Functional diversity and connectivity of hippocampal inhibitory cells

Ph.D. thesis

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1. INTRODUCTION

Complex behaviors require fast and efficient information processing, which is achieved in the mammalian brain by precise communication between specialized neuronal circuits. These circuits consist of distinct cell types endowed with highly specific connectivity rules enabling unique information processing capabilities. However, the number of available circuit components sets an upper limit to the ability of the central nervous system for responding to the myriad of arriving inputs. These theoretical limitations are partly overcome by the nervous system by employment of functionally diverse cell types. Neuronal diversities are well documented at the level of local circuitry, as in their innervation target and connectivity, and subcellular level as well, which ranges from the number, length and complexity of subcellular processes, to passive electrical properties and voltage or ligand gated channels expression, as well. These heterogeneities can lead to substantial differences in the information processing capabilities of distinct cell types, which multiplies the computational power of the whole nervous system. Therefore, to understand the inner workings of the nervous system and more specifically, the physiological roles of certain brain circuits, it is imperative to characterize two key features: the cellular diversity and the connectivity of these circuit components.

During my PhD studies, we investigated the circuit heterogeneities within the hippocampus, which area plays a key role in learning, coding and storage of spatial information and several pathophysiological processes.

In the first project, with my colleagues we investigated hippocampal feedforward inhibition (FFI), which is a fundamental wiring scheme in several cortical areas and is necessary for accurate neuronal information transfer. The dentate gyrus (DG)-CA3 interface within the hippocampus is a prototypical FFI circuit due to its anatomical and physiological features, as the axons of the principal cells of the DG, called mossy fibers (MF) establish morpho-functionally specific synaptic connections with excitatory and inhibitory cells in the CA3 region. Briefly, MFs contact pyramidal cells (PCs) of the CA3 with exceptionally large synapses, which are able to discharge their postsynaptic target due to the robust short-term facilitation. On the other hand, MFs evoke depressing responses in inhibitory cells of the CA3 region via regular sized axonal boutons. MF form connections with about ten-times more inhibitory cells than PCs. Therefore, although the CA3 region receives potent excitation from the DG, the activity of the MF pathway results in the powerful activation of local inhibitory cells, which enables dynamic control of transferred information. It is essential to know the manner in which FFI is wired at the level of individual cells, as different single-cell connectivity arrangements can underlie different FFI function, however, no information was available regarding the elementary FFI connectivity rule at the DG-CA3 interface.

The second project of my doctoral thesis focused on the functional heterogeneity of inhibitory cells of the DG-CA3 feedforward inhibitory network. It is known, that there are several different types of GABAergic interneurons in the hippocampus, which play distinct roles in regulating neuronal activity within the network. In the past few decades, these cell types have been

rigorously characterized based on anatomical and physiological features. New technologies relying on sequencing the complete RNA content of individual cells suggest that the number of GABAergic types may be higher than the number of morphologically determined classes However, the links between the conventional and transcriptional categories are not yet clear. To address this question, we investigated one of the most numerous and diverse inhibitory cell classes of the CA3 region, the cholecystokinin-expressing (CCK+) interneurons. Although these cells are heterogeneous in anatomical terms, including their axonal arborization and molecular marker content, their *in vitro* firing characteristics are considered to be homogeneous and distinct from other inhibitory cell types. However, *in vivo* recordings suggested that individual CCK cells may be active during different oscillatory states. Because of these complexities, hippocampal CCK+ interneurons (CCK+INs) are ideal to address the question of boundaries of cell classes from a broader perspective, including genes, molecular content, morphology, excitability and potential contributions to specific physiological functions.

As most of my work during my PhD studies relies on direct patch clamp recordings, often from small neuronal processes, in the third project we investigated the limitations of this technique using a computational approach. These limitations mostly arise from the physical properties of the recording pipettes, namely from their high resistance and substantial capacitance. The presence of these electrical components can distort electrical signals in two ways: by low-pass signal filtering and by directly interfering with the native electrical environment of the cellular structure (“observer effect”). However, theoretically it is possible to recover the native

electrical signal from heavily disturbed voltage measurements, if the precise morphological and electrical features of the recorded structure and the phenomenologic behaviors of the recording pipettes and other related instrumentation are known.

