Counteracting Neuronal Alterations in Space and on Earth - Novel Hydroxynorketamine Derivatives are Potent Agents to Enhance Synaptic Plasticity *in vitro*

[1]Kalinski L., [2]Weber H., [2]Drouvé N., [1]Peter C., [1]Lichterfeld Y., [1]Hemmersbach R., [2]El Sheikh S. and [1]Liemersdorf C.

[1]German Aerospace Center, Institute of Aerospace Medicine, Gravitational Biology, Cologne, Germany
[2]Cologne University of Applied Sciences, Faculty of Applied Natural Sciences, InnovAGe Institute, Leverkusen, Germany

Introduction

Space conditions impact neurons and synaptic activity. Thus, the development of neuroprotective agents is necessary for future missions. Loss of synaptic plasticity in the brain has been linked to cognitive deficits, thus providing a promising target for therapeutic interventions. Ketamine, a drug applied in treatment-resistant depression, rapidly induces synaptic plasticity via a BDNF-dependent mechanism. Ketamine features substantial side-effects due to its NMDA receptor interaction. Its most relevant metabolite, hydroxynorketamine (HNK), has been attributed a stimulating effect on synaptic plasticity, while its weak affinity to NMDA receptors leads to a mitigation of the psychotropic side effects. Aiming to utilize this enhancing effect of HNK on synaptic plasticity for astronauts and patients on Earth, novel HNK derivatives were synthesized. These derivatives are investigated for their use as preventative agents or neuro-stimulants while omitting psychotropic side-effects.

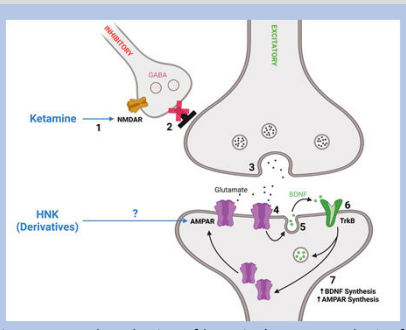

Fig. 1: Proposed mechanism of ketamine´s pro-neuroplastic effect. Blockage of NMDAR-mediated GABA-release leads to AMPAR-dependent transmission, inducing BDNF expression and AMPAR translocation. HNK derivatives evade NMDA receptor binding and should act directly on AMPARs, ultimately enhancing synaptic plasticity.

Methods

Hippocampal neurons were isolated from E17 mice and cultured until synaptic maturity in co-culture with astroglial cells. Cells were treated with 19 different HNK derivative candidates in total. After incubation, treated neurons were either fixed with PFA for immunostainings, lysed in RIPA buffer for Western blot or recorded employing Multielectrode Array (MEA) technology.

Results & Conclusion

We applied ketamine, HNK and 19 different newly synthesized HNK derivatives at a broad range of concentrations (0.1-100 µM) and incubation durations (1h-72h) to hippocampal neurons *in vitro*. Evaluation of pre-synapse counts by VAMP2 immunostaining revealed that several candidate compounds enhanced synaptic plasticity in a concentration-dependent manner after incubation of 48h. HW-774 stood out as the best candidate compound with the highest stimulatory effect at the lowest dose (50x more potent than standard ketamine).

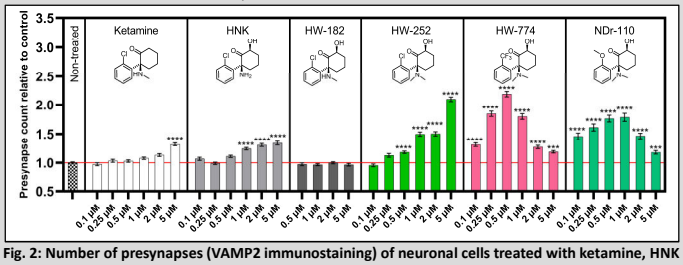

Fig. 2: Number of presynapses (VAMP2 immunostaining) of neuronal cells treated with ketamine, HNK and the best-performing derivatives at different concentrations relative to non-treated cells, Treatment for 48h, HW-182 cannot be matabolized and serves as a negative control, Mean + SEM, n ≥ 75 dendrite segments, Ordinary One-way ANOVA and multiple comparisons vs. non-treated

Enhancement of synaptic plasticity was verified by STED super-resolution imaging of HW-774-treated neurons. Dendritic spines showed increased numbers of mushroom-shaped structures, which are commonly seen as mature, functional spines and are primarily responsible for signal transmission.

