Warsaw, March 15, 2025

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Dear Professor Agnieszka Dobrzyń

I am posting my review of the doctoral dissertation below.

A review of the doctoral thesis titled:

"Long-term loss of visual field in ophtalmological patients with central or peripheral degeneration of photoreceptors - fMRI analysis of visual cortex" prepared by mgr Marco Ninghetto. The thesis supervisor is dr hab. Kalina Burnat.

The dissertation concerns the mechanisms of brain plasticity based on studies of the functional effects of visual field loss. The author presented two experimental models; the study was conducted in patients with degeneration of photoreceptors in the central retinal area (Stargardt disease) and with degeneration of peripheral retinal area (retinitis pigmentosa). Observational results were compared with those obtained in healthy volunteers, in whom the visual field was restricted, respectively.

I believe that the choice of the dissertation topic is appropriate and justified. This topic is a continuation of the work carried out by the team of dr K. Bernat. The knowledge of the plasticity mechanisms of visual perception becomes not only relevant to science, but also to medical practice.

The dissertation is written in accordance with the accepted principles. It consists of three main parts: the first part deals with a common, comprehensive introduction and basic information on the methodology of the study, the second part is devoted to the results of studies on

adaptation of receptive fields - pRF size analysis - after loss of the peripheral or central visual field, while the third part deals with studies on motion-based visual acuity in both group of patients.

In the "Introduction", the author comprehensively described the anatomical and functional structure of the visual system, paying attention to the retinal representation in the visual cortex. The author also presented basic information about the retinotopic organization. These data are important for a better understanding of the results obtained. The author also referred to previous studies on the organization of visual reception, emphasizing the importance of both electrophysiological and autoradiographic methods using [^{14}C]C-2-DG. These methods provided insights into how visual information is spatially organized and processed across different cortical layers. It is worth noting that autoradiogaphic methods using [^{14}C]C-2-DG, pioneered by Sokolov, were also introduced at the Nencki Institute. Currently, these methods are being replaced by non-invasive procedures, of which the fMRI technique plays a crucial role. This method has been used for animal studies, but its primary advantage is its applicability to humans.

I believe that an important advantage of the reviewed work is the application of this very research method in human studies. This technique made it possible to estimate the pRF location in the visual field by measuring RF eccentricity.

I would like to emphasize that the author cites the basic article by Keliris et al. confirming the correlation between electrophysiologically recored responses of single RF neurons with fMRI responses to flickering checkerboards of different spatial frequentities. I believe that the choice of this research method is correct.

The author concludes this section by presenting the rationale behind the choice of the population of study volunteers: the study was conducted in patients with Retinitis pigmentosa and Stargardt disease. The first group of subjects represented the peripheral visual field damage model, while the second population represented the central visual field damage model. I believe that the choice of these two groups of patients is valid and fully justified.

In the next chapter: "State of art and aims", the author presented the main objectives of the study: the importance of the dorsal and ventral subdivisions of the early visual areas and the role of peripheral vision in ophalmological acuity.

In the chapter on research methods, the author presented the criteria for recruiting STGD and RP patients and healthy volunteers for the study. I believe that this methodological element is particularly important and may result in inconsistent results. I believe that the patient groups studied are sufficiently large and homogeneous. I have no comments on this paragraph.

The parameters of the fMRI study and the study protocol are not objectionable. The statistical methods used are also not objectionable.

The author proved that the patterns of cortical reorganization are different in the case of peripheral visual field restriction and central visual field restriction: in V1, all the groups presented a significant bilateral increase in pRF size compared with that of the controls. But in V2 a bilateral decrease in pRF size was noted in case of peripheral visual field restriction and increase in pRF was noted in case of central visual field restriction. A similar pattern was observed in V3 field.

The author also presented interesting and original results on the role of dorsal and ventral division in the reorganization of the visual cortex in case of visual field restriction.

In the "Discussion", the author compared his results with those of previous studies. He presented possible reasons for the differences in observations. I agree with the presented interpretation.

In conclusion, the presented findings demonstrated that the response to visual field loss is dependent on dorso-ventral cortical subdivisions and provides insights into the neural mechanisms underlying transient and long-term visual field loss.

The author rightly pointed out that the results presented can be helpful in practice in developing rehabilitation methods for patients with limited visual field.