2. OBJECTIVES

The first aim of my doctoral work was to reveal the connectivity rule that governs FFI from individual granule cells of the DG onto CA3 PCs (Aim I/1).

In the second part of my PhD work, we have examined the functional heterogeneity of CCK+ INs in the hippocampal CA3 region. During this study, my specific questions were:
II/1: Are there differences in the excitability of CA3 CCK+INs if the recording conditions are extended to broader, physiologically relevant membrane potential (MP) ranges?
II/2: What conductances are responsible for the different excitability of CCK+INs?
II/3: How do functionally heterogeneous CCK+INs behave under realistic *in vivo*-like network conditions?
II/4: What are the underlying molecular differences in the distinct excitability properties of individual CCK+INs?

Finally, we examined how current clamp recording instrumentation influences voltage recordings from small neuronal structures. My specific questions were:
III/1: Can native signals be recovered from distorted recordings using a precise model of the recording

instrumentation and the morphological features of the recorded structure are known?
III/2: What are the contributions of pipette filtering and the observer effects to the measurement error in voltage recordings?

3. METHODS

3.1 Slice preparation and electrophysiology

Adolescent Wistar rats (P21-P36) from both sexes were used for slice preparation. Animals were deeply anesthetized with isoflurane and after decapitation 350 μm thick slices were cut in ice-cold cutting solution. Slices were transferred to a recording chamber of an upright microscope continuously perfused with oxygenated recording solution at a flow rate of 1-2 ml/min. Experiments were performed at near physiological temperature (35 °C). 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) were measured using patch-clamp electrophysiology. Recording pipettes were fabricated from borosilicate glass capillaries and filled with a K-gluconate based internal solution.

Several patch clamp recording configurations were used to investigate the electrical properties of neuronal cells and their subcellular processes as well. To explore the connectivity rule of FFI (1st project), paired recordings were made from CA3 PCs and either CA3 granule cells or giant MF boutons in the stratum lucidum. During the recordings, the large MF boutons were first

assessed in cell-attached configuration to evoke presynaptic APs from intact boutons, then whole-cell configuration was established for intracellular labeling for posthoc identification. In case of somatic (1st and 2nd project) and axonal whole-cell recordings (1st and 3rd project), voltage responses of the cell to square pulse current injections were recorded. To assess the firing behavior of CCK+INs (2nd project), current clamp recordings were carried out at two different MPs (slightly depolarized MP: -60 mV and hyperpolarized MP: -80 mV).

Active conductances in the somatic and dendrites were investigated in whole-cell voltage clamp mode, nucleated patch configuration and in outside-out patches made from the somatic and dendritic membrane surface (2nd project). In these recordings, voltage protocols were calibrated for investigating the activation and inactivation kinetics and voltage dependence of transiently activating potassium currents. Briefly, cells were held at -120 mV for 300 ms, after which more positive voltage steps were applied (ranging from -120 to -20 mV with a 10 mV increment for 300 ms to investigate activation kinetics and voltage dependence as well as inactivation kinetics), followed by a fixed voltage step to -30 mV for 100 ms (for addressing inactivation voltage dependence). Leak and capacitive current components were subtracted during potassium current recordings using online P/−4 method.

3.2 Computer simulations

Simulations were performed using the NEURON 7.5 and 7.6 simulation environments. For the 2nd project, morphologically detailed reconstructions were made from five CCK+INs to assess the behavioral consequence of

transient potassium channels with distinct voltage dependence and kinetics. Two potassium conductance models were constructed based on whole-cell voltage clamp recordings using a specific blocker of Kv4.2 and Kv4.3 channels and dendritic/somatic outside-out recordings made for examining the dendritic distribution of transient potassium channels. First, a right shifted potassium conductance model was constructed for regular spiking cells (RS, see Results). Based on this model, an additional leftward shifted potassium conductance model was made which, in combination with the right shifted model was able to capture voltage responses and voltage-clamp recordings from cells showing transient outward rectification (TOR cells, see Results). Additional active conductances were selected based on previous publications, to reproduce critical features of CCK+IN voltage responses. Excitatory and inhibitory synaptic events were placed on the dendritic and somatic surface of the reconstructed cells, with amplitudes constrained on whole-cell recordings and temporal distribution mimicking network oscillations at pre-defined frequencies. In order to investigate the contribution of the two potassium conductance models, each simulation was run two times, with replaced transient potassium conductances, in otherwise identical conditions and the firing responses of the two simulations were compared.

