Effect of intra-hippocampal lead injection on affective and cognitive disorders in male WISTAR rats: Possible involvement of oxidative stress.

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Abstract

This paper is focused on affective and cognitive disorders induced by direct exposure to lead chloride (Pb) and the possible implication of oxidative stress in male WISTAR rats. Using stereotaxic surgery, a group of male wistar rats received an intracerebral injection of 3 µL of lead chloride (1 mg / L) into the right hippocampus or 3 µL of 0.9% Nacl for shame operated controls groups. After 4 days of rest, a series of benchmark neurobehavioral tests were made to evaluate affective and cognitive behaviors, such as open field test (OFT) and Elevated plus maze (EPM) for anxiety like behavior; Forced swim test (FST) for depressive like behavior; finally Y maze (Y-Maze) and Morris maze (MWM) tests for working memory, spatial learning and spatial memory. The data showed that Pb induces anxiogenic and depressogenic effects but does not induce a significant effect in memory. Then by measuring oxidative stress markers using the hippocampal assay of lead-treated rats, it's appears that Pb can increase nitrogen monoxide, lipid peroxidation and reduces significantly the activity of superoxide dismutase. Therefore, our results showed that Pb is able to induce affective disorders and increase highly oxidative stress but has an insignificant effect on cognitive impairment.

KEY WORDS: Depression; Anxiety; oxidative stress; chloride lead; short-term memory; spatial memory; WISTAR Rats

Introduction

Affective and cognitive disorders are one of the main causes of social disability in the world, they are part of the most famous range of mental disorders and they have a high prevalence and represent a considerable global burden in terms of suffering and suffering. In 2002, WHO stated that out of 450 million people in the world suffered from neuropsychiatric conditions, 26.9% were depressed and obviously other disorders[1].

The sources of these complex pathologies can be caused directly or indirectly by environmental pollution, pollutants such as heavy metals are not excluded. Lead is recognized today as a nerve agent, a potential factor that can cause affective and cognitive disorders[2]–[4] (Cory-slechta; 1997- Patrick L; 2006- Kosnett; 2007) In rats, Pb induces anxiogenic and depressive effects clearly observed in previous studies in our laboratory[5]. The alterations caused by lead on neurotransmitter systems have been demonstrated in different regions of the brain (nucleus accumbens, hippocampus, septum, etc.) [6].

Based on these considerations, our work is focused on the implication of lead in affective and cognitive disorders in WISTAR rats, by proceeding with an intracerebral injection of 3µl of Pb of 1mg / l (5µmol / l) in the right ventral hippocampus.

1. Materials and Methods

1.1 Wistar Rats and Group Distributions

18 Wistar rats aged 15 and 18 weeks were divided into groups of 5 in transparent cages and had free access to water and food (30g / rat / day) throughout the procedure, the rats are divided into 3 groups:

• A group of 5 rats: called the "White control" group, which will not undergo any treatment to ensure that the surgical procedure does not influence the expected results.

• Another group of 5 rats: called the "Positive control or sham-operated" group of rats, they received 3µl of 0.9 % Nacl by intracerebral injection.

• A group of 8 rats: separated into a group of 2 cages (4 rats per cage), this is the "lead" group which will receive 3µl of lead chloride by intracerebral injection (5µmol / l).

1.2 Stereotaxic procedure

The assigned rats for surgery were each given 7% chloral with the 1ml / 100g of rat weight rule as anesthesia. After sterilization of the operating field and materials, the rats were placed in the stereotaxic frame according to the protocol adapted by Ferry[7], an incision was made to expose the skull so as to clearly observe the Bregma of each rat, since bregma is used as reference[8]. A hole of approximately 1 mm in diameter was drilled with an electric drill on the skull, according to the following stereotaxic coordinates: Antero-posterior (AP): - 2.40 mm. Medio-lateral (ML): + 1.6 mm. Dorso-ventral (DV): - 3.5 mm.

