Differential Synaptic Vesicle Priming States Determine Synaptic Strength at Hippocampal Glutamatergic Synapses

A Short Thesis Book by:

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1. INTRODUCTION

The hippocampal formation, located in the medial temporal lobe, plays a vital role in spatial and episodic memory, as well as cognitive map formation and attentional control. Information within its subfields is precisely regulated by various types of principal neurons and interneurons. This thesis focuses on synaptic transmission within the hippocampal CA1 region, specifically examining the connections between pyramidal cells (PCs) and two distinct types of GABAergic interneurons (INs).

Synaptic transmission, the fundamental process of neuronal communication, occurs at chemical synapses. When an action potential (AP) reaches presynaptic boutons, synaptic vesicles (SVs) fuse with the active zone (AZ) membrane, releasing neurotransmitters into the synaptic cleft. These neurotransmitters then bind to postsynaptic neurotransmitter receptors, causing them to open and generate a postsynaptic current. This intricate process involves SV tethering, docking and priming at the AZ, followed by Ca^{2+}-triggered fusion. The strength of

this communication, or synaptic efficacy, depends on the probability and amount of neurotransmitter release, alongside the number of activated postsynaptic receptors.

Synaptic efficacy is dynamic; it undergoes activity-dependent changes known as short-term plasticity (STP), which can manifest as either facilitation or depression. A key observation in cortical networks is that PCs form strong synapses characterized by high SV release probability (*Pv*) and short-term depression (STD) when they innervate parvalbumin-expressing fast-spiking interneurons (FSINs). In contrast, the same PC axons form weak synapses with low *Pv* and short-term facilitation (STF) when they innervate somatostatin-expressing interneurons (SST-INs), such as oriens-lacunosum moleculare interneurons (O-LM INs). Despite their distinct functional roles, the precise molecular mechanisms driving these differences in *Pv* and STP at hippocampal CA1 PC – FSIN and PC – O-LM IN synapses are largely unknown. This study aims to shed light on these mechanisms.

2. OBJECTIVES

Despite extensive research, the exact molecular mechanisms underlying differences in *Pv* and STP remain incompletely understood. This study investigated the distinct functional properties of hippocampal CA1 PC to FSIN connections (characterized by high *Pv*, STD) and PC to O-LM IN connections (characterized by low *Pv*, STF). This research aimed to answer the following key questions about these mechanisms:

- **Target-cell-specific localization of presynaptic molecules:** To investigate the target-cell-specific localization of presynaptic molecules, particularly Munc13-2, at AZs of PC axons targeting O-LM INs, and determine its influence on *Pv* and STF.

- **Differential Effective [Ca2+] Reaching Docked SVs:** To examine if differences in the effective [Ca^{2+}] reaching SVs at their release sites (RSs) contribute to the *Pv* disparity. This could be due to variations in the number or conductance of voltage-gated Ca^{2+} channels (VGCCs), or the

coupling distance between these channels and the Ca^{2+} sensor for SV fusion.

- **Differential Occupancy of RSs by SVs:** To assess whether a lower occupancy of RSs by SVs at PC – O-LM IN synapses contributes to their low *Pv*. *Pv* can be viewed as the product of the probability that a docking site is occupied by an SV (P_{occ}) and the probability with which a docked vesicle is released (P_{fusion}) upon AP arrival.

- **Priming State of Docked SVs:** To determine if differences in the priming state of docked SVs, specifically a lower proportion of fusion-competent vesicles at O-LM IN-innervating synapses, are the primary cause of *Pv* differences.

3. METHODS

This study employed a multi-faceted approach combining electrophysiology, pharmacology, high-resolution immunolocalization, and mathematical modelling to investigate synaptic properties in hippocampal CA1 PCs.

- **Animals:** Adult male and female transgenic mice (Chrna2-Cre-tdTomato for O-LM IN identification, Elfn1-KO, and Munc13-2 conditional KO) were used. C57BL/6J mice were used for SDS-FRL and immunofluorescent experiments. All experiments adhered to Hungarian Act of Animal Care and Experimentation.
- **Virus Injection:** AAVs expressing Cre-recombinase were injected into the dorsal hippocampus to achieve conditional gene knockout of Munc13-2 in CA1 PCs.
- **Slice Preparation:** Acute coronal hippocampal slices (250-300 μm thick) were prepared from anesthetized mice using a vibratome and

incubated in oxygenated artificial cerebrospinal fluid (ACSF).

- **Electrophysiological Recordings:** Whole-cell patch-clamp paired recordings were performed at 32-33°C. Presynaptic PCs were held in current-clamp, and postsynaptic INs (FSINs or O-LM INs identified by fluorescence/morphology/firing pattern) were held in voltage-clamp. Action potentials were evoked in PCs using depolarizing current pulses, and excitatory postsynaptic currents (EPSCs) were recorded. Various stimulation protocols (e.g., 3-5 APs at 40 Hz, trains at 5, 20, 100 Hz, and complex protocols for modelling) were applied.

- Pharmacological agents, including ω-Conotoxin GVIA (N-type Ca^{2+} channel blocker), 4-aminopyridine (4-AP, a K^{+} channel blocker), and Phorbol 12,13-dibutyrate (PDBU, a Munc13 activator), were used to assess their effects on EPSC amplitudes and STP.