In the 3rd project, we recovered the native electrical behavior of a recorded MF axon from heavily distorted current clamp recordings through four consecutive fitting steps. First, we used voltage clamp data for tuning the actual pipette parameters. After connecting the model instrumentation with the detailed morphology of the recorded structure, we created the passive structure of the cell and adjusted the access

resistance according to the current clamp measurements. Next, we equipped the model structure with active sodium and potassium conductances and tuned their properties to reproduce the experimentally recorded action potential waveform. Finally, having established the appropriate conductance set, we were able to obtain the native behavior of the axon.

3.3 Constraining the model amplifier

We systematically collected data about the behavior of the amplifier using three different test configurations to emulate the amplifier features in the NEURON. In the first test we assessed real voltage clamp responses of the isolated head stage in open circuit, that is, without electrode holder, pipette and connection to the ground. This configuration was sufficient to characterize both the high frequency boost unit and capacitance compensation of the voltage clamp circuitry. Similar open circuit measurements are not possible in current clamp mode therefore we closed the circuit through a resistor. The third test circuit was a modified 1U model cell circuit (Molecular Devices) which contained passive electrical circuits representing a cell and a measuring pipette therefore it is suitable to emulate whole-cell recording conditions.

4. RESULTS

4.1 Feedforward inhibition is randomly wired from individual granule cells onto CA3 pyramidal cells (Aim I/1.)

To distinguish between the possible wiring arrangements of the FFI circuit of the DG-CA3 interface and to gain insight into the preferred tripartite connectivity between individual MFs, feedforward interneurons (FF-INs), and PCs, we recorded disynaptic inhibitory postsynaptic currents (diIPSCs) in PCs evoked by a single MF input. The diIPSCs reflect the reliable activation of an intermediate, not recorded FF-IN by the simulated MF. This locally activated FF-IN, in turn, provides characteristic inhibitory events to the recorded PC within a time window that is consistent only with two synaptic steps. Convergence of monosynaptic excitatory and disynaptic inhibitory connections in a single PC indicates that the individual GC innervates both the recorded PC and a FF-IN and the latter also innervates the same PC. In MF/GC to PC pairs often only diIPSCs were recorded. These indicate the reliable activation of an FF-IN which innervates the recorded PC. However, the PC does not receive direct EPSC from the individual GC. Thus, comparison of the connection probability of diIPSCs in directly connected and not connected MF/GC-PC pairs allow to predict the prevalent FFI innervation scheme.

We found that the probabilities of observing single-MF-elicited diIPSCs were similar in PCs with or without direct excitation (5 diIPSC couplings out of 42 tested pairs with monosynaptic inputs, 11.9%; 34 diIPSC couplings out of 321 tested pairs without monosynaptic inputs, 10.6%). Thus, the directly innervated PCs are neither spared nor preferred by single MF-evoked FFI. This is consistent with the idea that the FFI is randomly distributed by individual GCs, which potentially allows for the adjustment of general excitability of the CA3 network based on the activity of the DG.

4.2 Half of the CA3 CCK+INs show state-dependent firing (Aim II/1.)

To explore potential differences in the excitability of individual CCK+INs in the CA3 region of the rat hippocampus, first we characterized their firing properties in two different conditions. Specifically, CCK+INs were stimulated from slightly depolarized (range: -60 – -65 mV, relative to rest: -64.7 ± 0.4 mV) and hyperpolarized MPs (between -75 to -85 mV). When spiking was evoked from depolarized MPs, AP firing always showed characteristic spike-frequency accommodation. However, when firing was evoked from hyperpolarized MPs, in half of the recorded cells the spiking was strongly inhibited and its onset was delayed (252 ± 15 ms silent period). We categorized these cells as Transient Outward Rectifying cells or TOR cells. The rest of CCK+INs were characterized as regular spiking or RS cells as they fired regularly irrespective of their MP.

