DIVERSE SUBCELLULAR DISTRIBUTION PATTERNS OF PROTEINS AND THEIR FUNCTIONAL CONSEQUENCES AT MULTIPLE SPATIAL SCALES

Ph.D. thesis

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Introduction

The diversity of neurons in the central nervous system (CNS) originates from their distinct morphological build-up (the number, length and complexity of their dendrites and axon; Figure 2.1.1), the heterogeneity of voltage- and ligand-gated ion channels expressed in their cell membranes with different subcellular axo-somato-dendritic distributions (Golding et al., 2005; Kerti et al., 2012; Kirizs et al., 2014; Hu and Jonas, 2014) as well as their passive electrical properties. This diversity leads to substantial differences in the information processing capabilities of the distinct cell populations. Another level of complexity is introduced by the broad variety of synaptic plasticity mechanisms. At the nanoscale level, synaptic plasticity can be manifested by a spatial reorganization of synaptic proteins (Tang et al., 2016; Pennacchietti et al., 2017). For example, distribution of voltage-gated Ca^{2+} channels (VGCC) governing Ca^{2+} influx necessary for presynaptic vesicle fusion and their spatial arrangement in relation to the docked synaptic vesicles within the active zone (AZ) critically affects vesicular release probability (P_r; Eggermann et al., 2012).

These features of the CNS inspired my doctoral work and prompted me to investigate the diverse protein distributions governing information processing both at subcellular and subsynaptic spatial scales with a combination of experimental and computational tools.

Objectives

1 Develop and validate a combined methodological approach to determine subcellular ion channel distributions of neurons. I used *in vitro* dendritic patch-clamp recordings from acute brain slices of male Wistar rats, morphological reconstruction of the recorded cells and *in silico* multi-compartmental modelling of the recorded V_m changes. To validate this combined approach, I aimed to replicate the known subcellular distribution of hyperpolarization-activated cyclic nucleotide–gated (HCN) ion channels in hippocampal CA1 pyramidal neurons.

2 Determine the contributions of dendritic and gap junctional filtering to signal processing in electrically coupled cerebellar Golgi cell networks and the factors that diversify the strength of electrical coupling between these inhibitory interneurons.

3 Find and implement methods that are able to quantify the distribution patterns of distinct pre- and postsynaptic proteins.

Methods

Electrophysiology: horizontal slices (300 μm) of the ventral hippocampus were cut from male Wistar rats (16 – 22 days old). Rats were deeply anesthetized by isoflurane (Abbott Laboratories) and killed by decapitation, in accordance with the Hungarian Act of Animal Care and Experimentation (1998, XXVIII, section 243/1998)

and with the ethical guidelines of the Institute of Experimental Medicine Protection of Research Subjects Committee. The brain was quickly removed and placed into an ice-cold cutting solution containing (in mM): sucrose, 205.2; KCl, 2.5; $NaHCO_3$, 26; $CaCl_2$, 0.5; $MgCl_2$, 5; NaH_2PO_4, 1.25; glucose, 10; saturated with 95% O2 and 5% CO_2. Hippocampal slices were prepared using a Leica vibratome (VT1200S; Leica Microsystems), incubated in submerged holding chamber in ACSF containing (in mM): NaCl, 126; KCl, 2.5; $NaHCO_3$, 26; $CaCl_2$, 2; $MgCl_2$, 2; NaH_2PO_4, 1.25; glucose, 10; saturated with 95% O_2 and 5% CO_2 (pH = 7.2 – 7.4) at 34 °C that was then gradually cooled down to room temperature (~1 hour). Recordings were carried out in the same ACSF at 24 °C, slices were kept up to 5 hours in the holding and recording chambers.

Cells were visualized with a Nikon Eclipse FN-1 microscope using infrared differential interference contrast (IR-DIC) optics and a water immersion objective (40x, 0.8 NA, Nikon). Current clamp whole-cell recordings from the soma and apical dendrite of CA1 PCs were performed using a MultiClamp 700B amplifier (Molecular Devices). Traces were filtered at 10 kHz and digitized online at 50 kHz using a Digidata1440A interface (Molecular Devices). Patch pipettes were pulled (Zeitz Universal Puller; Zeitz-Instrumente Vertriebs or P-1000 Micropipette Puller, Sutter Instruments) from thick-walled borosilicate glass capillaries with an inner filament (1.5 mm outer diameter, 0.86 mm inner diameter; Sutter Instruments). Tip resistance was 4–7 MΩ for somatic and 8–14 MΩ for dendritic recordings when filled with intracellular solution containing 130 mM

