# GENERAL AND NERVOUS SYSTEM EFFECTS OF THE NEUROTOXIC METAL MANGANESE UNDER VARIOUS CIRCUMSTANCES OF APPLICATION

**Summary of PhD Thesis**

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# Introduction

The role of metals in the development of human culture and technology became significant around 4000 B.C., when people discovered how they can extract copper from its ores and manufacture objects of everyday use, and the copper era began. With changing way of life, humans started to use other easily extractable metals from the environment, such as tin or lead. As time went by, metals became vital for everyday life but they also presented a source of environmental pollution and harmful human exposure.

Several terms are used for the classification of metals, such as light or heavy metals, essential, beneficial, toxic and trace metals. It has been customary to group metals into light and heavy ones based on their density, but over the years, no consistency was achieved in this density-based definition. Recently it was suggested that heavy metals should be rather defined based on their position in the periodic table, because this position refers to the chemical properties and hence the possible toxic effect. According to this classification, transition elements, such as chromium (Cr), manganese (Mn) and iron (Fe), as well as rare earth metals, post-transition metals and metalloids should be considered as heavy metals.

Heavy metals as elements do persist in the environment. A lot of metals are xenobiotics, substances which can be neither utilized nor neutralized by the organism. This is so because they used to have minimal presence (and, hence, bioavailability) during the evolution of life, before anthropogenic emission into the environment had begun; and because they are either completely useless or toxic for the human organism (e.g. Hg, Pb or Cd) or essential as micronutrients but toxic when overdosed (Mn, Cr, Cu, etc.).

Emission of heavy metals into the environment can be of natural or anthropogenic origin. The most important man-made sources are ore processing and combustion processes (power generation or waste incineration) raising the concern of occupational health hazard. Due to the environmental transport of metals through air, water and the food chain, distant areas can be polluted leading to exposure of the general population.

The concentration of essential elements in human and animal organism is under homeostatic control, and the uptake from the environment (via food and/or drinking water) is regulated by the nutritional demand. Disturbances in the regulatory mechanisms can result in either insufficient (deficiency) or excess (toxicity) metal uptake.

Ingested heavy metals are absorbed from the intestine to around 10%. Common metal transport mechanisms, responsible for the uptake of essential metals, are involved in that, which explains why individuals with Fe or Ca deficiency absorb more of the toxic metals (e.g. excess Mn absorption in anaemic individuals, or lead absorption in children requiring more Ca for bone growth). Trivalent ions of toxic metals like Mn or Cr use transferrin (Tf) for transport from the blood, to pass the blood-brain barrier (BBB) and to reach the central nervous system (CNS).

Inhalational exposure occurs predominantly in the occupational environment. Miners and metal workers are exposed to airborne metal particles first of all, but inhabitants around industrial sites are also at risk. Metal-containing dusts and fumes are generated in the whole life cycle of metals, from ore mining through smelting and final

product manufacturing to waste management and recycling, and are found in the workplace atmosphere sometimes at hazardous concentrations.

One of the most common sources of such metal aerosols is welding. Welding fume is a complex mixture of metal oxides and materials of the electrode. The most common elements in steel that welders are primarily exposed to by inhalation are Fe, Mn and Cr. Airborne particles, released into the environment by welding, have major adverse health effects, causing among others neurological disorders and respiratory illnesses. Welders who are at excessive exposure may be at risk of developing early Parkinson's disease or manganism caused by overexposure to Mn. The neurotoxicity of Mn has been described extensively but the role that other agents (such as Cr or Fe) peculiar to welding may play in the causation of the above mentioned neurological disorders are still unknown.

The extent of metal exposure is influenced by numerous factors including chemical form in which the metal is present in the particles and the particle size. Much of the particles derived from the welding process have a diameter below 100 nm, i.e. they are nanoparticles (NPs). The potential health impact is influenced by the site of deposition (for NPs, mostly nasopharyngeal and alveolar). Also, the surface-to-mass ratio of these particles is extremely high, which means a small mass fraction within a complete aerosol sample, but with a high number of particles and a very high and reactive overall surface. The composition of NPs is determined by the composition of the materials worked with or burnt. Inhaled NPs have various interactions in a living system and can contribute to adverse health effects in the respiratory tract as well as in extrapulmonary organs. Extrapulmonary effects of NPs depend on several factors including particle solubility, particle or aggregate size, the site of deposition, and the integrity of the alveolar epithelial lining. Inhaled NPs deposited in the nasopharynx can pass a healthy blood-brain barrier (BBB) and reach the CNS via direct transport along the olfactory pathway. Particles that reach the alveoli are translocated directly to the blood and transported further. Pulmonary circulation continues to supply the brain with significant amounts of welding-related metals long after the exposure has been terminated. Also for the general population, inhalation may be a significant source of NP exposure, e.g. from car exhaust because most of the metals leaving the engine with the exhaust gases are in form of microscopic and submicroscopic oxide particles. Extensive use of NPs means that, besides inhalation, routes of dermal and oral uptake should be also considered.

