The Applicant's Publications referred to in the Thesis

I. **Lukács A.,** Szabó A., Nagymajtényi L. 2005. Behavioural and neurotoxicological effects following acute and subchronic administration of MK-801 in rats. Proceedings of 12th Symposium on Analitical and Environmental Problems pp. 201-205.

II. **Lukács A.,** Szabó A., Nagymajtényi L. 2005. Changes in neurobehavioural parameters and spontaneous cortical activity in rats after acute single and combined exposure by 3-nitropropionic acid and MK-801. Homeostasis 43: 169-172.

III. **Lukács A.,** Szabó A., Lengyel Zs. 2005. Central nervous effects of 3-nitropropionic acid and MK-801 elicited by acute single and combined administration in rats. Centr Europ J Occup Environ Med 11: 319-325.

IV. Szabó A., **Lukács A.,** Nagymajtényi L. 2005. Neurobehavioural and electrophysiological alterations in rats acutely treated with the mitochondrial toxin 3-nitropropionic acid and its functional antagonist, MK-801. Homeostasis 43: 173-178.

V. Szabó A., **Lukács A.,** Nagymajtényi L. 2005. Neurobehavioural and electrophysiological alterations in rats acutely treated with the neurodegenerative 3-nitropropionic acid and its functional antagonist, MK-801. Centr Europ J Occup Environ Med 11: 327-336.

VI. **Lukács A.,** Szabó A., Vezér T., Papp A. 2006. The acute effects of 3-nitropropionic acid on the behavior and spontaneous cortical electrical activity of rats. Acta Neurobiol Exp 66: 227-233. *Impact factor: 1.209*

VII. **Lukács A.,** Szabó A., Papp A., Nagymajtényi L. 2006. Behavioural alterations induced by acute and subacute administration of 3-nitropropionic acid in rats. Centr Europ J Occup Environ Med 12: 309-316.

VIII. **Lukács A.,** Szabó A. 2006. A 3-nitropropionsav akut és szubkrónikus adagolásával kiváltott magatartás-toxikológiai változások vizsgálata patkányban. Egészségtudomány 3-4: 202-208. (Az MHT Fiatal Higiénikusok II. Fórumán II. díjat nyert előadás alapján, felkérésre megírt közlemény.)

IX. Szabó A., Lengyel Zs., **Lukács A.,** Papp A. 2006. Studies on the neurotoxicity of arsenic in rats in different exposure timing schemes. Trace Elem Electroly 23: 193-198. *Impact factor: 0.571*

X. **Lukács A.,** Lengyel Zs., Institóris L., Szabó A. 2007. Subchronic heavy metal and alcohol treatment in rats: changes in the somatosensory evoked cortical activity. Acta Biol Hung 58 (3). Accepted for publication. *Impact factor: 0.636*

Summary

The term, neurodegenerative disorders, implies that there is no exact knowledge of the cause or pathogenesis of these diseases. These diseases have been attracting great research interest, because drugs, able to influence their pathogenesis, are not available, and because their pathomechanism can be studied in animal models.

In the pathogenesis of Huntington's disease (HD; an autosomal dominant hereditary neurodegenerative disorder) excitotoxicity likely plays an important role. Excitotoxicity includes a dysfunction of excitatory amino acid neurotransmission, usually a hyperstimulation of glutamate receptors. In the central nervous system, the striatum is the most glutamaceptive region and is thus predisposed to excitotoxicity. Excess activation of N-methyl-D-aspartate (NMDA) receptors induces apoptosis or necrosis of the cells expressing them, which will result in behavioural and functional disorders. Prior to cell death, dopaminergic neurons can be over-excited and can release dopamine (DA), which itself can be also neurotoxic.

Mitochondrial toxins – such as 3-nitropropionic acid (3-NP) – lead to a pattern of striatal atrophy similar to that seen in HD, and constitute so a tool in studying the disease in animal models. Beyond the histological effects themselves, bilateral striatal degeneration was shown to have neurobehavioural consequences. These included alteration of locomotor activity, startle reflex abnormalities, and reduced sensorimotor gating. In patients with HD, first hyper- then hypoactivity, and impaired pre-pulse inhibition (PPI), can be observed. Beside the pattern of striatal atrophy similar to that of HD, neurobehavioural alterations induced by 3-NP also underline its important role in modelling the disease.

