ANATOMICAL INVESTIGATIONS OF THE SOURCE OF PERISOMATIC EXCITATORY INPUTS ON PARVALBUMIN-EXPRESSING INTERNEURONS IN THE DENTATE GYRUS AND THE DISTRIBUTION OF TRPC6 CHANNELS IN THE HIPPOCAMPUS

PhD thesis

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Introduction

The hippocampal formation plays a fundamental role in several cognitive processes such as episodic memory formation or spatial navigation. It is an anatomical and functional unit formed by the entorhinal cortex, subiculum, parasubiculum, presubiculum and the hippocampus. The hippocampus itself is a 3-layered, allocortical structure that is built up by the hippocampus proper and the dentate gyrus (DG).

Similarly to other cortical regions, most of the neurons in the hippocampus are categorized as either principal cells (PCs) or interneurons (INs), two groups that are functionally and morphologically distinct. In terms of their abundance and connectivity patterns, PCs make up the majority of the local neurons and also give rise to projections that target different brain regions. In contrast to PCs, INs arborize locally and are less abundant than PCs.

The GABAergic INs show great anatomical and physiological heterogeneity and play a fundamental role

in the precise spatiotemporal regulation of the neuronal activity. Functionally relevant IN populations have been defined using a combination of morphological, physiological, developmental properties and neurochemical markers. Based on which subcellular domain they innervate; two large populations of the INs are the perisomatic region-targeting INs and the dendrite-targeting INs. The perisomatic region-targeting INs innervate the perisomatic region of the PCs (proximal dendrites, somata, axon initial segments), while the dendrite-targeting INs form their synapses on the distal dendrites of the PCs.

Perisomatic inhibition is provided by axo-axonic cells (AACs) and basket cells (BCs) in the hippocampus. The AACs exclusively innervate the axon initial segments of the PCs, while the BCs form synapses on their proximal dendrites and somata. These INs exert strong control over the spiking activity of their postsynaptic partners. In the DG, BCs receive inputs from the commissural-associational fibers, the ipsilateral CA3 and from the

perforant path. The AACs also receive inputs from the perforant path and commissural fibers.

However, there are still open questions about the inputs of these neurons in the DG. A perisomatic region-targeting IN population expresses parvalbumin (PV-IRs). A circuit motif that is present in other cortical areas as well is the presence of perisomatic excitatory synapses on PV-IRs. In the DG, excitatory synapses have been described on the perisomatic region of these INs, however their source has remained unknown.

Previous results show that the perisomatic region of PV-IRs are heavily contacted by Zn^{2+}-containing boutons in the DG. The source of this input was not known and has been assumed to be the PCs of the DG, the granule cells (GCs), although, they do not send axonal collaterals to the *stratum granulosum*, where many PV-IRs are present.

Another type of excitatory neurons in the DG are the semilunar granule cells (SGCs). SGCs differ from GCs in anatomical, physiological, developmental and functional features. Similarly to GCs, the terminals of the SGCs also

contain high levels of Zn^{2+}. Therefore, these neurons may be a probable source of the perisomatic excitation of PV-IRs in the DG. In our work presented in the first part of my dissertation, we aimed to test the hypothesis that SGCs provide the perisomatic excitation to the DG PV-IRs.

In the second part of my dissertation, I present our work focusing on the cellular and subcellular distribution of TRPC6 channels in the hippocampus.

Ion channels are fundamental in neuronal computations. In animals, there are hundreds of ion channel genes. The orchestrated cellular and subcellular expression patterns of these diverse molecules are necessary for the functioning of neural circuits. Learning the precise cellular and subcellular distribution of these channels on the highly polarized neurons greatly facilitates our understanding of their possible functions.

The TRPC6 channels are transient receptor potential (TRP) channels, which are a conserved group of cation channels. In the brain TRPC6 channels are present only in a few regions, such as the hippocampus or the cerebrum

and they are involved in several physiological and pathological phenomena for example in spatial navigation, synapse formation, or epilepsy.

TRPC6 channels can be activated through multiple mechanisms. Phospholipase C (PLC) is activated by several G-protein coupled receptors, such as mGluR1/5 and receptor tyrosine kinases, such as the TrkB receptors. PLC hydrolyzes phosphoinositol-diphosphate-2 (PIP2) into diacylglycerol (DAG) and inositol-triphosphate-3 (IP3). The IP3 can bind to its receptors on smooth endoplasmic reticulum, which serve as intracellular Ca^{2+}-stores. The depleted Ca^{2+}-levels of the ER activate STIM2, which in turn could activate the TRPC6 channels. Meanwhile, the hydrophobic DAG remains in the membrane and can directly activate TRPC6 receptors. DAG-lipase, mGluR1/5 and PLC are all has been shown to be present in excitatory synapses closely localized to each other in the perisynaptic zone in the hippocampus.

