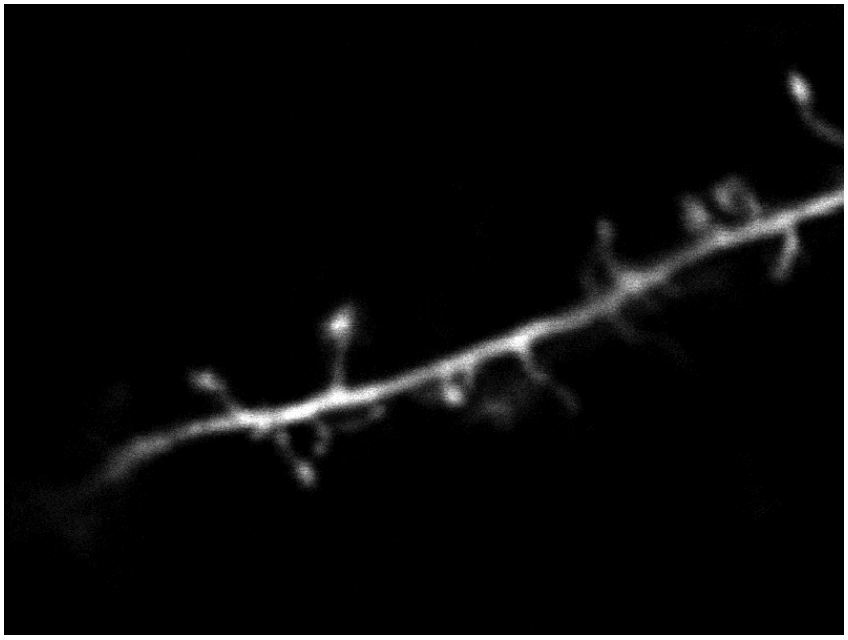
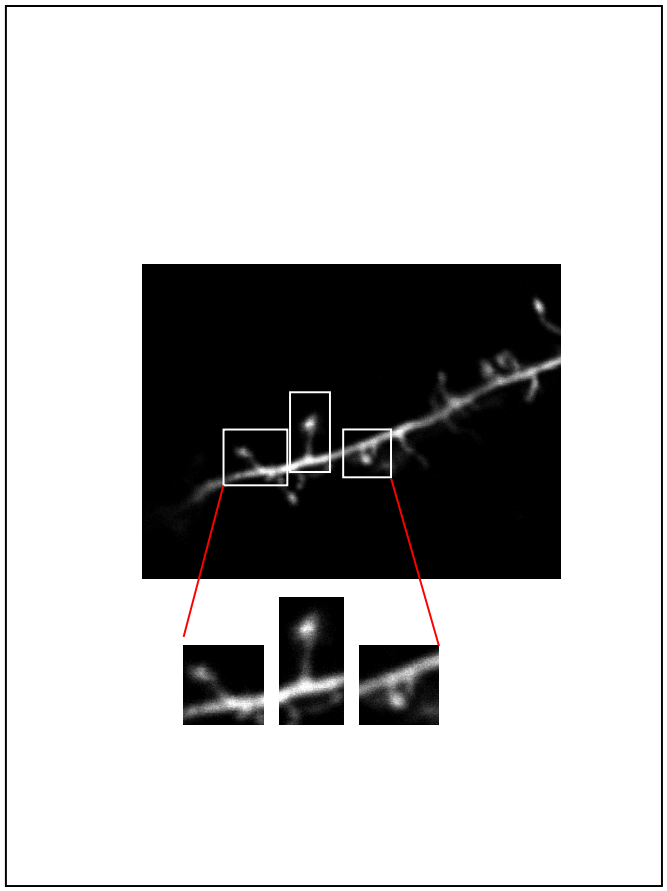
Lab Meeting Oct 2

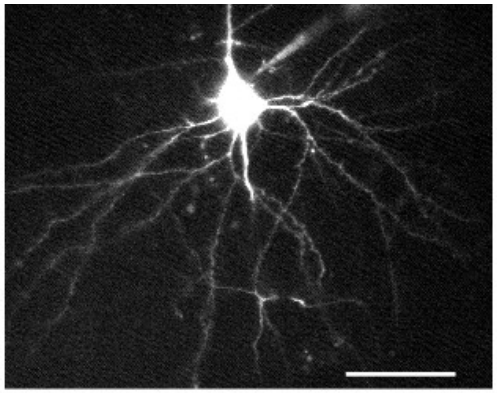


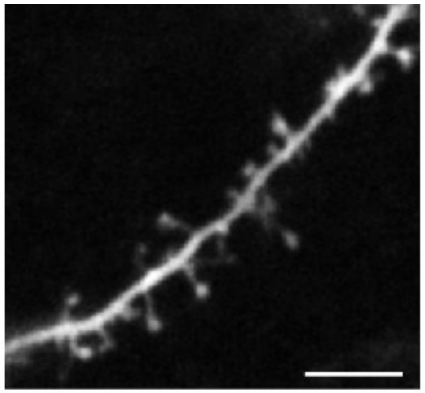
- **The fundamental operation of any neuron is to integrate synaptic inputs in order to decide when to fire an action potential**
- **How neurons integrate thousands of excitatory inputs in order to make this decision?**
- **Dendritic spines mediate most excitatory inputs in the brain.**
- **Passive cable theory predicts that *"The somatic depolarization due to an excitatory synapse on a spine is a very sensitive function of the spine neck length and diameter" C. Koch and T. Poggio***



- - 1) Dendritic spine neck geometry: The Spine Neck and the Transmission of Membrane Potentials
- - 2) Role of Sodium Channels in Spine Uncaging Potentials
- - 3) The spatio-temporal integration of evoked-EPSP

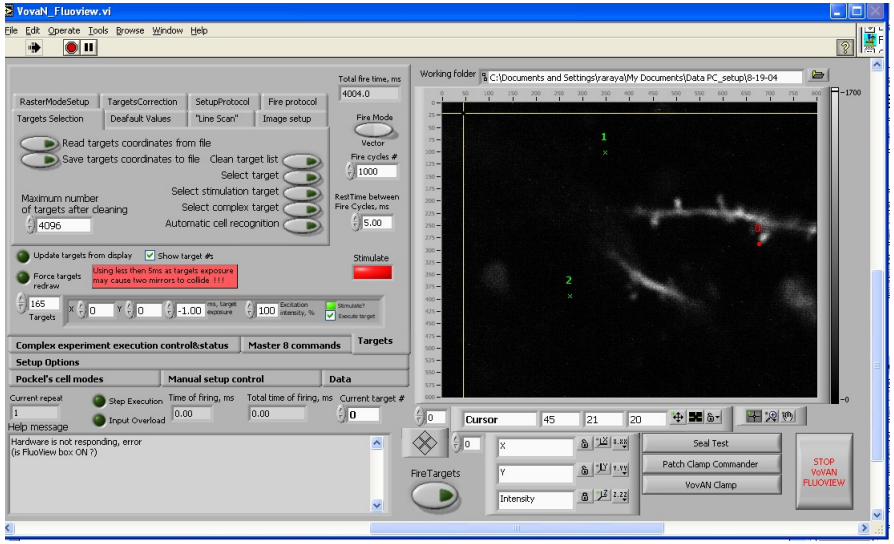
Two-photon uncaging of MNI-glutamate at the level of the spine

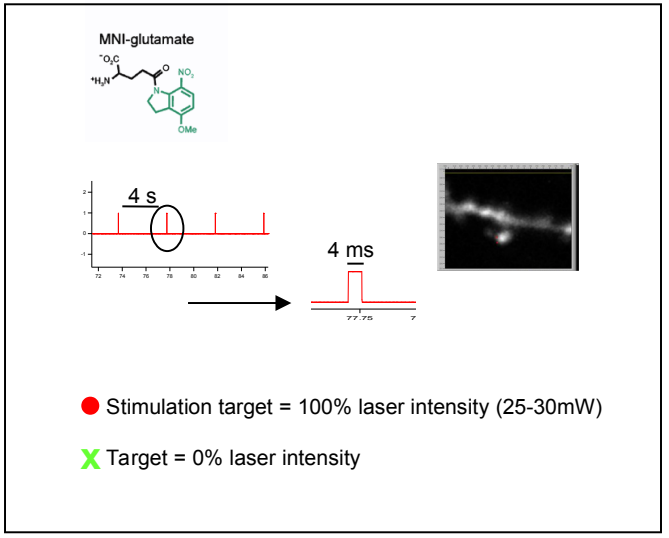




- 300 μm thick coronal slices of visual cortex (P14-20 mice)
Loaded with Alexa488

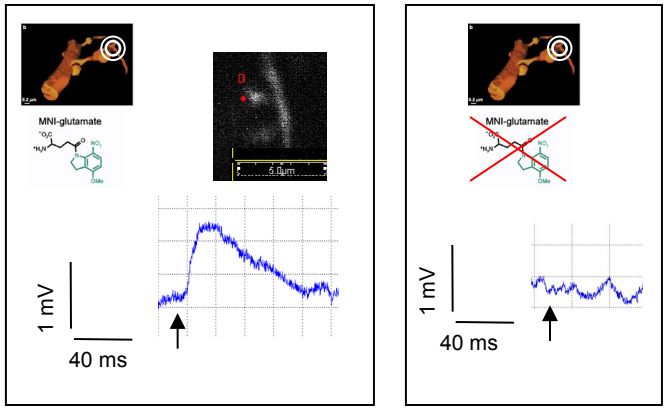
- A custom-built two photon microscope was controlled by Vovan's software
Imaging and uncaging were performed at a wavelength of 725nm

