

In order to study memory engrams, one approach is to permanently label engram neurons of one specific memory event with ChR2; and then to reactivate that specific memory event experimentally by light activation of those ChR2 labeled neurons.

To permanently label engram neurons of one specific memory event with ChR2, a “fos-CreER” line is often used, and AAV is often injected into the brain area of interest to achieve Cre-dependent ChR2 expression.

- a. Please explain why a “fos” promoter instead of the “fos” coding sequence is used in the “fos-CreER” line.

The fos promoter is thought to bind transcription factors in response to neural activity, for example during memory formation. By preserving this promoter and replacing the coding sequence with CreER, they are able to express CreER and, in turn, ChR2 in response to neural activity during memory formation.

- b. Please explain why CreER instead of Cre has to be used in such studies.

CreER is a fusion between Cre and a mutant form of ligand binding domain of estrogen receptor that keeps the Cre protein in the cytoplasm via interactions between ER and Hsp90 [1]. CreER entry into the nucleus can be controlled using drug treatment and without tight control over CreER entry, the experimenter can be certain that ChR2 is only being expressed in cells involved in the formation of memory of one specific event.

- c. Please explain how Cre-dependent ChR2 expression is achieved.

A stop codon with loxP sites on either side is placed upstream of a ChR2 gene which is then excised in the presence of Cre. Thus ChR2 is only expressed in the presence of Cre.

- d. Can another transgenic line be used to achieve Cre-dependent ChR2 expression – without using AAV? Why (or why not)?

**Explain how “tracing the relationship between input and output (TRIO)” was achieved in the published study in Beier et al. Cell 162: 622.**

Tracing the relationship between input and output (TRIO) is a process used to classify populations of cells based on which brain regions they receive their inputs from and which brain regions they project their outputs.

TRIO was achieved by injecting CAV-FLEX<sup>loxP</sup>-Flp into two subdivisions of the ventral striatum (lateral or medial nucleus accumbens), medial prefrontal cortex, and the amygdala which had been identified previously. They also injected AAV-CAG-FLEX<sup>FRT</sup>-TC and AAV-CAG-FLEX<sup>FRT</sup>-G into the VTA along with G-deleted rabies virus expressing GFP (RVdG). The expression of GFP in this rabies construct is dependent on TC and G expression which is, in turn, dependent on the retrograde transport of CAV-FLEX<sup>loxP</sup>-Flp. In this way, the authors can be confident that the only cells expressing GFP are those that output to one of these four brain regions. Also, since the rabies virus can propagate to VTA inputs and be “activated” by these VTA neurons, GFP expression can be achieved in the inputs and thus they have established an input output relationship.

**From homology screening, you have identified a previously unknown protein that is expressed in mammalian neurons with significant overlap in amino acid sequence with the SNARE protein syntaxin. Describe two experiments that could help demonstrate whether this novel protein is involved in vesicle docking and/or fusion. Please include diagrams of expected results.**

I will design one experiment that will show if this protein is involved in vesicle docking and another that will show if this protein is involved in vesicle fusion. These experiments together will show whether this protein is required for neither, one, or both. They are similar in nature to those used by Geppert et al. in their work on synaptotagmin.

For both experiments, I will use a mouse line in which a mutation has been introduced into the gene of interest. This would involve generation of a targeting vector with appropriate sequences for positive-negative selection (such as a Neomycin and HSV).

### **Experiment 1: Gene Editing & Electron Microscopy**

Since I already have the amino acid sequence from homology screening it would be possible to develop siRNA that would inhibit the translation of mRNA transcripts of the target protein. If this protein is involved in vesicle docking I would expect to see a change in the number of docked vesicles in electron micrographs. If it is involved in fusion

### **Experiment 2: Gene Editing & Measuring Post-Synaptic Response**

Using patch-clamp electrophysiology, I could measure the post-synaptic responses in the neurons of mutant mice and wild-type mice and compare the results.

**You recently discovered a compound extracted from a tropical plant that improves memory. Based upon the growing body of evidence demonstrating that memory is in part mediated by changes in synaptic strength, you set out to explore how this substance might alter excitatory synaptic transmission in the hippocampus. First, you'll need to come up with a catchy name for the molecule – in anticipation of its possible use in the clinic. Second, outline a hypothesis regarding the effects of this molecule on excitatory synapses and describe three experiments that will provide mechanistic insights into the effects of this drug on synaptic strength. Be clear about the proposed experiments, including enough detail to ensure that the experiment is feasible. Be sure to explain strengths and limitations of your approaches.**

**Molecule Name:** Remembrin

In principle, Remembrin could operate at several stages of the putative pathway for synaptic plasticity. I will hypothesize that Remembrin operates early in this pathway by increasing release probability of glutamate thereby facilitating the formation of stronger synapses. To prove or disprove such a hypothesis, I will design three experiments that provide evidence for the stage in this pathway the molecule operates. I will assume that my recent discovery consisted of enough research that I don't need to design an electrophysiology experiment that shows increased postsynaptic response after treatment with Remembrin. The experiments will be mainly based on fluorescence imaging.

### **Experiment 1: Release probability**

Enhanced plasticity could occur via the release of higher amounts of glutamate into the synapse. To determine if Remembrin acts at this stage, I would utilize quantal analysis. Quantal analysis defines several parameters of interest: the readily releasable pool, probability of vesicle fusion, quantal content, and quantal size (post-synaptic response to a single quanta). By using a glutamate sensor such as recently developed iGluSnFR which is an intensity-based glutamate fluorescence reporter with sub micromolar resolution. Cultured hippocampal neurons could be treated with the drug and iGluSnFR fluorescence measured before and after treatment.

### **Experiment 3: Remembrin as a neuromodulator**

Enhanced post synaptic response could also be result from neuromodulatory activity of Remembrin. The molecule could be act as an agonist for NMDA receptors or activate GPCRs that are known to be involved in synaptic plasticity. To rule out this possibility and show that it is a result of increased release probability, I will

### **Experiment 2: Trafficking of AMPA receptors**

To determine if the drug has an effect on the post synaptic density of AMPA receptors, I will fluorescently tag AMPA receptors and estimate their density from the integrated fluorescent signal. Perhaps this could be done through treatment with the drug followed by fixation and immunofluorescent imaging.

**You are recording synaptic transmission in a tissue slice preparation from brainstem where you expressed channelrhodopsin in a nucleus that sends dense axonal projections to the nucleus solitaries, a key center for adrenergic signaling and autonomic control. With the knowledge that this synaptic input likely influences adrenergic signaling in the CNS, you are particularly interested in examining the nature of this synaptic connection onto these adrenergic neurons.**

- a. Describe experiments that would help you understand the excitatory vs inhibitory effects of fast ion channel effects that you observe following activation of the synaptic terminals with 473 nm light flashes.
- b. Along with the rapid effects on membrane potential, you also note that high frequency stimulation results in a hyperpolarization of the neurons that persists for >20 min after stimulation. This persistent voltage effect involves a decrease in input resistance for the postsynaptic neuron. Illustrate the data that demonstrates the change in input resistance and present a hypothesis to explain the underlying mechanisms. Outline 2-3 experiments that would provide insight into how this persistent change is induced and maintained. It is important that you consider and incorporate intracellular along with intercellular mechanisms in your answer.