# Gender-dependent behavioural impairment in young adult rats exposed to low lead levels.

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**Abstract**

The behavioural effects induced by exposure to lead acetate (Pb) producing blood levels below from those considered “acceptable” (<40 µg/dl) for adult population was studied in adult rats. In order to assess gender differences, we performed parallel behavioral experiments in male and female rats. Exposure to lead acetate (50 ppm) for 30-45 days induces subtle neurological alterations consisting in hyperactivity in a novel environment and impairment of spatial memory. The effects were observed only in male rats. Object recognition, motor coordination were unaffected by lead exposure. Magnetic resonance spectroscopy allows *in vivo* assessment of main brain metabolites (glutamate/glutamine, creatine, myoinositol, n-acetylaspartate and choline) whose changes have been demonstrated in several central nervous system pathologies. Exposure to low lead level does not affect metabolites profile in the striatum and increase myoinositol signal in the hippocampus of male rats. The increase in myoinositol in hippocampus suggests early lead-induced alteration in glial metabolism and may represent a potential marker of early neurological dysfunction during low lead exposure.

Key words: lead, magnetic resonance spectroscopy, hippocampus, n-acetylaspartate, myoinositol, rat

## Introduction

Lead (Pb) is a non-essential toxic heavy metal widely distributed in the environment, and chronic exposure to low levels of Pb has been a matter of public health concern in many countries. Despite improvements in public health policies and substantial reductions in blood lead levels in adults, lead exposure remains an important health problem worldwide.

For adult population, acceptable blood Pb levels are those below 40μg/dl (Occupational Safety and Health Administration (OSHA), 2008). Several studies demonstrated that cumulative exposure to lead, is associated with accelerated cognitive decline at older age (Wijngaarden et al., 2009; Weisskopf et al., 2004, 2007; Weuve et al., 2009; Wright et al., 2003) but the impact of subacute lead exposure at lower doses on cognitive and motor performance in adult population needs further studies. The U.S. Department of Health and Human Services recommends that blood concentration among all adults should be reduced to <25 μg/dl (CDC, 2009) suggesting that lead level below the acceptable limit might have adverse effects on health. The impairment of cognitive and motor functions after prenatal and postnatal (during lactation and just after weaning) exposure to moderate-level (blood concentration 15–39 μg/dl) and high-level (blood concentration ≥40 μg/dl) of lead have been studied in rodents (Cory-Slechta 1997; Crofton et al., 1980; Kuhlmann et al., 1997; Mansouri and Cauli, 2009) but the effects of low lead level exposure in adult male and female rats have not been investigated. Gender-related differences have received surprisingly little attention to date in lead-induced neurotoxicity, particularly in studies that aimed to address the effects of lead exposure during adulthood. Gestational exposure impairs spatial learning more in male than female offspring (Yang et al., 2003), an effect was not due to the disturbances in motor performance and visual function. De Souza et al. (2005) demonstrated that Pb

exposure during both pregnancy and lactation induced increment of emotionality state in males detected in the open-field test and depressive-like behavior in females detected in the forced swimming test. Postnatal exposure to 500 ppm from weaning induced anxiogenic effect only in males (Soeiro et al., 2007).

For this reason, we have performed the studies in parallel in male and female rats.

Magnetic resonance spectroscopy (MRS) is a non-invasive tool that provides information about biochemical aspects of neurological diseases *in vivo*. The development of spatially localized MRS that sample the relative levels of metabolites from the volume of tissues defined from magnetic resonance imaging (MRI) scanning has provided the basis for integrating the biochemical information with anatomical information obtained from the MRI (Cox, 1996; Ross et al., 1991). Moreover, MRS allows analyze simultaneously different brain areas in the same individual. As far as we know, MRS has not been used to analyze brain metabolism during lead exposure.

In this work we assessed:

(1) whether low lead exposure (0 or 50 ppm) in adults rats alters motor and/or cognitive functions

(2) different gender-vulnerability

(3) whether lead exposure alters the profile of the main brain metabolites using *in vivo* MRS technique. We performed MRS studies in the striatum and hippocampus since these brain regions play a main role in the modulation of motor and cognitive functions.

Motor activity and coordination were assessed by open-field and rota-rod tests, respectively. Spatial and object memory were evaluated by Morris water maze and novel object recognition memory test.

## EXPERIMENTAL PROCEDURES

### Animals and Pb exposure protocol

All experiments have been conducted in accordance with the guidelines for care and use of experimental animals of the European Communities Directive (86/609/EEC; D.L., 27.01.1992, number 116).

Adult Wistar male and female rats (220±20 g) were used at the beginning of the study. The animals were subdivided in two groups (control and exposed animals), which were housed four per cage, at 24±2 °C, with a light-dark cycle of 12 h. Food and water were freely available to all the animals throughout the study.

Ten days after receiving to the laboratory, the rats were divided randomly in four groups: (1) 50ppm of $Pb^{2+}$ male, (2) 50ppm of $Pb^{2+}$ female, (3) control male, and (4) control female. All experimental groups contained 8 rats each.

Lead-exposed rats received a 50 ppm solution of lead acetate for at least one month via their ad lib water supply, 54.7mg/L lead acetate trihydrate from Sigma (St. Louis, MO) dissolved in distilled water. Half of control rats received tap water and the other half received water solution containing sodium acetate (50 ppm). Lead exposure was maintained throughout the course of experiments. Drinking solution containing lead or sodium acetate was freshly prepared every week. Data from control rats exposed to tap water or to 50 ppm of sodium acetate produced similar results in each of the measurements performed in the study so the data were pooled together.

