Bidirectional Conversation Between Neural Nuclei and Synapses

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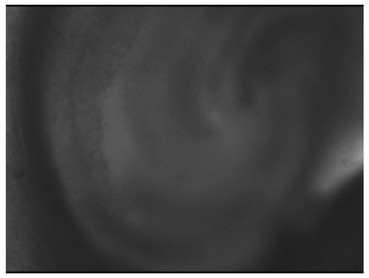
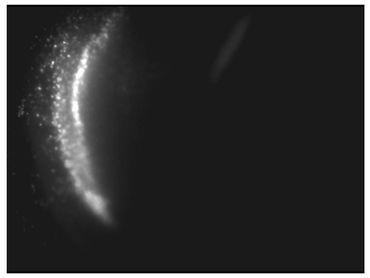
Regulation of Inhibitory Activity by Npas4

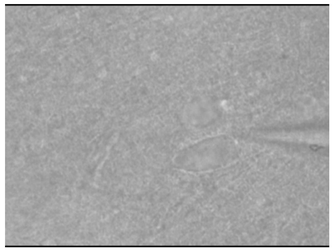
Npas4 is an immediate early gene and transcription factor, which is expressed in the brain and regulates inhibitory synaptic activity in response to excitatory synaptic activity. In wild type mice, Npas4 is known to increase inhibition on the soma, while decreasing inhibitory activity in the proximal dendrites. To identify the inhibitory neuron subtypes that form Npas4 regulated inhibitory synapses onto pyramidal cells, conditional knock out mice were stereotaxically injected with a Cre-expressing AAV virus, using coordinates for CA1 of the hippocampus. Sparse infection with AAV allows us to compare wild type and knock out cells, identified by GFP expression, within the same mouse as a control. Electrical recordings are made from interneurons and pyramidal cells and connectivity is evaluated. During recording, cells are filled with biocytin so that cell type can be resolved.



