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The review

of the PhD thesis entitled:

The involvement of the hypothalamic paraventricular nucleus in the neuronal mechanism of relaxin-3 orexigenic action in the rat
by Alan Kania (M.Sc.)

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Precise regulation of complex neural circuits in the hypothalamus governs essential autonomic processes and associated behaviors such as metabolism, growth, and feeding, stress responses, arousal, and locomotor activity, as well as reproduction and social/sexual behavior. These intrinsic and often interacting neural circuits utilize various hormonal releases.

Just over a decade ago, the final member of the relaxin and insulin-like peptide superfamily was discovered and named H3 relaxin or relaxin-3 (RLN3), in line with the prior discovery and characterization of two other relaxin genes in humans. The cognate receptor for relaxin-3 is the relaxin family peptide receptor 3 (RXFP3) present in various brain regions including the cortex, hippocampal formation and hypothalamic region.

RXFP3 has been localized in various regions of the hypothalamus in the rat. The highest densities are present in the paraventricular hypothalamic nucleus (PVN) and adjacent medial (MPO) and lateral preoptic (LPO) nuclei, which is consistent with a putative role of relaxin-3/RXFP3 signaling in the control of a range of homeostatic and autonomic behaviors via modulation of related hypothalamic networks.

The PVN is one of two hypothalamic nuclei which tightly regulate alimentary behavior including food intake *per se* and energy homeostasis. Relaxin-3

immunoreactivity and RXFP3 mRNA and binding sites have been identified in the PVN and extensive research has demonstrated that relaxin-3 can alter the feeding and appetite in rats. PVN is also considered as a major hub of homeostatic control in mammals. Composed of functionally distinct cell populations: magno- and parvocellular cells (MNCs and PNCs respectively), the PVN contributes to the hypothalamic neurosecretory system.

The reviewed PhD thesis addresses the electrophysiological properties of MNCs and PVN involvement in the control of food intake in rats. The study of Mr. Alan Kania was completed in the Department of Neurophysiology and Chronobiology at the Jagiellonian University under supervision of Prof. Grzegorz Hess and co-supervision of Prof. Anna Błasiak.

The aim of the study was precisely and clearly explained at the end of the introduction. It was focused on two distinct, but related subjects: 1/ the investigation of RLN3/RXFP3 signaling in rat PVN as a putative neuronal mechanism of RLN3 orexigenic action which involved its characteristics, sex differences and its involvement in binge eating behavior, 2/ the investigation of MNCs in the PVN: their properties and sex differences in the neurosecretory function.

At the beginning of this dissertation, prior to the introduction, lists of abbreviations and symbols used in the manuscript were introduced. I recognize this as a very helpful editorial procedure. The thesis is 138 pages altogether, and enriched by a number of figures, diagrams and tables. The theoretical principles of the project presented in the dissertation were clearly presented in the introduction chapter, which is excellently written in polished English and contains the background of the presented problems. In this chapter the author addresses, in a most interesting way, a number of issues concerning the relaxin-3 signalling system, its expression in the central nervous system and the role of relaxin-3 in food intake. In separate subchapters the author characterizes paraventricular nucleus of hypothalamus and the brain's control of food intake. Next, the author focuses on the binge eating disorder (BED) as a phenomenon most commonly affecting women twice as frequently as men and whose neuronal mechanism is still completely unknown. The chapter ends with two very clearly written, specific goals.

In the second chapter (Material and Methods), Mr. Alan Kania describes a broad spectrum of advanced neurobiological techniques and methods used in the

experiments. Considering the sex differences in the relaxin-3 orexigenic action, the majority of experiments were conducted on male and female rats to investigate a putative source of sex differences in the sensitivity to RLX3. In this chapter the author presents a number of very tough and sophisticated electrophysiological, molecular, anatomical and behavioral techniques including patch-clamp recordings, single cell reverse-transcription polymerase chain reaction, immunofluorescent and immunohistochemical staining and finally behavioral tests. **On page 27, the author mentions that the initial part of the research was conducted on Wistar rats and next on Sprague Dawley rats. Was that premeditated? Maybe the author changed the strategy because of relative resistance to binge eating behavior displayed by Wistar rats (?).**

On p. 29, the author describes in detail the animals used in the experiments. **Interestingly, the patch-clamp experiments, histochemical experiments, neural tract tracing, behavioral tests and single cell examination precisely defined that different ages of animals were used. In the same paragraph the author informs the reader that: "*The ages of the animals for each experiment were determined to assure credible and interpretable results*". I would expect some additional comments from the author. Why would animals of different ages provide credible data?**

On p.35, the author describes electrophysiological identification and classification of PVN neurons. PVN neurons were identified based on their unique electrophysiological properties. Specifically, in contrast to the neuron of type II, type I neurons (probably MNCs) were recognized by the presence of a delay to the first potential action when a neuron is depolarized from a hyperpolarized membrane state. This is a spectacular difference between these two neurons. **I wonder; what are the putative mechanisms of this electrophysiological pattern?**

All the methods and techniques applied in Mr. Alan Kania's dissertation were very precisely and clearly described. I am greatly impressed that Mr. Kania - a short time into his PhD study – has so quickly mastered a number of advanced and difficult techniques.

The results were carefully presented in detail on 42 pages along with many of the figures which show the particular results obtained during the experiments. All the experiments performed are logically linked and create a very positive picture of Mr. Kania as a PhD candidate. Studies on the RLN/RXP3 signalling in the PVN showed its

inhibitory effect on the electrical activity of putative MNCs which can underline a plausible neuronal mechanism of the RLN3 orexigenesis. This inhibitory action of RXFP3 requires the activation of an M-like current. No sex difference was revealed in the RLN/RXP3 signalling of the PVN. Among other interesting data obtained in the experiment I would like to emphasize the study, which identified that RLN3 fibers are far less abundant in the PVN somatic area than in the perinuclear region, suggesting an RLN3 action through non-somatic sites and/or volume transmission. Interestingly, using the model of binge eating behavior in female rats, the present study showed that the blockage of RCFP3 in PVN prevents this pattern of behavior.

In summary, the dissertation thesis of Mr. Alan Kania represents a high level of scientific work in the field of neurobiology. All experiments were very well-designed, well-arranged and the measurements, techniques and methods were correctly applied. The dissertation in general is accurately presented and interesting to read. All the references (more than 250) were correctly selected and cited. The explanations are appropriate and focused on the relevant topics.

In my opinion, the reviewed thesis fulfils all requirements related to theses with the aim of obtaining a PhD degree (article 13, act of 14. 03. 2003). This PhD thesis by Mr. Alan Kania is ready to be defended orally in front of a scientific committee.

Additionally, since the data presented in the dissertation were previously presented in an oral or poster form, as well as published in a good international journal from the JCR list, I suggest a commendation of this work with the appropriate award approved in the Faculty of Biology of the Jagiellonian University.

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