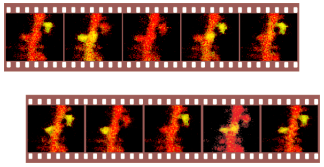
Part 1: ***Optical studies of single synapses***

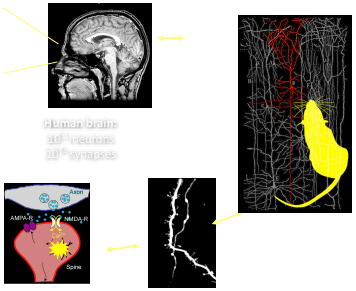


Karel Svoboda

Group Leader

HHMI,

Janelia Farm Research Campus



We interact with the world through our sensory organs.

Perception

Circuits – cell types; connected in a highly specific manner

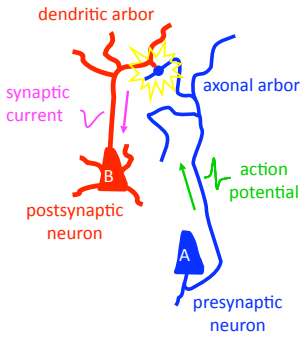
there are indications that we will learn a lot about circuits in the next few years. How do we learn what neurons in circuits do?

We need to be able to observe

And perturb – ideally genetically identified neurons

Do a kind of genetics of Neural Circuits

Synapses and neural circuits



There is a lot of evidence that learning-induced changes occur at synapses, the connections between neurons.

Synapses occur between axons, the output side of neurons and dendrites, the input.

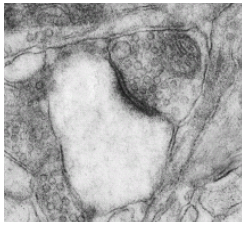
Synapses are specialized junctions consisting of a presynaptic terminal and a postsynaptic spine.

These dendritic spines have a postsynaptic density PSD, which contains the receptors for neurotransmitter, glutamate for the case of excitatory synapses.

There is a one-to-one correspondence between dendritic spines and synapses

Synaptic structure

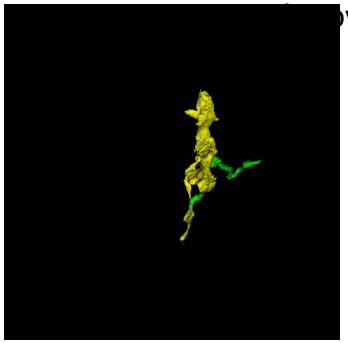
Electron microscopy



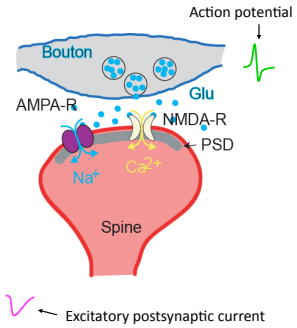
0.5 μm

Knott *et al* 2006

There are many things we would like to know about synapses:



Transmission at a canonical excitatory synapse



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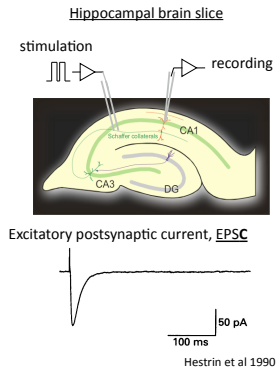
Measuring synaptic transmission



Hestrin et al 1990

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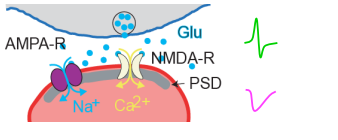
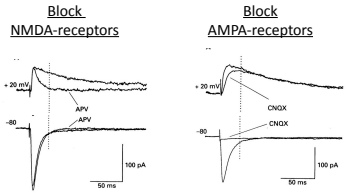
Two major types of synaptic glutamate receptors





