Behavior Alterations of Contaminated Lead Mice Exposed to Ansiogenic Stimulus

Liliana Noemí Fracchia[1]

Profesora Asociada a Cargo de la Cátedra Metodología de la Investigación, Facultad de Medicina y Docente Investigador Categorizado, CIUNT. Universidad Nacional de Tucumán. Tucumán, Argentina.

Nora Martínez Riera

Profesora Asociada a Cargo Cátedra de Toxicología y Docente Investigador Categorizado CIUNT. Facultad de Medicina. Universidad Nacional de Tucumán. Tucumán, Argentina.

Norma Soria y María José Gandur

Jefes de Trabajos Prácticos de la Cátedra de Toxicología y Docentes Investigadores Categorizados CIUNT. Facultad de Medicina. Universidad Nacional de Tucumán. Tucumán, Argentina.

Patricia Mercedes Vargas

Profesora Adjunta de la Cátedra Metodología de la Investigación y Becario Doctoral CIUNT. Facultad de Medicina. Universidad Nacional de Tucumán. Tucumán, Argentina.

Nora Riera de Martínez Villa[2†] †

Profesora Titular de la Cátedra de Toxicología y Docente Investigador Categorizado CIUNT. Facultad de Medicina. Universidad Nacional de Tucumán. Tucumán, Argentina.

Correspondence: Liliana Noemí Fracchia. Lamadrid 875 – 1º piso - San Miguel de Tucumán, Tucumán C.P. 4000, Argentina. Teléfono: +54 381 4244152, +54 381 4107236, Fax: +54 381 4248024. Correo electrónico: fracchia@arnet.com.ar, metodol@fm.unt.edu.ar

Aknowledgments

We owe our deep gratitude to Professor Doctor Nora Lilia Riera de Martinez Villa, who permanently guided and lead us through the paths of science and life. This project was developed within the "Lead poisoning: conduct /behavior, metabolism and nutrition. Physiopathologic, clinic and therapeutic aspects". Direction: Professor Doctor Nora Lilia Riera de Martinez Villa. Arbitrated and subsidized by the "Consejo de Investigaciones de la Universidad Nacional de Tucumán" (CIUNT), (Council of Investigations of the National University of Tucuman), Argentina.

[1] Directora del proyecto: "Neuroquímica de la conducta antisocial y agresión en ratas contaminadas con plomo". Actual Directora del Programa: "Intoxicación plúmbica: conducta, metabolismo y nutrición. Aspectos fisiopatológicos, clínicos y terapéuticos" dentro del cual se desarrolla el proyecto. Proyecto evaluado, acreditado y subsidiado por el Consejo de Investigaciones de la Universidad Nacional de Tucumán (CIUNT).

[2] Directora del programa: "Intoxicación plúmbica: conducta, metabolismo y nutrición. Aspectos fisiopatológicos, clínicos y terapéuticos".

† Fallecida

Resumen

En estudios previos, se encontraron alteraciones de la conducta exploratoria no conflictiva y de la dopamina cerebral en animales tratados con bajas concentraciones de plomo. Al evaluar la conducta social y agresión como componentes de la reactividad emocional en los mismos, se encontró diferencias altamente significativas en el desarrollo de conductas agresivas con respecto a los controles. Los objetivos de este estudio fueron evaluar las respuestas de los animales tratados con plomo en un modelo de ansiedad como el Laberinto en Cruz Elevado (LCE) y determinar los niveles de Serotonina plasmática. Se trabajó con ratones macho, cepa C3H, mantenidos en condiciones controladas de laboratorio. El grupo experimental fue tratado con acetato de plomo en el agua de bebida en una concentración de 0,5 ppm. Se empleó el LCE, estructura que se encuentra suspendida a 60 cm del suelo, con una plataforma central y cuatro brazos, dos abiertos y dos cerrados donde se evaluaron las actividades conductuales: Tiempo de latencia, frecuencia de entrada a cada brazo y actividad aérea e inmovilidad. Al comparar ambos grupos se encontró un menor tiempo de latencia, junto a un incremento significativo de la actividad aérea. La frecuencia de entrada a los brazos abiertos se vio aumentada, con una alteración de la Serotonina plasmática. Se evidencia una disminución significativa de la sensibilidad a los procedimientos ansiogénicos en los animales expuestos al plomo que en relación a los cambios bioquímicos podrían corresponderse con una participación en el desarrollo de conductas agresivas y antisociales.

