

THE EFFECTS OF THE ADMINISTRATION OF TWO DIFFERENT DOSES OF MANGANESE ON SHORT-TERM SPATIAL MEMORY AND ANXIETY-LIKE BEHAVIOR IN RATS

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Abstract – Manganese is a very well known neurotoxic agent. It has been mainly linked to impaired motor skills and disturbed psychomotor development. However, very few aspects are known about the cognitive deficits and behavioral consequences of chronic manganese exposure. In this context, we report herein our findings regarding short-term spatial memory, motor and anxiety-like behavior assessments in male Wistar rats exposed for 45 days to two different doses (3 mg/kg b.w., i.p. and 10 mg/kg b.w., i.p.) of manganese. Behavior testing (Y-maze task and elevated plus maze) was performed after 45 days of manganese administration. Chronic manganese exposure in Wistar rats led to behavioral alterations consisting of cognitive deficiencies in the Y-maze task and anxiety/compulsive-like behaviors in the elevated plus maze, but no motor disturbances as tested by the number of arm entries in the Y-maze. Additional work is necessary to understand the long-term effects of different doses and dosing regimens of manganese on cognitive/affective and motor functioning.

Key words: Manganese, short-term spatial memory, motor activity, anxiety-like behavior

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INTRODUCTION

Manganese (Mn) has been known as a neurotoxic compound for more than 150 years. Various studies have been reported subsequently providing analyses of neurological disturbances arising as a result of manganese poisoning, also referred to as manganism (Tanaka, 1994, Bowler et al., 2006). Basically, the neuropathological alterations due to manganese in humans mainly refer to impaired motor skills (Parkinson-like syndrome) and disturbed psychomotor development (Vezér et al., 2007, Vukojević et al., 2009). However, it was also demon-

strated that manganese toxicity includes psychiatric disturbances, such as irritability, impulsiveness, agitation, obsessive-compulsive behavior, hallucinations and cognitive deficits such as memory impairment, reduced learning capacity, decreased mental flexibility and cognitive slowing (He et al., 1994, Mergler et al., 1997, Woolf et al., 2002, Shukakidze et al., 2003, Klos et al., 2006, Schneider et al., 2006, Blond et al., 2007, Vezer et al., 2007). Still, results from epidemiological studies have been inconsistent in describing the nature of such cognitive deficits and very few things are known about the behavioral consequences of chronic manganese

exposures (Schneider et al., 2009). Moreover, contrasting results regarding these aspects were previously reported, with many variables in the nature of the cognitive deficit produced by manganese exposure or with studies showing little or no dose-effect relationships on several measures of motor and cognitive functioning (Zoni et al., 2007, Schneider et al., 2009).

In this context, we report here our findings regarding short-term spatial memory, motor and anxiety-like behavior assessments of male Wistar rats exposed for 45 days to two different doses of manganese.

MATERIAL AND METHODS

Experimental animals

Male Wistar rats (n=18), weighing approximately 200-250 g at the beginning of the experiment were used. The animals were housed in a temperature- and light-controlled room (22°C, a 12-h light/dark cycle starting at 08:00 h) and were fed and allowed to drink water *ad libitum*. The rats were treated in accordance with the guidelines of animal bioethics from the Act on Animal Experimentation and Animal Health and Welfare Act of Romania, and all procedures were in compliance with the European Communities Council Directive of 24 November 1986 (86/609/EEC). This study was approved by the local Ethics Committee and efforts were also made to minimize animal suffering and to reduce the number of animals used.

Drug treatment

As mentioned, manganese (Sigma, USA) was injected intraperitoneally (i.p.) in two separate groups of rats at doses of 3 mg/kg/day and 10 mg/kg/day for 45 consecutive days. Other animals received an injection of saline, with the same procedure. A sample size of n=6 for each experimental group was used. Behavior testing (Y-maze task and elevated plus maze) was performed after 45 days of manganese administration.

Y-maze task

Short-term memory was assessed by spontaneous alternation behavior in the Y-maze task. The Y-maze used in the present study consisted of three arms (35 cm long, 25 cm high and 10 cm wide) and an equilateral triangular central area. The rat was placed at the end of one arm and allowed to move freely through the maze for 8 min. An arm entry was counted when the hind paws of the rat were completely within the arm. Spontaneous alternation behavior was defined as entry into all three arms on consecutive choices. The number of maximum spontaneous alternation behaviors was then the total number of arms entered minus 2, and the percent spontaneous alternation was calculated as (actual alternations/maximum alternations) x 100. Spontaneous alternation behavior is considered to reflect the spatial working memory, which is a form of short-term memory (Gurzu et al., 2008, Hritcu et al., 2009).