- **Two-Photon Laser Scanning Microscopy:** Ca^{2+} imaging was conducted in local axon collaterals of CA1 PCs to measure AP-evoked Ca^{2+} transients using Fluo5F, with postsynaptic targets (parvalbumin+- or mGluR1α+-targeting boutons) identified *post hoc*.

- **Tissue Processing and Immunolabeling:** After electrophysiological recordings, slices were fixed, sectioned, and biocytin-filled cells were visualized. Multiplexed postembedding immunolabeling was performed to investigate the colocalization and density of synaptic proteins (e.g., Munc13-2, mGluR1α, mGluR7, PSD95, AMPA receptors, Bassoon, Cav2.1 VGCC subunit, Rim1/2).

- **EM Tomography:** Electron microscopy tomography was used to analyse the ultrastructure of synapses and quantify the density and spatial distribution of docked SVs in AZs targeting FSINs or O-LM INs.

- **SDS-Digested Freeze-Fracture Replica Labeling (SDS-FRL):** This technique was used to investigate the nanoscale distribution of $Ca_V2.1$ (P/Q-type VGCC) and Munc13-1 (as a marker for RSs) in AZs targeting FSINs or O-LM INs. The mirror replica method allowed for simultaneous labeling of pre- and postsynaptic components.
- **Modelling Short-Term Plasticity:** The sequential two-step priming model (Lin et al., 2022) was implemented in Berkeley Madonna. This model includes loosely docked (LS) and tightly docked (TS) states, with an additional labile tightly docked state (TSL) for high-frequency release. Model parameters were optimized to fit experimental EPSC data, and root-mean-square deviation (RMSD) was calculated to quantify the goodness of fit.

4. CONCLUSIONS

This study investigated the mechanisms underlying postsynaptic target cell type-dependent *Pv* and STP at two distinct hippocampal CA1 PC connections: those to FSINs and those to O-LM INs.

Our key findings are as follows:

- **Differential Synaptic Strength and STP:** PC – FSIN connections exhibited strong synapses with a *Pv* more than ten times greater than PC – O-LM IN connections, displaying STD. Conversely, PC – O-LM IN connections were weak and displayed STF.

- **Munc13-2 Localization and Function:** Presynaptic Munc13-2 was selectively enriched at low-*Pv* PC – O-LM IN synapses in an Elfn1-dependent manner. However, conditional knockout of Munc13-2 in CA1 PCs did not significantly affect the evoked EPSC peak amplitudes or STP patterns, indicating that Munc13-2 is not the primary determinant of the

low *Pv* at these synapses. This suggests that the functional effects of Elfn1 removal (increased EPSC amplitude and reduced STF) are solely due to the loss of mGluR7.

- **Calcium Channel Distribution and Influx:** High-resolution immunolocalization revealed that while the overall spatial distribution of Cav2.1 (P/Q-type VGCC) around RSs was similar, the density of these channels was significantly higher in FSIN-targeting AZs compared to O-LM IN-targeting AZs. While presynaptic AP-evoked Ca^{2+} influx was approximately 40% greater at PC – FSIN connections, this difference alone was insufficient to explain the ~10-fold disparity in synaptic strength. Experimentally matching Ca^{2+} influx with 4-AP only resulted in a 2.7-fold increase in evoked EPSCs at PC – O-LM IN connections, which still remained ~7-fold smaller than PC – FSIN synapses. This argues that P_{fusion} is not the sole, or even primary, limiting factor for *Pv*.

- **Synaptic Vesicle Priming States:** The phorbol ester analog PDBU, which enhances Munc13 activity and SV priming, caused a significant ~4.5-fold augmentation of evoked EPSCs at PC – O-LM IN synapses, compared to only a 1.7-fold increase at PC – FSIN synapses. This differential sensitivity strongly indicates distinct SV priming states between the two synapse types. Electron microscopy analysis showed similar densities of docked SVs in AZs innervating both FSINs and O-LM INs, ruling out differences in RS occupancy by morphologically docked vesicles. This points to incompletely primed, yet docked, vesicles limiting PC – O-LM IN synapse output.

- **Mathematical Modelling of Priming:** Using a sequential two-step SV priming model, we accurately reproduced the complex STP patterns observed at both PC – FSIN and PC – O-LM IN synapses. The model revealed that the primary difference underlying the distinct *Pv* at these connections lies in a substantial difference in the

fraction of well-primed SVs (TS fraction), rather than a major difference in the P_{fusion} of these SVs. Specifically, PC – FSIN connections had a P_{fusion} of 0.6 and a TS fraction of 0.44, while PC – O-LM IN synapses had a P_{fusion} of only 0.36, but a remarkably lower TS fraction of 0.07. The model demonstrated that modifying only three key parameters related to the second priming step (k_{2_0} and *s2*) and P_{fusion} was sufficient to transform the depressing PC – FSIN-like release dynamics into facilitating PC – O-LM IN-like dynamics.

In summary, this study provides compelling evidence that the profound differences in synaptic strength and STP between two hippocampal glutamatergic synapses targeting distinct IN types are primarily governed by the differential proportion of SVs residing in a fusion-competent, well-primed state, rather than variations in Ca^{2+} influx or the intrinsic P_{fusion} of these vesicles. These findings not only clarify the fundamental principles governing synaptic strength but also open new avenues

for understanding and potentially modulating neuronal circuit function in health and disease.

5. BIBLIOGRAPHY OF PUBLICATIONS

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