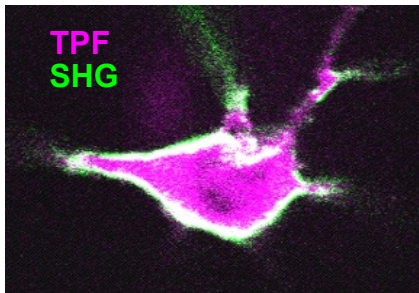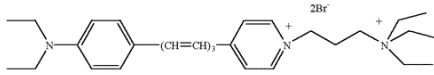# Electrical Properties of Dendritic Spines
Probed by the Second Harmonic Generation Imaging

Lab Meeting
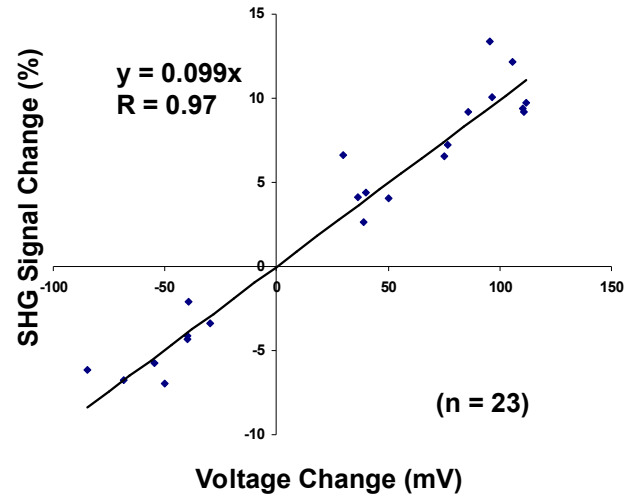10/16/06

Mutsuo Nuriya

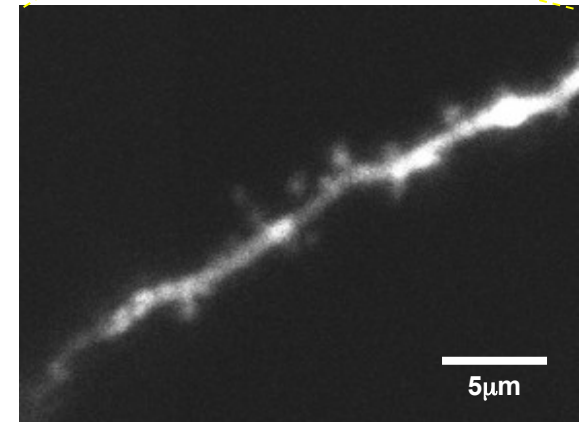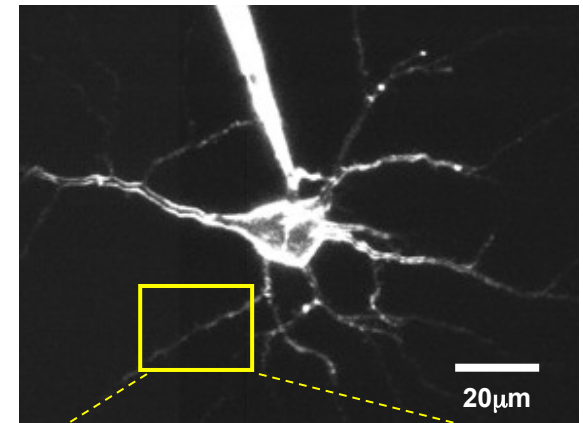# Second Harmonic Generation (SHG) as a Probe for Membrane Potential

• Plasma Membrane Selective

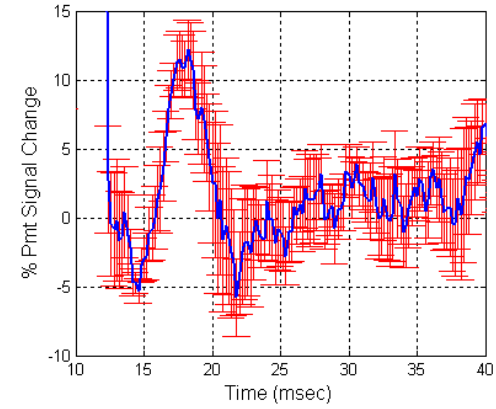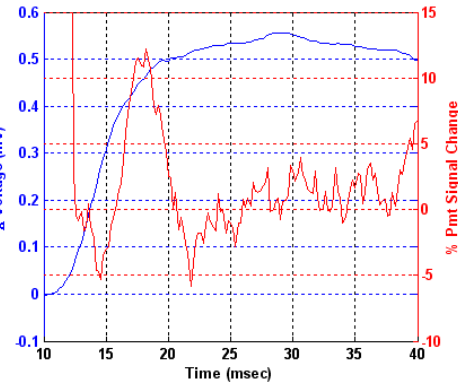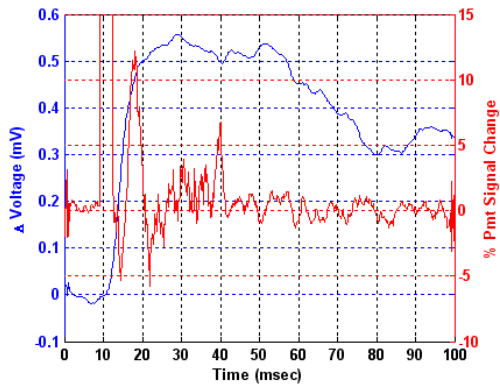**• Linear Response to Membrane Potential**
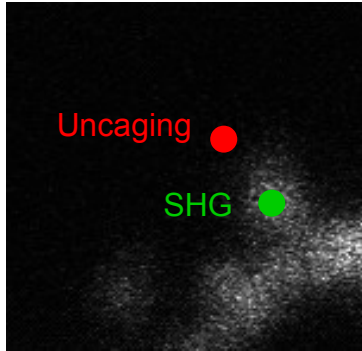

y = 0.099x
R = 0.97

(n = 23)

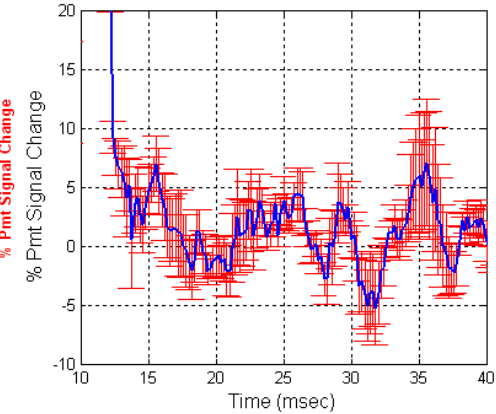**• Visualization of Spines**

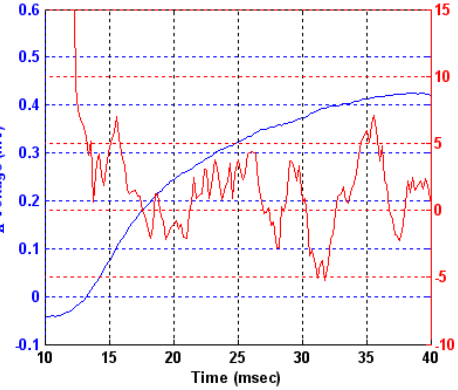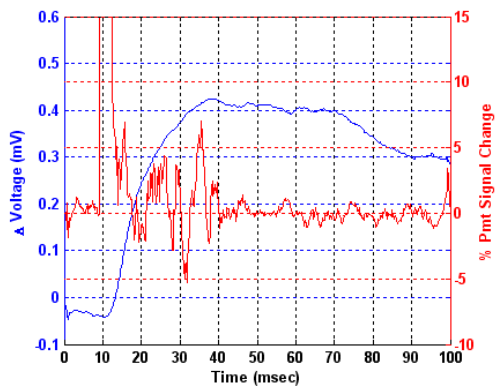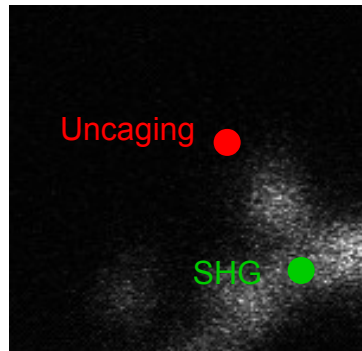**• Fast Recording Capability**
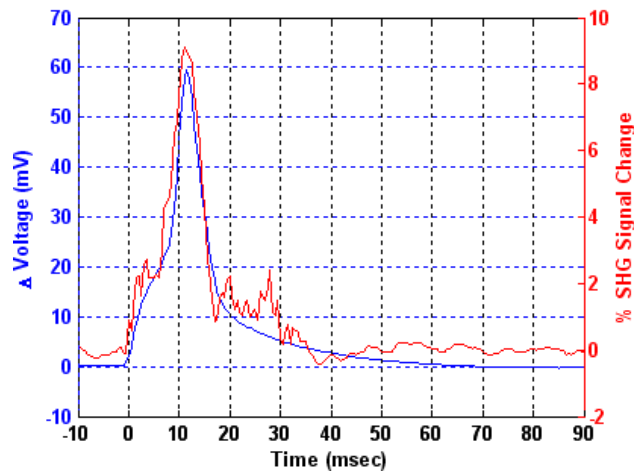
Spine SHG with Glutamate Uncaging

Spine SHG Measurement

(n = 5)

Shaft SHG Measurement

(n = 5)

Goals

- Reproduce the uncaging data.