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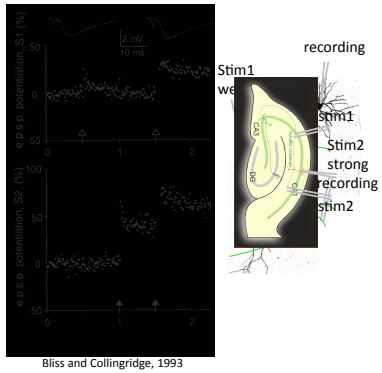
LTP in brain slices



Bliss and Collingridge, 1993

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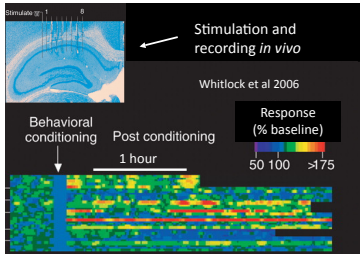
Learning induces long-term potentiation (LTP) of synaptic transmission in vivo

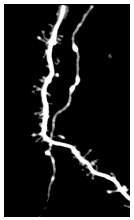


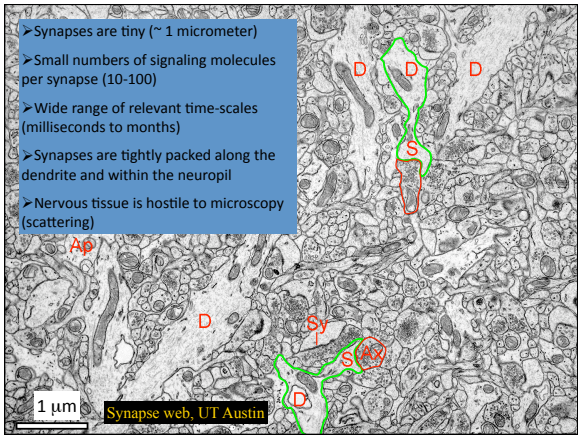
- LTP / learning require functioning NMDA-Rs
- LTP requires intact Ca^{2+} signaling in spines

one-trial inhibitory avoidance learning in rats produced the same changes in hippocampal glutamate receptors as induction of LTP

Some fundamental questions …

- How stable are synapses in the intact brain?
- How local are synaptic transmission and synaptic plasticity?
- How local is Ca^{2+}-dependent synaptic signaling at synapses?



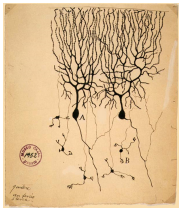


There are many things we would like to know about synapses:

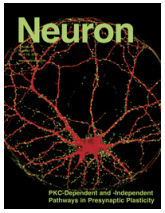
Imaging tools to probe single synapses

- **Two-photon excitation laser scanning microscopy** – image single synapses in scattering tissues
- **Ca^{2+} imaging –** measure synaptic transmission and Ca^{2+} signals
- **Two-photon glutamate uncaging** - activate single synapses
- **Fluorescence lifetime imaging** - measurement of protein-protein interactions in single synapses

