The role of location-dependent mechanisms in the dendritic information processing of hippocampal granule cells

PhD. thesis

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1. INTRODUCTION

Granule cells of the dentate gyrus actively contribute to hippocampus-related neuronal information processing. These complex neuronal roles require multifarious dendritic signal processing in granule cells. Therefore, detailed knowledge about the underlying dendritic mechanisms – the so called dendritic toolbox – is crucial for the understanding of the neuronal functions of granule cells and hippocampus. This thesis is the short summary of two studies that revealed such fundamental dendritic mechanisms.

In the first project of my thesis we studied the role of dendritically localized group II metabotropic glutamate receptors (mGluII receptors) in hippocampal granule cells. The mGluII receptors are known to be involved in the presynaptic forms of short- and long-term regulation of synaptic strength in granule cells. However, mGluII receptors are also present in the somatodendritic region of certain cell types, but the function of these dendritic receptors is less clear than the presynaptic effects. Because dendritic mGluII receptors had been described in granule cells as well, the goal of the first study of my

thesis was the characterization of the functions of these dendritic receptors.

In the second project we asked, whether dendritic back-propagating action potentials (bAPs) are capable of transmitting analog information about the overall state of the cells. Action potentials (APs) were classically regarded as digital signals, because – according to the all or none law – their size is constant and they are generated according to an all-or-none rule. However, it has been discovered recently that axonal APs also carry analog information about the somatic membrane potential, which can modify the AP shape and the primarily digital information content. Therefore, APs convey not only the rate and timing of neuronal activity, but they also provide information about the overall state of the cell. Thus, axonal APs can be considered as hybrid signal carrying both analog and digital information. bAPs have different biological roles than axonal APs, as they provide feedback signals to the dendrites about the electrical activity of the cell.

However, it was not known whether dendritic bAPs function as hybrid or pure digital signals. Therefore, we

examined whether bAPs are capable of carrying analog information content about the soma in granule cells.

2. OBJECTIVES

The aim of our first study was to reveal the physiological roles of dendritic mGluII receptors in hippocampal granule cells. We addressed the following specific questions:

I/1: What are the cellular effects of dendritic mGluII receptor activation in the granule cells?

I/2: What are the contributions of mGluII receptor functions to dendritic information processing of granule cells?

I/3: Are the dendritic mGluII effects and functions specific to hippocampal granule cells among the diversity of cells types?

In the second study, we examined whether bAPs in granule cells are capable of carrying analog information content or they act as a purely digital signal. During this study our specific questions were the following:

II/1: Are the shapes of bAPs dependent on the somatic membrane potential?

II/2: Do bAP-associated dendritic calcium signals reflect the analog information content encoded in the bAP shape, and if so, what mechanisms provide the link between the analogous state of the granule cells and the bAP-related calcium signals?

II/3: Do the analog information carried by the bAPs contribute to the regulation of dendritic synapses in granule cells?

3. METHODS

3.1 Slice preparation and electrophysiology

350 μm thick hippocampal slices from adolescent Wistar rats (P23-P36) were used for the studies. Experiments were performed either at near physiological temperature (35 °C, 1st project) or at room temperature (23-28 C°, 2nd project). Recordings were done under the guidance of infrared differential interference contrast optics. The electric activity of cells (and specific subcellular compartments like dendrites and axon terminals) was measured using patch-clamp electrophysiology.

Recording pipettes were filled with a K-gluconate based internal solution. After the recordings, slices were fixed in 2% paraformaldehyde solution for subsequent morphological and immunocytochemical characterization of the recorded cells.

3.2 Computer simulations

Simulations were performed using the NEURON 7.2 simulation environment. We used three morphologically and physiologically realistic passive granule cell models (ModelDb accession number is: #95960) to investigate dendritic mGluII function. The conductance model showed the experimentally observed voltage dependence of mGlu2-activated conductance (1. aim). We applied single compartment simulations to understand the behavior of calcium currents activated by the dendritic bAPs (2. aim). An N-type conductance served as template for the construction of the non-inactivating, high-voltage activated calcium current model (N_{I_Ca}), whereas the behavior of the inactivating model (I_{I_Ca}) was based on the properties of R-type currents. Combination of the two model currents sufficiently described the behavior of experimentally measured calcium currents.