Using a Hamilton syringe, we gently and succely injected either 3 µl of Pb chloride (1 mg / l) for the "lead" group or 3 µl of 0.9% Nacl for the "positive control" group. In order to avoid scattering, the injection speed was 1µL per min. after the injection of the entire volume, 3min of rest was added and the syringe was removed slowly to limit reflux at the injection site. An injection of intramuscular analgesic (Ketoprofen 50mg / ml) was performed to minimize the pain that the rats might feel when they wake up, the volume injected was determined according to the following rule: 5mg is equivalent to 1000g of weight rat. The neurobehavioral tests began after 4 days of rest following the surgery.

2. Neurobehavioral tests

2.1 Open Field Test (OFT)

For this study, the OFT model used was similar to that reported by Durand[9]. The rat is placed in the arena and allowed to roam freely for 10 minutes under white lighting while being recorded. Entrances to the central area and the time spent in by the rats is inversely proportional with the level anxiety, the most relevant parameters evaluated are: Time spent in central area (TCA), number of visit in the central area (NVC) and number total of visited squares (NTS) which is a reliable index of locomotor activity general[10].

2.2 Elevated plus Maze Test (EPM)

The elevated maze test is one of the most popular tests among all animal anxiety models currently available, to define mechanisms underlying anxiety-related behaviors[11]. The model used for this study is the same model used by Pellow[12]. The animal is placed in the center of the intersection square and is allowed to explore the labyrinth freely for 5 min. The recorded behavior makes it possible to analyze the following parameters: The numbers of entries in the open arms (EOA) as well as the time spent in open arms (TOA) they correlate inversely with the level of anxiety and the number of entries in all arms (NEA). reflate motor impairment[9].

2.3 Forced Swimming Test (FST)

The forced swimming test was initially proposed by Porsolt[13], this test is used to assess and highlight a potential depression, the parameter analyzed are the struggling time (ST) considered as an escape attempt, it's the time during which the animal swims actively or only floats in order to keep the head out; and the immobility time measured (IT) is considered as a state of "behavioral hopelessness," which occurs when the rat lose hope on escaping and stop swimming, the depressive behavior is characterized by a significant reduction of ST or an increase of IT[14].

2.4 Y-maze test

This test allows the evaluation of spatial working memory, short-term memory as well as general locomotor activity[15]. Spontaneous alternation was assessed using a Y-maze consisting of three equidistant arms (120 ° intersection, 41cm long and 15cm high, 5cm wide). The data presented results from an analysis of this distribution to count the alternations and is expressed as a percentage of alternation, it is calculated according to the formula:

% alteration = [Number of alternation / (Number of visits - 2)] X 100 [16].

Spontaneous alternation in rodents has been described as dependent on the hippocampus. It has important functions that govern short and long-term memory, as well as navigation in space [17].

2.5 Morris water maze Test

This test was designed by R.G.M. Morris[18] to assess the abilities of rodents to memorize and manage spatial information, the abilities of spatial and learning memory [19], spatial orientation and vision-motor guidance were assessed using the Morris pool test according to the protocol described by Wong and Brown[20]. The probe test is carried out on the 5th day after 4 days of training. The time spent in the quadrant where the platform was located during the training phase (N-W) is described as spatial memory.

