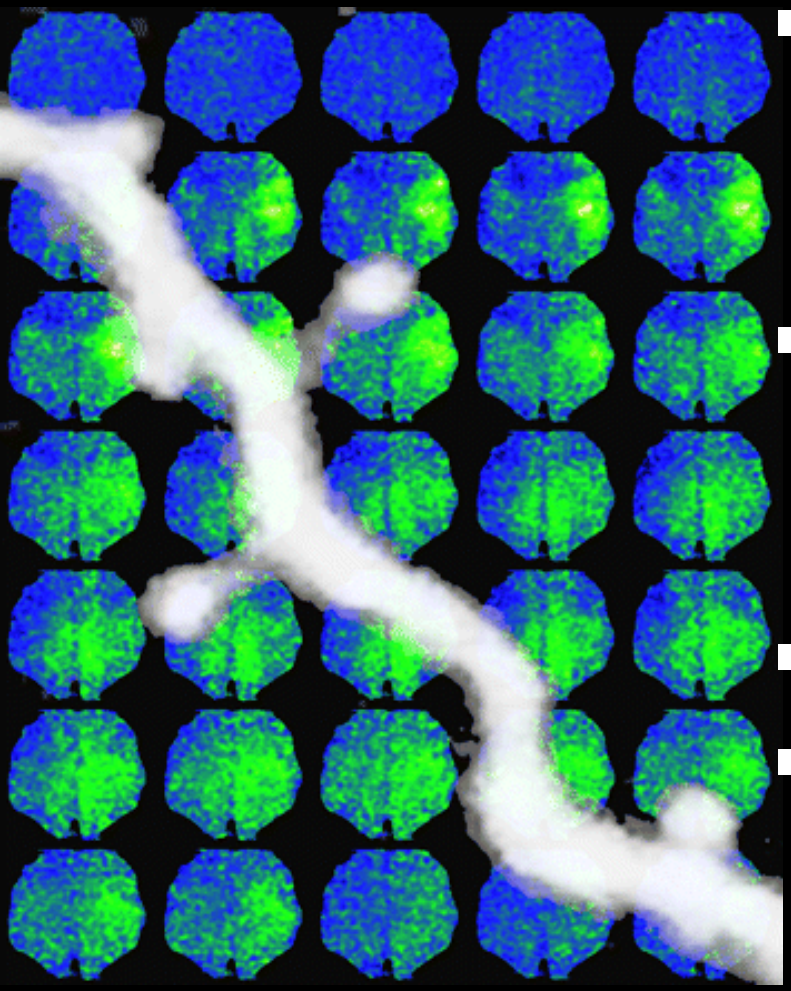
“Synapse and circuit level plasticity after stroke.”

Timothy H. Murphy
Univ. of British Columbia



- Acute stroke and dendrite structural damage.
- Stroke recovery circuit plasticity, over weeks/hrs.
- Optogenetic motor mapping
- methods for stroke.

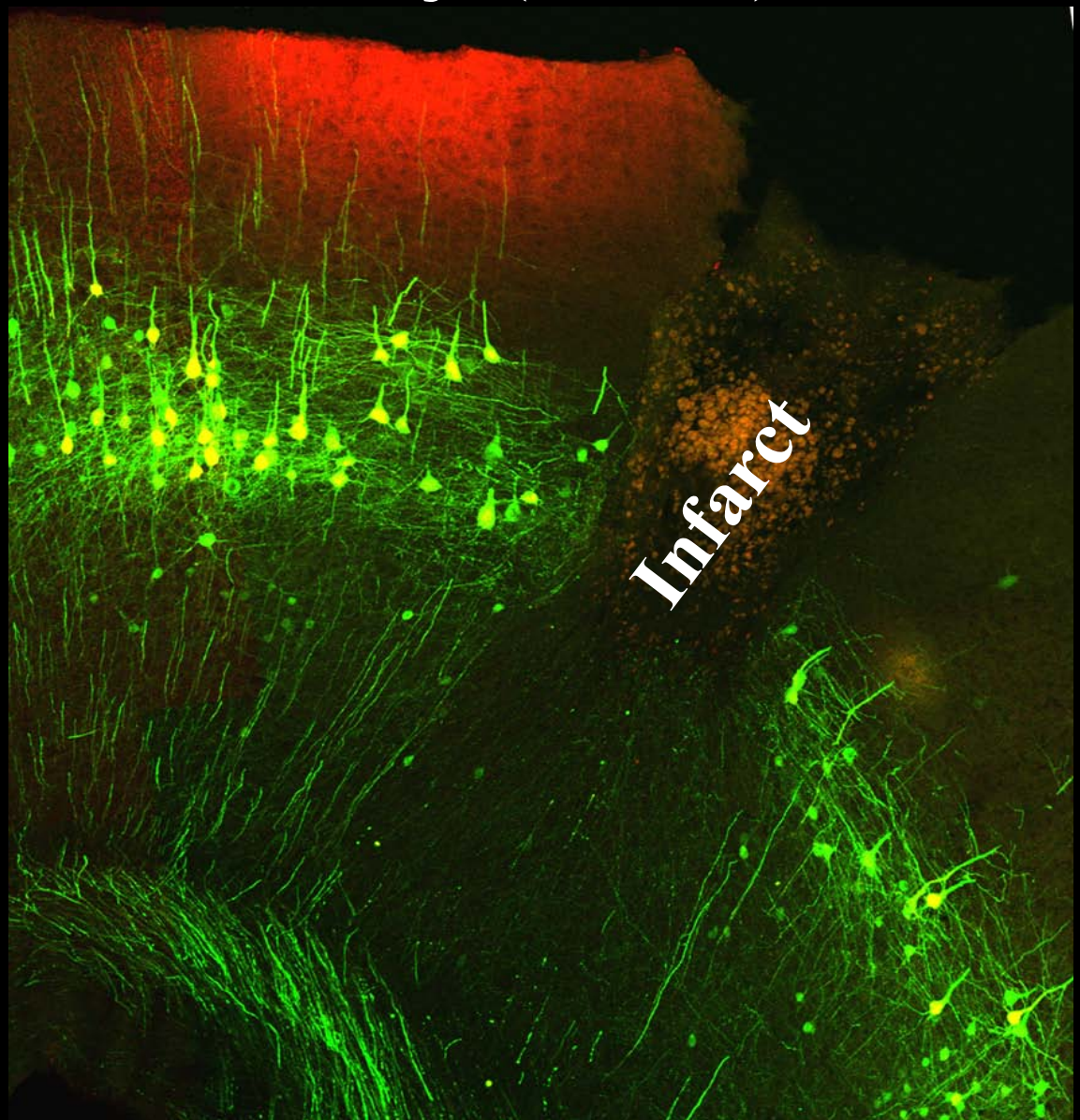
HEART AND STROKE FOUNDATION OF B.C. & YUKON