Mn is an essential micronutrient, a component of several metalloenzymes, but toxic when overdosed. One of the most important applications of Mn is steelmaking, and the coating of welding rods. In the production of dry cells, $MnO_2$ is used. Maneb and Mancozeb represent the use of Mn in agricultural fungicides; while the contrast agent trisodium mangafodipir (Mn-DPDP, Teslascan) is used in magnetic resonance imaging for diagnostic purposes. Most of the inhalable Mn found in (both workplace and residential) environment is of anthropogenic origin. The typical form of occupational Mn exposure is chronic inhalation (usually but not exclusively as $MnO_2$), which may result in severe neurological disorders; starting with apathy, asthenia and headache, and ending in Parkinson-like syndrome. Inhalational Mn exposure for the general public may result from the Mn-containing petrol additive, but ingestion may be equally important for them. Abnormally high Mn levels in the drinking water were reported in Japan, in Greece and in the USA, causing CNS symptoms in the residents of the polluted areas, but cases of Mn overexposure among patients receiving Mn-supplemented parenteral nutrition were also reported. Disorders with

electrophysiological signs after Mn exposure include myoclonus in welders and epileptic activity in over-exposed children. EEG and visual evoked potential alterations were observed in persons with elevated blood Mn.

Fe is of great value in biology: it is essential for both plant and animal life, e.g. by carrying oxygen and thus enabling vital functions, but due to its advantageous technical properties, Fe is also of high importance in numerous alloys, first of all in steels. In case of overload, Fe can deposit in certain mitochondria rich organs, and causes disruption of the mitochondrial energy production by decreasing enzyme activity, and leads to generation of ROS responsible for the toxic effect. In certain neurodegenerative diseases, such as Parkinson's or Alzheimer's disease, accumulation of Fe in the basal ganglia and induction of oxidative stress is held responsible. Another possible mechanism of Fe toxicity involves rupture of lysosomes and release of digestive enzymes into the cytoplasm, which may end in cell death. After intratracheal (it.) instillation of $Fe_2O_3$ NPs pulmonary toxicity was studied, but no neurological or behavioural tests were conducted. Fe oxide NPs are, on the other hand, of considerable interest for biomedical applications, which is yet another reason for examining their toxicity.

Cr, used in the production of pigments, in leather tanning and in alloy formation, is naturally present in the environment and in the living organisms as well. The main forms of Cr are the trivalent ($Cr^{3+}$) and the hexavalent ($Cr^{6+}$) state. $Cr^{3+}$ is a component of the glucose tolerance factor, thus regulating glucose, protein and fat metabolism. ). Environmental $Cr^{6+}$ originates almost totally from human activity; it is more toxic than $Cr^{3+}$, is a strong oxidant, carcinogen, allergen and acute irritant. The natural sources of Cr in the environment are weathering of rocks and leaching from soils, but exposure of the general population is negligible compared to occupational exposure. Occupational exposure to $Cr^{6+}$ and $Cr^{3+}$ compounds and their respiratory effects have been described several times, but information about the neurological effects is scarce, though it causes free radical formation, reduces mitochondrial activity, and studies prove that Cr can also reach the brain. Cytotoxicity of nanoparticulate $Cr_2O_3$ is higher compared to fine particles. Cr NPs were found in in vitro experiments to increase intracellular ROS level and to cause oxidative stress.