3. Biochemical analysis: oxidative stress.

After one day of rest following neurobehavioral tests, the right hippocampus was carefully removed, kept at a low temperature (4°C) and used for biochemical analyzes after being homogenized in a 50Mm phosphate buffet (ph7) and centrifuged at 1500 rpm / 10min. Nitrogen monoxide (NO) evaluation was made using the Griess reaction with the protocol of Grisham, the protocol consists of mixing 500 µl of the hippocampus solution with 500 µl the same volume of Griess solution composed of 1% sulfanilamide (1ml), the absorbance of the solution obtained in the spectrophotometer at 540nm after 30 minutes of incubation under room temperature conditions[21]. Lipid peroxidation is evaluated by measuring the amount of the thiobarbituric-acid-reacting substance (TBARS) which represent the concentration of Malondialdehyde (MDA) in the cells of the hippocampus. The principle of the protocol consists in mixing the supernatant of the hippocampus with 1 ml of trichloroacetic acid 10% (TCA) and 1ml of thiobarbituric acid 0.67% (TBA), the solution obtained is heated in Bain Marie for 15min, then mixed with butanol (2: 1 v/v) and centrifuged at 800g / 5min, the absorbance of the solution is measured at 535nm[22]. The activity of superoxide dismutase was measured by evaluating the ability of the enzyme to inhibit the reduction of nitroblue-tetrazolium (NBT) by the superoxide anion. The reaction mixture is composed of: 0.94 ml 50 mM phosphate buffer (pH 7.4) containing 12 mM methionine, 75 µM NBT, 0.1 mM EDTA, 0.025% Triton X-100 plus 2 µM of riboflavin and 0.06 ml of supernatant of hippocampus. The absorbance was measured at 560 nm after the test tubes containing the mixture were placed in yellow light for 10 min [23].

4. Statistical analysis

The statistical analysis was carried out by analysis of variance (Anova), via the SPSS software, The results are represented as a mean plus or minus standard deviation (m ± s), illustrated by graphs produced by the GraphPad Prism 6 software.

5. Results

The difference between the 2 control groups is not significant for all the tests. Meaning that the Stereostatic surgery was successful and does not influence the results of this work in any way.

5.1 Effect of intrahippocampal injection of Pb on anxiety-like behavior measured in OFT

Fig1 reflect the level of anxiety measured in the OFT test, the TCA parameter decreased in the group of rats treated with Pb compared to controls with $p < 0.01$ ($p = 0.005$) ***fig (1.a),*** it's continues with ***fig (1.b)*** compared to the controls, the NVC parameter was reduced ($p < 0.001$) in the group of rats treated with Pb. In addition, the NTS parameter is reduced in the rat group treated with Pb compared with the controls ($p<0.001$) ***fig (1.c).*** Therefore, intrahippocampal injection of Pb is able to induce anxiety in rats.

5.2 Effect of intrahippocampal injection of Pb on anxiety-like behavior measured in EPM

Fig2 shows the level of anxiety measured in EPM, which shows that intrahippocampal injection of Pb is able to induce anxiety, there is a decrease of the TBO parameter in the group of rats treated with Pb compared to controls ($P < 0.001$ and $P < 0.01$) ***fig (2.a),*** a very significant decrease of the EOA parameter in the group of rats treated with Pb compared to the controls ($p < 0.01$ and $p<0.05$) ***fig (2.b).*** Unlike the EOA parameter, ***fig (2.c)*** describe an increase of the NEA parameter with a value $P > 0.05$ ($p = 0.47945$) so, the locomotor activity is not influenced compared to the controls.

5.3 Effect of intrahippocampal injection of Pb on depression-like behavior measured in (FST)

The immobility time presented in ***fig (3.a)*** is significantly increased compared to the groups of control rats with a value of $p < 0.001$, while ***fig (3.b)*** shows a significantly decrease of the ST parameter in the group treated with Pb, $P < 0.001$ ($p = 0.0003$). Fig.3 shows that the intrahippocampal injection of Pb strongly influences the level of depression.

5.4 Effect of intrahippocampal injection of Pb in Y-labyrinth test

Opposing to what expected, the hippocampal injection of Pb did not affect working memory, the percentage of alternations in the group treated with Pb compared to controls ($P > 0.05$, $p = 0.3072$) is no significant, as shown in **fig.4.**

5.5 Effect of intrahippocampal injection of Pb in Morris water maze test

During the 4 days of learning, the latency time was used in this work to assess learning memory, ***fig (5.a)*** shows that the time decreased each day in all groups of rats studied with a difference not significant (P> 0.05; p = 0.99555). Intrahippocampal injection of Pb did not affect the learning phase or the spatial memory, as shown in ***fig (5.b)*** the difference of time spent looking for the platform removed at the Northwest quadrant is not significant between the group of rats treated with Pb and the control groups with P> 0.05; p = 0.7546.

