AXONAL PROPERTIES UNDERLYING SIGNAL TRANSMISSION MODULARITY IN THE PRIMATE SOMATOSENSORY CORTEX

PhD thesis

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1. Introduction

Cortical connectivity at the neuronal population or mesoscale level exhibits a strong structure–function relationship in the primate somatosensory cortex. The long-range cortico-cortical axonal processes of nearby pyramidal neurons distribute in a patchy pattern. Likewise, cortical microstimulation results in a patchy activation pattern similar to the distribution of long-range connectivity. In the primary visual cortex orientation tuning of the regions of axonal patches are biased towards that of the parent soma of the axons, which support the functional significance of the patches. In the somatosensory cortex, tactile modalities are mapped in a columnar manner, similar to orientation domains in the visual cortex. Also, patchy organization of the lateral connectivity is present throughout the cerebral cortex, which suggests that it plays a fundamental role in cortical processing in primates.

At the neuronal level, axonal patches are formed by recurrent collaterals of pyramidal cells bearing high-density bouton clusters. These distal clusters are connected to the parent soma by bouton forming linear

axonal segments that can establish rich connectivity. Bouton-forming linear axonal segments cross an array of neuronal populations with diverse tuning properties. Understanding the mechanisms of specificity and diversity of neuronal connections is a central question in deciphering cortical computation. How the information propagated by axonal patches and no-patch linear axonal segments contribute to cortical computation is not known.

The morphological properties of axons, such as thickness, tortuosity and bouton spacing can influence signal transmission in various ways by determining conduction velocity (delay) and synaptic integration via single or multiple synapses. In addition to individual axonal properties, the collective nature, i.e., the convergent and divergent characteristics of bouton-forming axonal branches also plays a crucial role in cortical functioning by supporting synchronization or providing redundancy. Based on these observations, the major goal of this study was to unravel the computational role of unmyelinated bouton-forming axons of the gray matter within and outside the patches using tract tracing and quantitative morphological approaches. Specifically,

we aimed to identify individual and collective axonal properties that distinguish the role of patch and no-patch axonal connections in cortical function and organization.

In our study of the axonal connections in non-human primates (NHP), we applied biotinylated dextran amine tracing, which labels axonal processes with high detail. However, traditional tracers including BDAs are transported both in the anterograde and retrograde directions with a preference in one direction. In contrast to BDA and other traditional tracers, recent advancement in the application of neurotropic viruses allows the investigation of the neuronal circuits with unprecedented specificity. The use of a virus as a self-replicating tracer to track brain circuits is known as viral neuronal tracing. It was shown that during anterograde tracing, both AAV and BDA labeled the bundles of fibre tracts and surrounding axon branches projecting into the gray matter. However, more axon branches were labeled in the AAV injections as compared with those in the BDA injections. Also, one of the greatest advantages of viral tracing is its use to manipulate neuronal phenotype. In

addition to genetic modifications making viruses highly specific to certain neuronal types and allowing the mapping of specific neuronal subcircuits, viruses are used as vectors of gene delivery. Most notably, in the neurosciences viral vectors play essential role in optogenetics studies via delivering the channelrhodopsin genes into the targeted neurons. Therefore, another objective of my studies was to investigate the functional role of connections formed by mediodorsal thalamic neurons with vasoactive intestinal polypeptide expressing interneurons (VIP + INs) of the prefrontal cortex in the rodents.

2. Objectives

The connection of the somatosensory areas BA3b and BA1 allowed us to compare feedforward and feedback patch and no-patch axonal domains in addition to intrinsic connections. Accordingly, the major goal of the presented studies was to unravel the functionally relevant morphological properties of bouton forming axons within patch domain and no-patch domain in BA3b and BA1 of squirrel monkeys (*Saimiri sciureus*) by using tract tracing

and quantitative morphological approaches. We measured individual axonal features regarding tortuosity, bouton spacing and axon thickness as well as collective axonal features indicating convergence, divergence. Multivariate analyses tools were used to determine the role of the variables in pathway specific properties including patch designation (patch vs. no-patch), intrinsic vs. inter-area connections, feedforward vs. feedback inter-area connections and laminar termination (supra vs. infragranular).

We hypothesised that

1) Patch designation is the most prevalent organizational feature of axonal morphology in the cerebral cortex and

2) Patches (P) are formed by the convergence of highly tortuous axonal branches with high bouton densities whereas no-patch (NP) axons are less tortuous, and have parallel course and low bouton densities.

3) In addition to BDA tracing, the 3rd objective of my studies was to explore the connectivity of a specific type

of neurons namely vasoactive intestinal polypeptide expressing interneurons (VIP + INs) in the anterior insular cortex (aIC) of mice, by using viral tracing.

A note on my role in the studies performed

The experimental part of the primate studies was done at Vanderbilt University followed by histology at Semmelweis University. I was not involved in the experimental (animal surgeries and histology) part of the study because I joined the laboratory later on and started my project by reconstructing axonal segments with Neurolucida followed by qualitative and quantitative analysis of already prepared brain sections. However, I performed double labelling experiments using viral tracing in mice at the Medical University of Innsbruck, Austria.