## Aims

This thesis is the continuation of a long line of antecedent research at the Department on the neurotoxicity of Mn, in dissolved and more recently in nanoparticulate form and on interactions of neurotoxic metals. The overall goal, modelling human heavy metal exposure – which is often inhalational but can also be oral – more adequately, led to more elaborate experimental designs, one involving the combination of several industrially relevant metals, and the other involving more than one physicochemical form of one metal. Varying the way and site of administration was included also. From all that, the aims of the present PhD work were specified as follows:

- To examine the effects on general toxicological, CNS electrophysiological and behavioural parameters exerted by nanoparticulate Mn, Fe and Cr in an identical experimental setup;

- To repeat these experiments with double and triple combinations of the mentioned metals, in order to reveal possible interactions and to have a better model of welding fumes and similar industrial emissions;
- To examine the relationship of external and internal doses in dependence of the physicochemical form and site of application of Mn, and on co-application of the other two metals;
- To test whether analysis of the second:first ratio, a method used previously to study the CNS effect of the mitochondrial toxin 3-nitropropionic acid and the acute effect of Mn, is applicable for detection of neuro-functional alterations induced by subacute application of Mn and other metals.

## Materials and Methods

Young adult male Wistar rats were obtained from the university's breeding centre. The different experiments were started with different initial body weights, see Table 1. The animals were housed, with up to four rats in one cage, under GLP-equivalent conditions (22±1 ºC, 30-60% relative humidity, 12-h light/dark cycle with light on at 06:00), and had free access to tap water and standard rodent chow (Bioplan, Isaszeg).

The rats were exposed to Mn by per os (po.) and it. administration. In po. administration, aqueous solution of $MnCl_2$ was applied, while in it. administration the rats were instilled either with aqueous solution of $MnCl_2$, or with a suspension of $MnO_2$, $Fe_3O_4$ or $Cr(OH)_3$ NPs or their combinations (the treatment schemes are given in Table 1).

$MnCl_2$ was of >99% purity, available commercially (Sigma-Aldrich Hungary). The $MnO_2$, $Fe_3O_4$ or $Cr(OH)_3$ NPs for Experiment III and IV were synthesized at the Department of Physical Chemistry and Materials Science, University of Szeged, Faculty of Science and Informatics.

**Experiment I**: For po. administration, 2.5 mg/ml $MnCl_2$ was dissolved in tap water, and given as drinking fluid to the 12 animals of the treated group for 6 weeks. This amount of Mn was, according to literature data, a small, but at the same time relevant dose considering possible Mn intoxication of environmental origin. Animals of the control group (n=8) consumed clear tap water during the exposure period. Mn content of the tap water used was negligible (0.03 μg/ml), therefore it was disregarded at the calculation of cumulative dose. In order to prevent Mn precipitation from tap water, 0.25 mg/ml citric acid was added. The pH of the drinking fluid, containing $MnCl_2$ and citric acid in the above mentioned concentrations, was 6.7. Based on the water consumption of the animals, the solution had no unpleasant taste.

During the treatment period, body weight and water consumption of the animals was measured daily (for the sake of the latter, the animals were housed alone). From the data, cumulative Mn dose (per kg body weight) could be calculated. At the end of the 6-week treatment, after preparation for electrophysiological investigation (see below) two recordings of somatosensory (SS) evoked potentials (EPs) per animal were made in urethane anaesthesia.

**Experiment II**: In this experiment the rats had it. instillations of $MnCl_2$ (0.5 mg/kg b. w., instillation volume: 1 ml/kg b. w.), once a day, five days per week, for 5 weeks. The applied dose was based on previous experience and on literature data. The rat was put in a glass jar with air-tight lid, saturated with ether vapour. The completely anesthetized rat was suspended on a board tilted to 60° from horizontal, by hanging its upper incisors in a wire loop. Keeping this way the rat in place and its mouth open, the trachea was illuminated transdermally by means of a fibre optic light guide brought into direct contact with the animal's neck. The tongue was pulled forward with a pair of non-traumatic forceps, and a custom-made laryngoscope was used to gain access to the glottis. The $MnCl_2$ solution (or distilled water for the vehicle controls) was instilled into the trachea by means of a 1 ml syringe and 1.2 mm diameter plastic tubing, inserted between the vocal chords. The electrophysiological recording at the end of the 5-week treatment was identical to that done in Experiment I.

