**Independent Study Proposal**

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**Lab:** Klann lab
**Credits**: 4.0
**Num hours at lab per week:** 20-25
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The amygdala has been shown to be the main loci of fear memory acquisition and processing, specifically the consolidation of newly formed fear memories[1.]. For memories to be stored into long term, extensive studies have also shown that *de novo* protein synthesis is necessary[2]. The goal of this project is to develop an inducible protein synthesis inhibition system in the amygdala with temporal and spatial specificity in order to elucidate the mechanisms underlying not only normal memory processes, but also better understand malfunctions of the system leading to memory-related diseases, in our case fear-related memories such as phobias or PTSD.

Protein synthesis has been shown to be tightly regulated at the translation initiation step. Our pharmacogenetic protein synthesis inhibition system approach (will be referred to as PSI) therefore targets initiation. It does so by pharamacologically inducing the kinase domain of protein kinase R, PKR.

PKR is one of the four kinases that can phosphorylate eIF2α, a key protein in initiation. In its phosphorylated form at the α subunit, eIF2 interacts with eIF2B and is unable to recycle back to its active GTP-bound state, therefore halting the translation machinery and shutting down global protein synthesis. Our PSI system engineered a constantly active PKR kinase domain (PKRkin*) with a target sequence for NS3/4A protease. NS3/4A protease is better known as the main drug treatment for Hepatits C and has not been shown to be toxic. In the presence of NS3/4A protease, engineered PKRkin* levels go down but non-transfected normal PKRkin levels in the cell are not affected. NS3/4A effectively inhibits PKRkin*. The drug Asunaprevir (ASV) however inhibits NS3/4A. As such, when the drug is injected, PKRkin* is disinhibited and therefore expressed, which we have shown is then correlated with an increase in phosphorylated eIF2alpha and markedly lower translation. This strategy has therefore allowed us to develop a successful new protein synthesis inhibition system without off-target effects.

Novel double transgenic mice were then generated using that protein synthesis inhibition system transfected downstream of a floxed STOP cassette, crossed with a Nestin Cre driver line, allowing our PSI knockin system to be expressed in Nestin expressing cells. This therefore allows us to specifically express that inhibition pan neuronally throughout the amygdala when ASV is locally delivered to the amygdala.

I am currently quantifying de novo protein synthesis levels (as well as eIF2α and other protein levels associated with different pathways regulating fear memory) biochemically and correlating it with behavioral data examined in fear conditioning experiments where transgenic mice injected with ASV exhibit markedly lower freezing response when the conditioned stimulus is presented.

Recent studies have shown the complexity of the circuit involved in fear memory consolidation: most principle neurons in the lateral and centrolateral amygdala responsible for the acquisition and processing of memory are under regulatory control of different subtypes of inhibitory interneurons[3]. As such we're also currently directing our system to specific inhibitory neural cell type populations by selectively expressing our PSI system in PV and Som expressing inhibitory cells in the amygdala. The goal of this part of the project is to examine the role and activity of each of these interneuron populations in fear memory consolidation.

1- McLaugh J.L. (2004). Amygdala and memory consolidation. Annual Review Neuroscience. 27, 1-28

2- Flexner J.B. (1963). Memory in mice as affected by intracerebral puromycin. Science. 141, 57–59.

3- Wolff S.B. (2014). Amygdala interneuron subtypes involved in fear learning. Nature. 7501, 453-8.