Also, the next part of the article contains original observations. The purpose of this part of dissertation was to analyze the reorganization of the visual cortex in motion perception and visual acuity in cases of visual field restriction (central/peripheral). In the Method section, the author, briefly characterized the recruited RP and STGD patients. I have no objections to the group of subjects presented. The chapter accessibly and comprehensively presented the study protocol. I, in particular, want to highlight the description of fMRI preprocessing. I recognize that the protocol adopted and the methods of statistical analysis are appropriately chosen.

The main observation is that RP patients displayed impaired motion-acuity threshold and different brain activation patterns with significant reduced responses in peripheral primary

visual areas V1-3. However, limitation of the peripheral vision showed reduced activation in the bilateral motion-sensitive MT+ region. The author suggests that this symptom may be a result of central visual processing. It seems that this interpretation is correct.

In conclusion, the author presented, based on two models - peripheral and central visual field reduction - how the visual system adapts to visual field reduction.

I would like to emphasise the very carefully prepared layout of the work and especially graphic documentation. The dissertation also has the merit of including all individual test results in tabular form. The paper also contains information on the limitations of the study.

I would like to ask the candidate to elaborate more on the practical application of the results obtained in the clinic. Have the results been of interest to ophthalmologists ?

Since fMRI and pRF analysis, described originally in 2008 by Duomulin and Wandell, are basic research methods. Therefore I would like to ask for a broader discussion of the advantages and limitations of these techniques.

I believe that the dissertation presents the candidate's general theoretical knowledge of the main topic of the dissertation. This is evidenced by the data in the Introduction and Discussion.
The dissertation demonstrates that the candidate is able to formulate the scientific problem, properly select research tools suitable for conducting the research, perform this research, and provide an appropriate interpretation of the obtained results. Thus, the dissertation demonstrates that the candidate shows the ability to conduct scientific work independently. The dissertation also presents original results and their interpretation. This is especially true for the presentation of various mechanisms of plasticity of the nervous system in patients with visual field restriction in the course of retinal diseases.

In my opinion the doctoral dissertation meets the conditions specified in Article 187 of the Act of 20 July 2018 - Law on Higher Education and Science.
In connection with the above, I request the Scientific Council of the Institute of Experimental Biology to admit Marco Ninghetto, M.A., to further stages of the proceedings for awarding the degree of doctor.

I believe that the dissertation is distinguished and I submit an application for its distinction

Kraków, piątek, 18 kwietnia 2025

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Doctoral thesis review

"Long-term loss of visual field in ophthalmological patients with central or peripheral degeneration of photoreceptors - fMRI analysis of visual cortex"

Author: Mr. Marco Ninghetto

Supervisor: dr. Kalina Burnat, PhD, D.Sc.

The scientific objective of the thesis presented by Mr. Marco Ninghetto is to understand how the long-term loss of visual field—resulting from central or peripheral degeneration of photoreceptors—affects the functional organization of the human visual cortex. By employing advanced neuroimaging methods and behavioral paradigms, Mr. Ninghetto seeks to delineate how sensory deprivation alters cortical responses in early visual areas (V1–V3), and how these changes are modulated by the type and duration of visual field loss.

This line of research is of major significance both for fundamental neuroscience and clinical translation. At a basic level, it addresses enduring questions about the plasticity of the adult brain—specifically, whether and how cortical areas retain the capacity to reorganize when deprived of their primary sensory input. Understanding whether such reorganization is adaptive or maladaptive has profound implications. On an applied level, the thesis speaks directly to the growing need for diagnostic and rehabilitative strategies in ophthalmological care. As novel therapies and prosthetic interventions for inherited retinal diseases move toward clinical implementation, it becomes crucial to understand the state of cortical function in affected patients—whether the cortex is still

functionally viable, how it has reorganized, and whether it can integrate restored input. Mr. Ninghetto's work thus sits at a productive intersection of theoretical inquiry and translational relevance

This doctoral thesis is a sophisticated research project. It combines theoretical insight, technical innovation, and methodological rigor, and is structured around two complementary studies: the first examining cortical receptive field reorganization using pRF (population receptive field) mapping, and the second investigating perceptual thresholds and cortical activation using a custom-designed motion-acuity task.