In order to assess whether behavioural changes were linked to gender and no to the higher lead concentration found in male as compared to female rats, we performed additional behavioural experiments in female rats exposed to a higher dose of lead acetate dose (70 ppm) or vehicle (70 ppm sodium acetate). Behavioural and magnetic

resonance experiments were conducted between days 30-45 after starting of lead exposure.

**Motor activity**

Spontaneous motor activity was assessed by placing rats in the open-field box (76 x 76 cm, divided by 8 lines into 25 squares). Each rat was individually placed in the centre of the open-field and allowed to explore it for 10 min. During this time the motor activity was recorded by videotaping the rat in the open-field. Ambulatory activity was measured as "lines crossed" (horizontal movement crossing the floor lines). Other elements of exploratory activity such rearing, sniffing and grooming were carefully observed and time spent performing each behavior was recorded. These three parameters were defined as follows: rearing (standing on hind legs with paws pressed against the wall of the arena); sniffing (continuous placing nose against floor for at least 2s); grooming (using paws or tongue to clean/scratch body) (Cauli and Morelli, 2002). The arena was carefully cleaned with 70% ethanol solution between animal tests to eliminate any olfactory cue derived from the previous rat located in the box.

**Motor coordination test (Rotarod)**

The rotarod test assesses the ability of rats to stay on a rotating drum to evaluate motor coordination functions. An accelerating 4-lane rotarod was used (Rotomex 5, Columbus Instruments, Columbus, OH). Two consecutive days before testing, each rat was placed on the rotarod which was switched off for 3 min. On the day of measurements, rats were placed on the rod, then the apparatus was switched on and the speed progressively increased from 2 to 30 r.p.m. over 300 s. The starting speed was set to 0, and the speed was increased by 2 rpm every 5 seconds up to 30 rpm. The time at which each animal fell off the rungs was recorded, with a maximum cut-off of 600 s. Each animal was given 3 trials, and the mean latency of three trials was calculated (Monville et al., 2006).

**Object recognition memory test**

This test exploits the tendency of rats to preferentially explore novel elements of their environment. Thus, when a rat is presented with both a novel and recently presented familiar object, it will spend significantly more time exploring the novel object. The familiar object was presented in a previous training session 2 h before the test. The percentage of time exploring the non-familiar object in the training session over total exploration time (exploration time of the familiar plus the non-familiar objects) was represented. Rats were habituated to the test cage for 1 hour the day before the start of the test (Garcia-Ayllon et al., 2008)

**Morris Water Maze Test**

***Apparatus***

The water maze task was a black circular tank 136 cm in diameter and 60 cm in height. The tank was filled with water (20±1°C) to a depth of 25 cm. The maze was located in a room containing extra-maze cues (posters). The maze was divided geographically into four quadrants [northeast (NE), northwest (NW), southeast (SE), southwest (SW)] and starting positions [north (N), south (S), east (E), west (W)] that were equally spaced around the perimeter of the pool. A hidden circular platform (diameter: 13 cm) was located in the center of the NW quadrant, 1 cm below the surface of the water. A video camera was mounted directly above the water maze to record the rats' swim paths. A computer-based video tracking system (Ethovision Noldus, Waningen, The Netherlands) was used to assess escape latency, swimming speed and also the percentage of traveled distance and the time spent in the target quadrant (Morris et al., 1982).

***Procedure***

Thirty-two rats in the four groups of animals as described were used. The rats were given four training trials each day on 4 consecutive days. The rat's escape from the water reinforces its desire to quickly find the platform, and on subsequent trials (with the platform in the same position) the rat is able to locate the platform more rapidly. This improvement in performance occurs because the rat has learned where the hidden platform is located relative to the conspicuous visual cues. For each training trial, the rats were placed in the water facing the pool wall at one of the four starting positions (north, south, east, or west pole) in a different order each day and allowed to swim until they reached the platform located in the NW quadrant of the maze in every trial. The latency to reach the platform was recorded for up to 90 s. They remained on the platform for 30 s before being removed. The recall test trial (90 s) with the platform removed from the pool was conducted 24 h after the last training trial to assess memory. After completing the trials, rats were dried with a towel and placed in a holding cage under a heating lamp before it was returned to the home cage.

**Determination of lead in blood and brain**

After the exposure period, to prepare the sample for blood Pb measurement, a 0.1-ml aliquot of whole blood was mixed well with 3.9 ml of 0.5 N nitric acid containing 0.01% Triton X-100. After centrifugation the supernatant was taken. The blood Pb values were expressed as µg/dL of whole blood.

The brain Pb levels were determined by graphite furnace atomic absorption spectrometry (Yun et al., 2000). For measurement of Pb in the brain, animals were anesthetized and then perfused transcardially with 100 ml of normal saline to remove blood from the brain tissue then the whole brain was collected. To prepare 10% (w/v) brain homogenates, the whole brain was homogenized at an appropriate mixture of 0.5

N nitric acid, 0.5 N perchloric acid and 0.01% Triton X-100. To determine Pb concentration in samples, the same volume of each samples and 0.2% magnesium nitrate (as a modifier) was mixed and 10 µl was injected into graphite furnace of atomic absorption spectrophotometer (Perkin–Elmer 3030). Because the homogeneity of regional Pb concentration in the adult rat brain has been reported (Widsowski and Cory-Slechta, 1994), we measured Pb level in the whole brain (expressed as ng of Pb/g wet tissue).