Palabras clave: Plomo, conducta, agresión, ansiedad, serotonina, ratones.

Summary

Previous results, have found alterations in the non-conflictive exploratory behavior and brain dopamine in mice treated with low doses of lead. Evaluations of social interactions and aggression, as emotional reactivity components, show significant differences between contaminated and the control groups in the aggressive behavior development. A relation between anxiety basal levels and antisocial aggressive behavior development is proposed. To study the response of mice treated with lead in a model of anxiety: the elevated plus maze, and to determine blood serotonin levels. The test was done with C3Hs adult mice fed on a standard diet and water *ad libitum*. The animals were divided into two groups: an experimental one, treated with lead acetate (0.5 ppm) in drinking water, and a control group, which drank fresh tap water. The elevated plus maze was used. Latency time, frequency of entries into each arm, rears and serotonin levels were determined. A significant diminution of latency time and freezing, as well as an increase in the number of rears were observed in the experimental group. Contaminated mice, showed a high percentage of entries into open arms ($p \leq 0.05$). Serotonin levels were altered. The results showed a significant diminution of sensibility to the ansiogenic method in the lead contaminated animals; in this group an inhibition of aversive normal response behaviour and fear to stimulus expositions were observed. These results support the hypothesis that alterations in the anxiety basal level in animals exposed to lead, which in relation to the biochemical changes, could be related to the development of antisocial and aggressive behaviour.

Key words: Lead, behaviour, aggression, anxiety, serotonin, mice.

Introduction

Health problems due to lead exposure in adults have largely been recognized and studied both, in the labor field and as an environmental pollutant. It is important to highlight the fact that, even though the thresholds that cause neurotoxicity have not been defined yet, blood lead levels which are below the reference limits, could be associated to behavior disorders, cognitive deficit, manifestations of aggressiveness (Canfield et al., 2003; Ntoxment Workshop, 2006; Villeda Hernandez, 2002; Wakefield, 2002), which could be related to alterations in the levels of neurotransmitters such as dopamine (D) and serotonin (5-HT) (Anguiano Rodriguez, Faria Velasco, Gonzalez Burgos, & Olivera Cortes, 2004; Canfield, Kreher, Cornwell, & Henderson, 2003; Lasley & Gilbert, 2000; Lewendon, Kinra, Nelder, & Cronin, 2001).

These findings have been experimentally evaluated in animal models; Kala and Jadhav (1995) report that animals treated with doses of 25, 50 and 500 ppm of lead acetate in their drinking water, present dose-dependant modifications of serotonin, dopamine and metabolites in regions of their brains. In preliminary studies this working group has determined alterations in the non conflictive exploratory conduct and a decrease in the concentration of brain dopamine in mice treated with small doses of lead (0.5 ppm) (Fracchia, Martinez Riera, Soria, Gandur, & Riera, 2003; Martínez Riera, Gandur, Soria, & Riera De Martinez Villa, 2001). Possible alterations of social conduct and aggression as part of emotional reactivity were also studied (Fracchia, Martinez Riera, Soria, Gandur, & Riera, 2002). The evaluated social interaction components (duration and frequency of contacts) as well as the aggressive episodes were significantly increased in the group contaminated with lead. This increase in aggressiveness agrees with the results obtained by other authors, in which lead is related to the development of aggression, lack of control of impulses and criminal conduct in humans (Davidson, Putnam, & Larson, 2000; Liu & Wuerker, 2005; Garza Almanza, 2001; Masters, Hone, & Doshi, 2001; Matte, 2003; Moreira & Vassilieff, 2001; Sepulveda, Vega, & Delgado, 2000). In addition, the levels of 5-HT in the experimental group were found to be significantly diminished, which agrees with the literature about the existent relationship between this neurotransmitter, alterations in conduct and personality disorders (Huertas, Lopez Ibor Aliño, & Crespo Hervas, 2005; Muller, 1997; Nelson & Chiavegatto, 2001).