5.6 Effect of intrahippocampal injection of Pb on oxidative stress in the right hippocampus.

Nitrogen monoxide (NO).

The difference of concentration is highly significant between the group of rats treated with Pb and the group of control rats (p <0.001; p <0.01) as shown in ***fig 6.a***, Pb significantly increased the concentration of monoxides of nitrogen (NO) expressed in µmol / g of hippocampus, in the group treated with Pb.

Thiobarbituric-acid-reacting substance (TBARS)

The variation of the concentration of TBARS in nmol /g according to the different groups of rats studied is shown in ***fig 6.b*** which shows that the intrahippocampal injection of Pb increased their concentration in the group of rats treated with the Pb and the difference compared to the controls is significant with p <0.01 (p = 0.0077) and p <0.05 (p = 0.0324).

Superoxide dismutase (SOD).

The activity of the enzyme superoxide dismutase (SOD) is expressed in mmol / min / g of hippocampal tissue, the difference is highly significant between the group treated with Pb and the control groups with p <0.001 as shown in fig 6.c, intrahippocampal injection decreased the activity of the SOD enzyme in the group of rats treated with Pb, the intrahippocampal injection of lead increases nitrogen monoxide and lipid peroxidation but also decreases the activity of the enzyme superoxidase.

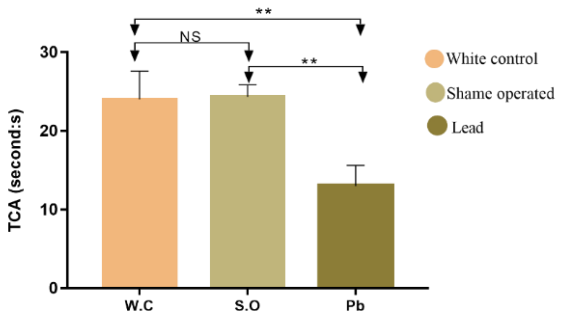

Fig 1.a : Time spend in Central Area in second (TCA).

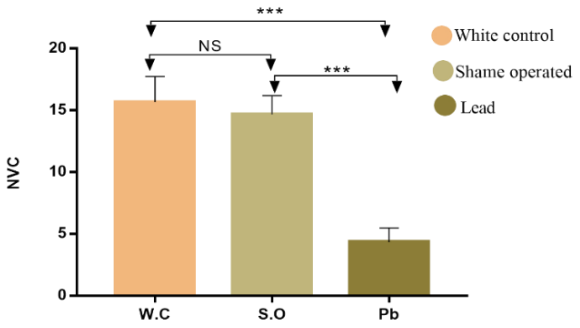

Fig 1.b : Number of Visit in Central area (NVC)

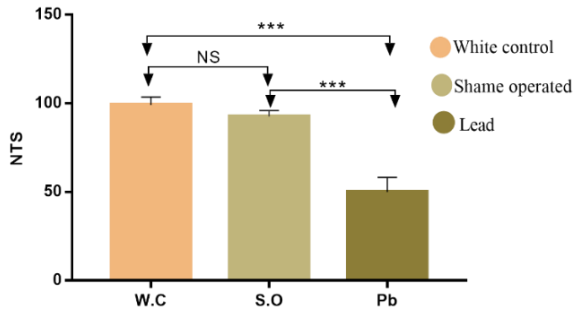

Fig 1.c: Number of Total visited Squares (NTS)

Figure 1: Presentation of the Parameters evaluating the effect of Pb on anxiety-like behavior measured in OFT, 4 days after an intracerebral injection of 3 µL of Pb (1 mg / l) for the lead group (n = 8) or 3 µL of 0.9% Nacl for the group for shame operated control (n = 5) The results are represented on average ± SEM 05, the significance level is 0.05: NS = not significant; * = p <0.05: weakly significant; ** = p <0.01: moderately significant; *** = p <0.001: highly significant.