**Magnetic Resonance Imaging (MRI) experiments**

The magnetic resonance (MR) experiments were performed on a Bruker Pharmascan system (Bruker Medical Gmbh, Ettlingen, Germany) using a 7.0-T horizontal-bore superconducting magnet, equipped with a $^{1}H$ selective birdcage resonator of 38 mm and a Bruker gradient insert with 90 mm of diameter (maximum intensity 36 G/cm). All data were acquired using a Hewlett-Packard console running Paravision software (Bruker Medical Gmbh, Ettlingen, Germany) operating on a Linux platform. Anesthesia was initiated by inhalation of oxygen (1 l/min) containing 4 % isofluorane and maintained during the experiment employing a mask and 2% isofluorane in $O_2$. Animal temperature was maintained at approx. 37 ºC with a heated probe. The physiological state of the rats was monitored using a Biotrig physiological monitor (Bruker, Germany) that controlled the respiratory rate and body temperature. T2 weighted spin-echo images of the whole brain were acquired in axial orientation to localize the region where carrying out the later spectroscopic studies.

***In vivo* $^{1}$H Magnetic Resonace Spectroscopy (MRS)**

$^{1}H$-MRS studies were performed in two brain regions: striatum and the hippocampus. The in vivo spectroscopy protocol used a Point-Resolved Spatially Spectroscopy (PRESS), combined with VAPOR water suppression and employed the following

parameters: TR=3000 ms, TE=35 ms, Av=128, voxel volume=3 $mm^3$. Metabolites were quantified by measuring the area of the peaks by using the software MestRecC (Mestrelab Research, Santiago de Compostela, Spain). The analysis of metabolites was performed by selecting the following resonances: *N*-acetylaspartate (2.01 ppm), creatine/phosphocreatine (Cr) (3.02 ppm), choline-containing compounds (3.20 ppm), and myoinositol (mIns) (3.55 ppm). Signal intensity for each resonance is referred to the protons of the methyl group of the Cr signal at 3.02 ppm. Values for each metabolite in lead-exposed groups are expressed as percentage of the corresponding metabolite values in the control-exposed groups.

**Statistical analysis**

The results are presented as mean±SEM. The data were analyzed by one-way ANOVA followed by Dunnett's test. *P* values lower than 0.05 were considered statistically significant. Statistical analysis was performed using the Graph Pad Prism4 software (GraphPad Software Inc., San Diego, USA).

**RESULTS**

Body weight was measured at the beginning of experiments and at the time of sacrifice. During the treatment all animals showed a normal and gradual increase of the body. Two-way ANOVA showed a significant effect of time $P<0.0001$, of group $P<0.0001$ and interaction $P<0.01$. At the end of experiment the weight of female rats were significant different from male ($P<0.05$). No significant effect of Pb exposure was observed in the weight gain (Table1).

**Lead concentration in blood and brain**

As expected, Pb-exposed rats showed significant more lead in the blood and in the brain. Two-way ANOVA showed a significant effect of treatment $P<0.0001$, of gender $P<0.05$ and interaction $P<0.05$ for both blood and brain (Fig. 1). In control groups there

was no statistical difference of lead content in the blood and brain between female and male rats. In contrast, Pb-exposed female rats showed significant less lead concentration than Pb-exposed male rats either in blood (6.8±0.5 μg/dl and 8.8±0.7 μg/dl, $P<0.05$) (Fig. 1A) and in the brain (204±7 ng/g and 239±9 ng/g, $P<0.01$) (Fig. 1B). In female rats exposed to 70 ppm, lead concentration in blood was 8.6±0.4 μg/dl and in brain 250±16 ng/g.

**Motor activity after 50 ppm Pb exposure**

***Ambulatory activity***

Two-way ANOVA showed no significant effect of treatment ($P=0.052$), significant effect of gender ($P<0.001$) and no significant treatment X gender interaction ($P=0.44$). Female rats in the control groups showed significant more ambulatory activity counts than male rats (296±12 and 238±10 respectively, $P<0.01$). Female rats exposed to lead show significant more ambulatory activity counts than male Pb-treated rats (272±11 and 311±13 respectively, $P<0.05$). Male rats exposed to Pb display significantly increased ambulatory activity counts (272±12) than the corresponding control ($P<0.05$). There was no significant difference in ambulatory activity counts between female rats exposed to lead (311±14) as compared to corresponding control ($P=0.06$) (Fig. 2A).

***Rearing***

Two-way ANOVA showed no significant effect of treatment ($P=0.07$), significant effect of gender ($P<0.001$) and no significant treatment X gender interaction ($P=0.65$). Female rats in the control groups showed significant more rearing counts than male rats (40±2 and 29±2 respectively, $P<0.01$). Female rats exposed to Pb showed significant more rearing counts than male Pb-treated group (44±2 and 35±3 respectively, $P<0.01$). No significant differences were observed in rearing activity between control and lead-exposed rats (Fig. 2B).

***Grooming***

Two-way ANOVA showed no significant effect of treatment ($P$=0.52), significant effect of gender ($P$<0.01) and no significant treatment X gender interaction ($P$=0.41). Female rats in the control groups showed significant more grooming activity than male rats (48±3 and 38±2 respectively, $P$<0.05). No significant difference was observed in grooming activity between female and male rats exposed to lead (48±2 and 42±3 respectively $P$=0.12). No significant differences in grooming activity between control- and lead-exposed rats were observed (Fig. 2C).

***Sniffing activity***

Two-way ANOVA showed no significant effect of treatment ($P$=0.14), of gender ($P$=0.46) and no significant treatment X gender interaction ($P$=0.51) for sniffing activity. No significant effect of Pb exposure was observed in sniffing activity (control male 38±3; Pb male 43±3; control female 41±2; Pb female 43±2) (Fig. 2D).

