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Impact of Lead Sub-Chronic Toxicity on Recognition Memory and Motor Activity of Wistar Rat

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Abstract: The aim of this research was to investigate the impact of lead nitrate administered in drinking water during 90 days (sub-chronic toxicity), on body weight gain, motor activity, brain lead accumulation and especially on recognition memory of Wistar rats. Two groups of young female Wistar rats were used. Treated rats received 20 mg L^{-1} of lead nitrate diluted in drinking water, while control rats received drinking water only, for 3 months. An evolution of body weight, motor activity, object recognition memory and measure of brain lead levels has been evaluated. The body weight was taken weekly, whereas the memory abilities and the motor activity are measured once every fortnight alternatively, by submitting rats to the Open Field (OF) test and to the Novel Object Recognizing (NOR) memory test. The results have shown a non significant effect in gain of body weight. However, a high significance was shown for horizontal activity ($p<0.01$), long memory term ($p<0.01$), at the end of testing period and for brain lead levels ($p<0.05$) between studied groups.

Key words: Lead, sub-chronic, toxicity, rat, NOR test, open field

INTRODUCTION

Lead is a ubiquitous toxicant and most of the high levels found throughout the environment come from human activities. Sources of lead in surface water include deposits of lead-containing dust from the atmosphere, waste water from industries that handle lead, the absence of sewage treatment station, urban runoff and mining piles. All these factors cause an increase of lead contents in this environmental compartment and therefore expose humans to a serious health problems. It is admitted that a crucial aspect of human exposure is neurotoxicity. In deed, humans and laboratory animals exposed to high levels of either inorganic or organic forms of lead develop neurologic disorders (Louis *et al*., 2003; Coulehan *et al*., 1983; Booze *et al*., 1983; Konat and Clausen, 1974).

It is reported that the effect of chronic low-level exposure to lead has been linked with developmental problems, deficits in intellectual performance and decreased stature in children (Brody *et al*., 1994) and poorer performance on cognitive tests in adults (Payton *et al*., 1998; Muldoon *et al*., 1996).

Furthermore, lead exposure has been shown to produce impairments in the learning process, memory consolidation attention, spatial memory, hyperactivity and also in motor skills in rats (Trombini *et al*., 2001; Jett *et al*., 1997; Cory-Slechta, 1995; Luthman *et al*., 1992; Murphy and Regan, 1999).

The aim of this study is to contribute on elucidation of the impact of lead nitrate administered in drinking water during 90 days (sub-chronic toxicity), on body weight gain, motor activity, brain lead accumulation and especially on recognition memory of Wistar rats, because in our knowledge, all the studies realized, in this field, investigated the lead effect on spatial memory only.

MATERIALS AND METHODS

This study was performed in the year 2003 in Ibn Tofail University, Faculty of Science, Kenitra, Morocco.

Animals and treatment: Female Wistar rats, 3 months of age and 179.6±4.28 g in weight (Mean±SEM, n = 14) at the beginning of the treatment, were used in this study. They were reproduced in colony room of Biology Department, Faculty of Sciences, Kenitra, Morocco. The rats were housed in propylene cages under standards conditions (20°C, 50-70% humidity and 12L: 12D cycle). They were given free access to food (SNV, Temara, Morocco) and tap water. The control rats (n = 7) were given tap water and the lead intoxicated rats (n = 7) received 20 mg L^{-1} of lead nitrate (Merck). Lead nitrate was diluted in tap water and given to animals during 90 days.

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Behavioral experiments

Open field behavior: An open field test was conducted between 9:00 and 11:00 am to examine the possible effect of sub chronic lead intoxication on behavior in a novel environment every fortnight from the beginning to the end of intoxication period.

Apparatus consisted of an open top wooden box (100×100×40 cm) covered by white consistent plastic. Floor area was marked into 25 squares and illuminated in the center by a 60 W halogen bulb suspended 100 cm above.

Animals were placed in the center of the open field and behavior was videotaped for 7 min for each rate. Open field behaviors were scored by a trained observer who was blind to the treatment conditions. The measures scored consisted on horizontal rat activity (number of squares crossed).

Novel Object Recognition (NOR) task: The apparatus and procedures for NOR training have been described elsewhere (De Lima *et al*., 2005; Ennaceur *et al*., 2004). The task took place in a 40×50 cm^2 open field surrounded by 50 cm high walls, made of plywood covered by black fine plastic layer. All animals were given a habituation session where they were left to freely exploring the open field for 5 min.