Canadian Stroke Network
Réseau Canadien contre les accidents cérébrovasculaires

CIHR IRSC

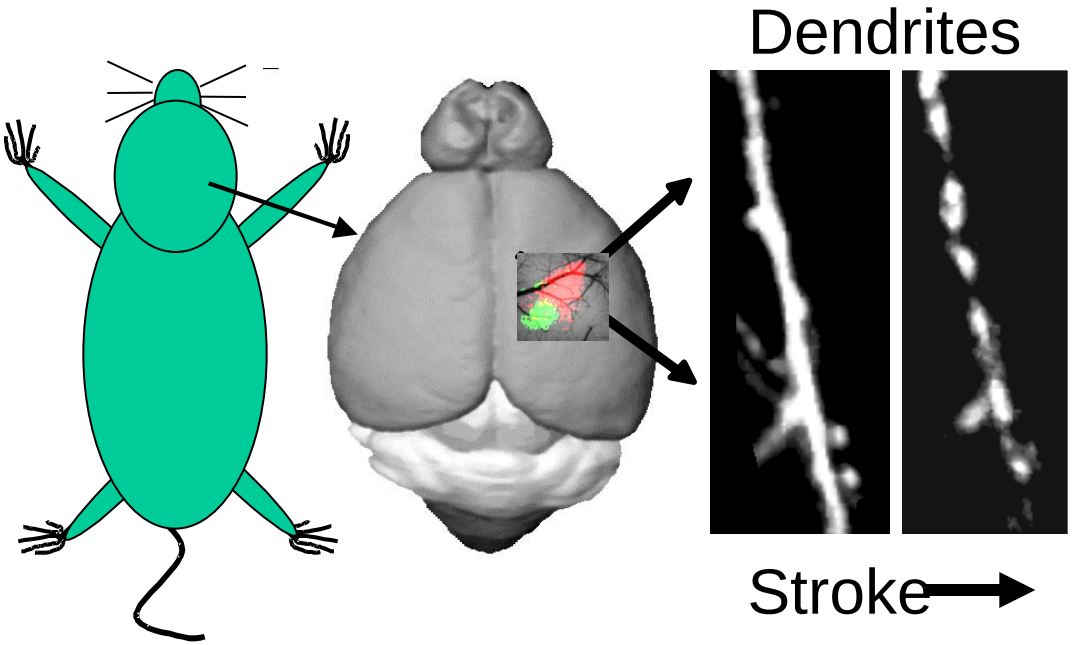
Brain Research Centre

How do synapses and circuits respond acutely (hours) to stroke ?



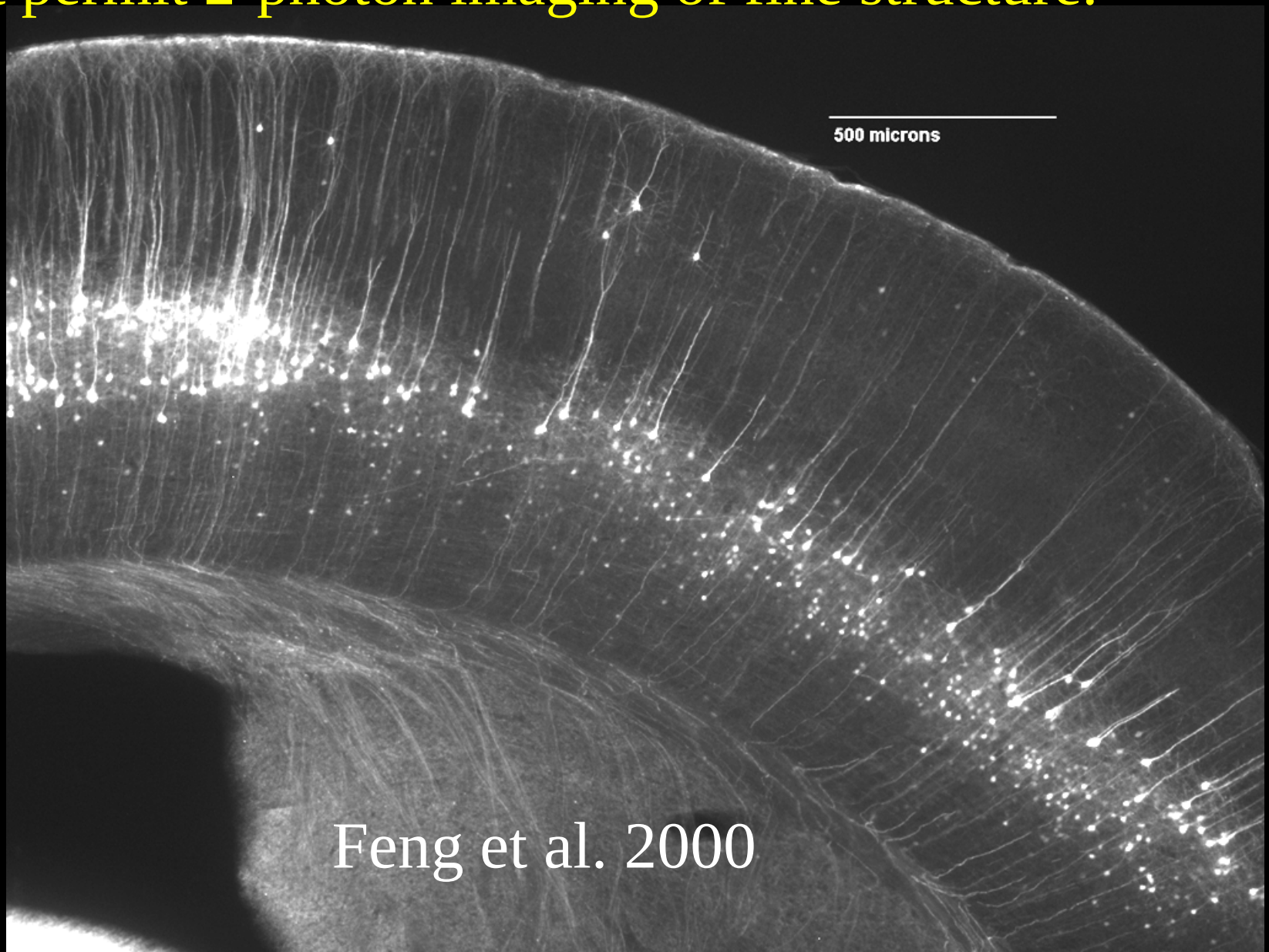
Can we understand stroke pathogenesis by imaging its early events at the level of individual synapses in vivo using 2-photon?