The introduction (Chapter 1) is extensive and well-structured, guiding the reader through the complex architecture of the visual system. Subchapters 1.1 to 1.4 offer a comprehensive overview of the visual field and its cortical representation, the anatomical and functional properties of the retina, the concept of receptive fields, and the evolution of techniques used to measure retinotopic organization. The explanations provided are rooted in both classical and modern literature and demonstrate the author's solid grasp of the field. Particular emphasis is placed on the hierarchical organization of visual processing and the dorsal-ventral distinction in the cortical visual pathways. **As a whole, it testifies that the author has mastered the general knowledge in his discipline.**

Importantly, the author convincingly argues why the study of visual system plasticity in response to sensory deprivation is not only timely but also essential. In subchapter 1.7, and further elaborated in Chapter 2, Mr. Ninghetto lays out a strong rationale for focusing on the differential effects of peripheral and central visual field loss. He notes that, despite substantial advances in neuroimaging and vision science, most prior studies of cortical plasticity in ophthalmological patients have been limited by small sample sizes, lack of systematic comparisons between disease types, and minimal behavioral validation. He then formulates his goal. which is to address these gaps by combining advanced functional MRI methods with behavioral testing in larger, genetically characterized cohorts of patients with Retinitis Pigmentosa (RP) and Stargardt disease (STGD), each of which affects distinct regions of the retina and thus distinct regions of visual space.

The five specific research questions posed in Chapter 2 are clearly formulated and guide the structure of the empirical work that follows. Mr. Ninghetto thus asks: Can peripheral vision loss be effectively modeled in healthy participants? How does long-term visual deprivation affect the size and location of population receptive fields in visual areas V1–V3? Do dorsal and ventral cortical subdivisions respond differentially to the type of loss (central vs. peripheral)? Can a novel motion-acuity test distinguish between transient and long-term deprivation? And finally, do these changes translate into meaningful differences in brain activation patterns during task performance?

What is especially commendable in this dissertation is not only the clarity and depth of the theoretical framing but also the precision and diligence with which the author conducts his experiments. In the first study, population receptive field mapping is employed using high-resolution 3T fMRI data and a mapping approach grounded in the work of Dumoulin and Wandell (2008). This approach uses rotating wedges and expanding rings to construct retinotopic maps, The author uses an excellent but challenging software package (mrVista) to derive estimates of pRF size and eccentricity. These techniques represent the current gold standard and are used here with exceptional attention to detail and reliability.

Moreover, Mr. Ninghetto must have displayed admirable scientific and logistical skill in dealing with rare and vulnerable populations. Recruiting and interacting with patients with Stargardt disease and Retinitis Pigmentosa—both of which are low-prevalence genetic disorders—is a challenge in itself. He not only succeeds in forming sizeable and well-characterized research populations but also carefully matches them with control participants and employs rigorous exclusion criteria and pre-screening protocols that ensure the scientific validity of the data.

In Study 1 (Chapter 4) the author demonstrates that in RP patients and in controls subjected to a transient restriction of visual input, pRF size increases in V1 but decreases in V2 and V3, suggesting a hierarchy-specific adaptation. In contrast, in STGD patients with central visual loss, pRF sizes are significantly increased in all three areas (V1–V3), particularly in the dorsal subdivisions. These results are supported by extensive statistical modeling, including nested GLM analyses and voxel-wise comparisons, and are accompanied by clear, well-annotated figures and graphs.

The discussion of these findings (Chapter 4.4) is also noteworthy. Mr. Ninghetto goes beyond a simple interpretation of results and engages with multiple explanatory frameworks—from retinocortical feedback mechanisms and neuroplasticity models, to hemispheric asymmetries and attentional control. He also contextualizes his findings with reference to prior electrophysiological and neuroimaging studies in both human and animal models, citing the work of Burnat, Haak, Baseler, and others. His interpretation of the dorsal-ventral dissociation in the STGD group—emphasizing the role of dorsal cortex in spatial attention and compensatory visual strategies—is both compelling and well-supported.

The second study (Chapter 5) further strengthens the scientific contribution of the thesis. Here, the author introduces an innovative behavioral paradigm—a motion-acuity task using random-dot kinematograms under varying contrast and velocity conditions. This paradigm is not only novel but also ecologically valid, simulating the challenges faced by individuals with visual field deficits in everyday perception. The task is used both outside and inside the MRI scanner, allowing the author to correlate behavioral thresholds with patterns of brain activation.