**Rotarod test after 50 ppm Pb exposure**

Two-way ANOVA showed no significant effect of treatment ($P$=0.11), significant effect of gender ($P$<0.001) and no significant treatment X gender interaction ($P$=0.77). Female rats in the control group showed significant reduced latency to fall than male rats in the control group (216±9 and 265±15 respectively, $P$<0.01). Female rats exposed to Pb showed significant reduced latency to fall than male rats exposed to lead (202±8 and 245±7 respectively, $P$<0.01). No significant effect of Pb exposure was observed in the rota-rod test (Fig. 3A).

**Object recognition memory test after 50 ppm Pb exposure**

Two-way ANOVA showed no significant effect of treatment ($P$=0.80), of gender ($P$=0.17) and no significant treatment X gender interaction ($P$=0.90). No significant

effect of Pb exposure or gender was observed in the object recognition memory (control male 62±6; Pb male 63±8; control female 72±9; Pb female 75±8) (Fig. 3B).

**Morris water maze after 50 ppm Pb exposure**

Learning, assessed by the reduction in latency to find the hidden platform over days, was clearly evident in all groups. Two-way ANOVA show significant effect of days ($P$<0.01), significant effect of group ($P$<0.001) and no significant days X group interaction ($P$=0.89). Rats learn the task, needing every day less time to find the platform. During the learning phase of the spatial task the performance of the female rats (control or Pb group) is significantly reduced at day 3 of testing as compared to that of male rats in the corresponding group ($P$<0.01 between female and male in control group and $P$<0.05 between female and male of Pb group). The ability of female rats in both control and Pb group to find the platform is significantly reduced at day 3 of testing as compared. At day 1, 2 and 4 there were no significant differences in any treatment group between female and male rats (Table 2 & Fig. 4A). No significant effect of Pb exposure was observed in the learning of the spatial navigation task in both genders. Twenty-four hours after the last trial, the platform was removed from the pool and the rats were allowed to swim. The percentage of time spent swimming in the quadrant where the platform was located during the learning phase (days1-4) was measured as an index of memory recall. Two-way ANOVA show significant effect of treatment ($P$<0.01), significant effect of gender ($P$<0.05) and no significant treatment X gender interaction ($P$=0.40). In the control group, female rats spent significant less time in the target quadrant than male (28±2 and 35±1.5 respectively, $P$<0.05). In the Pb-treated groups, no significant difference was observed between female and male rats (24±2 and 27.8±2.5 respectively, $P$=0.20). Pb exposure significantly decreased spatial memory in male rats as compared to the corresponding control ($P$<0.05, Fig. 4B).

For the swimming speed, two-way ANOVA show no significant effect of treatment ($P$=0.1), significant effect of gender ($P$<0.001) and no significant treatment X gender interaction ($P$=0.46) (data not shown). In the control groups, female rats swam with less significantly speed than male (18.4±1.1 and 25±1.4 respectively, $P$<0.01). In Pb-treated groups, female tend to swim with less speed compared to male but it did not reach statistical significance (17.1±1.5 and 21.2±1.3 respectively, $P$=0.06). Pb exposure tend to decrease swim speed in male rats but it did not reach statistical significance ($P$=0.08).

**Behavioural experiments in female rats exposed to 70 ppm lead acetate**

No significant differences were observed between female rats exposed to 70 ppm lead acetate or vehicle in none of the behavioural tests performed. In particular those behaviours altered in male rats after exposure to 50 ppm lead acetate were not affected in female rats exposed to 70 ppm lead acetate. No significant difference was observed in ambulatory activity between female rats exposed to 70 ppm Pb and to vehicle (257±14 and 265±18 respectively) (data not shown). The time spent swimming in the right quadrant during the test of Morris water maze was not significantly different between female rats exposed to 70 ppm Pb and to vehicle (37±5 and 35±4 respectively) (data not shown).

**Magnetic resonance spectroscopy**

***Striatum***

Magnetic resonance spectroscopy performed in the striatum revealed no significant differences between male and female rats in any of the metabolites investigated. No significant effect of Pb exposure was observed in both genders (Fig. 5A).

***Hippocampus***

Magnetic resonance spectroscopy performed in the hippocampus revealed no significant differences between male and female rats in any of the metabolites investigated.

Exposure to 50 ppm Pb increased myoinositol concentration in the hippocampus of male rats as compared to control group (134 ±8 and 100±9, respectively $P<0.05$) (Fig. 5B). Myoinositol concentration in the hippocampus was not significantly different between female rats exposed to 50 ppm Pb or to vehicle (113±11 and 100±12, respectively).

**DISCUSSION**

Our results showed that exposure to lead during adulthood, producing blood levels included in the ”acceptable range”, induces subtle behavioural alterations. These data support the following main ideas: (i) blood lead levels around 10 μg/dL induce behavioural alterations in some behavioural tests related to psychomotor and cognitive functions, (ii) the effects depend on gender: male rats are affected while female are not. The behavioural impairment is not due to a gender-dependent lead accumulation in brain or blood, (iii) magnetic resonance spectroscopy in striatum and hippocampus reveals selective metabolic alterations induced by low lead exposure in adult rats consisting on myoinositol increase in the hippocampus of male Pb-exposed rats.

