



ACADEMIC EVALUATION STATEMENT

by Assoc. Prof. Dr. Stoyan Pavlov Pavlov, MD, PhD, Department of Anatomy and Cell Biology,
Faculty of Medicine, Medical University "Prof. Dr. Paraskev Stoyanov" – Varna

Regarding: doctoral dissertation for the acquisition of the educational and scientific degree "Doctor" in the scientific specialty "Anatomy, Histology, and Cytology", professional field "7.1 Medicine", area of higher education "7. Health Care and Sports" by Dr. Radoslav Hristov Spasov, a regular PhD student at the Department of Anatomy and Cell Biology, Faculty of Medicine, Medical University "Prof. Dr. Paraskev Stoyanov" – Varna, under the doctoral program "Anatomy, Histology, and Cytology," on the topic: "Role of Transcription Factor Pax6 in the Development of the Cerebellum in Mice" with scientific supervisor Prof. Dr. Anton Bozhidar Tonchev, DSc, Department of Anatomy and Cell Biology, Faculty of Medicine, Medical University "Prof. Dr. Paraskev Stoyanov" – Varna.

Public defense procedure:

In my capacity as a member of the Scientific Jury according to Order No. P-109-182/08.04.2025 of the Rector of Medical University "Prof. Dr. Paraskev Stoyanov" – Varna, based on a proposal from the Faculty Council of the Faculty of Medicine, and pursuant to Protocol from the meeting of the Scientific Jury No. 1/22.04.2025, I was appointed to prepare an academic evaluation statement in a procedure for the public defense of a doctoral dissertation on the topic "Role of Transcription Factor Pax6 in the Development of the Cerebellum in Mice" for the acquisition of the educational and scientific degree (ESD) "Doctor" in area of higher education 7. Health Care and Sports, professional field 7.1. Medicine, doctoral program "Anatomy, Histology, and Cytology." The author of the dissertation is Dr. Radoslav Hristov Spasov, Department of Anatomy and Cell Biology, Faculty of Medicine, Medical University "Prof. Dr. Paraskev Stoyanov" – Varna.

1. Subject of evaluation

In accordance with the Procedure for Acquiring the Educational and Scientific Degree "Doctor" at MU – Varna, the candidate has submitted a set of documents on paper and electronic media, which includes the following:

1. Doctoral Dissertation.
2. Extended Abstract of the Dissertation.
3. Application to the Rector of MU – Varna.
4. Curriculum Vitae (CV).
5. Copy of Higher Education Diploma.
6. Order for Enrolling the PhD Student in the Doctoral Program at MU – Varna, signed by the Rector of MU – Varna.

7. Protocol from the Departmental Council Meeting for the Completion of the Doctoral Minimum.
8. Protocol from the Departmental Council Meeting for Discharge with the Right to Defend and Composition of the Scientific Jury.
9. Order for Discharging the PhD Student from the Doctoral Program at MU – Varna, signed by the Rector of MU – Varna.
10. Declaration of Originality of the Submitted Documents.
11. List of Scientific Publications Related to the Dissertation Topic.
12. The Scientific Publications Themselves Related to the Dissertation Topic.
13. Declaration of Authenticity of the Submitted Documents.
14. Declaration for Active Profiles in Scientific Databases.
15. Similarity Report Prepared by the Publishing Department at MU – Varna.
16. Academic Reference for Active Scientific Profiles in Google Scholar and ORCID Issued by the Library at MU – Varna.

The PhD student has also included 3 publications and 1 conference participation related to the dissertation work. The submitted documents meet the requirements of the Law on the Development of the Academic Staff in the Republic of Bulgaria and the Regulations of Medical University "Prof. Dr. Paraskev Stoyanov" – Varna for admission to public defense for the acquisition of the ESD "Doctor."

Structure of the dissertation

The presented doctoral dissertation consists of 144 pages, including 71 figures (most of them are panels from more than one illustration), 33 diagrams, and 3 tables. Eight (8) of the figures are included in the "Literature Review," seven (7) in "Materials and Methods," 55 in "Results," and one (1) in "Discussion." A total of three (3) tables are presented: two (2) in "Materials and Methods" and one (1) in "Discussion." A total of 124 literary sources are cited.

The text follows the usual structure for a doctoral dissertation:

1. Introduction – 2 pages.
2. Literature Review – 21 pages.
3. Aim and Objectives – 1 page.
4. Materials and Methods – 23 pages.
5. Results – 74 pages.
6. Discussion – 7 pages.
7. Conclusion – 1 page.
8. Findings – 1 page.
9. Conclusions and Contributions – 1 page.
10. Bibliography – 9 pages.
11. Publications and Reports – 1 page.
12. Abbreviations Used – 3 pages.
13. Acknowledgments – 1 page.

