Research Interests

Advanced cognitive learning is encoded in distributed circuits that span multiple forebrain areas. Further, synaptic plasticity and neural network theories hypothesize that essential information for specific discriminations is encoded in specific neuronal ensembles, neurons, and synapses. However, for a distributed forebrain circuit, the critical components that encode a specific discrimination, and the encoding mechanisms, remain unknown.

We are studying the encoding of an advanced cognitive task, visual object discrimination learning, using a genetic approach for neuronal circuit analysis. This learning is encoded in a distributed rat neocortical circuit that spans a critical multimodal associative area, postrhinal (POR) cortex, which is required for this learning. POR cortex neurons receive afferents from specific visual areas, and project to more than ten neocortical areas.

The genetic intervention we are using is activation of protein kinase C (PKC) pathways in small numbers of spatially localized neurons, via a virus vector. Specific PKC genes are required for specific learning tasks. Further, constitutively active PKCs occur naturally from calpain cleavage, and constitutively active PKMζ is genetically encoded, although it is not required for learning. Our approach is that activating PKC pathways affects plasticity to enhance learning, consistent with known roles of PKCs. The hypothesis is that neurons that contain activated PKC pathways increase their response to incoming activity representing a specific discrimination, thereby enhancing learning.

We showed that genetically modifying several hundred neurons in a critical multimodal associative area, POR cortex, alters synaptic plasticity and enhances visual learning. We delivered a constitutively active PKC (PkcΔ; via a virus vector) into several hundred spatially-grouped glutamatergic and GABAergic neurons in POR cortex. This intervention activates PKC pathways and increases activation-dependent neurotransmitter release. Of note, the rats learn new visual object discriminations faster and to higher accuracy (Fig. 1), but performance is not altered on control discriminations learned before gene transfer.

![Fig. 1. Enhanced visual object learning following delivery of a constitutively active PKC (PkcΔ), or controls (PkcΔGG, a point mutation lacking activity, PBS, or no surgery (wild type)), into POR cortex neurons. (A) Experimental design, (B) [] vs. +, and (C) / vs. \.](image)

Importantly, some of the essential information for performance is encoded in the genetically-modified circuit. After both the gene transfer and learning, creation of small neurochemical lesions that ablate the genetically-modified circuit (~21 % of POR cortex) selectively reduces performance for only discriminations learned after gene transfer (Fig. 2).
Fig. 2. The genetically modified circuit encodes some of the essential information for performance. Rats learned two image sets; PkcΔ or a control lacking activity (PkcΔGG) was injected via cannulas; rats were retested on one control image set, and then trained on two new, experimental image sets ([] vs. + & / vs. \). Rats received a neurotoxin, NMDA, via the cannulas, or no NMDA; the chosen dose ablated ~21% of POR cortex. After 10 days for lesioning, rats were tested on both image sets learned after gene transfer, and a control image set learned before gene transfer (|| vs. |||). Experimental group, PkcΔ/lesion; lesion-control, PkcΔGG/lesion; learning-control, PkcΔ/no lesion. The graphs show the changes in accuracy for each group on specific image sets after the lesion/delay, compared to before lesion/delay. (A) [] vs. +, (B) / vs. \, (C) || vs. |||.

During learning, the genetically-modified circuit is preferentially activated (Fig. 3). Activity-dependent gene imaging showed that before learning, this circuit exhibits minimal activity; the constitutively active PKC, alone, does not increase activity. Visual learning increases activity throughout POR cortex, and, importantly, the genetically-modified circuit exhibits larger increases in activity, but only during performance of discriminations learned after gene transfer. This critical circuit contains ~500 neurons and is sparse-coded, with a coding density of ~3%.

Specific discriminations are encoded in characteristic and different neuronal ensembles, and different discriminations are encoded in intermingled ensembles, as shown by mapping the positions of the active neurons in the critical circuit.
An identified ensemble in the circuit, the transduced neurons, is required for both learning and subsequent performance. To show activity in the transduced neurons is required for learning, PKC pathways were activated and neurotransmitter release was blocked, by coexpressing PkcΔ and a Synaptotagmin I (Syt I) siRNA (Fig. 4). To show activity in the transduced neurons is required for performance, after gene transfer and learning, Syt I siRNA expression was induced from a regulated promoter, resulting in deficits in performance. Correlatively, during learning, dendritic protein synthesis and three learning-associated signaling pathways; CaMKII, MAP kinase, and CREB; are preferentially activated in the transduced neurons.
The activity of PkcΔ-transduced neurons is required for enhanced learning. After gene transfer, rats were tested on [] vs. +.