Crash test dummy



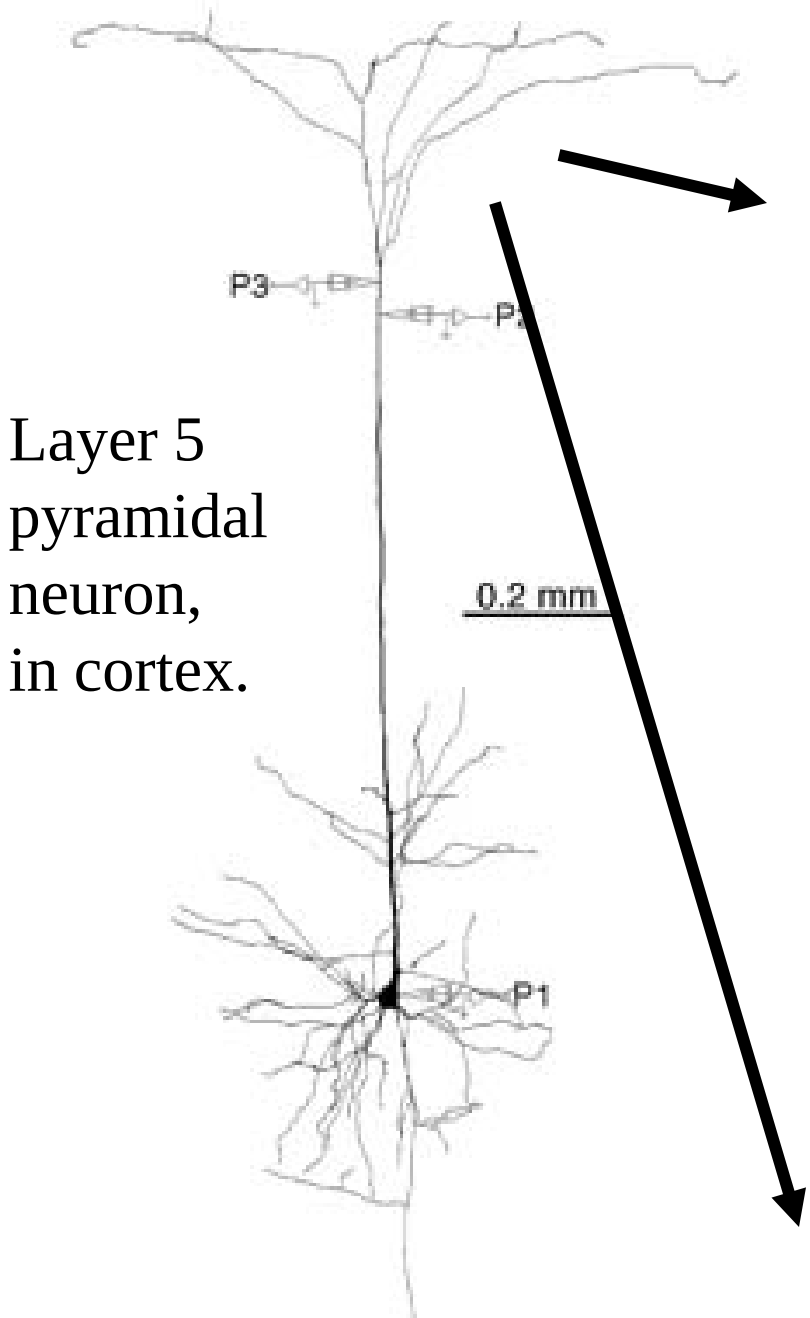
Mouse in vivo imaging during stroke (adult C57 bl6 mice)

Stroke models in GFP-M/YFP-H transgenic mice that permit 2-photon imaging of fine structure.