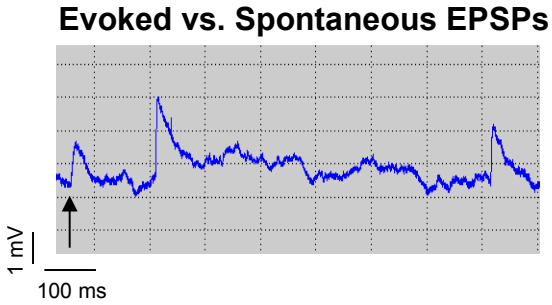


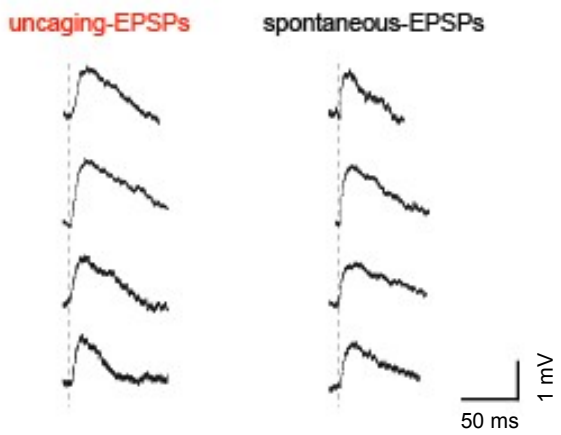


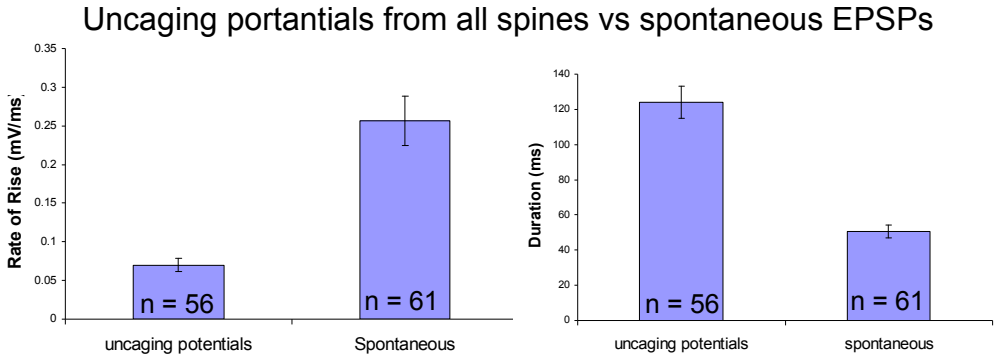
-Voltage deflections due to glutamate uncaging (uncaging potentials) in **basal dendrites** were recorded from the soma in whole cell current clamp

2-photon uncaging of MNI-glutamate allows the release of glutamate within a small focal volume so that activation of glutamate receptors is limited to a region within ~ 1μm of the uncaging beam









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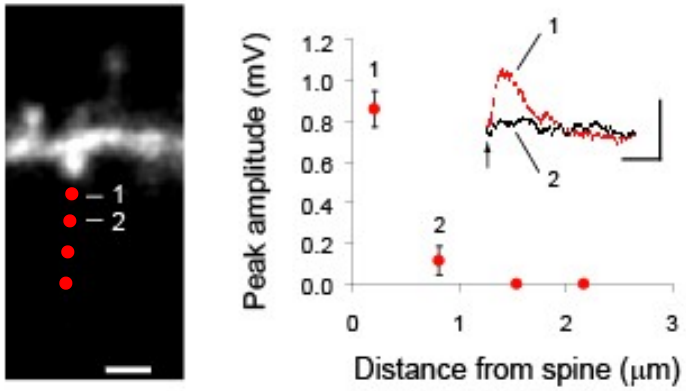
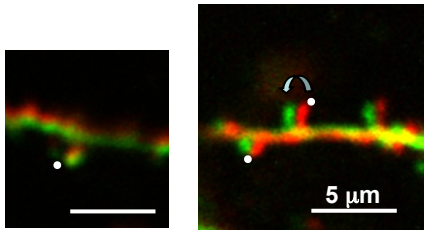


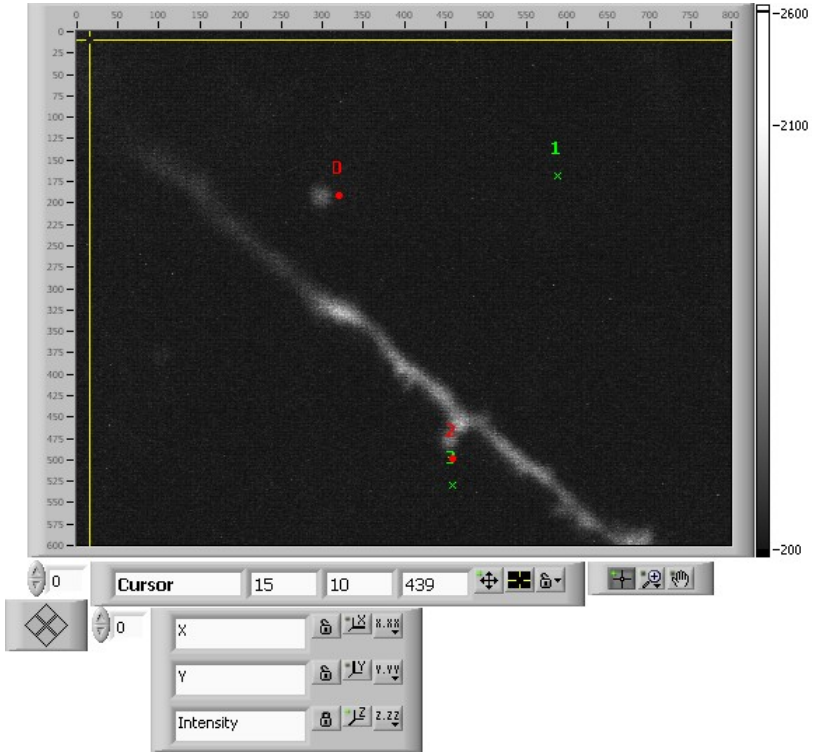


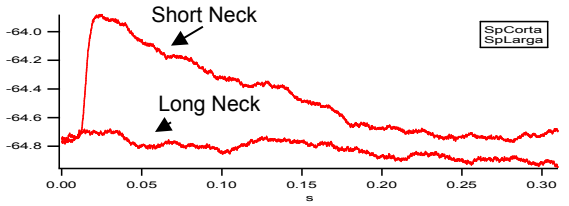
Before

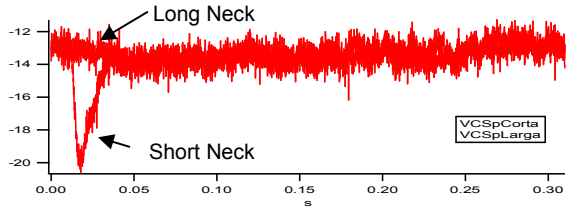
After

Stim 1 μm away From spine head

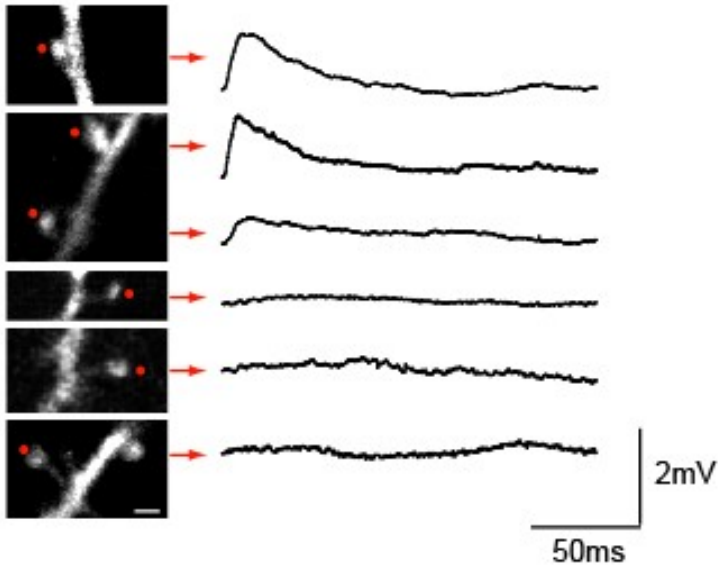
Is the somatic depolarization due to an excitatory synapse on a spine a very sensitive function of the spine neck length?

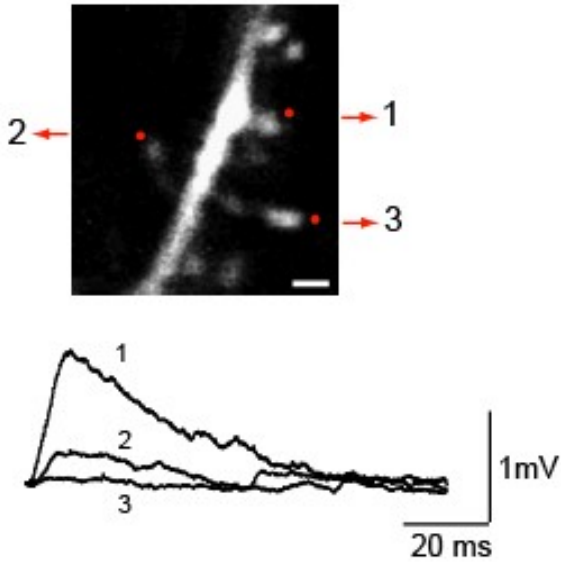






The somatic depolarization due to MNI-glutamte uncaging on a spine is a very sensitive function of the spine neck length.

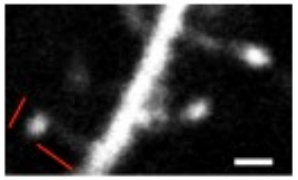




Spine neck length, head diameter and spine distance from soma measurements

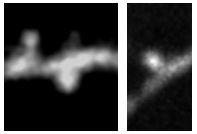
Neck length

from proximal edge of Sp head to edge dendrite
or shortest orthogonal distance between the base of the spine head and the edge of the dendrite

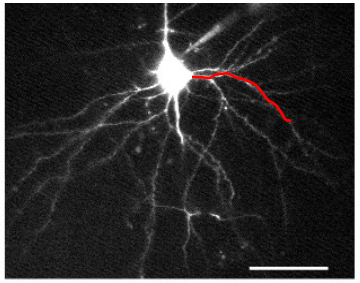


For spines with no discernible necks

(<0.25 µm) we arbitrarily chose a value of 0.2 µm.