**Experiment III**: The rats, divided into 10 groups, were intratracheally instilled with a suspension of $MnO_2$, $Fe_3O_4$ or $Cr(OH)_3$ NPs (2 or 4 mg/kg b. w.; for details see Table 1). Treatment was performed once a day, 5 times per week, for 4 weeks. This short period was chosen because experience showed that the nanoparticulate form of Mn (and other metals) tends to be more toxic than other (dissolved etc.) forms. $MnO_2$ and $Fe_3O_4$ NPs were suspended in PAA, the vehicle also used to treat the *FMVC* vehicle control group. $Cr(OH)_3$ NPs were suspended in normal saline, so the *CrVC* control group was saline treated. An untreated control (*Cont*) group was also used because of the expectable own effects of the treatment procedure. It. treatment was performed as described in Experiment II. At the end of the treatment, open field (OF) test and electrophysiological recording was conducted (methods are described below).

**Experiment IV**: In this experiment, animals were treated with the double and triple combinations of $MnO_2$, $Fe_3O_4$ or $Cr(OH)_3$ NPs. The treatment lasted for 4 weeks; it. instillation was performed once a day, 5 times per week (it was supposed that during 4 weeks the effects will develop but animal loss due to excessive toxicity will be minimal). Five groups of animals were set up as detailed in Table 1. The vehicle control (*VC*) group was treated with PAA and normal saline with several hours delay. Fe and Mn NPs were both suspended in the PAA medium, and could be administered together. Administration of NPs in different vehicles (combinations containing Cr: *FC, MC* and *FMC*) was separated in time by several hours in order to decrease burden put on the lungs by the instillation (that is, to avoid the administration of double volume). It. treatment was performed as described in Experiment II. At the end of the treatment, OF test and electrophysiological recording was conducted (methods are described below).

Preparation and electrophysiological measurement was done after anaesthetizing the animals with urethane (1000 mg/kg b. w. intraperitoneally; Reanal, Hungary). The head of the rats was fixed in a holder frame, the skin was opened by a mid-sagittal cut, and the muscles and connective tissues attached to the skull were removed. Wounds were sprayed with 10% Lidocaine, then the left temporal bone was cut along its inner circumference by a dental drill bit attached to a mini drill, the bone was lifted, and the left hemisphere was thus exposed. The exposed dura surface was covered with a thin layer of petroleum jelly in order to protect it from dehydration. The prepared animals were wrapped in a warm cloth to maintain body temperature and were put aside for at least 30 min for recovery.

After the recovery period, the rat was placed into the stereotaxic frame of the electrophysiological setup. For recording spontaneous and evoked cortical activity, ball-

tipped silver recording electrodes were placed on the dura over the primary SS projection area of the whiskery pad (barrel field), and over the primary visual (VIS) and auditory (AUD) focuses. A stainless steel clamp was attached to the cut skin edge as indifferent electrode.

Stimulus-evoked activity (sensory EPs) was recorded in each of the experiments. SS stimulation was done by electric pulses given through a pair of needles inserted into the whiskery skin (3 4 V; 0.05 ms). VIS stimulation was performed by flashes delivered by a high-luminescence white LED directly into the contralateral eye of the rat. For AUD stimulation, clicks (ca. 40 dB) were applied into the contralateral ear of the rat from a mini earphone through the hollow ear bar of the stereotaxic frame.

In Experiment I-II, only SS EPs were recorded, using double stimulation. In that, the first stimulus was followed by the second one after 300, 240, 180, 120 and 60 ms interstimulus intervals (ISIs). A complete record involved 20 "runs"; and in one run each ISI occurred once in decreasing order (5 "sweeps"). Recording of 20 "runs" (a complete record) took 6 min. Such complete recordings followed in 20 min intervals thus the animals had enough time (14 min) for recovery. This stimulation scheme was applied, and found sensitive for neurotoxic influences, in earlier experiments of the Department. Stimulation, recording of EPs, and analysis of the records (described below) was governed by the software Clampex 8.0 (Axon Instruments Inc., USA).

The EPs were averaged automatically by the software from the recorded 20 "runs", for each double stimulation. On playing them back, cursors were positioned manually on the positive and negative peaks of the EPs, generated by the first and second stimuli. The cursor positions defined numerical data which were used to calculate peak-to-peak amplitude and the latency and amplitude of the positive (first) and negative (second) peak of the SS EP. In Experiment I-IV, two recordings per rat were made at the end of the treatment period. Data from these two records were averaged, then group means were calculated and compared.

A novel and important part of the evaluation was the calculation of second:first ratio. To do that, each parameter of the second EP was divided by the corresponding parameter of the first EP. Second:first ratio calculation was performed in case of each animal one by one and from these data, group means were calculated. The second:first ratio was interpreted as a measure of dynamic interaction between the two excitation processes.

