UNIVERSITY OF PÉCS

Doctoral School of Biology and Sportbiology

In vivo electrophysiological investigations of different cognitive enhancer compounds on glutamatergic and cholinergic neurotransmission in the rat hippocampus

PhD Thesis

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1. Introduction, objectives

The number of people living with various neurocognitive disorders has increased dramatically in the recent decades. This growing trend affects not only patients but also their environments, the healthcare system and also has economic implications at a global level. In order to address this problem, numerous research programs have focused worldwide on elucidating the pathological background of neurocognitive disorders and the possible pharmacological treatments for the diseases. At present, however, it can be said that there are still unknown underlying mechanisms in the background of each identified disease and the range of available pharmacotherapeutic treatments options are not satisfying. The aim of the experiments in the present research project and PhD thesis was to investigate the effects of pharmacological compounds which are already on the market or involved in preclinical research as potential neurocognitive enhancers. The tested compounds had different targets at the receptor level: NMDA receptor antagonist memantine, alpha7 nACh receptor agonist (PHA-543613), antagonist (Methyllycaconitin – MLA) and PAM compounds (NS-1738, PNU-120596, CPDX), as well as D-serine, a compound that binds to the glycine binding site of the NMDA receptor, and a DAAO inhibitor compound that affects the extracellular level of D-serine. The brain area investigated in the experiments was the hippocampal region of the rat, which is essential for memory formation and consolidation, and further mapping and understanding of this brain region is crucial for the development of effective pharmacological therapies in neurocognitive disorders.

2. Methods

Both the local and systemic effects of the tested compounds were investigated by measuring the extracellular firing activity of pyramidal cells in the CA1 region of the hippocampus of anaesthetized rats. We measured how the different test compounds affect the glutamatergic and cholinergic neurotransmission, as the applied experimental protocols were based on iontophoretic delivery of endogen neurotransmitters (NMDA

and ACh). In addition to NMDA and ACh-induced excitations, the spontaneous firing activity of the pyramidal neurons was also investigated. A summary of the instruments used is shown in **Figure 1**.

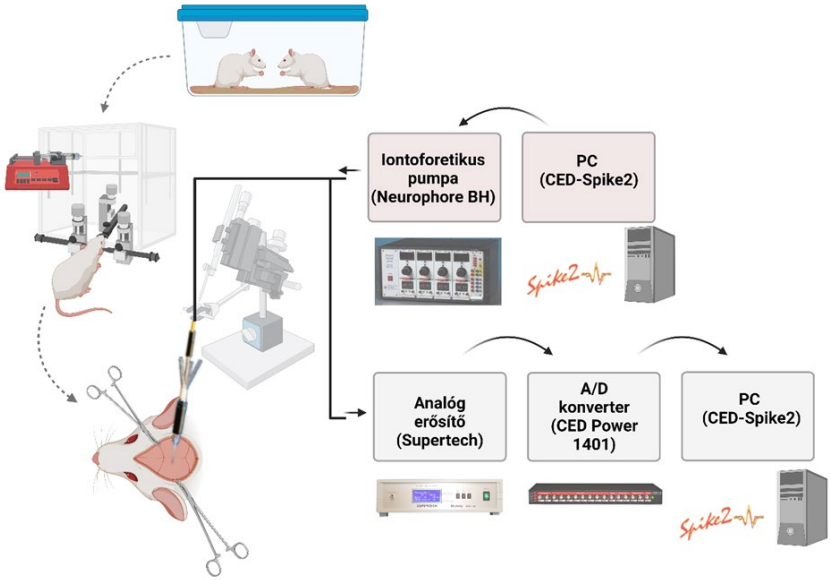

Figure 1.

Applied in vivo experimental design
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3. New scientific results

I. Thesis: Alpha7 nACh receptor agonist and PAM compounds differentially affect spontaneous and NMDA-evoked firing activity of hippocampal pyramidal cells.

