

STATEMENT

By **Prof. VANYA GORANOVA STEFOVSKA, MD, PhD**

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REGARDING: Defense of the dissertation work: “**Proliferation and differentiation of progenitor cells in the subventricular zone of the telencephalon of adult monkeys**“ of **dr. Martin Nikolaev Ivanov**, regular PhD student in the doctoral program „Anatomy, histology and cytology” at the Department of „Anatomy and cell biology” of MU – Varna for awarding of the educational and scientific degree "**Doctor**" in the field of higher education 7. Health and sport, professional field 7.1. "Medicine" and scientific specialty: „Anatomy, histology and cytology”, code: 03.01.02.

I. PROCEDURE

With a decision of the FC of the MF of MU – Varna, Prot. № 11/23.10.2023, and an order of the Rector of MU – Varna № R-109-469/09.11.2023 I have been assigned as a **member of the Scientific Jury**. According to Prot. № 1/21.11.2023 of a meeting of the SJ, I have been chosen to prepare a **statement** under the procedure of acquiring the ES degree "**Doctor**" with a candidate **dr. Martin Nikolaev Ivanov**. He is enrolled by an order № R-109-81/01.02.2019 of the Rector. He passed the exam for the doctoral minimum on 25.01.2022 and is deducted with the right of defense by order № R-109-469/09.11.2023. He has accurately presented all necessary documents according the Rules of MU - Varna.

II. BIOGRAPHIC DATA AND CAREER DEVELOPMENT OF THE CANDIDATE

Dr. Martin N. Ivanov was born on 24.02.1994 in Sofia. In 2018 he graduated the specialty of „MEDICINE" with the EQD of MASTER and professional qualification MASTER OF SCIENCE IN MEDICINE from the Medical University “Prof. dr. Paraskev Stoyanov” – Varna (diploma reg. № 005881/01.11.2018). Since September 2018 for one year he has been appointed as part-time assistant, then as regular assistant at the Department of „Anatomy and cell biology” of MU – Varna. He has conducted seminars in cell biology and anatomy with Ist and IInd year medical and dental medicine students. He has participated as a lecturer in FChD "Neurobiology". He has been visiting researcher in Max-Plank Institute of Biophysical Chemistry in Göttingen, Germany for 3 months in 2019 and for 2 weeks in August 2020, then in the department of Neuroanatomy and molecular neurobiology in the Ruhr University in Bochum, Germany for 1 week in April-May 2022. He acquired a specialty in „Anatomy, histology and cytology”

(certificate reg. № 026540/08.08.2023, № 4927) in August 2023. He has published 2 articles in English and has participated in 8 national and international scientific meetings. In 2023 he has been honoured with the prize „Prof. Dimitar Kadanov“ of the BAS.

Dr. Ivanov is proficient in English language. He has got an active profile registered in Google Scholar, Research gate and Orcid. He possesses a high computer literacy and microscopic competence. He has very good leader and communicative abilities.

III. ACTUALITY AND SIGNIFICANCE OF THE TOPIC

The topic of the dissertation work is from the field of neurosciences and refers to the formation of new neurons in the stem-cellular niche of the anterior horn of the lateral ventricle by adult individuals. It is actual, extremely interesting and relevant mainly from fundamental aspect. In the dissertation work the spatial localization of genes with a significant elevation of their expression after short-term global brain ischemia in adult primates is investigated. A comparison with two other types of mammals is done. The results obtained might contribute to gain insight into some fundamental mechanisms by patients after ischemic brain disorders.

IV. STRUCTURE AND ANALYSIS OF THE DISSERTATION WORK

1. Volume and distribution

The dissertation work is structured according to the conventional requirements. It contains 147 standard pages, one of them title-page, contents (3 pages), dedication (1 page), abbreviations (3 pages), introduction (2 pages), literature review with 5 figures (18 pages), goals and tasks (2 pages), materials and methods with 8 figures and 3 tables (29 pages), results with 46 figures (51 pages), discussion (15 pages), disadvantages (2 pages), conclusion (1 page), conclusions - 10 items (1 pages), reference of the contributions - 6 items (1 page), publications - 2 items (1 page), reports (1 page), bibliography - 179 references (14 pages), acknowledgements (1 page). The figures are well selected, demonstrative and presented in details, a part of them are author's.