We discuss these ideas below:

(i) Although the mean blood lead levels of the entire population is relatively low (1-3 μg/dL), thousands of adult workers continue to be exposed to higher concentrations of lead in many industries including battery manufacturing, painting, non-ferrous smelting, radiator repair, brass and bronze foundries, pottery production, scrap metal recycling, firing ranges, and wrecking and demolition (Rybicki et al., 1997). Hobbies such as recreational target shooting (Svensson et al., 1992), home remodeling, casting bullets and fishing weights (Reynolds et al., 1999), making stained glass and ceramics (Landrigan et al., 1980), use of traditional remedies (Saper et al., 2004), and drinking homemade alcoholic brews (Mushak et al., 1989) can also be sources of lead exposure

in a non-industrial setting. CDC recommended as a preventive health measure, a reduction of the concentration of the metal below <25 μg/dl in subjects occupationally exposed (U.S. Department of Health and Human Services, 2000). In this study, we demonstrated that sub-acute exposure to 50 ppm of lead acetate in young adult rats producing blood lead levels around 10 μg/dL induce mild but significant behavioural alterations. It should be noted that behavioural alterations were observed only in two tests, open-field and spatial memory test. Pb exposure did not elicit behavioural impairment in the rota-rod, object recognition memory and spatial learning suggesting that Pb-induced alterations are not due to overal impairment in the general activity or learning and memory process but rather induced by impairment of selective processes in the brain.

One can argue that these differences might be due to a selective regional accumulation of Pb but our group and others have demonstrated that, at least under conditions of postnatal Pb exposure, this is not the case for the brain (López-Larrubia and Cauli, 2011; Widsowski and Cory-Slechta, 1994). Difference in brain regions sensitivity to Pb could be likely due to differences in the interactions of Pb with biochemical or cellular targets unique or enriched in a given brain region. Similarly, prenatal exposure to lead induced behavioural alterations in adult offspring that was dependent on the behavioural test (Leasure et al., 2008; Moreira et al., 2001) suggests that different brain circuits are differently affected by lead also in the case of prenatal exposure. The impairment in some behavioral tasks has been reported also in human exposed to lead (Cincinnati lead study). Ris et al. (2004) evaluated different neuropsychological endpoints (memory, learning/IQ, attention, visuoconstruction, and fine-motor activity) in mid-adolescence prenatally exposed to lead, and found a significant effect of lead exposure only in fine-motor function, attention and visuoconstruction but not in other neuropsychological

functions. The reason why only specific behaviours are altered by lead exposure clearly needs future studies.

(ii) Only male rats are affected by postnatal lead exposure whereas female appear resistant to disruptive effects motor activity and spatial memory at this exposure dose. We found that blood and brain lead levels in female rats are significant lower than those in male rats. Gender differences in lead distribution in other organs and species have been observed (Lanszki et al., 2009; Nwokocha et al., 2011; Sobekova et al., 2009). Similarly to animal models, gender differences in lead metabolism/accumulation have been observed in humans. Young or premenopausal women retain Pb more avidly or release Pb more slowly than do men (Popovic et al., 2005; Theppeang et al., 2008), whereas this distinction is lost for postmenopausal women (Popovic et al., 2005). The 2007-2008 NHANES found that overall males have higher blood Pb levels than females. In our study, the behavioral impairment observed in male rats was not related to an increased accumulation of lead compared to females. In fact exposure to 70 ppm Pb acetate in female rats, despite producing Pb levels similar to those achieved after exposure to 50 ppm Pb acetate in male rats, does not elicit any behavioral alteration, confirming the finding of increased vulnerability of male rats to lead-induced behavioural impairment.

(iii) This animal study is, to our knowledge, the first to examine the association between lead exposure and metabolic changes in brain as measured with MRS. Exposure to lead in young adult rats does not induce metabolic alterations in the striatum indicating that at this exposure level, biochemical pathways regulating the concentration of the most abundant metabolites are not altered in this brain area. In contrast, lead exposure increases mIns content in the hippocampus while the concentration of other metabolites is unaffected. The increase in mIns alone suggests that subacute lead exposure alters

metabolism mainly in glial cells (Brand et al., 1993). Some studies suggested that lead accumulation in the brain occurs preferentially in glia cells rather than in neurons (Lindahl et al., 1999; Tiffany-Castiglioni et al., 1989) thus supporting the interpretation of early insult to glia cells. The astroglial response to lead exposure may be an initial attempt to protect neurons from injury (Struzynska et al., 2005). Although the pathological significance of elevated mIns is not completely clear, it has been proposed to reflect increased numbers of glial cells (Brand et al., 1993; Ross et al., 1997) or osmotic stress. The increase in the number of glial cells could be likely, in fact, postnatal lead exposure induces astrogliosis (Selvin-Testa et al., 1994; 1997), increases the expression of astrocyte marker glial-fibrillary acidic protein (GFAP) (Selvin-Testa et al., 1994) or mRNA for GFAP (Stoltenburg-Didinger et al., 1996) in the hippocampus. Astrocyte differences could account for the different sensitivity between hippocampus and striatum in mIns changes (Black et al., 1993). Regional heterogeneity of astrocytes has been observed with respect to receptor expression (Ruzicka et al., 1995). Recently, a community-based cohort of elderly U.S. men reported an association between bone lead and increased myoinositol-to-creatine ratio in 31 people with a mean age of 77 years (Weisskopf et al., 2007).

No changes on N-acetylaspartate (NAA) in hippocampus or striatum have been observed after lead exposure in adult rats. NAA levels in the brain decrease as a consequence of neuronal damage (Ross et al., 1997) or severe mitochondrial dysfunction (Stork, 2005). The NAA/Cr ratio in the hippocampus was found to be decreased after exposure to high lead levels (Weisskopf et al., 2004). A study in China of 6 children with blood lead >27 μg/dl found lower NAA/Cr ratios in both the frontal cortex and hippocampus associated with the higher lead exposure while mIns levels were not determined (Meng et al., 2005). A study in the United States of 16 children

with blood lead levels between 23 and 65 μg/dl and 5 children with blood lead <10 μg/dl also found reduced NAA/Cr, but no change in mIns/Cr, in the frontal cortex associated with the higher lead exposure (Trope et al., 2001). In all these studies the exposures were higher than ours.

