PROJECT OVERVIEW

Dynamic memory management includes acquisition, consolidation, and eventual storage for further recall or removal by active forgetting. These processes, together, enable optimal adjustment to an ever-changing environment, rational decision making, and removal of troubling memories. Active forgetting is thus necessary for optimal cognitive function and is as important as memory acquisition for optimal human performance and cognitive and emotional well-being. *As extensive progress is made towards understanding memory acquisition, the mechanistic understanding of forgetting is largely elusive.*

The RhoGTPase Rac1 is a major regulator of the actin cytoskeleton in dendritic spines. Spines serve as the functional receivers of synaptic input where the reorganization of spine actin cytoskeleton underlies the functional outcomes of synaptic plasticity such as AMPAR trafficking and spine size change. Intriguingly, recent evidence suggests that Rac1 is a critical regulator of both the forms of synaptic plasticity, LTP and LTD. However, what determines Rac1 activity towards one form of plasticity over the other remains largely unknown. Rac1 requires activation by guanine nucleotide exchange factors (GEFs). In one well-characterized pathway, once activated, Rac1 instigates a phosphorylation cascade culminating in the phosphorylation of Cofilin, a complex regulator of the actin cytoskeleton, that depolymerizes actin. Many Rac1 GEFs are intimately associated with either LTP or LTD. The Rac1 GEFs Tiam1 and Kalirin7 respond to CaMKII following NMDAR activation and lead to spine enlargement and cell-surface insertion of AMPARs. Conversely, the Rac1 GEF, P-Rex1, mediates AMPAR removal following NMDAR activation during LTD. Direct activation of Rac1 using optogenetics in select dendritic spines induced spine shrinkage, manifestation of spine weakening.

Recent studies have begun to shed light on the molecular mechanism for active forgetting and have shown that expression of LTD underlies this 'neglected' aspect of memory management. The molecular principles of forgetting are largely unknown, nevertheless, studies have begun to show the critical role of Rac1 in this process. Over-activation of Rac1 was shown to accelerate forgetting of hippocampal dependent memory while inhibiting its function led to increased retention of that memory. Moreover, an insight into the mechanisms that might modulate Rac1-mediated forgetting found that the ***scaffold protein Scribble*** forms a signalosome with Rac1 in facilitating forgetting in fly mushroom body neurons, whereby genetic deletion of Scribble led to enhanced memory recall. Further investigations into Scribble, including our preliminary data, showed similar enhanced memory retention in mice when Scribble is mutated or knocked down in the mouse hippocampus **(Fig 1, 2 and 3)**. Scribble serves as a facilitator of molecular interactions at specific subcellular locations due to its multidomain structure and specified localization patterns. Scribble associates with the endocytic protein AP2 to recycle NMDAR subunits during basal synaptic activity. Scribble also influences spine development via the GEFs β-pix and adaptor protein NOS1AP. These studies highlight the significant impact of organizational scaffold proteins in orchestrating cell signaling at the synapse.

We propose that during LTD, Scribble associates with Rac1 GEFs which activate Rac1 and mediate the endocytosis of GluA2-containing AMPARs, thus underlying LTD and behavioral correlates of forgetting.

Our Specific Aims are as follows:

Aim1: Characterization of association between Rac1 GEFs and Scribble during LTD

We will determine the Rac1 GEFs that bind to Scribble during LTD and determine the subcellular localization of these proteins during LTD. We will use genetic manipulations and cLTD methods to induce LTD in cultured hippocampal neurons and in hippocampal slice and track protein localization to study protein translocation and interaction during LTD.

Aim1.1) Determine the binding of Rac1 GEFs and Scribble in ectopic cell lines, cultured hippocampal neurons and in hippocampal slice following cLTD.

Aim1.2) Determine Scribble and Rac1 GEF subcellular localization in spines following cLTD in cultured hippocampal neurons and in hippocampal slice.

Aim 2: Investigate the effect of Scribble on Rac1 activity during LTD

We will determine the role of Scribble on Rac1 activity during LTD by combining live imaging techniques with genetic manipulations. We will also utilize Scribble KO mice to determine the effect of Scribble on activity of Rac1 during LTD in hippocampal slices.

Aim2.1) Determine Rac1 activation induced by cLTD in cultured hippocampal neurons using FRET biosensor

Aim2.2) Determine the effect of Scribble on Rac1 activity during cLTD in cultured hippocampal neurons

Aim2.3) Determine the activity of Rac1 in Scribble$^{FLox/Flox}$ mice hippocampal slices during LTD

Aim 3: Determine the molecular mechanisms by which Scribble interacts with and regulates GluA2 containing AMPARs during cLTD

We will determine the role of Scribble in facilitating the removal of AMPARs during LTD. We will also determine the molecular mechanism by which Scribble might interact with AMPARs during LTD.

Aim3.1) Determine the effect of Scribble on AMPAR endocytosis in cultured hippocampal neurons

Aim3.2) Elucidate the molecular mechanism by which Scribble effects AMPAR endocytosis