Elevated plus maze

Behavior in the elevated plus maze is utilized to assess exploration, anxiety and motor behavior. The elevated plus maze (Coulbourn Instruments) consists of four arms, 49 cm long and 10 cm wide, elevated 50 cm off the ground. Two arms were enclosed by walls 30 cm high and the other two arms were exposed. Rats were placed at the juncture of the open and closed arms and the amount of time spent on the open arms was recorded during a 5-min test. Time spent on the open arms is considered to be an index of anxiety (Ciobica et al., 2009, 2010).

Data analysis

The animal's behavioral activities were tracked and recorded using ANY-maze behavioral software (Stoelting Co., USA, version 4.5) and then statistically analyzed using one-way analysis of variance (one-way ANOVA). All results are expressed as mean \pm SEM. *Post hoc* analyses were performed using Tukey's honestly significant difference test

in order to compare the two different doses of manganese used. F values for which $P < 0.05$ were regarded as statistically significant (Hritcu et al., 2011).

RESULTS

The effects of manganese administration on short term spatial memory and motor activity in the Y-maze task

The performance of the rats in the Y-maze task was analyzed by spontaneous alternation percentage and number of arm entries. Therefore, each of these criteria measures a specific aspect of Y-maze performance (Hritcu et al., 2008, Foyet et al., 2011).

The administration of 10 mg/kg b.w., i.p. manganese resulted in a significant decrease ($F(1,10)=16$, $p = 0.002$) of spontaneous alternation percentage compared with the control rats, suggesting significant effects on spatial memory, especially on short-term memory. (Fig. 1) This effect could not be attributed to a decreased motor activity because the number of arm entries was not significantly changed ($F(1,10)=1$, $p = 0.3$), as compared to the controls (Fig. 2). The administration of 3 mg manganese/kg b.w. (i.p.) resulted in a decrease of spontaneous alternation percentage compared with the control rats, but this was not statistically significant ($F(1,10)=3$, $p = 0.1$) (Fig. 1). Also, in this case we did not find any significant modifications regarding the number of arm entries, in comparison with the control group ($F(1,10)=0$, $p = 0.98$) (Fig. 2).

More importantly, *post-hoc* analysis revealed significant differences between the 3 mg/kg and 10 mg/kg groups ($p = 0.05$) in terms of spontaneous changes in percentage (Fig. 1), which could also suggest a dose-effect relationship. Also, *post-hoc* analysis showed no significant differences between the 3 mg/kg and 10 mg/kg groups ($p = 0.447$), regarding the number of arm entries in Y maze task (Fig. 2).

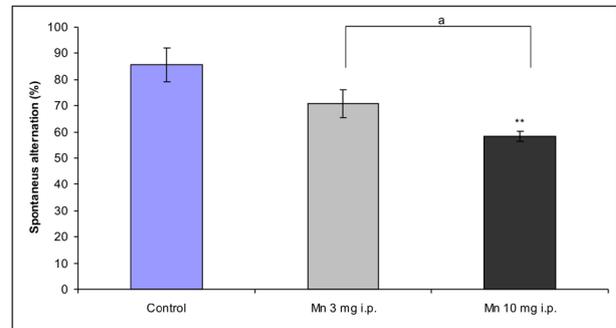


Fig. 1. Percentage of spontaneous alternations induced by manganese (Mn) exposure (3 mg/kg b.w., i.p. and 10 mg/kg b.w., i.p.) in the Y-maze task. Values are means \pm S.E.M. ($n=6$ per group). ** $p=0,002$ vs. sham-operated group. For post-hoc analysis - a (3 mg/kg vs. 10 mg/kg) : $p = 0.05$.

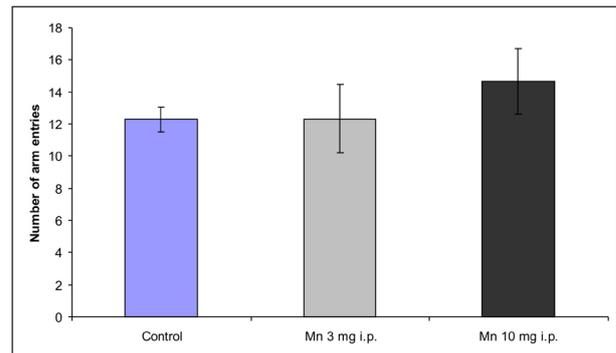


Fig. 2. Number of arm entries induced by manganese (Mn) exposure (3 mg/kg b.w., i.p. and 10 mg/kg b.w., i.p.) in the Y-maze task. Values are means \pm S.E.M. ($n=6$ per group).

