampus and cortex of rats treated with the 0.1 mg/kg dose of lead for up to 8 weeks during postnatal development. Exposure to this dose of the heavy metal for 4 weeks resulted in a 45% increase in the noradrenaline content of the hippocampus, while at 6 weeks the concentration of the monoamine in the same brain region was reduced by 47% relative to controls. Following 8 weeks of lead treatment, no changes were observed in either of the brain regions examined.

With regard to striatal and hippocampal dopamine levels concentrations measured in rats treated with 0.1 mg lead/kg, no significant changes in the levels of this monoamine were observed in either areas of the CNS (Table 6).

We further investigated the effects of low level lead exposure on noradrenergic neurotransmission in the CNS by measuring the turnover rates of noradrenaline in the hippocampus of rats treated with 0.1 mg lead/kg for 6 weeks (Table 7). This was the only region in which changes in catecholamine content was observed following chronic lead exposure. In the hippocampus of animals treated with this heavy metal, the rate constant (k) of noradrenaline depletion following the administration of α-methyl tyrosine (α-MT) was found to be reduced by 36% relative to controls. By utilizing the test of parallelism, it was determined that the slope of noradrenaline depletion in lead treated rats was significantly [illegible] flatter than that derived from controls. Since k is equal to the slope [illegible] constant x (-1), we concluded that fractional rate constant of noradrenaline depletion was also statistically significantly reduced in the lead poisoned rat. From this comparison of rate constants of catecholamine

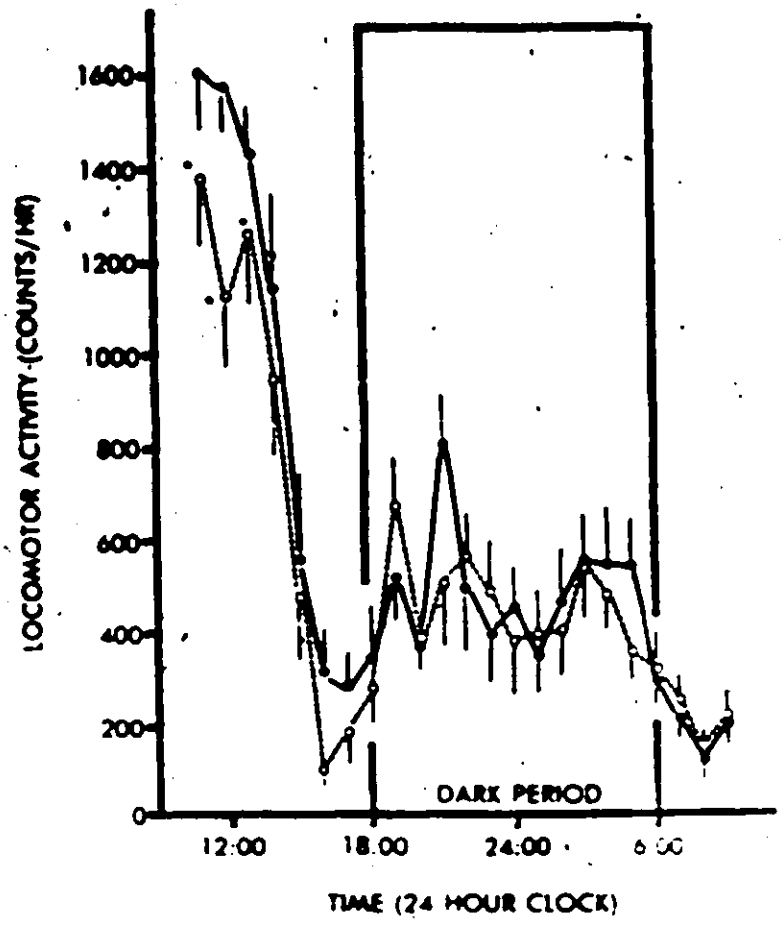


FIG. 11: The effect of d-amphetamine on the circadian spontaneous locomotor activity cycle of rats exposed to 0.1 mg lead/kg for 8 weeks (o---o). Pups were given 0.1 mg lead/kg daily by gastric intubation from 3 days until 8 weeks of age. Controls (●——●) received an equal amount of sodium acetate. d-Amphetamine was administered intraperitoneally to control and lead treated rats then their locomotor activity was recorded over an 11 hour light/12 hour dark illumination cycle by an electronic activity meter. Each point is the mean ± S.E.M. of 6 rats. *Significantly different from the control value; $p<0.05$

TABLE 5

Concentration of noradrenaline in the hippocampus and cortex of rats treated daily with 0.1 mg lead/kg during postnatal development

Age (Weeks)	Treatment†	Brain Region	
		Hippocampus	Cortex
4	Control	0.121±0.013‡ (100)	0.089±0.008 (100)
	Lead	0.173±0.018* (143)	0.063±0.011 (70)
6	Control	0.196±0.025 (100)	0.118±0.006 (100)
	Lead	0.106±0.011* (53)	0.165±0.020 (138)
8	Control	0.475±0.063 (100)	0.312±0.030 (100)
	Lead	0.448±0.072 (94)	0.247±0.023 (79)
10	Control	0.272±0.056 (100)	0.170±0.016 (100)
	Lead	0.220±0.041 (81)	0.217±0.023 (128)

†Beginning at 3 days and continuing until 4, 6 or 8 weeks of age, 0.1 mg lead/kg was administered to rat pups daily by gastric intubation. A group of rats that had been exposed to 0.1 mg lead/kg for 8 weeks were withdrawn from treatment and kept for an additional 2 weeks (i.e. until 10 weeks of age) at which time neurochemical measurements were made. Controls received equal amounts of sodium acetate.

‡Values are the mean ± S.E.M. of 5-8 rats. The concentration of noradrenaline is expressed as µg/g tissue weight. Data are also given as percentages (in parentheses) taking the value of controls as 100 percent.

*Significantly different from the control values ($p<0.05$).

TABLE 6

Concentration of dopamine in the striatum and hippocampus of rats treated daily with 0.1 mg lead/kg during postnatal development

Age (Weeks)	Treatment†	Brain Region	
		Striatum	Hippocampus
4	Control	7.67±0.33‡ (100)	2.68±0.20 (100)
	Lead	6.86±0.38 (89)	2.65±0.18 (99)
6	Control	4.26±0.81 (100)	4.96±0.24 (100)
	Lead	4.93±1.33 (116)	4.96±0.25 (100)
8	Control	8.67±0.64 (100)	2.14±0.15 (100)
	Lead	8.26±0.96 (95)	2.37±0.41 (111)
10	Control	8.12±1.14 (100)	1.85±0.35 (100)
	Lead	8.63±1.09 (106)	1.47±0.10 (79)

†Beginning at 3 days and continuing until 4, 6 or 8 weeks of age, 0.1 mg lead/kg was administered to rat pups daily by gastric intubation. A group of rats that had been exposed to 0.1 mg lead/kg for 8 weeks were withdrawn from treatment and kept for an additional 2 weeks (i.e. until 10 weeks of age) at which time neurochemical measurements were made. Controls received equal amounts of sodium acetate.

‡Values are the mean ± S.E.M. of 5-8 rats. The concentration of dopamine is expressed as µg/g wet tissue weight. Data are also given as percentages (in parentheses) taking the value of controls as 100 percent

*Significantly different from the control values ($p<0.05$).

TABLE 7

Turnover rate of noradrenaline in the hippocampus of rats treated with 0.1 mg lead/kg for 6 weeks

Treatment†	K (hr^{-1})‡	T.R. (µg/g/hr)§
Control	0.721 (100)	0.148 (100)
Lead	0.459* (64)	0.066 (42)

†Rats were treated daily with 0.1 mg lead/kg via gastric intubation from 3 days until 6 weeks of age. Controls received an equal amount of sodium acetate.