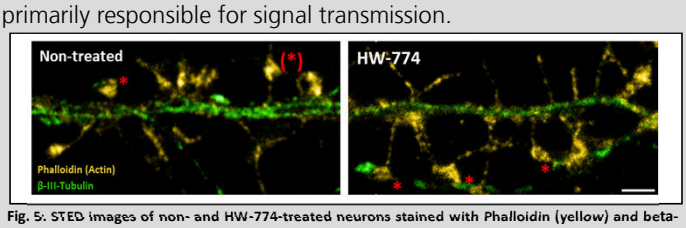

Fig. 5: STED images of non- and HW-774-treated neurons stained with Phalloidin (yellow) and beta-III-Tubulin (green), red asterisks mark mushroom-shaped synapses, HW-774 treatment: 0.5 µM, 48h, Scale bar: 2 µm

Furthermore, significantly elevated levels of the neurotrophic factor BDNF could be detected in HW-774-treated cells via Western blot.

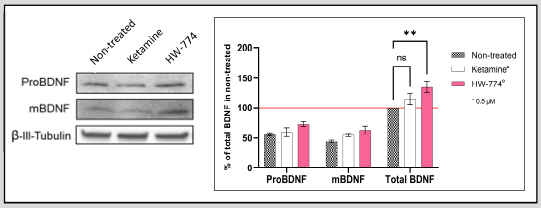

Fig. 3: Western blot analysis of BDNF in lysates of non-treated and treated hippocampal primary neurons (48h), Mean + SEM, n = 3 , Ordinary 2way ANOVA and multiple comparisons vs. non-treated

Additional experiments using MEA technology were carried out to assess effects on the functional electrophysiological activity of treated cells. A stimulating effect on firing activity was observed in neuronal cells treated with the compounds. For HW-774, stimulation reached its peak after incubation of 48 h.

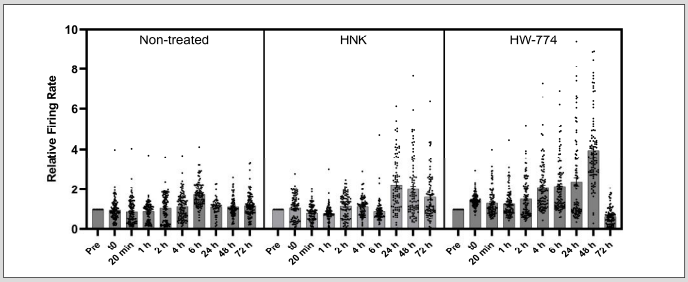

Fig. 4: Firing rate profiles for non-, HNK- and HW-774-treated neuronal cells over time relative to firing rates pre treatment (Pre). HNK and HW-774 concentration: 0.5 µM

In conclusion, our study resulted in the identification of highly potent novel HNK derivatives, which effectively enhance functional neuronal synaptogenesis in a concentration-dependent manner. Thus, the beneficial effect on neuronal plasticity could pave the way for the development of new neuroprotective therapies in the future.

Outlook:

These results indicate that the novel compounds could be used as a countermeasure against synaptic loss induced by space conditions and especially microgravity. Therefore, several studies are envisioned that utilize HNK derivatives in simulated and real microgravity. Experiments utilizing MEA measurements during parabolic flights and in the MAPHEUS rocket program are scheduled for 2024 and 2025.

Institut für Luft- und Raumfahrtmedizin

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Contact
Laura Kalinski
Email: Laura.Kalinski@dlr.de
Phone: +49 2203 601 5244