To understand how TRPC6 channels could contribute to physiological and pathological neural computations in the hippocampus, our aim was to determine which exact

neural elements of the hippocampus express these channels and to determine their subcellular distribution.

Objectives:

The aims of this PhD dissertation were:

I/

1. Reveal the source of the perisomatic asymmetric synapses on the PV-IRs in the DG.

II/

1. Provide an overview of the expression of TRPC6 channels in the hippocampus of WT mice and rats and confirm the specificity of the TRPC6 antibody used in this study using Trpc6 KO mice
2. Determine which neuronal elements of the hippocampus express TRPC6 channels in rats and mice.
3. Investigate the density of TRPC6 channels in different compartments of the dentate GCs.
4. Determine the distribution of TRPC6 channels on the dendritic spines of the dentate GCs.

5. Determine the subcellular localization of TRPC6 channels in the mossy fibers and mossy terminals.
6. Identify the interneuron populations in the hippocampus that expresses TRPC6 channels.
7. Determine the subcellular localization of TRPC6 channels in interneuron dendrites.

Methods:

To reveal the source of the perisomatic asymmetric synapses on the PV-IRs in mice we have filled GCs and SGCs in in vitro whole cell patch-clamp recordings with biocytin. Fixed slices were re-sectioned to 60 μm thick sections and then processed for biocytin detection using avidin-biotin complex (ABC). The reaction was visualized using Ni-intensified diaminobenzidine (DAB-Ni). Those cells that presented collaterals with varicosities in the inner molecular layer and granule cell layer were further processed for parvalbumin immunoreactivity using diaminobenzidine (DAB), either for light- or electron microscopy.

A subset of the fixed slices was developed with Alexa Fluor 488-conjugated streptavidin. Once imaged, slices containing collaterals with varicosities in the GC layer were demounted, re-sectioned to 60 µm thick sections, developed using DAB-Ni, and processed for parvalbumin immunoreactivity as described previously. An additional batch of slices was developed with Cy3-conjugated streptavidin, and subsequently immunostained for parvalbumin and visualized using an A488-conjugated antibody. The results were evaluated using light- and electron microscopy. To label Zn^{2+} - positive terminals Timm-staining has been used.

To determine the distribution of TRPC6 channels in the hippocampus in rats and mice we have performed immunostainings using an anti-TRPC6 polyclonal antibody. To get an overview of the TRPC6 expression we stained the slices using immunoperoxidase technique, developed the immunoreaction with DAB and evaluated the results with light microscopy.

To evaluate the specificity of the used antibody we performed immunostaining on slices from Trpc6

knockout mice. Most of the immunopositive profiles were absent in the hippocampus. However, cytoplasmic and glial immunolabeling were present in the Trpc6 knockout samples in all layers of the hippocampus, therefore these were considered aspecific signals and were excluded from the analysis. To determine the subcellular distribution of TRPC6 channels we used pre-embedding immunogold labeling and evaluated the immunostaining with electron microscopy. To determine the identity of the IN populations expressing TRPC6 we performed double immunostainings against mGluR1a or PV and TRPC6.

Results:

Our light- and electron microscopic data shows that a distinct excitatory cell type of the DG, the SGCs, give rise to the perisomatic excitation of the PV-IRs in the DG. In our dataset, we identified morphological differences between GCs and SGCs and found the SGC axons contact the perisomatic region of PV-IRs. Our subsequent electron microscopic analysis of these close contacts confirmed that SGCs form asymmetric synapses on the PV-IRs. The morphology of these boutons was strikingly

similar to the previously reported Timm-positive boutons contacting the perisomatic region of PV-IRs. Taken together, our results underline that the asymmetric synapses are partially if not fully are formed by SGCs on the perisomatic region of PV-IRs in the mouse DG.

Our results presented in the second part of the thesis show that TRPC6 channels are expressed in all compartments of the dentate GCs: somata, dendrites, dendritic spines, axons and axon terminals. In the dendrites, spines and somata the TRPC6 channels are located in the plasma membranes. On the dendritic spines the TRPC6 channels are somewhat enriched in a perisomatic *annulus* around the synapse but are evenly distributed otherwise. In contrast, in the axons of GCs, the TRPC6 channels are frequently located intracellularly, however, in the axon terminals they are often associated with membrane *cisternae* while eluding the vesicular clusters. Moreover, we found that both mGluR1a-expressing and PV-expressing IN populations express TRPC6 channels in the plasma membranes of their dendrites.

Conclusions:

In our studies presented in this PhD dissertation we have 1) revealed the source of the perisomatic excitatory synapses on PV-IRs in the mouse DG and 2) determined the cellular and subcellular distribution of TRPC6 channels in the rat hippocampus.

