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# Influence on Metallomic Distribution in Brain by Prolonged Consumption of Mn in Drinking Water: Modulation of Elemental Levels in Subcellular Fractions

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**Abstract.** Manganese (Mn) toxicity can arise from a variety of environmental exposure and it differentially affects the nervous system more severely. Our recent finding that chronic and life-span treatment of Mn induces organ-specific and brain region-specific changes in their distribution of trace and major elements prompted us to investigate the hypothesis brain accumulation of Mn induces changes in subcellular levels of trace and major elements. Our results demonstrated chronic and life-span treatment of Mn induced changes in the subcellular distribution of all trace elements studied, especially Br, Co, Cr, Cu, Fe, Mn, Se and V. In particular, Mn induced increases in 8 elements and decreases in 8 other elements in synaptosomes while it induced increases in 13 elements but decreases in 3 other elements in nuclei. Our findings may have pathophysiological implications in neurodegenerative diseases such as Alzheimer's disease and Parkinson's disease. They may also be pathophysiologically relevant in hepatic encephalopathy.

**Keywords:** Manganese toxicity, elements, metallomic distribution, brain, subcellular fractions.

## 1. Introduction

Manganese (Mn) toxicity can arise from exposure to elevated levels of the metal in air, water and diet [1]-[3]. Manganese toxicity in humans is associated with an acute phase of psychiatric signs and symptoms known as "Mn madness" and humans with chronic Mn toxicity show signs and symptoms reminiscent of Parkinsonism and dystonia [2], [3]. Even though Mn toxicity also affects peripheral organs such as liver and reproductive systems, the Mn-induced toxicity in the nervous system is by far the more severe [2], [3]. However, the cellular and molecular mechanisms underlying Mn neurotoxicity are still incompletely understood [1]-[3]. Nevertheless, there is recent evidence that Mn interacts with other trace and major elements [2], [3]: conceivably, the Mn-induced target organ toxicity could be attributed, at least in part, to the Mn interactions with other trace and major elements [2], [3].

Employing a life-span rat model of chronic Mn exposure [1], we have previously demonstrated that adding Mn in their drinking water can induce dose-related and region-specific increases in brain levels of Mn [2], [3]. These Mn-induced changes Mn brain levels are associated with selected changes in brain regional distribution of trace and major elements [2]-[4]. Because Mn's interactions with other metal ions in health and disease are still poorly understood [2]-[4], this study, therefore, investigates the hypothesis that brain accumulation of Mn induces changes in subcellular levels of trace and major elements.

## 2. Materials and Methods

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Our chronic and life-span rat model of Mn toxicity involved exposing Wistar rats to Mn (10 mg $MnCl_2.4H_2O$/ml of drinking water) at mating: thus, the rats were exposed to Mn in utero via their mothers' circulation and postnatally via their mothers' milk [1]. From around weaning (20-22 days postnatal) onward, they were directly exposed to Mn in the drinking water [1]. Control rats did not have Mn added to their drinking water [1]. In each experiment, the hypothalamus, striatum, midbrain and hippocampus from five adult (i.e., 120-day old) female rats were pooled for preparation of the tissue homogenate which was then employed for the isolation of subcellular fractions employing the procedure of Lai and Clark [5]. The subcellular fractions isolated from the brain tissue homogenate were: mitochondria, synaptosomes (i.e., nerve-ending particles), nuclei, myelin, capillaries, microsomes, and cytosol. The elements in the isolated subcellular fractions were determined employing instrumental neutron activation analysis (INAA) as described by Chan et al [6]. The element levels in each subcellular fraction were normalized with respect to the total protein content in the fraction. For data comparisons, the fractional change of each element determined in each subcellular fraction was calculated according to the following equation: element's fractional change = (element's level in a subcellular fraction derived from Mn-treated rats – element's levels in the corresponding subcellular fraction derived from control rats)/(element's level in the corresponding subcellular fraction derived from control rats).

## 3. Results and Discussion

Because of space limitations, this paper only very briefly discusses a few general trends of the Mn-induced changes in the trace and major elements determined in the various subcellular fractions studied. Additionally, the Mn-induced changes in the levels of elements in two of the subcellular fractions, namely synaptosomes and nuclei (see Figs. 1 and 2 below), are selected for a more detailed discussion regarding their broader implications in health and disease. These two subcellular fractions are selected because they showed particular range of Mn-induced changes in their levels of elements and they play important and somewhat special functional roles in neurology, neurobiology and neural cell biology.