K-Gluconate, 5 mM KCl, 2 mM $MgCl_2$, 0.05 mM EGTA, 10 mM HEPES, 2mM NaATP, 1 mM NaGTP, 10 mM creatine phosphate titrated to pH = 7.3 with KOH, with 7 mM biocytin. Access resistance was < 15 MΩ for somatic and ≤ 62 MΩ for dendritic (range: 17 – 62 MΩ, average: 37 ± 13 MΩ, n = 22) recordings. CA1 PCs were held between -65 mV and -70 mV (baseline V_m, without correction for the liquid junction potential), at both somatic and dendritic sites. For passive dendritic recordings, the standard ACSF contained additionally 3 mM kynurenic acid (Tocris), 20 μM SR95531, 50 μM Cd^{2+}, 1 μM TTX (Tocris), 5 mM 4-AP (Tocris) and 40 μM ZD7288 (Tocris). All drugs were purchased from Sigma unless stated otherwise.

Computer simulations: CA1 PCs were filled with biocytin during the electrophysiological experiments through the recording pipette, and visualized by a DAB reaction for detailed morphological reconstruction using the Neurolucida software. Multi-compartmental models were constructed in NEURON (Carnevale and Hines, 2006) and simulations were run in NEURON (version 7.4).

For fitting apical dendritic recordings of CA1 PCs individually, the R_a parameter was kept at 150 Ω*cm (lower bound of the range determined by Golding et al., 2005), while the R_m and C_m parameters were iterated until NEURON's built-in Praxis fitting algorithm found the best fit of the experimental data. For simultaneous fitting of n = 6 apical dendritic recordings of CA1 pyramidal neurons, NEURON's MulRunFitter was used as in the individual case, but the summed error of all cells was used as a

feedback for the fitting algorithm. Spatial discretization was applied as parameters changed according to the *d_lambda* rule (Carnevale and Hines, 2006), with a value of 0.1. Simulations were run on a desktop PC under Windows 10 using variable time step integration method 'CVODE'.

Software development for modelling and analysing spatial point patterns: A software (GoldExt) was developed in Python (version 2.7, 64-bit), with which performed the generation of uniform and clustered patterns; their comparisons to random distributions; and cluster analysis were performed. GoldExt uses the following dependencies: numpy, scipy, matplotlib, scikit-learn (Pedregosa et al., 2011), xlsxwriter, openpyxl and PyQt4 (the latter for graphical user interface (GUI), which was drawn using Qt Designer). GoldExt is developed, tested and ran on 64-bit Windows environment (Windows 10). The software is available at https://github.com/nusserlab/GoldExt.

Results

Subcellular HCN and leak conductance distribution of hippocampal CA1 PCs: I performed *in vitro* dendritic whole-cell patch-clamp recordings from CA1 PCs' apical dendrite. In order to be able to model dendritic current injection-evoked V_m changes of hippocampal CA1 pyramidal neurons with the fewest possible variables, I included a set of antagonists and ion channel blockers in the ACSF to render the dendritic cell membrane passive. The control

solution lacked the HCN blocker ZD7288, while the passive solution contained this drug as well. After the electrophysiology experiments, I reconstructed the 3D morphology of the recorded neurons with the Neurolucida software. Using the *in vitro* electrophysiological experiments and 3D morphological reconstructions of the recorded neurons, as the last step, I sought to determine the distance-dependent subcellular distribution of HCN channels along the apical dendritic tree of CA1 PCs with multi-compartmental modelling. The 3D morphology and V_m traces of the cells were imported into the NEURON simulation environment to first determine the passive electrical properties (R_m, C_m and R_a) of individual neurons. To this end, I fitted the passive V_m responses (i.e. those recorded in passive conditions) of the cells with a purely passive model, which had a leak conductance (G_{leak}) inserted into the cell membrane with a uniform density. To estimate the local conductance of HCN channels (G_h), I also fitted G_h on the experimental data recorded in control conditions. The distribution of both G_{leak} and G_h were in good agreement with previous publications (e.g. Magee, 1998) when fitting individual cells, however, potentially due to the prominent diversity present among CA1 PCs, population-wise fitting of the data only partially reproduced the experimentally recorded V_m responses of these neurons.

