ERK-mTOR interactions in the lateral, basolateral, and central amygdala during fear memory consolidation

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Introduction

The amygdala receives projections from the thalamus and hippocampus and is generally considered a critical locus of neural plasticity following fear conditioning. The different subnuclei within the amygdala interact through intrinsic connections and have different roles during the consolidation and expression of aversive learning. The details about how the lateral, basolateral, and central nuclei interact during long-term memory (LTM) formation are not well understood. ERK and mTOR are two major signaling pathways that regulate synaptic plasticity supporting memory consolidation in the amygdala. Phosphorylated p70s6k can be used as a readout of mTOR complex 1 activity, which regulates downstream translational machinery. The phosphorylation of ERK regulates transcriptional and translational processes. There is some evidence supporting an interaction between ERK- and mTOR-mediated translation during activity-dependent synaptic plasticity (e.g., Doakas et al., 2007). The current study focused on amygdala-subnuclei specific interactions between ERK and mTOR during LTM consolidation after fear conditioning. Consistent with our prior work, results suggest that bilateral microinjections of pharmacological inhibitors of ERK (U0126) or mTOR (rapamycin) phosphorylation into the lateral amygdala, leaving the central nucleus of the amygdala unaffected, is sufficient to prevent memory formation when assessed 24-hours after training. Conversely, blocking ERK phosphorylation within the central nucleus of the amygdala did not impact fear memory. Immunohistochemistry results revealed that mTOR inhibitor reduced phosphorylated ERK in the lateral amygdala, phosphorylated p70s6k in the lateral and basolateral amygdala, and increased phospho-ERK immunopositive cells in the central amygdala. Additionally, inhibition of ERK resulted in a significant reduction of both phosphorylated p70s6k in the basolateral and ERK in the lateral amygdala. This effect suggests a bi-directional interaction between the ERK and mTOR pathways that is dependent on the specific population of cells within the amygdala.

Antibodies

Primary antibodies purchased through commercial vendors were phosphorylated p70s6k (1:100, Cell Signaling) and phosphorylated ERK (1:500, Cell Signaling).

Procedure

Fear Conditioning

| 30-min UCS | 1-hr Sacrifice | Test | 4x UCS 24-hr |

Regions of interest were determined based on Ph-aut measurements to ensure equal diameters across tissue slices. Individual cells were counted and averaged bilaterally across 3 slices based on amygdala anatomy and cannulae placement.

Antibodies

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Cell counts

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ERK and mTOR phosphorylation is required in the basolateral region of the amygdala during consolidation

Conclusions

- Phosphorylation of ERK and p70s6k are required in the lateral complex of the amygdala following delay fear conditioning for memory consolidation.
- Infusions of the mTOR inhibitor rapamycin into the amygdala impaired trained phosphorylation of p70s6k in the LA and BLA.
- Local application of U0126 also showed the predicted attenuation of training induced phosphorylation of ERK although the sensitivity of the effect differed in LA versus BLA.
- The results establish a molecular profile within amygdala subnuclei during fear memory consolidation.

References


