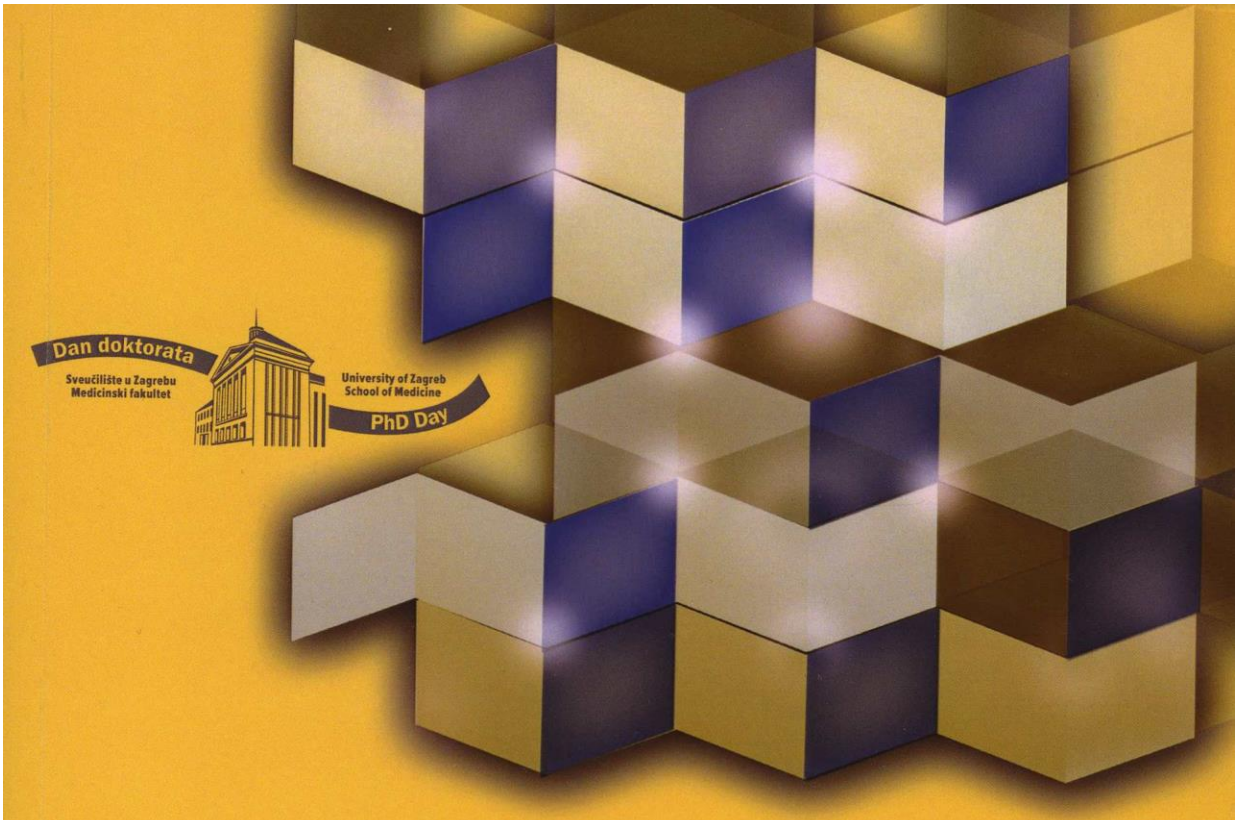

DAN DOKTORATA

KNJIGA SAŽETAKA

PhD DAY

ABSTRACT BOOK

2019.