2. Introduction, literature review, goals and tasks

The introduction properly introduces into the process of neurogenesis in the brain of adult individuals in normal state and in ischemia, which explains the interest in the topic.

In the literature review the role and the stages in the development of the neuronal stem cells in the subventricular zone of the lateral ventricle and subgranular zone of *gyrus dentatus* of the hippocampal formation by rodents and primates are thoroughly analyzed. Activation and shift in the transcriptome of neuronal progenitor cells after ischemic brain injury and recent methods of neurogenesis investigation are presented. It becomes apparent the ability of the candidate very well to discover, analyze and summarize necessary information from the available references.

The goals and tasks are adequately and correctly formulated.

3. Materials and Methods

Adult Japanese monkeys, distributed into control and ischemic groups are used. One animal was injected with the thymidine analogue BrdU as a marker for newly generated cells. Tissues of normal human brains are used for studying the apelinergic system in the neurogenic niche in humans. Modern methods of tissue investigation and phenotypic analysis of selected genes: immunofluorescent and *in situ* hybridization stainings, image acquisition, image processing and statistical presentation. The materials and methods applied are suitable to achieve the goals set through the tasks marked.

4. Results, discussion, conclusion and disadvantages of the dissertation work

The results are the most significant part of the study. A thorough characterization and phenotypic analysis of selected genes are performed using well chosen micrographies, schemes, graphs and tables by the control and ischemic animal groups. Using appropriate double and triple stainings coexpression by some genes is demonstrated. Documentation and statistical data are correctly, clearly and consequently presented in respective figures, especially by the fluorescent micrographs.

In the discussion, the results obtained regarding the phenotype of APLNR, TNC, CD38 and GJA1 in normal and ischemic monkey brain and of APLNR in normal human brain are properly analyzed.

The conclusion makes the statement that the present study has demonstrated for the first time an increased expression of the genes TNC, APLNR, GJA1, CD38 following global brain ischemia in mammals and shows their rostro-caudal expression.

As disadvantages the insufficient animal number and limitations of the available marker accessible data base are self-critically pointed out.

5. Conclusions and contributions

The conclusions and contributions correspond to the results. They are exactly formulated and have scientific relevance. Most of the conclusions are original, the rest have supplemental or confirmative meaning.

6. Publications and participations in scientific meeting

The PhD student has got 2 publications in English on the topic of the dissertation work. In one of them, which is in an international periodic refereed edition, he is the second author, in the other one, that is in a national journal, he is the first author. He has participated with reports and posters in 3 national and 5 international scientific meetings without indicating the titles.

7. Bibliography

The bibliography includes 179 references, 178 in Latin and 1 in Cyrillic. The prevailing part of the titles are in prestigious editions. Approximately ¼ of the papers are published during the last 5 years. They substantially cover contemporary aspects of the problem studied.

8. Abstract

The abstract presents substantially, clearly and authentically the significant part of the dissertation work. It is prepared on 72 pages, distributed according the requirements into a title-page, description, contents, introduction, materials and methods, results, discussion, conclusion, conclusions, contributions, publications, abbreviations and acknowledgements. Goals and tasks are omitted. Three tables and 29 representative figures of schemes and micrographs, combined mainly with graphs are included.

V. CONCLUSION

The dissertation work: **“Proliferation and differentiation of progenitor cells in the subventricular zone of the telencephalon of adult monkeys“** presented by **dr. Martin Nikolaev Ivanov** for defense at the **Scientific Jury** is well structured, correctly prepared and completed. It demonstrates high competence, deep interest and excellent methodological training on the subject investigated. The dissertation work is written in an accurate and concise scientific style. It contributes to elucidation of fundamental aspects related to neurogenesis following ischemia in primates. According to the actual Law on the Development of the Academic Staff in the Republic of Bulgaria and the present Rules of MU - Varna the present dissertation work corresponds to all procedural requirements.

As a member of the **Scientific Jury** fully convinced I give **my positive vote** for awarding of the educational and scientific degree **"Doctor"** in the field of higher education 7. Health and sport, professional field 7.1. "Medicine" and scientific specialty: „Anatomy, histology and cytology”, code: 03.01.02 to **dr. Martin Nikolaev Ivanov**.

Date: 11.12.2023

Varna



Signature:

(Prof. Vanya Goranova Stefovska, MD, PhD)