This structure and volume of the doctoral dissertation comply with the norms for awarding the ESD "Doctor."

2. Biography and career profile

Dr. Radoslav Spasov was born in Kyustendil and graduated from the Natural Sciences and Mathematics Gymnasium "Prof. Emanuil Ivanov" in Kyustendil in 2007. He obtained a Master's degree in the specialty "Medicine" at Medical University "Prof. Dr. Paraskev Stoyanov" – Varna in 2014. From 2014 to 2015, he worked in the Pediatric Intensive Care Unit at UMMC "St. Marina" EAD. Since September 2015, he has been a lecturer on a civil contract at the Department of Anatomy and Cell Biology at Medical University – Varna, and since March 2016, after successfully passing a competitive exam, he has been appointed as a regular assistant in the same department. After successfully participating in a competition in January 2018, he was enrolled in the regular doctoral program in "Anatomy, Histology, and Cytology" at the Department of Anatomy and Cell Biology of Medical University – Varna. In May 2021, after successfully passing the State Exam, he acquired the specialty in "Anatomy, histology, and cytology." In 2022, he was elected as the favorite lecturer of the graduating class in Bulgarian language medical training at Medical University – Varna. He speaks English. Dr. Spasov's scientific interests are focused in the field of neurobiology. He is a member of the Bulgarian Medical Association and the Bulgarian Anatomical Society.

3. Relevance of the Topic and Appropriateness of the Set Goals and Objectives

The cerebellum plays a role in controlling the higher executive functions of the central nervous system: motor control, motor and other types of procedural memory, and others. In recent years, evidence has emerged of its involvement in processes such as emotions and decision-making. The cerebellum contains over 70% of all neurons in the CNS. According to some studies, specific cerebellar impairments are observed in developmental disorders, such as those on the autism spectrum. Additionally, medulloblastoma – the most common brain tumor in childhood – develops due to impaired control mechanisms of proliferation of cerebellar granular neurons. All this necessitates detailed study and clarification of the mechanisms of cerebellar development. An important tool for accumulating new scientific data in this area are transgenic mouse models. Given the above, I believe that the topic of the doctoral dissertation is current and would significantly contribute to improving contemporary knowledge in the field of morphological neuroscience.

The aim and objectives are clearly formulated, and the carefully selected methodology makes the goal achievable.

4. Knowledge on the topic

The need for research in the field of the doctoral dissertation is clearly and logically argued in the Introduction. The Literature Review covers 21 standard pages and demonstrates the doctoral student's in-depth knowledge and awareness of the available literature on the subject of the structure of the cerebellum, the mechanisms of its formation, and the control chains of transcription factors that guide its development. The transcription factor Pax6 plays a particularly significant role in the described control mechanisms, and the

doctoral student has logically and consistently justified the choice of this gene as the target molecule of the present dissertation.

The doctoral student demonstrates the ability to work with a large number of publications on the subject of the mechanisms of cerebellar development, summarizing the findings presented in over 120 bibliographic sources.

5. Research methodology

The object of scientific research in the doctoral dissertation are tissues from experimental animals (mice) created and raised in Germany with permission from the local ethics committee in Lower Saxony, adhering to the principles of minimizing animal suffering. For selective genetic modification of animals in vivo, the Cre/LoxP technology was used, where the enzyme Cre-recombinase, located behind the regulatory element of the Pax6 gene, ensures tissue selectivity of expression in the cerebellum, recognizing specific (flox) sequences around the target gene – Pax6. This allows the gene to be specifically and selectively silenced in the cerebellum, enabling the animals to survive perinatal lethality, characteristic of global knockout of Pax6. Overcoming perinatal lethality is crucial because neurogenesis of cerebellar granule neurons – the most numerous cell type in the cerebellum and the entire nervous system – occurs postnatally. Therefore, the global knockout of Pax6, leading to death at birth (due to reasons related to dysfunction in other organs), is not applicable for studying cerebellar neurogenesis of granule neurons. Successfully deactivating Pax6 in the cerebellar cortex allows, for the first time, observing in vivo the results of reduced Pax6 function for the cerebellum.

The section "Materials and Methods" details the histological, histochemical, and immunohistochemical methods of processing, visualization of samples, and statistical methodologies for analysis.