- Improve the data quality (signal to noise ratio).

- Establish a stable system for further characterizations (e.g. pharmacology).

Changes in the System

Lasers:

  • Uncaging Laser: Chameleon → Chameleon Ultra
  • SHG Laser: HighQ Laser → Fianium Fibre Laser

Detection:

  • Mode: PMT Analogue → Photon Counter
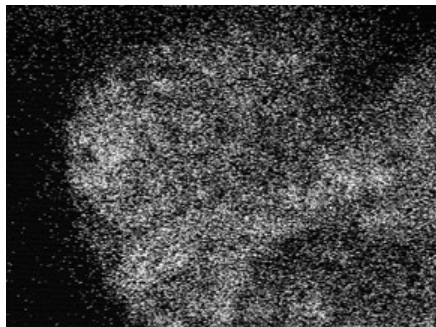
Uncaging Protocol:

  • MNI-Glu Concentration: 50mM → 20mM (to avoid pipette clogging)
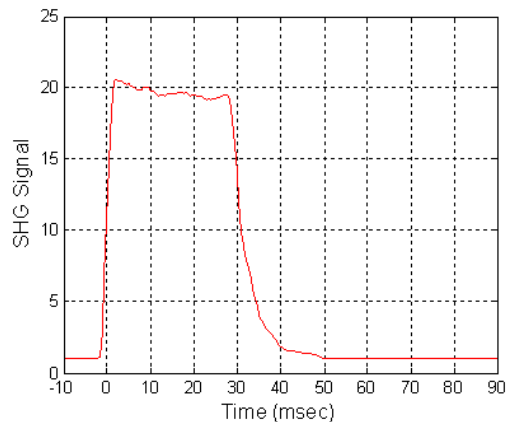 • Puffing: Picospritzer → Manual (for a better control)

Acquisition:

  • Program: MATLAB → LabVIEW
  • Sampling: 10kHz after 3kHz low pass filter → 2kHz direct

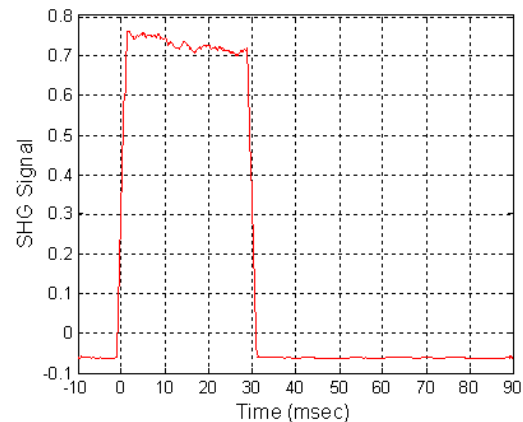# Comparison of SHG Signal Noises between Photon Counter and PMT Analogue Recordings
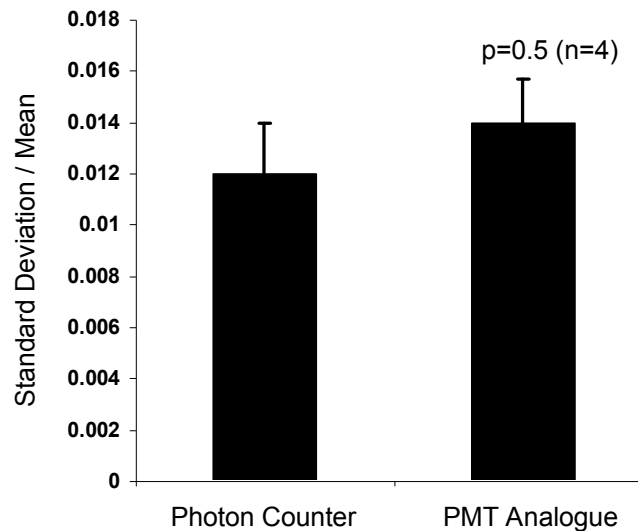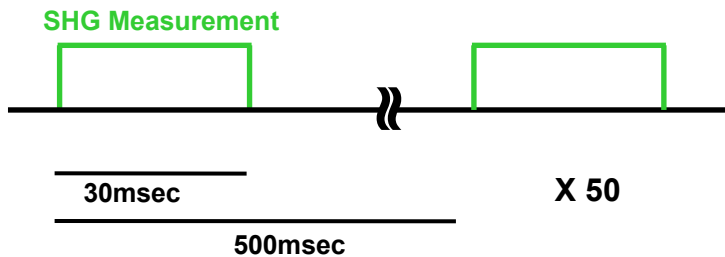
SHG Signal from Pollen Grain

Photon Counter Recording (n=1)

PMT Analogue Recording (n=1)

Recording Protocol

SHG Measurement

30msec

500msec

X 50

p=0.5 (n=4)
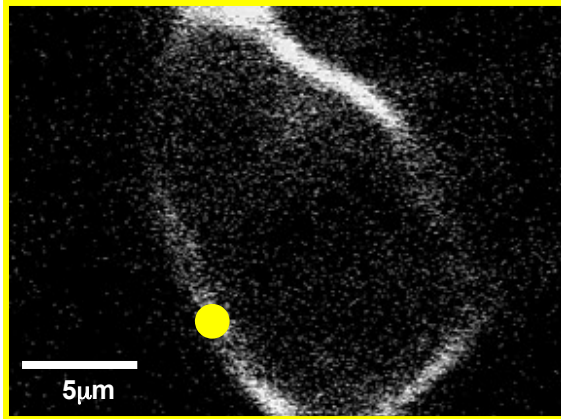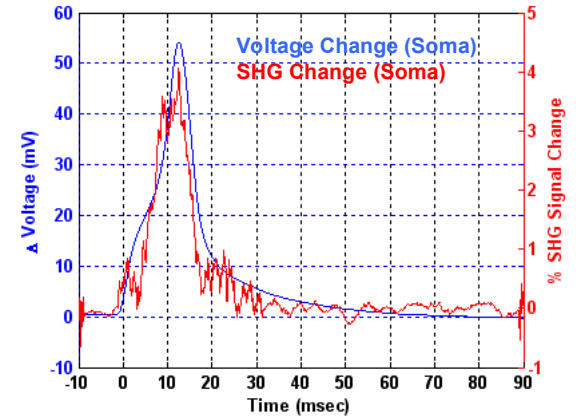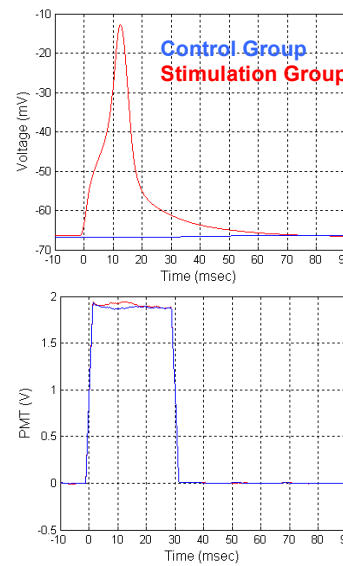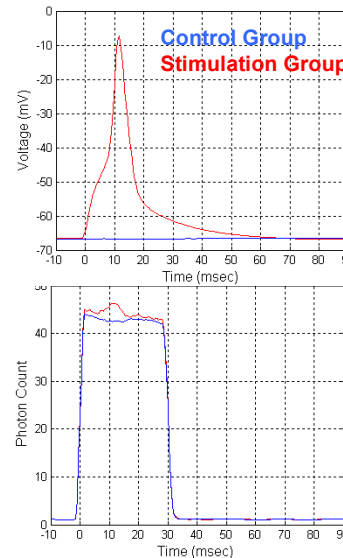
Comparison between PMT and Photon Counter
- AP Recording at Soma -

SHG Signal at Soma

5μm

PMT Recording

Control Group
Stimulation Group

Voltage Change (Soma)
SHG Change (Soma)