‡Fractional rate constant (K) of noradrenaline decline was calculated from the rate of amine depletion after inhibition of its synthesis by α-methyl-p-tyrosine. Data are also given as percentages (in parentheses) taking the value of controls as 100%.

§Turnover rate (T.R.) of noradrenaline was calculated by multiplying the fractional rate constant and the initial concentration of noradrenaline measured in the hippocampus. Data are also given as percentages (in parentheses) taking the value of controls as 100 percent.

*The slope of noradrenaline depletion obtained from lead treated rats was significantly less than that from controls; $p<0.05$.

depletion following the administration of α-MPT, it was concluded that exposure of rats to 0.1 mg lead/kg for 6 weeks resulted in a reduced rate of noradrenaline turnover in the hippocampus.

We further reduced the dose of lead administered down to 0.025 mg/kg. Analysis of the lead concentration in the stomach contents of two one-week old pups (mean body weight equal to 13 g) revealed that in controls the total quantity of lead in the stomach amounts to 44 ng. With the 0.025 mg lead/kg dose of metal, a total of 0.325 ug of lead is given to each of the pups which weighed 13 g. We therefore estimated that during the preweaning period animals treated with this dose of lead were given a quantity of metal that was equivalent to at least 5 times the amount of lead in the stomach contents of controls.

As can be seen in Table 8 treatment with 0.025 mg lead/kg for 8 weeks resulted in 25% reduction in the noradrenaline content of the hippocampus but did not significantly alter the concentration of this monoamine in the other brain regions. On the other hand, this dose of lead treatment did not significantly alter the levels of dopamine in any of the brain areas examined.

Concentration of Lead in Blood and Brain Regions

Presented in Figure 12 is the concentration of lead in blood and brain regions of rats treated with 0.1 mg lead/kg for 4 weeks. This dose of exposure resulted in a 78% increase in blood lead level and a significantly elevated concentration of lead in the cortex (by 125%) and the hippocampus (by 522%) of these animals. Note that in treated rats the lead content of the hippocampus was about 4 times greater than the amount of metal deposited in the cortex and was significantly greater than the lead content of the three other brain areas examined (ANOVA: $F(3,31)=6.50$, $p<0.05$).

The mean concentration of lead in the blood of those rats treated with the same dose of metal (0.1 mg/kg) for 6 weeks was 13.8 ug/dl, an increase of 76% over control values (Fig. 13). This duration of treatment resulted in a significant elevation of lead levels in three of the brain regions examined,

TABLE 8

Concentration of noradrenaline and dopamine in discrete brain regions of rats treated daily with 0.025 mg lead/kg for 8 weeks

Catecholamine	Brain Region	Treatment†	
		Control	Lead
Noradrenaline	Hippocampus	0.274±0.009‡ (100)	0.206±0.18* (75)
	Cortex	0.116±0.010 (100)	0.137±0.007 (118)
Dopamine	Striatum	10.33 ±0.857 (100)	11.27 ±1.28 (109)
	Hippocampus	5.07 ±0.137 (100)	5.59 ±0.654 (100)

†Beginning at 3 days and continuing until 8 weeks of age 0.025 mg lead/kg was administered to rat pups daily by gastric intubation. Controls received an equal amount of sodium acetate.

‡The values are the mean ± S.E.M. of 6 rats. The concentrations of noradrenaline and dopamine are expressed as µg/g wet tissue weight. Data are also given as percentages (in parentheses) taking the value of controls as 100 percent.

*Significantly different from the control values, $p<0.05$.

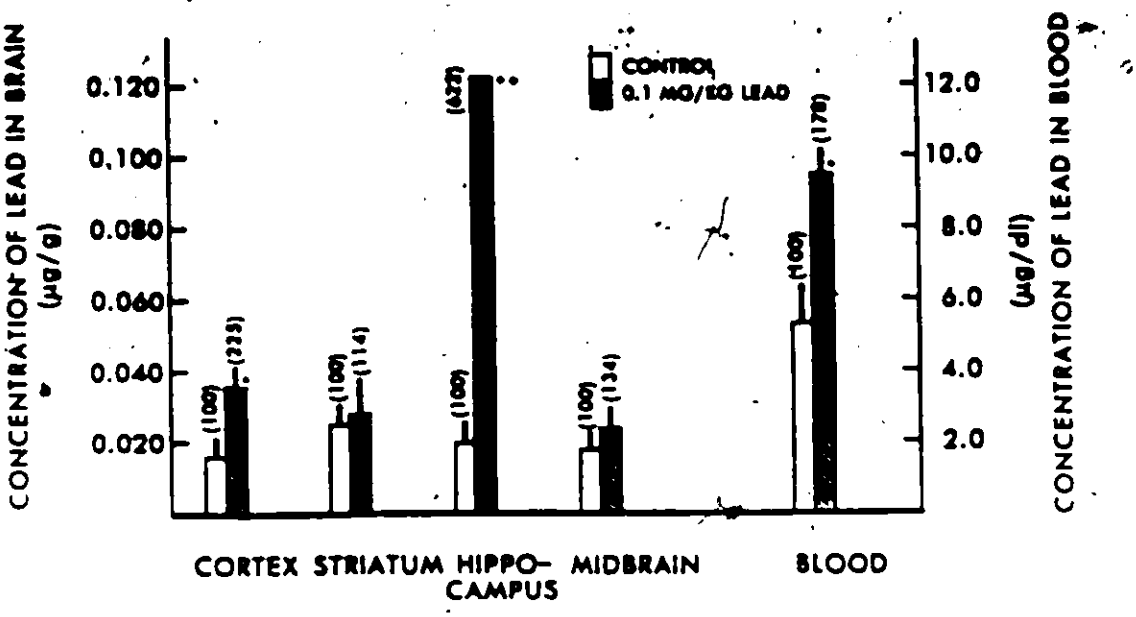


FIG. 12: The concentration of lead in blood and various brain regions of rats treated with 0.1 mg lead/kg for 4 weeks. Pups were given 0.1 mg lead/kg daily by gastric intubation from 3 days until 4 weeks of age. Controls received an equal amount of sodium acetate. The concentration of lead in tissues was determined by flameless atomic absorption spectrophotometry. Each point is the mean ± S.E.M. of 5-7 animals. Data are also given as percentages (in parentheses) taking the value of controls as 100 percent. *Significantly greater than control; $p<0.05$. +Significantly different from all other regions; $p<0.05$.

