The role of neuronal networks and inhibition in the processing of tactile information at early stages in the primate somatosensory cortex

Doctoral thesis

Emese Pálfi

Semmelweis University

Szentágothai János Doctoral School of Neuroscience

Supervisor: László Négyessy, Ph.D.

Official reviewers: Zoltán Kisvárday, Ph.D., D.Sc.
Tibor Kovács, M.D., Ph.D.

Chairman of examination board:
Béla Vígh, Ph.D., D.Sc.

Members of examination board:
Krisztina Herberth-Minkó, Ph.D.
Zoltán Vidnyánszky, Ph.D., D.Sc.

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1. Introduction

In humans and non-human primates, the hands, especially the fingertips, can be considered as the organs of touch. The primary somatosensory cortex (SI) is located on the postcentral gyrus and consists of four areas including Brodmann's area 3a, 3b, 1 and 2 (BA3a, BA3b, BA1, BA2).

BA3b and BA1 are hierarchically related and have special importance in tactile perception. The hierarchical relationship between these areas is demonstrated by the different size and complexity of the receptive fields (RF) being larger in BA1. In contrast, the cortical magnification factor (CMF) quantifying the size of cortical area representing a unit size of skin, is larger in BA3b than in BA1. The hierarchical relationship also plays an important role in the spatial organization of the RFs via the distinctive distribution of the feedforward (FF), feedback (FB) and the horizontal connections within the area.

Our previous qualitative studies have shown that the injection of a bidirectional tracer in a column size area of SI results in a strong reciprocal connectivity within and between BA3b and BA1. Connectivity between BA3b and BA1 is localized to the homotop finger representations, whereas within the areas connections were formed between the neighbouring distal finger pad representations. These observation suggested that tactile information is integrated and processed along two main pathways: an associative (inter-areal), responsible for exchanging information between the representations of the same fingertip, and an intrinsic circuitry, which is responsible

for integrating information across the fingertip representations. However, this model of connectivity does not meet our expectations of the hierarchical organization of cortical connections and cannot explain the appearance of extra classical RF properties, the emergence of complexity and increasing the size of RFs in BA1 as compared to that in BA3b. Using column-size injections allows the investigation of the connectivity of neuronal populations, which play an important role in the spatial organization of RF.

The integration of intrinsic and inter-areal processing is one of the fundamental functions of the cortical network. Exploring the relationship of the distribution of intrinsic and inter-areal connections help understanding the anatomical basis of the functioning of the cortical network. In the present thesis we aimed to investigate the circuitry formed by cortical neuronal populations within and between BA3b and BA1. In addition, the role of inhibition in these connections was also studied.

2. Aims

We studied the organization of the neuronal connections of BA3b and BA1 and the role of the connectional distribution in the integration of tactile informations in the squirrel monkey *(Saimiri sciureus)*. Our study was focused on the distal finger pad representations, which play pivotal role in tactile perception. The following hypotheses were tested:

- Do the intrinsic connections of BA1 exhibit larger lateral spread and lower somatotopic specificity than that of BA3b, which could result in larger and more complex RF properties?
- Does the consideration of the magnification factor changes the pattern of connections mentioned in the previous point?
- Do the connections exhibit stronger clustering in BA3b than in BA1?
- How are the horizontal distribution of FF and FB connections between the areas related a) to the intrinsic connections, b) to each other, c) to the population activity, d) exhibit anisotropic distribution similar to the intrinsic connections?
- Are the interactions of cortical areas based on the communication between similar sized tissue volumes as it was observed in the visual cortex? Is sensory cortical processing based on a similar connectional motif?
- What kind of GABAergic interneurons are the postsyanaptic targets of the anterogradely labeled axons in the SI?

3. Materials and methods

3.1 Division of labor

The experiments were carried out in co-operation with the Psychology Department of the Vanderbilt University (Nashville, TN, USA). Physiological and tracing experiments were made in our partner's laboratory with our participation. The histological experiments have been carried out in our laboratory at the Semmelweis University Department of Anatomy, Histology and Embryology.