The molecular mechanisms by which lead exposure affects astrocytes of the hippocampus only in male rats needs further studies.

Future studies should address the impact of occupational exposure to low lead levels on cognitive and motor functions in both males and females.

Our findings are of importance in public health worldwide because raise questions how occupational lead exposure producing blood levels around 10μg/dL has to be considered safe.

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**Conflict of Interest Statement**

The authors declare that there are no conflicts of interest

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## LEGENDS TO TABLES AND FIGURES

**Table 1.** The record of weight gain shown for one month of 50 ppm lead exposure rats. Data are expressed as mean ± SEM for n=8/group. There was no statistically significant effect of lead acetate on body weight (P>0.05).

**Table 2**. Time spent by rats to find the platform during the acquisition of spatial learning tasks. Data are expressed as mean ± SEM for n=8/group. *: differs from corresponding control; [a]: differs from male rats, Dunnett's test.

**Fig. 1.** Content of Pb in the blood (A) and in brain (B) of control and Pb-exposed (50 ppm) rats. Values are mean ± SEM (μg/dl for blood, ng/g wet weight for brain) for n=8/group(n=8). *: differs from corresponding control; [a]: differs from male rats, Dunnett's test.

**Fig. 2.** Effect of exposure to 50 ppm Pb on motor and exploratory activity: (A) Ambulatory activity; (B) frequency of rearing response; (C) frequency of grooming response; and (D) frequency of sniffing response. Data are expressed as mean ± SEM for n=8/group. *: differs from corresponding control; [a]: differs from male rats, Dunnett's test.

**Fig. 3.** Effect of exposure to 50 ppm Pb on motor coordination and object recognition memory: (A) Latency time to fall from rota-rod test. (B) time spent exploring the new object. Data are expressed as mean ± SEM for n=8/group. *: differs from corresponding control; [a]: differs from male rats, Dunnett's test.

**Fig. 4.** Effect of exposure to 50 ppm lead on learning and memory. (A) Time to find the platform during the acquisition of the spatial navigation task. (B) Memory retrieval as indicated by time spent in the target quadrant. Data are expressed as mean ± SEM for

n=8/group. *: differs from corresponding control; [a]: differs from male rats, Dunnett's test.

**Fig. 5.** Effect of exposure to 50 ppm lead on metabolic profile in striatum (A) and in hippocampus (B) by magnetic resonance (MR) spectroscopy. Metabolites were quantified as described in Methods session. Representative MR spectrum is shown in panel (C). F = female; M = male. Data are expressed as mean ± SEM for n=8/group. *: differs from corresponding control, Dunnett's test.

**Table(s)**

**Table 1**

| | Before starting experiment | After finishing experiment |
|---|---|---|
| Control (male) | 218±3 | 319±4*** |
| Pb (male) | 213±3 | 316±5*** |
| Control (female) | 208±4 | 284±4 *** a |
| Pb (female) | 216±4 | 289±8*** a |

Table(s)

## Table 2

| | Control (female) | Pb (female) | Control (male) | Pb (male) |
|---|---|---|---|---|
| Day 1 | 79±4 | 84±8 | 77±5 | 80±6 |
| Day 2 | 73±3 | 76±4 | 62±2 | 65±4 |
| Day 3 | 67±2* | 72±6 | 52±4** | 58±4** |
| Day 4 | 40±3*** | 45±4*** | 35±2*** | 40±5 |

Figure(s)

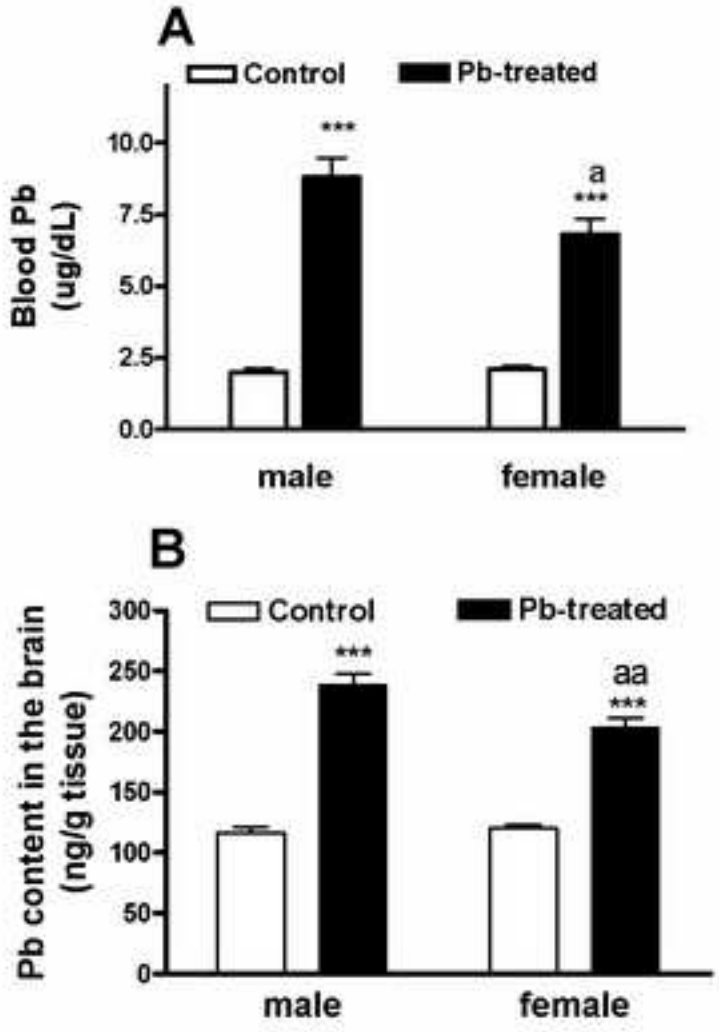


Figure 1

Figure(s)

