To the Scientific Board of School of Medicine University of Belgrade

At the meeting held on 7th May 2015, the Scientific Board of School of Medicine University of Belgrade nominated the Commission for the evaluation of doctoral dissertation entitled:

„ The hypothalamic oxytocin receptors in the paraventricular nucleus (PVN) in autonomic cardiovascular control"/"Oksitocinski receptori u paraventrikularnom jedru hipotalamusa u autonomnoj kontroli kardiovaskularnog sistema "

of the candidate Maja Lozić Đurić, MD, employed as a teaching assistant at the Institute of Pharmacology, Clinical Pharmacology and Toxicology, School of Medicine University of Belgrade. Mentor of the thesis is Prof. dr Nina Japundžić-Žigon, co-mentor is Prof. dr David Murphy.

The members of the nominated Commission for the evaluation of doctoral thesis are:

1. Doc dr Aleksandar Trbović, assistant professor, School of Medicine University of Belgrade
2. Prof dr Miroslav Radenković, associated professor, School of Medicine University of Belgrade
3. Prof. dr Branko Beleslin, associated professor, School of Medicine University of Belgrade
4. Prof dr Dragana Bajić, full time professor, Faculty of Technical Sciences, University of Novi Sad
5. Prof dr Mike Ludwig, full time professor, The University of Edinburgh, United Kingdom

Based on the thorough evaluation of doctoral dissertation , the Commission delivers following

REPORT

A) Dissertation contents overview

Doctoral dissertation of Maja Lozić Đurić, MD is divided into seven chapters written in English. The chapters are: Introduction, Hypothesis and aims, Materials and Methods, Results, Discussion and Literature. Short overviews of introduction and results, hypothesis and conclusions are presented in Serbian as well. Doctoral dissertation also contains abstracts in English and Serbian, biography of candidate, names and titles of Commission members and list of abbreviations used in text.

Introduction provides necessary information about the paraventricular nucleus of the hypothalamus, its morphology, function, projections and its role in autonomic control of cardiovascular system. Another part of introduction is dedicated to oxytocinergic system. The multiple nature of oxytocin as a hormone, neurotransmitter and neuromodulator is described, along with oxytocin receptors-their structure, signaling cascade, distribution.

Hypothesis and aims are well defined and focused with strong rationale.

The chapter entitled as **Materials and Methods** contains information about animal species used in experiments, resolves potential ethical concerns regarding working with animals and provides with the description of surgical procedures e.g. implementation of radiotelemetry device and stereotaxic procedures-implementation of chronic cannula in the region of paraventricular nucleus and *in vivo* gene transfer. The construction of adenoviral vectors is carefully described, along with methods for detection of gene expression, namely qRT-PCR procedures for identification of oxytocin receptor mRNA and immunohistochemistry for detection of oxytocin receptor. Experimental protocol is described in both textual and schematic form.

Cardiovascular signal processing and analysis is described, with special view on heart rate variability parameters and baroreceptor reflex functioning assessment. Separate parts of the text resume statistical approach used in experiments.

Results are meticulously and logically presented in the form of text, tables (3) and figures (6).

Discussion gives thorough and critical comparison of results obtained in this work with the relevant studies in the field. It is soundly written and accentuates the most important findings of the study .

Conclusions are concise, clearly based on outcomes and present the highlights of the study.

Literature contains 93 references**.**

B) Short overview of the results

The findings of the present work show for the first time that OTRs in the PVN tonically modulate autonomic cardiovascular control both under baseline and stressful physiological conditions. Central neural control of the cardiovascular system by the oxytocinergic system in the PVN was studied by microinfusions of exogenous oxytocin, selective antagonist of oxytocin receptors and gain-of-function approach was used.

Over-expression of OTRs in the PVN causes increase in mean values of SBP, MBP and BRS and decrease in VLF-BP and total BP variability during baseline conditions. The most striking result in animals with higher density of OTRs in the PVN exposed to stressful conditions is significantly higher value of BRS compared to controls along with decrease in LF-SBP and LF-DBP and increased overall HR variability.

Administration of selective antagonist in animals over-expressing OTRs confirmed the functionality of ectopic OT receptors by reducing BRS and de-buffering cardiovascular short-term variability under baseline and stressful conditions.

