Ph.D. Thesis

Experimental investigation of the behavioural toxicity of an environmental pollutant heavy metal, manganese

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THE APPLICANT'S RELEVANT PUBLICATIONS

I. *Vezér, T.,* Schulz, H., Nagymajtényi, L.: Memory effect of neurotoxic lead compunds in subacute animal experiments. *Centr. Eur. J. Occup. Environ. Med.,* 6: 209-16 (2000)

II. *Vezér, T.*, Papp. A., Nagymajtényi, L.: Behavioural alterations induced by tetraethyllead treatment in rats. In: Galbács, Z. ed.: *Proceedings of the 7th Symposium on Analytical and Environmental Problems*, Szeged, pp. 146-150 (2000)

III. Papp, A., *Vezér,* T., Nagymajtényi, L.: A complex study on the neurophysiological and behavioural effects of inorganic lead exposure in rats. In: Garban, Z., Dragan, P., eds.: *Metal Elements in Environment, Medicine and Biology*, Vol. IV, Timisoara, pp. 239-244 (2000)

IV. *Vezér, T.,* Papp, A., Nagymajtényi, L.: Chronic exposure by $HgCl_2$ in male Wistar rats: effects on behaviour performance. In: Galbács, Z. ed.: *Proceedings of the 8th Symposium on Analytical and Envrironmental Problems*, Szeged, pp. 34-39 (2001)

V. *Vezér, T.*, Papp, A.: Subchronic Exposure to Methylmercury in Male Wistar Rats: Effects on Neurobehavioral Performance. *Centr. Eur. J. Occup. Environ. Med*., 8: 131-141 (2002)

VI. Pecze, L., *Vezér, T.*, Papp, A. Effects of acutely administered heavy metals on the evoked activity recorded from the cortical ad thalamic somatosensory center in rats. *Fiziologia,* 12: 34-38 (2002)

VII. *Vezér, T.*, Papp, A., Hoyk, Z., Varga, C., Náray, M., Nagymajtényi, L.: Behavioral and neurotoxicological effects of subchronic manganese exposure in rats. *Environ. Toxicol. Pharmacol.,* 19: 797-810 (2005)
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VIII. *Vezér, T.*, Papp, A., Kurunczi, A., Párducz, Á., Náray, M., Nagymajtényi, L.: Behavioral and neurotoxic effects seen during and after subchronic exposure of rats to organic mercury. *Environ. Toxicol. Pharmacol*., 19: 785-796 (2005)
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IX. Papp, A., Nagymajtényi, L., *Vezér, T.*: Subchronic mercury treatment of rats in different phases of ontogenesis: functional effects on the central and peripheral nervous system. *Food Chem. Toxicol*., 43: 77-85 (2005)
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ABSTRACTS

I. *Vezér, T.,* Schulz, H., Nagymajtényi, L.: memory effects following subchronic organic and inorganic lead administration in rats. J. Physiol. (London) 526: 162P-163P (2000)
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II. *Vezér, T.,* Papp, A., Nagymajtényi, L.: Behavioral and neurotoxicological alterations induced by subchronic inorganic lead and mercury treatment in rats. *Neurobiology* 9: 273 (2001)

III. *Vezér, T.,* Papp, A., Nagymajtényi, L.: Behavioral alterations in male Wistar rats induced by subchronic treatment with organic lead and mercury compounds. *Toxicol. Lett.* 123 Suppl 1: 77 (2001)
Impact factor: 1,587

IV. Papp, A., *Vezér, T.,* Nagymajtényi, L.: The effect of subacute mercury exposure on behavioural and neurophysiological parameters in rats. *NeuroToxicol.* 222: 543 (2001)
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V. *Vezér, T.,* Papp, A., Nagymajtényi, L.: Subchronic exposure by methyl mercury in male Wistar rats: effects on neurobehavioral performance. *Pflügers Arch. Eur. J. Physiol.,* 442 Suppl: 369 (2002)
Impact factor: 1,695

VI. *Vezér, T.,* Papp, A.: Behavioral and neurotoxic effects obtained in rats by subchronic inorganic manganese exposure. *Clin. Neurosci., 56* Suppl 2: 96 (2003)

SUMMARY

Behavioural toxicology is becoming increasingly important in risk assessment of exposure to neurotoxic substances, due to the high sensitivity of behaviour towards neurotoxic action, and to the integration of several underlying processes and neural functions in behavioural phenomena.