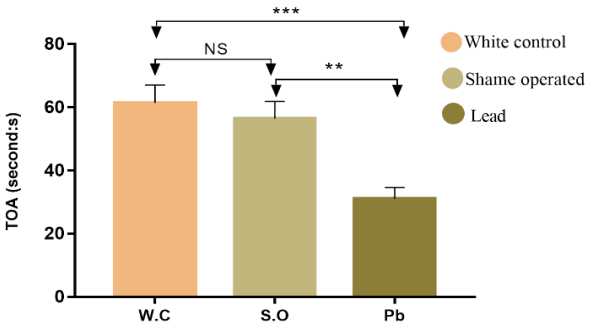

Fig 2.a : Time spent in Open Arms in second (TOA).

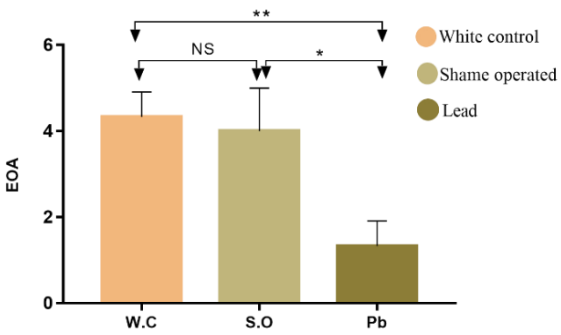

Fig 2.b : Numbers of Entries in the Open Arms (EOA).

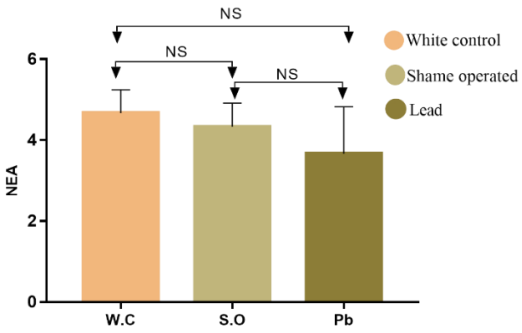

Fig 2.c : Number of Entries in All Arms (NEA)

Figure 2: Presentation of the Parameters evaluating the effect of Pb on anxiety-like behavior measured in EPM 5 days after an intracerebral injection of 3 µl of Pb (1 mg / l) for the lead group (n = 8), 3 µL of 0.9% Nacl for the group of patients operated on for shame (n = 5) The results are represented on average ± SEM 05, the significance level is 0.05: NS = not significant; * = p <0.05: weakly significant; ** = p <0.01: moderately significant; *** = p <0.001: highly significant.

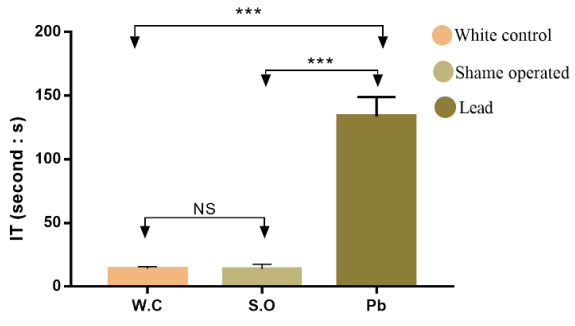

Fig 3.a: Immobility Time measured in FST, in second (IT).