In the background of 3-NP induced neurobehavioural alterations, both glutamatergic and dopaminergic functions are supposed. In order to investigate the action of 3-NP on these neurotransmitter systems, drugs which are known to act on these were chosen: the NMDA receptor antagonist, dizocilpine maleate (MK-801); the D_2 receptor agonist, quinpirole (QP); and the D_2 antagonist, sulpiride (SP).

The aim of the study was to reveal the mechanism of neurobehavioural alterations more in detail, thereby contributing to the improvement of the behavioural aspect of the

HD model, so that the potential protective agents could be investigated on behavioural, and not only histological, endpoints.

Adult male Wistar rats were used in the behavioural experiments. Administration of the drugs and neurobehavioural investigations were done in two, "immediate" and "acute", experimental schemes. The behavioural tests: open field (OF), rotarod, and acoustic startle response without (ASR) and with (PPI) pre-pulse stimulus were performed before drug application, and were repeated 30 after injection in the immediate, and 24 after it in the acute scheme. 3-NP (20 mg/kg) and MK-801 (0.8 mg/kg) was given the rats by intraperitoneal injection, alone or combined. In the combination groups, 30 minutes were left between injecting the first and the second drug. QP (5 mg/kg) or SP (80 mg/kg) was injected subcutaneously 15 min before the administration of 3-NP or MK-801. Controls were injected with saline.

The rats' spontaneous exploratory activity was investigated in the OF apparatus (Conducta 1.0 System, Experimetria Ltd.) in 10-minute sessions. Ambulatory, vertical and local activity was computed on the basis of infrared beam interruptions detecting the animal's movements.

For investigating the effects of drugs on motor coordination, the rats' rotarod performance was tested. Before the beginning the experiment, the rats received a preliminary training on the rotarod (ROTA-ROD for rats 47700, Ugo Basile, Italy) on 5 consecutive days. In this test, we measured the time in seconds that the rats were able to spend on the rod, during the 300 sec session.

ASR was recorded for assessing the drugs' effects on sensorimotor reactivity. The rats were put into a sound-proof plexiglas chamber (Responder X System, Columbus Instrument, Ohio, USA) and, following a 10-15 min accommodation period, were exposed to a series of 10 startling stimuli (5 kHz, 110 dB, 200 ms, 15 s interval). The vertical force associated with the startle response (whole-body muscle twitch) was measured by a piezoelectric force transducer. Following a 15 minutes recovery time, another session of 10 stimuli was started. This time a low-amplitude stimulus, a pre-pulse (1 kHz, 73 dB, 500 ms) was presented just before the startling stimulus (inter-stimulus time: 200 ms). This "warning" pulse attenuates the level of the startle response, and this normal suppression of the ASR by a preceding stimulus is termed pre-pulse inhibition

(PPI). The number of noise-positive responses; and some numeric parameters like latency, peak amplitude, and time to peak, of noise-positive responses were measured.

The number of noise-positive responses in the ASR and PPI test was evaluated by Chi^2 test. Distribution of OF, rotarod and other ASR/PPI data was checked for normality by Kolmogorov-Smirnov test. In case of normal distribution, all behavioural data were tested by one-way analysis of variance (ANOVA) (*post hoc* Scheffe test, $p<0.05$. At non-normal distribution, by Kruskal-Wallis (*post hoc* Mann-Whitney test, $p<0.05$) was used. The software pack SPSS 9.0 was used to the statistical analysis. During the whole study, the principles of the Ethical Committee for the Protection of Animals in Research of the University were strictly followed.

In the immediate experimental scheme, both 3-NP and MK-801 significantly decreased the total open field locomotor activity compared to the pre-administration self-control. Similar trend of motility was also observed in the combination groups. When given to the rats before 3-NP administration, both QP and SP caused further decrease of ambulatory activity compared to control. On the vertical activity, the effect of QP+3-NP and SP+3-NP was similar. QP+3-NP caused an increase in local motility, which was significant versus control and versus 3-NP group. QP and SP, combined with MK-801, significantly decreased the vertical activity versus control.