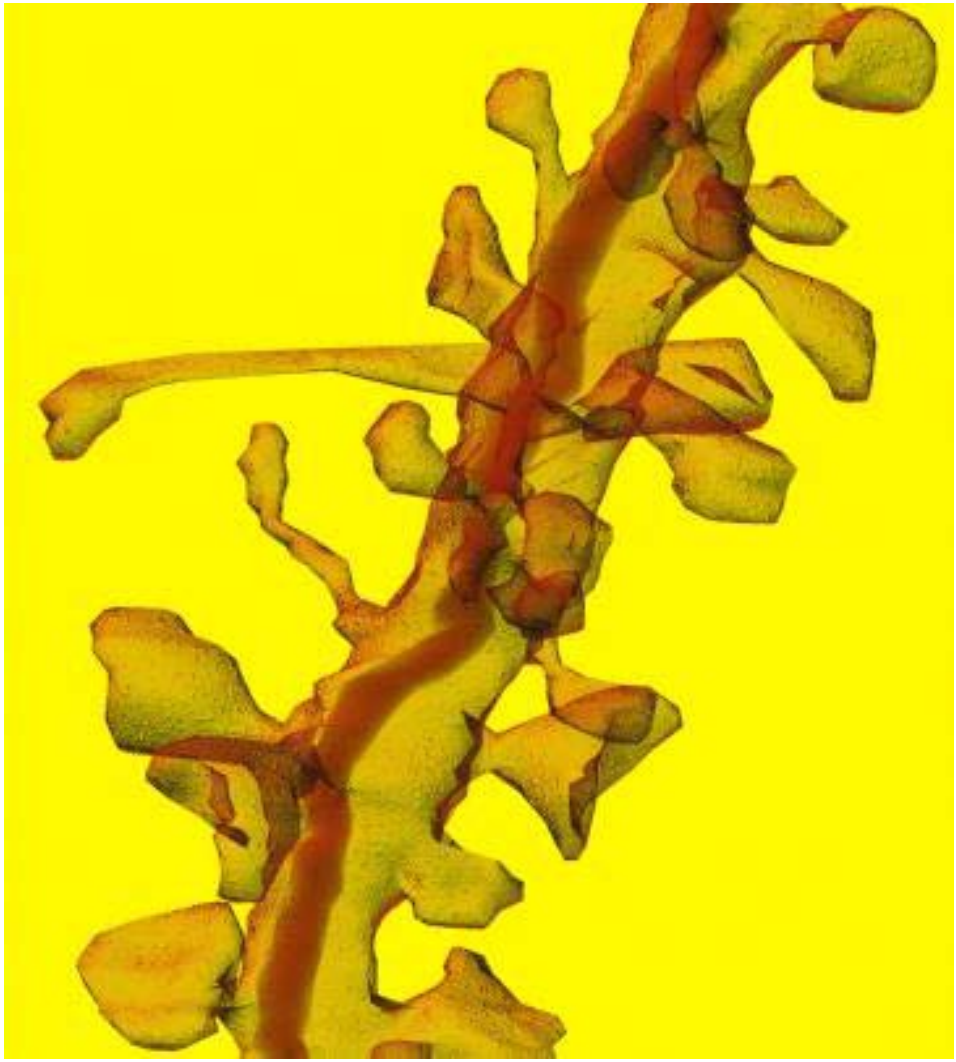


Spines are the receiving end of cortical excitatory synapses.

Layer 5 pyramidal neuron, in cortex.



From Larkum and Zhu JNS 2003

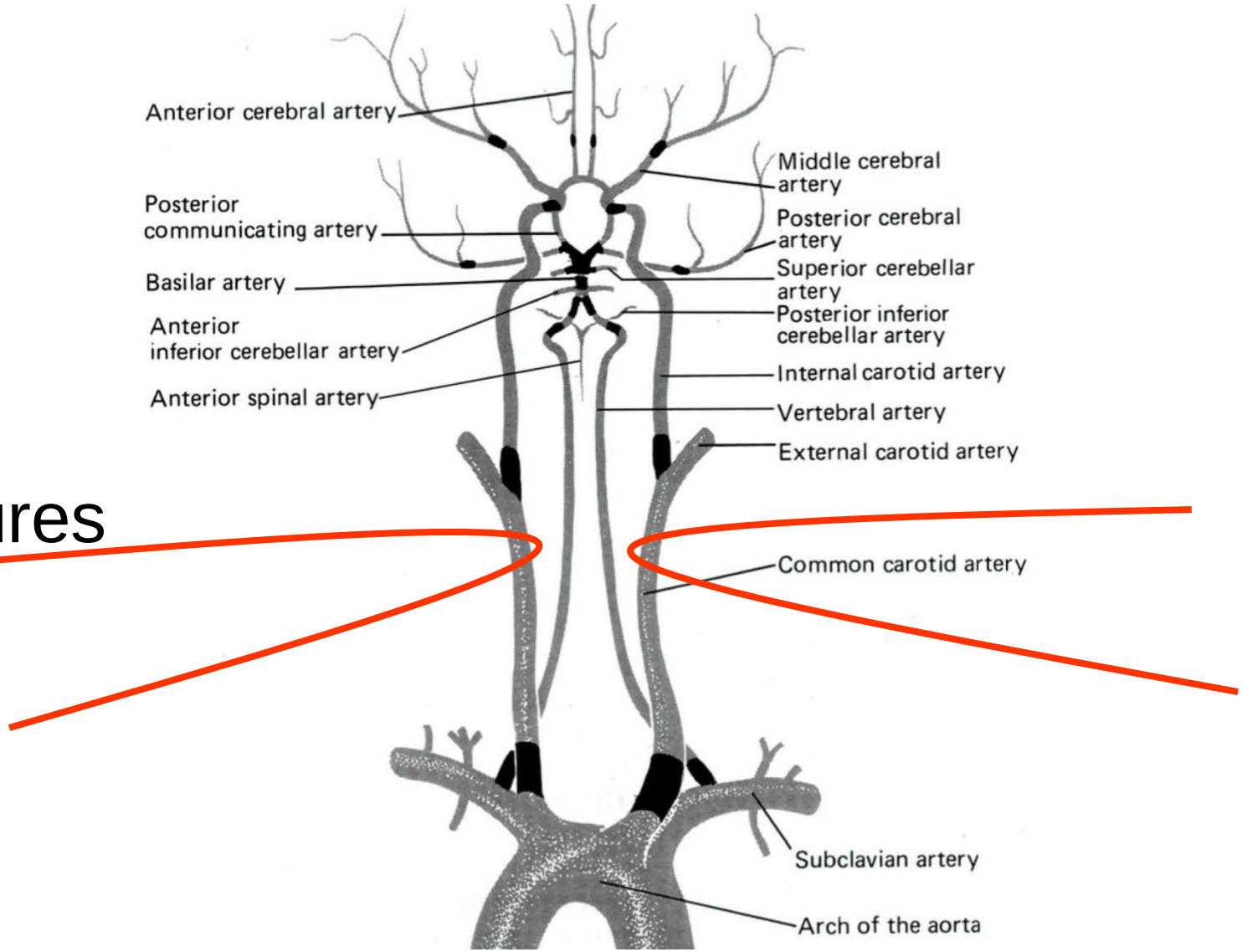


From Kristin Harris Lectures.
http://synapses.mcg.edu/lab/harris

Develop rapid and reversible models of ischemia for two-photon imaging during stroke induction.