Needelman et al. (1996) studied the students with low IQ, hyperactivity, attention disorders, aggressiveness, anxiety, depression and criminal conduct at a public school. On analyzing de factors that could have caused these alterations in this group and finding high concentrations of lead in their bones, he highlighted the importance of evaluating lead as another factor that contributes to trigger them.

These findings led this working team to use a standardized anxiety-model, the elevated plus maze (Carobrez & Bertoglio, 2005; Hata, Nishikawa, Itoh, & Funakami, 2001; Rodgers & Dalvi, 1997; Schwarting & Bortoa, 2005), as an answer to the aggression and antisocial conduct found. It is known that in natural conditions, an animal exposed to an unknown environment

reacts in either of two ways, exploring the environment or looking for a sheltered spot. It has long been considered that these conducts are somewhat influenced by reactions to fear or anxiety. From this point of view, an animal that is extremely frightened or anxious will not explore, while a fearless animal will show a distinctive exploratory conduct (Alvarez, Ruarte & Gargiulo, 1999; Van Meer & Raber, 2005). Anxiety is the psycho-biological mechanism that allows us to deal with those situations or events that we reckon as dangerous; it acts as an alert signal in order to preserve life. Therefore, it is regarded as a form of surveillance that enables the system to be alert to changes in the surroundings and ready for any emergency that might occur (Bonet, 2006). In certain types of environments this process is made clearly evident, so the exploratory activity under these conditions is called "conflictive exploration", since the animal must resolve the "conflict" of whether or not to explore. This is the basis of the Elevated Plus Maze, a device that is widely used to quantify fear or anxiety in experimental animals (Alvarez et al., 1999). On the basis of the foregoing, the objectives of the present study were to evaluate conduct alterations in mice treated with small doses of lead acetate in an experimental model of anxiety and to investigate the possible relationship with the levels of plasmatic 5-HT.

Methods

Four month old (adult) male mice of the C_3 H strain were used. They were fed on a standard diet and ad libitum water and kept in a constant room temperature, humidity and light-dark cycles. The mice were divided into two groups. The experimental one was treated with 0.5 ppm lead acetate in drinking water from weaning until three months of age. The control group drank fresh tap water.

The study was conducted in accordance to the ethic principles of the National Health Institute, which states the points to be taken into account when using animals in research in the Guide to the Care and Use of Experimental Animals, edited by Canadian Council on Animal Care (1993).

Animals. Behaviour Evaluation

The elevated plus maze was used. It is a plus-shaped structure with a central platform 10 cm wide and 10 cm long, and four arms. Two of them are open while the other two are enclosed with 10cm high walls. Before being exposed to the maze, the animal was placed into a transparent box for 5 minutes and then it was placed in the centre of the platform, facing one of the closed arms.

The observations were simultaneously carried out by three observes who were unaware of the treatment. The activity was observed under strict controlled conditions. Each experimenter recorded the following conduct parameters:

Latency Time: the time, measured in seconds, which the animal takes to start activity after it has been placed in the centre of the platform.

Entries in each arm: number of entries into each open and close arm (frequency). The results were expressed as the total number of entries into the open arms over the total number of entries into both arms, the same criterion being applied to close arms.

Rearing: the number of times the animal rears on its back legs by itself or onto a wall for more than two seconds (frequency)

Freezing: the time during which the animal remains still without engaging in exploratory activities, measured in seconds.

Biochemical Evaluation

After conduct evaluation, the animals were sacrificed by decapitation and their blood, collected directly from the trunk into heparinised tubes and immediately processed: To determine erythrocytary ALA-D (delta-Aminolevulinic Acid Dehydratase) as a biological parameter of lead exposure, the spectrophotometric method of Burch, H.B. and Siegel, A.L was used, reference values: 15 to 3 U/1 . To determine serotonin levels the Elisa method (Serotonin Enzyme Immunoassay kit – IMMUNOTECH Ref. 1749)) was used. Concentrations were expressed as nM.

Statistical Analysis

The groups were compared, as applicable, with the Mann-Whitney non-parametric test or the t-test. $P < 0.05$ was considered as statistically significant.