Recording Protocol

Stimulation Group   **Control Group**

SHG Measurement
Current Injection

15msec
30msec
500msec

X 50 Sets

Photon Counter Recording

Control Group
Stimulation Group

Voltage Change (Soma)
SHG Change (Soma)
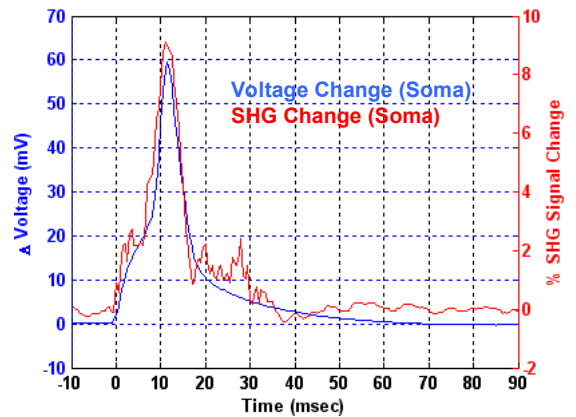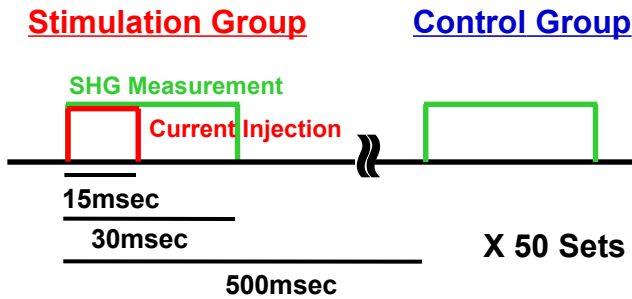
Standard Deviation / Mean = ~0.0093 (1%)

SHG Recordings at Spines with bAP
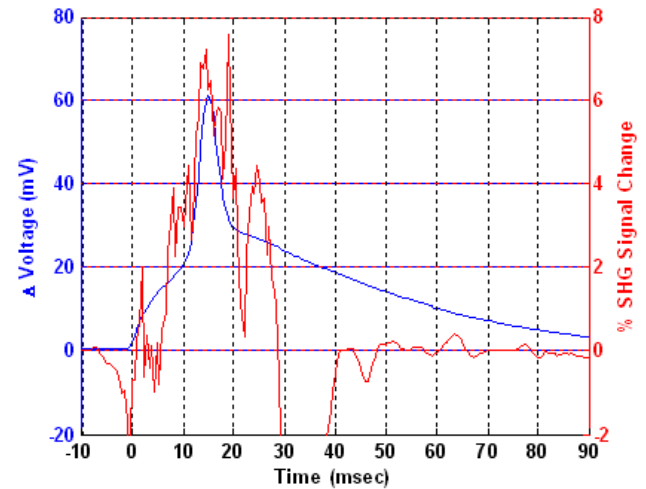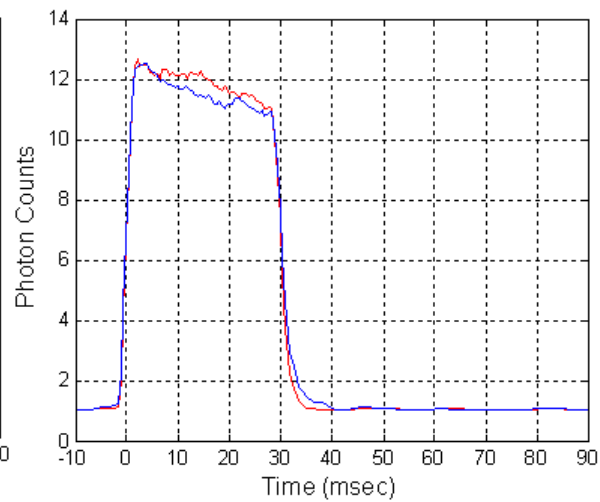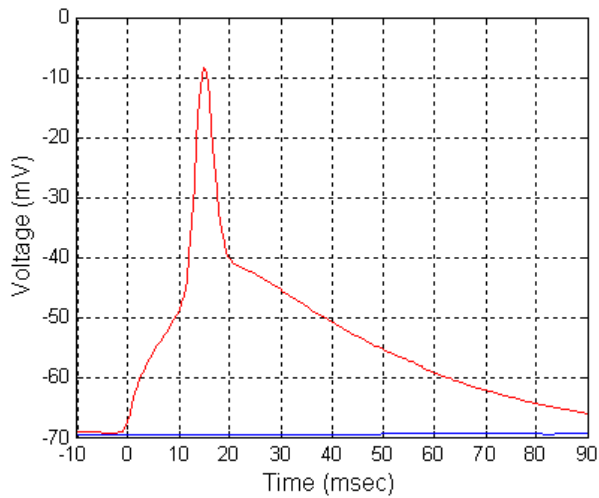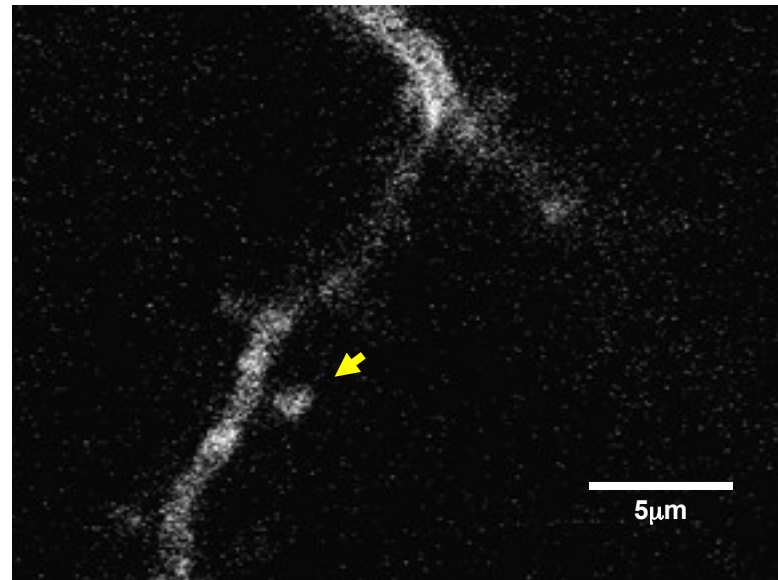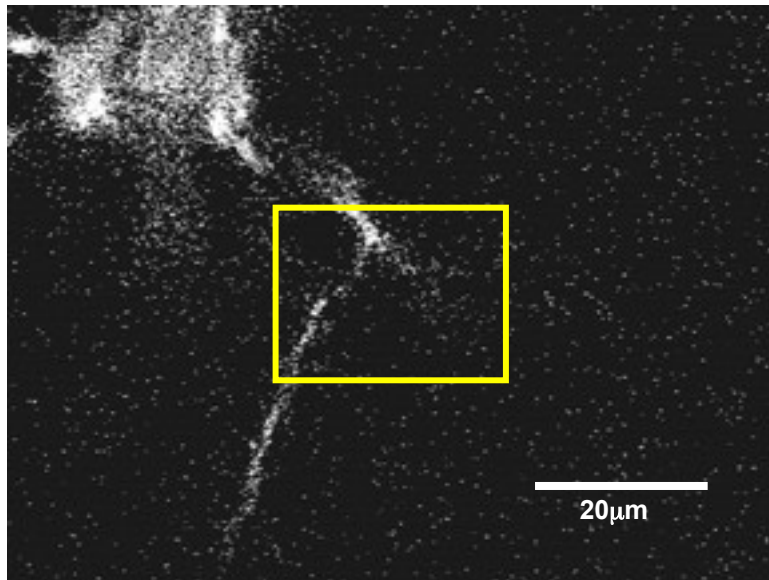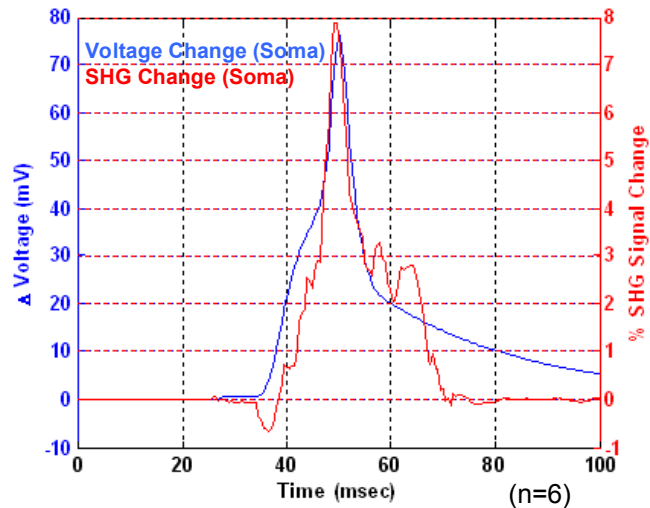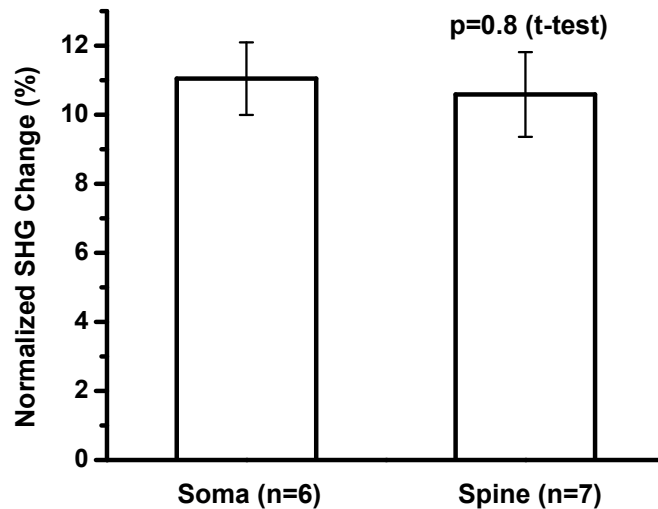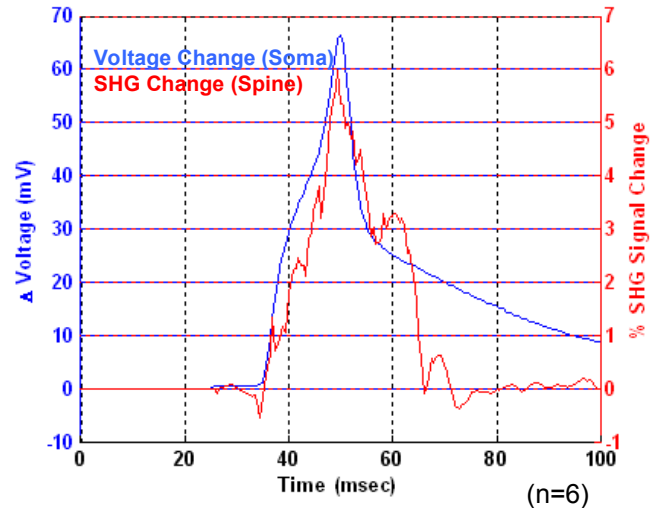