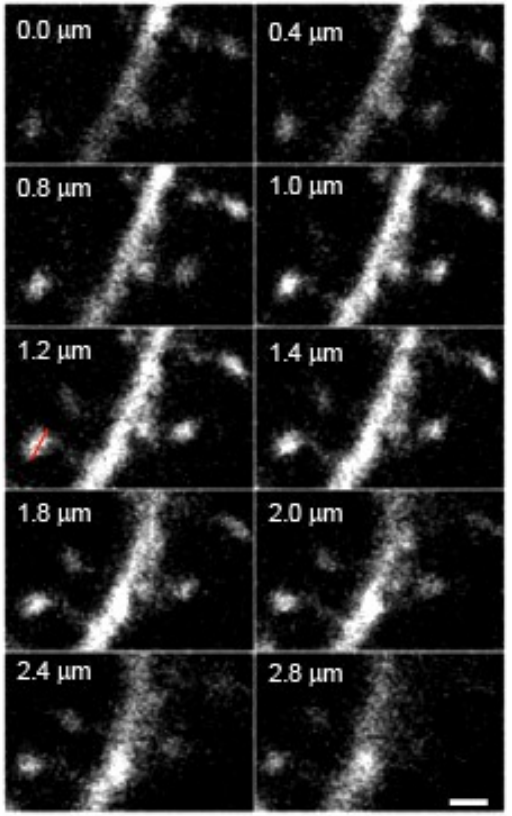


Distance from soma

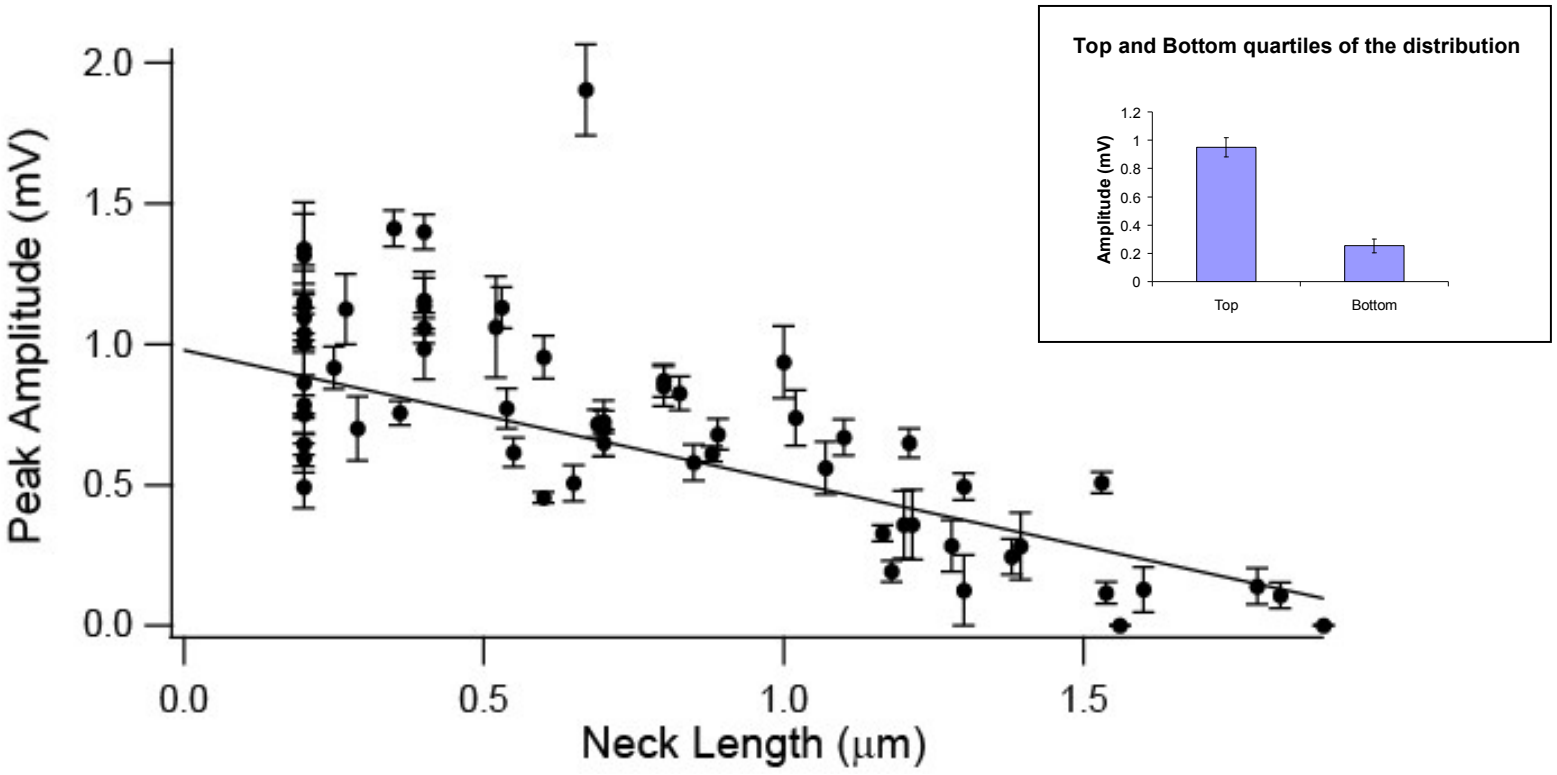


Sp head diameter

longest possible axis at any of the frames in the z-stack of images

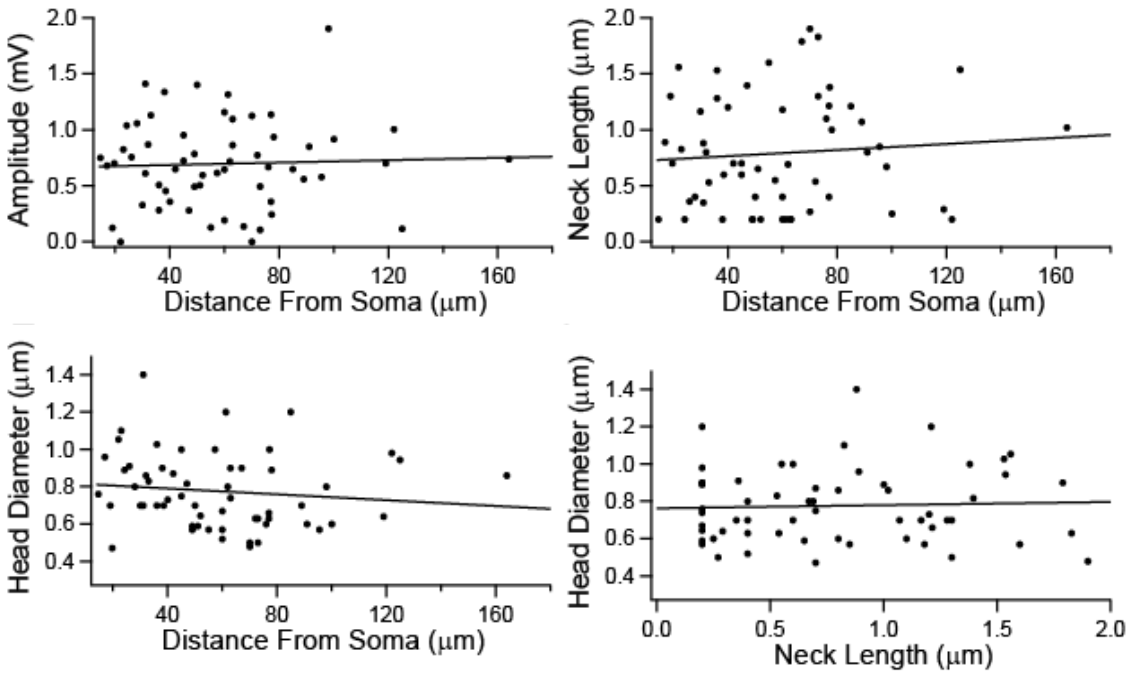


The somatic depolarization due to MNI-glutamte uncaging on a spine is a very sensitive function of the spine neck length.

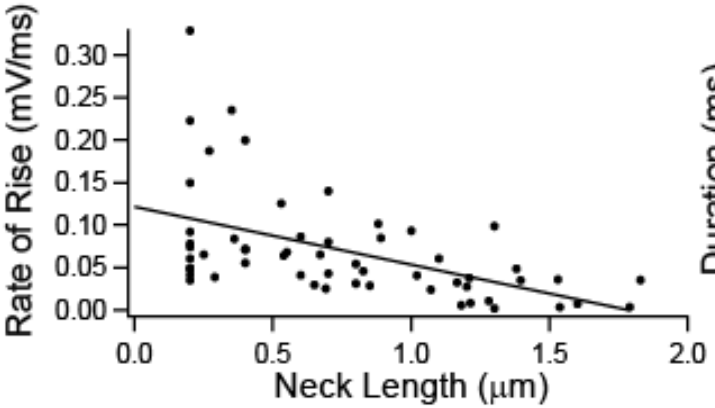


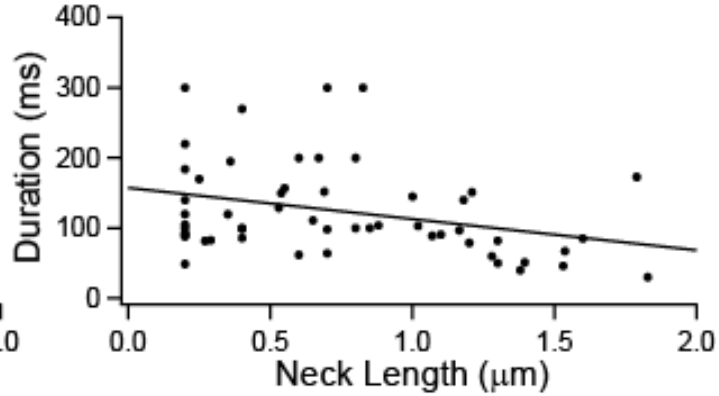
A linear fit to the function gave a R of - 0.75 and a slope of - 0.46 ± 0.01 mV/μm
p<0.001