Functional properties of dendritic gap junctions in cerebellar Golgi cells: I aimed to determine the underlying mechanisms of the diversity observed in the electrical coupling between these inhibitory cerebellar INs. To characterize the passive electrical properties of

GoCs, I modelled the V_m responses of these cells acquired by *in vitro* two-photon targeted dual soma-dendritic patch-clamp recordings in passive conditions after the *post hoc* LM reconstructions of those cells where the morphology was sufficiently preserved. I iterated freely the R_m, C_m and R_a parameters to obtain the best fit of the experimental data, resulting in an R_m of 3.5 ± 1.6 kΩ*cm^2, a C_m of 4.3 ± 1 μF/cm^2 and an R_a of 92 ± 115 Ω*cm (n = 5). To directly address the individual contribution of R_a and G_{GJ} to voltage attenuation between the electrically interconnected GoCs, I constructed multi-compartmental models of the reconstructed cell pairs. The sequential fitting of R_m and G_{GJ} was iterated until their values changed by less than 5%. This approach resulted in a mean R_m of 32 ± 7 kΩ*cm^2 and a mean G_{GJ} of 0.94 ± 0.35 nS (n = 4 GoC pairs). Finally, my simulations suggest that the main determinant of coupling strength between these cerebellar interneurons is the number of GJs between them.

Objective quantification of nanoscale protein distribution patterns: It is of great importance to quantify the distribution (whether it is random, clustered or uniform) of synaptic proteins in an objective manner. To this end, I investigated the efficacy of five different measures in distinguishing clustered patterns of localization points from random distributions. Nearest neighbour distance (NND) and a 2D autocorrelation function (2D ACF) proved to be the best. These metrics can also differentiate between uniform and random patterns on both single synapse and population levels. Once a point pattern is determined to be clustered, an obvious expectation is to investigate

whether clusters could be identified or not in such patterns. I applied four clustering algorithms, where the number of expected clusters does not need to be pre-defined. From these four algorithms, DBSCAN (Ester et al., 1996) outperformed the others throughout the whole tested localization point density range.

Conclusions

I have been participated in three main projects during my Ph.D. studies. All three have a common property that a multidisciplinary approach was applied to tackle the questions. The first project aimed to develop a method with which the subcellular distribution of ion channels could be determined. It is based on *in vitro* dendritic patch-clamp recordings combined with pharmacological manipulations of the ion channels of interest, morphological reconstruction of the recorded neurons and *in silico* multi-compartmental modelling of the recorded membrane voltage responses. To validate the hypothesis, I tested this method against the subcellular distribution of HCN channels in the apical dendritic region of hippocampal CA1 pyramidal neurons. The results showed similar distribution as has previously been reported (Magee, 1998; Lőrincz et al., 2002), and the models with linearly increasing G_h were in good agreement with the experimental data.

During the second project, we determined the GJ plaque conductance (0.94 nS), open channel probability of a GJ plaque

(18%) and the number of GJs between coupled GoC pairs as the main source of variability observed in coupling strength in a joint effort with Dr Andrea Lőrincz and Dr Frederic Lanore. We have also determined that signal attenuation is more severe along the dendrites than through GJs. These investigations implied the collective need of physiological, anatomical and computational tools as well.

A third project was about to find and test (on simulated datasets) pattern recognition algorithms that can objectively quantify the nanoscale distribution patterns of proteins. Two simple metrics, spatial autocorrelation function and the nearest neighbour distance distribution of the sample were able to distinguish between random, uniform and clustered patterns. Four clustering algorithms were also tested on clustered patterns to further investigate the organizational principles of the protein in question. I have implemented these algorithms in an open source Python software to provide an integrated surface for protein distribution analysis to a wider neuroscience community.

The bibliography of the candidate's publications

Publications related to the Ph.D. dissertation

- Szoboszlay M*, Kirizs T*, Nusser Z
  Objective quantification of nanoscale protein distributions.
  ***SCIENTIFIC REPORTS* 7:**(1) p. 15240. (2017)
  *Equal contribution

  IF: 4.259 (2016)

- Szoboszlay M*, Lorincz A*, Lanore F*, Vervaeke K, Silver RA, Nusser Z
  Functional Properties of Dendritic Gap Junctions in Cerebellar Golgi Cells.
  ***NEURON* 90:**(5) pp. 1043-1056. (2016)
  *Equal contribution

  IF: 14.024

Other Publications

- Olah VJ, Szoboszlay M
  CA2 Pyramidal Neurons: Biophysically and Anatomically Predisposed Integrators of Cortical Sensory Information.
  ***JOURNAL OF NEUROSCIENCE* 37:**(32) pp. 7564-7566. (2017)
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