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Chronic exposure to low mercury chloride concentration induces object recognition and aversive memories deficits in rats

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ABSTRACT

This work examines the effects of chronic exposure to low inorganic mercury (mercury chloride, $HgCl_2$) concentration on the recognition and aversive memories. Forty male Wistar rats were divided into 4 groups treated during 30 or 60 days with saline (control) or $HgCl_2$ doses. After treated the animals were tested considering object recognition and inhibitory avoidance behavioral memory paradigms. Elevated plus maze, open field and tail flick tests were used to assess anxiety, locomotor and exploratory activity and pain thresholds. Only exposure for 60 days to $HgCl_2$ induced in memory deficits quantified in the object recognition task. In the inhibitory avoidance all the animals exposed to mercury (for 30 or 60 days) presented worst performance than control animals. Our results suggest that chronic exposure to low mercury chloride concentrations impairs memory formation.

1. Introduction

In a variety of human activities there is frequent contact with heavy metals. This condition has significantly increased both the professional and environmental exposure and therefore poisoning risks (Vassallo et al., 2011). According to Clarkson et al. (2003), the human contact with heavy metals compounds are correlated with high levels of toxicity, even at low concentrations.

Most of knowledge on the toxicological effects of heavy metals for the humans has been obtained from catastrophic poisoning episodes. Concerning mercury exposure, the major mass health disasters occurred in Minamata and Niigata, Japan, in the 1950s and in the 1960s, respectively, after human consumption of fish from waters severely polluted with mercury from the local industrial discharges (Castoldi et al., 2001). These disasters, as well others such as in Iraq in the 1970s, have shown the toxic effects of metals like mercury in different organs and systems of humans.

The concern about exposure to mercury has increased, especially in the last decades. Effects of acute and chronic exposure to low mercury concentrations have been described mainly for the kidneys and cardiovascular system, and somewhat discussed regarding its neurological effects (Azevedo et al., 2012). The effect of mercury exposure would rely on that, after reaching the bloodstream, mercury easily passes through the blood brain barrier reaching the central nervous system (CNS) (Chang and Hartmann, 1972). When in contact with the CNS mercury may promote changes in the ganglion neurons, increasing the number of mitochondria and decreasing rough endoplasmic reticulum (Chang and Hartmann, 1972). It leads to behavioral effects, such as irritability, fatigue, tremors, headaches, hearing and cognitive loss, dysarthria, incoordination, hallucinations, and death (Azevedo et al., 2012). Additionally, organic and inorganic forms of mercury were recently described as neurotoxic and somewhat related to memory loss and attention deficits (Bernhoft, 2012).

Recent studies demonstrated that different forms of mercury (methylmercury, mercury chloride, mercury vapor) caused neurotoxicity in rats (Fujimura et al., 2012; Yoshida et al., 2011; Chehimi et al., 2012). Considering that the most common form of mercury encountered is the inorganic one (Rosales et al., 2005) and that toxicity of this mercury form has been much less studied than the toxicity of methylmercury (Chehimi et al., 2012), added to the fact

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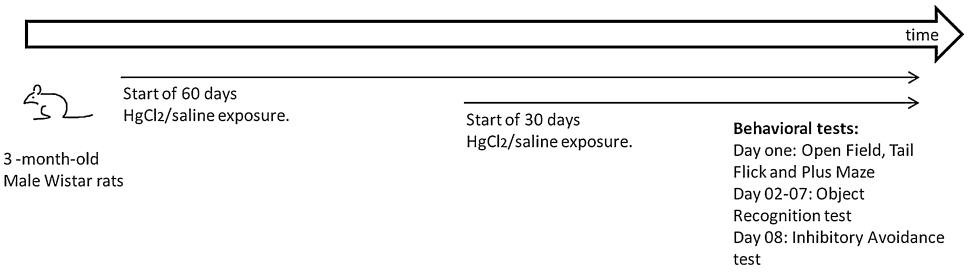

Fig. 1. Experiment design. 3-month-old male Wistar rats were exposed to $HgCl_2$ or saline for 30 or 60 days. After 30 or 60 days of treatment animals were submitted to behavioral tests.

that the most of studies that investigated the effects of mercury considered models of exposition during the brain development period (gestation, prenatal or postnatal period), using a single or repeated doses for a limited period, therefore not representing the conditions for a contaminated environmental, we decided investigated here the effects of the exposure to low $HgCl_2$ concentration during 30 and 60 days on different types of memory in adult rats.

2. Materials and methods

2.1. Experimental design

Forty male *Wistar* rats (3-month-old, 250–280 g) bought at UFSM vivarium (Federal University of Santa Maria, Rio Grande do Sul, Brazil) were used. They were housed five to a cage and kept with freely access to food and water under a 12 h light–dark cycle, with light onset at 7:00 AM. The temperature of the animal's room was maintained at 22–24 °C.

Rats were organized in four groups: control (vehicle-saline 0.9% solution intramuscular) and treated, for 30 or 60 days. Treated group received mercury chloride ($HgCl_2$) for 30 or 60 days using a model described previous by our group (Wiggers et al., 2008): 1st dose 4.4 μg/kg and subsequent 0.07 μg/kg/day intramuscular. In this model, the first dose was calculated to obtain a blood concentration of 20 nM, calculated from the loss of urine and feces, and the subsequent doses were administered for the maintenance of the initial concentration. After 30 or 60 days of treatment animals were submitted to behavioral tests, as follow: control behavioral experiments (open field, plus maze and tail flick; day 01), object recognition test (02–07 days) and inhibitory avoidance test (day 08). During these days the animals continued to receive $HgCl_2$/saline (Fig. 1).