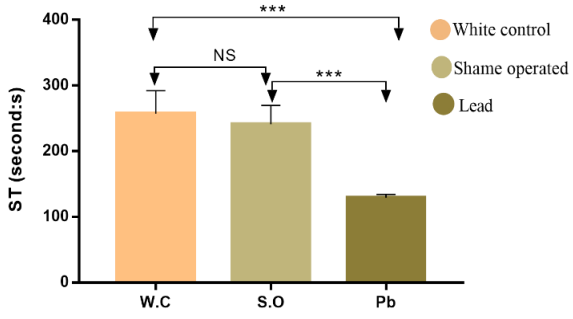

Fig 3.b: Struggling Time measured in FST, in second (ST)

Figure 3: presentation of the parameters reflecting depressive-like behavioral measured in the Forced Swimming test after 6 days following an intracerebral injection of 3µl of Pb (1 mg / l) for the lead group (n=8), 3 µl of 0.9% Nacl for the group of shame-operated (n=5). The results are represented as means ± SEM 05, the significance level is 0.05: NS= not significant; * = p <0.05: weakly significant; ** = p <0.01: moderately significant; *** = p <0.001: highly significant.

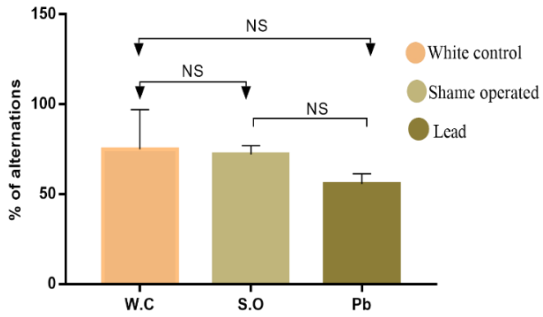

Figure 4: Percentage of triplet alternation evaluated on the Y-maze test which reflects working memory, 7 days after an intracerebral injection of 3 l of Pb (1 mg / l) for the lead group (n = 8), 3 l of Nacl at 0.9% for the group of patients operated on for shame (n = 5). The results are represented as a mean ± SEM 05, the significance level is 0.05: NS = not significant; * = p <0.05: weakly significant; ** = p <0.01: moderately significant; *** = p <0.001: highly significant.

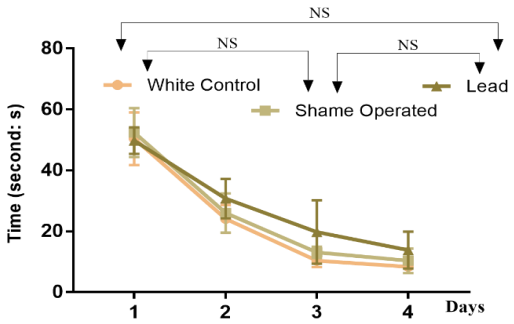

Fig 5.a :.latency time measured during the learning phase in second

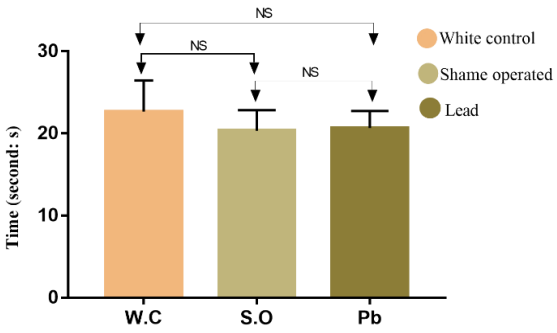

Fig 5.b : time spent in the correct quadrant without the platform in second

Figure 5: presentation of parameters reflecting spatial memory and learning memory measured in the Morris water maze,8 days after an intracerebral injection of 3 l of Pb (1 mg / l) for the leader group (n = 8), 3 l of Nacl at 0.9% for the group of patients operated on for shame (n = 5). The results are represented as a mean ± SEM 05, the significance level is 0.05: NS = not significant; * = p <0.05: weakly significant; ** = p <0.01: moderately significant; *** = p <0.001: highly significant.

