CENTRAL NERVOUS EFFECTS OF MANGANESE IN RATS INVESTIGATED BY REPEATED SIMULTANEOUS ELECTROPHYSIOLOGICAL AND BEHAVIORAL RECORDING

Summary of PhD Thesis

Szabolcs Takács

Supervisor: András Papp PhD

Department of Public Health
University of Szeged
Szeged
2012

CENTRAL NERVOUS EFFECTS OF MANGANESE IN RATS INVESTIGATED BY REPEATED SIMULTANEOUS ELECTROPHYSIOLOGICAL AND BEHAVIORAL RECORDING

Summary of PhD Thesis

Szabolcs Takács

Supervisor: András Papp PhD

Department of Public Health

University of Szeged

Szeged

2012

INTRODUCTION

Neurotoxic studies represent an especially important part within the whole complexity of investigating the biological interactions of living organisms and their environment.

Nervous system plays an essential role in perception of relevant external stimuli and in shaping the organism's adequate response to these in order to secure its survival. In case of humans, an improperly functioning nervous system may greatly affect the quality of life at individual level, and cause a loss of mental power, being perhaps the most valuable human resource at population level.

Human environment (including workplaces, homes, outdoor areas etc.) can contain various neurotoxicants, being of both natural and synthetic origin, and exposing humans by inhalation, by consumption of foods and drinking water, or via the skin. The major groups of relevant toxicants include both organics and inorganics, mainly metals and metal compounds including manganese (Mn).

In pure state, manganese (Mn) is a silvery-grey, hard and brittle metal. In such form it is not used but its alloys and compounds are found in numerous practical applications from steelmaking to nano-electronics. Mn shows various oxidation states between 2 and 7; which is of toxicological importance. It is an essential trace element for all living organisms but has toxic effects when overdosed. Ingested Mn is absorbed to 5-15% only, in a regulated process where Mn overload leads to decreased absorption rate. Parenteral absorption, e.g. after inhalation, can be much more efficient which also has toxicological relevance.

One of the Mn containing enzymes, glutamine synthetase, is a glia-specific Mn-protein that catalyses the conversion (that is, inactivation) of the excitatory transmitter glutamate to glutamine. This enzyme requires Mn but is inhibited by Mn excess, resulting in neurotoxicity.

Mitochondrial complex II and complex III can also be inhibited by Mn excess, leading to energy shortage and oxidative stress. The latter is counteracted (among others) by another Mn-enzyme, Mn-containing superoxide dismutase (Mn-SOD). Inhibited mitochondrial function probably blocks tyrosine hydroxylation which contributes to dopaminergic dysfunction, besides Mn-induced autooxidation of dopamine.

Occupational Mn exposure typically means chronic inhalation of metal dusts and fumes (in jobs like welding, steel casting, ferromanganese production etc), and causes a state called manganism. This disease starts with nonspecific symptoms including apathy, anorexia, asthenia, headache, hypersomnia, spasms etc., and ends with a Parkinson-like syndrome. One of these symptoms is bradykinesia but in the early phase behavioral disinhibition with

hypermotility also occurs. And, in spite of the similar symptoms, the site of damage in manganism and in Parkinson's disease is different, since Mn affects the striatal, and not the mesencephalic, dopaminergic neurons. Parkinson-like disorder caused by Mn was also observed in patients undergoing maintenance hemodialysis, or high oral doses e.g. from high-Mn drinking water.

The neurotoxic spectrum of Mn is variable. Beyond classical manganism it includes, among others, epileptic disorders, observed primarily in Mn-overexposed children or young adults. So, Mn is among the environmental factors occasionally causing epilepsy. Epilepsy is, defined by the International League Against Epilepsy, a brain disorder characterized by the prolonged or steady predisposition to produce epileptic seizures. These are in turn defined as transient occurrences of signs or symptoms of abnormal – excessive or hypersynchronous – activity of brain neurons. In exposed workers with elevated blood Mn level, electroencephalographic (EEG) and visual evoked potential alterations were also observed.