Results

When determining ALA-D (delta-Aminolevulinic Acid Dehydratase) levels, the following values were found: 10,2 ± 2,8 U/L in the group treated with the metal and 17,0 ± 1,11 U/L ($p< 0,0001$) in the control group.

Discussion

The elevated plus maze is a test in which the animal's anxiety is reflected by an unconditioned aversion to heights and open spaces, entry rates and exploration time in open arms provide information about inhibition of exploratory activity due to fear, this activity increases with the administration of anxiolytic agents and decreases with anxiogenic agents (Carobrez & Bertoglio, 2005; Hata et al., 2001; Ruarte, Orofino & Alvarez 1997; Van Meer & Raber, 2005). Reduced latency time (Figure 1), increase in the number of rearing (Figure 3), decrease in duration of freezing behaviour (Figure 4) and mainly, increase in the rates of entries into the open arms of the elevated plus maze (Figure 2) resulting from this study, show that animals exposed to low concentrations of lead could be classified as no anxious, that is, their normal aversion responses (escape) to an unconditioned anxiogenic stimulus are diminished. Antisocial conduct and aggression observed in previous studies in the same line of work could be interpreted as a reflection of lack of fear conditioned by medium or moderate stress agents such as the ones used in this research.

This lack of emotive reactivity would support the hypothesis about lead influence on the development on antisocial and impulsive aggressive conducts, and this, in turn, is consistent with what has been described by other authors such as Hahn et al 1991 and Delville, 1999.

It is known that serotonin is a neurotransmitter responsible for modulating complex conducts such as irritability, emotional instability, lowering of self-esteem and aggressiveness; Valzelli and Coccaro relate the decrease in serotoninergic activity to the presence of aggressive conducts (Gandhi & Venkatakrishna-Bhatt, 1999; Huertas et al., 2005; Hughes, 2001; Nelson & Chiavegatto, 2001; Nevin, 2000; Stretesky & Lynch, 2001).

The emergence of aggressive behaviour in the neurobehavioral tests used in previous studies, alteration in alert states as well as lower anxiety observed in the elevated plus maze, led to testing plasmatic serotonin

levels which as a result, showed a decrease in the circulating levels of this neurotransmitter (Figure 5), which would evidence that small doses of lead could be involved in the complex mechanism that triggers the various types of aggression. Serotonin would play the role of a key or trigger increasing irritability and impulsiveness, a finding that would match with what has been observed by other authors (Escobar & Gomez Gonzalez, 2006; Hughes, 2001; Mata, 2000; Van Heeringen, 2003).

The relevance of this study lies on the fact that this toxic metal, abundant in the environment as industrial process waste, even in cases of lead exposure at low concentrations, might be causing alterations in the nervous functions, which leads to a reduction of brain ability to use serotonin and dopamine, neurotransmitters directly associated with conduct disorders and aggressiveness.

Current researches are exploring these new and specific variables as risk factors to explain criminality.

Elevate Plus Maze:

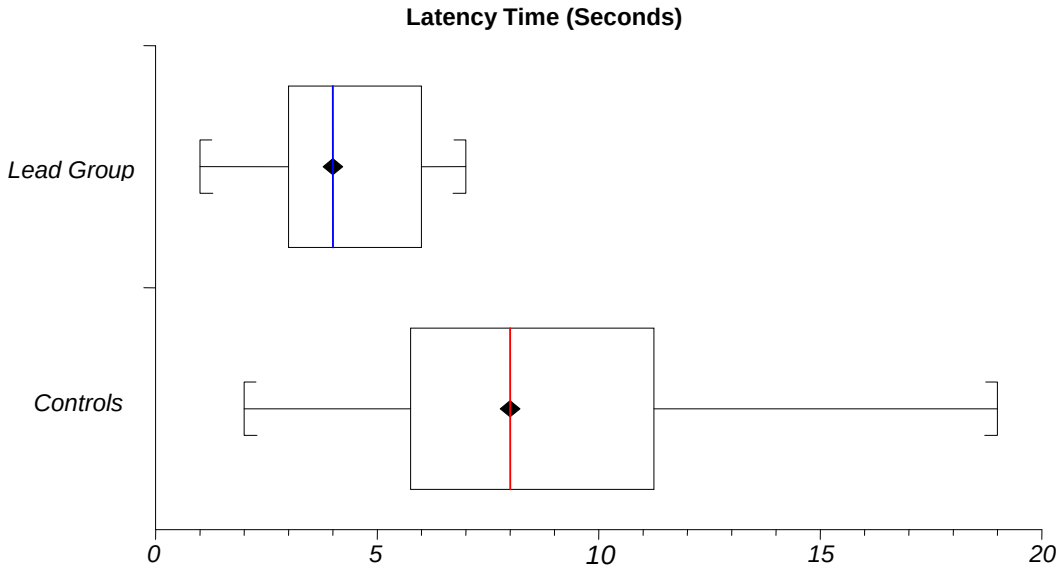

Figure 1. Controls: 8.33±0.74; Median = 8 (n = 30). Lead Group: 4.05±0.35; Me= 4 (n = 28). p = 0.0001 Mann Whitney U Test.

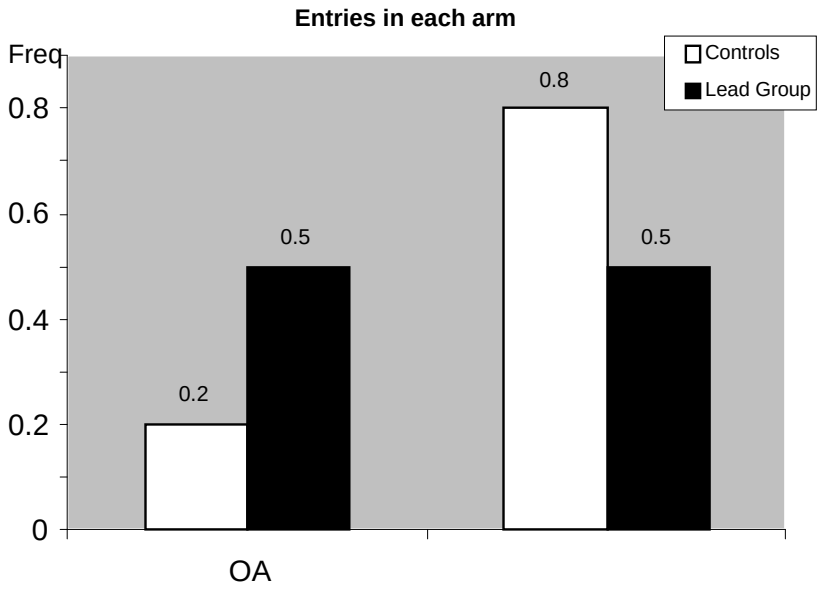

Figure 2. Open Arm (OA): Controls: Total entries: 24. Total entries both arms: 115. 0.8 ± 0.1; Me: 1 (n = 30). Lead Group: Total entries: 41. Total entries both arms: 81. 1.46 ± 0.1; Me: 1 (n = 28). p = 0.0004 Mann-Whitney U Test. Closed Arm (CA): Controls: Total entries: 91. Total entries both arms: 115. 3.14 ± 0.2; Me: 3 (n = 30). Lead Group: Total entries: 41. Total entries both arms: 81. 1.43 ± 0.2; Me: 1.5 (n = 28). p = 0.0001 Mann-Whitney U Test.