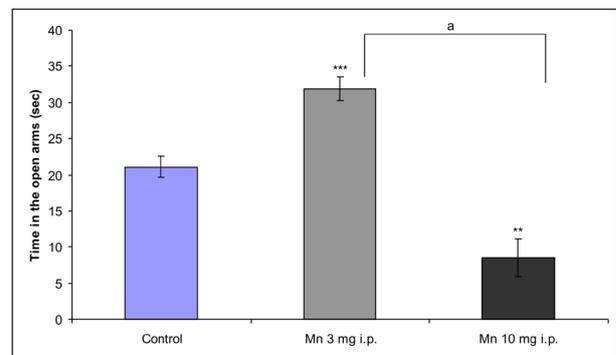


Fig. 3. Effects of manganese (3 mg/kg b.w., i.p. and 10 mg/kg b.w., i.p.) on the time spent in the open arms of the elevated plus maze vs. control. The values are mean \pm SEM. ($n=6$ per group). ** $p=0,002$ vs. sham-operated group, *** $p=0,0005$ vs. sham-operated group. For post-hoc analysis - a (3 mg/kg vs. 10 mg/kg) : $p < 0.0001$.

Effects of manganese administration on elevated plus maze behavior

Behavior in the elevated plus maze is mainly used to assess exploration and anxiety status (Ciobica et al., 2011a,b). The administration of 3 mg manganese/kg b.w. caused the rats to spend significantly more time ($F(1,10)=24$, $p=0.0005$) in the open arms of the elevated plus maze, as compared to the control group, suggesting that this dose of manganese could diminish anxiety-like behavior (Fig. 3).

Interestingly, the administration of 10 mg manganese/kg b.w. caused the rats to exhibit an increased anxiety state, as can be deduced from the significant reduction ($F(1,10)=17$, $p=0.002$) of the time spent in the open arms of the elevated plus maze, in comparison to the control group (Fig. 3).

Also *post-hoc* analysis revealed significant differences between the 3 mg/kg and 10 mg/kg groups ($p<0.0001$), in terms of time spent in the open arms of the elevated plus maze (Fig. 3).

DISCUSSION

In the present study we describe short-term spatial memory and anxiety-like behavior abnormalities resulting from manganese exposure in Wistar rats. At the level of manganese exposure used in the present study (3 mg/kg b.w.(i.p.) and 10 mg/kg b.w.(i.p.)), important cognitive effects were observed (a decrease in spontaneous alternation in the Y-maze task), along with behavioral changes suggestive of anxiety or compulsive-like behaviors (an alteration of time spent in the open arms of the elevated plus maze task).

It was previously demonstrated that microinjection of manganese chloride into the rat substantia nigra pars induced an increase in the open arm exploration in the elevated plus maze, suggesting an anxiolytic effect (Ponzoni et al., 2000). Also, in a recent study by Schneider et al. on macaque monkeys, manganese exposure resulted in mild deficits in spatial working memory, more significant defi-

cits in non-spatial working memory and no deficits in reference memory (Schneider et al., 2009). However, the Pappas group reported that neither the 2 nor the 10 mg/ml perinatal manganese-exposed rats differed from the controls on the elevated plus apparatus or on the Morris water maze and the radial arm maze (Pappas et al., 1997). Another recent study reported that manganese-treated rats showed hypoactivity, decreased memory performance, and diminished sensorimotor reaction (Vezer et al., 2007).

In our study no significantly effects on motor activity were observed in the Y maze task, as shown by the number of arm entries. Other authors have reported little or no dose-effect relationships on several measures of motor and cognitive functioning (Zoni et al., 2007), while others have reported findings such as poor hand steadiness, difficulty in performing fast alternating movements, muscle rigidity, postural instability, poor memory, slow reaction time and decreased cognitive flexibility after moderate but long-term manganese exposure (Iregren, 1999). The reasons for such inconsistent results are not entirely clear but may be related, at least in part, to differences in methodology and choice of neuropsychological assessments across the studies, as well as the uncertainties surrounding the estimation of manganese dose and exposure duration, the presence of confounding health issues, exposure to other potentially toxic agents, and the timing of the cognitive evaluation in relation to the actual exposure (Bouchard et al., 2007, Schneider et al., 2009).

Also, the Schneider group recently demonstrated that individual animals had different sensitivities to manganese administration and developed different degrees of cognitive and motor impairment at different times post manganese exposure (Schneider et al., 2009). Such differences in individual sensitivity to manganese make group analyses of some cognitive data difficult, especially with the current small number of animals. Nonetheless, data from individual animals suggested disruptive effects of chronic manganese exposure on aspects of cognition and anxiety-like behavior.

CONCLUSIONS

In summary, chronic manganese exposure in Wistar rats led to behavioral alterations consisting of cognitive deficiencies in the Y-maze task and anxiety/compulsive-like behaviors in the elevated plus maze, but no motor disturbances as tested by the number of arm entries in the Y-maze. Additional work is necessary to understand the long-term effects of different doses and dosing regimens of manganese on cognitive/affective and motor functioning.

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