Standard Deviation / Mean = ~0.03 (3%)

SHG Changes in Spines by Backpropagating Action Potential
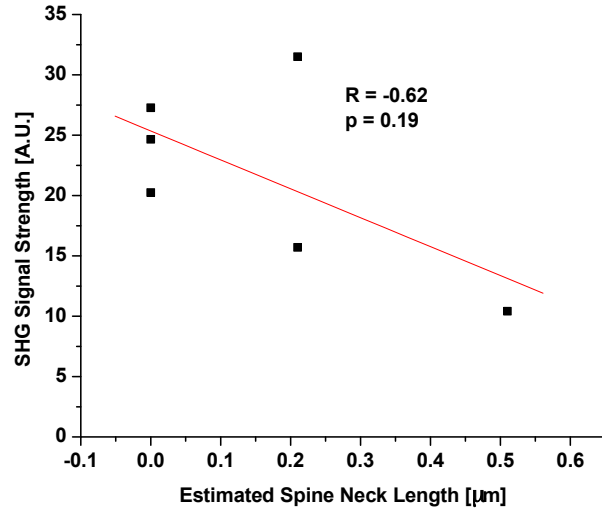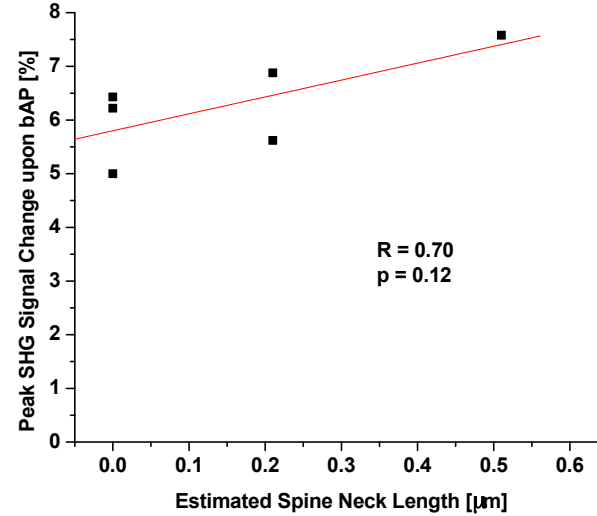
SHG Signals at Soma

(n=6)

SHG Signals at Spines

(n=6)

SHG Signal Change at Spines upon bAP
– Relationship with Spine Neck -

Spine Neck Length and SHG Signal Strength

R = -0.62
p = 0.19

Spine Neck Length and SHG Signal Change upon bAP

R = 0.70
p = 0.12

SHG Signal Strength and SHG Signal Change upon bAP
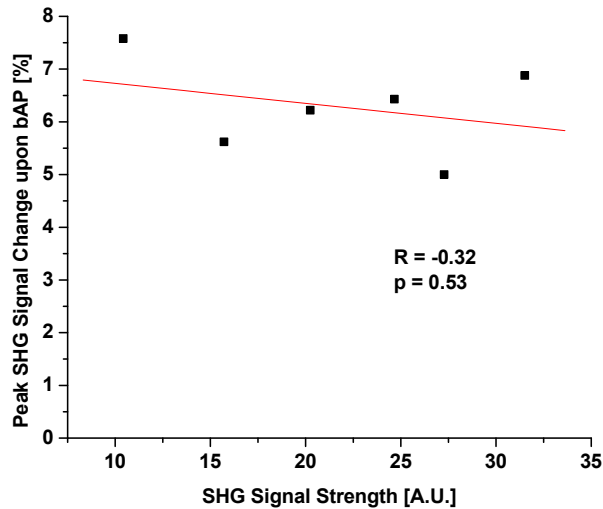
R = -0.32
p = 0.53

(n=6)

SHG Measurement at Spines after Glutamate Uncaging

Uncaging

EO Modulator 2

Ti:Sapphire Femtosecond Laser (725nm)

SHG

EO Modulator 1

Nd:Glass Femtosecond Laser (1064nm)

Fluoview Scanhead

PMT

PMT

Band-Pass Filter (530/20)

Spine SHG Measurement

Uncaging

SHG
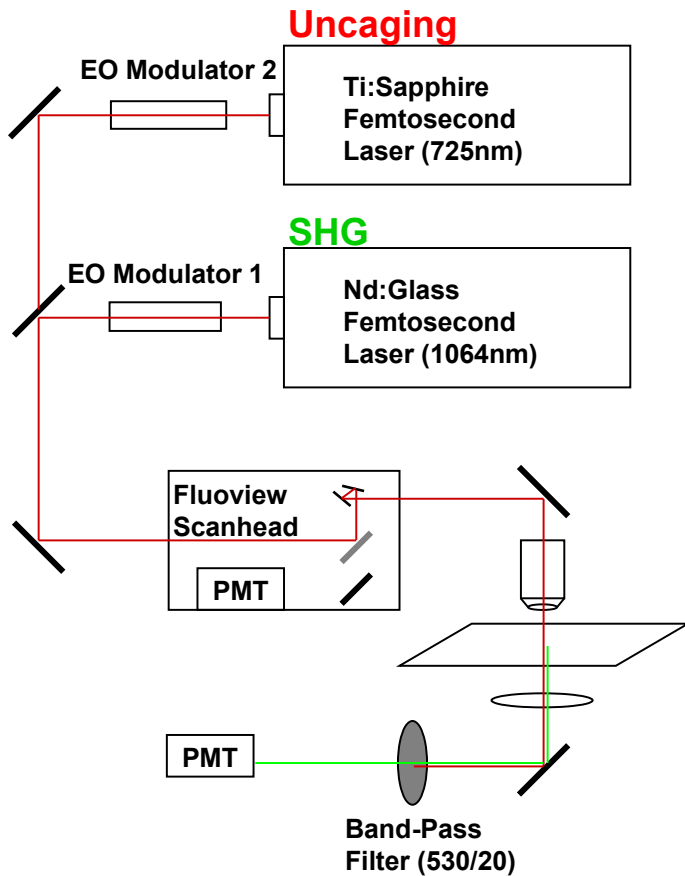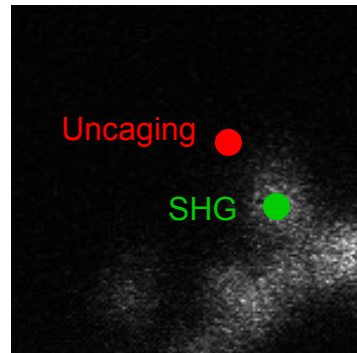
Shaft SHG Measurement