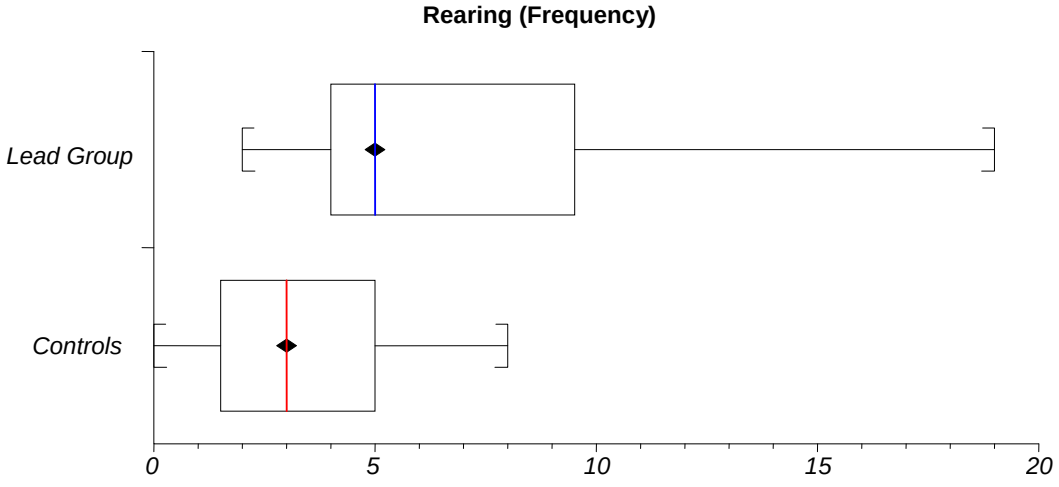

Figure 3. Controls: 3.23 ± 0.41; Median: 3 (n = 30). Lead Group: 7.39 ± 1.01; Median: 5* (n = 28). p = 0.005 Mann-Whitney U Test.

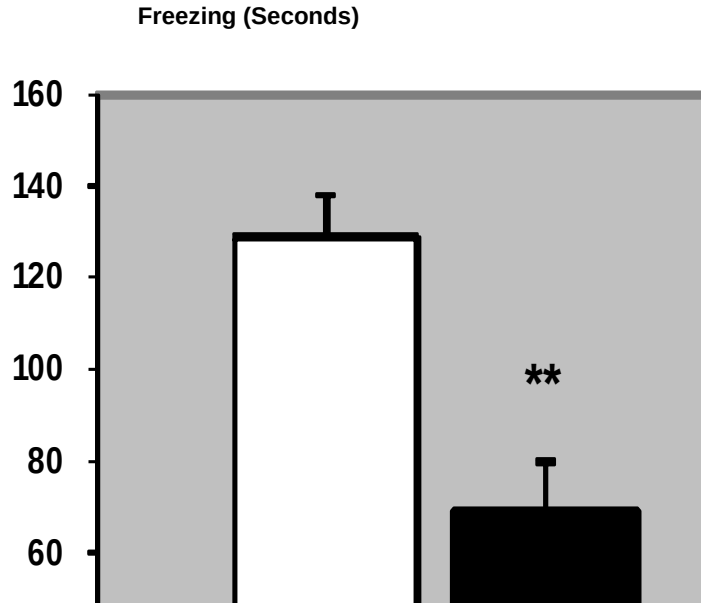

Figure 4. Controls: 128.83 ± 9.1 (n = 30). Lead Group: 69.39 ± 10.44 (n = 28). p<0.001 Test t: 5.12

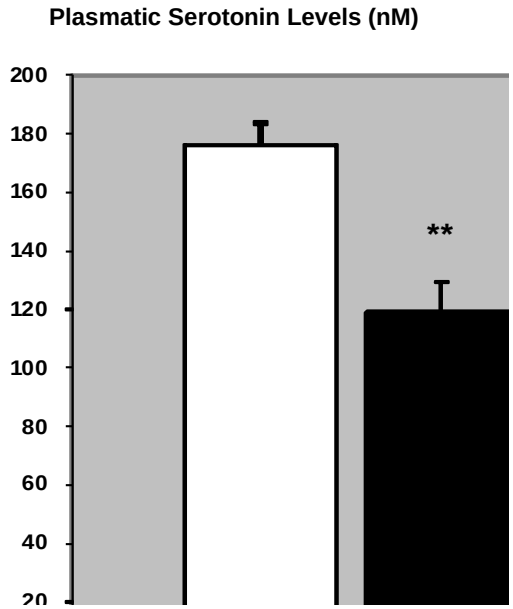

Figure 5. Controls(C): 176.2±7.48 (n=30). Lead Group (Pb):119.0±10.1 (n = 28). p <0.001 Test t: 4.38.

Referencies

Alvarez, E., Ruarte, M., & Gargiulo, P. (1999). Neurofarmacología de los procesos cerebrales de la motivación y la emocionalidad. *Neuropsicofarmacología Clínica, 4*(29), 5-11.