3. Materials and Methods

3.1 Primate studies

3.1.1. Animals

Three male (Mac, Mo, Pe) and three female (J, M, V) adult squirrel monkeys (*Saimiri sciureus*) were used in this study.

3.1.2. Functional mapping

After craniotomy and durotomy, the homologous hand representation area of the pre- and postcentral gyrus (BA1, BA3b) was revealed. Using the end of the central sulcus as a landmark, the electrophysiological mapping was performed with tungsten microelectrodes in the explored areas. intrinsic signal optical imaging (IOS) was also used to map the hand's finger pad representations.

3.1.3. Neuronal tract tracing

Distal finger pad representations of BA3b and BA1, localized by IOS and electrophysiology, were injected with 1:1 mixture of BDA (preferentially anterograde) and. Injections were made via iontophoresis.

3.1.4. Histology

50 μm thick sections were cut in the tangential plane. BDA was revealed by the avidin–biotin peroxidase protocol using Ni-intensified diaminobenzidine as the chromogen. Sections were osmicated, dehydrated and flat embedded in resin.

3.1.5. Alignment of sections

Alignements and the reconstructions were made with a motorized Olympus research microscope and Neurolucida (MicroBrightField Europe, E.K.). Sections were scanned with a 4x objective by a written in-house software.

3.1.6. Data collection and analysis

3.1.6.1. Reconstruction of axonal segments

Using Neurolucida, three BDA-labeled axonal segments were reconstructed (at 100X) in 3D with patch domains. Three labeled axonal segments were also selected in each cortical region between the injection site and the patches and reconstructed (no-patch domains). Only bouton forming axonal segments were examined.

3.1.6.2. Measurements and data analysis

Length was measured from the starting point, where the axon appeared on the top of the section. Inter-bouton interval was defined as the axonal distance to the preceding bouton. For axonal thickness measurements digital images were captured with 100x objective magnification by a 2-megapixel CCD camera built in the Neurolucida setup. Thickness measures were made by ImageJ at three different locations taken by chance along an axon, and then averaged for each axon separately.

Bouton density, i.e. the number of boutons in a unit length, was calculated for the full length of an axonal segment. The variability of bouton distribution along an axon was characterized by the standard deviation (SD) of the distances between boutons. Bouton clustering was measured by counting the number of boutons that were farther from each other (in terms of inter-bouton intervals) than a separation length along an axon as a function of separation length.

Tortuosity is a measure of the length of an axon relative to the straight line connecting the endpoints. To determine

the length value that best distinguished between the patch and no-patch axonal segments, we plotted the cumulative change of tortuosity as a function of a fixed 10 μm incremental increase of the axonal length. Based on the graphical representation of the measures from the patch and no-patch axons, we used as an estimate of the tortuosity of patch and no-patch axons, the tortuosity of the first 40-μm segment.

Directional differences in the projection of axons within individual patch and no-patch domains were determined using ImageJ in 2D, ignoring section depth. The direction was determined in the $0\text{-}180^0$ area relative to a reference line drawn between the injection site and the patches in each section. A measure of the convergence of boutons (bouton-convergence) was determined in Neuroexplorer. The mean distance of a bouton of a reconstructed axon segment from the two nearest BDA-labeled boutons of other axons was measured for each patch and no-patch axon.

Statistical analyses were performed in Statistica (version 13. TIBCO Software Inc. (2018),

http://tibco.com.) and MS Excel. ANOVA, Principal Component Analysis (PCA) and stepwise logistic regression were done in Statistica. If not mentioned otherwise, measurements and computations were made in MATLAB and Python.

3.2. Viral tracing in mice

Two male (VIP-cre: Ai9 double heterozygous) mice were used in this part of the study, which were unilaterally injected with a AAV5.CamKIIa-hChR2(HI34R)-mCherry into the mediodorsal thalamus (MD) in a volume of 300 nL. The sections were extensively washed with TBS, mounted on glass slides and coverslipped with Vectastain (Vector Laboratories) or ProLong Diamond (Thermo Fisher Scientific). Images were acquired using either an epifluorescence microscope (Axio Imager, Carl Zeiss, Oberkochen, Germany) and the Openlab software (Version 5.5.0). Raw confocal images were channel dye separated and deconvolved using Huygens software (Scientific Volume Imaging, Hilversum, The Netherlands). Image processing was

performed using the IMARIS 9.7.0 software (Oxford Instruments, Bitplane, Zurich, Switzerland).

For the quantification of VIP+ INs, free-floating 50 μm thick sections were used. ABC followed by DAB was to reveal VIP+INs. Sections immunolabeled for VIP were used to assess the number of VIP + INs (N = 4) by using the Neurolucida software (MBF Bioscience). Borders of the insular cortex (IC) were outlined with the help of consecutive Nissl stained sections according to a mouse brain atlas.