Imaging neurons and their synapses – a long story



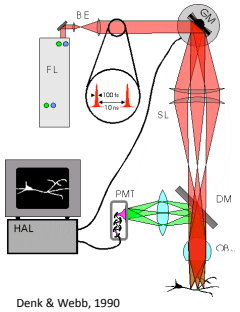
Ramon Y Cajal, 1899



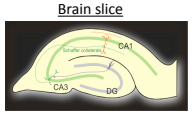
Neuron, 2007

Two-photon microscopy

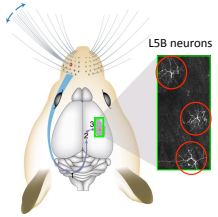
Two-photon laser scanning microscope



Brain slice



Intact mouse

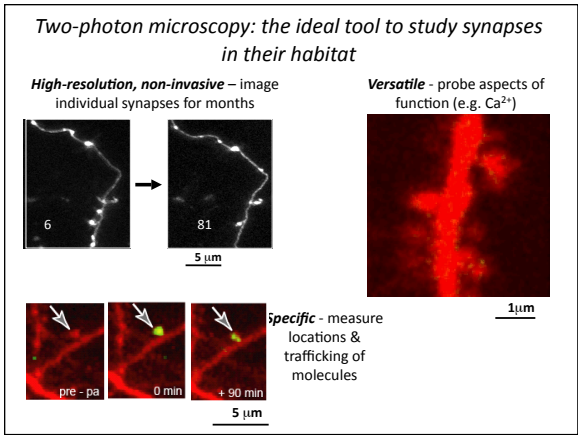


Denk & Webb, 1990
Reviewed in Svoboda & Yasuda, 2006

To begin to address these issues we use 2-photon laser scanning microscopy (2PLSM). 2-photon microscopy brings all of the advantages of fluorescence microscopy to scattering tissue.

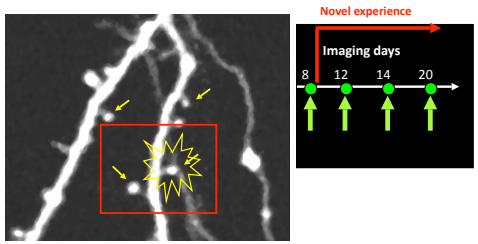
We use mice in which a subset o neurons are fluorescent.

Barrel cortex ..

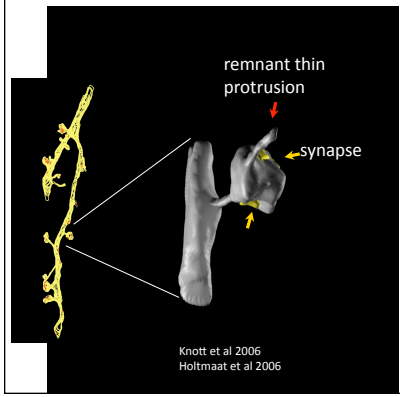


For this reason we use in vivo time lapse fluorescence microscopy to follow individual synapses in the brain. High-resolution fluorescence microscopy is perfectly suited to study plasticity because:

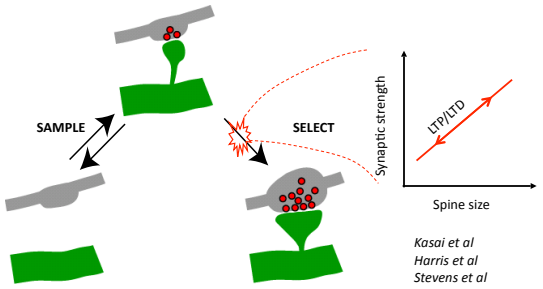
1. It has the resolution to track the fates of individual synapses
2. Non-invasive -- need to track the fates of individual synapses over time -- weeks and months
3. Fluorescence microscopy is specific -- depending on what you tag you can watch the distributions of tagged proteins.
4. Fluorescence microscopy is versatile.



After whisker trimming we see stabilization of transient spines.
Here is an example of what we see in time-lapse images after deprivation.
On the left is a new thin spine that had just formed; looks like a transient spine that is likely to disappear in the next imaging session. However, after deprivation this spine acquires a spine head, and now looks like a bona fide persistent spine. It then grows further, and there is another thin spine in the neighborhood, which then also gorws to acquire a spine heads. These spines then persisted to the end of the imaging session, 20 days after deprivation.
We see this very rarely under control conditions.
These sorts of images are at least consistent with stabilization of transient spines as a mechanisms of plasticity.



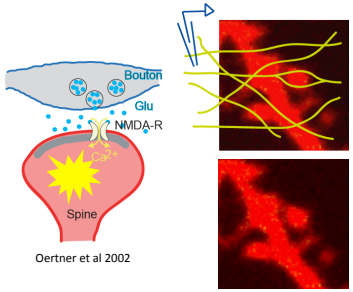
'Sampling' and experience-dependent 'selection'



Kasai et al
Harris et al
Stevens et al

What could be role of transient spines?
Our hypothesis is that transient spines grow to sample presynaptic partners. Most of these events lead to subsequent retraction.
In the presence of an imbalance of acitivity, for example after novel sensory experience, a fraction of these transient spines may then be converted to persistent spines, with an enlargement
This would be an elegant mechanism for plasticity. This would allow wiring of previously unconnected neurons, unleashing some of that flexibility and memory capacity that I have previously mentioned.