The functional neurotoxicity of Mn has been investigated at the Department of Public Health for ca. 15 years. Electrophysiological and behavioral methods both brought a substantial amount of new information. However, comparison of effects on electrical activity and open field motility was encumbered by the fact that electrophysiological recording was done in anaesthesia and hence its data could not be directly put in parallel with the behavioral effects. In the meantime, it became possible to perform repeated simultaneous recording of cortical electrical activity (electrocorticogram, ECoG) and open field (OF) motility in awake rats. In the research work described in this thesis, rats prepared for such chronic recording were used, and Mn was administered to them for 4 to 8-10 weeks orally by drinking water or via the airways by intratracheal instillation. The particular aims of the work were:

- To observe the development of the CNS effects of Mn in time;
- To examine to what extent changes in motor behavior and in cortical electrical activity develop in parallel, and what relationship can be detected between the two; and
- To examine whether the epileptogenic effect of Mn, mentioned in the literature, can be observed under the experimental conditions applied.

METHODS

To implement these aims, four experiments (summarized in the table 1) were performed on adult male rats. In Experiment 1, 2 and 3 these were Wistars of 10-11 weeks of age, with ca. 350 g body weight, and were obtained from the breeding centre of the University of Szeged.

Rats of this age were needed because their skull bones are strong and no more growing. In Experiment 4, WAG/Rij rats were used (representing a strain specially developed to model human temporal lobe epilepsy, and showing short bursts of spike-and-wave discharges, SWD, in their cortical electrical activity). A stock of parent males and females was obtained from Charles River, Germany. They were bred several times with the maximal exclusion of in-breeding, and the male offspring were used in the experiments.

Table 1

Experiment No.	**Rat strain**	**Manganese treatment**			**Number of rats evaluated**			
		Substance	Dose	Time	Open Field and general tox.		ECoG	
					Treated	*Control*	*Treated*	*Control*
1	Wistar	$MnCl_2$, oral, by drinking water	2.5 mg/mL water	4 weeks	4	4	3	3
2	Wistar	$MnCl_2$, oral by drinking water	7.5 mg/mL water	8 weeks	4	4	4	3
3	Wistar	MnO_2 nano intratracheal	2.63 mg/kg. b.w.	8 weeks	7	4 (UnT) 3 (VT)	5	3 (UnT) 2 (VT)
4	WAG/Rij	$MnCl_2$, oral by drinking water	7.5 mg/mL water	8 weeks	4	3	4	3

UnT, untreated control; VT, vehicle-treated control in Experiment 3.

For repeated recording of ECoG, the rats were implanted with chronic electrodes and a connector "crown", in an operation carried out under aseptic conditions in general anesthesia (isoflurane in 100% O_2, 4.5% for induction and 2-3% for maintenance).

After ca. 10 days recovery, one recording session per week was made. The first two were control sessions with one week interval and without any treatment. Then, Mn administration for the treated rats (and vehicle administration for the controls in Experiment 3) was started, and lasted as given in Table 1 with further recording sessions in one week intervals. The sessions lasted 30 (Experiment 1) or 60 (Experiment 2, 3 and 4) minutes. $MnCl_2$ was dissolved in normal tapwater and given in the drinking bottle. In Experiment 3, MnO_2 nanoparticles of ca. 30 nm diameter were instilled in the treated rats' trachea.

OF activity of the rats was recorded in a black plastic box with 48x48x40 cm inner space, equipped with an array of infrared light gates at floor level. The instrument recorded and

analyzed the rat's horizontal motor activity based on the interruptions of the infrared beams, using the software Conducta 1.3 (Experimetria Ltd, Hungary). Detection of vertical activity (rearing) was deliberately omitted because of the false signals the crown and cable would have produced. The room used for recording was lit dimly. The software automatically processed beam interruption data to numerical description of motility (ambulation, local activity or immobility; total time and event counts, plus ambulation distance). The data were obtained and evaluated for the whole 30 or 60 min session, and also in 3 min periods within a session to see short-term changes.