Next, we investigated whether the two MP-dependent firing phenotypes can be linked to previously known subtypes of CCK+INs. We found that both TOR and RS firing types occurred similarly among basket cells, mossy-fiber associated cells, and Schaffer collateral associated cells as well. Furthermore, as CCK+INs are known to heterogeneously express several molecules, we compared their molecular content by performing single cell RNA sequencing. Our mRNA data, which was validated by immunohistochemistry suggest that these firing phenotypes are not related to previously known subtypes of the CCK+IN class.

4.3 Differences in low-voltage-activated (I_{SA}) potassium currents underlie the heterogeneity of CCK+IN firing (Aim II/2.)

Next, we investigated the conductances that are responsible for the difference of the two firing types in CCK+INs. We focused on near-threshold potassium currents, due to the outward rectification of the firing pattern of TOR cells. Our voltage clamp experiments showed that TOR cells had a substantial amount of potassium currents below firing threshold, in contrast to RS cells, in which potassium currents activated at more positive voltages. Importantly, the majority of I_{SA} was available in TOR cells at hyperpolarized MPs (91.3 ± 1.6% at -80 mV), where the inhibition of firing was clearly observable. But at -60 mV, where the inhibition of spiking was not prominent, the majority of outward currents in TOR cells were inactivated; only 35.7 ± 3.4% of the current was available. Thus, the MP-dependence of the steady-state inactivation of I_{SA} can explain the TOR firing phenotype.

4.4 TOR cells are selectively silenced by I_{SA} in a narrow range of oscillatory states (Aim II/3.)

Based on whole-cell recordings and the rigorous investigation of the somato-dendritic distribution of I_{SA}, we constructed realistic multicompartmental single cell CCK+IN models, which were able to accurately represent both firing types. Using these models, we simulated the activity of RS and TOR firing cells during *in vivo*-like oscillating network conditions. When we compared the average spiking of the same model equipped with either TOR or RS I_{SA} conductance, we found that in most conditions, the firing rates we comparable. However, CCK+INs were efficiently silenced by TOR I_{SA}

conductances in 8-15 Hz input frequency regimes. On average, 39.1 ± 0.6% fewer APs were evoked. Thus, modifications in the properties of I_{SA} enabled distinct functions in individual cells that otherwise belong to the same neuronal class.

4.5 Different sets of Kv4.3 potassium channel auxiliary subunits are responsible for the functional specification of CCK+INs (Aim II/4.)

Finally, to address the molecular background of the different excitability of CCK+INs, we pharmacologically dissected their potassium currents, and found that the blockade of Kv4 channels selectively inhibits the TOR phenomenon. However, both our immunohistochemical data and mRNA data suggested that the Kv4.3 content of TOR and RS cells is comparable. Importantly, it has been shown that the properties of I_{SA} currents not only depend on the core channel, but on auxiliary subunits as well. After reanalyzing the RNA results at the level of individual exons, the data revealed that TOR and RS cells contain different sets of potassium channel interacting protein isoforms; KchIP4e was prevalent in TOR cells, KChIP1 in RS cells. The disparate KChIP content of CCK+INs explains crucial features of I_{SA} in TOR and RS cells, as in contrast to most KChIPs, including KChIP1 that is present in RS cells and other KChIP4 types, KChIP4e does not promote the surface expression of Kv4.3 channels, slows down the inactivation kinetics and it may help the left shifting of the voltage-dependence caused by an other auxiliary subunit, DPPs (which is also present in different isoform in TOR and RS cells).Taken together, we showed that the different functionality of CCK+INs is

caused only by apparently small differences, by different availability of splicing isoforms of few auxiliary subunits.