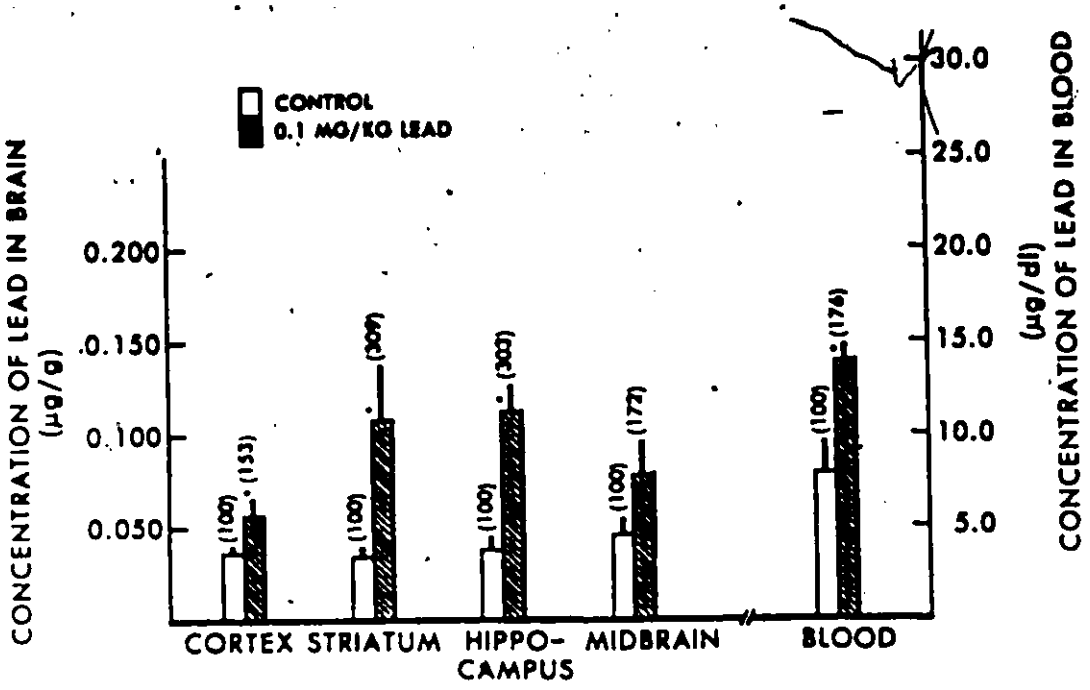


FIG. 13: The concentration of lead in blood and various brain regions of rats treated with 0.1 mg lead/kg for 6 weeks. Pups were given 0.1 mg lead/kg daily by gastric intubation from 3 days until 6 weeks of age. Controls received an equal amount of sodium acetate. The concentration of lead in tissues was measured by flameless atomic absorption spectrophotometry. Each point is the mean ± S.E.M. of 5 rats. Data are also given as percentages (in parentheses) taking the values of controls as 100 percent. *Significantly greater than control; $p<0.05$.

the cortex (by 53%), striatum (by 209%) and the hippocampus (by 203%). Statistical analysis indicated that there were no significant differences in lead concentrations among the various brain regions.

In rats receiving 0.1 mg lead/kg for 8 weeks the mean blood lead concentration (12.7 µg/dl) was 38% greater than that measured in the untreated group (Fig. 14). In these lead treated animals the lead content of all four brain regions examined was significantly elevated relative to their respective controls but there were no significant inter-regional variations in lead content.

The distribution of lead in blood and brain regions was also examined after the withdrawal of chronic lead exposure. When the administration of 0.1 mg lead/kg was discontinued at 8 weeks of age and measurements made two weeks later, blood lead levels were found to be within the range of control values. However, the concentrations of the metal in the striatum, hippocampus and midbrain were still significantly greater than those measured in their respective controls (Fig. 14

Illustrated in Figure 15 and 16 is the change in concentration of lead with age in the blood and brain regions of controls and of rats exposed to the 0.1 mg/kg dose of metal. Blood lead levels of treated animals increased between 4 and 6 weeks of age and reached a maximum value of 13.8 µg/dl, but between 6 and 8 there was no significant change in the concentration of lead in blood. Note that the lead content in the blood of controls at 8 weeks of age was significantly greater than that at 4 weeks, indicating that the blood lead level of untreated rats has also increased between 4 and 8 weeks of age.

Whereas the small age related changes in lead content were similar in the brain regions of control animals, there were significant inter-regional differences in the time course of lead distribution within the brains of rats exposed to the metal (Fig. 16). In contrast to other brain regions examined, the concentration of lead in the hippocampus was at its maximum value (0.122 µg/g) after only 4 weeks of lead administration. Furthermore, whereas the levels of

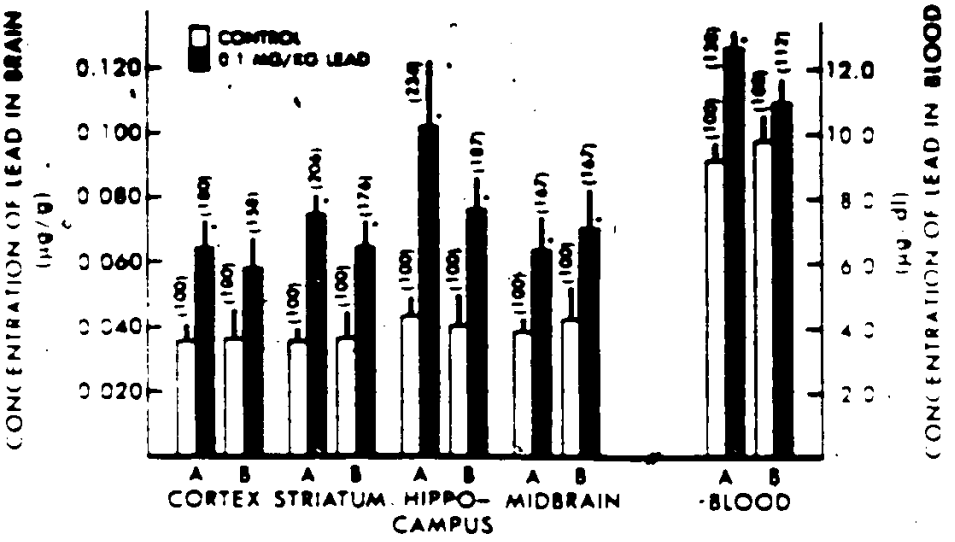
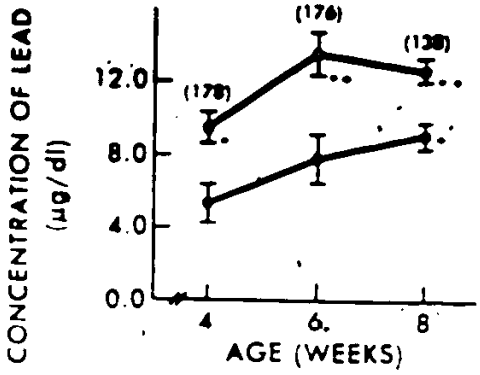


FIG. 14: The concentration of lead in blood and various brain regions of rats treated with 0.1 mg lead/kg for 8 weeks (A) and of rats that were subsequently withdrawn from treatment for 2 weeks (B). Pups were given 0.1 mg lead/kg daily by gastric intubation from 3 days until 8 weeks of age. Controls received an equal dose of sodium acetate. At 8 weeks a group of control and lead-treated rats were withdrawn from further treatment and kept for an additional 2 weeks. The concentration of lead in tissues was determined by flameless atomic absorption spectrophotometry. Each point is the mean ± S.E.M. of 5-7 animals. Data are also given as percentages (in parentheses) taking the value of control as 100 percent. *Significantly greater than control; $p<0.05$.



[illegible] The concentration of lead in whole blood of control (●—● and lead [illegible] (○—○) at 4, 6 and 8 weeks of age. Pups received by gastric [illegible] mg lead/kg daily from 3 days until 4, 6 or 8 weeks old. Controls were given an equal amount of sodium acetate. The concentration of lead was measured by atomic absorption spectrophotometry. Each point is the mean ± S.E.M. of 5-7 rats. Data are also given as percentages (in parentheses) taking the value of controls as 100 percent. *Significantly greater than control, $p<0.05$. Significantly different from value at 4 weeks, $p<0.05$.