The rat's cortical electrical activity was taken up by a preamplifier mounted on the end of the flexible lead-off cable connected to the crown. The two lead-off points on the left and right hemisphere, respectively, provided one bipolar channel each. Overall amplification was 10^4 x with high- and low-pass filters set to 1.6 and 75 Hz. The ECoG signals were visualized on the monitor of a PC in real time and stored on the hard disk, using the software Neurosys EEG v1.1.0.72 of Experimetria. No major difference between the electrical activity on the two channels was seen. So, signals of channel 1 (left hemisphere) were used for analysis of ECoG. From the ECoG records, the software calculated the continuous power spectrum with 0.5 Hz resolution and the total power between 1 and 49 Hz. The analysis was based on FFT technique. Evaluation was based on the shape of the power spectrum curve, and on difference spectra obtained as the ratio of a spectrum from a given phase of treatment and a control spectrum. Band spectra were obtained to see any correlation with variables of motility. The band limits were set as follows: delta , between 1 and 3.5 Hz; theta, between 4 and 7.5 Hz; alpha, 8-12 Hz; low beta, 12.5-15; mid beta, 15.5-18; high beta, 18.5-31, and gamma, 31.5-44 Hz. The lower part of beta was cut in two bands to see better any change around 13-15 Hz where the epileptogenic effect of Mn typically appeared. Correlation between OF, ECoG and tissue Mn level data was tested by the "linear fit" function of Excel after plotting corresponding data pairs in an X-Y plot.

Spectrum calculations were done for the whole, 30 or 60 minutes long, recording session, or for shorter periods. Three minute periods were used to see the change within one 30 or 60 min session; and 1 s resolution was used in Experiment 4 to obtain numerical data of the visually detected epileptic SWD burst. For that, the maximum and minimum of ECoG power in the 12.5-18 Hz range (low and mid beta) was determined for every 1 s period, and the value of *[(max-min)/2 + min];* called peak indicator, calculated. This value was then used to decide which seconds are to be regarded as belonging to a burst. The numerical criteria were set to achieve maximal agreement between the bursts observed on the ECoG and the calculations

Body weight data were plotted against the days of the experiment to obtain weight gain curves. Tissue Mn level determination was done by inductively coupled plasma mass spectrometry.

Due to the small data pool, a simple statistical evaluation was done. Corresponding data of different groups (treated or control) were taken as one data set each and were compared by means of two-sample t-test (Experiment 1, 2 and 4) or one-way ANOVA (Experiment 3). Significance was accepted at $p<0.05$.

RESULTS

Body weight gain of the rats was not affected by Mn exposure in Experiments 1, 2 and 4. In Experiment 3, there seemed to be a minimal lag in weight gain in the treated rats which might have been due partly to the treatment procedure. There was, however, significant Mn deposition in the treated rats' tissue samples (Table 2).

Table 2

		Tissue Mn level, µg/kg		
		Blood	**Brain**	**Liver**
Exp. 1	Control	181.46±60.17	984.71±132.56	7132.24±471.74
	Treated	281.35±63.62*	1931.88±156.93***	8022.97±609.25*
Exp. 2	Control	181.46±60.17	984.71±132.56	7132.24±471.74
	Treated	502.42±114.98**	3394.55±358.72***	11304.12±3042.25*
Exp. 3	Control (VT)	285.43±133.21	2072.53±358.16	7369.43±1837.86
	Mn-treated	979.08±389.31**	11210.89±1393.34***	12309.07±1553.89*
Exp. 4	Control	385.86±131.78	2269.57±680.70	6865.94±920.33
	Treated	440.95±88.18	4983.24±2051.48*	11671.06±3192.37*

Mean±SD, n: see Table 1. *, **, ***: $p<0.05$, 0.01, 0.001 vs. Control.

It was one of the major findings of the work that these Mn levels were caused by highly dissimilar summed doses (Experiment 1: ca.1100 mg Mn in 4 weeks, oral; Experiment 2: ca. 5700 mg Mn. in 10 weeks, oral; Experiment 3: ca. 40 mg Mn in 8 weeks, intratracheal).