All procedures were conduct in accordance with the "Principles of laboratory animal care" (NIH publication N. 85-23, revised 1996). All possible effort was made to reduce the number of animals used and for minimize their suffering. This work was approval by the local Ethics Committee.

2.2. Object recognition task

To analyze the effect of $HgCl_2$ on object recognition (OR) long term memory (LTM) consolidation, male Wistar rats treated for 30 or 60 days with $HgCl_2$ or saline were trained in an OR learning task involving exposure to two different stimuli objects. Training and testing in the object recognition (OR) were performed in the light period, with low brightness and using an open-field arena (50 cm × 50 cm × 50 cm) built in polyvinyl chloride plastic, plywood and transparent acrylic, as described elsewhere (Myskiw et al., 2008). As depicts in Fig. 2A, the animals were first habituated to the open-field apparatus by being placed in the apparatus for 20 min per day to freely exploration during 4 consecutive days before the training. In the training day, two different objects (X and Y) made of metal, glass, or glazed ceramic were placed in the apparatus; animals were allowed to explore them freely for 5 min. Exploration was defined as sniffing or touching the objects with the nose and/or forepaws (sitting on or turning around the objects was not considered exploratory behavior). Therefore 24 h later, on test phase, one of the objects was randomly changed for a novel object (Z). Rats were reintroduced into the apparatus for a 5 min period freely to explore (Fig. 2A illustrates the OR design). To avoid confounds by lingering olfactory stimuli and preferences, the object and the arena were cleaned with 70% ethanol after testing each animal. The experiments were performed by an observer blind to the treatment condition of the animals.

Data from OR task was converted in percentage of total exploration time and analyzed using one sample *t*-test, considering the theoretical mean equal to 50%.

2.3. Inhibitory avoidance task

After different times of $HgCl_2$/saline exposure, rats were trained in a one-trial step-down inhibitory avoidance (IA) task as previously described (Mello et al., 2009). The training apparatus was a 50 cm × 25 cm × 25 cm plexiglass box with a 5 cm-high, 8 cm-wide, and 25 cm-long platform on the left end of a series of bronze bars which made up the floor of the box. For training, animals were gently placed on the platform facing the left rear corner of the training box. When they stepped down and placed their four paws on the grid, received a 2 s, 0.5 mA scrambled footshock. This test was performed in the light period and with low brightness. Memory retention was evaluated through a retest session 24 h after training (Fig. 3A illustrates the IA design). The step down latency was measured.

For IA results, considering that a ceiling of 180 s was imposed for step-down latencies, data were presented as median and interquartile range with comparisons between training and test conditions accomplished non-parametric statistic, using Wilcoxon's test to comparing training and test latencies in each group, and to compare each mercury exposure group test latency with its control test latency was used Mann–Whitney test.

2.4. Open field, plus maze and tail flick

Open field (OF), plus maze (PM) and tail flick (TF) were control experiments for exploratory and locomotor behavior, anxiety and pain threshold.

The OF test were performed in the light period, with low brightness; 30 or 60 days after of treatment rats were placed on the left quadrant of a 50 cm × 50 cm × 39 cm open field made of wooden painted in white, with a frontal glass transparent wall. Black lines were drawn on the floor to divide it into 12 equal quadrants. Crossings and rearing, as measures of locomotor and exploration, respectively, were measured over 5 min as described elsewhere (Barros et al., 2006).

To assess the anxiety state after 30 or 60 days of treatment, the rats were exposed to an elevated PM as detailed in Pellow et al. (1985). The maze had a central platform (5 cm × 5 cm), two open arms (50 cm long × 10 cm wide, 0.5 cm high borders) and two enclosed arms (50 cm deep × 10 cm wide, with 10 cm-high walls), elevated 50 cm above the ground. The animal was placed in the center of the apparatus facing the open arm and its locomotion was observed for 5 min. Total number of entries in the open and closed arms and time spent in each one were recorded over a 5 min session.

The pain threshold after 30 or 60 days of treatment with $HgCl_2$ or saline was determined using the TF test (Tjolsen et al., 1989). For the TF test, pain was induced by giving infrared light on the tail of the mice 5 cm away from the tip of the tail. Reaction time (tail-flick latency) was noted by observing the interval between placing the tail on the infrared light source and the withdrawal of the tail.

OF, PM and TF data were compared between the four groups using one way ANOVA.

2.5. Data presentation

All data were expressed as mean ± standard deviation (except for IA, median ± interquartile range). The sample size (*n*, number of animals in each group) for each experiment is stated in the figures' captions. Statistically significant differences were established at $P < 0.05$.

3. Results

3.1. Object recognition task

To analyze the effect of $HgCl_2$ on object recognition long term memory (LTM) consolidation, male Wistar rats treated during 30 or 60 days with $HgCl_2$ or saline were trained in the OR learning task, when they explore for a similar time the two new objects (X and Y; Figs. 2B and 3C, training).

24 h after training, in the LTM testing session, control and $HgCl_2$ treated for 30 days spent a significantly longer time exploring the new object (Z; control 66.81 ± 16.81% and $HgCl_2$ 67.44 ± 17.44% of total exploration time exploring the new object; Fig. 2B).