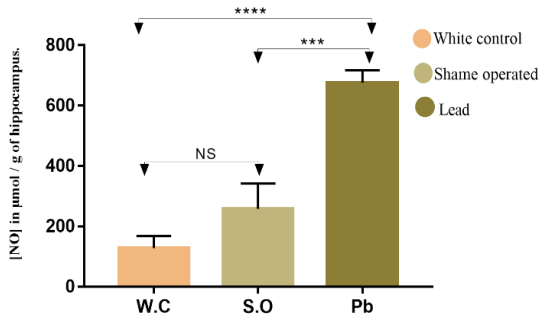

Fig 6.a: Nitrogen monoxide [NO] concentration in µmol / g of hippocampus tissue.

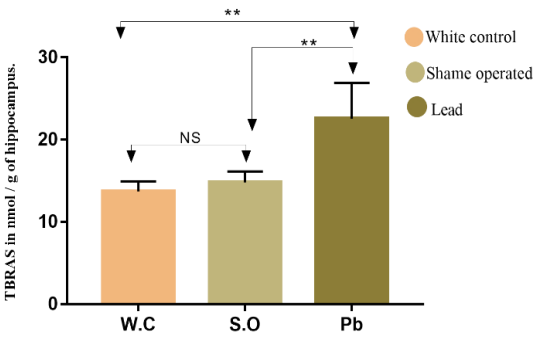

Fig 6.b: Concentration of TBRAS in nmol / g of hippocampal tissue.

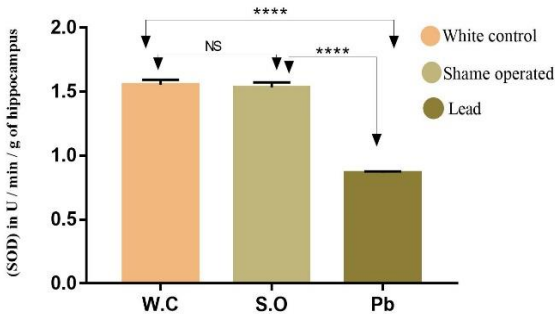

Fig 6.c: Activity of the enzyme superoxide dismutase (SOD) in U / min / g of hippocampal tissue

Figure 6: Presentation of indicators parameters reflecting oxidative stress in the hippocampus, 12 days after an intracerebral injection of 3 µl of Pb (1 mg / l) for the lead group (n=8), 3 µl of 0.9% Nacl for the group of shame-operated (n=5). The results are represented as means ± SEM 05, the significance level is 0.05: NS= not significant; * = p <0.05: weakly significant; ** = p <0.01: moderately significant; *** = p <0.001: highly significant.

6. Discussion

This study aimed to demonstrate the effect of direct exposure of Pb (1mg/1) in the hippocampus on affective and cognitive disorders as well as its effect on markers of oxidative stress. To our best knowledge, this study is the first to use a concentration of 1mg / l of Pb for an intracerebral injection targeting a specific structure, the right ventral hippocampus. The hippocampus plays an essential role in spatial and episodic[24] Modulates the cognitive aspects of depression[25], [26] and codes the contextual signals associated with anxiety [27]. It is shown that in the FST, lead strongly induce a depressogenic effect under these conditions and our results are in agreement with the current scientific literature as well as other studies, depressive behavior was detected in FST following early and chronic exposure to Pb in female wistar rats following administration by gavage to mother rats with Pb acetate, during pregnancy and lactation at levels of approximately 5µg / dl of Pb in residual blood[28]. In fact, Pb induces a significant decrease in TCA and NVC parameters in the OFT test. induces a reduction in the EOA and TOA parameters in the EPM test in the group of male rats treated with Pb, It can be concluded in these circumstances that Pb exerts an anxiogenic effect. As the results reported by Winneke G. Showing that lead induces anxiety disorders and which are a consequence of neurobehavioral lesions in Wistar rats subjected to chronic and early exposure to Pb[29]. Sansar study also showed that Pb can induce anxiety after chronic exposure for 3 months to 0.5% lead acetate in drinking water in male wistar rats[30]. Surprisingly the Y-maze test alternation shown that working memory is not affected, also In Morris maze test, it is observed that Pb does not affect learning and does not affect also there is no deficit of spatial memory. This study suggests that Pb causes either short-term memory or spatial memory impairment. Many previous studies have shown that the contrary that Pb can induces short-term and long-term memory problems as well as learning problems in both rats and humans[31], [32]. The most recent study which showed that in the wistar rat, exposure to Pb altered the formation of dendritic spines in the pyramidal neurons of the hippocampus. Resulting in decreased performance of rats in the Morris water maze and decreased amplitudes of postsynaptic currents excitatory (EPSC) in the CA3-CA1 regions of the hippocampus[33]. Pb decreases the AChE activity of the crude homogenate in several regions of the rat brain such as the hippocampus and cerebellum[34], [35]. Related to oxidative stress, this present study shows that a dose of 1mg/l of Pb administered directly by intracerebral injection greatly accelerated oxidative stress in the hippocampus. The results shows the variations of strong indicative parameters of the level of oxidative stress such as the concentrations of nitrogen monoxide (NO) and TBARS which are significantly high, while the activity of the SOD enzyme was reduced. This study is consistent with other studies. Bokara's study showed that there was a strong increase in lipid peroxidation in the hippocampus in rats exposed to Pb[36]. Chronic exposure to Pb can induce oxidative