Importantly, the results reveal that RP patients, in contrast to healthy controls, exhibit significantly impaired performance in motion-acuity tasks involving fast motion and negative contrast—conditions that mimic real-world vision in complex scenes. In terms of brain activation, the patients display reduced responses in the motion-sensitive MT+/V5 area and in anterior salience network regions (e.g., dorsal anterior cingulate cortex, frontal operculum). In contrast, healthy controls with transiently limited vision show increased activation in these regions, reflecting a flexible engagement of attentional resources. This contrast is interpreted as a possible marker of long-term adaptation vs. transient compensation and opens new avenues for rehabilitation strategies.

The thesis speaks of both the author's capacity of independent scientific investigation and of his achievement: an original solution of a scientific problem. The second study is already published in a high-impact, peer-reviewed journal:

Ninghetto, M., Kozak, A., Gałecki, T., Szulborski, K., Szaflik, J. P., Ołdak, M., Marchewka, A., & Burnat, K. (2024). Good.vision.without.peripheries¿behavioral.and.fMRI.evidence. Scientific Reports, 14(1), 26264.

The first study is currently under review:

Ninghetto, M., Keliris, G. A., Szulborski, K., Gałecki, T., Kossowski, B., Panneman, D., Cremers, F. P. M., Ołdak, M., Szaflik, J. P., & Burnat, K. (under.review)¡ Cortical response to transient and long-term visual field loss. Cerebral.Cortex.

Additionally, the author has an outstanding list of other publications (see page 165), including co-authorship in several international articles spanning TMS, a causal method, ophthalmology, and functional imaging. This record reflects both his productivity and collaborative capacity.

In conclusion, Mr. Marco Ninghetto has produced an excellent and original doctoral dissertation that addresses a scientifically relevant and clinically meaningful topic. It demonstrates his competence in theoretical reasoning, technical execution, statistical modeling, and scientific communication.

Therefore, I have no hesitation in evaluating this work positively. This doctoral dissertation entitled "Long-term loss of visual field in ophthalmological patients with central or peripheral degeneration of photoreceptors - fMRI analysis of visual cortex" meets the requirements specified in Article 187 of the Act of July 20, 2018 – Law on Higher Education and Science, and I recommend the admission of M.Sc. Marco Ninghetto by the Scientific Council of the Nencki Institute of Experimental Biology PAS to the next stages of the procedure for the award of the doctoral degree.

(Prof. Marcin Szwed)

PhD thesis from **Marco Ninghetto**, Laboratory of Brain Imaging of the Nencki Institute of Experimental Biology, Polish Academy of Sciences

Long-term loss of visual field in ophthalmological patients with central or peripheral degeneration of photoreceptors - fMRI analysis of visual cortex

Review by Elvire Vaucher, PhD, School of optometry, University of Montreal, Canada

This thesis from Marco Ninghetto is written as a standard thesis with 2 chapters depicting 2 experiments. Numerous appendices provide detailed results and raw data. 2 first authors and 2 co-authorship articles related to this work are found in Pubmed but are not annexed here. The candidate lists 9 published articles + 1 submitted (4 as first author) in the appendix 9, since 2019. The work is highly original and up-to-date, using the powerful fMRI technology paired to perceptual assessment. The articles and results are of excellent quality, with a definite innovative impact for patients and the vision science community. The interpretation of the results is adequate and elaborated. The thesis is very well written, well organized, well-illustrated, well documented and complete in terms of bibliography. There are virtually no spelling or typos errors. The doctoral dissertation demonstrates the Ph.D. student's overall theoretical knowledge of the field and ability to conduct independent scientific work.

In this Thesis, Marco Ninghetto describes two experiments to determine the effect of Stargardt disease, STDG, or retinitis pigmentosa, RP, on the visual processing. He especially measured effects on population receptive field (pRF) size and shape/motion perception of these central vs peripheric visual deficit, respectively, compared to age-matched visually healthy participants. A new and original visual task previously designed was validated in this study to assess visual acuity in both central and periphery of the visual field in visually deficient persons. Visual plasticity of the pRF in response to an acute restriction of central or peripheric vision was also investigated. The second aim was to validate the use of a new a motion/shape discrimination task as a tool for ophthalmological oculo-visual exams. 46 visually healthy participants and 45 RP + 23 STDG patients were first recruited but 23 of the visually-deficient were excluded due to incompatibility of their visual condition with the need of the experiments. STDG patients were not included in the second study for the same reasons. In spite of this, the candidate was able to recruit a large cohort of visually deficient patients, which recruitment is usually very limited in other studies.