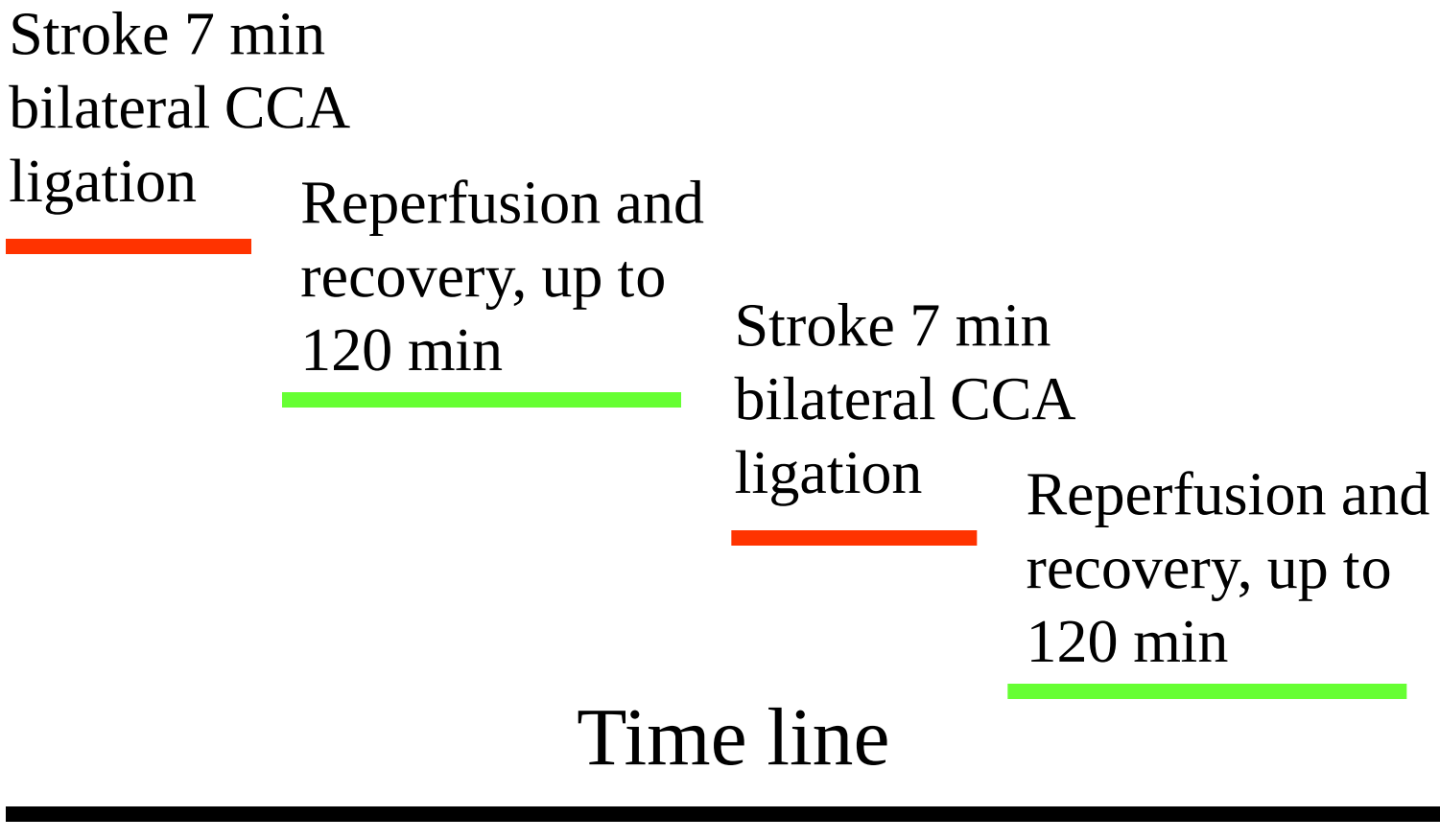
- **Focal middle cerebral artery occlusion model.**
- Global ischemia model, bilateral occlusion of the common carotid arteries in the C57-bl6 mouse (GFP-H or YFP-M), typically 6-8 min of occlusion.
- Photo-thrombosis, irreversible but targeted.
- How is synaptic structure and function altered during ischemia and reperfusion?
- Which ionic events trigger loss of dendritic structure?

Sutures



Sutures placed around both common carotid arteries (CCAs) to induce global ischemia and tensioned while a urethane-anesthetized mouse is imaged in two-photon; this procedure works since C57-bl6 mice have poorly developed posterior communicating arteries.

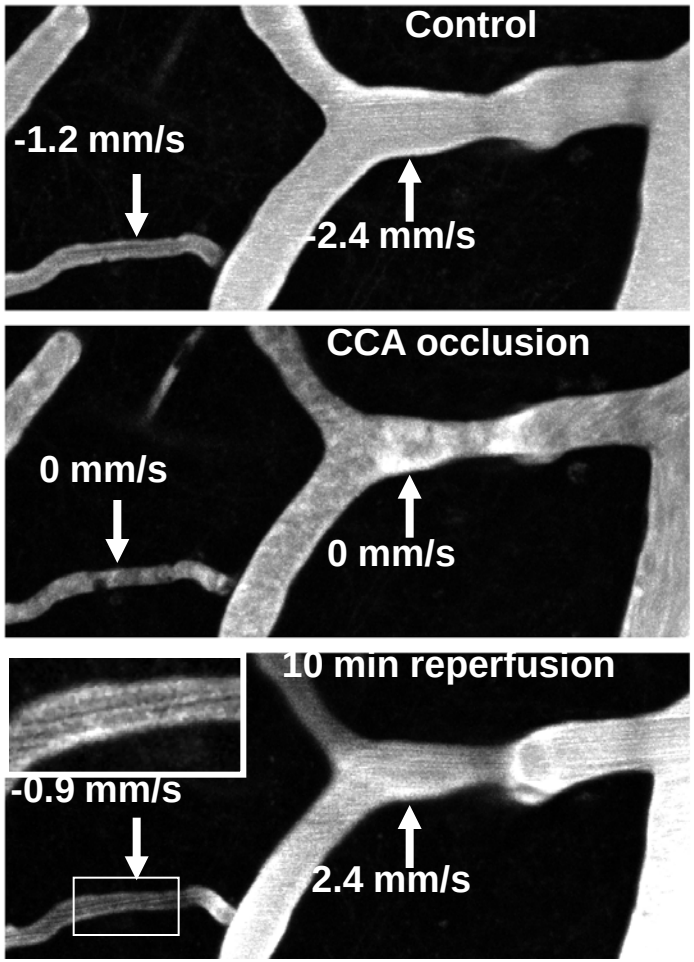
Structural and functional assessment of somatosensory cortex during repeated episodes of transient global ischemia.