Conclusion: The materials and methods are described in detail, allowing independent researchers to replicate the experiments

6. Characteristics and evaluation of the dissertation

The results are described over 70 standard pages and illustrated with 55 figures and 2 tables. The figures are of very good quality and clearly demonstrate the theses and findings of the scientific work. Many of the figures are multi-paneled, so the total number of figures is much larger than 55. The presented results support the theses of the dissertation.

The results of the study provide data on the selectivity of Cre-recombinase in the context of the specific experiment. By using a reporter line, it is proven that Cre has predominant, almost exclusive, expression in the cerebellum. This confirms that inactivation of Pax6 occurs in the target brain region, namely in the physiological zone of Pax6 expression in the external migratory stream. The work traces the morphological changes in the cerebellum upon suppressed Pax6 function. Detailed measurements were made of the areas of the cortex (gray and white matter), as well as individual layers, and the reduction in mutants compared to controls was calculated. Quantitative characteristics of cerebellar neuronal types (glutamatergic and GABAergic neurons) were established in the mutants. The author describes ectopic groups of neurons in the white matter in mutants, suggesting deficits in

migration of precursors not only of glutamatergic but also of GABAergic neurons upon inactivation of Pax6. The dissertation also shows some of the likely mechanisms through which the deficit of Pax6 can lead to the observed cerebellar malformations.

Conclusion: The presented results are of high quality and support the theses of the dissertation.

Dr. Spasov's good knowledge of the subject matter and analytical thinking are demonstrated in the Discussion section. His own results are summarized and discussed critically in depth in light of the data accumulated in the literature. As a result, well-founded hypotheses have been proposed for the molecular mechanisms through which Pax6 influences cerebellar morphology.

7. Scientific contributions and practical significance of the dissertation

The presented dissertation is an original scientific work with a significant contribution to clarifying the role of Pax6 in postnatal neurogenesis in the cerebellum. Most cerebellar neurons form after birth, but mice with a global knockout of the Pax6 gene die perinatally, due to which there was no model available until now that would allow the establishment of deficits in the cerebellum under reduced Pax6 function. This work describes for the first time in the world the consequences of reduced Pax6 function occurring postnatally in the brain of a mammal.

Conclusion: I believe that the contributions are sufficient for obtaining the ESD "Doctor."

8. Evaluation of the accompanying publications

To the documentation for the procedure for obtaining the ESD "Doctor," the doctoral candidate has attached 3 publications, complemented by 1 participation in a scientific forum on the topic. The attached publications have a methodological connection to the dissertation, but do not report its scientific results. Given the substantial scientific contributions of the dissertation, I recommend that Dr. Radoslav Spasov publish his results in an international peer-reviewed and refereed scientific journal.

9. Personal participation of the author

The described dissertation is the personal work of the doctoral candidate, and the formulated contributions and obtained results are the personal merit of Radoslav Spasov. Of course, in contemporary morphological science, the development of such a scientific project is the result of teamwork, whose contributions Dr. Spasov acknowledges and reflects (in the author collectives of the attached articles and in the Acknowledgments section). The transgenic mouse line was created at the Max Planck Institute in Göttingen.

10. Evaluation of the extended abstract

The extended abstract complies with the requirements and summarises the main results and contributions described in the dissertation.

11. Critical notes

In the text of the dissertation and the abstract, there are spelling and punctuation errors, which are likely the result of haste due to impatience to complete this important work for the career development of the doctoral candidate.

Conclusion

The dissertation of Radoslav Hristov Spasov from the Department of Anatomy and Cell Biology at Medical University "Prof. Dr. Paraskev Stoyanov" – Varna is dedicated to an up-to-date and interesting medico-biological problem. The doctoral candidate has presented a number of original results, obtained through the application of morphological and genetic methods. I believe that the dissertation fully meets the requirements of the Law on Academic Staff Development of the Republic of Bulgaria, its Rules for Application, and the Regulations for the Development of the Academic Staff at Medical University "Prof. Dr. P. Stoyanov" – Varna for obtaining the ESD "Doctor." **Therefore, I vote positively and allow myself to recommend the same to the other members of the esteemed Scientific Jury, appointed with Order No. P-109-182/08.04.2025 of the Rector of Medical University "Prof. Dr. Paraskev Stoyanov" – Varna.**

Statement prepared by:

Заличено на основание чл. 5,
§1, б. „В“ от Регламент (ЕС)
2016/679

Assoc. Pr

Department of Anatomy and Cell Biology
Medical University – Varna

Varna, 11.05.2025г.