We are currently determining the relationship between the gain in the circuit and learning, a critical parameter that controls learning in neural network theory. To this end, we are genetically modifying the action potential-dependent gain in neurotransmitter release during the learning. The results show that learning can occur only within a narrow range of the gain in release.

These results establish approaches for analyzing the encoding mechanisms and mapping the critical circuit. First, we will analyze encoding mechanisms by activating PKC pathways and coexpressing a gene that alters synaptic plasticity. Second, we will identify the neuron and synapse types that encode the learning. Using gene transfer to connected neurons, detailed below, the presynaptic neurons will receive the genetic modification that enhances learning, and, after learning, specific postsynaptic neuron types will receive a gene that blocks neuronal activity. Inhibiting critical postsynaptic neurons will cause learning deficits, identifying critical components. Third, for the critical ensembles, we will detail network architecture, which constrains encoding. We will use modern genetic tools to map the critical neurons and synapses.

For over two decades, my laboratory has pioneered helper virus-free Herpes Simplex Virus (HSV-1) vectors for gene transfer into neurons. We were responsible for the first direct gene transfer into neurons using a virus vector, the first use of a virus vector to alter neuronal physiology, and the first use of direct gene transfer to correct an animal model of a neurological disease. Further, we were responsible for the first temperature sensitive mutant packaging system, the first deletion mutant packaging system, and the first helper virus-free packaging system.

After developing the vector system, we realized that due to the complexity of the mammalian brain, a number of additional technological capabilities were essential for analyzing circuits. First, we developed promoters that support long-term expression in all neurons, or specific neuron types, including catecholaminergic, GABAergic, glutamatergic, or glutamatergic neuron subtypes. Second, we developed a general method to target gene transfer to specific neuron types, antibody-mediated targeting. We added the Staphylococcus A protein antibody binding domain to a vector particle protein. Complexes of these vector particles and specific antibodies target gene transfer to specific neuron types, such as neurons that contain NMDA receptor NR2A or 2B subunits. Third, we developed gene transfer to connected neurons, to deliver different genes into presynaptic neurons and a selected subset of their postsynaptic neurons, based on both projection area and synapse type (Fig. 5). The first gene transfer, into the presynaptic neurons, uses standard procedures. The vector expresses a synthetic peptide neurotransmitter that contains a dense core vesicle sorting domain, a neurotransmitter receptor binding domain, and the His tag. Upon release, this peptide neurotransmitter binds to the cognate receptors on the postsynaptic neurons. Antibody-mediated targeting to these postsynaptic neurons uses a His tag antibody, as the peptide neurotransmitter contains the His tag. Fourth, we recently developed HSV-Brainbow, which can label small numbers of neurons with unique hues. Additional technologies for circuit analysis will be developed, as indicated by results.
Fig. 5. The strategy for gene transfer to connected neurons, and the model system. POR cortex has a large projection to perirhinal (PER) cortex, shown as a neuron in POR cortex with an axon projecting to PER cortex. First, gene transfer into the presynaptic neurons, in POR cortex, uses standard procedures. This vector uses a glutamatergic-specific promoter to express a synthetic peptide neurotransmitter, containing i) a dense core vesicle (DCV) sorting domain, ii) a NMDA NR1 binding domain, and iii) the His tag. Second, upon release, the peptide neurotransmitter (solid ellipse with His) binds to NMDA receptors on the postsynaptic neurons in PER cortex. Third, targeted gene transfer to the postsynaptic neurons uses antibody-mediated targeting and anti-His tag antibodies. The postsynaptic vector uses a neuron-specific promoter (INS-TH-NFH) to express a dendrite-targeted GFP. This strategy supports an ~10-fold increase in gene transfer to connected neurons.

We have a long-standing interest in gene therapy for Parkinson's disease. We published the first report that corrected a rodent model of Parkinson's disease by direct gene transfer. Subsequently, we showed that coexpressing four dopamine biosynthetic and transporter genes supports high-level biochemical and behavioral correction of the rat model. This is a synthetic biology approach to gene therapy that recreates a biosynthetic and transporter pathway in heterologous neurons.

We developed a gene therapy approach to cognitive deficits. In aged rats, activating PKC pathways in hippocampal neurons corrects deficits in spatial learning. This approach may be applicable to cognitive decline due to aging, and potentially to Alzheimer's disease, as PKC reduces Aβ levels by directing processing of the amyloid precursor protein into the α-secretase pathway.