No objects were placed in the box during the habituation trial. Twenty-four hours after habituation, NOR training was conducted by placing individual rats for 5 min into the field, in which two identical objects (objects A1 and A2) were positioned in two adjacent corners, 10 cm from the walls. In a long-term retention test given 24 h after training, the same rats explored the field for 5 min in the presence of familiar object (A) and a novel object (B).

A single set of three objects was used for all animals. All objects presented similar textures, colours and sizes, but distinctive shapes. The index of recognition memory was defined as ratio of object B exploration number and the sum of object A and B exploration number. Between trials the objects were washed with 10% ethanol solution.

Exploration of an object was defined as directing the nose to the object at a distance ≤ 1 cm and/or touching it with the nose; conversely, turning around or sitting on the object was not considered as exploratory behaviour. NOR procedures were conducted in a presence of luminescent source (60 W) from 1 m in the top of the apparatus.

The test took place every fortnight alternatively with the open field test.

Brain lead evaluation: The day after the last test, lead concentration was estimated in control and treated rat's brain by graphite furnace atomic absorption spectrometry (Perkin Elmer 1100). Rats were anesthetized by the chloral 7% and killed by decapitation. The whole brain was extracted from the skull. Tissues samples (0.1-0.3 g) were dried milled and digested by HNO_3 acid 65%, Merck. All the analysis were performed in triplicate and the results were expressed in μg g^{-1} tissue wet weight. All the vessels and their caps used were previously washed in hydrochloric acid and then in 1% nitric acid (Merck) for a week and rinsed in ultrapure water, to prevent any contamination (Pinta, 1980).

Statistical analysis: Data obtained was expressed as Mean±SEM. To evaluate the differences between control and treated groups, the one-way ANOVA and the repeated one-way ANOVA parametric test were used. A p-value less than 0.05 was considered to reflect statistically significant difference.

RESULTS AND DISCUSSION

Body weight: The obtained results showed no significant difference in body weight ($p>0.05$) during 13 weeks of exposure suggesting that lead nitrate sub-chronic toxicity did not decrease food intake or weight gain (Fig. 1).

Motor activity in open field: The mean crossed squares exploring by both control (C) and treated rats (L) in (OF) test, during each fortnight, was not significantly different ($p>0.05$) in the first twelve weeks. However, high significant effect is registered at the end of testing period ($p<0.01$). Indeed, hyperactivity was shown in the treated rats (L) compared to the control ones (C) (Fig. 2).

Index of recognition: The mean index of recognition memory didn't show any significant difference between the experimented groups at the beginning of the testing period (week 2-6). This index decreased significantly in the treated group compared to the control at the end of the test (week 8 and 10) ($p<0.01$) (Fig. 3).

Brain lead levels: The lead levels showed significant difference between lead exposed (L) and control (C) rats ($p<0.05$) (Fig. 4).

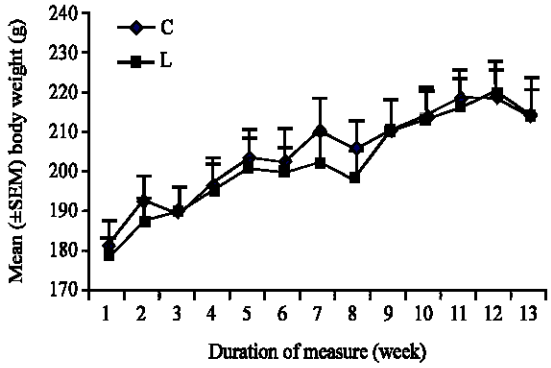

Fig. 1: The record of weight gain shown for the 13 weeks of lead exposure

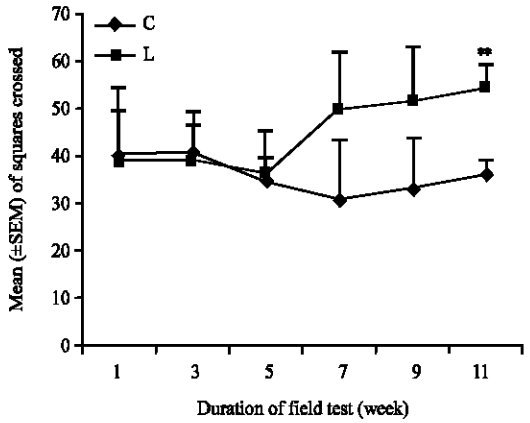

Fig. 2: Mean of squares crossed in the open field (Horizontal motor activity) every fortnight respecting alternation between open field test and recognition memory test. High statistically difference between rats groups (control (C) and lead treated (L)) was registered (**p<0.01) in the last week of test (week 11)

The current finding showed that lead sub-chronic exposure did not affect the body weight of intoxicated rates. These results are consistent with previous research proving that no gross differential body weight difference in adults' rates in normal physiological state (Salinas and Huff, 2002; Cory-Slechta *et al*., 1989) or during pregnancy and suckling (Trombini *et al*., 2001). Besides, the results of present study showed that the administration of lead to rats, during three months, affected the locomotor activity in the OF test. The registered rat's hyper activity began from the medium of intoxication period. Other studies realized during the postnatal period found the same results in the pup's rat treated by lead (Trombini *et al*., 2001; Ma *et al*., 1999).