Uncaging

SHG
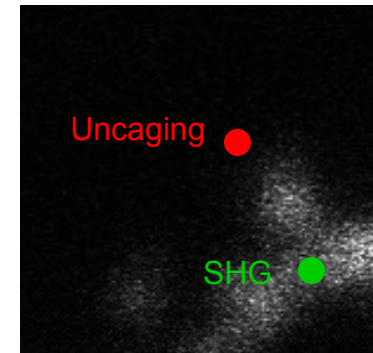
Uncaging Group

Control Group

Uncagng

SHG Measurement

1msec

30msec

500msec

X ~20 Sets
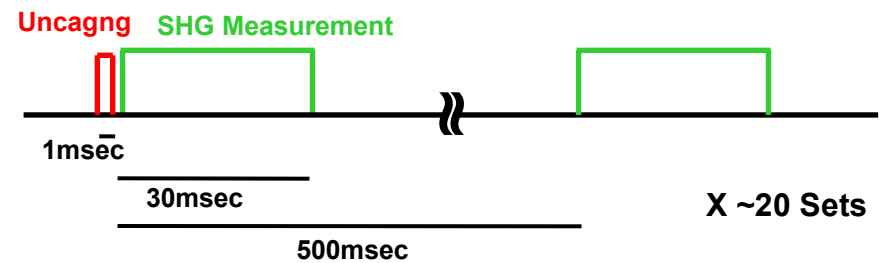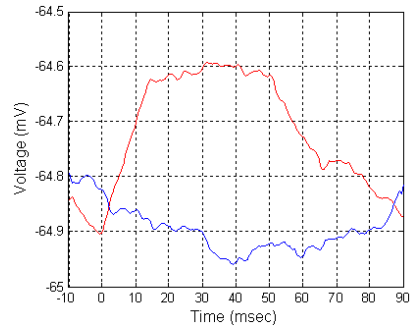
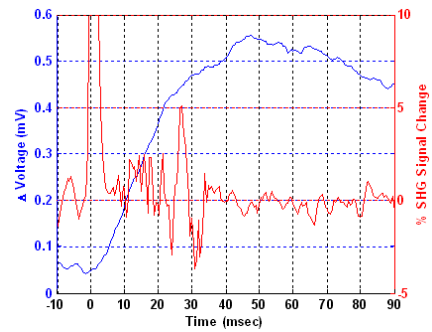# SHG Measurement at Spines after Glutamate Uncaging
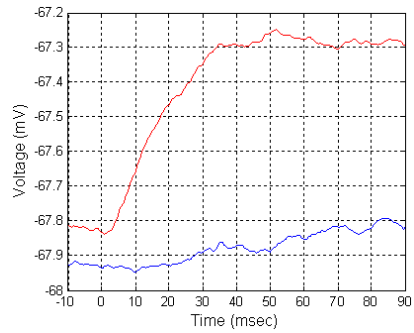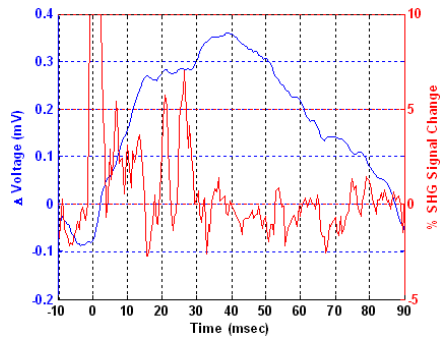

9/7/06, Cell2, 43min   9/7/06, Cell5, 65min   9/19/06, Cell3, 28min   9/21/06, Cell1, 63min
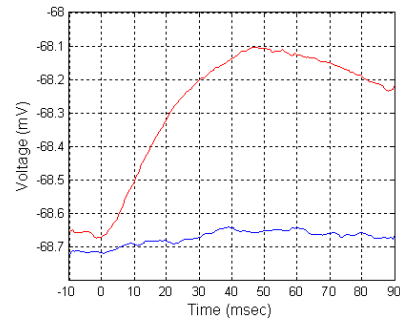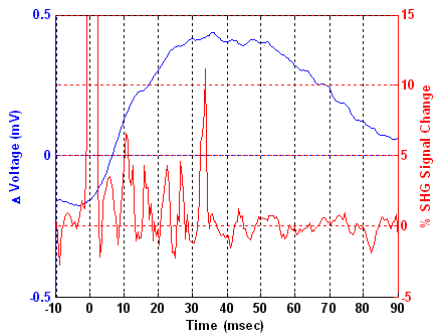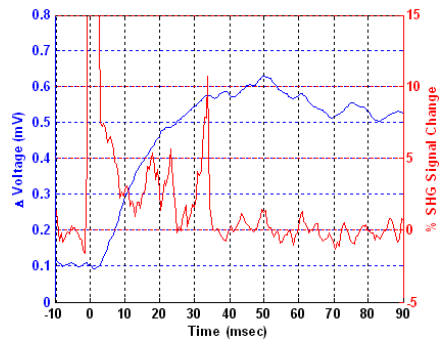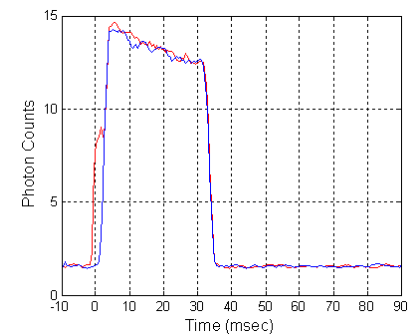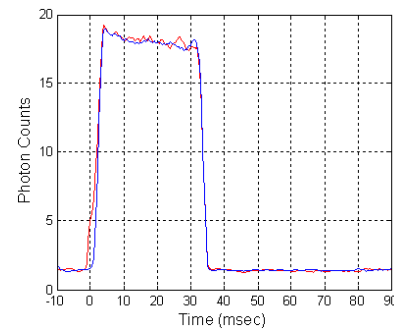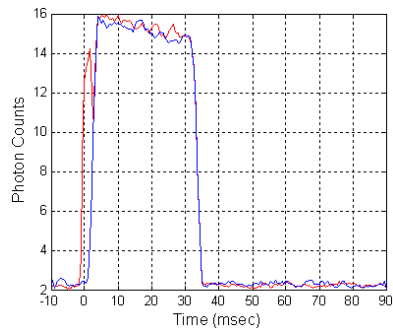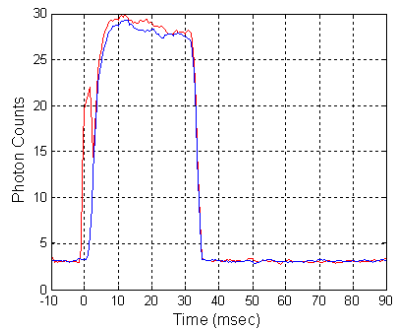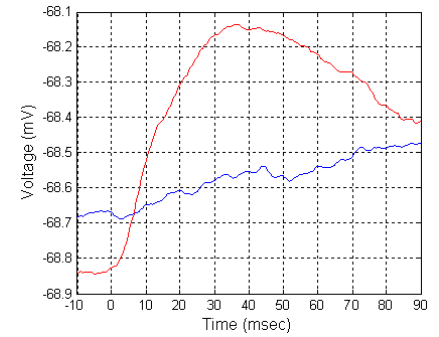
SHG Measurement at Spines after Glutamate Uncaging
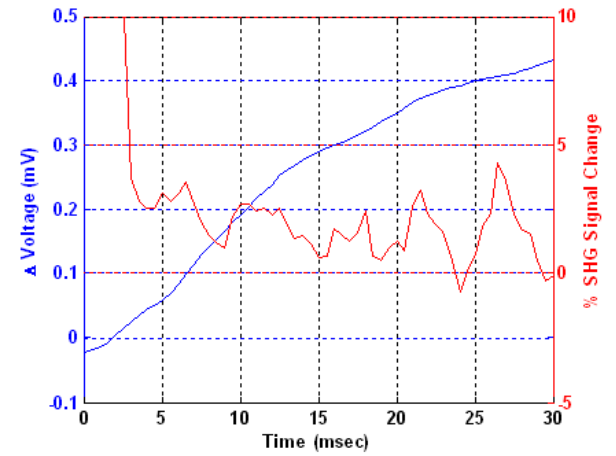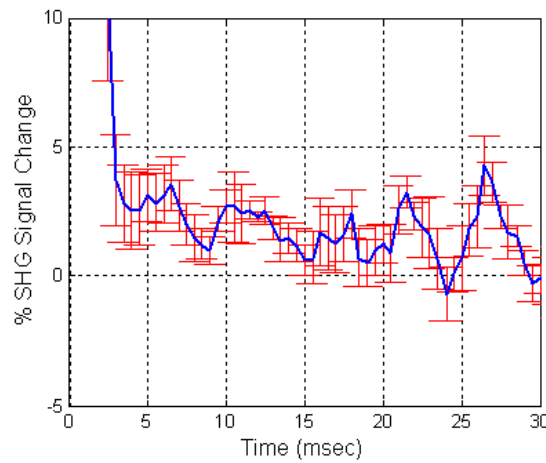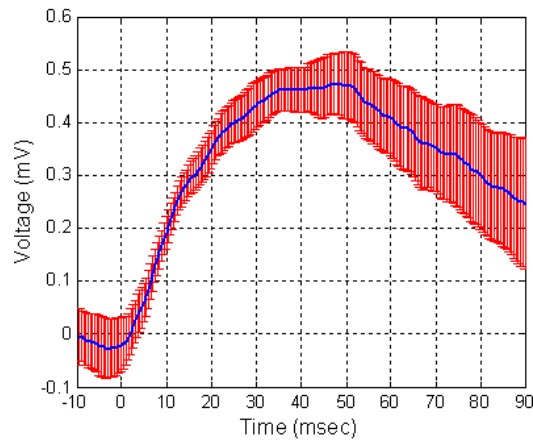
(n = 4)

SHG Recordings at Spines upon Glutamate Uncaging

Criteria for "Successful Experiments":

1) Successful Glutamate Uncaging.
   - Confirmation by electrophysiology recording at soma.