- An antagonistic-like effect of the agonist compound was observed in the study of local effects, which may be due to the receptor desensitizing effect of the

compound, which is also attributed to possible procognitive and neuroprotective effects.

- In contrast, PAM compounds had a clear stimulatory effect on firing activity. This finding may be due to the facilitating effects of PAM on the natural activity of the endogenous ACh agonist.

- Combined administration of the agonist and the PAM compound additively modulated neuronal activity compared to their single administration, and the inhibitory effect of the agonist was reversed by the combined administration of the agonist with PAM. The results obtained may provide further evidence for the influence of PAM compounds on desensitization.

Relevant own publication:

Bali, Z. K., **Nagy, L. V.**, Budai, D., & Hernádi, I. (2019). Facilitation and inhibition of firing activity and N-methyl-D-aspartate-evoked responses of CA1 hippocampal pyramidal cells by alpha7 nicotinic acetylcholine receptor selective compounds *in vivo*. *Scientific Reports, 9*(1), 9324. https://doi.org/10.1038/s41598-019-45796-7 **IF: 3,998**

II. Thesis: The alpha7 nACh receptor-sensitive compounds and memantine modulate the spontaneous, the NMDA- or ACh-evoked firing activity of hippocampal pyramidal cells differentially depending on the mechanism of action and the doses applied.

- The highest dose of the registered AK drug memantine tested had only an inhibitory effect on NMDA receptors, which is consistent with the NMDA receptor inhibitory effect reported in the literature. The other doses relevant to behavioural experiments did not modulate NMDA-induced excitations but did affect spontaneous and ACh-induced firing activity. This result questions the canonically accepted NMDA-receptor mediated antagonism of memantine behind its previously observed cognitive enhancer effects.

- The alpha7 nACh receptor agonist compound had mostly facilitating effect on spontaneous and ACh-induced firing activity and was characterized by an inverted U-shaped dose-response curve similar to that previously observed in behavioural experiments. The specific dose-response curve can be explained by that low doses of the agonist induce no effect in the central nervous system, whereas high doses do not induce a stimulatory effect on neuronal function due to receptor desensitization and receptor binding site occupancy.
- The tested alpha7 nACh receptor PAM compound had a strong stimulatory effect on ACh-induced firing activity, with no or less effects in the other two firing conditions. This finding may be due to the agonist-dependent mechanism of action observed when using PAM compounds.
- When analysing the combined doses, in line with the behavioural results, the combination of sub-effective doses produced significant increases in firing activity in every firing condition, while no significant difference in activity was observed for the high dose combinations. The results with high doses may be due to the off-target effects of the two compounds. It strongly suggested that clear that alpha7 compounds could modulate the effect of memantine when administered both at sub-effective low doses and at high doses, thus these results may provide evidence for an interaction between the two different compounds.

Planned own publication:

Nagy, L. V., Bali, Z. K., Hernádi, I. Modulation of spontaneous and N-methyl-D-aspartate or acetylcholine-evoked firing activity of hippocampal CA1 pyramidal neurons by systemically applied cognitive enhancer compounds in the anesthetized rat *(Under consideration for submission)*

III. Thesis: D-serine-induces facilitatory effect on firing activity in a large number of the hippocampal pyramidal cells, both when local and systemic effects are tested

- When the local effects of D-serine and DAAOI were investigated, the two compounds increased the firing activity of pyramidal cells in a similar manner. They were found to stimulate spontaneous firing activity in the majority of the tested neurons, and also NMDA-induced firing activity in half of the cases. This phenomenon may be due to the regulatory role of local neuronal connections within the hippocampus.
- As with the local administration, the systemic effects were found to be similar for D-serine and the compound affecting D-serine levels (DAAOI), which may be linked to the positive cognitive and pro-cognitive behavioural effects observed in experiments previously described in the literature. Thus, DAAOI compounds are highly suitable to replace D-serine as a cognitive enhancer, as D-serine is nephrotoxic and has a lower level brain penetration, when administered chronically

- No significant difference was found between the effects of D-serine and DAAOI compound, suggesting that increasing extracellular D-serine levels directly and via DAAOI modulated neuronal firing activity to a similar level, which may give DAAOIs an advantage for drug development because of the potential peripheral effects of chronic administration of D-serine.

