Resolving inhibitory connections in large-scale extracellular recordings by high-resolution optogenetics

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Learning from experience to drive behavior is one of the fundamental roles of the brain and relies upon changes in synapses efficacy. While current methods based on high-density extracellular recordings can detect excitatory connections, disclosing inhibitory synapses in behaving animals is far more challenging. Here we provide a method for resolving inhibitory synaptic connections based on low-density, high-resolution optogenetics in CA1. We reasoned that in the instant that an interneuron is directly inhibiting a pyramidal cell, optogenetic stimulation on the pyramidal cell will fail to evoke a response; namely, there will be a collision between an inhibitory postsynaptic potential (IPSP) and the light stimulation (Fig. A). First, we validated this principle in neural network simulations and in vitro pair recordings. Then we used high-resolution optogenetic probing (NeuroLight Optoelectrode, 20ms, 0.01-0.05µW) in the dorsal hippocampus of behaving mice (n=4 Camk2::Ai32 mice, Fig. B) to detect collisions between light stimulation of large numbers of pyramidal cells (822 pyramidal neurons) and the spontaneous spikes from the nearby inhibitory neurons (p-value cutoff less than 10-3 after 500 shuffled replicates). Preliminary analysis suggests a connection probability between putative inhibitory cells (including narrow waveform and wide waveform interneurons) and pyramidal cells ranging from 0.05–0.3 (0.01+/-0.02) (Fig. C).

In conclusion, we have developed an approach to resolve inhibitory connections in freely behaving mice from large-duration (>7 hours), large-scale, extracellular recordings. Monitoring the changes in the efficacy of these synapses during memory tasks will allow us to trace the synaptic plasticity dynamics underlying learning in the hippocampus.