In **Experiment 1**, the treated rats' OF motility was higher than that of the controls from the 2nd treatment week on, and their habituation to the OF environment, characterized by the decrease of ambulation during a 30 min recording session, was also less. The numerical data of immobility showed an opposite trend. Overall cortical electrical activity, quantified as the

total power in the 1-49 Hz range, decreased substantially in the treated rats by the end of the 4th treatment week but did not change much in the controls. The difference spectra showed that the ECoG power decrease was present over the whole 1-49 Hz frequency range in the treated rats but it appeared first around the 7 Hz maximum. The time course of the OF parameters and the ECoG power during the 6 weeks suggested a correlation between the two. In fact, $R^2>0.5$ was found for theta, high beta and gamma in the Mn-treated and for theta, alpha, mid beta and high beta in the control rats, for the correlation with the time of ambulation and immobility.

In **Experiment 2**, the rats were exposed to 7.5 mg $MnCl_2$/mL water for at least 8 weeks. Here, the Mn-treated rats showed mildly decreased motility towards the end of the exposure period (might be interpreted as transition to the later phase of Mn-induced Parkinson-like disorder). Ambulation time data of individual rats on the last week and Mn levels in the brain samples were correlated ($R^2>0.5$). Within the 60 min recording sessions, immobility in the treated rats decreased less steeply than in the controls. Decrease in the ECoG total power (like in Experiment 1) was seen also only in the last recordings. The difference spectra showed gradually developing decrease of ECoG power in the Mn-treated rats, around the 7 Hz peak and above 30 Hz.

In correspondence with the decreased gamma activity, correlation with good R^2 values was found between the time trend of ECoG gamma band power and several OF parameters in the treated rats – even if the OF parameters themselves did not change significantly. This indicated that the correlation of two functional parameters, supposedly influenced by Mn, might be more sensitive than the parameters alone. Local activity time and ECoG total power showed fair correlation to brain Mn level.

In this experiment, Mn dose and treatment time were apparently sufficient to provoke epileptiform activity. Towards the end of the treatment period, short series of spikes were observed in two of the four exposed rats, similar to those seen in the WAG/Rij rats used in Experiment 4, and were coincident with decreased motility and a typical peak on the ECoG spectrum around 13 Hz.

In **Experiment 3**, Mn treatment was done by intratracheal instillation of MnO_2 nanoparticles, 5 days per week, for 8-10 weeks. This resulted in higher inner Mn doses and stronger neuro-functional alterations. The inner Mn dose was sufficient to induce significant hypomotility: decrease of ambulation time was seen in the 3rd week of the experiment, after only 5 days of Mn administrations. The decrease developed further in the first 4-5 weeks of treatment but showed minimal change afterwards. A similarly manifest but opposite trend was seen in

immobility, and immobility also showed more intense increase during a 60 min recording session.

ECoG total power increased starting with the 4th week of Mn exposure. Maximal increase was seen in the 7th week; after that, some decrease followed. The direction of ECoG total power change was opposite to that seen in the previous experiments and supported what was suggested by the OF data, namely that with various inner Mn doses not only the strength but also the direction of the Mn-induced functional changes can vary. The difference spectra showed stability in untreated controls. In vehicle treated rats, there were deviations indicating alteration vs. the pre-treatment period but without a clear trend. In Mn-treated rats, however, the difference curves showed a gradually developing power increase which resulted in the relative reduction of the 7 Hz peak.

Ambulation distance, time and count had fairly good correlation to brain Mn levels. The correlation of ambulation distance and time, and immobility time, with low beta, mid beta and high beta power in the treated rats was strong (R^2>0.5).

From two treated rats in Experiment 3, tail vein blood was taken on the 4th and 8th week. The measured Mn level in these samples and in the final blood sample (10th week) were correlated to ambulation distance and to ECoG total power, suggesting that the non-linear time trend of OF and ECoG was due to a non-linear trend of Mn deposition in the rats' body.