Relevant own publication:

Nagy, L. V., Bali, Z. K., Kapus, G., Pelsőczi, P., Farkas, B., Lendvai, B., Lévay, G., & Hernádi, I. (2021). Converging evidence on D-Amino acid oxidase-dependent enhancement of hippocampal firing activity and passive avoidance learning in rats. *International Journal of Neuropsychopharmacology*, *24*(5), 434–445. https://doi.org/10.1093/ijnp/pyaa095 **IF: 5,678**

4. Summary

Our *in vivo* electrophysiological results support that cognitive performance enhancer compounds acting through different pathways can modulate the firing activity of hippocampal pyramidal cells. The investigated compounds differentially affected both NMDA and ACh sensitivity of the examined neurons and their spontaneous activity. Furthermore, we observed dose-dependent effects of the compounds on the firing activity of neurons in all three firing conditions tested. The experimental set-up and test protocol developed in our experiments may be suitable for electrophysiological investigation of the cellular mechanisms of potential cognitive performance enhancer compounds.

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6. Publication activity

Science metrics:

MTMT ID: 10061002

Number of publications in peer-reviewed journals: 8

Total impact factor: 33,247

Number of citations: 99 (independent: 89)

Peer-reviewed articles related to the thesis

Nagy, L. V., Bali, Z. K., Kapus, G., Pelsőczi, P., Farkas, B., Lendvai, B., Lévay, G., & Hernádi, I. (2021). Converging Evidence on D-Amino Acid Oxidase-Dependent Enhancement of Hippocampal Firing Activity and Passive Avoidance Learning in Rats. *International Journal of Neuropsychopharmacology*, *24*(5), 434–445. https://doi.org/10.1093/ijnp/pyaa095 **IF: 5,678**

Bali, Z. K., **Nagy, L. V.**, Budai, D., Hernádi, I. (2019). Facilitation and inhibition of firing activity and N-methyl-D-aspartate-evoked responses of CA1 hippocampal pyramidal cells by alpha7 nicotinic acetylcholine receptor selective compounds *in vivo*. *Scientific reports, 9*(1), 9324. https://doi.org/10.1038/s41598-019-45796-7 **IF: 3,998**

Nagy, L. V., Bali, Z. K., Hernádi, I. Modulation of spontaneous and N-methyl-D-aspartate or acetycholine-evoked firing activity of hippocampal CA1 pyramidal neurons by systemically applied cognitive enhancer compounds in the anesthetized rat. ***(Under consideration for submission)***

Poster presentations related to the thesis:

Nagy, L. V., Bali, Z. K., Hernádi, I. (2022). Modulation of firing activity of CA1 hippocampal pyramidal neurons by systemically applied alpha7 nicotinic acetylcholine receptor selective compounds and memantine in the anesthetized rat, *in vivo*. International Neuroscience Meeting, Budapest, Magyarország

Nagy, L. V., Bali, Z. K., Hernádi, I. (2019). In Vivo Glutamatergic And Cholinergic Interplay In The Rat Hippocampal CA1 Area: Interactions Of Alpha7 Nicotinic Acethylcholine Receptor Excitation With Spontaneous And NMDA-Evoked Firing Activity. FENS Regional Meeting, Belgrád, Szerbia

Nagy, L. V., Bali, Z. K., Kapus, G., Hernádi, I. (2018). Local and systemic effects of D-serine modulation on the firing activity of hippocampal CA1 neurons in vivO. 11th FENS Forum of Neuroscience, Berlin, Németország

Nagy, L. V., Bali, Z. K., Hernádi, I. (2018). The effects of alpha7 nAChR agonists and positive allosteric modulators on spontaneous and NMDA-evoked firing activity of rat hippocampal CA1 neurons in rats *in vivo*: implications for the treatment of neurocognitive disorders. Alzheimer's Research UK Conference, London, Egyesült Királyság