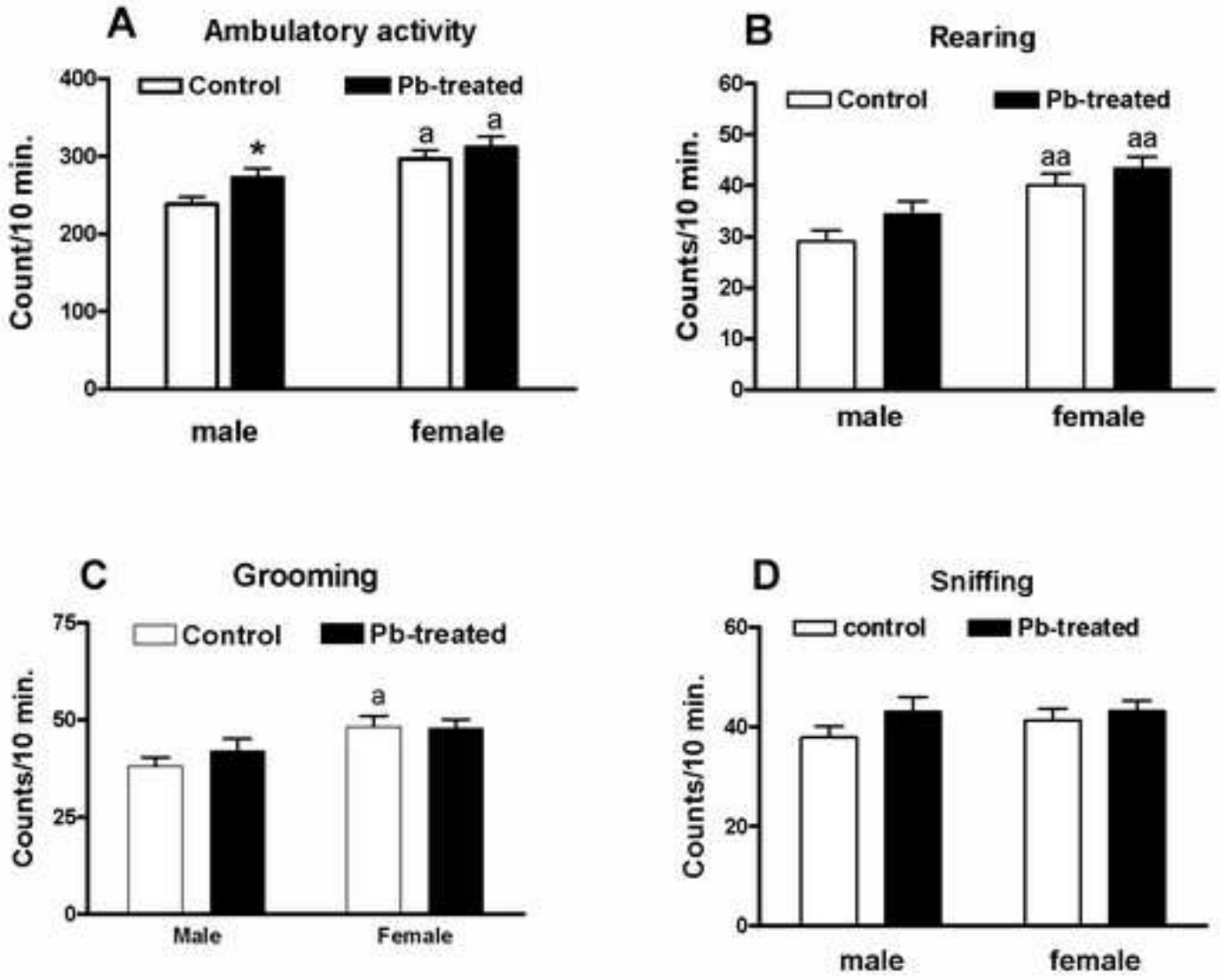


Figure 2

**Figure(s)**

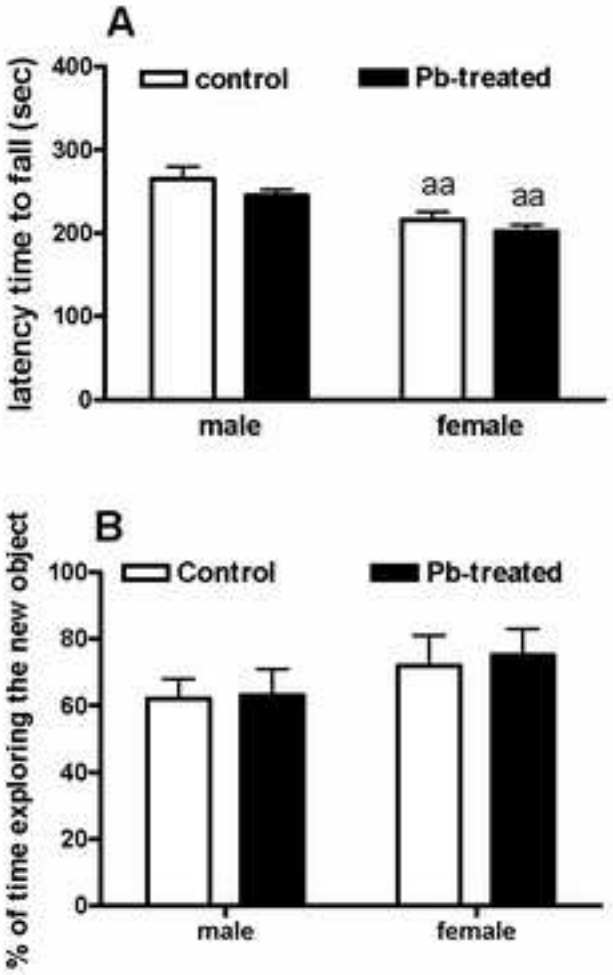


Figure 3

Figure(s)