Anguiano Rodriguez, P., Faria Velasco, A., Gonzalez Burgos, I., & Olivera Cortes, M. (2004) *Efecto de la manipulación de la serotonina hipocampal sobre la ejecución de pruebas de aprendizaje egocéntrico y la actividad theta subyacente en la rata.* XLVII Congreso Nacional de Ciencias Fisiológicas, 116.

Bonet, J. L. (2006). Síntomas corporales de la ansiedad y el pánico en la consulta médica. *Revisa del Simposio GEMTAP (Grupo de Estudio Multidisciplinario en Trastornos de Ansiedad y Pánico), 1*, 5-10.

Canadian Council on Animal Care (1993). *Guide to the Care and Use of Experimental Animals.* Recuperado Marzo 21, 2010, desde:http://74.125.155.132/scholar?q=cache:pcB0LwY02jwJ:scholar.google.com/+%22Guide+for+the+Care+and+Use+of+Laboratory+Animals%22+NIH&hl=es&as_sdt=2000&as_vis=1

Canfield, R. L, Henderson, C. R. Jr., Cory-Slechta, D. A., Cox, C., Jusko, T. A., & Lanphear, B. P. (2003) Intellectual impairment in children with blood lead concentrations below 10 microg per deciliter. *The New England Journal of Medicine, 348*, 1527-1536.

Canfield, R., Kreher, D., Cornwell, C., & Henderson, C. (2003). Low-level lead exposure, executive functioning, and learning in early childhood. *Child Neuropsychology, 9*(1), 35-53.

Carobrez, A., & Bertoglio, L. (2005). Ethological and temporal analyses of anxiety-like behavior: The elevated plus-maze model 20 years on. *Neuroscience and Biobehavioral Reviews, 29*, 1193-1195.

Davidson, R., Putnam, K., & Larson, C. (2000). Agression. *Crime Times, 6*(4), 1-4.

Delville, Y. (1999). Exposure to lead during development alters aggressive behaviour in golden hamsters. *Neurotoxicology and Teratology, 21*(4), 445-449.

Escobar, A., & Gomez Gonzalez, B. (2006). Violencia y cerebro. *Revista Mexicana Neurociencia, 7*(2), 156-163.

Fracchia L, Martinez Riera N, Soria N, Gandur M, Riera N. (2002). Anxiety basal levels alterations possible response of aggression and antisocial behaviour in contaminated lead mice. *Biocell, 26*(1), 192.

Fracchia, L., Martinez Riera, N., Soria, N., Gandur, M., & Riera, N. (2003). Agresión e interacción social en ratones contaminados con plomo. *Revista de la Facultad de Medicina. Universidad Nacional de Tucumán, 4*(1), 23-27.

Gandhi, D., & Venkatakrishna-Bhatt, H. (1999). Inhibitory effect of lead on 5-HT induced contractions of isolated ileum of rats. *Agent's Actions, 33*(3-4), 337-342.

Garza Almanza, V. (2001). Nexos entre conductas criminales y acumulación de sustancias neurotóxicas en el cuerpo. Contaminación y Violencia. Recuperado Marzo 21, 2010, desde http://www.jornada.unam.mx/2001/07/23/cien-almanza.html

Hahn, M., Burright, R., & Donovick, P. (1991). Lead effects on food competition and predatory aggression in Binghamton HET mice. *Phisiology and Behavior, 50*(4), 757-764.

Hata, T., Nishikawa, H., Itoh, E., & Funakami, Y. (2001). Axiety-like behavior in elevated plus- maze tests in repeatedly cold-stressed mice. *The Japanese Journal Pharmacology, 85*, 189-196.

Huertas, D., Lopez Ibor Aliño, J., & Crespo Hervas, M. (Eds.). (2005). Neurobiología. *Neurobiología de la Agresividad Humana* (2a. ed., pp. 43-75). Barcelona: Ars Médica.

Hughes, D. (2001). Control psicofarmacológico agudo del paciente psicótico agresivo. *RET, Revista de Toxicomanías, 26*, 16-20.

Kala, S. V.; & Jadhav, A. L. (1995). Region-specific alterations in dopamine and serotonin metabolism in brain of rats exposed to low levels of lead. *Neurotoxicology, 16*(2), 297-308.