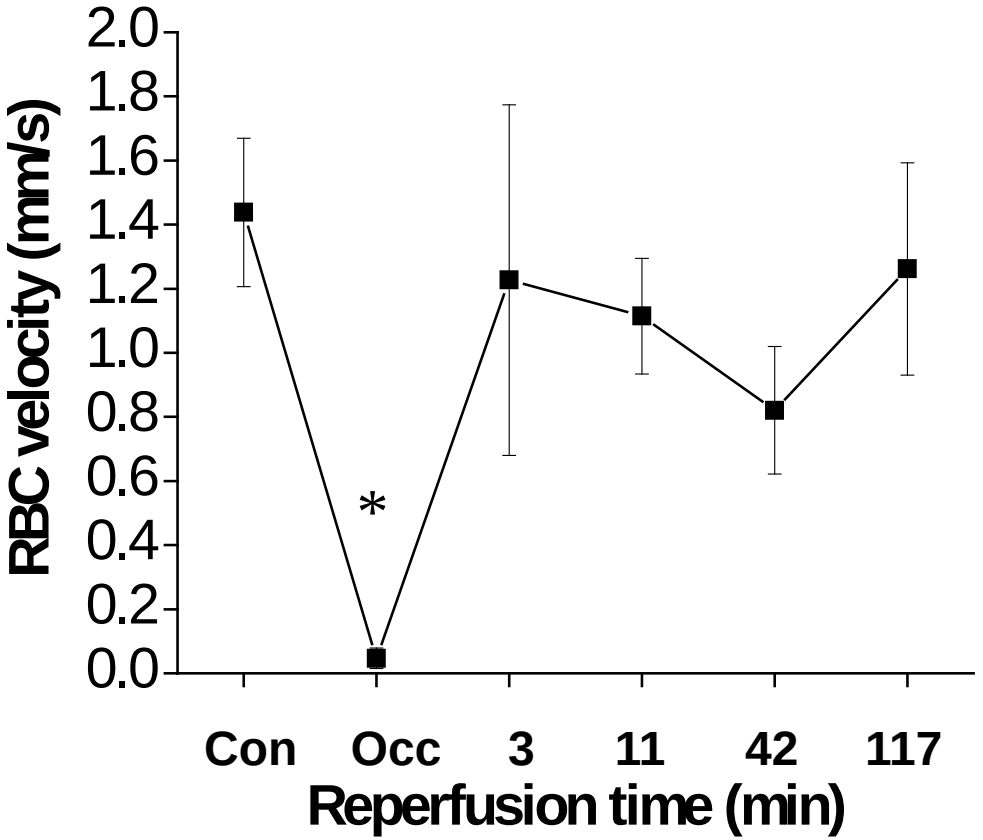


Assess structure (YFP/2-photon), monitor electrical events EEG, calcium imaging, and function (IOS imaging).

J. Neurosci. 2008 Murphy et al.

Bilateral common carotid artery occlusion reduces local blood flow.