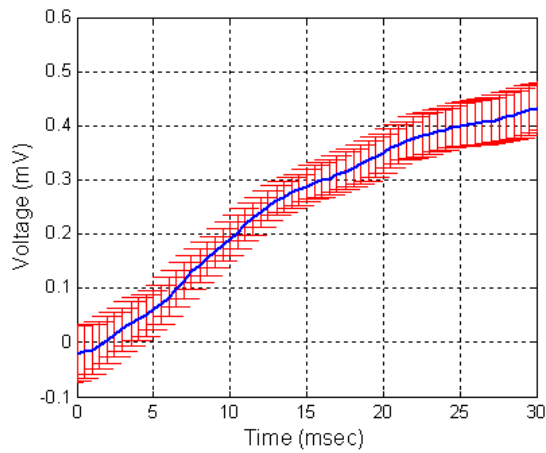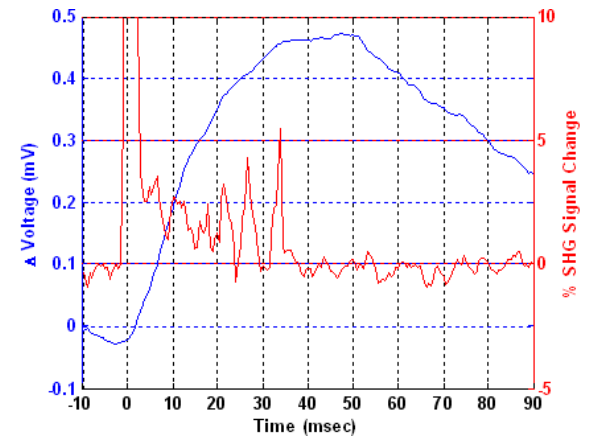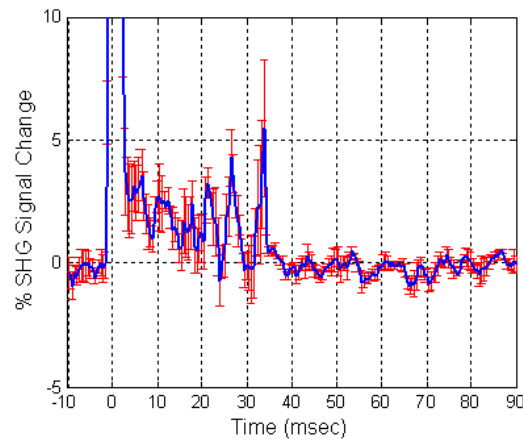2) Reliable spine SHG recording as a reporter of membrane potential change.
   - Confirmation by bAP induced SHG changes at spines.

3) Successful SHG Recordings.
   - SHG signals with decent signal to noise ratio.

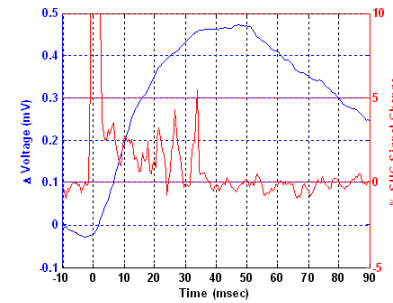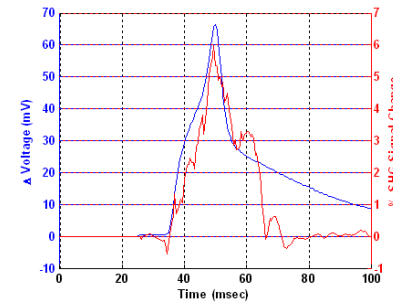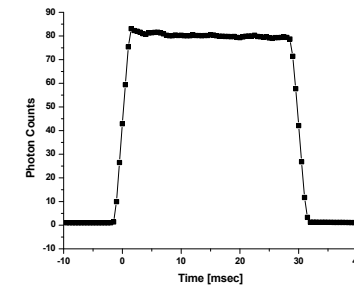# SHG Measurement at Spines after Glutamate Uncaging
- Comparison between Old and New Data -

Old Data

Average STD / Mean Value: 4.06%

New Data
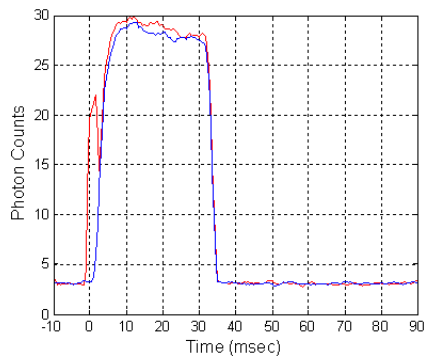
Average STD / Mean Value: 2.40%

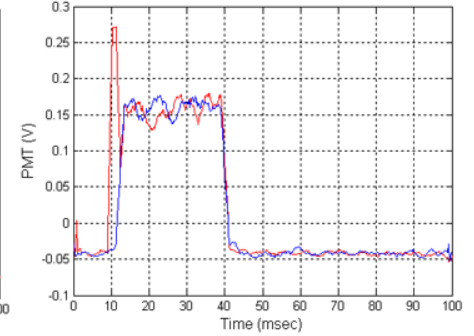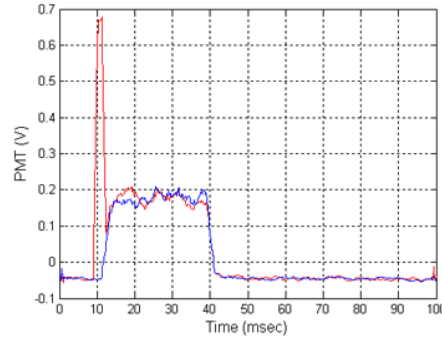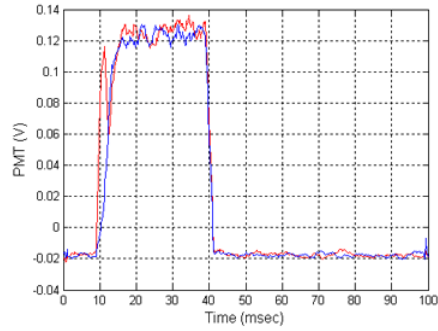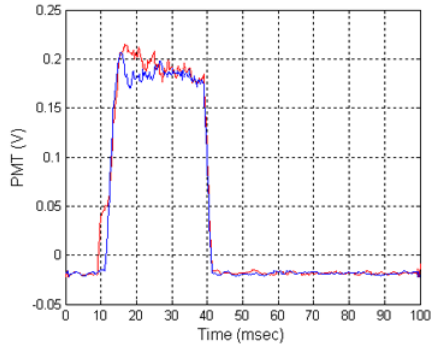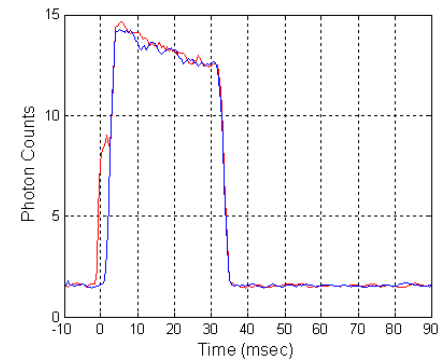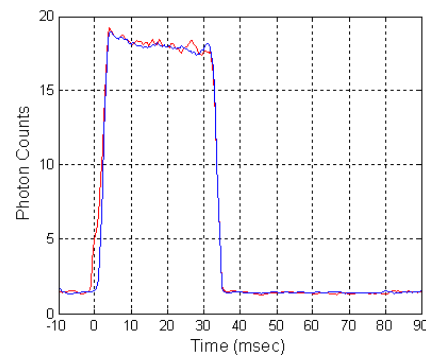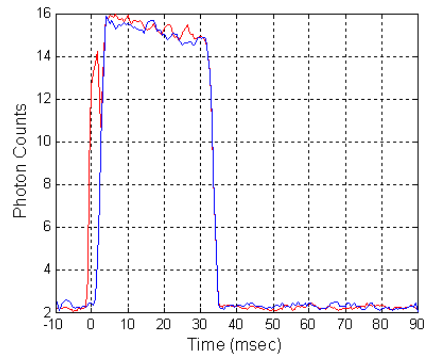# Toward Better Understandings of Electrical Properties of Spines
- Voltage Gated Ion Channel in Spines -
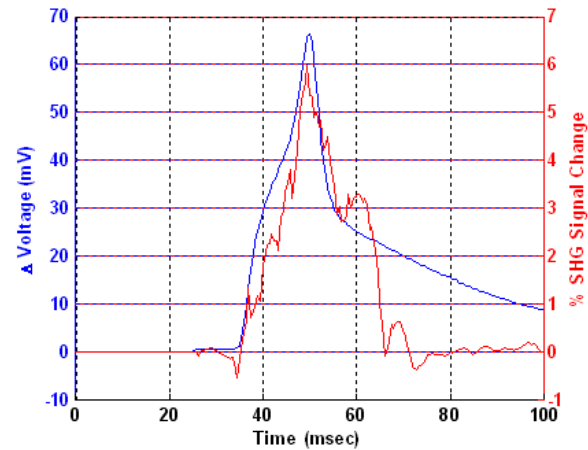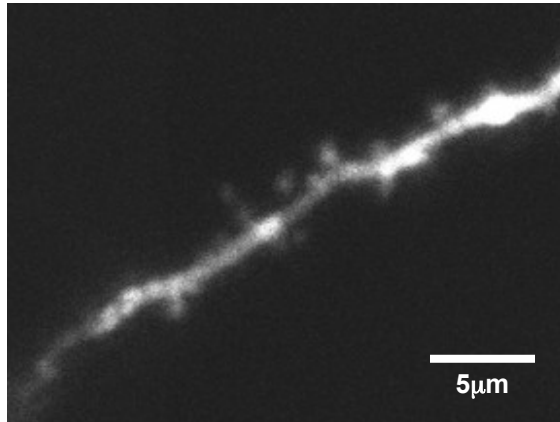
- Screening for ion channels.
    - Find ion channels that may play key roles in spine physiology.

