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. 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 to which they project their outputs. This group used the Cre-lox and Flp-FRT system to achieve input output tracing.

More specifically, TRIO was achieved by injecting CAV-FLEx^{loxP}-Flp which expressed a Cre dependent Flp recombinase into two subdivisions of the ventral striatum (lateral or medial nucleus accumbens), medial prefrontal cortex, and the amygdyla which had been identified previously. They also injected Flp-dependent AAV-CAG-FLEx^{FRT}-TC which expresses the avian EnvA envelope glycoprotein fused with mCherry and AAV-CAG-FLEx^{FRT}-G (Cre dependent rabies glycoprotein) into the VTA. G-deleted rabies virus expressing GFP (RVdG) was then injected into the VTA, being dependent on existing expression of TC and G for successful infection. In other words, the expression of GFP by the rabies virus can only occur in cells with existing TC and G expression which is, in turn, dependent on the retrograde transport of CAV-FLEx^{loxP}-Flp. In this way, the authors can be perform output tracing - the only cells expressing GFP are those that output to one of the four brain regions where CAV-FLEx^{loxP}-Flp was injected. As for the input tracing, the rabies virus can propagate to VTA inputs in a retrograde fashion after being activated by VTA neurons i.e. GFP expression can be achieved in VTA inputs and, in utlimately 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.

Diagram

Experiment 1: Gene Editing & Electron Microscopy

First, I would introduce a mutation into the wild-type gene of interest via homologous recombination in mice. This would involve synthesis of a targeting vector with appropriate sequences for positive-negative selection e.g. neomycin resistance cassette and HSV thymidine kinase gene. Alternatively, it may be possible to develop siRNA that would silence the translation of mRNA transcripts of the target protein. In any case, it would be necessary to generate a number of blots to confirm the desired expression profile had been achieved. Following genomic or transcriptomic modifications, I would prepare tissue samples for analysis via electron microscopy. Electron micrographs could be used to count the average number of docked vesicles in wild type versus mutant samples and therefore determine if the sequence identified using homology screening it involved in vesicle fusion.

Diagram

Experiment 2: Measuring Post-Synaptic Response

Using the same mutant mouse line discussed above, I would utilize patch-clamp electrophysiology to measure the response of post-synaptic neurons to stimuli artificially induced at the pre-synaptic neuron. Any notable impairment of the production of post-synaptic currents in mutant mice with respect to wild-type mice would suggest that expression of the sequence under consideration is important for fusion of synaptic vesicles and is potentially an alternate form of the syntaxin protein.

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 exocytosis 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 at which 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.

Experiment 1: Measuring pre-synaptic glutamate release

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). Here we would be considering the readily releasable pool, probability of fusion and quantal content. One method of precisely measuring glutamate in the synapse is by using a glutamate sensor such as iGluSnFR, which is an intensity-based glutamate fluorescence reporter with sub micromolar resolution. Cultured hippocampal neurons could be treated with Remembrin and iGluSnFR fluorescence measured before and after treatment. Note that this experiment would not reveal the exact parameter defined by quantal analysis that is being modified. Rather, this experiment would determine whether or not Remembrin has any effect at all on glutamate release.

Experiment 3: Remembrin as a neuromodulator

Enhanced post synaptic response could also be result from neuromodulatory activity of Remembrin. For example, the molecule could be act as an agonist for NMDA receptors or possibly 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 prepare samples treated and untreated with Remembrin. We can 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.

We can design paired pulse experiments that can be used alongside the existing optogenetic tools. For example, we might glue an optical fiber to the glass pipet and record the post-synaptic current of adrenergic neurons in the nucleus solitaries in response to activation of channelrhodopsin in the nucleus with 473 nm light. Since it is widely believed that residual calcium in the presynaptic cell increases release probability of synaptic vesicles containing neurotransmitter, comparing the amplitudes of paired pulses spaced on the order of milliseconds could reveal inhibitory or excitatory effects.

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.

The long-lasting hyperpolarization observed here suggests that these dense axonal projections to the nucleus solitaries are inhibitory synapses. Also, the fast ion channel effects indicate channels with high conductance (low resistance) that are highly sensitive to changes in voltage.

Since this effect is occurring over such long-time scales, it is unlikely that there is a presynaptic mechanism such as a change in the release probability of inhibitory neurotransmitter. Rather, it is likely a post-synaptic mechanism which could be an intracellular change in the postsynaptic cell e.g., modification of inhibitory neurotransmitter receptors. Intercellular mechanisms such as reduced reuptake of inhibitory neurotransmitter which would result in continuous activation of inhibitory receptors; however, this is unlikely to give the observed lasting effects. Both of these mechanisms would explain the increase in conductance at the postsynaptic cell. However, since the voltage effect lasts for such a long time, I hypothesize that tetanic stimulation induces plasticity of inhibitory connections resulting in spontaneous hyperpolarization of the postsynaptic cell.

Experiment 1:

The purpose of this first experiment is to prove that that there is an increase in the release probability by the presynaptic cell by measuring the paired pulse ratio in response to pulses of 473nm light on the nucleus expressing channelrhodopsin.

Experiment 2:

The purpose of this experiment is to determine whether or not this lasting voltage effect is due to intercellular changes in the post-synaptic cell.