Lasley, S., & Gilbert, M. (2000). Glutamatergic components underlying lead-induced impairments in hippocampal synaptic plasticity. *Neurotoxicology, 21,* 1057-1068.

Lewendon, G., Kinra, S. Nelder, R., & Cronin, T. (2001). Should children with developmental and behavioural problems be routinely screened for lead. *Archives of Disease in Childhood, 85*(4), 286-288.

Liu, J. & Wuerker, A. (2005). Biosocial bases of aggressive and violent behavior? Implications for nursing studies. *International Journal of Nursing Studies, 42*(2), 229–241.

Martínez Riera, N., Gandur, M., Soria, N., Riera De Martinez Villa, N. (2001). Evaluación de las alteraciones conductuales y dopaminérgicas en ratones con bajas concentraciones de plomo. *Revista de Toxicología, 18*(2), 87-91.

Masters, R., Hone, B., & Doshi, A. (2001). Environmental pollution, neurotoxicity, and criminal violence. Recuperado Marzo21, 2010, desde www.vrfca.org/files/vrf/article-masters-environmental-pollution.pdf

Mata, E. (2000). Violencia y agresión en el psicópata. *Alcmeon. Revista Argentina de Clínica Neuropsiquiátrica, Año XI, 9*(3), 42-44.

Matte, T. (2003). Efectos del plomo en la salud de la niñez. *Salud Pública de México, 45*(S2), 220-224.

Moreira, E., & Vassilieff, I. (2001). Developmental lead exposure: Behavioral alterations in the short and long term. *Neurotoxicology & Teratology, 23*(5), 489-495.

Muller, R. (1997). Trastorno de la personalidad antisocial. *RET, Revista de Toxicomanías, 12*, 22-28.

Needleman M, Riess J, Tobin P, Gretchen E, Biesecker J, Greenhouse J. (1996) Bone, lead levels and delinquent behavior. *Journal of the American Medical Association, 275*(5), 363-369.

Nelson, R., & Chiavegatto, S. (2001). Molecular basis of aggression. *Trends in Neurosciences, 24(*12), 713-719.

Nevin, R. (2000). How lead relates to temporal changes in IQ, violent crime, and unwed pregnancy. *Environmental Research, 83*(1), 1-22.

Ntoxment Workshop (2006). Declaración de Brescia sobre prevención de la neurotoxicidad de los metales. *Boletín de la Asociación Toxicológica Argentina, 20*(74), 8-10.

Rodgers, R., & Dalvi, A. (1997). Anxiety, defence and elevated plus-maze. *Neurocience & Biobehavioral Reviews, 21*(6), 801-810.

Ruarte, M., Orofino, A., & Alvarez, E. (1997). Hippocampal histamine receptors and conflictive exploration in the rat: studies using the elevated asymmetric plus-maze. *Brazilian Journal of Medical and Biological Research, 30*(12), 1451-146.

Schwarting, R., & Bortoa, A. (2005). Analysis of behavioral asymmetries in the elevated plus- maze and in the T-maze. *Journal of Neuroscience Methods, 141*(2), 251-260.

Sepulveda, V., Vega, J., & Delgado, I. (2000). Exposición severa a plomo ambiental en una población infantil de Antofagasta, Chile. *Revista Médica de Chile, 128*(2), 2-5.

Stretesky, P., & Lynch, M. (2001). The relationship between lead exposure and homicide. *Archives of Pediatrics & Adolescence Medicine, 155*(5), 579-582.

Van Heeringen, K. (2003). The neurobiology of suicide and suicidality. *Canadian Journal of Pshychiatry, 48*(5), 292-300.

Van Meer, P., & Raber, J. (2005) Mouse behavioural analysis in systems biology. *Biochemical Journal, 389*, 593-610.

Villeda Hernandez, J. (2002). Efectos neurotóxicos en niños intoxicados con plomo. *Archivos de Neurociencias (México), 7*(2), 90-98.

Wakefield, J. (2002). New centers to focus on autism and other developmental disorders. *Environmental Health Perspectives, 110*(1), A20-A21.