Consistent with the dose- and region-specific increases in Mn levels in brain upon chronic and life-span exposure to Mn in the drinking water [2]-[4], the Mn levels in all subcellular fractions isolated from brain homogenates of rats that had chronic and life-span exposure to Mn in their drinking water also showed Mn-treatment related increases (Table 1). However, the increases in Mn levels in the different subcellular fractions varied depending on their subcellular locations and were in the rank order: nuclei >>mitochondria>synaptosomes>cytosol>microsomes>myelin>capillaries (Table 1). Thus, these findings strongly suggest that nuclei, mitochondria and synaptosomes (nerve-endings) may be the key subcellular target for chronic Mn neurotoxicity.

Chronic and life-span treatment of Mn induced changes in the subcellular distribution of all trace elements studied, including Al, Br, Co, Cr, Cu, Fe, Mn, Rb, Se, V and Zn (Table 1). Nevertheless, the subcellular distributions of Al, Rb and Zn were the least affected by the Mn treatment among the trace elements examined (Table 1).

In contrast with the Mn-induced changes in subcellular distribution of trace elements, among the major elements and electrolytes studied, the subcellular distributions of Cl and Mg were most affected, that of Ca were somewhat affected and those of K and Na were virtually unaffected by the same Mn treatment *in vivo* (Table 1). These findings strongly suggest that chronic Mn neurotoxicity may significantly alter intracellular metabolism and dynamics of Cl and Mg and Ca to a lesser extent.

Chronic and life-span treatment of Mn induces increases in eight elements (Ca, Cl, Co*, Cr*, Cu*, Mn*, Na, and Se*) and decreases in eight other elements (Al, Br*, Fe, K, Mg, Rb, V*, and Zn) in synaptosomes isolated from the brains of Mn-treated rats and only those changes marked with * were significantly different ($p<0.05$) from corresponding levels in synaptosomes derived from control rats (Table 1 and Fig. 1). All of the elements (Co*, Cr*, Cu*, Mn*, and Se*) showing significant increased levels in synaptosomes are required trace or ultra-trace (i.e., Co*) metals that elicit biphasic physiological and pharmacological responses from cells including neural cells (i.e., they support normal cell physiology at lower levels but are toxic at higher levels). Because we and others (see Ref. 1 for details) have previously demonstrated that both

essential and non-essential metal ions can induces alterations in synaptic transmission (i.e., changes in nerve cells' communications) at pathophysiological and toxic levels.

Table 1: Fractional changes of elemental levels in subscellular fractions relative to those in the corresponding subcellular fractions derived from control animals. Data were derived from the mean of 3-5 separate experiments. Abbreviations were: H, homogenate; Mito, Mitochondria; Syn, Synaptosomes; Capi, Capillaries; Microsom, Microsomes; ND, not detected. *Element levels in subcellular fraction derived from Mn-treated rats were significantly ($p<0.05$ by t-test) from corresponding levels in in subcellular fraction derived from control rats.