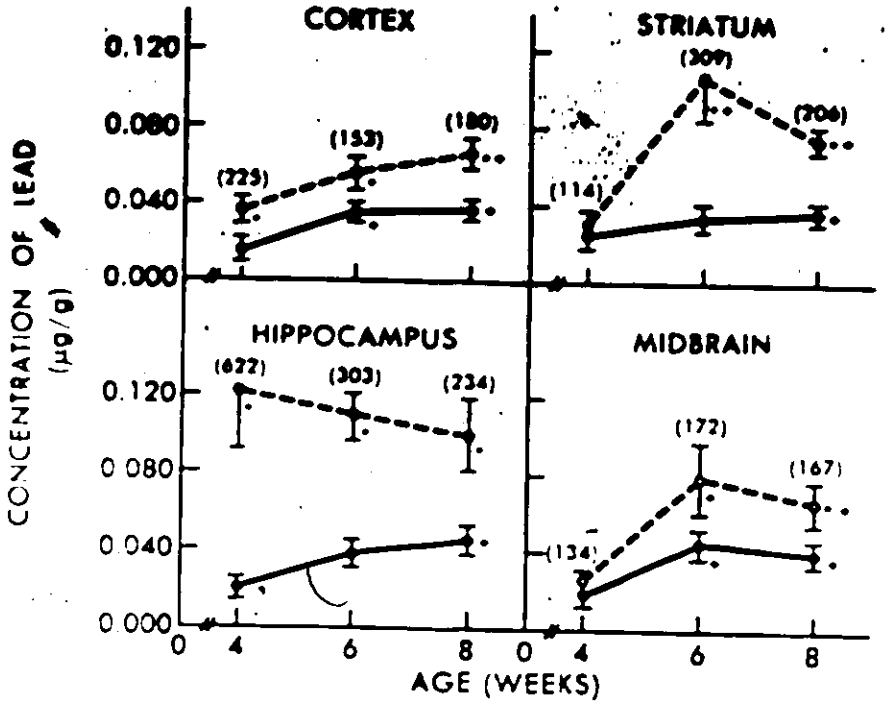


FIG. 16: The concentration of lead in the cortex, striatum, hippocampus and midbrain of control (●——●) and lead treated rats (o---o) at 4, 6 and 8 weeks of age. Pups received by gastric intubation 0.1 mg lead/kg daily from 3 days until 4, 6 or 8 weeks of age. Controls were given an equal amount of sodium acetate. The concentration of lead was measured by atomic absorption spectrophotometry. Each point is the mean ± S.E.M. of 5-7 rats. Data are also given as percentages (in parentheses) taking the value of controls as 100 percent. *Significantly greater than control; $p<0.05$. ‡Significantly different from value at 4 weeks; $p<0.05$.

the metal in cortex, striatum and midbrain after 8 weeks of lead treatment was significantly greater than the values at 4 weeks, a slight but non-significant reduction in the metal content of the hippocampus was observed as the duration of treatment increased.

The concentration of metal in the blood of animals that had received chronic exposure to the 0.025 mg/kg dose of lead for 8 weeks was not significantly elevated relative to controls (Fig. 17). However, at this dose level we did observe a two-fold increase in the lead content of the hippocampus. Moreover, since the concentration of lead was significantly elevated only in this region it appears that there was a preferential deposition of the metal in the hippocampus.

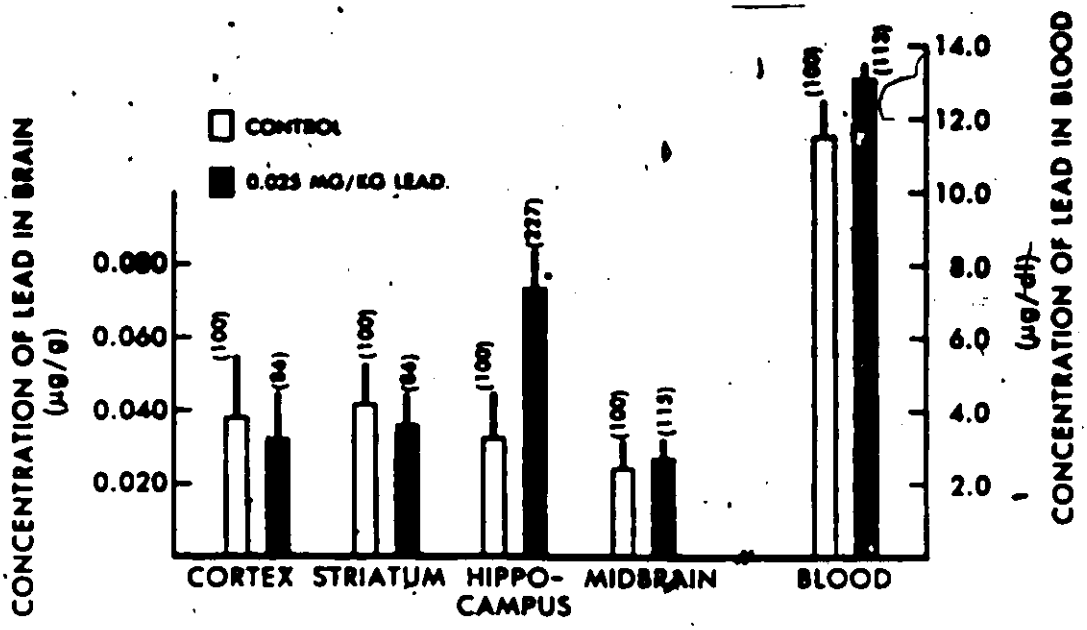


FIG. 17: The concentration of lead in blood and various brain regions of rats treated with 0.025 mg lead/kg for 8 weeks. Pups were given 0.025 mg lead/kg daily by gastric intubation from 3 days until 8 weeks of age. Controls received an equal amount of sodium acetate. The concentration of lead in tissues was measured by flameless atomic absorption spectrophotometry. Each point is the mean ± S.E.M. of 5 rats. Data are also given as percentages (in parentheses) taking the values of controls as 100 percent. *Significantly greater than control; $p<0.05$.

IV. DISCUSSION

Introduction

Research on the neurobehavioural consequences of subclinical lead poisoning has been the source of much discussion and controversy. Clinical studies have reported that moderate increases in body burden of lead in children may (David et al., 1972; Beattie et al., 1975; Baloh et al., 1975; Needleman et al., 1979) or may not (Kotok, 1972; Landsdown et al., 1974) be associated with various subtle neuropsychological deficits, including hyperactivity. Animal studies with rodents have had limited success in establishing a model of subclinical lead poisoning. Since it was first established that suckling rats are vulnerable to the neurotoxic effects of lead (Pentschew and Garro, 1966), numerous experiments have been conducted to investigate the neurochemical and behavioural consequences of chronic sublethal lead poisoning in the developing rodent (Michaelson and Sauerhoff, 1974; Silbergeld and Goldberg, 1974; Sobotka and Cook, 1974; Krehbiel et al., 1976; Dubas and Hrdina, 1978; Govoni et al., 1979; Jason and Kellogg, 1981). However, in most of these earlier studies the dose of lead administered was so large that their relevance with regard to subclinical lead poisoning is a point of question.

One of the ways in which the present study on chronic lead poisoning is uniquely different from most earlier investigations is that the doses of metal used (0.025 and 0.1 mg lead/kg) are among the lowest examined and may even be considered environmentally relevant levels of lead exposure. The total daily intake of lead by individuals of the general Canadian population has been estimated to be 0.175 mg (NRC, 1973). Assuming a standard 70 kg body mass, the 0.1 mg/kg dose of lead used in this study is equivalent to 40 times the total daily intake. The maximum blood lead level observed in rats exposed to 0.1 mg lead/kg dose of metal was 13.8 ug/dl and the largest concentration measured in the brain regions was 0.122 ug/g. Whilst these levels are significantly greater than those noted in controls they do not represent an excessive increase in blood and brain lead content. The values obtained at this dose are within the range

of blood and brain lead concentrations found in "normal" children less than 9 years of age (0.090 µg/g in blood and 0.070 µg/g in cortex) and slightly greater than that of children less than 1 year old (0.030 µg/g in blood and 0.030 µg/g in cortex) (Barry, 1975).