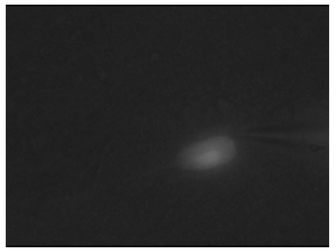
Infected Hippocampus under DIC Light



GFP in Hippocampus Infected with AAV

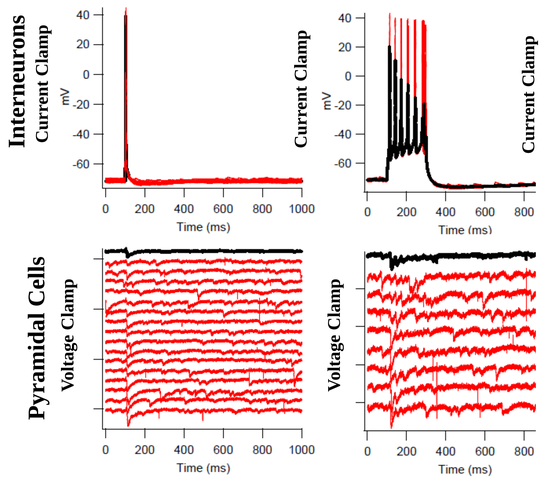


Intracellular microelectrode patching of interneuron in stratum radiatum

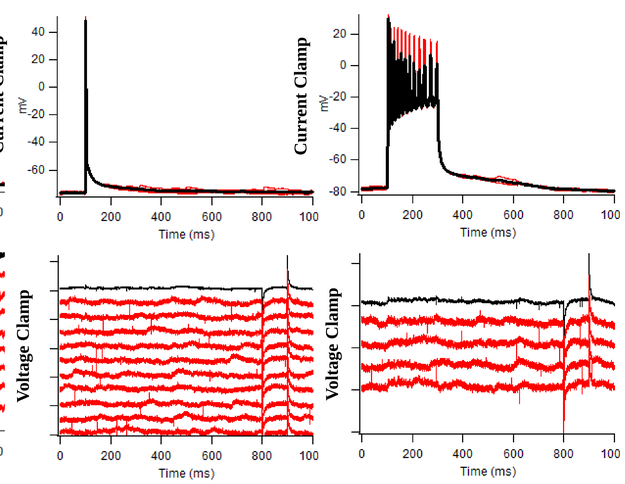


Biocytin-filled, fluorescent interneuron in stratum radiatum

Synaptically Connected Pair



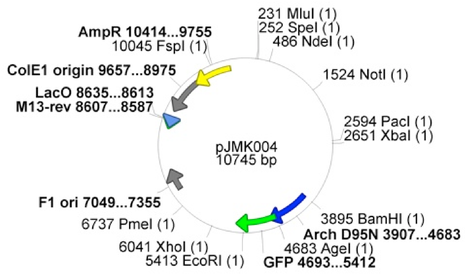
Unconnected Pair Synaptic Activity

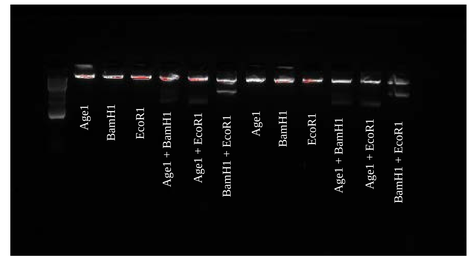


Testing Role of ER in Signaling Between Synapses and Nucleus

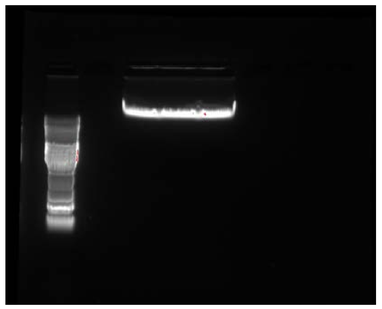
Synaptic activity must be communicated to the nucleus so that activity-dependent transcription can be initiated. This signaling is extremely rapid and the mechanism by which it is propagated is unknown. The ER is an ion channel –containing membrane that spans the entire cell, even extending into dendritic spines. The potential role of the endoplasmic reticulum in signaling between the nucleus and synapses is studied by use of the Arch D95N plasmid, a voltage-dependent, fluorescent , temporally specific reporter able to detect single action potentials. An ER retention sequence will be added to the plasmid for movement into and retention in the ER. Two different ER retention sequences are being used: RKR inserted at the Age1 cut site and KKSS at the EcoR1 cut site at the c-terminus of the ArchD95N sequence.

Arch D95N Plasmid Map

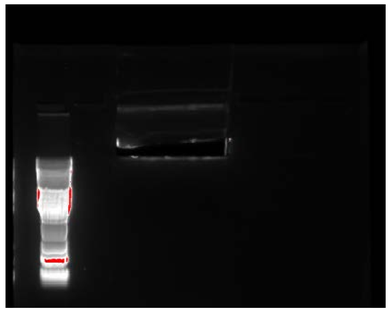




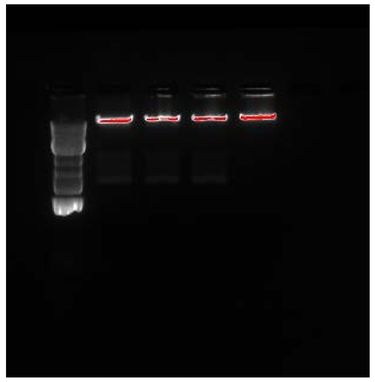
Restriction Enzyme Test Cuts
Through gel electrophoresis, the specificity of restriction enzymes Age1. BamH1, and EcoR1 was confirmed.



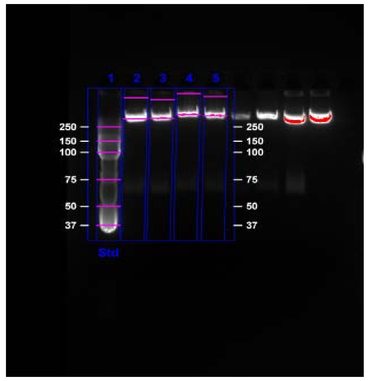
Vector Linearization Uncut Gel



Vector Linearization Cut Gel



ER Diagnostic Digest with Age1 and BamH1



ER Diagnostic Digest with Age1 and EcoR1