- Identification of ion channels in spines.
    - Identify the existence and localization of target channels in spines.

- Characterization of ion channels.
    - Manipulate the function of the target channels and assess the resulting effects.

Identification of Ion Channels in Spines
- Immunocytochemistry -

EGFP   GIRK2   Overlay
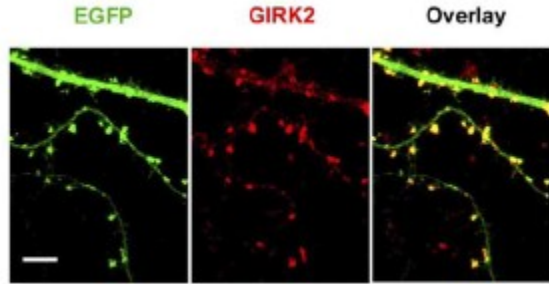
Huang CS et al. Cell (2005)

Kv1.4

Juiz JM et al. Eur.J.Nsci. (2000)

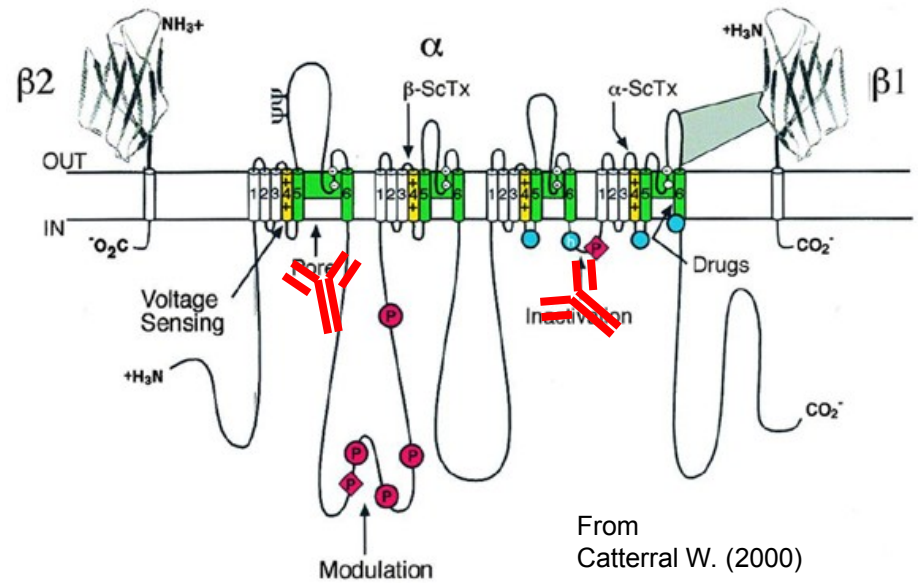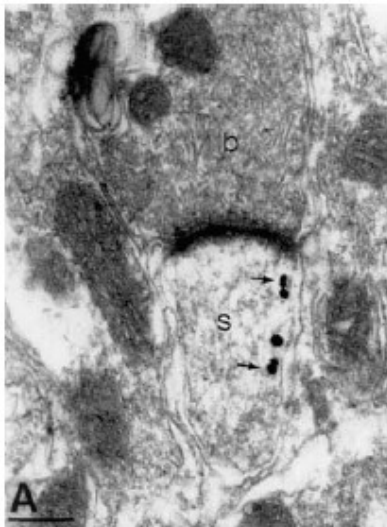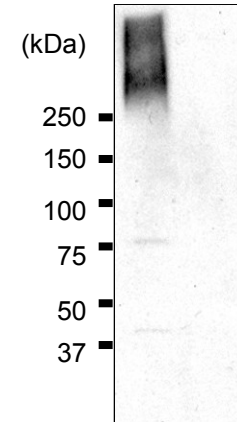**Nav1.5**
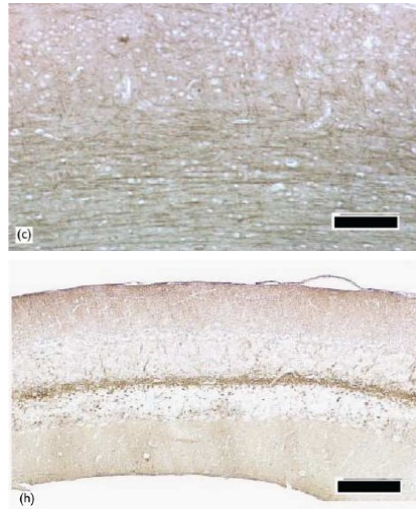
Wu L et al, NeuroReport (2002)

β2   NH₃+   α   +H₃N   β1

β-ScTx   α-ScTx

OUT

IN   ⁻O₂C   CO₂⁻

Voltage
Sensing   Pore   Inactivation   Drugs

+H₃N   CO₂⁻

Modulation

From
Catterral W. (2000)

(kDa)

250
150
100
75
50
37

Peptide Block: - +

Ab: PAN-Nav

Sample:
P2 Fraction from
Mouse Brain Lysate (P20)