3.2 Animals

4 female (J, V, M, R) and 4 male (Mc, Mo, P, T) adult squirrel monkeys *(Saimiri sciureus)* were used in this study. The animals were 2-9 years old and weighing 0,6-1,1 kg. Animal care and surgeries were performed according to NIH (National Institute of Health) regulations and were in compliance and approved by the Institutional Animal Care and Use Committee of Vanderbilt University.

3.3 Functional mapping

Initial anaesthesia was done by ketamine hydrochloride (10 mg/kg) and sustained with inhalation of isoflurane (0,9-1,3%). After craniotomy and durotomy the homologous area of the pre- and postcentral gyrus (BA1, BA3b, BA3a, M1) was revealed. The topographic structure of the finger-specific cortical areas was mapped by vibrotactile stimulation of hairless skin in the distal, middle and proximal phalanxes of the finger. Using the end of the central sulcus as a landmark, the electrophysiological mapping was performed with tungsten microelectrodes in the upper and middle

cortical layers in the explored areas. We also used intrinsic signal optical imaging (IOS) to map the hand finger pad representations.

3.4 Neuronal tract tracing

Distal finger pad representations of BA3b, BA3a and BA1, localized by IOS and electrophysiology, were injected with 1:1 mixture of BDA (biotinylated dextran amine) 10K (preferentially anterograde) and 3K (preferentially retrograde). Injections were made via iontophoresis.

3.5 Histology

3.5.1 Brightfield microscopy

50 μm thick sections were cut in the tangential plane (parallel with the cortical layers). 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.

3.5.2 Fluorescent microscopy

A series of 20 μm thick sections were collected for fluorescent immunhistology. Multiple fluorescence immunohistochemical labeling were performed on floating sections according to the standard protocol of our laboratory.

3.6 Mapping of the BDA labeling

The BDA labeling was mapped using Neurolucida® program (MicroBrightField Europe, E.K. Magdeburg, Germany).

In the case of retrograde labeling, the distribution of cell bodies identified the input regions of the injected locus.

In the case of anterograde labeling, three types of BDA labelled structures were distinguished: 1) long-range horizontally running

fibers, 2) terminal axon arborisation patches and 3) bouton-like structures.

3.7 Data analysis

Beside the analysis of the distribution of the retrograde and anterograde labeling, the data analysis was partly based on new and literature-based methods inlcuding kernel density analyses and density based cluster analysis (DBSCAN) of the retrograde labeling and analysis of anisotropy.

4 Results

4.1 Retrograde labeling

Injections of BA1 (three cases) and BA3b (three cases) were investigated. Most of the retrogradely labeled neurons were pyramidal cells, although, some "smooth" dendritic neurons without spines were also visible around the injection site. Neurons appeared in higher numbers within the injected area and in the supragranular layers in all cases.

The high density areas of retrograde labelling exhibited larger lateral spread in the injected areas than inter-areally.

The area of population activity determined by IOS (IOS-area) included larger number of neurons in BA3b than in BA1 irrespective of the injected area. Also, the ratio of the highest density area of retrograde labelling and the IOS-area were bigger in BA1 than in BA3b.

Retrogradely labeled neurons showed significantly higher clustering in BA3b than in BA1. Similarly, clustering of the intra-areal connections was higher than the inter-areal. However, cluster size distribution exhibited similar diversity in all cases of connections.

4.2 Anterograde labeling

Injections of BA1 (three cases) and BA3b (three cases) were investigated. BDA labeled terminal axon arborizations were more evenly distributed between the two areas than the retrograde labeling. However, BA3b injection resulted in a larger ratio of intra-areal labeling than the injection of BA1. Supragranular labeling

dominated in all cases without differences in the laminar distribution between the two investigated areas.

On the overlain series of tangential sections the majority (83-85%) of the individual terminal arborisations overlapped forming vertically arranged groups, which suggest a column-like distribution across the depth of cortex. For intrinsic connections 3-4 terminal arborisations formed groups compared to the 5 or more terminal arborisations of inter-areal labeling.

