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What Maintains "Engram Cells" in a Neuronal Network?

Protein kinase M zeta (PKMζ) is autonomously active PKC isoform that maintains synaptic long-term potentiation and long-term memory. Using the state-of-the-art technique, dual-eGRASP (dual-enhanced green fluorescent protein reconstitution across synaptic partners, we investigate the role of PKMζ in long-term memory maintenance. Engram cells refer to neurons that are transcriptionally activated during memory encoding, which have been shown to be necessary and sufficient for memory retention. However, it is unclear what molecular mechanisms maintain these cells in functional networks. In dual-eGRASP, two complementary mutant fluorescent fragments are delivered by adeno-associated virus (AAV) and expressed separately on pre- and postsynaptic membranes, which reconstitute at the synapse to form a functional fluorescent protein. Postsynaptic dendrites of engram (E) and nonengram (N) cells in hippocampal CA1 are labeled by post-eGRASP, together with mScarlet and near-infrared fluorescent protein 670, respectively. Fos promoter-driven reverse tetracycline-controlled transactivator with yellow fluorescence pre-eGRASP, and Cre recombinase with cyan fluorescence pre-eGRASP are delivered into contralateral CA3 presynaptic E and N neurons by AAV, respectively. Hence, four types of synapses, E-E, N-E, E-N, N-N, can be identified by confocal microscopy. After 14 days, doxycycline is injected 2 h prior to behavior training. We use spatial learning paradigm, active place avoidance to activate the engram cells. The mouse is placed in a rotating arena that has an invisible fixed shock zone and must use remote spatial cues to avoid shocks. Spatial memories are rapidly acquired after three 30-min training trials and are associated with persistent increase of hippocampal PKMζ. Two days after training, the brains are collected and imaged using confocal microscopy. We will examine the presence of E-E synapses and in the future determine the functional role of PKMζ at these synapses.