Title: Chasing the memory engram

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Memory engram refers to those changes in brain tissue that correlate with a learned experience. But gaining a deeper understanding of the nature of a memory engram has been challenging. A central question to us is: what is the architecture of a memory engram?

We focused our examination in the hippocampus, a structure that over decades of research has provided us with the molecular and functional foundations of memory. The research led to the formulation of the "synaptic plasticity hypothesis of memory", which proposes that neural activity that underlies experience changes the efficacy of appropriate synapses to create and store memory.

First, we looked for such memory-associated changes in synaptic function by measuring synaptic transmission at distinct synapses along the hippocampal circuit. We trained mice in a spatial memory task and one-day after the end of training we obtained brain slices and placed stimulation and recording electrodes in four set of synaptic pathways. In the dentate gyrus, we found reduced transmission at distal, but increased transmission at proximal, synapses that receive inputs from the lateral or medial entorhinal cortex (EC), respectively. In CA1, we found enhanced synaptic transmission at proximal synapses that receive inputs from CA3 neurons, but unchanged transmission at distal synapses that receive inputs from the EC. This nonuniform functional layout suggest that memory fine-tunes synaptic circuit weights to experience's demand.

Next, we next looked into the cellular organization of memory associated changes. We used an *in vivo* immediate early gene tagging system (Arc-Cre/flox-eYFP transgenic mouse) to measure the percentage of tagged cells in different regions of the hippocampus at memory acquisition (training) and memory retention (one-day after the end of training) stages. In the DG, we found that memory training increases the percentage of tagged cells in the upper but not the lower blade. This increase was also observed, albeit reduced, after memory retention test. In the CA1 region, we observed higher recruitment levels compared to the DG with memory training, and an even larger recruitment size after memory retention test. These data suggest that different cell recruitment (or ensemble sizes) may be a consequence of synaptic circuit function changes across the hippocampal network with memory.

We also speculated that if memory recruits cells into ensembles, can it also do it at the level of gene expression profiles? That is, form "memory-associated gene expression ensembles." Recently, we started using spatial transcriptomics -a methodology that yields RNA sequencing data that can be traced back to almost single-cell resolution to the tissue of origin- to inquire on the spatial organization of biological processes and transcripts associated with memory. We discovered that in memory trained animals, most of differentially expressed genes clustered within the biological process "regulation of trans-synaptic signaling". Within this process, we found homer 3 to be highly enriched in CA3 area. Homer 3 is key for the structural organization of synapses and communication between the dendritic and synaptic compartments. These data mark the beginning of an exploration into the landscape of gene expression programs associated with memory.

Our overarching goal is to link the maps for synaptic circuit function, neuronal tagging and gene expression profiles to define the functional and molecular topology of memory-associated ensembles across the hippocampal circuit.