In Experiment III and IV, the software NEUROSYS 1.11 (Experimetria Ltd., Hungary) was also used for recording cortical activity. In these experiments, spontaneous electrical activity (electrocorticogram, ECoG) was first recorded from the three cortical areas mentioned above, simultaneously for 6 min. EPs were also recorded from each cortical area (SS, VIS, AUD) in Experiment III and IV. Fifty stimuli of each modality per rat were applied.

From the ECoG records, relative spectral power of the frequency bands and the fast/slow activity ratio ("ECoG index") was determined. EPs were automatically averaged off-line, and their parameters (onset latency, duration) were measured manually by positioning the cursors of the software to specific points of the EP curve.

Previous studies demonstrated that by varying the frequency of stimulation the dynamic interaction of successive excitation processes in the sensory system can be assessed, which in turn reflects the actual state of the CNS. In theoretical basis and final aim, stimulating with varied frequencies was akin to double-pulse stimulation used in

the present experiments. Frequency dependence in the parameters of the SS EPs was determined by delivering stimuli to the whisker pad also with 2 and 10 Hz frequency, beyond the standard 1 Hz.

Body weight of the animals was regularly measured during the experiments, to follow-up weight gain and to determine the exact daily doses. Following electrophysiological recording, the animals were sacrificed by an overdose of urethane, were dissected, and blood was collected. Brain, lungs, heart, thymus, liver, kidneys, spleen and adrenal glands were removed and the organ weights were measured. From these data, relative weights were calculated by relating organ weights to brain weight or 1/100 of body weight. Metal level was determined from blood, brain and lung samples by inductively coupled plasma mass spectrometry at the Laboratory of the MOL Hungarian Oil and Gas Company.

General toxicological, behavioural and electrophysiological data were tested for significance with one-way ANOVA with post-hoc LSD test. Linear regression calculations between tissue metal levels and general- and neurotoxicological parameters were done by the “linear fit” function of MS Excel. The level of significance was set to $p<0.05$.

During the whole study, the principles of the Ethical Committee for the Protection of Animals in Research of the University were strictly followed. The methods used in the experiments were licensed by the authority competent in animal welfare issues under No. XXI./02039/001/2006.

**Table 1** Treatment schemes of the experiments.

| | **Experiment I** ***(Subacute per os)*** | **Experiment II** ***(Subacute intratracheal)*** | **Experiment III** ***(Subacute intratracheal)*** | **Experiment IV** ***(Subacute combined intratracheal)*** |
|---|---|---|---|---|
| **Duration** | 6 weeks | 5 weeks | 4 weeks | 4 weeks |
| **Groups (with code) and number of animals per group** | *Control*: 8<br>*Treated*: 12 | *Control* (untreated): 6<br>*Vehicle control*: 12<br>*Treated*: 12 | Untreated control (*Cont*): 6<br>Vehicle control for Fe and Mn treatment (*FMVC*): 8<br>Low dose $Fe_3O_4$ (*FeLD*): 8<br>High dose $Fe_3O_4$ (*FeHD*): 8<br>Low dose $MnO_2$ (*MnLD*): 8<br>High dose $MnO_2$ (*MnHD*): 8<br>Vehicle control for Cr treatment (*CrVC*): 8<br>Low dose $Cr(OH)_3$ (*CrLD*): 8<br>High dose $Cr(OH)_3$ (*CrHD*): 8 | Vehicle control (*VC*): 10<br>$Fe_3O_4$+ $MnO_2$ (*FM*): 10<br>$Fe_3O_4$+ $Cr(OH)_3$ (*FC*): 10<br>$MnO_2$+ $Cr(OH)_3$ (*MC*): 10<br>$Fe_3O_4$+ $MnO_2$ +$Cr(OH)_3$ (*FMC*): 10 |
| **Body weight at start** | 200±20 g | 200±20 g | 260-280 g | 260-280 g |
| **Substances, doses, and way of administration** | $MnCl_2$<br>2.5 mg/ml<br>po. via drinking water | $MnCl_2$<br>0.5 mg/kg b. w.<br>it. | *FeLD*: 2 mg/kg b. w.<br>*FeHD*: 4 mg/kg b. w<br>*MnLD*: 2 mg/kg b. w.<br>*MnHD*: 4 mg/kg b. w.<br>*CrLD*: 2 mg/kg b. w.<br>*CrHD*: 4 mg/kg b. w.<br>it. | *FM*: 2 mg/kg b. w.<br>*FC*: 2 mg/kg b. w.<br>*MC*: 2 mg/kg b. w.<br>*FMC*: 2 mg/kg b. w.<br>it. |
| **Vehicle** | Tap water | Distilled water | PAA (FMVC), normal saline (CrVC) | Normal saline and PAA |
| **Investigation** | Electrophysiology<br>Dissection<br>Organ weighing<br>Tissue metal level determination | Electrophysiology<br>Dissection<br>Organ weighing<br>Tissue metal level determination | OF activity<br>Electrophysiology<br>Dissection<br>Organ weighing<br>Tissue metal level determination | OF activity<br>Electrophysiology<br>Dissection<br>Organ weighing<br>Tissue metal level determination |