Ca^{2+} imaging to track synaptic transmission and Ca^{2+} signaling



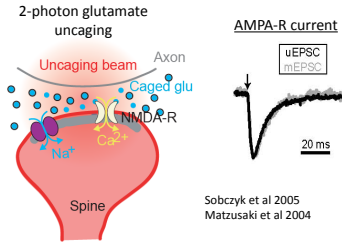
Oertner et al 2002

- Synaptic transmission is point-to-point
- Spine [Ca2+] signals are synapse-specific

Most of you have seen cartoons like these -- i want to point out that important aspects of synaptic function are associated with Ca^{2+} dynamics.
The technique that we use is Ca^{2+} imaging.

Imaging Ca2+ through NMDA –Rs:
Sensitive way to detect glutamate
Optical measurement transmission at single synapses

Synaptic responses evoked by 2-photon glutamate uncaging

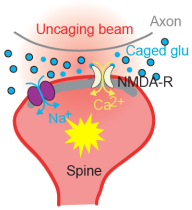


Sobczyk et al 2005
Matzusaki et al 2004

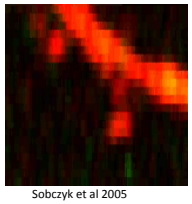
- Mimic synaptic transmission by uncaging glutamate in the synaptic cleft

Synaptic responses evoked by 2-photon glutamate uncaging

2-photon glutamate uncaging & imaging



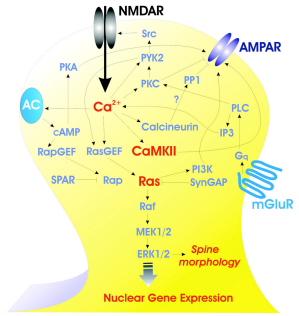
NMDA-R Calcium



Sobczyk et al 2005

•Spine [Ca^{2+}] signals are synapse-specific

Calcium-dependent signal transduction in spines

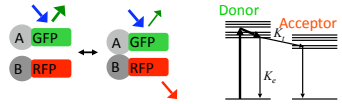


Sheng M & Kim MJ, 2002

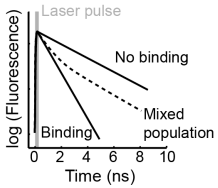
Downstream of NMDA-R activation and Ca2+ in neurons
Ras & GAPs, GEFs concentrated in spines
Critical for long-term potentiation
Critical for spine-enlargement
Mutants implicated in mental retardation
Amenable to analysis by FRET (Mochizuki et al *Nature* 2001)

*Measure protein-protein interactions using **fluorescence lifetime imaging (FLIM)***

Fluorescence resonance enegrgy transfer (FRET)

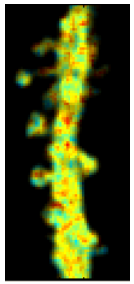


Fluorescence lifetime measurement



In order to study spatial signaling, we need a method to do this

FRET FLIM reports protein-protein interaction in neuronal microcompartments



Tune sensor for

signal level

biology

Yasuda et al, 2006

From this experiments and others we Cnclude that fras reports endogenous ras activation

Summary 1: Imaging tools

- Image single synapses in scattering tissues over milliseconds to months
- Measure synaptic transmission and Ca^{2+} signals in single synapses
- Activate single spines with high temporal and spatial precision
- Measure protein-protein interactions (quantitative biochemistry) in single synapses

Summary 1: Optical studies of single synapses

- Most spines persist for months; other spines appear and disappear over days
- New spines are stabilized after novel experience
- New spines have synapses
- Synaptic transmission is point-to-point
- NMDA-R mediated Ca^{2+} signals are limited to individual dendritic spines