The effect of the spine neck is independent of spine position and head diameter



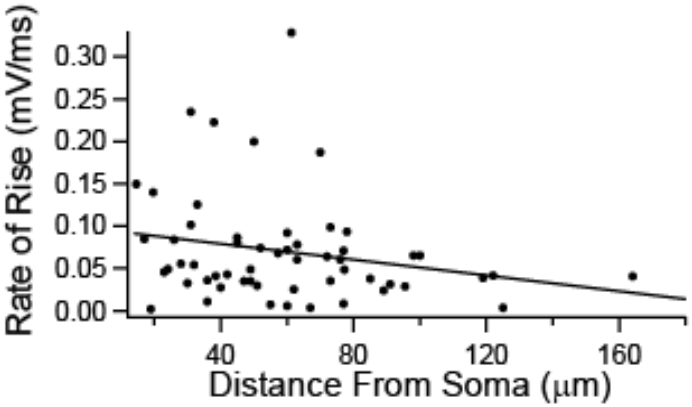
Analysis on the kinetics of the uncaging events

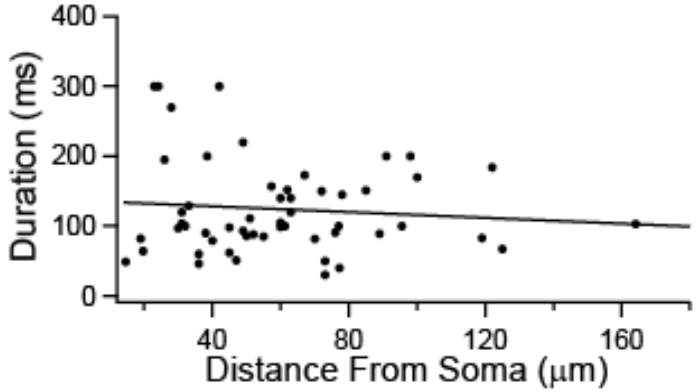




R = 0.5; slope = -0.068 ± 0.015 mVms/μm, p < 0.001

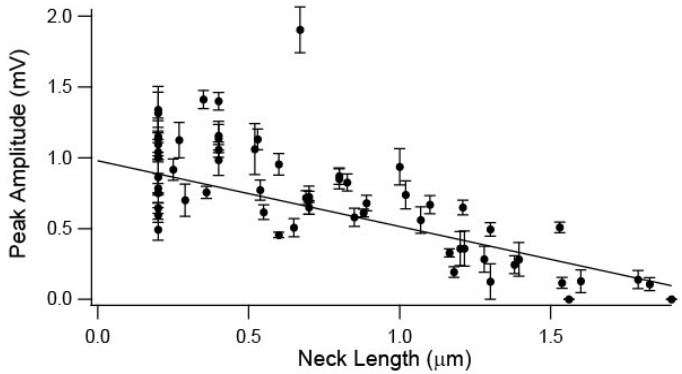
R = 0.3; slope = -44.42 ± 18.2 ms/μm, p = 0.015



