Exploration of unique physiological features of dentate gyrus granule cells

Ph.D. thesis

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1. Introduction

The hippocampus is an evolutionarily conserved brain region, which is involved in complex cognitive functions such as episodic memory and spatial navigation. Being a simple cortical structure with many generally applicable features, the hippocampus was extensively studied in the past decades. On the other hand, hippocampus also have some unique anatomical and physiological specializations, which support its essential role in important cognitive functions. Many of these features, despite the long history of gaining the interest of researchers, remain poorly understood. My Ph.D. studies aimed to address intriguing and unique features of dentate gyrus granule cells (GCs), one of the principal cell types in the hippocampus.

In contrast to most of the other neuron types, GCs are continuously generated during the postnatal life, therefore adult neurogenesis provides unique form of plasticity to the neural circuit of the dentate gyrus. After their integration into the hippocampal circuit, adult born granule cells (ABGCs) show a maturation process (between 3-10th postmitotic weeks), in which their physiological properties change. Currently, it is believed

that ‘young’ ABGCs have distinct physiological role than those that are already ‘matured’. According to the current hypothesis, the distinct excitability of ‘young’ ABGCs contributes to their distinct function. However, it was not clear, how the continuous maturation of physiological properties enables sustaining discrete functional states, instead of a broadly distributed continuum. Therefore, we characterized the functional maturation of ABGCs after their integration into the hippocampal circuit, to unveil their switch from ‘young’ to ‘old’ functional phenotype.

The *in vivo* reported activity of GCs is also unique. They are one of the most quiescent neurons with their remarkably low overall firing activity (below 1 Hz). However, when GCs are active, they either fire single action potentials (AP), or short, high frequency spike bursts (3-7 APs with 100-200 Hz). Such bursting activity occurs for instance, when the animal traverses the specific spatial location which is associated to the GCs (so called place fields). Multiple studies addressed the physiology of mossy fibers (MF), the characteristic axons of GCs projecting to the CA3 hippocampal region. Various plasticity mechanisms have been reported in MF synapses

- usually with prolonged, non-physiological stimulations - which demonstrate the complex functions of this unique synapse. However less is known about the operation of MF synapses during physiologically relevant activity patterns. It is not clear whether short, truly physiological high frequency GC bursts activate any specific synaptic plasticity mechanisms.

2. Objectives

My first project, aimed to understand how young and matured adult born granule cells can perform distinct neuronal functions, with the following specific questions:

- How do the various biophysical properties of ABGCs mature?
- Which specific intrinsic physiological properties enable the emergence of distinct functional populations?
- When and how do ABGCs switch their "young" functionality to "matured" operation state during their postmitotic development?

In the second project, I addressed the synaptic mechanisms operating in MF synapses during physiologically relevant activity of GCs:

- Do short, truly physiological GC bursts accompanied by distinct synaptic plasticity mechanism than single AP firing?
- How physiologically relevant activity patterns, containing single APs, short high frequency bursts and long quiescent periods are translated by the CA3 neurons?

3. Methods

Electrophysiological data were obtained by *in vitro* recordings from acute hippocampal slices prepared from adult (postnatal day 51-105, for recording ABGCs) or juvenile (postnatal day 21-45, for recording MF synaptic functions) Wistar rats. Cells were visualized with an upright microscope (Eclipse FN-1; Nikon) with infrared (900 nm) Nomarksi differential interference contrast optics. The standard recording solution was composed of (in mM): 126 NaCl, 2.5 KCl, 26 $NaHCO_3$, 2 $CaCl_2$, 2 $MgCl_2$, 1.25 NaH_2PO_4, and 10 glucose. The temperature

was held at 35 - 36°C during the experiments. During standard conditions, for whole-cell patch clamp recordings high [Cl^-] intracellular solution were used. In the experiments, where disynaptic connections were targeted, the postsynaptic cells were recorded with low [Cl^-] intracellular solution, which enabled the clear separation of excitatory and inhibitory postsynaptic responses (EPSCs and IPSCs, respectively). Electrophysiological recordings were acquired with Multiclamp 700B amplifiers (Molecular Devices) and pClamp10 software.