Growth and Physical Development

Reduced body weight gain has been reported in animals that have indirectly received chronic lead treatment via the nursing mothers milk (Silbergeld and Goldberg, 1973; Modak and Stavinoha, 1979). However, studies indicate that the metal itself does not directly cause retarded growth rate. This instead, seems to result from nutritional deprivation during the suckling period, which is secondary to malnutrition of dams raised on a diet treated with large amounts of lead (Michaelson and Sauerhoff, 1974; Michaelson, 1980). This explanation is consistent with the observations that relatively large doses of the metal (up to 90 mg lead acetate/kg) administered directly to developing rats via the oral route have not been associated with reduced growth rate (Sobotka and Cook, 1974; Golter and Michaelson, 1975; Overmann, 1977; Dubas and Hrdina, 1978).

In our results we observed little evidence of retarded physical development in lead treated rats especially during the preweaning period. Exposure to the largest (10.0 mg/kg) and the lowest (0.025 mg/kg) doses of lead did not significantly alter the body weight gain at any time during the 8 weeks of treatment and for the first three weeks of life the growth rate of rats receiving the 0.1 mg/kg dose of metal was also similar to that of controls. In addition, the time course of appearance of developmental landmarks for animals receiving 10.0 and 0.1 mg lead/kg was indistinguishable from that of untreated rats. However, at 4, 5 and 6 weeks of age slight (less than 10%) but statistically significant ($p<0.05$) reductions in body weight were observed in animals treated with the lower dose of metal.

Although there is some evidence to suggest that nutritional status during the early stages of development can influence the regulation of locomotor

activity and the brain neurochemistry of rodents (Loch et al., 1978; Shoemaker and Wurtman, 1973) these observations have been reported in animals that were malnourished during their suckling period and to such an extent that their body weights were reduced to as low as 50% of normal values. Furthermore, experiments on learning which use reinforcement schedules frequently employ food deprived animals that are maintained at 80% of their free feeding body weight without causing any dramatic alterations in behaviour (Ferster and Skinner, 1957; Zenick et al., 1979). Because the decrease in body weight that we had observed in rats receiving 0.1 mg lead/kg was less than 10% in all cases and since signs of physical development and growth during the critical preweaning period were normal, we reasoned that the biological significance of such a small reduction in body weight with regard to malnutrition induced behavioural changes is doubtful.

Effect of Chronic Lead Exposure on Circadian Locomotor Activity

The locomotor activity of rodents is characterized by its correlation with the dark/light illumination cycle in a circadian rhythm. Rodents are nocturnal animals and as such are always markedly more active during the dark period as compared to the light period in the cycle (Holmquest et al., 1966). Studies indicate that there are circadian phase dependent variations in the effects of centrally active drugs which alter locomotor activity (Lemmer and Berger, 1978). Furthermore, there is some evidence to suggest that the expression of lead induced behaviour changes may be influenced by the dark/light cycle of the circadian rhythm. Dolinsky and colleagues (1981) noted that in lead poisoned mice, hyperactivity was observed only during the dark period of the cycle. It is therefore apparent that measurement of SLA at one point of the circadian rhythm may yield a result which is not consistent with findings made at other times.

Previous studies on lead and locomotor activity have met with limited success. For the most part they have demonstrated that chronic lead exposure

can result in hyperactivity (Sauerhoff and Michaelson, 1973; Silbergeld and Goldberg, 1973; Dubas and Hrdina, 1978; Jason and Kellogg, 1981). However, this finding has not been consistent and there have been reports of unaltered (Sobotka and Cook, 1974; Krehbiel et al., 1976) as well as reduced activity (Driscoll and Stegner, 1976; Verlangieri, 1979) in rodents that have been chronically treated with the metal. One of the shortcomings of previous experiments has been in the timing of locomotor measurements. For the most part, the motor activity of control and lead exposed rats had been recorded either over relatively short periods of time (5 mins - 3 hrs) (Silbergeld and Goldberg, 1973; Sobotka and Cook, 1974; Brown, 1975; Petit and Alfano, 1979; Grant et al., 1980; Memo et al., 1980; Wince et al., 1980; Jason and Kellogg, 1981) or the cumulative SLA counts were compared over much longer time intervals (6-24 hrs) (Sauerhoff and Michaelson, 1973; Krehbiel et al., 1976; Overmann, 1977; Cutler et al., 1977; Modak and Stavinoha, 1979; Dolinsky et al., 1981). While these methods provide information on the locomotor activity of animals at some point during the circadian rhythm or on the overall trend of activity, neither of them are able to fully describe how chronic lead poisoning affects the locomotion of animals throughout their circadian cycle.

In order that we might determine what effect lead has on the circadian SLA of the rat we compared the hourly activity counts of control and treated animals over a 12 hour dark/11 hour light cycle. Our results indicate that chronic exposure to relatively large doses of the metal (10.0 mg lead/kg) is associated with a transient elevation in locomotor activity at 3 weeks of age. In these animals, exploratory behaviour was significantly enhanced and there was heightened locomotion during the last quarter of the dark cycle. These results are consistent with the observation of other authors who have reported that reactivity is significantly elevated in rodents that have received chronic treatment with relatively large doses of metal (Reiter, 1977; Petit and Alfano,

1979). That 10.0 mg lead/kg resulted in hyperactivity during the last quarter of the dark cycle but did not affect the SLA during the light period is in agreement with the observations of Dolinsky and coworkers (1981) who noted that lead induced hyperactivity in mice occurred primarily during the dark phase of the circadian cycle.

The 10.0 mg/kg dose of lead is relatively large, and as such the relevance of our observations at this dose level to subclinical lead poisoning in children is questionable. We therefore studied the behavioural consequences of the 0.1 mg lead/kg dose that results in significant but not excessive increases in blood and brain lead levels. Chronic treatment of newborn rats with 0.1 mg lead/kg for 4 and 6 weeks was found to have no effect on their circadian locomotor activity. However, exposure to this dose for 8 weeks did result in hyperactivity which was not evident upon withdrawal from treatment for 2 weeks. In animals receiving the 0.1 mg/kg dose for 8 weeks there was no significant difference between untreated and lead exposed rats during the initial hour of SLA measurement. However, while in controls the SLA counts subsequent to the initial period decreased steadily with time, indicating normal habituation to the novel environment, the activity of treated animals remained elevated. These findings suggest that while control and poisoned rats responded in a similar manner to the novel environment it took the lead exposed rodents about one hour longer to acclimate to the new surroundings. A similar disruption of the habituation mechanism was observed also in rats treated with the 0.025 mg/kg dose of lead. Exposure to this low dose of metal during postnatal development was found in 5 week old rats to result in a transient heightened reactivity as indicated by an almost 2-fold increase in SLA counts over controls during the first hour of activity measurement.

From the results of our experiments on chronic lead poisoning there appears to be some indication of a dose-response relationship with regard to circadian locomotor activity. Exposure of the newborn rats to relatively high doses of

the metal (10.0 mg/kg) was found to cause a heightened exploratory activity as well as a localized period of hyperactivity during the dark phase of the circadian rhythm. On the other hand, lower levels of lead treatment (0.1 and 0.025 mg/kg) seemed to alter only the habituation process but had no effect on the remainder of the circadian cycle. These observations are consistent with the hypothesis that in rats chronic lead exposure during postnatal development does not result in a generalized elevation in motor activity, instead the occurrence of hyperactivity is influenced by the circadian nature of their locomotor behaviour.

The results of the present study also indicate that the lead induced hyperactivity was a transient phenomenon. For those rats receiving chronic treatment with 10.0 mg lead/kg heightened motor activity was noted at 3 weeks of age but was not evident when measurements were again made during the following weeks of lead exposure. Similar observations were also made of those rats that were administered 0.025 mg lead/kg. This level of chronic lead exposure resulted in an elevated reactivity at 5 weeks of age which was not observed during subsequent recordings conducted following 6 and 7 weeks of treatment. Our observation that lead induced hyperactivity in rats is a transitory occurrence which does not persist with the duration of exposure is consistent with previous reports of transient elevations in locomotor activity of lead treated rats (Reiter, 1977; Dubas and Hrdina, 1978).