A single dose of MK-801 – both alone and in combinations – caused uncoordinated, ataxic gait in the rats, preventing any rotarod performance.

Significantly decreased number of ASR responses was seen in the MK-801 group compared to its own pre-administration control, and this diminishing trend was also seen following the combination treatments. The slight inhibition seen in the control group disappeared in the 3-NP treated group. When 3-NP was preceded by MK-801, some inhibitory effect was again observed, but in 3-NP+MK-801 group there was no inhibition at all. Latency of noise-positive acoustic startle responses was significantly decreased in the 3-NP treated rats. The changes induced by MK-801 alone were similar to those seen in the 3-NP group. In the MK-801+3-NP group, the effects were like and the decrease of time to peak was significant. In the test with pre-pulse stimulus, the peak amplitude was significantly lower in the 3-NP group versus the value obtained without pre-pulse.

When 3-NP was preceded by QP, significantly decreased, but when by SP, significantly increased number of ASR noise-positive responses was observed. The slight

inhibitory effect, which was observed in the control group, disappeared in the 3-NP treated rats. However, combined with SP, a stronger pre-pulse inhibition was again observed. Combined with MK-801, significant contrary effects of QP and SP on MK-801 altered noise-positive responses were not observed.

QP administration before 3-NP increased significantly the latency and time to peak of the ASR response compared to the 3-NP alone group, both without and with pre-pulse. ASR peak amplitude decreased significantly after QP+3-NP treatment versus 3-NP alone; but in the SP+3-NP group, significant increase was seen in the same comparison. Opposite effects of QP and SP on the ASR response parameters were also seen on combination with MK-801 group, except peak amplitude in the test with pre-pulse.

Similar to their immediate effects, both 3-NP and MK-801 decreased the total open field locomotor activity in also the acute experimental scheme, but the decrease of ambulatory activity was less than it was 30 min after treatment. The 3-NP+MK-801 combination had a similar effect, which was also significant. SP+3-NP treatment caused significant decrease of vertical activity versus control; and SP+MK-801, in the horizontal activity versus MK-801 alone.

The number of ASR responses showed increasing tendency in all groups. In the 3-NP and MK-801+3-NP treated rats, pronounced pre-pulse inhibition was seen.

In the 3-NP treated rats, latency of ASR decreased and peak amplitude increased significantly compared to the self-control groups. In the pre-pulse inhibition test, time to peak decreased significantly. These effects were also visible in the combined groups. The administration of QP or SP before 3-NP or MK-801 had minimal further effect the number of noise positive responses and on pre-pulse inhibition.

3-NP caused significant increase of peak amplitude, which was reduced significantly in the QP+3-NP group. In the pre-pulse inhibition test 3-NP decreased significantly the time to peak. When 3-NP was preceded by QP, peak amplitude decreased significantly.

The particular points of conclusion, derived from the above results, are the following:

- 3-NP-induced reduced locomotor behaviour is consistent with decreased release and action of DA in the caudate-putamen, which confirms the present histological HD model.
- Reduction of DA release in the caudate-putamen is mediated by an indirect inhibitory mechanism of corticostriatal axons, which exert a dual presynaptic influence on dopamine release.
- The hypomotility induced by the NMDA-antagonist MK-801 was, in contrast to what could be expected on the basis of literature data, not opposite to that of 3-NP.
- Behavioral effects of 3-NP seem to rely, at least partly, on not only glutamatergic, but on also other, that is, dopaminergic mechanisms.
- The positive effect of 3-NP on the startle response indicates increased DA release in the nucleus accumbens (NAC).
- When 3-NP was preceded by the postsynaptic D_2 antagonist SP, diminished PPI induced by 3-NP became normal, which indicates the stimulant effect of 3-NP via postsynaptic D_2 receptors in the NAC.
- 3-NP need not be applied so long as to destroy the striatal dopaminergic neurons for a behavioural effect, which is relevant in modelling HD and indicates that the effect generated immediately is also based on dopaminergic deficit.
- Behavioural methods may give opportunity to monitor the effects of agents, inducing or preventing HD-relevant damage, in a non-invasive way, contrary to the present, histological models, where the tests are not repeatable.
- Beyond modelling HD, the present results may gain importance in improving the therapy of other chronic neurodegenerative diseases.