3.3 Calcium imaging experiments

The bAP associated calcium signals were measured using the fluorescent calcium indicator, Fluo-5F (183 μM). The bAP-related fluorescent intensity changes were imaged either in line-scan mode at the selected dendritic locations (610 lines/s, using conventional confocal microscopy) or simultaneously over large dendritic areas (93.75 frames/s, using spinning disc microscopy).

3.4 Two electrode dynamic-clamp experiments

We interfered with the repolarization phase of bAPs by injecting an artificial conductance using a software based dynamic-clamp system. One recording pipette was used to monitor the membrane voltage and a second one for injecting the calculated currents. We measured the bAP-related calcium signals during conductance-clamping in a proximal dendritic branch.

3.5 MNI-glutamate uncaging

For evoking synaptic glutamate receptor-mediated voltage responses in granule cell dendrites we employed the MNI-glutamate photolysis. The spatio-temporally controlled glutamate uncaging was performed using the 405 nm laser line of a conventional confocal imaging system. The somatically measured amplitudes of the

photolysis-evoked responses were in the range that corresponds to a few native synapses.

3.6 Measurements of synaptic plasticity

Dendritic synaptic-like voltage responses were evoked by MNI-glutamate uncaging (uEPSPs) and these were paired with somatically evoked APs 300 times at 1Hz rate (timing range: ±4 ms, mean: -0.82 ± 0.4 ms). APs were evoked either from hyperpolarized or from depolarized membrane potentials during the pairing.

4. RESULTS I

4.1. Dendritic mGluR2 activation leads to G-protein mediated GIRK channel opening in granule cells (Aim I/1.)

First, we investigated the effects of mGluII receptor activation on the excitability of granule cells. Receptor-specific agonist application resulted in an outward current in voltage-clamp mode or hyperpolarization in current-clamp mode, suggesting an inhibitory effect for mGluII receptors in granule cells. We also performed pharmacological experiments to identify the contributions of the two members of mGluRII receptor family and

found that the somatically recorded hyperpolarization is mediated exclusively by type 2 metabotropic glutamate receptors (mGluR2s). Based on the voltage dependence and the pharmacological sensitivity of the mGluR2-activated outward current, we concluded that mGluR2s open GIRK channels through G-protein signaling. Direct recordings from isolated membranes of distinct cellular compartments, including large mossy fiber terminals, somatic membranes and dendrites, indicated that the mGluR2-evoked hyperpolarization originates from the dendrites of the granule cell.

4.2. mGluR2s activate GIRK channels in a short proximal dendritic segment and this spatial arrangement enables the selective and uniform control of individual dendrites (Aim I/2.)

The dendritic position of a potassium conductance determines its effects on the synaptic integration. Therefore, next we functionally mapped the location of GIRK channel opening within dendrites by measuring its effects on the dendro-somatic propagation of transient membrane depolarizations, which were evoked by glutamate uncaging at specific dendritic loci. In this experimental arrangement we took advantage of the

observation, which was verified in simulations, that differential spatial shunting profile should be associated with the potential dendritic mGluR2 localizations. Our results showed that the mGluR2-induced potassium conductance localized in a spatially restricted dendritic region of the granule cells at 100-130 μm distance from the soma. Importantly, our computational modeling suggested that the spatially restricted dendritic expression of mGluR2-coupled potassium conductance enables uniform and selective inhibition of individual dendritic branches in granule cells.

4.3. Only granule cells showed the mGluR2-activated hyperpolarizing effect among the tested hippocampal cell types (Aim I/3.)

To address the question whether the dendritic mGluR2 effect is a specific feature of granule cells, we recorded the effect of the mGluII specific agonist in CA1, CA2 and CA3 pyramidal cells and in three types of DG interneurons, including parvalbumin positive basket cells, regular-spiking perisomatic interneurons and neurogliaform cells. We found that none of the studied cell types showed mGluR2-activated outward current

suggesting that the dendritic mGluR2 effect is not a general cellular mechanism within the hippocampus.