In this experiment, the kind of epileptiform activity observed earlier (short series of spikes on the ECoG) were occasionally seen. The epileptogenic action of Mn was further investigated in the next experiment whether and how Mn exposure alters the spontaneous epileptic activity of WAG/Rij rats, in:

Experiment 4. In the Mn-treated WAG rats, decrease of ambulation and increase of immobility during the weeks of Mn exposure – due partly to habituation and partly to aging – was much less than in the controls. Also, the increase of immobility within one 60 min recording session became more pronounced in the controls as the weeks elapsed but in the treated rats the trend was opposite.

Total ECoG power increased massively in the treated rats on commencing the Mn exposure. This was not reflected in the general shape of power spectra but the difference spectra and their time trend were markedly dissimilar in the treated and control rats. The difference spectra of the Mn-treated WAG rats were partly similar to those in Experiment 2 and 3 which is another indication of the latent epileptogenic effect of Mn in those experiments.

In the treated rats, the time spent in ambulation and in immobility was strongly correlated to ECoG total power and to the power of the low beta band. These OF parameters were also

strongly correlated to brain Mn level, whereas the correlation of ECoG low beta power to brain Mn was weaker.

The ECoG of WAG rats is characterized by short (5-15 s) bursts of SWDs, during which a peak appears on the ECoG power spectrum around 13-15 Hz (approximately the the low beta band). The ECoG power spectrum underlined the importance of low beta. In silent phase, the peak around 7 Hz was visible, while in high-amplitude irregular activity the elevation of the spectrum curve right of the peak made the peak disappear. Typically this activity appeared when the initial exploratory reaction of the animal put into the OF box was over. During a burst, the 13-15 Hz peak was present. Hence, the power in the low and mid beta band was used to measure the intensity of epileptic activity. ECoG low beta power during the bursts increased more than the number of bursts itself. Especially the power for one bursting second increased in the treated rats, indicating that bursting – manifested in frequent and high-amplitude deflections from the isoelectric line – was more intense under Mn influence. The ratio of low beta power in bursting/silent seconds was, on the contrary, higher in the controls, probably because in the Mn-treated rats the high-amplitude irregular cortical activity was more abundant in non-bursting periods.

DISCUSSION

In all four experiments, administration of Mn in different chemical forms and doses resulted in significant tissue Mn deposition (inner dose) and in alterations of CNS functional parameters. Body weight gain and organ weights indicated no noteworthy general toxicity even at the highest inner doses in Experiment 3 which suggested there was no major general toxic effect of Mn that could be responsible for the observed neuro-functional effects.

Based on the correlations observed in each experiment, one can broadly state that the changes in OF motility and in cortical electrical activity developed in parallel. The neuro-functional effects were apparently highly dependent on the inner Mn doses and on the length of exposure; not only in terms of strength but also in direction. In Experiment 1 (where the dose and time of Mn exposure, and the resulting inner dose, was the lowest) treated rats had higher OF motility vs. the controls, which could be likened to the early phase of adult human manganism, characterized by behavioral disinhibition, and to attention deficit and hyperactivity observed in children consuming high-Mn drinking water.

In Experiment 2, and even more in Experiment 3, the dose and length of Mn exposure was longer and the final tissue Mn levels were sufficient to induce the late “established” stage of

Mn-induced Parkinson-like disorder. There were more expressed alterations in the first ca. 4 weeks of exposure than afterwards, possibly due to decreased absorption and/or increased excretion of Mn. In Experiment 3, the actual ambulation time and in ECoG total power data during the exposure period were correlated to the blood Mn levels, demonstrating directly what was suggested by earlier result of the Department, namely that the deposition of Mn during a longer treatment period was not linear in time and that the functional alterations depended directly on brain Mn level. Taking Experiments 1 to 3 together, an up-and-down shape of the dependence of OF motility on the (presumable) total inner Mn dose could be conceived, which was in some sense similar to the published time course of CNS effect of 3-nitropropionic acid, the mechanism of action of which is partly identical to that of Mn.

The typically fair correlations among changes in the OF or ECoG parameters and the measured brain Mn levels raise the question what beyond the presence of Mn in the brain could be the common background.