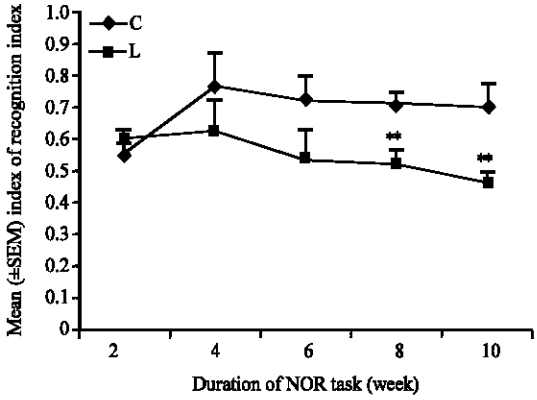

Fig. 3: Mean of recognition memory index (ratio of object B exploration number and the sum of object A and B exploration number) of every fortnight respecting the alternation between recognition memory test and open field test. High statistically difference between control rats (C) and lead treated group (L) was registered (**p<0.01) at the last weeks of the test (week 8 and 10)

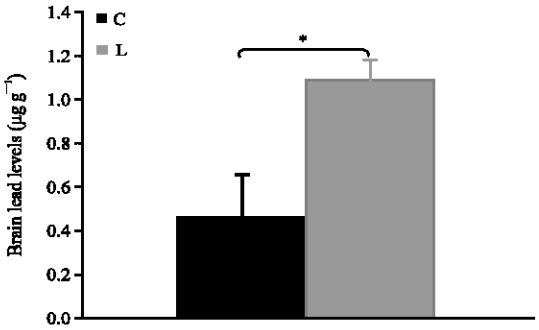

Fig. 4: Brain lead levels in control (C) and lead treated (L) rats. Significant difference was registered between both groups (*p<0.05)

With respect to the study of memory, several studies demonstrate the effect of lead on spatial memory even in human and animals (Winneke *et al*., 1996; Cory-Slechta, 1995; Tang *et al*., 1995). However, in our knowledge, no study investigates its effect on recognition memory. The current results showed high significant decrease in the long term recognition memory of intoxicated rats. The significant difference appears at the end of experimentation.

Both the registered effects, in the medium or in the end of testing period, as well in locomotor activity or in memory, can be explained by the lead accumulation in rat's brain. This data is proved by the significant difference between lead level in control and intoxicated rats founded in the whole brain.

The hyperactivity founded in present study could be explained by lead effects on dopaminergic system; it is known that the dopamine plays critical roles in the regulation of locomotor activity and it is demonstrated that lead can affects the synthesis, turnover and reuptake of dopamine; changes in levels of dopamine and its metabolites; as well as changes in the number of dopamine receptors (Cory-Slechta, 1995; Rossouw *et al*., 1987; Winder and Lazareno, 1985).

Furthermore, the decrease registered in long term recognition memory, in this study, could be due to the lead alteration of acetylcholine esterase activity (AchE) in hippocampus. Indeed, evidence showed hippocampal involvement and delay-dependent hippocampal involvement in object recognition memory (Hammond *et al*., 2004; Clark *et al*., 2000; Vnek and Rothblat, 1996). Besides, other findings demonstrated that the object recognition was impaired after larger hippocampal lesions that encompassed 75-100% of hippocampal volume (Broadbent *et al*., 2004). Earlier data have shown that early (perinatal) exposure to lead causes alterations in AchE of hippocampus especially in the latter periods of postnatal development. The data from AchE histochemistry suggested that Pb exposure causes localized changes in the brain, with a considerable decrease in the granule cell layer of dentate gyrus of hippocampus (Reddy *et al*., 2003).

The obtained data showed that lead exposure sub-chronically causes hyperactivity and decrease in recognition memory in exposed rats. Some earlier studies have permit to elucidate the possible neurochemical causes of these effects, but the exact mechanisms remain unclear and require more investigations.

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