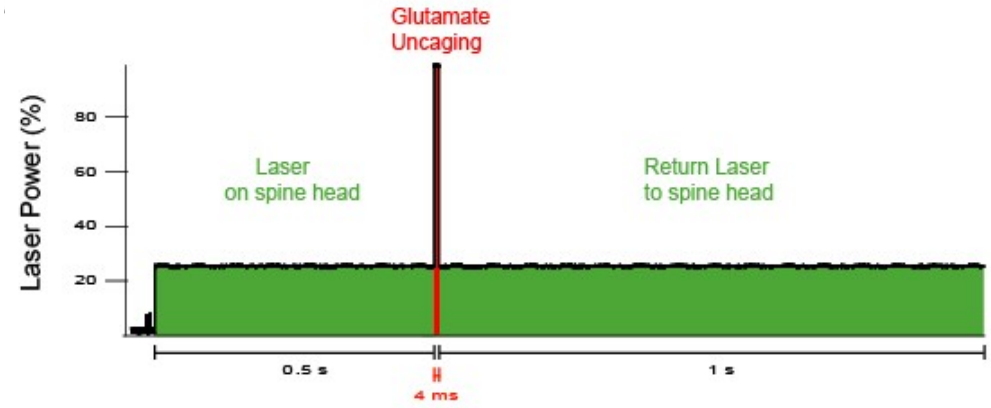


R = 0.22; slope = -0.00046 ± 0.00027 mVms/μm, p = 0.1

Calcium signals in spines

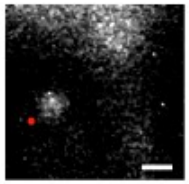


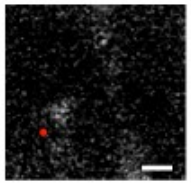
200 μM Ca-green-1

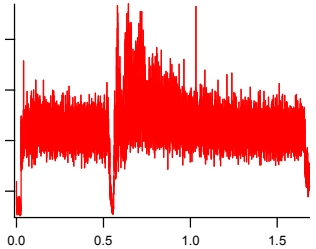


Pointscan calcium signals were collected with a PMT

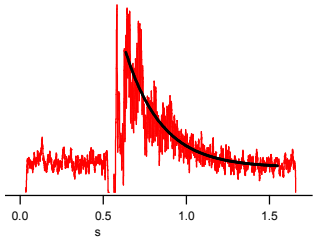
Long neck spines

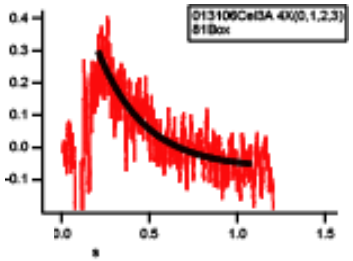


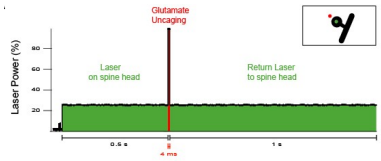




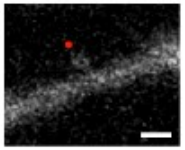
Window smoothing filtration

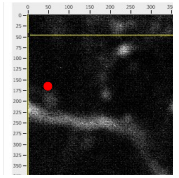


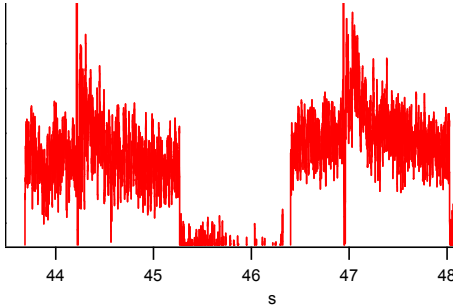


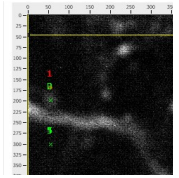


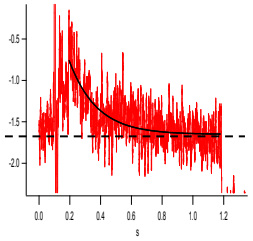
Short neck spines

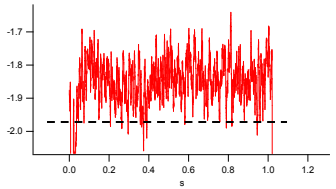


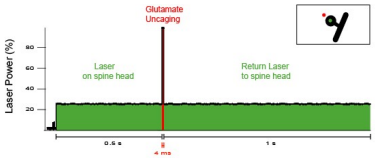


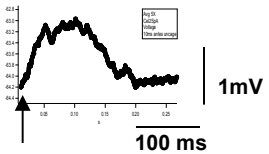




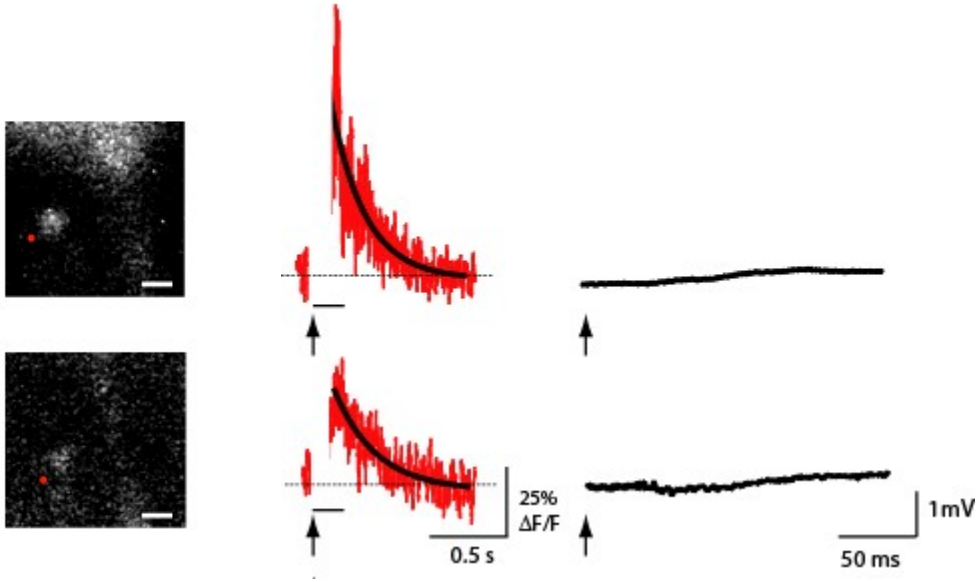


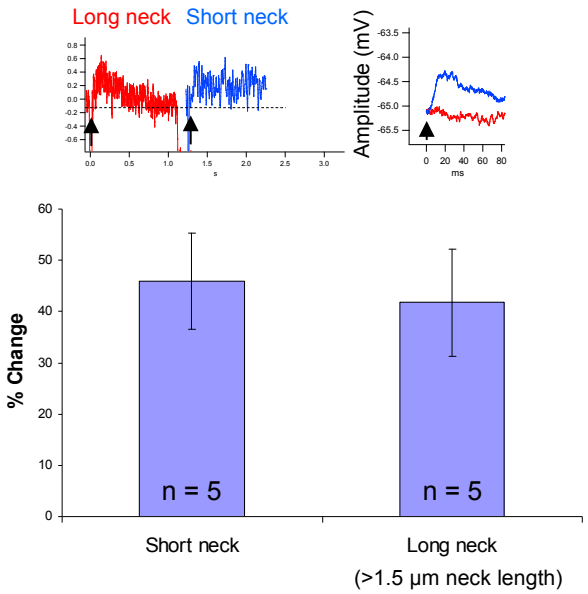




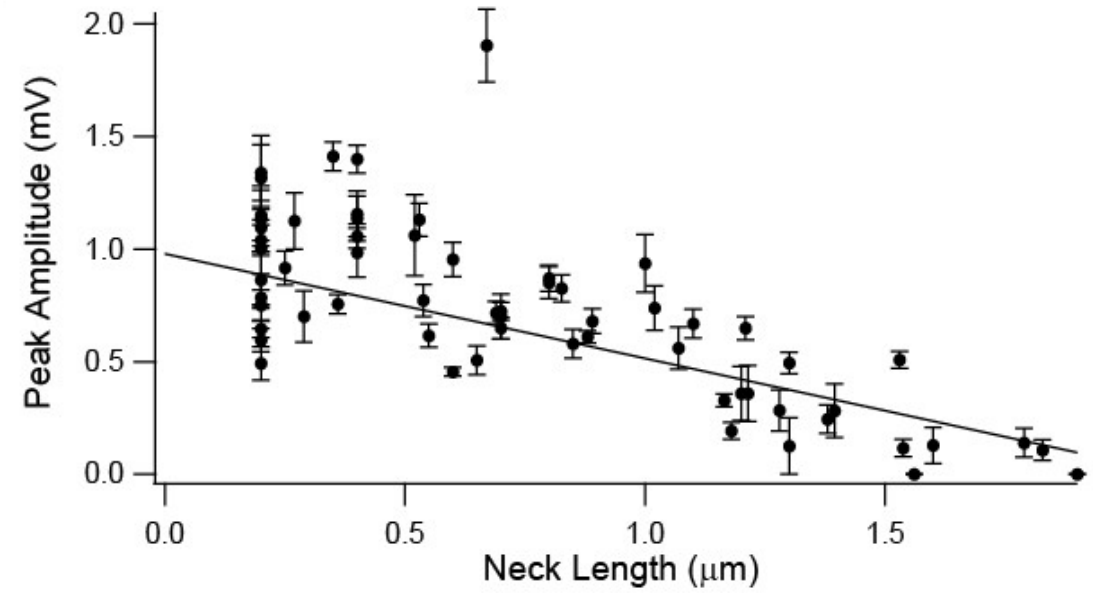


Long-necked spines are activated by glutamate uncaging





Can this be explained only by passive properties of spines?

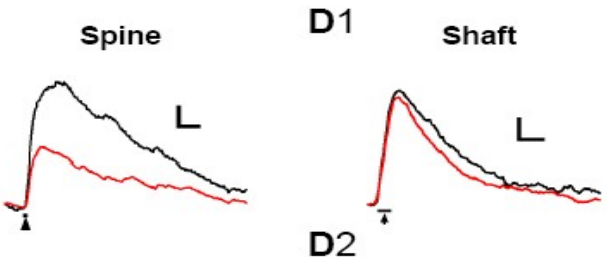


Pharmacological blockade of Na channels

•High neck resistance could make possible the amplification of synaptic potentials at the spine head. Possible generation of Spine AP

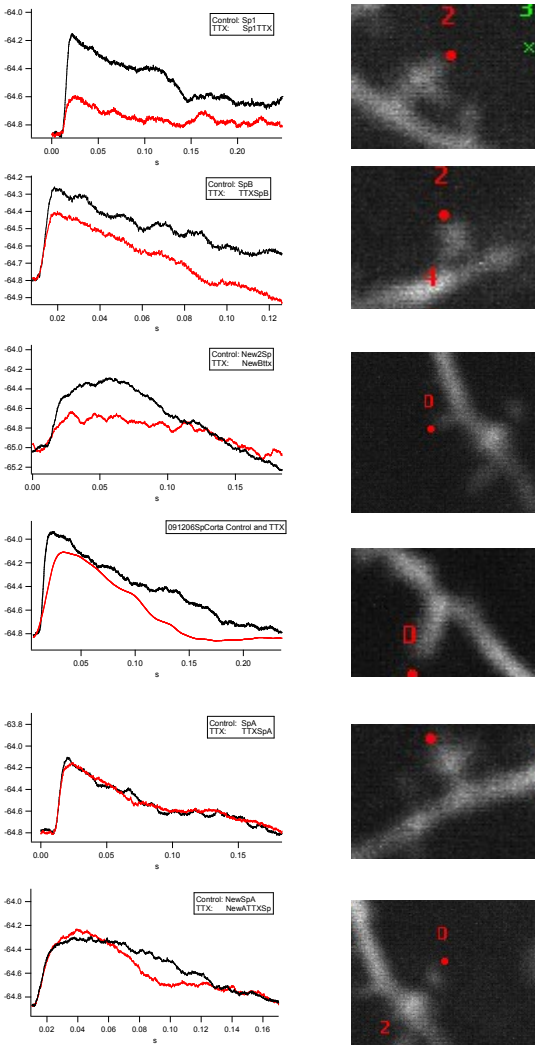
Active properties of dendritic spines: Na channels