In the first study, the candidate characterized the effects of STGD or RP on population receptive fields size in different areas of visual cortice (V1, V2 and V3) using fMRI and a visual restriction paradigm. Actually, participants experience tunnel vision (for RP) or central blindness (for STDG). Tunnel vision was reproduced in control participants by means of modified googles. At final, 45 controls and 22 RP + STDG patients were used. The results show that pRF size increased with visual deficiency including control deprived of peripheral vision in V1, but decreased in V2V3, except for STDG patients in whose central vision loss induced increase in V2 and V3 pRF. This alteration of the pRF size was dependent on dorsal vs ventral visual streams.

In the second study, the candidate associated the behaviorally assessed visual deficit with cerebral regions activation, measured by fMRI. RP patients and controls with limited peripheral vision showed reduced activation in the V1, and bilateral motion-sensitive MT+ region when compared to controls with full vision. STDG were not able to perform the task (central fixation). This study is already published in scientific reports. The results are impressive both in terms of quantity and quality of analyses performed. This study provides a new understanding of the visual circuitry adaptation to visual loss.

Overall, the studies add new knowledge in the field of visual loss and rehabilitation. There are few studies that have been investigating the cerebral effect of visual loss. Moreover, the large amount of patients tested is impressive. Unlike most other studies that have focused on static visual stimuli to characterize the visual loss and plasticity, the current work is novel because it dives into changes in functional plasticity using a new assessment of motion perception and the powerful fMRI technique. The work delineates characteristics of the visual loss and visual processing in the different levels of visual hierarchy. These studies are thus prone to help defining strategies for visual rehabilitation of this patients.

Although I think this thesis and work is excellent and could be accepted as is, I have some minor comments, especially for the study #1 since the second one is already published (and has gone through a thorough review):

1. In the study # 1 visual stimuli should be better described. Even if the candidate seems to use standard stimuli there is no description of those in the methods, except in the figure 3 legend.

2. In the study # 1 the number of control participants is greater than of visually deficient group. How this non-symmetrical number in different groups may affect statistics?

3. It appears that the same control participant has been tested with a normal visual field then a restricted visual field using googles. It is said that different testing sequences were used for the wear of googles and there was no difference between them. However, applying a patch for monocular deprivation for 1hour induce a strong plasticity in binocular vision that come back to normal within 1hour. I expect that such adaptative change might exist when wearing glasses for the first time, especially if there is no habituation to the google wear, and thus might affect the level of reactivity when the entire visual field is tested in a second step. Also, the total time of googles wear was not given. The candidate should give more details on the acute effects of these googles wear and discuss the possibility of slight changes perception when pRF for normal field of view are measured after googles wear.

4. The result showing similarity of the pRF change in RP and transient visually deprived controls is particularly interesting and has potent outcomes since it shows that transient deprivation might predict long-term changes. It would have been nice

to test central visual loss to mimic STDG disease to see if other types of visual deprivation might result to a similar conclusion. If there is no test performed with central vision limitation, the candidate should discuss this point and the possibility to predict changes in visual processing after transient visual loss.

5. Related to the precedent point, it is not really discussed whether the results of this research work could be used to elaborate a diagnostic tool to better detect STDG or RP patients or any retinal disease before the damage is too much advanced.

6. It's a pity that V5/MT -or other associative areas- was not investigated in the first study. This would add interesting insights on the involvement of this cortex in long-term processing changes after the deficit, in the different visual streams. What is the rationale for choosing only V1 to V3 areas. Why other areas were not investigated?

7. In the figure 10 it is difficult to see the direction of the moving dots (arrowhead not clear), please increase image resolution.

In conclusion, I highly recommend the acceptance of this thesis and attest that it should be admitted for the subsequent stage of thesis defense. I recommend outstanding status as the candidate as developed a method to investigate the cerebral effects of long term as well as acute effect of deprivation that can be used for developing visual rehabilitation strategies, and given that he has published 9 publications in high level journals. The mastering of fMRI technique is also an asset in the research work performed and represent a huge amount of work.