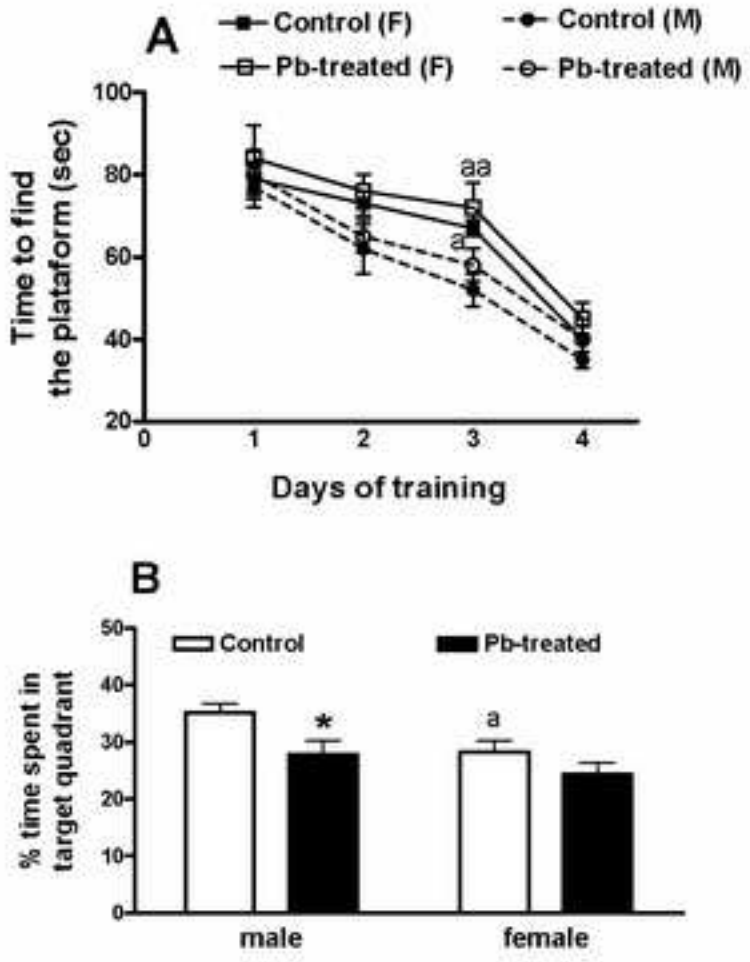


Figure 4

**Figure(s)**

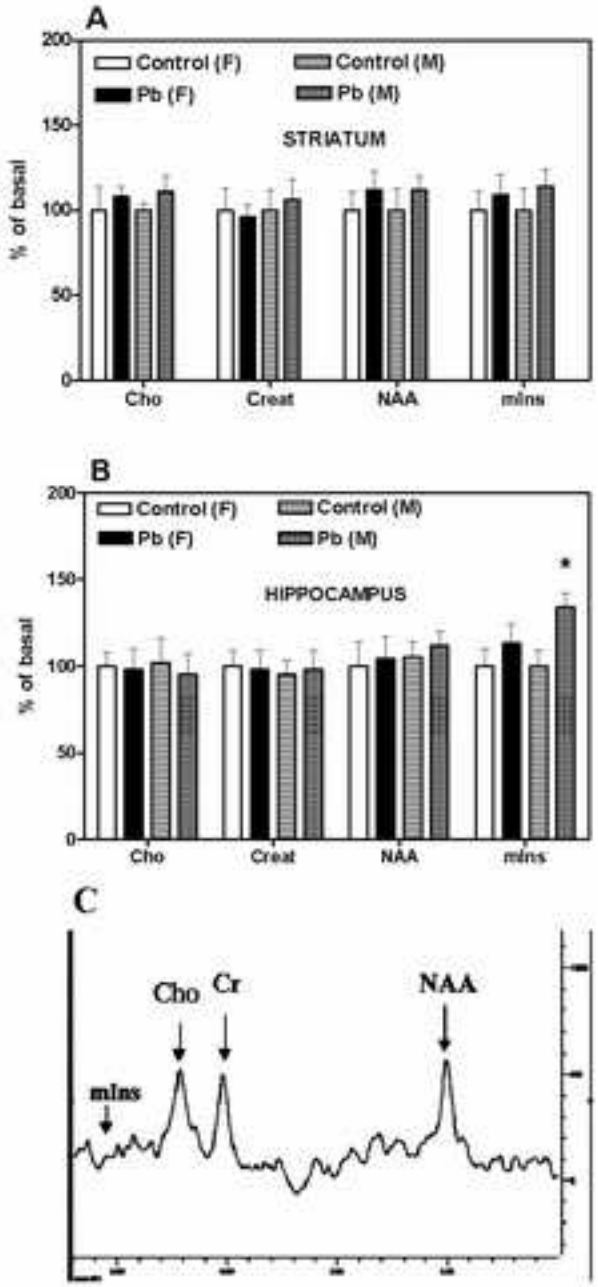


Figure 5