Nagy, L. V., Bali, Z. K., Hernádi, I. (2017). Az α7 nAChR agonista PHA-543613 és a pozitív alloszterikus modulátor NS-1738 együttes hatásai a CA1-es hippocampalis piramissejtek tüzelési aktivitására és NMDA-érzékenységére. A Magyar Kísérletes és Klinikai Farmakológiai Társaság Gyógyszerinnovációs Kongresszusa, Velence, Magyarország

Nagy, L. V., Bali, Z. K., Hernádi, I. (2017). Alpha7 nAChR agonist and positive allosteric modulators differntially modulate the spontaneous and NMDA-evoked firing activity of rat hippocampal CA1 neurons *in vivo*. 5th FENS Regional Meeting, Pécs, Magyarország

Other peer-reviewed articles:

Bali, Z. K., **Nagy, L. V.**, Bruszt, N., Bodó K., Engelmann P., Hernádi Z., Göntér K., Tadepalli S.A., Hernádi I. Increased brain cytokine level associated impairment of vigilance and memory in aged rats can be improved by alpha7 nicotinic acetylcholine receptor agonist treatment. ***(Under consideration for submission)***

Bruszt, N., Bali, Z. K., **Nagy, L. V.**, Bodó K., Engelmann P., Némethy Z., Lendvai B., Hernádi I. Combination of memantine and alpha7 nicotinic acetylcholine receptor ligands exerts superior efficacy over monotreatments to improve cognitive performance of aged rats. ***(Under consideration for submission)***

Bali, Z. K., Bruszt, N., Kőszegi, Z., **Nagy, L. V.**, Atlasz, T., Kovács, P., Csupor, D., Csupor-Löffler, B., Hernádi, I. (2022). *Aconitum* Alkaloid Songorine Exerts Potent Gamma-Aminobutyric Acid-A Receptor Agonist Action In Vivo and Effectively Decreases Anxiety without Adverse Sedative or Psychomotor Effects in the Rat. *Pharmaceutics*, *14*(10), 2067. https://doi.org/10.3390/pharmaceutics14102067 **IF: 6,525**

Bruszt, N., Bali, Z. K., Tadepalli, S. A., **Nagy, L. V.**, Hernádi, I. (2021). Potentiation of cognitive enhancer effects of Alzheimer's disease medication memantine by alpha7 nicotinic acetylcholine receptor agonist PHA-543613 in the Morris water maze task. *Psychopharmacology*, *238*(11), 3273–3281. https://doi.org/10.1007/s00213-021-05942-4 **IF: 4,415**

Tadepalli, S. A., Bali, Z. K., Bruszt, N., **Nagy, L. V.**, Amrein, K., Fazekas, B., Büki, A., Czeiter, E., Hernádi, I. (2020). Long-term cognitive impairment without diffuse axonal injury following repetitive mild traumatic brain injury in rats. Behavioural brain research, 378, 112268. https://doi.org/10.1016/j.bbr.2019.112268 **IF: 3,332**

Bali, Z. K., Bruszt, N., Tadepalli, S. A., Csurgyók, R., **Nagy, L. V.**, Tompa, M., Hernádi, I. (2019). Cognitive Enhancer Effects of Low Memantine Doses Are Facilitated by an Alpha7 Nicotinic Acetylcholine Receptor Agonist in Scopolamine-Induced Amnesia in Rats. Frontiers in pharmacology, 10, 73. https://doi.org/10.3389/fphar.2019.00073 **IF: 4,225**

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Bali, Z. K., **Nagy, L. V.**, Hernádi, I. (2017). Alpha7 Nicotinic Acetylcholine Receptors Play a Predominant Role in the Cholinergic Potentiation of N-Methyl-D-Aspartate Evoked Firing Responses of Hippocampal CA1 Pyramidal Cells. Frontiers in cellular neuroscience, 11, 271. https://doi.org/10.3389/fncel.2017.00271 **IF: 2,298**