Response to d-Amphetamine

In addition to an elevation in basal locomotor activity, it has been reported that rodents chronically exposed to lead display a locomotor response to amphetamine and other CNS stimulants that is unlike that seen in untreated animals. Amphetamine is a sympathomimetic agent which upon administration to normal rodents results in a dramatic increase in locomotion (Maj et al., 1972b). On the other hand lead treated rats and mice have been found to display a paradoxical response to the stimulant. In animals that have been exposed to the

metal the elevation in locomotor activity resulting from the administration of amphetamines or other CNS stimulants is not as great as that observed in controls (Sobotka and Cook, 1974; Kostas et al., 1978). Furthermore, it has also been reported that amphetamine can actually subdue the heightened activity observed in lead treated hyperactive mice (Silbergeld and Goldberg, 1974; 1975).

In our study, chronic exposure of rats to 10.0 mg lead/kg for 7 weeks during postnatal development was found to result in a suppressed locomotor response to d-amphetamine. During the one hour interval following injection of the stimulant, the percent increase in locomotion above baseline activity level in rats that had received this dose of lead was about one-half of the response observed in untreated animals. There was no difference in basal motor activity between control and lead treated rats during the one hour period preceeding the administration of amphetamine. Therefore the observed variation in locomotor response between the lead exposed and untreated groups was not secondary to an already elevated basal SLA which may result from lead exposure. Our findings are in agreement with the results of other researchers who have reported a suppressed response to amphetamine by lead poisoned non-hyperactive rats (Sobotka and Cook, 1974; Reiter, 1977; Yamamoto and Kutscher, 1981).

We observed little evidence of a reduced locomotor response to d-amphetamine in rats that had been treated with 0.1 mg lead/kg for 4 and 6 weeks. However, those animals that had received this dose of metal for 8 weeks did display an altered response to the stimulant. During the first three hours following the intraperitoneal injection of d-amphetamine the SLA of chronically lead exposed rats did not increase to the same extent as that of untreated animals. The suppressed motor response to d-amphetamine is consistent with our earlier observations that exposure to this dose of the metal for 8 weeks was also associated with a heightened basal locomotor activity. Moreover, in both experiments the lead induced alterations in locomotion were confined to the initial 3 hours of measurement and the overall pattern of circadian SLA did not differ signifi-

cantly between control and lead exposed rats. These results therefore confirm the notion that the low level of chronic lead exposure (0.1 mg/kg) used in this study can cause a subtle disruption in the regulation of locomotor activity in rats.

Neurochemical Consequences of Lead Toxicity

There is evidence to suggest that the functional activity of noradrenaline and dopamine in the hippocampal and striatal regions of the brain may influence the locomotor behaviour of rodents. Noradrenergic pathways which terminate in the hippocampus and cortex of the brain have been described (Moore and Bloom, 1979) and microinjection of noradrenaline into the hippocampus of the rat has been found to result in an elevated open field activity as well as heightened reactivity to stimuli (Gage and Springer, 1981). A major dopaminergic system in the CNS is the nigrostriatal pathway. These neurons have their origins in the substantia nigra and terminate in the neostriatum where the fibers are distributed throughout the caudate and putamen. The importance of this pathway in the regulation of locomotor activity is well documented (Hornykiewicz, 1966). Stimulation of this system in rodents by intrastriatal injection of dopaminergic agonists have been reported to cause heightened body movement in the form of dyskinesia, stereotypic behaviour or a more generalized hyperactivity (Costall et al., 1974; Costall et al., 1975).

In the present study, examination of the steady state levels of catecholamines in the hippocampus, striatum and cerebral cortex revealed that chronic exposure to relatively low doses of lead (0.1 and 0.025 mg/kg) selectively alter the noradrenergic system in the hippocampus. No significant changes in the dopamine levels in the striatum and hippocampus were noted, and the noradrenaline concentration in the cortex was also unaffected by these levels of lead exposure. However, the noradrenaline content of the hippocampus was found to be significantly altered in those rats that were treated with 0.1 mg lead/kg for 4 and 6 weeks as well as in those that had received the 0.025 mg/kg dose of metal

for 8 weeks. In addition, upon further investigation, we observed that the turnover rate of noradrenaline in the hippocampus was significantly reduced in those animals exposed to 0.1 mg lead/kg for 6 weeks which confirmed that the functional activity of the noradrenergic system in the hippocampus was impaired by this dose of metal.

Previous investigations on the neurochemistry of lead treated rodents have reported that lead induced hyperactivity may be associated with alterations in the steady state levels of noradrenaline and dopamine in the CNS (Sauerhoff and Michaelson, 1973; Golter and Michaelson, 1975; Silbergeld and Goldberg, 1975; Hrdina et al., 1976; Dubas and Hrdina, 1978) and further studies suggest that chronic lead treatment may result in changes in the release (Wince et al., 1980), uptake (Jason and Kellogg, 1981) and turnover (Govoni et al., 1979) of these neurotransmitters. In many of the earlier studies attempts were made to correlate the observed neurochemical changes with lead induced hyperactivity. It has been shown that altered brain regional concentrations of noradrenaline and dopamine sometimes occur in temporal phase with transient hyperactivity observed in rats receiving chronic lead exposure (Dubas and Hrdina, 1978). In addition, Govoni and others (1980) demonstrated that in lead treated hyperactive rats dopamine synthesis was elevated in the nucleus accumbens. This observation is in agreement with the report by Pijnenburg and colleagues (1975) that bilateral administration of dopamine into the nucleus accumbens enhances locomotion. However, neurochemical changes observed following lead exposure have been inconsistent and there is little concensus on a characteristic neurochemical change in the brains of lead exposed hyperactive rodents (see Introduction). While some earlier studies suggest a correlation between elevated motor activity and alterations in central catecholamine function, a cause-effect relationship has not yet been established. In this study we noted no changes in brain regional concentrations of noradrenaline and dopamine in lead exposed

hyperactive rats. This observation suggests that the elevated locomotor activity noted in our treated rats was not associated with disturbed catecholaminergic pathways in the striatum, hippocampus and cortex. Our results thus do not support the proposed relationship between lead induced hyperactivity and alterations in noradrenergic and dopaminergic neuronal systems in the brain.

It is important to note that most of the earlier studies on lead toxicity employed relatively large doses of lead. For example, in the experiment by Dubas and Hrdina (1978) the concentration of lead in brain regions ranged from 1.27 to 2.88 μg/g; the blood lead level and brain lead content reported by Govoni and colleagues (1980) was 85 μg/dl and 0.87 μg/g respectively. These values are at least 5 times greater than the maximum concentrations of lead measured in blood and brain regions of treated rats in our study. Because of the large amounts of metal found in the brains of treated animals in previous investigations, it is possible that the observed catecholamine changes may have been non-specific neurochemical consequences of a greatly elevated lead exposure. With the relatively small doses of metal used in our study we have reduced the likelihood of this occurring. Indeed, the only alterations in brain regional catecholamine concentrations observed in the present study, was in the noradrenaline content of the hippocampus, a brain region that was found to store greater amounts of the metal than other areas of the CNS.