Poster Title: The possibility for development of non-transgenic rat tauopathy model by application of tau oligomers into the entorhinal cortex

PhD candidate: Lea Langer Horvat

Part of the thesis: The possibility for development of non-transgenic rat tauopathy model by application of tau oligomers into the entorhinal cortex

Mentor(s): Professor Goran Šimić, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Sporadic Alzheimer's disease (AD) is the most common secondary tauopathy characterized by progressive loss of cognitive functions and behavioral impairment. The accumulation of hyperphosphorylated tau and the disruption of microtubules positively correlate with neuropathological changes, which progress in a stereotypical manner with the first lesions in the locus coeruleus and the entorhinal cortex from where they spread to the hippocampus and high-order neocortical regions. With this research we aimed to explore if intracerebral injection of tau oligomers and tau fibrils will induce aggregation and trans-synaptic spread of pathological tau proteins from the site of injection and will those changes be associated with expected cognitive impairment.

Materials and methods: Three to four months old male Wistar rats (n = 96) randomly divided into three groups were stereotaxically injected into the entorhinal cortex with tau oligomers (4 μg), preformed tau fibrils (4 μg) or phosphate-buffered saline. Animals were analyzed 3 days, 4 months, 6 months and 9 months post-injection (10 rats per group). Cognitive performance was tested using open field, T-maze rewarded alternation task, novel object recognition (NORT) and object-location test (OLT). To specifically detect tau protein changes and perform staging of tau protein pathology in the rat brain, we used anti-tau antibodies T22, AT8, HT7, and PHF1. Tentative amyloid changes were assessed using anti-amyloid antibody 4G8. Proteins isolated from the entorhinal cortex and hippocampus were analyzed by immunoblotting using anti-tau antibodies HT7, AT8, PHF-1, and Tau5.

Results: We present preliminary results obtained on 3 groups of animals sacrificed 4 months after intracerebral injection. Percentage of total investigation time of novel object as well as discrimination index showed no significant differences between groups ($p = 0.60$ for novel object investigation time). Long-term (24 h) object recognition memory was intact in both TO and TF animals at 3 months of age. In the object location test comparison of percent total investigation time of the moved object during the training trial versus the testing time showed no significant increase in investigation of the object after it is moved thus suggesting that rats didn't remember where the object was located during training ($p = 0.25$). Data obtained from T-maze rewarded alternation tasks was used to assess the working memory of rats. Tau fibril group of animals displayed impaired performance compared to their respective control in T-maze (-25%, $p < 0.05$). Immunohistochemistry revealed positive HT7 signal in the brainstem and transentorhinal region only in the group injected with tau fibrils, but not in the group injected with tau oligomers or PBS. Oligomeric tau was present both ipsilaterally and contralaterally to the injection site, but not in the control rats. The Ser202/Thr205 phosphorylated tau epitope visualized by using AT8 antibody showed weak immunoreactivity in both tau fibril and tau oligomer group of rats. PHF and HT7 immunoreactivity were much higher in animals injected with tau oligomers in comparison to control groups.

Discussion: Our preliminary results indicate that stereotaxic injection of tau oligomers or tau fibrils induces phosporylation of AT8 epitope of tau protein and tau oligomers in rat brain 4 month post-injection. Using antibody HT7, which recognizes human (and not murine) tau, revealed a signal present in the brainstem 3 days and 4 months after intracerebral injection of tau fibrils. Evaluation of hippocampal function as well as function of other cortical regions involved in object recognition showed no obvious deficit in all 3 groups of animals tested. T-maze under a food reward alteration, used to assess the working memory, showed that there was much slower learning curve with more incorrect choices in rats injected with tau fibrils in comparison to the control and the tau oligomer group. Understanding the role of tau oligomers and tau fibrils in neurodegeneration has a great importance for revealing mechanisms underlying development and progression of AD and other tauopathies.

Acknowledgments: This work is funded by the Croatian Science Foundation HRZZ (IP-2014-09-9730) and by the European Union through the European Regional Development Fund, Operational Programme Competitiveness and Cohesion, grant agreement no. KK.01.1.1.01.0007, CoRE- Neuro.

MeSH/Keywords: Alzheimer disease, rats, tau proteins, neurofibrillary tangles