Na$_v$ Immunocytochemistry with Rat Cortex

anti-PAN-Nav (CU868)
1:500
40x Objective, 1x Zoom

100μm

anti-PAN-Nav (CU868)
1:500, Peptide Block
40x Objective, 1x Zoom

100μm

anti-PAN-Nav (CU868)
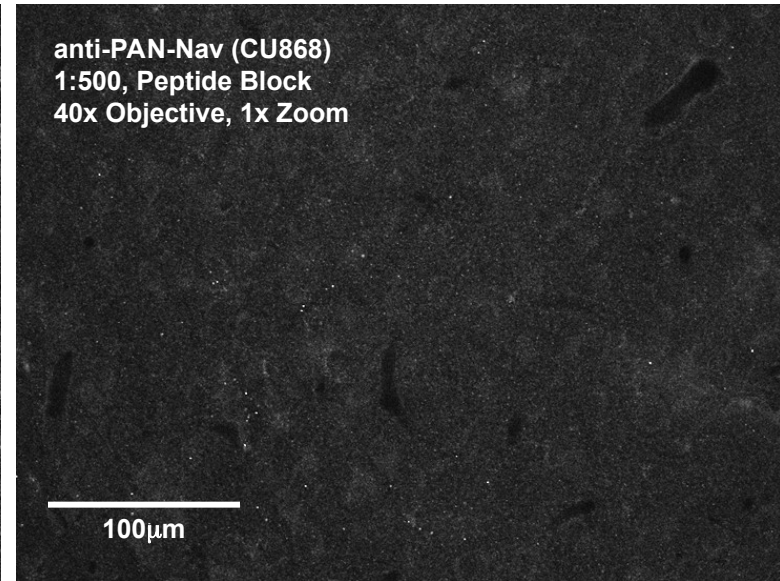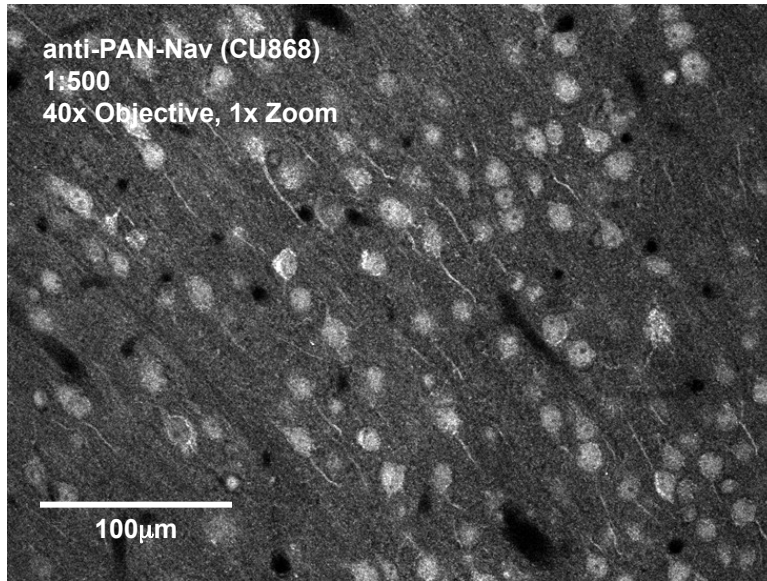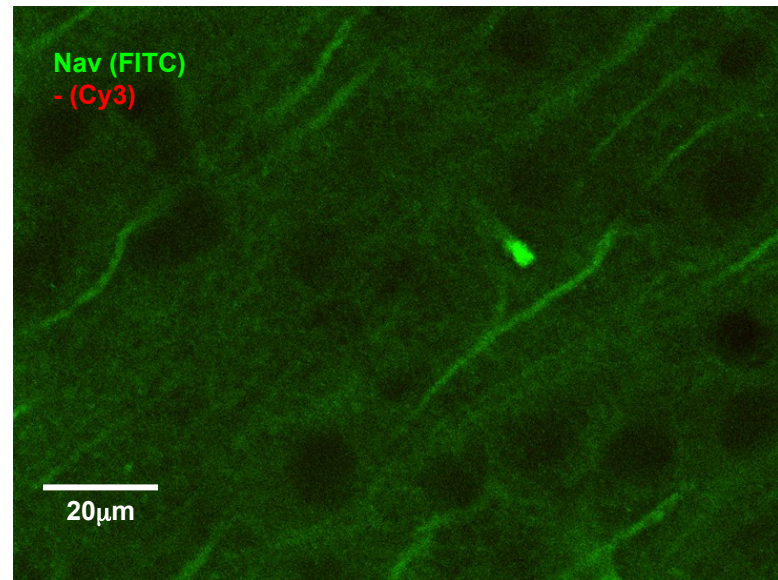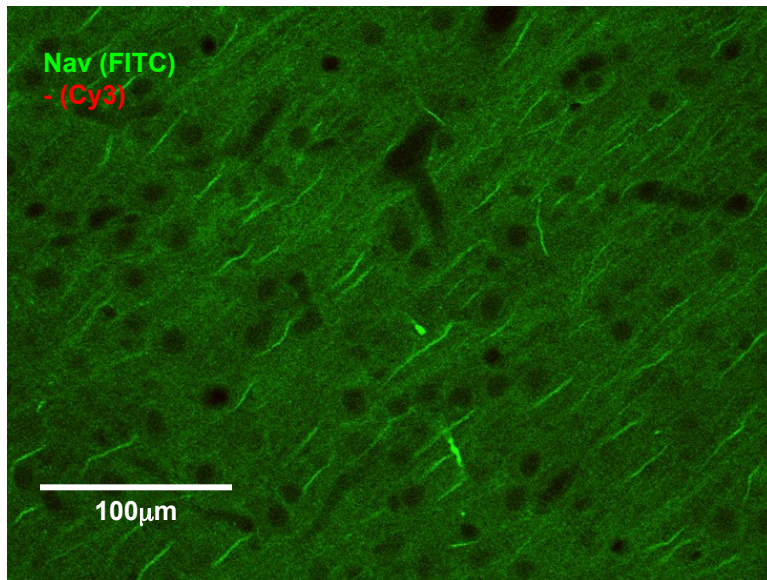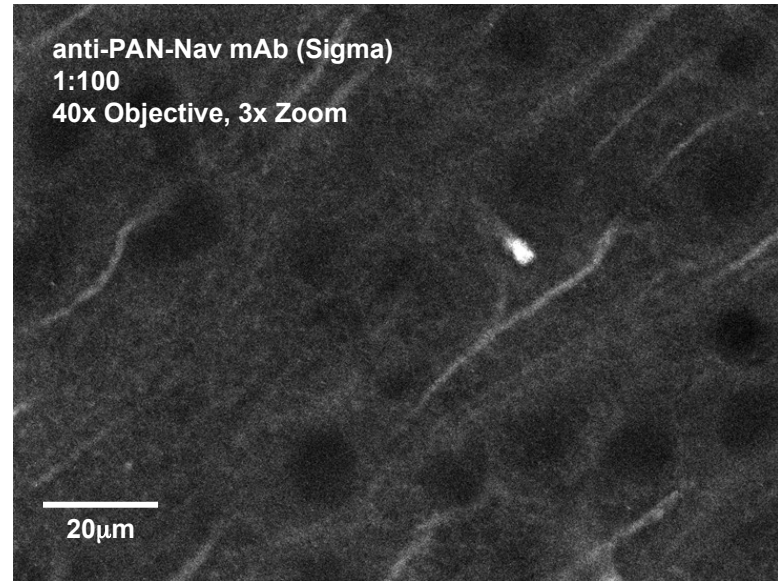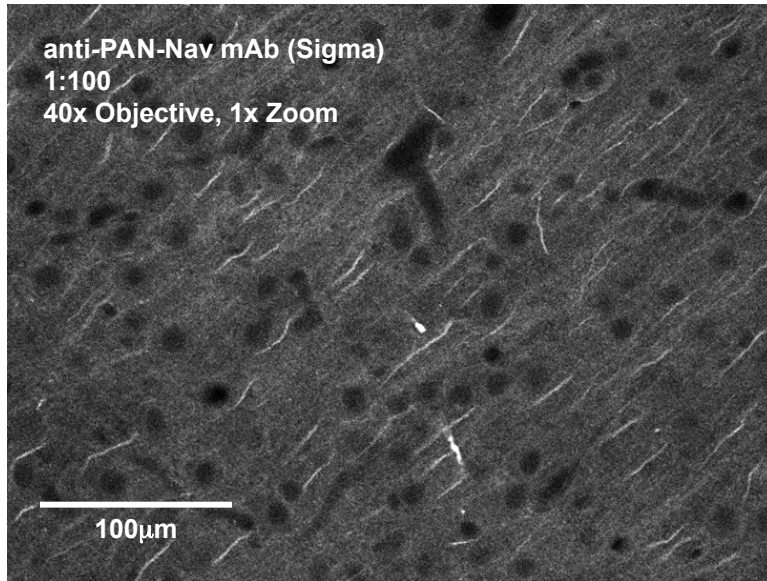1:500
40x Objective, 3x Zoom

20μm

anti-PAN-Nav (CU868)
1:500, Peptide Block
40x Objective, 3x Zoom

20μm

Na$_v$ Immunocytochemistry with Rat Cortex

anti-PAN-Nav mAb (Sigma)
1:100
40x Objective, 1x Zoom

100μm

anti-PAN-Nav mAb (Sigma)
1:100
40x Objective, 3x Zoom

20μm

Nav (FITC)
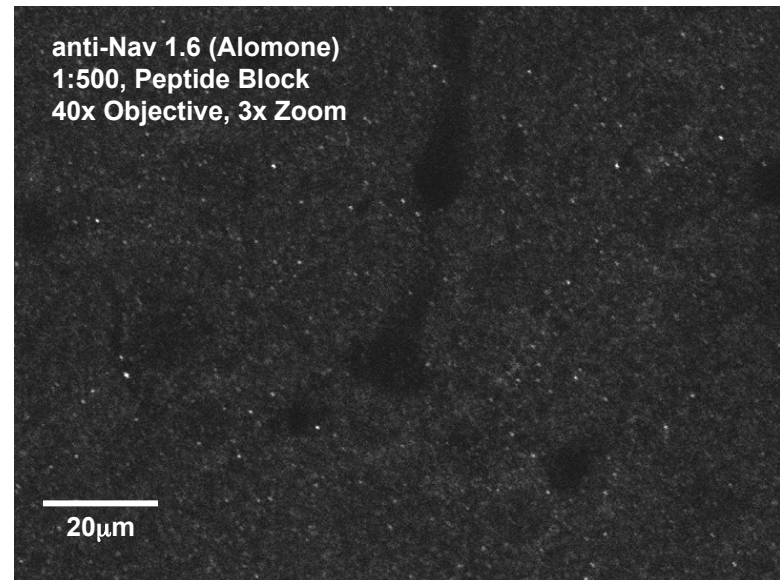- (Cy3)

100μm

Nav (FITC)
- (Cy3)

20μm

Na$_v$ Immunocytochemistry with Rat Cortex

Na$_v$ Immunocytochemistry with Rat Cortex

anti-Nav 1.5 (Alomone)
1:100
40x Objective, 1x Zoom

100μm

anti-Nav 1.5 (Chemicon)
1:100
40x Objective, 1x Zoom

100μm

anti-Nav 1.5 (Chemicon)
1:100
40x Objective, 3x Zoom

20μm