Correlation Between the Neurotoxic Effects of Lead and Brain Regional Distribution of the Metal

It has been previously reported that lead does not accumulate equally in all brain areas. Fjerdingstad and colleagues (1974), and more recently Fox et al. (1982) have demonstrated that in the rat brain more lead is stored in the hippocampus than in other regions. Our results on brain regional lead distribution confirm and extend these earlier observations. Exposure of newborn rats to 0.1 mg lead/kg for 4 weeks was found to result in a selective deposition

of the metal in the hippocampus. In the brains of these younger animals, the concentration of lead in the hippocampus was about 3 times greater than the levels measured in other brain areas examined. Similarly, we noted that in those rats that had been exposed to the 0.025 mg/kg dose of metal for 8 weeks, the hippocampus was the only region with a significantly elevated lead content. Following 4 and 6 weeks of chronic exposure to 0.1 mg lead/kg, significant alterations in the steady state levels of noradrenaline were also observed only in the hippocampus. Furthermore, chronic exposure to the lowest dose of lead used in this study (0.025 mg lead/kg) was found to result in a reduced noradrenaline concentration in the hippocampus but had no effect on the other brain areas that were examined. These incidences of selective neurochemical alterations in the hippocampus of rats chronically exposed to low doses of lead correlates well with the greater concentrations of lead deposited in this region. It would thus appear that the preferential accumulation of this heavy metal in the hippocampus may render this area of the brain more vulnerable than other regions to the neurotoxic effects of lead. Indeed, our results are supported by morphological studies which suggest that the hippocampus may be a major site of toxic lead action (Petit and Alfano, 1979; Le Boutillier et al., 1980).

Kinetics of Lead in Blood and Brain Region

Our data suggest that the distribution of lead to various brain regions is influenced by the amount of lead administered to the rats. Whereas a smaller dose of lead (0.025 mg/kg lead for 8 weeks) resulted in a significant increase in the lead content of only the hippocampus, similar concentrations of the metal were found in each of the brain regions of rats given a larger dose (0.1 mg/kg lead) for the same period of time. In addition to the dose of lead administered, it appears that the duration of exposure also influences the distribution of this metal to various brain regions. Treatment of rats with 0.1 mg lead/kg for 4 weeks resulted in significant preferential accumulation of the metal in the hippocampus, but in animals receiving the same dose of lead for 6 or 8 weeks,

no significant inter-regional differences in lead distribution were observed.

The kinetics of lead in blood and various organs has been previously shown to be influenced by age (Momčilović and Kostial, 1974; Mykkänen et al., 1979; Jugo, 1980; Keller and Doherty, 1980). In the present study, the blood lead levels of rats treated with 0.1 mg/kg dose increased between 4 and 6 weeks of age but then plateaued between 6 and 8 weeks. The levelling off of blood lead levels observed in this experiment is at variance with the findings of Mykkänen and coworkers (1979) who reported that there was a pronounced decrease in the concentration of lead in blood between 5 and 7 weeks of age in rats receiving chronic lead treatment. They attributed their finding to a declining lead absorption from the gastrointestinal tract with increasing age (Forbes and Reina, 1972). It should be noted however, that in the study by Mykkänen et al. (1979) lead (2% lead acetate) was administered via the food. It could therefore be argued that in addition to the age dependent reduction in lead absorption, the marked decrease in blood lead levels observed with age might have also been due to decreased lead intake resulting from a reduced food consumption by lead-poisoned animals (Michaelson and Sauerhoff, 1974). In the present experiment the lead intake was kept relatively constant since a fixed dose of lead relative to body weight was administered directly to the animal. This difference in the method of lead administration may account for the less pronounced decrease in blood lead levels observed in this study. Although lead absorption from the gastrointestinal tract is reduced in adult rats, some lead is still absorbed from the gastrointestinal tract of mature animals (Aungst et al., 1981). This observation is supported by our results which show that upon withdrawal of lead treatment for two weeks, blood lead levels decreased by 16%. Furthermore, in our control animals blood lead levels were found to increase with age until they were 8 weeks old probably due to the consumption of food containing small but measurable amounts of lead (0.211 ppm).

There was a notable difference in the kinetics of lead distribution to

various brain regions between control and lead-treated rats. In controls, the change in the concentration of lead with age was similar for all brain regions examined and was not unlike the time course of changes in the blood. On the other hand, in rats receiving 0.1 mg lead/kg significant regional differences were observed in the kinetics of lead distribution. In contrast to the cortex, striatum and midbrain, the concentration of lead in the hippocampus attained a maximum value (0.122 μg/g) after 4 weeks of lead treatment at which time it was even greater than blood lead levels (9.4 μg/dl). Furthermore the lead content of the hippocampus remained virtually unchanged as the animal grew older, whilst the concentration of metal in other brain regions increased between 4 and 8 weeks of age.

The mechanism by which lead selectively accumulates in the hippocampus remains unknown. However, the unusual kinetics of lead distribution to this brain region may help explain why following exposure to 0.025 mg lead/kg for 8 weeks, the lead content of the hippocampus was 2-fold greater than that measured in untreated rats yet at the time blood lead levels were similar to control values. As can be seen from our results, the preferential deposition of lead in the hippocampus of rats receiving the 0.1 mg/kg dose occurred in 4 week old rats and was not evident at 6 and 8 weeks of age suggesting that this phenomenon occurs primarily in younger animals. We propose that with the 0.025 mg lead/kg dose, the moderate increase in lead intake (estimated to be about 5 times greater than the intake of controls during the suckling period) was sufficient to raise blood lead values above control levels during the early states of development and result with a greater accumulation of lead in the hippocampus of lead exposed rats as compared to untreated animals.

If one can assume that the time course of change in blood lead concentration is the same during chronic exposure to 0.025 mg lead/kg as it was for the 0.1 mg/kg dose. Then blood lead levels during exposure to 0.025 mg lead/kg would probably level off following 6 weeks of treatment. Hence, while the concentration of lead

in the blood of rats treated with 0.025 mg lead/kg may have been greater than that of controls in younger animals the resulting steady state blood lead level at 8 weeks of age may have been too low to be significantly greater than the lead content of blood from controls at 8 weeks of age.

Little is known about the changes in regional lead distribution within the rat brain following the withdrawal of chronic lead treatment. A study by Momčilović and Kostial (1974) in which the amount of lead radioisotope (203 Pb) in rat brain was followed for 8 days after a single injection (i.p.), showed that lead is retained in the brain even after blood lead levels had declined. More recently, Jason and Kellogg (1981) reported that after oral lead treatment was discontinued, the concentration of the metal in the neo-striatum did not decrease as rapidly as did the blood lead level. This is now confirmed and extended by our results which show that after the withdrawal of chronic lead treatment for 2 weeks, the concentration of lead in most brain regions remains significantly greater than their respective controls even though blood lead levels are within the range of control values. Thus, the washout time of lead from some brain regions of rats that have received chronic lead treatment must be relatively slow.

V. SUMMARY AND CONCLUSIONS

In conclusion, the present study has shown that chronic exposure of rats to relatively low doses of lead (0.1 mg lead/kg, for 8 weeks; 0.025 mg lead/kg for 5 weeks) influences the regulation of motor activity. The lead induced effects included hyperactivity and a depressed motor response to the CNS stimulant, d-amphetamine. The elevated locomotion observed in animals receiving these low doses of metal occurred primarily during the initial exploratory period of the circadian locomotor activity cycle, while the remainder of the cycle was unaltered. A larger dose of lead (10.0 mg/kg for 3 weeks) produced in addition to this hyper-reactivity, an increase in nocturnal locomotor activity. Our results thus demonstrate that lead does not cause a generalized increase in locomotion throughout the entire circadian cycle and indicate that the extent to which hyperactivity occurs during the circadian cycle is dependent on the dose of chronic lead exposure.