| Element | H | Mito | Syn | Nuclei | Myelin | Capi | Microsom | Cytosol |
|---|---|---|---|---|---|---|---|---|
| Al | -0.370* | -0.214 | -0.629 | -0.010 | -0.206 | 0.313 | 1.908* | 0.200 |
| Br | 0.013 | -0.427* | -0.355* | 0.356 | -0.316* | -0.221* | -0.172* | -0.181* |
| Ca | -0.064 | 0.141 | 0.073 | -0.090 | -0.101 | -0.259 | 0.156 | 0.439* |
| Cl | 0.258 | -0.463 | 0.015 | 2.936* | -0.112 | -0.634* | -0.617* | 0.068 |
| Co | -0.385* | 2.340* | 0.751* | 0.156 | 1.134* | ND | ND | -0.484* |
| Cr | 0.526* | 1.597* | 1.111* | 0.514 | 0.209 | ND | ND | 0.452* |
| Cu | 0.357 | -0.046 | 0.698* | 1.021* | -0.068 | -0.512* | -0.128 | 0.022 |
| Fe | 0.442* | 0.078 | -0.152 | 1.919* | -0.346 | ND | ND | -0.585* |
| K | -0.204 | 0.429 | -0.517 | 0.119 | -0.192 | 0.231 | -0.262 | -0.231 |
| Mg | -0.083 | -0.881 | -0.084 | 0.723* | 0.845* | 2.063* | 0.760 | 0.808* |
| Mn | 0.926* | 0.700* | 0.611* | 1.223* | 0.437* | 0.249 | 0.505* | 0.543* |
| Na | 0.090 | 0.288 | 0.108 | 0.285 | -0.138 | -0.326 | 0.102 | 0.153 |
| Rb | 0.167 | 0.498 | -0.331 | -0.085 | 0.011 | ND | ND | 0.468* |
| Se | 0.747* | 0.051 | 1.224* | 0.186* | -0.311 | ND | ND | 1.030* |
| V | 0.439* | -0.422* | -0.545* | 1.136* | -0.845* | -0.440 | -0.283 | 0.505* |
| Zn | 5.959* | 0.297 | -0.180 | 0.382 | 0.278 | 0.117 | ND | 1.488* |
| Decreases | 5 | 6 | 8 | 3 | 10 | 6 | 5 | 4 |
| Increases | 11 | 10 | 8 | 13 | 6 | 5 | 5 | 12 |

Taken together, our previous [1] and current (Table 1 and Fig. 1) findings suggest the elevation of Co*, Cr*, Cu*, Mn*, and Se* may interfere with normal nerve cell communications in rats exposed to chronic and

life-span Mn treatment in their drinking water. Co is known to induce chemical hypoxia and oxidative stress in neural cells. Similarly both Cu and Mn can induce decreased oxidative metabolism but elicit oxidative stress in neurons and other neural cells [2]. Toxic metals such as Cu and Mn are known to be elevated in the brains of patients who died of neurodegenerative diseases such as Alzheimer's disease (AD) and Parkinson's disease (PD) [2]. Consequently, these metals have been proposed to induce death of neurons in AD and PD [2]. Furthermore, Mn is implicated in the pathophysiology of hepatic encephalopathy: thus, its increase in synaptosomes in Mn-treated brain may be pathophysiological relevant in hepatic encephalopathy. On the other hand, Br and V are not essential elements and their effects on the nervous system remains to be elucidated.

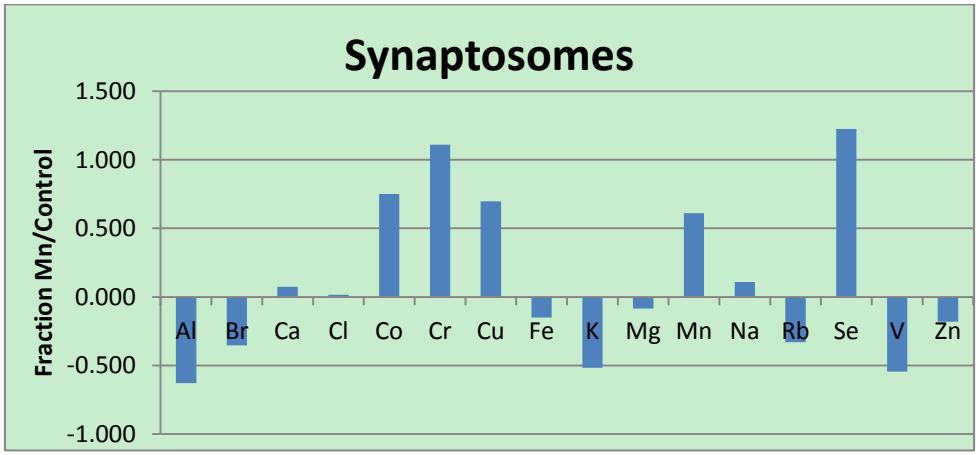


Fig. 1: Fractional changes in levels of elements in synaptosomes derived from Mn-treated rats relative to the corresponding levels of elements in synaptosomes derived from control rats. Data were derived from the mean of 3-5 separate experiments.