4. Results and Conclusions

4.1. Primate study

In the S1 of the squirrel monkey (*Saimiri sciureus*), anterogradely labelled axonal segments were reconstructed in three-dimension in BA3b and BA1. The morphological properties of these axonal segments, such as tortuosity, bouton clustering, bouton distances, bouton density, thickness, convergence, and directionality, were measured and compared within patch and no-patch domains, intrinsic and inter-area (FF and FB) connections and according to laminar localization. Axons exhibited difference only according to the patch classification. We found that except the tortuosity, which is an invariable property, the distribution of varicosities within the patch and no-patch domains varied substantially. In comparison to no-patch segments, we discovered that bouton densities are larger, clustering is more prevalent, and inter-bouton intervals are less variable within patches. Furthermore, boutons of disconnected axonal segments are much closer to one another within patches than outside patches. The distance between boutons of disconnected axonal

segments, which indicates convergence, is found to be the most powerful patch identity predictor. Aside from bouton convergence, bouton density has a significant influence on predicting accuracy of axon patch identification. Based on the findings described, the following conclusions are drawn:

The patch and no-patch domains of horizontal, long-distance axons exhibit unique morphological properties. All the variables studied were similar when compared according to pathway or laminar designations (BA3b vs. BA1, intrinsic vs. inter-area, FF vs. FB, supra vs. infra). The patch-like organization implies the parallel processing via divergence in the no-patch domains, whereas patch domains can promote convergence and functional cortical modularization due to the significantly variable path and higher densities of boutons of the axons.

Despite the thickness differences of axonal segments in patch and no-patch domains, the neural activity can travel at similar velocities. The distal branches are short, and it is reasonable to assume that the larger

bouton density within the patch domains compensates for the slower conduction of speed. Hence, the activity may be distributed with similar dynamics throughout the two axonal domains of the cerebral cortex.

Considering the functional significance of these findings, it is proposed that increased bouton density accompanied by extensive convergence could result in a highly efficient way of signal transmission via signal summation and neuronal synchronization in terminal arborization patches of the cerebral cortex. In contrast, long-range thick axons outside of patches could subserve quick dissemination of information. The patchy pattern is compatible with cortical circuitry that enables neuronal populations to operate together and boosting the effectiveness of signal transmission across the neural network.

4.2. Rodent study

By means of immunohistochemistry, I investigated the laminar distribution of VIP+ INs across transgenic VIP: Ai9-tdTomato mice insular cortex subdivisions and along its entire rostro-caudal axis. The highest percentage of VIP+ INs was found in cortical layer II/III.

Anterograde viral tracing approach was utilised to identify the projection from mediodorsal thalamus (MD) to VIP+INs of the anterior insular cortex (aIC). The bilaminar distribution of thalamocortical afferents. Notably, the distribution of anterograde labelling and VIP+ INs overlapped in the superficial layers. Anterograde tracing identified direct inputs to aIC VIP+ INs from the MD as shown by the close appositions between anterograde labelling and VIP immunolabeling. This arrangement supports the synaptic relationship between thalamocortical axons and VIP+ INs that was later confirmed by optogenetic assessment.

6. Publication List

5.1. Publications of the thesis

Mir et al. (2022). Modular Organization of Signal Transmission in Primate Somatosensory Cortex. *Front Neuroanat*. (16): 1-17. doi: 10.3389/fnana.2022.915238. **IF: 3.267**

Ramos-Prats A, Paradiso E, Castaldi F, Sadeghi M, **Mir MY**, Hörtnagl H, Göbel G, Ferraguti F. (2022). VIP-expressing interneurons in the anterior insular cortex contribute to sensory processing to regulate adaptive behavior. *Cell reports*.*39*(9):1-24. doi.org/10.1016/j.celrep.2022.110893

IF: 9.423

5.2. Conference publications and presentations related to the thesis

Mir Y, Pálfi E, Roe A, Friedman R, Négyessy L. (2019). Structural correlates of modular organization of activity propagation in the primate somatosensory cortex. *IBRO Reports*. Volume 6, Supplement 1(2019), Page S540, ISSN 2451-8301. https://doi.org/10.1016/j.ibror.2019.07.1681.

Mir Y, Pálfi E, Roe A, Friedman R, Négyessy L. (2021). Structural correlates of modular organization of signal transmission in the primate somatosensory cortex. *European Neuropsychopharmacology*. Volume 44, Supplement 1 (2021) Pages S23-S24, ISSN 0924-977x. https://doi.org/10.1016/j.euroneuro.2021.01.040.

Mir Y. Modular organization of cortical connectivity does not affect the structural basis of axonal transmission, International Medical Conference (2018), Pécs, Hungary.

Mir Y. Connectional modularity in primate somatosensory cortex, Meeting of Hungarian Neuroscience Society (2019), Debrecen, Hungary.

Mir Y. 'Structural correlates of modular organization of signal transmission in the primate somatosensory cortex', IBRO Conference (2019), South Korea.

5.3. Other publications

Baba, Abu Imran, **Mohd Yaqub Mir**, Riyazuddin, Ágnes Cséplő, Gábor Rigó, and Attila Fehér. 2022. "Plants in Microgravity: Molecular and Technological Perspectives. *International Journal of Molecular Sciences*.23(18):1-18. https://doi.org/10.3390/ijms231810548. **IF: 6.205**