The results presented in the first part of this thesis show that in the DG SGCs give rise to the perisomatic excitatory synapses on PV-IRs. Thus, this sparse excitatory cell type is in an influential position within the DG network, likely contributing to pattern separation by activating feed-forward inhibition.

Our results presented in the second part of the dissertation show that in the hippocampus the DAG-sensitive, Ca^{2+}-permeable TRPC6 channels are expressed by the dentate GCs and two functionally distinct populations of INs.

In the dendrites, dendritic spines and cell bodies of the GCs the TRPC6 channels are expressed in the plasma membranes. In the spines the TRPC6 channels are evenly distributed showing an enrichment at the edge of the postsynaptic densities, where these channels overlap with

proteins involved in the regulation of DAG levels. In the mossy fibers TRPC6 channels are localized intracellularly, whereas these channels are often associated to membrane *cisternae* in the axon terminals.

We also show that in the hippocampus TRPC6 channels are expressed in the dendrites of two IN populations, characterized by their mGluR1a and parvalbumin expression. These IN populations have been suggested to fulfil feed-forward and feed-back inhibition, respectively.

Taken together our results show that TRPC6 channels are in position to participate hippocampal information processing through regulating the input and output of GCs and through modulating feed-forward and feedback inhibition.

Bibliography of the candidates's publications:

Related to the thesis

Nagy, G. A., Botond, G., Borhegyi, Z., Plummer, N. W., Freund, T. F., & Hájos, N. (2013). DAG-sensitive and Ca2+ permeable TRPC6 channels are expressed in

dentate granule cells and interneurons in the hippocampal formation. *Hippocampus*, *23*(3), 221-232. https://doi.org/10.1002/hipo.22081

Rovira-Esteban, L., Hájos, N., **Nagy, G. A**., Crespo, C., Nacher, J., Varea, E., & Blasco-Ibáñez, J. M. (2020). Semilunar granule cells are the primary source of the perisomatic excitatory innervation onto parvalbumin-expressing interneurons in the dentate gyrus. *Eneuro*, *7*(4). https://doi.org/10.1523/ENEURO.0323-19.2020

Unrelated to the thesis

Robinson, H. L., Todorova, R., **Nagy, G. A.**, Gruzdeva, A., Paudel, P., Oliva, A., & Fernandez-Ruiz, A. (2026). Large sharp-wave ripples promote hippocampo-cortical memory reactivation and consolidation during sleep. *Neuron*,*114*(2),226-236. 10.1016/j.neuron.2025.10.003

Fernández-Ruiz, A., Oliva, A., Soula, M., Rocha-Almeida, F., **Nagy, G. A**., Martin-Vazquez, G., & Buzsáki, G. (2021). Gamma rhythm communication

between entorhinal cortex and dentate gyrus neuronal assemblies. *Science*, *372*(6537). https://doi.org/10.1126/science.abf3119

Fernández-Ruiz, A., Oliva, A., **Nagy, G. A.**, Maurer, A. P., Berényi, A., & Buzsáki, G. (2017). Entorhinal- CA3 dual-input control of spike timing in the hippocampus by theta-gamma coupling. *Neuron*, *93*(5), 1213-1226. https://doi.org/10.1016/j.neuron.2017.02.017

Veres, J. M., **Nagy, G. A.**, & Hajos, N. (2017). Perisomatic GABAergic synapses of basket cells effectively control principal neuron activity in amygdala networks. *Elife*, *6*, e20721. https://doi.org/10.7554/eLife.20721.001

Vereczki, V. K., Veres, J. M., Müller, K., **Nagy, G. A.**, Rácz, B., Barsy, B., & Hájos, N. (2016). Synaptic organization of perisomatic GABAergic inputs onto the principal cells of the mouse basolateral amygdala. *Frontiers in neuroanatomy*, *10*, 20. https://doi.org/10.3389/fnana.2016.00020

Veres, J. M., **Nagy, G. A**., Vereczki, V. K., Andrási, T., & Hájos, N. (2014). Strategically positioned inhibitory synapses of axo-axonic cells potently control principal neuron spiking in the basolateral amygdala. *Journal of Neuroscience*, *34*(49), 16194-16206. https://doi.org/10.1523/JNEUROSCI.2232-14.2014

Holderith, N., Németh, B., Papp, O. I., Veres, J. M., **Nagy, G. A**., & Hájos, N. (2011). Cannabinoids attenuate hippocampal gamma oscillations by suppressing excitatory synaptic input onto CA3 pyramidal neurons and fast spiking basket cells. *The Journal of physiology*, *589*(20), 4921-4934. https://doi.org/10.1113/jphysiol.2011.216259