Inhibitory Connections Probed by High-Resolution Optogenetics Structure the Hippocampal Spatial Code

Hippocampal CA1 neuronal dynamics support episodic memory formation and consolidation. Inhibitory circuits play a fundamental role in controlling these dynamics, including place cell expression and replay. Yet, inferring inhibitory connections in vivo remains challenging, as inhibition does not produce reliable extracellular signatures and is embedded within complex circuit architectures. Here, we present a method to infer inhibitory connections in vivo using high-resolution optogenetics. We reasoned that inhibition reduces pyramidal cell excitability; thus, inhibitory connections can be inferred from a decrease of pyramidal cell responses to external stimulation while being monosynaptically inhibited. We first validated our approach using neural network simulations and whole-cell paired recordings in vitro. We then scaled up this approach using chronic silicon probes with miniaturize LEDs for long-term monitoring of hundreds of inhibitory pairs during behavior. Combined with excitatory connection detection and cell-type classification based on ground-truth data, this approach enables the generation of a comprehensive functional connectivity map of the recorded hippocampal circuit. The weight of the excitatory connection correlates better with the spatial information content of the inhibitory postsynaptic cell rather than that of the pyramidal cell. This suggest that interneurons integrate spatial information from multiple excitatory sources. Conversely, inhibitory connections balance spatial information between interneurons and pyramidal cells, indicating that inhibition imposes integrated spatial codes onto selected pyramidal populations. Together, this work establishes a novel approach to study circuit connectivity and reveals a hierarchical organization of spatial coding in hippocampal CA1, with interneurons integrating spatial information through excitation and structuring its expression through inhibition.