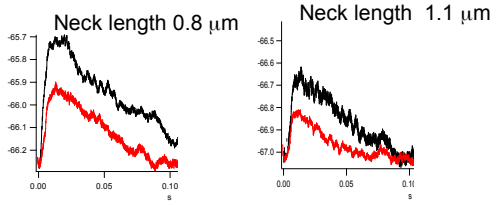
VoVAn's results

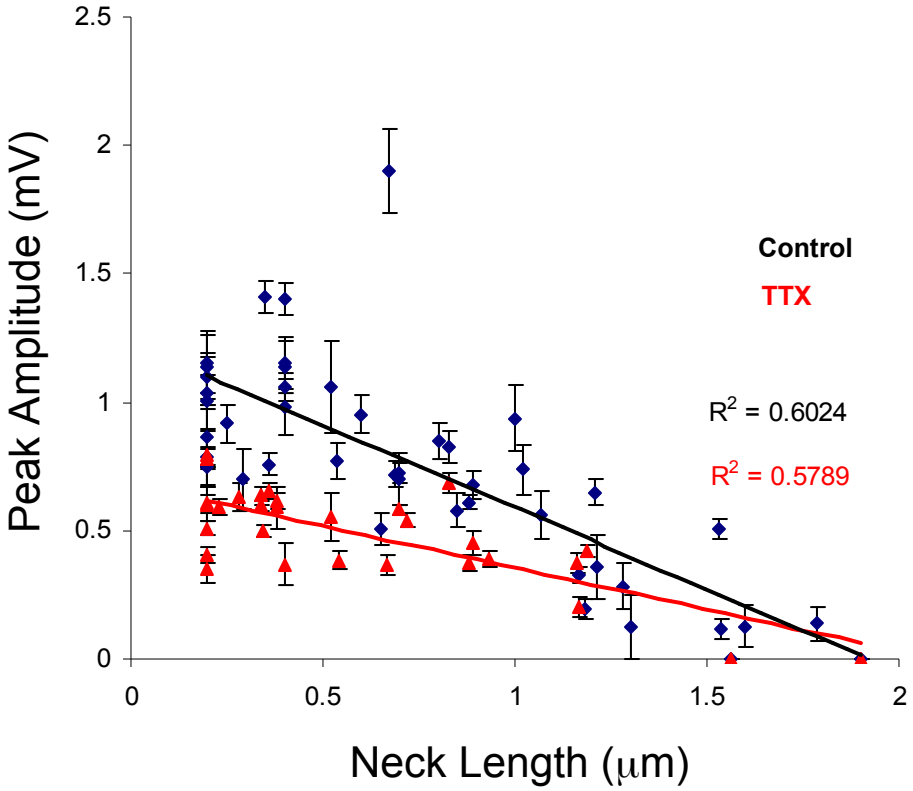


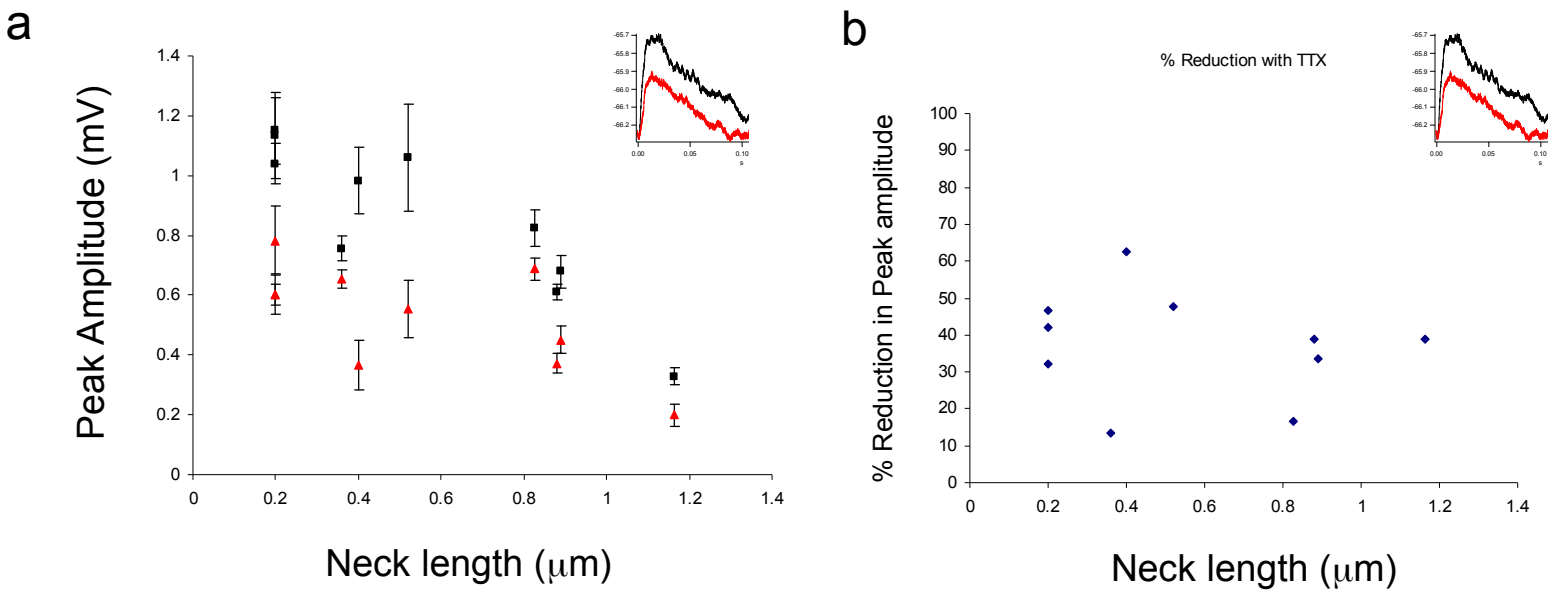
Spine

TTX reduces the amplitude of spine uncaging potentials



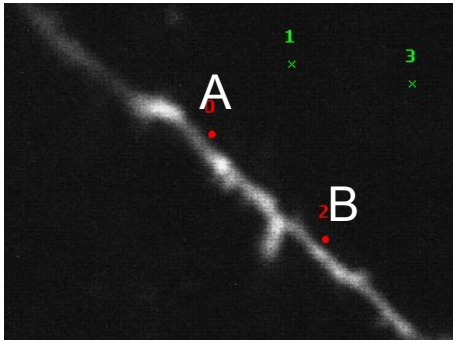


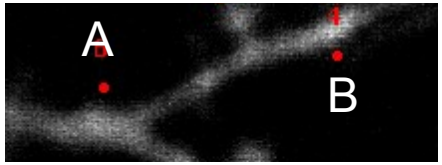


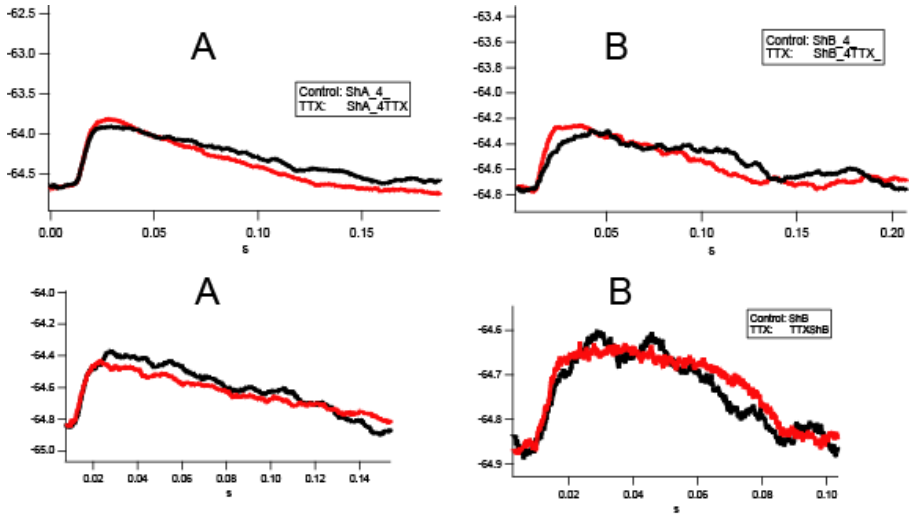


a) Peak amplitude vs neck length in 10 different spines before (black) and after (red) addition of TTX (1 μm). b) Percentage reduction in the peak amplitude after addition of TTX in the spines showed in **a**.

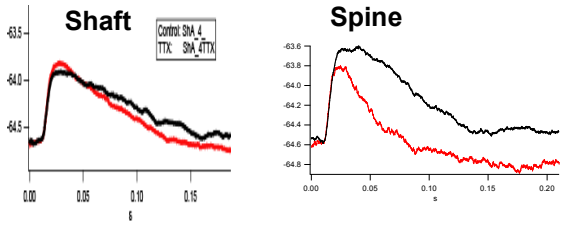
Shaft
TTX effect is restricted to spines





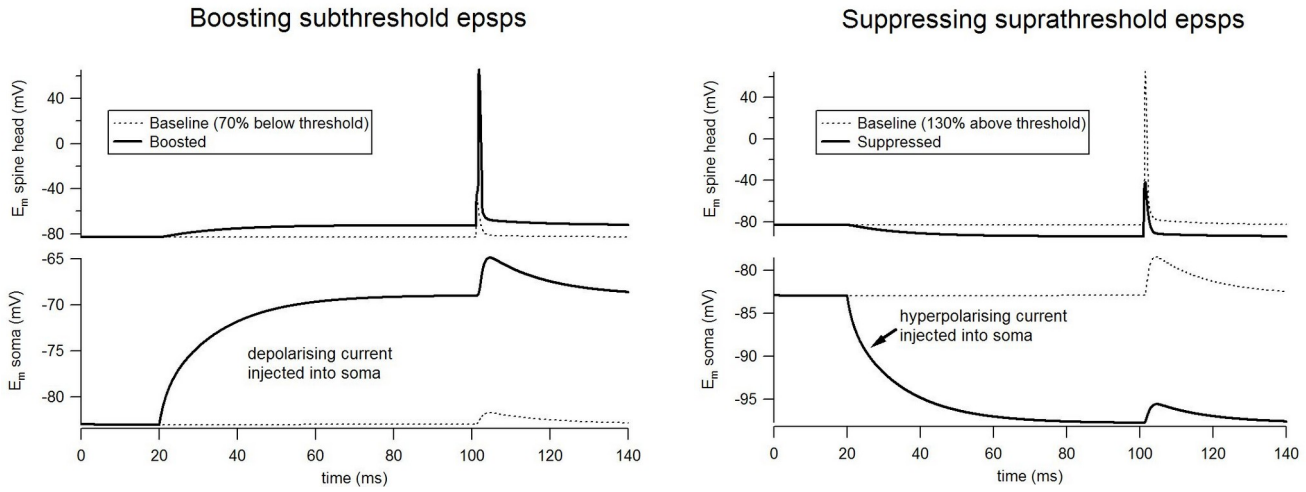


These results suggest that sodium channels responsible for the amplification of spine uncaging potentials were located in the dendritic spine.



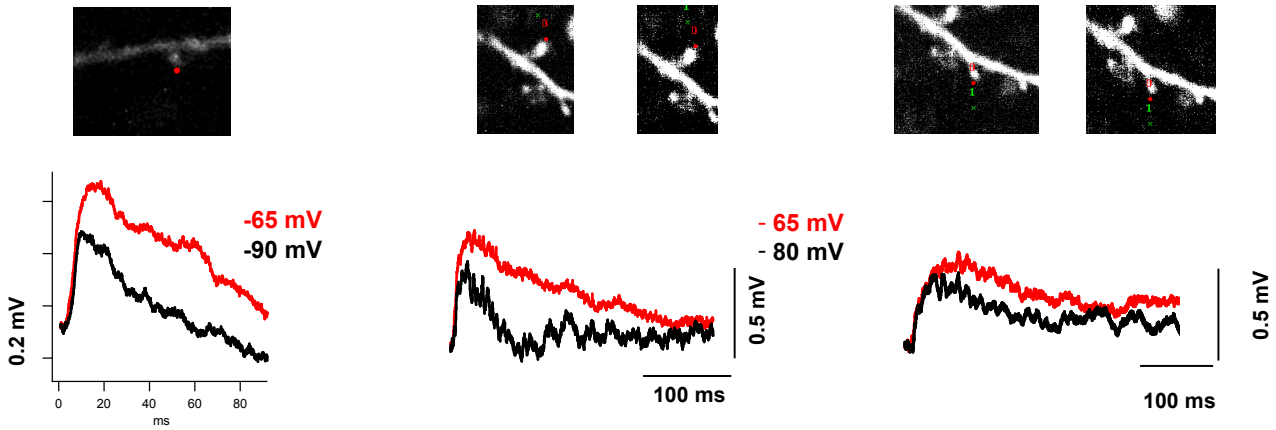
Modelling (Andy):

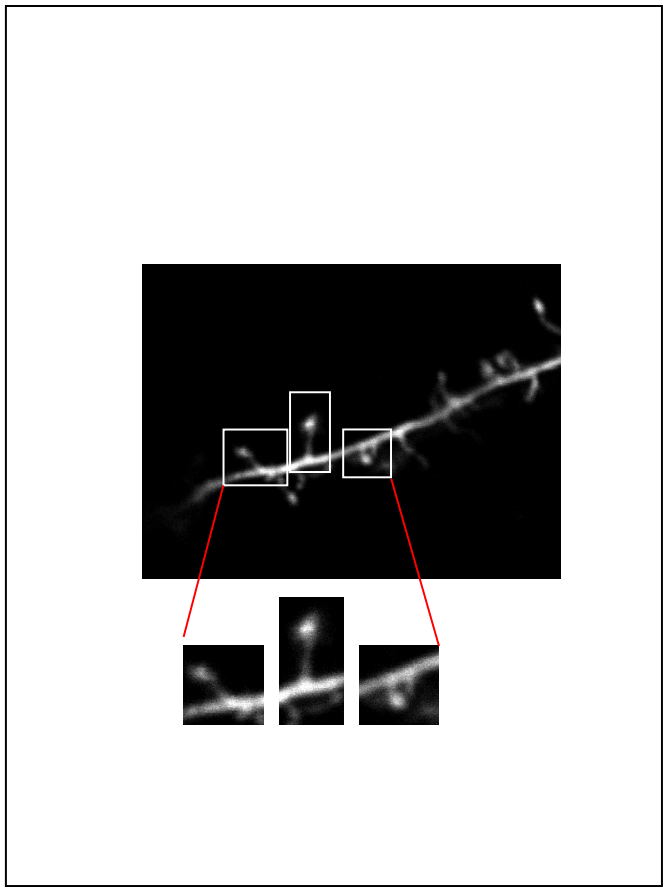
The threshold should be demonstrable simply by hyperpolarizing / depolarizing the soma



Experimentally:

Somatic hyperpolarization





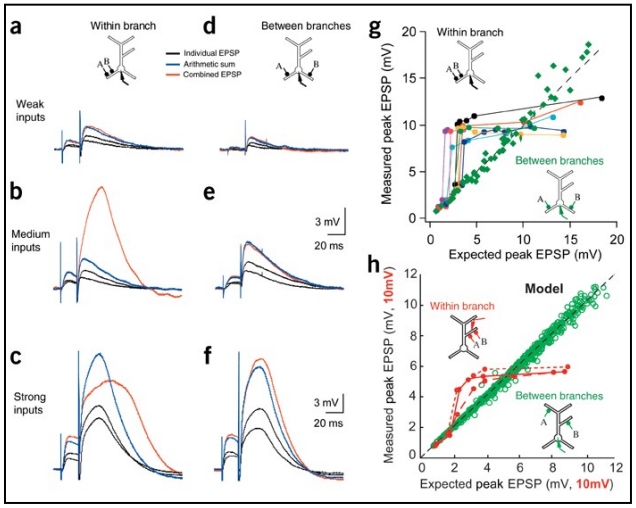
- - 1) Dendritic spine neck geometry: The Spine Neck and the Transmission of Membrane Potentials
- - 2) Role of Sodium Channels in Spine Uncaging Potentials
- - 3) The spatio-temporal integration of evoked-EPSP

How neurons integrate thousands of excitatory inputs in order to make this decision?