One such mechanism is the effect of Mn on astrocytic glutamate metabolism. Astrocytes not only take up glutamate and convert it to glutamine but supply that as transmitter precursor for both glutamatergic and GABAergic neurons. On action of Mn^{2+}, both glutamate uptake and transformation to glutamine are reduced. The result may be the imbalance between excitation and inhibition, not only because of excess free glutamate but because of loss of negative feedback between glutamate and GABA if glutamate-uptake induced GABA release from the astrocytes is lost. This feedback is supposed to act mainly when excitation is elevated, such as in (true or modelled) epilepsy, and might provide explanation to the increased intensity of bursting in the Mn-treated WAG rats in Experiment 4 – but the case is more complex because in WAG rats systemically given GABA agonists do strengthen, and not suppress, bursting.

The source of the bursts in WAG rats is apparently the perioral area within the somatosensory cortex of the rats where the motor impulses to drive the exploratory sweeping movements of the whiskers, with 7-12 Hz frequency, are generated. Under natural (no stimuli, no motion) or artificial (diminution of sensory input, this oscillatory tendency can spread the synchronic activity to the whole cortex. In Experiment 2 and 3, the occasional bursts appeared always when the animal was beyond the initial exploratory phase and was more or less motionless. Thus, Mn could promote bursting also by reducing the treated rats' motility, besides the effect on transmitters. Inhibited function of astrocytic glutamine synthetase is probably involved also in human CNS disorders, including various forms of epilepsy. There are a few reports on the epileptogenic effect of abnormally high Mn levels in children or young adults. At this point it is of interest that absence seizures, to which WAG rats serve as a model also occur

typically during childhood. High extracellular glutamate level is universally deteriorative for the CNS – due to imbalance between excitation and inhibition, to excitotoxicity, and to oxidative stress. The dopaminergic system is especially sensitive to oxidative stress, due to the autooxidizing tendency of dopamine and to the presence of monoamine oxidase. Spontaneous locomotor activity in the OF is regulated by mesolimbic/mesocortical dopaminergic structures. Increased immobility in Experiment 2 and 3 was in line with the symptoms of heavily Mn-exposed welders suffering from Parkinson-like syndrome.

The neuro-functional alterations observed in the Mn-treated rats were to some extent analogous to the effects in exposed humans, regarding both cortical electrical activity and motor behavior. As blood or urine Mn levels as routine measurements do not adequately characterize CNS damage, functional biomarkers of Mn effect, to be developed in the future on the basis of experimental results similar to those presented in this Thesis, may be more suitable for this purpose.

The particular points of aims could finally be answered as follows:

- The CNS effects of Mn developed in a non-monotonous way. Depending on the dose and length of exposure, the direction of changes varied (Experiment 1 vs. Experiment 2 and 3); and in case of longer (8-10 weeks) exposure, the effects developed mostly in the first 4-5 weeks and showed little change thereafter.
- The changes in motor behavior and in cortical electrical activity developed in parallel in time. Usually there was a fair correlation between numerical parameters of OF motility and of ECoG (total or band power), and inner Mn dose (brain Mn level) at the end of Mn treatment. In a few cases, the parallel changes of OF and ECoG parameters with blood Mn level during the treatment period could be showed. This raises the possibility to develop neuro-functional biomarkers of effect for human Mn poisoning.
- The epileptogenic effect of Mn was occasionally observed in Wistar rats in Experiment 2 and 3. In the epilepsy-prone WAG/Rij rats in Experiment 4, Mn treatment intensified bursting activity.
- Beside the above points, a further noteworthy conclusion could be drawn, namely that intratracheal administration of Mn in form of MnO_2 nanoparticles was far more efficient in causing high inner Mn doses than dissolved Mn applied via the drinking water.

