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PhD Day

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PhD DAY

ABSTRACT BOOK

2018.

ANALYSIS OF TRANS-SYNAPTIC SPREAD AND AGGREGATION OF TAU PROTEIN AFTER THE INTRACEREBRAL INJECTION OF TAU OLIGOMERS INTO THE RAT ENTORHINAL CORTEX

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Part of the thesis: Analysis of trans-synaptic spread and aggregation of tau protein after the intracerebral injection of tau oligomers into the rat entorhinal cortex

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Introduction: Sporadic Alzheimer's disease (AD) is the most common secondary tauopathy characterized by progressive loss of cognitive functions and behavioral impairment. The two major pathological hallmarks of AD are senile plaques composed of amyloid- β protein and neurofibrillary tangles composed of tau protein. 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. Recent studies on transgenic animal models of AD provide initial evidence that pathogenic protein aggregates could propagate from neuron to neuron, and then self-replicate by templated misfolding of monomeric tau proteins thus igniting further spreading.

Hypothesis: Within the time period of 12 months, the intracerebral injection of tau oligomers into the rat entorhinal cortex will induce aggregation and trans-synaptic spread of pathological tau proteins from the site of injection to the entorhinal projecting cortical regions and these changes will match with animals' cognitive impairment.

Aims: The goal of this study is a critical appraisal of the pathogenic tau protein spread hypothesis of neurodegeneration in wild-type Wistar rats. We will inject a single dose of soluble oligomeric form of human tau protein (4 μ g in a total volume of 4 μ l) into the entorhinal cortex to assess: 1) whether such an intervention will cause a neuron-to-neuron physical spread of tau oligomers and tau aggregates, and 2) will those changes be associated with expected cognitive impairment.

Materials and methods: The study will include 3-4 months old male Wistar rats ($n=108$) divided into three groups. Rats from the first experimental group will be stereotaxically injected with tau oligomers into the entorhinal cortex, the second group will be injected with preformed tau fibrils and the third, control group, with phosphate-buffered saline. Animals will be tested and analyzed 3 days (6 rats per group), 3 months, 6 months and 12 months post-injection (10 rats per group). The following cognitive and behavioral test will be used: open field test, passive-avoidance learning task, T-maze task, novel object recognition task and object-location memory task. To document neurofibrillary changes Bielschowsky silver staining, Gallyas-Braak silver impregnation, Nissl and Thioflavin S staining will be used. To specifically detect tau protein changes in brain sections, we will use immunohistochemistry using the following anti-tau antibodies: T22, TOMA-1, AT8, HT7, CP27, and MC1. Tentative amyloid changes will be assessed using anti-amyloid antibodies. Proteins isolated from the entorhinal cortex and hippocampus will be analyzed by immunoblotting using anti-tau antibodies HT7, AT8, T22 and Tau5. For quantitative measurement of A β levels, the adequate enzyme-linked immunosorbent assay (ELISA) will be used.

Expected scientific contribution: We anticipate to better understand mechanisms of spreading of the pathological changes in AD brain and to develop a model that will mimic early events underlying those changes in human disease. The ultimate goal would be to define possible new therapeutic targets and interventions aimed at blocking tau protein oligomerization, aggregation, and spread.

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

Poster code: T-01-08-117