The size of the terminal arborization groups was significantly larger inter-areally.

In BA3b, the size of the cortical area covered by intrinsic terminal arborization groups was greater than in the inter-area.

The nearest neighbours analysis of terminal arborization groups showed a looser distribution after BA1 injection.

The ratio of the terminal arborization groups and the IOS-areas were around 1 in BA1.

Comparing the distribution of retrograde labelling density and that of the terminal arborizations indicated that intra-areal axonal patch groups spread over a larger range of neuronal labeling densities than inter-areal patch groups.

4.3 Comparision of the represented skin surface of the retrograde and anterograde labeling

In the case of retrograde labeling, the highest density areas, represented similar skin surface areas in BA1 and BA3b injection.

The area of skin represented by a terminal arborization group was smaller in BA3b. The lateral spread of the terminal arborizations was

not different between intra- and inter-areal labeling after BA3b injection. However, in case of BA1 injection, the intra areal terminal arborizations represented larger skin area. Among the skin areas covered by injected areas, BA1 represents three times larger areas than BA3b.

4.4 Anisotropy of the retrograde and anterograde labeling

We found that both retrograde and anterograde labelings are oriented. Furthermore, the distribution of inter-areal labeling is also anisotropic. Connections distributed across the finger representations.

4.5 Distribution of the horizontally running thick and smooth axonal fibers

We found BDA labeled thick, presumably myelinated axons forming reciprocal connections between BA3b and BA1, which may be responsible for the fast conduction of information between the two areas.

4.6 Distribution of GABAergic interneurons targeted by the BDA labeled afferents in the somatosensory cortex

Injections of BA1 (three cases) and BA3a (two cases) were investigated. Selectivity of the inter-areal (BA1-BA3b and BA3a-M1) and intrinsic connections were examined for parvalbumin (PV) and somatostatin (SOM) containing interneurons.

5,6% of the mapped boutons formed close appositions with GABAergic interneurons. After BA1 injection 5,6%, after BA3b injection 5,8% of the BDA labeled boutons formed close appositions with GABAergic interneurons. 53% of the close appositions were found in the supragranular layers.

In case of PV positive cells there was no difference between the intra- an inter-areal distribution. After BA1 injection the anterogradely labeled axons formed close appositions with PV positive interneurons in a similar number in the two areas, whereas after BA3a injection close appositions were found only in M1. There was no difference between the supra- and the infragranular layers in the number of close appositions.

Close appositions with SOM interneurons resulted in a higher proportion in the injected area after both kinds of injections. After BA1 injection close appositions were observed only on dendrites, whereas after BA3a injection the ratio shifted to cell bodies.

5 Conclusions

5.1 Intra- and interareal connections between column size areas of BA3b and BA1

In this study we compared the lateral spread of inputs (projection neurons) and targets (efferent axonal patches) of column size cortical sites in the distal finger pad representations of the reciprocally connected and hierarchically organized somatosensory cortical BA3b and BA1. Several hierarchically relevant characteristics of the anatomical basis of lateral cortical interactions were explored, which were not studied before in the SI, by comparing the spread of intrinsic, feedforward and feedback connections.

5.1.1 Lateral spread of the connections

The lateral spread of the feedforward connections are the most restricted and can form the connectional basis of RF hotspots in SI. On the contrary, the large lateral spread of the feedback and intrinsic connections suggest their role in formation of the ecRF and, more generally, in tactile integrative processing both in terms of somatotopy and submodality in SI.

We also showed, that although the cortical spread of intrinsic connections is not remarkably different in BA3b and BA1, the smaller CMF results in a more widespread spatial integration of the peripheral input from the skin in BA1 than in BA3b.

5.1.2 Clustered distribution of the projection neurons

A further remarkable difference between the two areas were found in the clustering of the projection neurons, which can be related to the different size of the RFs in BA3b and BA1.

5.1.3 Anatomical foundations of the cortical population response

The spread of the intra-areal feedforward and feedback connections relative to the IOS-area also reveals that these connections contribute to the cortical population response in a different extent.