ABGCs were birth-dated by Moloney murine leukemia virus vector. The intrinsic physiological properties of ABGCs at their age between 3-10th postmitotic weeks were recorded. We measured the conventional biophysical properties (such as the input resistance, the membrane time constant, the AP threshold, the maximum rate of rise of the AP, the maximum firing capability and the whole cell capacitance) of all recorded ABGC. We also measured their excitability by characterizing their input-output properties in response to sinusoidal current injections at various frequencies (mimicking temporally organized input patterns). All ABGC were individually

characterized, i.e. similar age groups were not averaged during the analysis.

To address MF synaptic connections, paired recordings were performed either by direct axonal recordings from MF terminals or somatic recordings from GCs in the CA3 as presynaptic source. The postynaptic partners were various interneurons (INs) or pyramidal cells (PCs) of the CA3 region. The presynaptic protocol contained single APs, high frequency bursts (usually 15 APs at 150 Hz) and various length inactive periods to mimic the *in vivo* activity of GCs. The effect of GC bursts on single AP evoked EPSCs were analyzed by comparing the unitary responses before, and with various time delays after single bursts. Recruitment of feed-forward INs during GC activity was indirectly measured by analyzing the probability of disynaptic IPSCs in PCs. Similarly, disynaptic EPSCs were also recorded in INs, indicating effective driving of intermediate PCs by presynaptic GCs.

During the recordings cells were filled by biocytin and identified by *post hoc* anatomical analysis.

4. Results

Functional maturation of adult born granule cells:

In the first project, we characterized the functional maturation of adult born granule cells (ABGCs) after their integration into the hippocampal circuit, to unveil their switch from 'young' to 'old' functional phenotype. We recorded birth dated ABGCs with various age (3-10 weeks), and characterized their conventional biophysical properties as well as their excitability. By considering their excitability parameters, ABGCs formed two distinct population. The first group of ABGCs were particularly *sensitive* for certain input intensity ranges (hence we named the group as S-group), whereas ABGCs in the second group were characterized by *linear* input-output properties (hence we named the group as L-group). Strikingly, these two integrative states were present during the entire maturation period, only the proportion of ABGCs with 'old' phenotype increased by time, suggesting quick functional transition in a surprisingly broad time window.

The difference in the excitability parameters of S- and L-groups argues that they potentially serve different

function. Our results indicate that a substantially heterogeneous cell population, (concerning their age and multiple physiological properties) might serve similar functions.

The effect of granule cell bursts in the CA3 circuit:

The second project addressed the physiological effects of GCs short high frequency bursts on the postsynaptic CA3 circuit. We identified a new form of synaptic plasticity of the hippocampal MF pathway that substantially contributes to the determination of the synaptic impact of single GCs during different physiological activity patterns. Specifically, we found that single MF AP evoked EPSCs in postsynaptic feedforward INs (FF-INs) robustly increase after single, short, high frequency presynaptic bursts. Intriguingly, the effect of presynaptic MF bursts was remarkably similar in all the tested postsynaptic FF-IN groups (including ivy cells, axo-axonic cells, regular spiking / CCK expressing interneurons and fast spiking / parvalbumin expressing basket cells), but it was different in postsynaptic PCs and septum-projecting spiny lucidum cells, which do not contribute to the local inhibition. Importantly, robust burst

effect was evoked even by as short as 3-5 AP (at 150 Hz) containing bursts. The described plasticity phenomenon has a unique temporal profile (which is different from the characteristics of post tetanic potentiation), it needs about a second after the burst to develop, then the amplification persists at a similar level for 6-8 seconds before decaying.