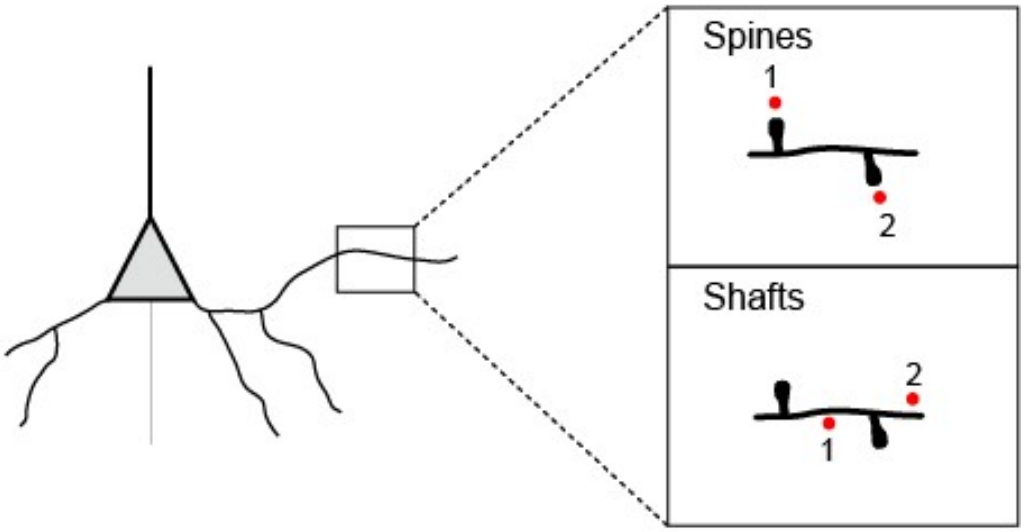
- Linear Summation
- Excitatory inputs:
  - Cash and Yuste 1998 and 1999 (Glutamate iontophoresis in CA1 pyramidal cells)

- **<u>Polsky et al., 2004</u>**
- Neocortical slices
- Whole cell patch-clamp - layer 5 pyramidal cells
- Stimulation with theta patch pipette: Basal and Oblique dendrites.

Problem: Other synapsis activated elsewhere in dendritic arbor by itinerant axons passing near stimulating electrodes



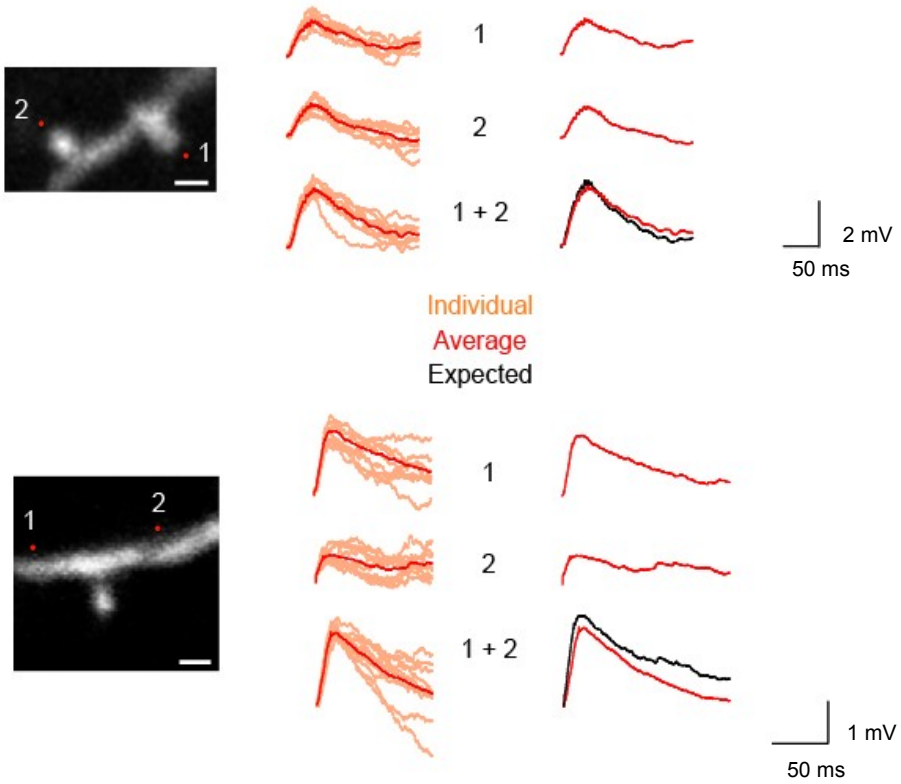
The spatio-temporal integration of uncaging potentials



-pairs or triplets (total 26 spines 8 cells)
-~15 -125 μm away from soma

-Two shaft locations (total 18 locations, 7 cells)
-~20 -80 μm away from soma

Uncaging potentials onto spines summed linearly whereas potentials on shafts summed sublinearly



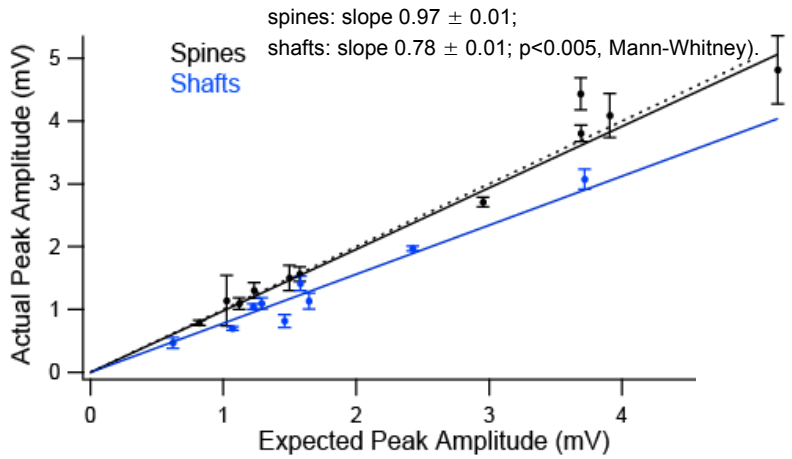
Theoretical predictions:

Integration in the shaft: *Passive properties: Cable theory predicts that the influence of an input will vary with its LOCATION and will shunt each other if they are close (Rall and Rinzel, 1973).*

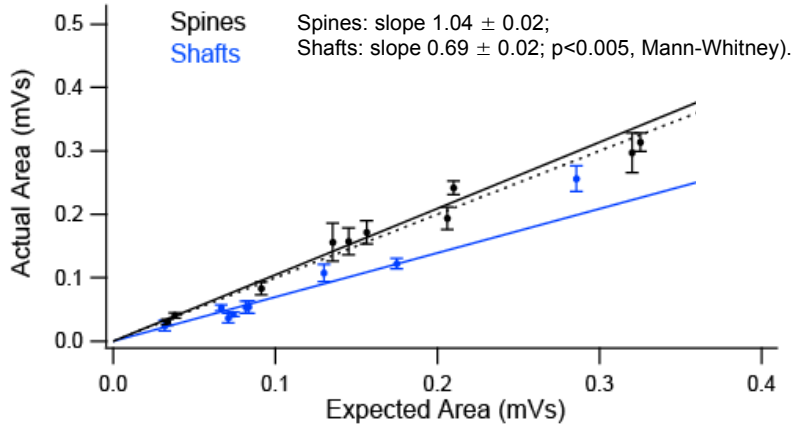
Integration in the spine: "The isolation of each spine synapse from others ending on the neurone would lead to very little Interaction between different excitatory inputs. This would result in nearly linear summation in the parent dendrite" (Jack)

Uncaging potentials onto spines summed linearly whereas potentials on shafts summed sublinearly