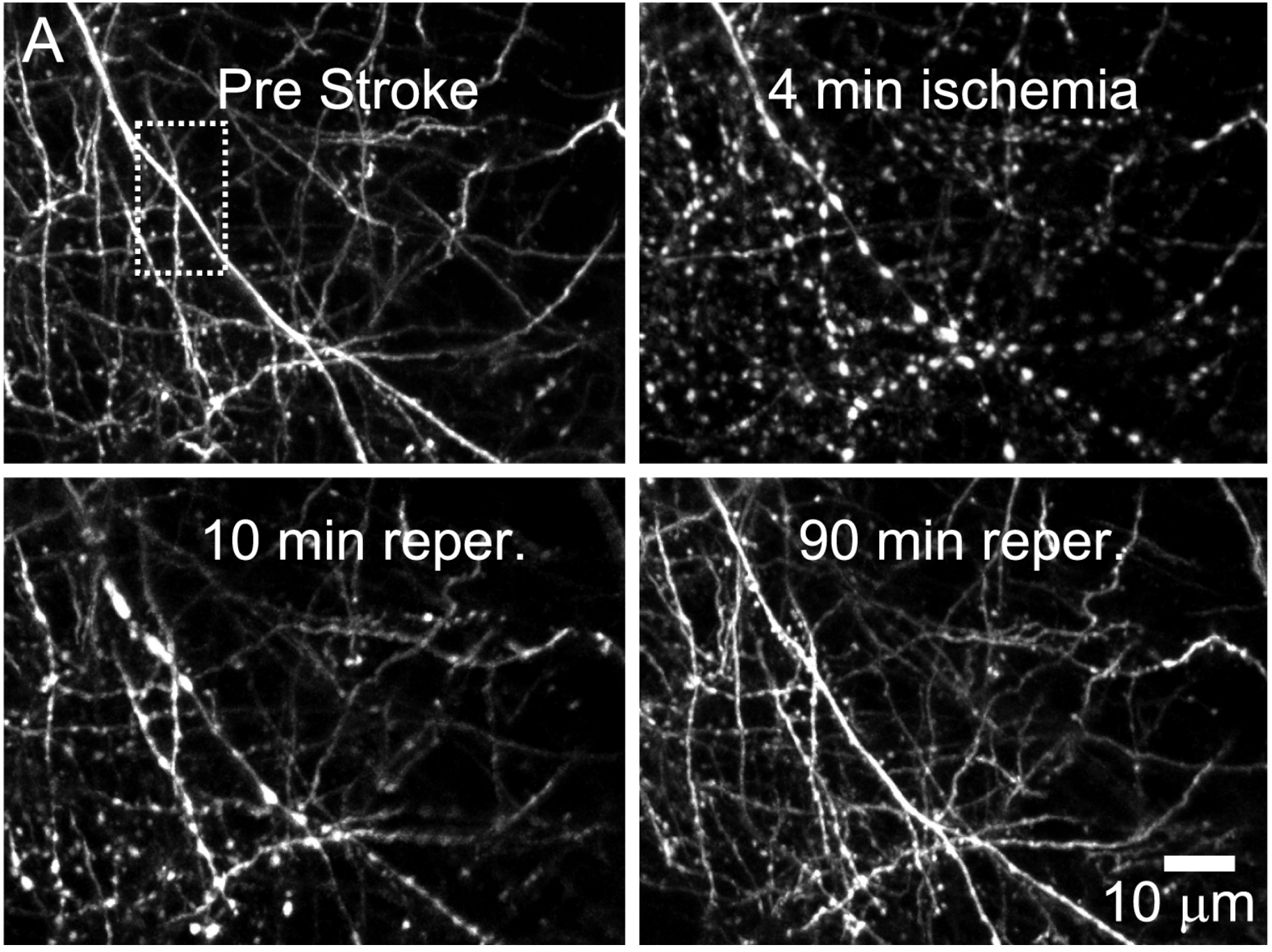




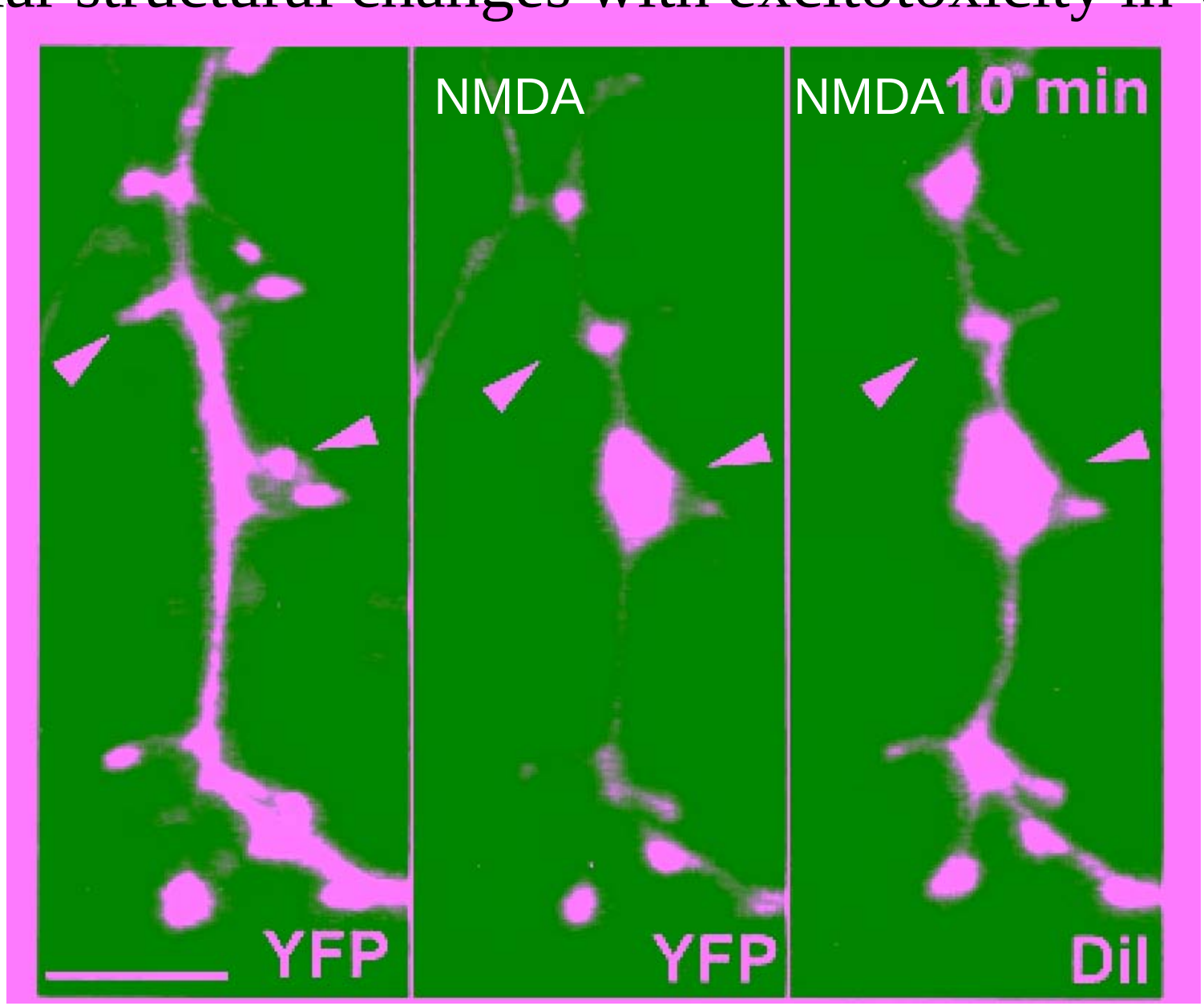
n=7 animals

J. Neurosci. 2008 Murphy et al.

Dendrites during ischemia and reperfusion.