In previous works of the Department it was found that heavy metals, including inorganic forms of Pb, Hg and Mn, induced marked alterations in the spontaneous and stimulus-evoked cortical activity of rats, which, among others, indicate changes of plastic representation at the cortical and subcortical level. Due to that, it was reasonable to include behavioural methods in the investigation of environmental xenobiotics, so that a more comprehensive access to the toxic effects and the underlying mechanisms can be achieved.

In human intoxication with heavy metals, higher order functions of the CNS are important aspects. Lead causes learning difficulties and IQ loss. With mercury (both inorganic and organic) a broad spectrum of sensory, motor and behavioural effects were described. The parallels of such human effects were observed in animal experiments including those done earlier at the Department. It turned out that combination of the methods available at the Department allow state-of-the-art investigation of heavy metal effects on higher order CNS functions in animal experiments. At the same time, environmental data in the literature indicated that other metals, such as manganese, deserve to be included into neurobehavioral experimentation.

Manganese is an essential micronutrient, but a potential environmental neurotoxicant in higher doses, causing childhood hyperactivity disorder, Parkinson-like extrapyramidal dysfunction, psychosis etc., which were modelled in animals.

The present experiment involved a complex behavioural test battery, supplemented by Mn level determination in blood, cortex and hippocampus, and by histological examinations. All investigations were performed both during and after the period of Mn administration. It was also attempted to prove the involvement of the dopamine (DA) system by applying a dopaminergic agonist in the elimination period.

The questions to be answered in these experiments were as follows:

- What alterations are induced by oral Mn administration in the learning and memory processes, spontaneous exploratory activity, and acoustic startle response?

- How long does it take for the alterations in higher order CNS functions to appear, and are these constant or increasing during the period of exposure to Mn?
- Is there any detectable increase in the blood Mn level, and deposition of Mn in peripheral tissues and CNS structures responsible for the behavioural effects?
- Are the Mn-induced behavioural alterations reversible upon cessation of the metal administration?
- After cessation of Mn administration, can behavioral effects, indicating altered plasticity, be elicited by means of decreasing cortical inhibition using the indirect dopaminergic agonist amphetamine?

These problems were investigated in young adult male Wistar rats. The experiments were started with 48 rats (16 animal/group). One group received the high dose (59 mg/kg b.w., 1/25 LD_{50}) and another, the low dose (15 mg/kg b.w., 1/100 LD_{50}) of Mn, for 10 weeks (treatment period) in a 5 days per week scheme, per os by gavage. The control group received distilled water. In the 12 weeks post-treatment period, no more metal was given in order to see the effects of a possible elimination. The rats' body weight was regularly measured.

In the 10 week treatment period, the rats first acquired the skill to find food in an 8-arm maze, then their short- and long-term working and reference memory was tested, and the tests were repeated in the post-treatment period. Spontaneous exploratory activity in the open field (OF) was tested before treatment, in the 5^{th} and 10^{th} treatment week, and in the post-treatment period. Acoustic startle response (ASR) and its pre-pulse inhibition (PPI) was tested in the 10^{th} treatment week and 7^{th} post-treatment week. Blood and tissue samples for Mn level determination were taken several times before, during and after Mn administration, and brain samples for immunhistochemistry, at the end of the treatment period.

In the 5^{th} and 10^{th} $MnCl_2$ treatment weeks, and in the early post-treatment period, Mn in the blood and peripheral tissues of the high dose group was significantly elevated vs. control. The elimination of Mn from the kidneys and femur was slower than form the blood. The cortical and hippocampal Mn level was also significantly elevated by the 10^{th} week in the high dose rats but returned to control by the end of the post-treatment period.

During acquisition (2^{nd} week of treatment), in the short term 4 hours working memory (5^{th} treatment week), reference memory (4^{th} treatment week), and in all of the long term memory retention tests (9^{th}-10^{th} treatment and 4^{th}-5^{th} post-treatment weeks), both Mn-treated groups showed significant, dose dependent decrease in the average memory performance.

After two weeks without Mn, memory return was greatly improved in the high dose group compared to the 8th week. In the long-term retention tests, however, no noteworthy change in any of the treated groups was seen.