Measurement of the steady state levels of noradrenaline and dopamine in the striatum, hippocampus and cortex revealed no evidence of a relationship between elevated motor activity and altered catecholamine mechanisms in these areas of the CNS. For the brain regions examined the only changes in steady state levels of catecholamines observed were in the hippocampus where relative to controls noradrenaline content was significantly altered following treatment with 0.1 mg lead/kg for 4 and 6 weeks as well as 0.025 mg lead/kg for 8 weeks. The turnover rate of noradrenaline was also significantly reduced in the hippocampus of rats exposed to the 0.1 mg/kg dose of metal for 6 weeks. These selective neurochemical changes correlate well with the greater deposition of metal observed in the hippocampus as compared to other areas of the CNS in lead treated rats. Thus these findings suggest that the preferential accumulation of lead in the hippocampus may render this brain region more vulnerable to the neurotoxic effects of the heavy metal. Further analyses of brain regional lead

content indicated that the kinetics of lead distribution to the cortex, striatum, hippocampus and midbrain is varied and is influenced both by the dose of lead administered and the duration of chronic treatment.

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RESUME

There has been much discussion about the neurotoxic effects of low level lead exposure in developing organisms. Clinical investigations report that hyperkinesis may be a symptom of subclinical lead poisoning in children with slightly elevated body burdens of lead. Some of these children display a paradoxical response to central stimulants such as amphetamine and methylphenidate, in that these drugs actually alleviate their hyperactive behaviour. Experiments with rodents have had limited success in establishing an animal model of subclinical lead poisoning. While it has been reported that in rodents chronic lead exposure can cause hyperactivity and a paradoxical locomotor response to centrally active stimulants, these observations have not been confirmed by all researchers. Neurochemical investigations reveal that noradrenergic and dopaminergic systems in the brain which are thought to play a role in the central regulation of locomotor activity may be altered by chronic lead treatment. However, there is little concensus on the characteristic neurochemical changes in the brains of lead exposed hyperactive rodents. A drawback of many of the earlier animal studies is that the dose of chronic lead treatment is so large that the relevance of observations made to subclinical lead poisoning is questionable. In the present study the effects of chronic low level lead exposure on the circadian locomotor activity rhythm and on brain regional concentrations of noradrenaline and dopamine were investigated in the rat. In addition, the kinetics of lead distribution in blood and various areas of the central nervous system (CNS) were examined. Beginning at 3 days of age and continuing for various periods of time during postnatal development, lead (10.0 mg, 0.1 mg and 0.025 mg/kg/day) was administered directly to the animals by gastric intubation. None of the treatments were found to have an effect on growth and physical development during the critical preweaning period.

Examination of the circadian spontaneous locomotor activity (SLA) of rats

that had received 10.0 mg lead/kg until 3 weeks of age revealed that their initial exploratory activity was elevated by 191% as compared to untreated animals. Furthermore, the SLA during the last quarter of the dark phase was also significantly greater (164%) than that of controls. The altered locomotion observed at this dose was transitory and was not present after an additional week of lead treatment (i.e. at 4 weeks). Rats exposed to 10.0 mg lead/kg for 7 weeks displayed a reduced locomotor response to d-amphetamine. In rats that were exposed to 0.1 mg lead/kg for 8 weeks, the duration of elevated motor activity associated with the habituation period was prolonged. However, treatment with this dose of metal for 4 and 6 weeks had no effect on the SLA cycle of developing animals. Further evidence that chronic exposure to 0.1 mg lead/kg for 8 weeks had altered the regulation of locomotor activity came from a comparison of the motor responses of control and lead treated rats to amphetamine. Rats that were treated with this dose of metal for 8 weeks displayed a lower motor response to d-amphetamine. However, no alterations in response to amphetamine were observed in those animals that had received the 0.1 mg/kg dose of lead for 4 and 6 weeks.

Chronic treatment of newborn rats with a lower (0.025 mg/kg) dose of lead during postnatal development was also found to result in hyperactive behaviour during the habituation period. Reactivity to a novel environment by animals receiving this dose of metal for 5 weeks was 91% greater than that of untreated rats. This elevation in SLA of lead exposed rats (0.025 mg/kg for 5 weeks) was a transient phenomenon, since it was not observed when measurements were again conducted following 6 and 7 weeks of treatment.

In rats which were chronically exposed to relatively large doses of lead, elevations in SLA occurred both during the habituation period and during the dark phase of the circadian cycle. On the other hand, hyperactivity of rats treated with lower doses of lead (0.1 and 0.025 mg/kg) was found to occur exclusively during the initial exploratory period while the remainder of the circadian locomotor activity cycle was unaltered. These results demonstrate

that lead does not cause a generalized increase in locomotion throughout the circadian SLA rhythm, rather, only specific periods of the circadian cycle are affected. Moreover, the extent of damage is dependent on the dose of chronic lead exposure.

Measurement of the steady state levels of noradrenaline and dopamine in the striatum, hippocampus and cortex indicated no significant changes in brain regional catecholamine content of rats that were administered the 0.1 mg/kg dose of lead for 8 weeks. This observation suggests that the elevated locomotor activity noted at this level of lead exposure was not associated with alterations in the catecholaminergic pathways of the brain regions. However, exposure to 0.1 mg lead/kg resulted in selective alterations of noradrenaline levels in the hippocampus (a 43% increase at 4 weeks but a reduction by 47% at 6 weeks of age). The turnover rate of noradrenaline in the hippocampus of rats receiving the 0.1 mg/kg dose of metal for 6 weeks was also significantly reduced (by 24%). At the lowest dose of lead used in our study (0.025 mg lead/kg), a significant reduction (by 25%) in the noradrenaline content of the hippocampus was observed after 8 weeks of exposure. No significant alterations in the steady state levels of dopamine in the striatum or the hippocampus and of noradrenaline content of, the cortex were found. Our results on brain regional lead concentrations demonstrated a preferential storage of metal in the hippocampus. In rats that were treated with 0.1 mg lead/kg for 4 weeks or with the 0.025 mg/kg dose of metal for 8 weeks, the lead content of the hippocampus had increased 3-fold and 2-fold, respectively over that found in other brain areas. From these observations we concluded that the selective neurochemical changes noted in the hippocampus may have resulted from the preferential deposition of lead in this brain region of rats receiving chronic lead treatment.

The blood lead levels of rats treated with 0.1 mg lead/kg for 4, 6 and 8 weeks was found to initially increase with the duration of exposure, reach a maximum value of 13.8 µg/dl at 6 weeks and level off thereafter. Whereas

exposure to small amounts of lead (0.025 mg/kg) resulted in a preferential accumulation of lead in the hippocampus, a larger dose of metal (0.1 mg/kg) administered for the same period of time produced significant increases in the lead content of all four brain areas examined; the cortex (by 80%), striatum (by 106%), hippocampus (by 134%) and midbrain (by 67%) but no significant inter-regional differences in lead content were noted. In addition to the dose of metal, the duration of treatment was also found to influence the distribution of lead. Exposure to 0.1 mg lead/kg for 4 weeks resulted in a significant increase in the lead levels of only the cortex (by 125%) and hippocampus (by 522%), while the same treatment for 8 weeks resulted in a significant accumulation of lead in all of the regions examined. These data indicate that in the case of chronic low level lead poisoning brain regional lead distribution is influenced by both the dose of metal administered and the duration of exposure. Significant amounts of lead were found to persist in brain regions for as long as two weeks after the withdrawal of lead treatment even though by this time blood lead levels were in the range of control values.

In conclusion, our results indicate that chronic exposure of rodents to relatively low doses of metal results in heightened locomotor activity which occurs primarily during the habituation period but does not alter the overall pattern of the circadian SLA rhythm. The observed lead induced hyperactivity was not accompanied by any changes in the steady state levels of noradrenaline and dopamine in discrete regions of the CNS. However, there was a correlation between selective alterations in the noradrenergic system of the hippocampus and a preferential accumulation of lead in this brain region.