THE APPLICANT'S RELEVANT PUBLICATIONS

I. *Takács Sz*, Bankó S, Papp A (2010) Effect of mitochondrial toxins on evoked somatosensory activity in rats. **Central European Journal of Biology** 5, 293–298. IF: 0.685

II. *Takács Sz*, Szabó A, Oszlánczi G, Paulik E, Papp A (2011) A pilot study with simultaneous recording of changes in motility and cortical electrical activity of rats during four weeks of oral manganese exposure. **International Journal of Environmental Health Research** DOI:10.1080/09603123.2011.643228. IF: 1.090

III. *Takács Sz.,* Papp A (2011) Neurotoxicity of manganese analysed by a novel combined electrophysiological-behavioral system. In Galbács Z ed.: **17th International Symposium on Analytical and Environmental Problems,** Szeged. pp. 210.214.

IV. *Takács Sz*, Szabó A, Oszlánczi G, Pusztai P, Sápi A, Kónya Z, Papp A (2012) Repeated simultaneous cortical electrophysiological and behavioral recording in rats exposed to manganese-containing nanoparticles. **Acta Biologica Hungarica**, accepted. IF: 0.793

Abstracts:

Takács Sz, Bankó S, Nagymajtényi L, Papp A (2009) Acute and subacute functional neurotoxicity of manganese in rats. **FEPS 2009**, Ljubljana, Szlovénia, 2009. november 12-15. Book of Abstracts p. 254.

Takács Sz, Papp A (2010) Changes of cortical electrical activity and open field motility in WAG rats during several weeks of oral manganese exposure. **IBRO International Workshop 2010**, Pécs, 2010. január 21-23. Frontiers in Neuroscience DOI 10.3389/conf.fnins.2010.10.00067.

Takács Sz, Papp A (2010) Antiepileptikumok és anesztetikum hatása a kérgi alapaktivitásra és a spontán motilitásra egy epilepszia hajlamos patkánytörzsben. **Magyar Élettani Társaság LXXIV. Vándorgyűlése és a Magyar Kísérletes és Klinikai Farmakológiai Társaság II. közös konferenciája**. Szeged, 2010. június 16-18. Acta Physiologica Hungarica 97, 480-481.

Papp A, *Takács Sz* (2010) Cortical electrical activity and open field motility of WAG rats during subacute oral manganese exposure. **7th Forum of European Neuroscience**, Amszterdam, 2010. július 3-7. FENS Abstracts vol.5, 164.26.

Takács Sz, Máté Zs, Horváth E, Papp A (2010) Behavioral and electrophysiological effects of intratracheal manganese exposure recorded in rats with chronically implanted cortical electrodes. **11th International Symposium Interdisciplinary Regional Research,** Szeged, 2010. október 13-15. Abstracts, p.108.

Takács Sz, Papp A (2011) Elektrokortikogram és open field motilitás változások patkányban 8 hetes orális mangán expozíció során. **Magyar Farmakológiai, Anatómus, Mikrocirkulációs, Élettani Társaságok Közös Tudományos Konferenciája (FAMÉ)**, Pécs 2011. június 8-11. Acta Physiologica 202 Suppl 684, 116-117.

ACKNOWLEDGEMENT

First, I would like to thank to Dr. László Nagymajtényi for providing the opportunity to perform my experiments at the Department of Public Health, and for always improving my work with his wise foresight and useful advices.

I am especially grateful to my supervisor, Dr. András Papp, who was always willing to help, continually smoothed the way for the completion of my thesis, and always guided my progress to the right direction. His support and guidance was vital in the thesis coming into existence.

I am thankful to my colleagues, Dr. Andrea Szabó, Dr. Edina Horváth, Zsuzsanna Máté and Dr. Gábor Oszlánczi, for their support and help in the experimental work. Many thanks to Dr. Zoltán Kónya, Dr. András Sápi and Péter Pusztai at the Department of Applied Chemistry, University of Szeged, for providing the applied nanoparticles, and to Dr. Attila Szőke, József Koszta and Edit Pálinkás at the laboratory of the MOL Hungarian Oil and Gas Company for the metal level determinations.

I also would like to thank to Mihályné Németh, Anita Balázs, Gyuláné Kiss and János Kis for their assistance in handling the animals, and to Imre Gera and Lászlóné Szalai for their help in technical questions and documentation.