We also show that similar to the intrinsic connections feedforward and feedback connections are also anisotropically distributed.

Regarding the dynamics of interactions, the results show the existent of a fast conducting inter-areal pathway, similar to what have been described in the visual cortex.

5.2 Inhibition in the somatosensory cortex

Regarding PV and SOM containing inhibitory interneurons we found that the target specficity of the connections exhibit similar patterns to that shown in the rodents and primates.

6 Publication list

6.1 Publications of the dissertation

Pálfi E , Zalányi L , Ashaber M , Palmer C , Kántor O , Roe AW , Friedman RM , Négyessy L. Connectivity of neuronal populations within and between areas of primate somatosensory cortex. *Brain Struct Funct.* 223: (6) 2949-2971. (2018) **IF: 4.698 (2016)**

Pálfi E , Ashaber M , Palmer C , Friedman RM , Roe AW , Négyessy L. Neuronal connections within the hand representation in areas 3b and 1 of the somatosensory cortex in primates ˇ"Neuronalis összeköttetések a szomatoszenzoros kérgi área 3b és área 1 kézreprezentációs területén foemlosökben". *Orvosi hetilap*. 157:(33) pp. 1320-1325. (2016) **IF: 0.349 (2016)**

Ashaber M , Palfi E , Friedman RM , Palmer C , Jakli B , Chen LM , Kantor O , Roe AW , Negyessy L. Connectivity of somatosensory cortical area 1 form an anatomical substrate for the emergence of multifinger receptive fields and complex feature selectivity in the squirrel monkey (Saimiri sciureus). *J Comp Neurol.* 522:(8) pp. 1769-1785. (2014) **IF: 3.225 (2014)**

Negyessy L , Palfi E , Ashaber M , Palmer C , Jakli B , Friedman RM , Chen LM , Roe AW. Intrinsic horizontal connections process global tactile features in the primary somatosensory cortex: Neuroanatomical evidence. *J Comp Neurol.* 521:(12) pp. 2798-2817. (2013) **IF: 3.508 (2013)**

6.2 Conference publications of the dissertation

Pálfi E, Kántor O, Ashaber M, Roe AW, Friedman RM, Dávid Cs, Nitschke R, Négyessy L. Areal and laminar distribution of Interneurons targeted by somatosensory cortical afferents in the non-human Primate *Samiri sciureus.* IBRO Workshop 2014, Debrecen, Magyarország.

Pálfi E, Kántor O, Ashaber M, Roe AW, Friedman RM, Dávid Cs, Nitschke R, Négyessy L. Selective targeting of inhibitory interneurons by sensorimotor cortical afferents in the non-human primate *Saimiri sciureus.* IBRO Workshop 2016, Budapest, Magyarország.

6.3 Other publications

Fekete Z , Pálfi E , Márton G , Handbauer M , Bérces Zs , Ulbert I , Pongrácz A , Négyessy L. Combined in vivo recording of neural signals and iontophoretic injection of pathway tracers using a hollow silicon microelectrode. *SENSORS AND ACTUATORS B-CHEMICAL* 236: pp. 815-824. (2016) **IF: 5.401 (2016)**

Kantor O , Benko Z , Enzsoly A , David C , Naumann A , Nitschke R , Szabo A , Palfi E , Orban J , Nyitrai M , Nemeth J , Szel A , Lukats A , Volgyi B. Characterization of connexin36 gap junctions in the human outer retina. *Brain Struct Funct.* 221: pp. 2963-2984. (2016) **IF: 4.698 (2016)**

Kantor O , Varga A , Toth R , Enzsoly A , Palfi E , Kovacs-Oller T , Nitschke R , Szel A , Szekely A , Volgyi B , Negyessy L , Somogyvari Z , Lukats A. Stratified organization and disorganization of inner plexiform layer revealed by TNAP activity in healthy and diabetic rat retina. *Cell Tissue Res*. 359:(2) pp. 409-421. (2015) **IF: 2.948 (2015)**