



Committee (Prof. Dubovy) of the habilitation procedure: Anatomy, Histology and Embryology  
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Evaluation of the habilitation procedure in Anatomy, Histology and Embryology, Faculty of Medicine in Pilsen, Department of Science and Research, Alej Svobody 76, 323 00 Pilsen, Charles University, Czech Republic on the thesis of Mgr. Yaroslav Kolinko, Ph.D. entitled "Brain Microvascularization and Cerebellar Disorders in Rodent Models Using the Stereology Approaches".

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The Habilitation thesis consists of a review of 82 pages, 8 figures, 388 references and 10 publications. Yaroslav Kolinko is first author on six publications and have contributed significantly to the remaining four publications by making the stereological estimates, data analysis, literature search and writing/editing of manuscript. The combined number of pages of the Habilitation thesis is 281. All ten papers are published in international peer-reviewed journals with impact factors ranging from 1.1 to 4.9 and an average impact factor of 3.5.

The topic of the dissertation is histological, immunohistochemical and stereological investigations of microvascularization in mice and rat transgenic models of neurodegenerative diseases: cerebellar ataxia (Purkinje cell degenerated mice and Lurcher mice) and Alzheimer's disease (TgF344-AD rats), with a focus on cerebellum as well as potential therapeutic interventions. This choice is highly relevant, as the number of patients with neurodegenerative diseases in the world is constantly increasing and the option of treatment is typical none or very limited. New ideas and options are therefore very welcome!

Yaroslav Kolinko has chosen to use histological, immunohistochemical, stereological and light microscopical methods to study microvascularization. This is a strong bouquet of methods and it is very encouraging that he is very aware of the "reference trap", which describes the situation, where wrong conclusions have been drawn from densities alone. Examples of dubious conclusions based solely on the estimation of densities are very frequent in the literature.

Yaroslav Kolinko is carefully estimating the reference volume with the Cavalieri method and then he is multiplying this with the length densities or number densities to obtain total length or total number. As length densities and number densities are ratios, conclusions on total length and total number based on densities become dubious, however, this is skilfully avoided in this Habilitation thesis. It is also important to note that the diffusion distance is estimated directly as an absolute number and therefore is not affected by the reference trap.

As design-unbiased stereology typically estimates 3-dimensional entities from 2-dimensional sections based on rigorous sampling strategies and small samples obtained from the whole reference volume, it becomes important to estimate the precision or sampling variance of the obtained data. Yaroslav Kolinko manages to estimate the coefficient of error of the Cavalieri method with the older Gundersen & Jensen equation from 1987, however, he does not do the same for length or number estimation. He shows that he is aware of the distinction between unbiasedness and precision and can use that to design his stereological studies properly.

The results in the Habilitation thesis supports the conclusions and to a great degree the expected aims of the Habilitation thesis have been obtained. Further discussions on tissue deformation, equation for diffusion distance, choice of "Space Balls" instead of thin IUR sections and profile counts, thickness of paraffin and frozen sections etc could be very interesting to pursue.

In conclusion: Yaroslav Kolinko demonstrates scientific competence by addressing methodological challenges and effectively discussing findings within existing literature and he has shown that he is competent in working scientifically and is able to process and solve methodological problems very well. This includes the development of research questions based on scientific literature within structural microvascularization as well as the discussion and classification of the results in the current state of research. The thorough bibliography reflects the exploration of international scientific publications in stereology, microvascularization and experimental neurodegenerative diseases in relation to cerebellum. Yaroslav Kolinko uses a precise writing style to present the topics in an understandable manner, uses technical terms appropriately and explains complex topics clearly when necessary. Besides a few typos, the language used in the thesis is of publication standard throughout. In my opinion, the Habilitation thesis meets international standards for dissertations in the field of health sciences.

I recommend: "Be accepted for defence".

Your sincerely,

Jens Randel Nyengaard  
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