5. RESULTS II

5.1. Distance-dependent modulation of dendritic bAP shape by somatic membrane potential (Aim II/1.)

Can the overall state of soma be reflected by the shape of bAPs? We answered this question by dual simultaneous somato-dendritic recordings from the granule cells. Our data revealed that the bAP shape depends on the somatic membrane potential in a location dependent way. In the proximal dendrites the speed of the repolarization of thebAPs , whereas in the distal segments their peak reported the physiological changes of somatic membrane potential.

5.2. The dendritic distance-dependent effects of somatic membrane potential are reflected by the bAP-evoked local calcium influx (Aim II/2.)

We examined whether the analog information stored in the bAP shape is also reflected in the dendritic calcium influx by imaging bAP-related fluorescent dendritic calcium signals. We found that the effects of somatic

membrane potential changes on dendritic calcium signals depends on dendritic distance. In proximal dendrites, bAPs evoked at hyperpolarized somatic membrane potentials resulted in larger calcium signals than calcium signals triggered at depolarized voltages. In contrast, somatic hyperpolarization had an opposite effect on calcium signals in distal dendritic regions, where bAP-evoked calcium signals were smaller at hyperpolarized states. Our data suggested that in the proximal dendrites the fast inactivating R-type calcium channels link the hyperpolarization-induced bAP-shortening with the enhancement of bAP evoked calcium influx. We also showed that the smaller calcium influx in distal dendrites is a direct consequence of the more negative bAP peak voltage during hyperpolarization. Furthermore, our data show that dendritic hybrid signaling is able to follow the physiologically relevant dynamic membrane potential changes of the soma.

5.3. The analog content of hybrid bAPs modulates dendritic synaptic plasticity (Aim II/3.)

Finally, we investigated whether the dendritic synapses are able to detect the analog content of hybrid bAPs. Pairing the glutamate-uncaging-evoked dendritic

potentials with bAPs resulted in the long-term potentiation of these uEPSPs. However, the potentiation was depended on the somatic membrane potential preceding the bAP-uEPSP pairing. Thus, dendrites are capable of utilizing the analog information encoded in hybrid bAPs at the level of synapses.

6. CONCLUSIONS

The results of the dissertation are as follows:

- Dendritic mGluR2 activation leads to G-protein mediated GIRK channel opening in granule cells.
- mGluR2s activate GIRK channels in a short proximal dendritic segment
- The strategic location of the mGluR2 effect enables the selective and uniform control of individual dendrites.
- The mGluR2-activated hyperpolarizing effect is specific to granule cells among hippocampal principal cell types

- Analog information about the somatic membrane potential is retained by the bAP waveform and by local dendritic calcium signaling.
- The analog modulation of bAPs in the dendrites is mediated by multiple mechanisms that are coordinated in a location-dependent manner.
- Analog information content of hybrid bAP signaling can be utilized by the dendritic synapses

7. LIST OF PUBLICATIONS

I. Publications on which the dissertation is based:

Brunner J , Szabadics J
Analogue modulation of back-propagating action potentials enables dendritic hybrid signalling
NATURE COMMUNICATIONS 7: Paper 13033. 13 p. (2016)

Brunner J , Ster J , Van-Weert S , Andrási T , Neubrandt M , Corti C , Corsi M , Ferraguti F , Gerber U , Szabadics J
Selective Silencing of Individual Dendritic Branches by an mGlu2-Activated Potassium Conductance in Dentate Gyrus Granule Cells
JOURNAL OF NEUROSCIENCE 33:(17) pp. 7285-7298. (2013)

II. Publications, which were not included in the dissertation:

Brunner J , Neubrandt M , Van-Weert S , Andrasi T , Kleine Borgmann FB , Jessberger S , Szabadics J
Adult-born granule cells mature through two functionally distinct states.
ELIFE 3: Paper e03104. 12 p. (2014)

Szabadics J , Varga C , **Brunner J** , Chen K , Soltesz I
Granule cells in the CA3 area
JOURNAL OF NEUROSCIENCE 30:(24) pp. 8296-8307. (2010)