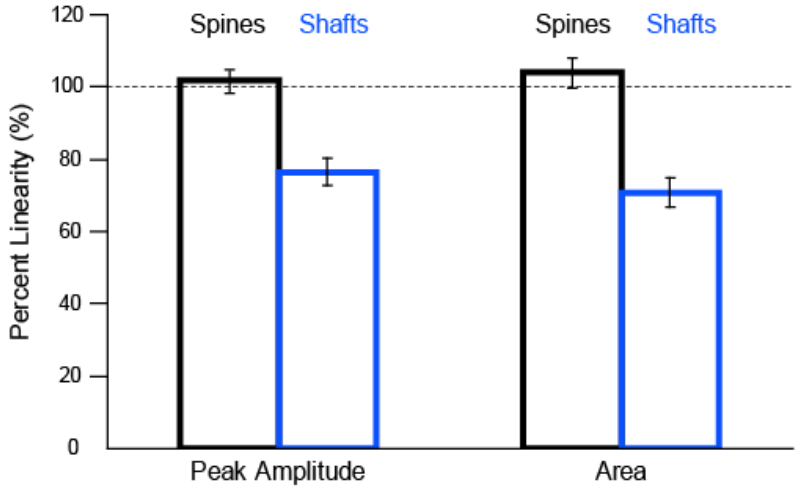
A



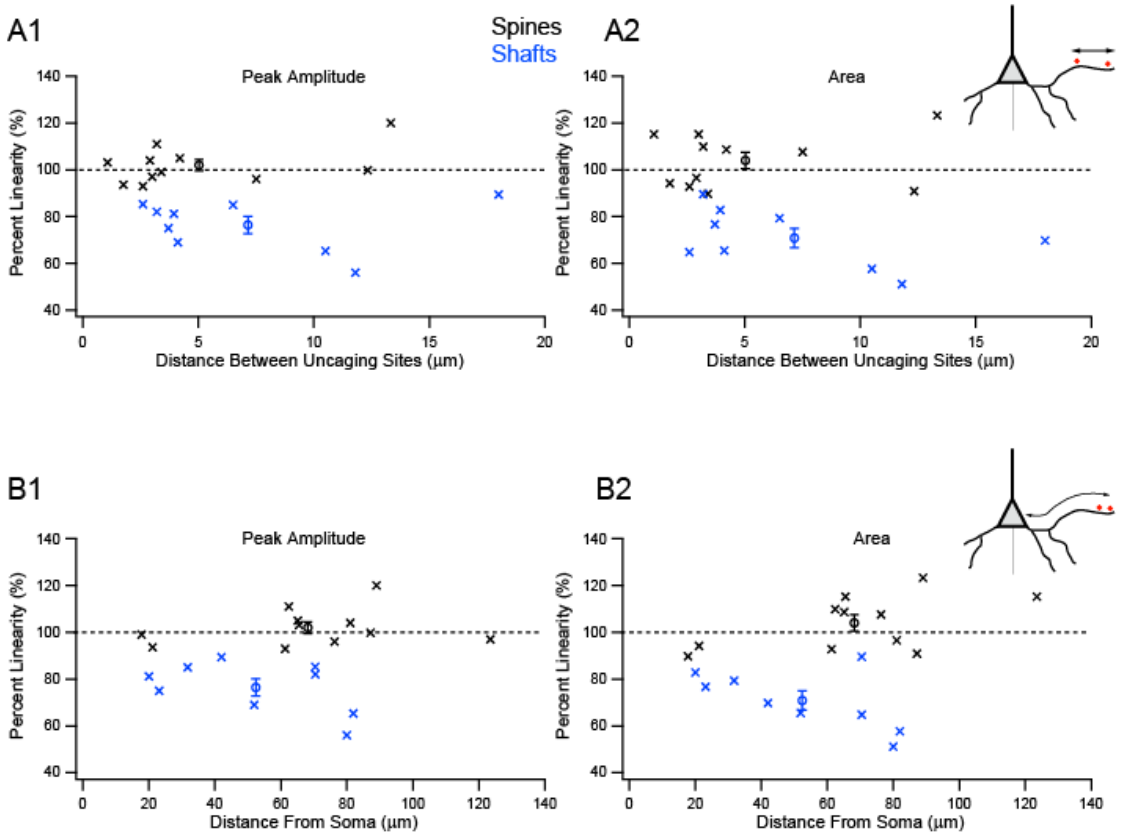
B

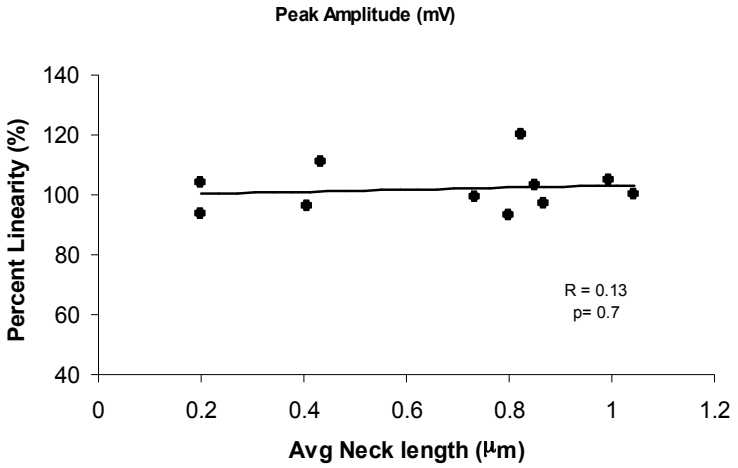


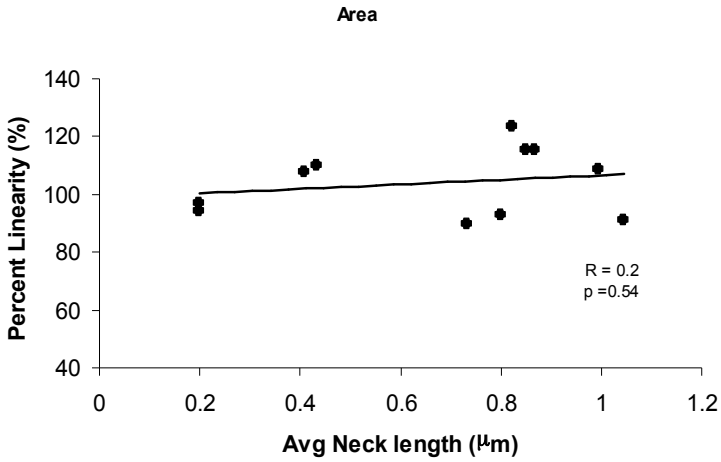
C



At both small and large amplitudes inputs onto spines added linearly, but inputs onto shafts sublinearly


A1
Spines
Shafts
Peak Amplitude
Percent Linearity (%)
Distance Between Uncaging Sites (µm)
A2
Area
Percent Linearity (%)
Distance Between Uncaging Sites (µm)
B1
Peak Amplitude
Percent Linearity (%)
Distance From Soma (µm)
B2
Area
Percent Linearity (%)
Distance From Soma (µm)

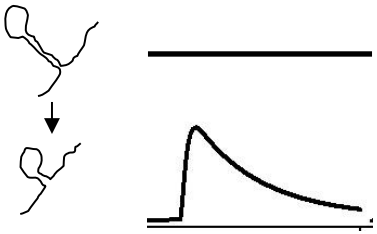

Peak Amplitude (mV)
Percent Linearity (%)
140
120
100
80
60
40
0
0.2
0.4
0.6
0.8
1
1.2
Avg Neck length (μm)
R = 0.13
p= 0.7


Area
Percent Linearity (%)
140
120
100
80
60
40
0
0.2
0.4
0.6
0.8
1
1.2
Avg Neck length (μm)
R = 0.2
p =0.54

Future directions

Do dendritic spines twitch?

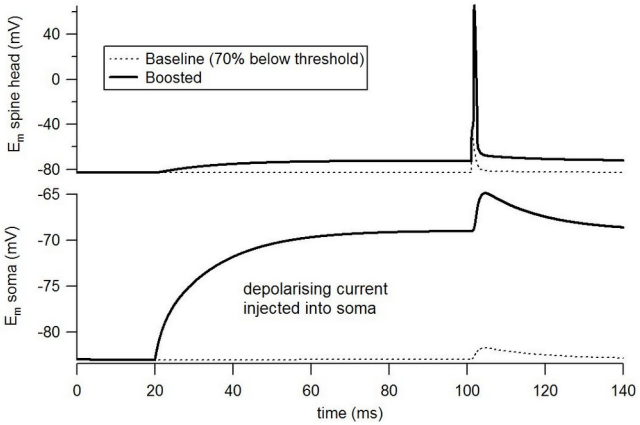
To see whether there is plasticity in the spine neck:
Protocol 0 magnesium, 1Hz or 100Hz stim.
Neck and voltage response
-Myosin IIB (Sheng's lab. Neuron) critical in spine morphology



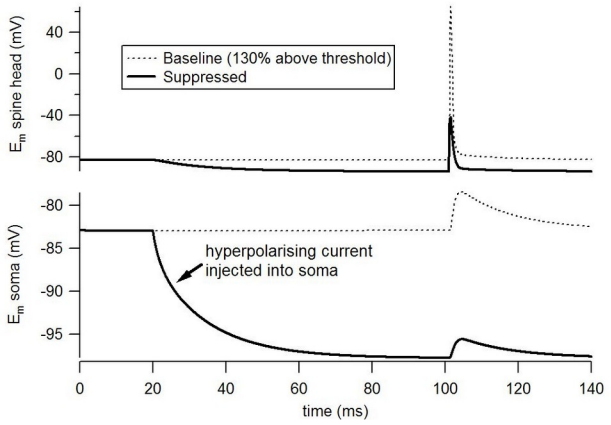
Integration in Up-states:

Such depolarizations can cause a marked change in the subpopulation of spines which could produce SpineAP

Boosting subthreshold epsps



Suppressing suprathreshold epsps



JJ's Results

500 μM FM 4-64 (Biotium Inc., Hayward CA) intracellularly via the patch pipette. SHG imaging was started when cells were stained with the dye for 30-60 min after breaking in, and performed using a second custom-made two-photon laser scanning microscope {Nikolenko, 2003 #2384} with a Nd:glass laser at 1064 nm (IC-100, HighQ Laser). SHG signals were collected with a photomultiplier tube (Hamamatsu H7422P-40) after a narrow band-pass filter (530/20). Slow somatic DC voltage pulse (30-50 mV in amplitude, 5-25 s in duration) were delivered by the patch pipette in voltage clamp mode, while the SHG intensity of dendritic shaft and spines was collected at a frame rate of 1-5 s/frame and averaged online with Olympus FluoView. This voltage pulse presentation was repeated 5-10 times in order to calculate the SHG changes of the dendritic shaft and spine head. Spines were selected based on their signal to noise. There was no statistical correlation between the neck length and SHG baseline intensity (R=0.026, p=0.91), effectively ruling out potential artifacts due to systematic differences of chromophore diffusion into long spines.