stress resulting in affective and cognitive disorders [37] in mice. The different mechanisms which Pb increases oxidative stress are well known and they all lead to an imbalance hypothesis between the generation and elimination of free radicals and affects membrane lipids[38], [39] A weakening of C-H bonds in fatty acids by the presence of double bonds[40], can be formed following exposure to Pb because it can cause elongation and changes in fatty acid composition of the membrane[41] That change increase the sensitivity of lipids in the membrane to peroxidation[42] The presence of Pb in tissues has been proved to cause accumulation of δ - aminolevulinic acid (δ-ALA) by inhibiting the enzyme δ-aminolevulinic acid dehydrase (δ-ALAD)[43].This accumulation can induce the generation of ROS[44],[45]. This study report a significant decrease of oxidoreductase capable of removing superoxide anion, which is the first toxic species formed from oxygen. The realization of this reaction, the SOD requires the fixation of copper and zinc[46],the presence of Pb inhibits the activity of ferrochelatase and prevents the incorporation of iron into protoporphyrin to the binding of zinc into protoporphyrin, in place of iron and therefore there is a decrease of zinc and an increase of iron (Blumberg;) The access of ferrous ions initiates the peroxidation of membrane lipids[38] CA1 and CA3 pyramidal cells and DG granule cells have been reported to be very sensitive to oxidative damage. As consequence it can decrease cell proliferation, alter remodeling capacity, structural plasticity, collectively disrupting synaptic neurotransmission as well as neurogenesis factors (BDNF)[47], [48]. It is therefore reported in this present study that a right ventral intrahippocampal injection of 1 mg /l of Pb in male wistar rats is capable of inducing depressive-like and anxiety-like disorders and the results presented can justify the possible implication, however no memory disorders were observed, the fact that the results are insignificant does not mean a lack of effect of Pb, the effects of Pb are very dependent on the concentration , the duration of exposure and also depending on the stage of brain development[5], [49].In our case, the duration of exposure of Pb in the hippocampus and the developed brain of the rat used may explain the “no significant” effect of Pb in memory.

Acknowledgements

The LGNB laboratory family of the Ibn Tofail University wishes to present our deepest condolences to Professor Ali Ouichou’s family and friends, he’ll always be part of us through our scientific and life knowledge and in our memories.

Compliance with Ethical Standards

The authors of this study declare that they have no conflicts of interest and the ethics committee approved and authorized the contribution of all the animals used in this study and all the experiments were in accordance with the national guide for use and of care for laboratory animals established and approved by the National ethics committee (local research institute committee).

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