## Results and Conclusion

In **Experiment I** (subacute po. treatment with $MnCl_2$ solution) neither the body weight gain nor the relative organ weights were affected by the treatment. The calculated daily Mn intake of the rats in the *Treated* group was equivalent to 141 mg $MnCl_2$, which meant that the cumulative dose of Mn was 1633 mg/rat by the end of the 6th week. Upon $MnCl_2$ treatment, significant Mn deposition evolved in the treated animals' brain, but not in blood. Second:first ratio of the EPs was shifted to lower values in case of the peak-to-peak amplitudes, and of the positive and negative peak latencies (the latter two being significant). The absolute latency values had no significant change which suggested that the ratio may be a more sensitive indicator.

In **Experiment II** (subacute it. treatment with $MnCl_2$ solution) no significant effect on the body weight gain in the *Treated* group was seen, however, relative weight (related to 1/100 body weight) of certain organs such as heart and lungs was significantly increased. At the end of the treatment, the calculated cumulative dose of Mn was merely ca. 1 mg/rat; all the same, Mn concentration in both brain and lungs – but not in blood – was significantly elevated. The (non-physiological) absorption of Mn from the airways (Experiment II and III) was apparently much more efficient in causing toxic internal doses than absorption from the gastrointestinal tract (Experiment I). The second:first ratio of the peak-to-peak amplitude showed significant decrease in the *Treated* group (at 240 and 180 ms ISI) which change was basically similar to the alteration observed in po. treatment (Experiment I). As to the positive and negative peaks, the second:first ratio was lower at all ISIs (significantly at 300 and 240, and 240 ms respectively). Significant correlation of brain Mn level with the second:first ratio of the negative peak amplitude was found at 240 ms ISI.

In **Experiment III and IV** (subacute it. treatment with Mn, Cr or Fe NPs and with their combinations) body weight gain of the animals treated with Mn and Cr NPs (or their combination) was significantly reduced, and so was the weight gain in case of Fe+Cr combination (*FC)*, however, Fe-only treatment lessened the negative effect of the treatment procedure. As for the organ weights, the most prominent alteration was the increase of the lungs' relative weight (related to both calculation bases) upon treatment with Mn and Cr NPs, and each of the metal NP combinations. In the OF test, local activity and immobility was increased and vertical activity was decreased by Cr and Mn and by Cr-containing combinations. The effect of Fe NP treatment was opposite. All three metal studied tend to be involved in redox cycling, leading to oxidative damage of the dopaminergic neurons crucial in motor control; and the observed decreased motility could be comparable to symptoms of welders suffering from manganism. In the ECoG, application of metal NPs caused a shift to higher frequencies, and this effect of Mn and Cr on the SS ECoG was significant, but the alterations caused by the metal NP combinations were more pronounced. The observed alterations on the VIS and AUD ECoG were similar to those of the SS ECoG. The SS EPs showed significantly increased latency on the action of Mn and Cr NPs (and their combination, *MC*), and of *FC*. In Fe NP treated groups, however, latency was decreased significantly. VIS and AUD latency were increased by both doses of Mn, high dose of Cr, and the combinations of *MC* and *FC*. In case of SS EPs elicited by double pulse stimulation, peak-to-peak amplitudes were increased significantly by Mn at almost each ISI. The effect of Cr treatment was opposite to Mn, and Fe NP treatment had no noteworthy effect alone. The second:first ratio of the peak-to-peak amplitudes was significantly