Results in wild-type rats demonstrate that administration of OTX into the PVN during baseline physiological conditions reduces BRS without affecting mean values of BP and HR and causes un-buffering of BP variability manifested as an increase in LF and HF domains. Exposure to acute emotional stress shows that pretreatment of wild-type rats with OTX induces less pronounced elevation of mean values of MBP, DBP and HR, prevents decrease of BRS and increases HR variability, especially in LF frequency domain, pointing to the domination of sympathetic control of the heart.

C) Comparative analysis of doctoral dissertation with currently available literature

It is well established that OT receptors are normally expressed in the PVN (Van Leeuwe *et al.,* 1985; Freund-Mercier *et al*., 1987; Tribollet*et al*., 1988; Yoshimura *et al*., 1993; Adan *et al*., 1995) where they have an important function as a part of an endogenous autocontrol mechanism (Richard *et al*., 1997). Anatomical and electrophysiological studies have shown that approximately 40% of PVN neurons projecting to spinal cord contain OT mRNA, indicating possible important role of oxytocinergic system in autonomic regulation of cardiovascular system (Pyner, 2009). Standing in line with previously mentioned, the results of this dissertation show for the first time that the change of density of receptors can modulate neurogenic control of circulation.

Microinjections of oxytocin in the region which contains specific and dense concentration of OT-immunoreactive terminals such as solitary-vagal complex

(NTS/DVN) enhance baroreflex functioning via the stimulation of OT receptors (Higa *et al*., 2002), while the results of studies in oxytocin-deficient mice by Michelini and collaborators reported blunted BRR in response to pressure changes. Experiments in reported dissertation show that over-expression of OTRs in the PVN enhanced BRS during baseline conditions, and this effect on baroreceptor reflex was unmasked by OTX administration.

The author observed that over-expression of OTRs in the PVN buffers the stress-induced BP variability response mediated by increased sympathetic outflow to blood vessels (LF) and stimulation of respiration (HF). Microinfusion of OTX in the PVN of non-transfected rats also prevented stress-induced DBP increase, reduced but did not prevent tachycardia and provoked the increase in HR variability. The increase of vagal influences to the heart during stress was reported to be useful in protecting the heart against sympathetic over-stimulation involving cholinergic NO synthesis in ventricles (Brack*et al*., 2012). This protective effect of the vagus is lifesaving during cardiac ischemia, when sympathetic over-stimulation triggers life threatening arrhythmias and sudden death. This assumption is further supported by the work of Wsol and associates (2009).The findings of this work are in line with a number of animal studies that suggest that OT activates an anti-stress response (Grippo *et al*., 2009; Lee *et al*., 2005; Windle *et al*., 1997; 2004). Clinical findings in humans also support a role for OT as an anti-stress hormone. Altemus and collaborators (2001) reported that lactating women have greater parasympathetic control of the heart, and Grewen and Light (2011) found that plasma OT in lactating women is correlated with lower cardiovascular reactivity to stress.

D) Published papers

Lozic M, Greenwood M, Sarenac O, Martin A, Hindmarch C, Tasic T, Paton J, Murphy D, Japundzic-Zigon N (2014). Overexpression of oxytocin receptors in the hypothalamic PVN increases baroreceptor reflex sensitivity and buffers BP variability in conscious rats. British Journal of Pharmacology 171(19): 4385-98

E) Conclusion

Doctoral dissertation of candidate Maja Lozić Đurić entitled:

"The hypothalamic oxytocin receptors in the paraventricular nucleus (PVN) in autonomic cardiovascular control" represents original scientific contribution in the attempts of understanding complex mechanisms underlying autonomic control of cardiovascular system. Findings of this study show for the first time that oxytocin receptors in the paraventricular nucleus (PVN) are involved in neurogenic control of circulation.

Results presented in this doctoral dissertation may be of use in the development of novel therapeutic strategies in the prevention and treatment of cardiovascular diseases caused by autonomic disbalance.

This is the study of strong rationale, with focused purposes and appropriate detail in description of state-of-the-art methodology and results. Discussion is concise and engaging and conclusions clearly based on outcomes. The dissertation is written with clarity and precision and narrative is logical and coherent.

Having in mind all previously written, the Commission for evaluation of doctoral dissertation of candidate Maja Lozić Đurić suggests to Scientific Board of School of Medicine University of Belgrade that the dissertation **should be publicly defended**.

Belgrade, 4th June 2015.

Commission Members:

Doc. dr Aleksandar Trbović

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Prof dr Miroslav Radenković

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Prof. dr Branko Beleslin

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