In the open field activity, a general decreasing trend was observed which was due to the aging of the animals. Irrespective of that, $MnCl_2$ treated animals had decreased spontaneous activities. Horizontal activity of the treated animals decreased in the 5th and 10th week in a dose-and time-dependent manner. Decrease of the local activity became significant by the 10th week. The number of rearings was reduced in a dose-and time-dependent manner by the 5th week. After 7 weeks without treatment the difference between the control and treated groups was minimal. In the 20th experimental week, 1.5 mg/kg d-amphetamine was given ip., whereby local activity decreased, and horizontal and vertical activity increased, vs. control.

The number of ASR responses was dose-dependently decreased in he Mn treated rats by he end of treatment. In the 7th post-treatment week, the treated vs. control difference was nearly unchanged. Onset latency of the ASR response increased significantly in the 10th treatment week in both treated groups vs. control but in the 7th post-treatment week, only the high dose group showed significantly lengthened latency. At the end of treatment, PPI was significantly reduced in both treated groups (especially in the low dose group) vs. control, so that the inhibition turned to facilitation. In the 7th post-treatment week, this was no more seen.

Body weight gain was also affected by Mn. In the 6th to 10th treatment weeks, the weekly average body weight in the high dose group was significantly reduced compared to the controls. After the 8th treatment week, also the low dose group had significantly reduced body weight. In the high dose group, the difference did not disappear in the post-treatment period. By the end of treatment, only the adrenals showed significant relative weight difference. The density of GFAP immunoreactive structures was significantly and dose-dependently increased in the hilar part of the dentate gyrus, but not in the hippocampal CA1 region.

The results of the presented study can be summarized as follows:

- Oral administration of Mn for 10 weeks resulted in significant decrease of the maze learning performance, OF activity, and the number of ASR responses.
- The majority of the behavioural effects of Mn was detectable already after 5 weeks treatment, and these became more expressed by the 10th week.

- The Mn content in blood, peripheral tissue samples, and in the cortex and hippocampus, increased significantly by the end of the treatment period.
- Some of the Mn-induced behavioural alterations, first of all in the OF, were reversible on cessation of the administration, while others, like decreased working memory and the ASR effects, seemed to be permanent.
- Administration of d-amphetamine revealed a lasting effect of Mn on the mechanisms involved in the OF behaviour.

Heavy metals are xenobiotics with significant presence in the general and occupational environment and corresponding risk populations. This points to the necessity of a sensitive and informative, but non-invasive testing method, especially because a neurotoxic effect can have various indirect consequences. In the study presented here, several behavioural functions showed marked or significant alterations. The results of animal experiments cannot be directly transferred to man, but if an experimental approach is complex (including chemical analysis, neurophysiological recording and behavioural tests), standardizable, and reveals sensitive markers, it can provide the base of developing methods suitable for population level investigations. Our study, and its planned extensions, can be a contribution to developing such methods.

TABLE OF CONTENTS

ABBREVIATIONS

accumb	nucleus accumbens
ACh	acethylcholine
AChE	acethylcolinesterase
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid
ASR	acoustic startle response
cAMP	cyclic adenosyl monophosphate
CNS	central nervous system
CSPP	the limbic cortical-striatal-pallidal-pedunculopontine circuitry
d-A	d-amphetamine
DG	dentate gyrus
EC	entorchinal cortex
EP	entopeduncular nucleus
ff	fimbria fornix
GABA	γ-amino-butiric acid
GFAP	glial fibrillary acidic protein
GFAP-IR	glial fibrillary acidic protein-immunoreactivity
Glu	glutamate
HC	hippocampus
5-HT	serotonine
IQ	intelligence quotient
LTP	long-term potentiation
mf	mossy fibers
MMT	methylcyclopentadienyl manganese tricarbonyl
neostr.	neostriatum
NGS	normal goat serum
NA	noradrenaline
NMDA	N-methyl-D-aspartate
OF	open field
PB	phosphate buffer
PnC	nucleus reticularis pontis caudalis (caudal pontine reticular formation)
pp	perforant pathway
PPI	pre-pulse inhibition
PPTg	pedunculopontine tegmentum
RM	reference memory
Sc	Schaffer collaterals
SNr	substantia nigra
TBS	tris buffered saline
v. pall.	ventral pallidum
WM	working memory
WM R	repeated working memory