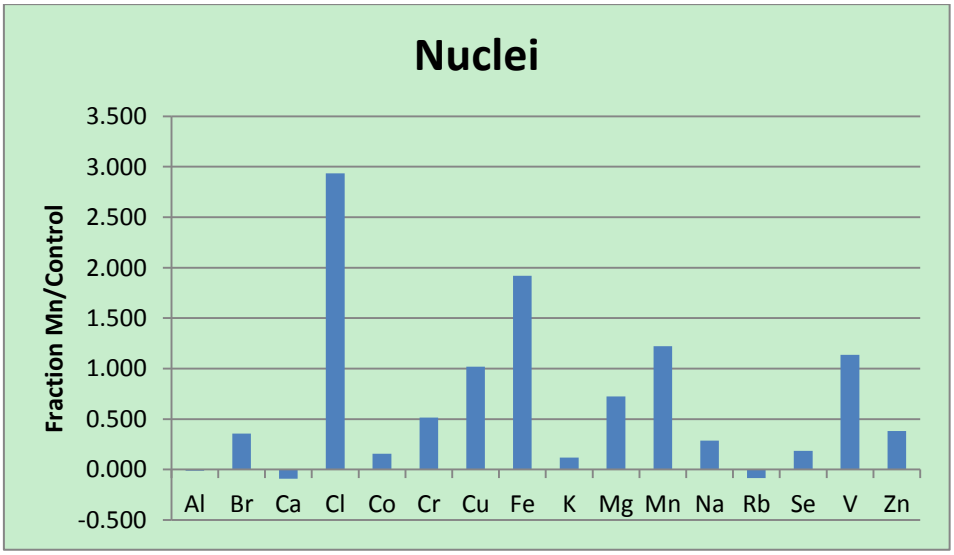


Fig. 2: Fractional changes in levels of elements in nuclei derived from Mn-treated rats relative to the corresponding levels of elements in nuclei derived from control rats. Data were derived from the mean of 3-5 separate experiments.

Chronic and life-span treatment of Mn induces increases in 13 elements (Br, Cl*, Co, Cr, Cu*, Fe*, K, Mg*, Mn*, Na, Se*, V*, and Zn) and decreases in three other elements (Al, Ca, Rb) in nuclei isolated from the brains of Mn-treated rats and only those changes marked with * were significantly different ($p<0.05$) from corresponding levels in nuclei derived from control rats (Table 1 and Fig. 2). With the exception of V, all the elements (Cl*, Cu*, Fe*, Mg*, Mn*, and Se*) showing significant increased levels in nuclei are required trace or major elements or electrolytes that elicit biphasic physiological and pharmacological responses from cells including neural cells (i.e., they support normal cell physiology at lower levels but are toxic at higher levels). Because Cu, Fe and Mn are known to induce oxidative stress [2], their elevated

presence in the nuclei of Mn-treated rat brain strongly suggests they may induce oxidative stress in that subcellular compartment. On the surface, such marked accumulation of divalent metals in nuclei in Mn-treated rat brain is somewhat puzzling and implies the presence of transporters or ion channels to facilitate their accumulation in nuclei. Indeed, new evidence derived from studies employing human embryonic kidney cells indicated that toxic divalent metal ions such as $Cd^{2+}$ can enter and be accumulated in nuclei through Ca channels [7]. Other divalent metal ion transporters may also be involved as well as Ca channels [7]. More importantly, the Mn-induced increases in accumulation of Cu*, Fe*, Mg*, Mn*, Se*, V* and Zn (although it did not reach statistical significance) may have impact on gene expression because Zn fingers are particularly important in regulating gene expression and presence of other divalent metals may interfere with Zn in Zn fingers. Furthermore, entry of toxic metal ions including Fe and Zn through voltage-gated calcium channels into neuronal cytoplasm and then into their nuclei may be implicated in neurodegenerative diseases such as AD and PD [8]. In addition to oxidative stress induced by the toxic metal ions in neurons, the presence of the toxic metal ions in their nuclei may also influence gene expression by interfering Ca signaling in regulation of gene expression [8]. On the other hand, the Mn-induced Cl accumulation in nuclei may be associated with its action as a counter-ion in conjunction with the entry of the positively charged cations such as Cu, Fe, Mg and Mn. However, effect of Mn treatment-induced accumulation of V in nuclei is presently unlike and awaits further investigation.

Taken together our results may assume pathophysiological and/or pathogenetic importance in mechanisms underlying devastating neurodegenerative diseases such as Alzheimer's disease and Parkinson's disease. They may also be relevant in the pathophysiology of hepatic encephalopathy. Clearly these are important areas that deserve further investigation as mechanistic findings in these and related areas are scant.

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