4.6 Reconstitution of the undisturbed membrane dynamics of a recorded axon (Aim III/1.)

In order to assess the native electrical behavior of small cellular structures without the influence of the instrumental distortions, we first constructed a measurement-based phenomenological model of the recording amplifier and implemented the patch clamp recording pipette with nonlinear passive parameters. To demonstrate the capability of the modelled instrumentation for predicting the undisturbed fast neuronal membrane responses, we choose APs recorded from a small bouton of a hippocampal MF axon. Our model revealed that the recorded APs were slower and smaller (absolute AP peak: 7.26 ± 0.24 mV, halfwidth: 0.5 ± 0.002 ms) than previously reported due to the extremely high resistance recording pipette, native spikes can be restored after the removal of the recording instrumentation from the model. Native APs were fast and large amplitude (absolute AP peak: 48.5 ± 0.24 mV, AP halfwidth: 0.297 ± 0.004 ms).

Next, we validated the predictive power of the model, by simulating the native AP waveforms of several recordings from the same axon, using different amounts of instrumental compensations, which resulted in different spike shapes in the recordings. Importantly, the model predicted similar native AP shapes irrespective of the experimentally used compensation and recorded AP shape, indicating that representation of the measuring system in the model is adequate to predict the correct native APs in a wide range of instrumentation settings (half-width of the native APs were 0.29 ± 0.006 ms,

0.297 ± 0.004 ms and 0.33 ± 0.005 ms for lower, moderate and high capacitance neutralizations respectively).

4.7 Instrumental and structural parameters cooperatively define signal distortion in recordings from small neuronal structures (Aim III/2.)

To characterize the contributions of the recording instrumentation to the filtering effect and the observer effect, in addition to recorded and native spikes we simulated the local AP waveforms as well, which is the AP *within* the cell when the pipette is present. The simulations were repeated with different structural sizes using a wide range of access resistances (R_{access}). Our results confirmed that the filtering effect increased sharply at higher R_{access} in both small and large structures. In contrast, while the observer effect caused significant measurement inaccuracy in low R_{access} ranges in small structures, larger structures remained relatively unaffected in a large range of R_{access}. Altogether, our results revealed target-size-dependent instrumental contribution to the measured voltage signals.

5. CONCLUSIONS

The results of the dissertation are as follows:

- Feed-forward inhibition is randomly wired from individual dentate gyrus granule cells onto CA3 PCs
- Half of the CCK+INs in the CA3 region show state-dependent firing (TOR cells) while the other half show regular firing patterns (RS cells). This novel type of heterogeneity does not correlate with previously known anatomical subpopulations of CCK+INs.
- A unique low-voltage-activated potassium currents (I_{SA}) underlies the state-dependent firing of TOR cells however a similar conductance is present in RS cells with different voltage dependence and kinetics.
- Realistic models of CCK+INs suggested that TOR cells are selectively silenced by I_{SA} in a narrow range of oscillatorions, between 8-15 Hz.
- Kv4 channels are responsible for both types of I_{SA} but these channels are differentially modulated by specific isoforms of auxiliary subunits.
- Reconstitution of undisturbed axonal membrane dynamics from voltage recordings can be achieved using *in silico* implementation of the patch-clamp amplifier and the detailed reconstruction of the target structure.

6. LIST OF PUBLICATIONS

I. Publications related to the dissertation:

Neubrandt M[1], **Oláh VJ[1]**, Brunner J, Szabadics J. (2017) Feedforward inhibition is randomly wired from individual granule cells onto CA3 pyramidal cells. Hippocampus, 27: 1034-1039.
[1] equal contribution

Oláh VJ, Lukacsovich D, Winterer J, Arszovszki A, Lőrincz A, Nusser Z, Földy C, Szabadics J. (2020) Functional specification of CCK+ interneurons by alternative isoforms of Kv4.3 auxiliary subunits. eLife, 2020;9:e58515.

Oláh VJ, Tarcsay G, Brunner J. Modelling the patch clamp experimental setup enables the recovery of native electrophysiological responses (*in preparation*)

II. Other publications:

Neubrandt M, **Oláh VJ**, Brunner J, Marosi EL, Soltesz I, Szabadics J. (2018) Single bursts of individual granule cells functionally rearrange feedforward inhibition. Journal of Neuroscience. 37: 1711-1724.