The observed plasticity phenomenon in mossy fibers was found to be a presynaptic process, characterized by increased release probability (indicated by decreased paired-pulse ratio). Strikingly, neither the potentially large calcium influx, invading the presynaptic terminal during the burst, was found to be essential in the burst induced potentiation (shown by experiments with presynaptic Ca^{2+} chelation using EGTA or reduced Ca^{2+} levels) nor the blockade of multiple signaling pathways (PKA, PKC, PLC, Munc13, mGluR2,3,7) could interfere with the amplification. Our results suggested that the priming of vesicles is transiently promoted during the plasticity phenomenon.

We tested the effect of the plasticity phenomenon on the recruitment of postsynaptic CA3 cells during GC activity by analyzing disynaptic connections. In

postsynaptic PCs, frequent disynaptic events in response for single GC AP, exposed the robust recruitment of feedforward inhibition after bursts of single GCs. In contrast, disynaptic EPSCs in inhibitory cells indicated effective activation of PCs only during the bursts, which is consistent with the well-known short-term plasticity features of the synapse.

The described synaptic mechanisms enable the hippocampal CA3 network to reliably detect the apparently small physiological differences between single action potentials and short GC bursts, and to also imprint these preceding activities on the subsequent periods of activity as different levels of CA3 network excitability.

5. Conclusions

My results provided important contribution to understanding the functions of a unique neuron type, the dentate gyrus granule cells. Below I summarize the main conclusions.

Conclusions on the functional maturation of adult born granule cells:

- The maturation of most of the conventional biophysical properties of ABGCs is continuous, however, their supra-threshold integrative properties mature through two discrete states. ABGCs in S-state are sensitive for certain input intensity ranges, while cells in L-state are characterized by linear input-output conversion.
- During the maturation process of ABGCs, both integrative states are present in a remarkably broad time window (5-9 weeks), during which the proportion of cells belonging to S- or L-group changes.
- Despite the highly variable biophysical properties of the S-, and L-group cells (due to their various age), the spiking output of the cells is efficiently tuned to similar integrative function.

Conclusions on the effect of granule cell bursts in the CA3 circuit:

- We identified a new plasticity form of hippocampal MFs. Postsynaptic responses in FF-INs evoked by a single MF spike triplicated seconds after a single, short, high frequency burst. The effect of the burst develops in the first second and remains up to 6-8 seconds.
- The potentiation can be evoked by as short as 2-7 AP-containing single high frequency (150 Hz) bursts, which is the physiologically relevant range for GC bursts.
- The phenomenon is postsynaptic cell type specific, only occurs in FF-INs. Single MF bursts did not have similar effect in postsynaptic PCs or septum-projecting spiny lucidum cells.
- The potentiation is clearly a presynaptic process, which acts as a promoted vesicle priming. The presynaptic changes probably do not involve the classical molecular pathways.
- The probability whether sporadic GC activity is eliciting APs in FF-INs strongly increases after

presynaptic bursts. Thus, single MF bursts effectively rearrange the recruitment of FFI for several seconds.

6. List of publications

Publications related to the thesis:

Neubrandt M[1], Olah VJ[1], Brunner J, Szabadics J Feedforward inhibition is randomly wired from individual granule cells onto CA3 pyramidal cells. *HIPPOCAMPUS. 2017 Jul 11.*

[1] equal contribution

Brunner J[2], Neubrandt M[2], Van-Weert S, Andrasi T, Kleine Borgmann FB, Jessberger S, Szabadics J Adult-born granule cells mature through two functionally distinct states.

ELIFE. 2014 Jul 24;3:e03104.

[2] equal contribution

Neubrandt M, Oláh VJ, Brunner J, Soltesz I, Szabadics J. Single bursts of individual granule cells rearrange feed-forward inhibition. *Under revision.*

Other publications:

Róna G, Borsos M, Ellis JJ, Mehdi AM, Christie M, Környei Z, Neubrandt M, Tóth J, Bozóky Z, Buday L, Madarász E, Bodén M, Kobe B, Vértessy BG
Dynamics of re-constitution of the human nuclear proteome after cell division is regulated by NLS-adjacent phosphorylation
CELL CYCLE. 2014;13(22):3551-64.

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
J NEUROSCI. 2013 Apr 24;33(17):7285-98.