decreased by Mn-only treatment, and the opposite effect of Cr was also seen here. The interaction of the two excitation processes, characterized by this ratio, may indicate changes in the neurons' energy supply and in transmitter release and removal In combination, the effect of *FC* and *FMC* on the second:first ratio was similar to that of Cr, whereas no noteworthy change was found in the peak-to-peak amplitudes. Mn and Cr content of the brain, lungs, and also blood was significantly increased by Mn and Cr NPs respectively, but the effect of Fe was only seen on increased lung Fe levels. In combinations, brain and blood levels of Mn were similar to that of caused by Mn alone in low dose. Cr levels were, however, influenced by the other metals applied, possibly due to interference on Tf and/or other binding sites. The correlation between brain Mn levels and SS and VIS ECoG index and SS and VIS latency was significant in Mn-only treated groups and also in combination with Cr in *MC* group. Brain Mn levels also correlated with the second:first ratio of the peak-to-peak amplitude.

Based on the results described and evaluated above, it can be stated that the attempt to model welding fume exposure in rats was successful. In a world where the large-scale use of various metals is daily reality and results in occupational exposure and environmental pollution the health effects in general, and in particular the effects on sensitive systems like the nervous system, are of primary concern. Nanotechnological application of metals is another new source of exposure to metal-containing particles, a new feature to the old problem. Especially in neurotoxicity, the study of functional alterations is important because classical biomarkers – such as levels of toxic metals in available human biological samples (blood or urine) – do not indicate well the damage to central or peripheral nervous system. This problem has been repeatedly raised in the literature. Animal model experiments can contribute to the development of neuro-functional biomarkers which may be better suited for this purpose.

The questions formulated in the particular point of aims can now be answered as follows:

- The general and nervous system effects of Mn were approximately identical to those found in previous experiments. The effects of Cr were, under identical conditions, partly similar to those of Mn. This similarity could be due to shared mechanisms of action. Data on neurotoxic effects of Cr are scarce in the literature so the findings described in this thesis may be novel. The effects of Fe were partly minimal, partly opposite to those of Mn.
- The interactions of the three metal studied were dissimilar on various parameters. The effect of Mn on body weight gain, and on electrophysiological and some OF parameters, were counteracted by Fe, but Cr was apparently not involved in such interaction. In the tissue metal levels, Fe acted on Cr but not on Mn. All that indicated that measured internal dose is not the sole determinant of the functional alterations and so, not an ideal biomarker of effect.
- Even when Mn was applied alone, the physicochemical form and site of application greatly influenced the resulting internal dose which may be of importance in case of various forms of human exposure.
- Electrophysiological tests, including double pulse stimulation and second:first ratio calculation, may well be suitable for detection and follow-up of functional damages in the nervous system but the particular form applied in the present work turned out not to be optimal.

# The Applicant's Relevant Publications

Horváth E, *Máté Zs*, Takács Sz, Pusztai P, Sápi A, Kónya Z, Nagymajtényi L, Papp A (2012) General and electrophysiological toxic effects of manganese in rats following subacute administration in dissolved and nanoparticle form. The Scientific World J, Article ID 520632. IF: 1.524

*Máté Zs*, Szabó A, Paulik E, Jancsó Zs, Hermesz E, Papp A (2011) Electrophysiological and biochemical response in rats on intratracheal instillation of manganese. Centr Eur J Biol 6, 925-932. IF: 0.685

*Máté Zs*, Szabó A, Paulik E, Papp A (2011) Experimental neurophysiological alterations caused by combined nano-manganese exposure. Fiziologia (Timisoara) 21, 17-20.

*Máté Zs*, Szabó A, Oszlánczi G, Papp A (2011) Per os Mn-expozíció modellezése és funkcionális idegrendszeri hatásának vizsgálata patkányban. Egészségtudomány 55, 71-81.

*Máté Zs*, Szabó A, Papp A (2008) Looking for parameters of early and delayed functional neurophysiological alterations caused by manganese. In: Galbács Z. (Ed.): 15th Symposium on Analytical and Environmental Problems, Szeged, 102-105.

## Abstracts

*Máté Zs*, Szabó A, Horváth E, Kovács K, Tombácz E, Papp A (2012) Nervous system effects after combined intratracheal metal exposure in rats. IBRO Internationaal Workshop, Pécs. Clin Neurosci 65 Suppl. 1, p. 44.

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