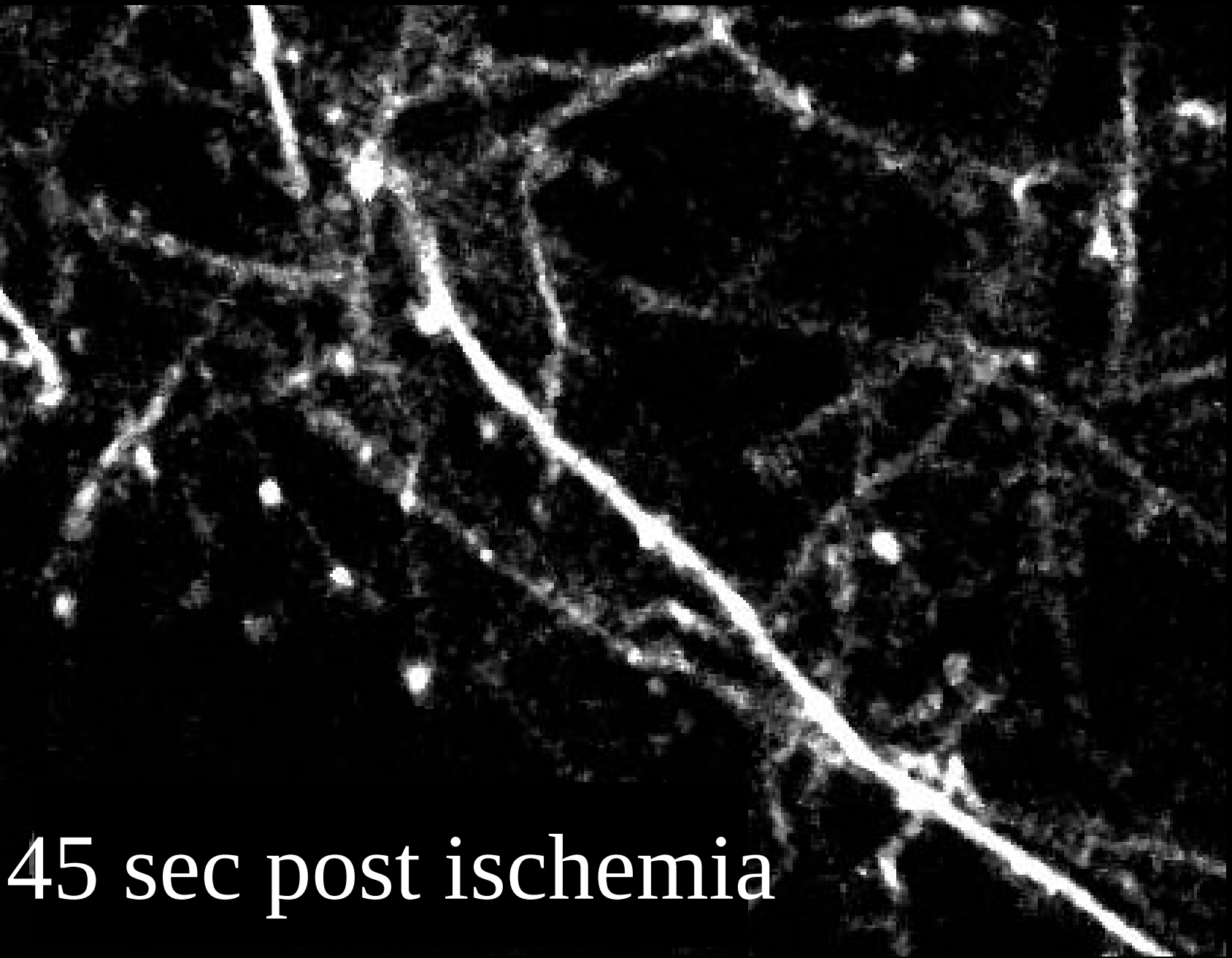


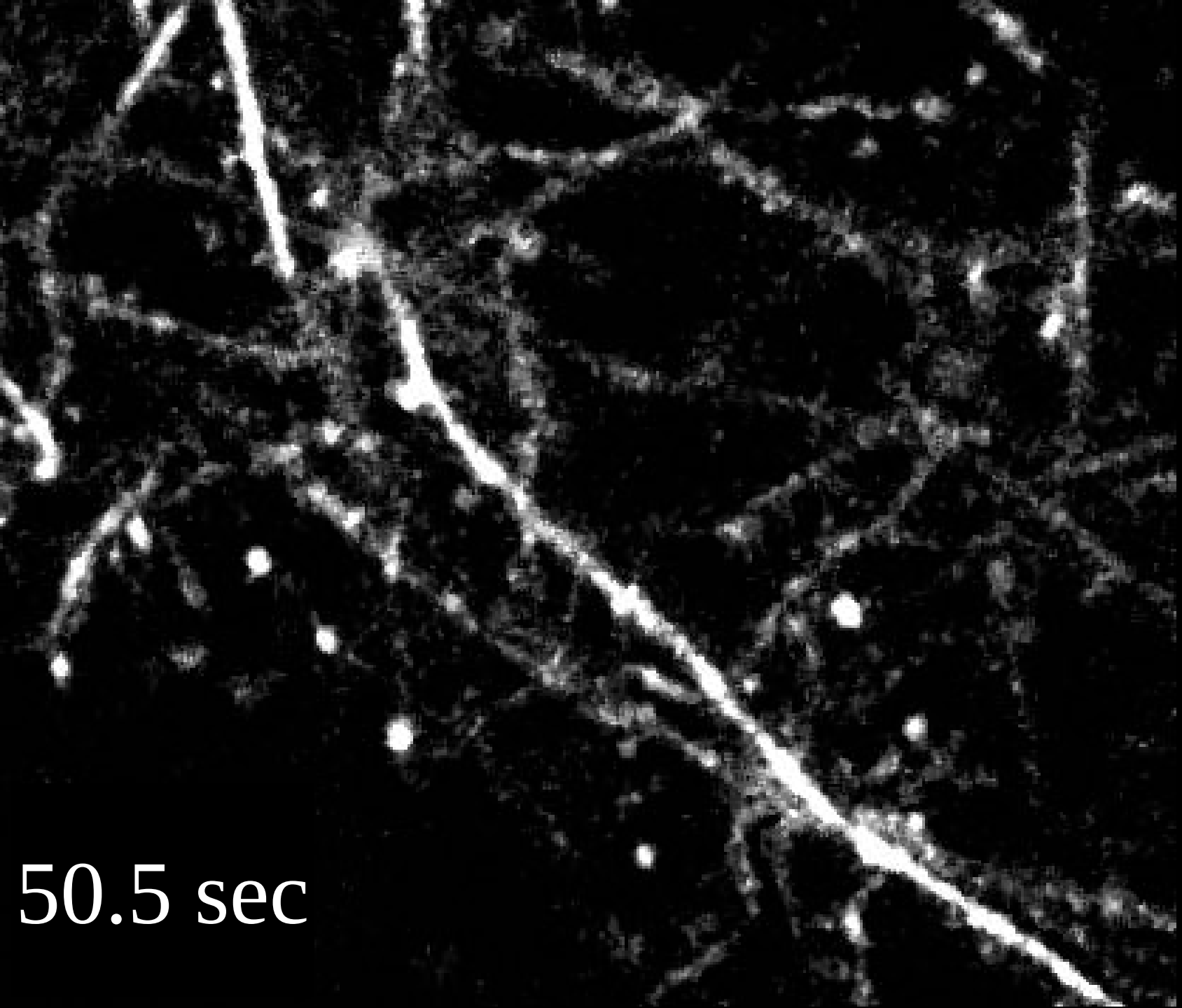
Similar structural changes with excitotoxicity in vitro.

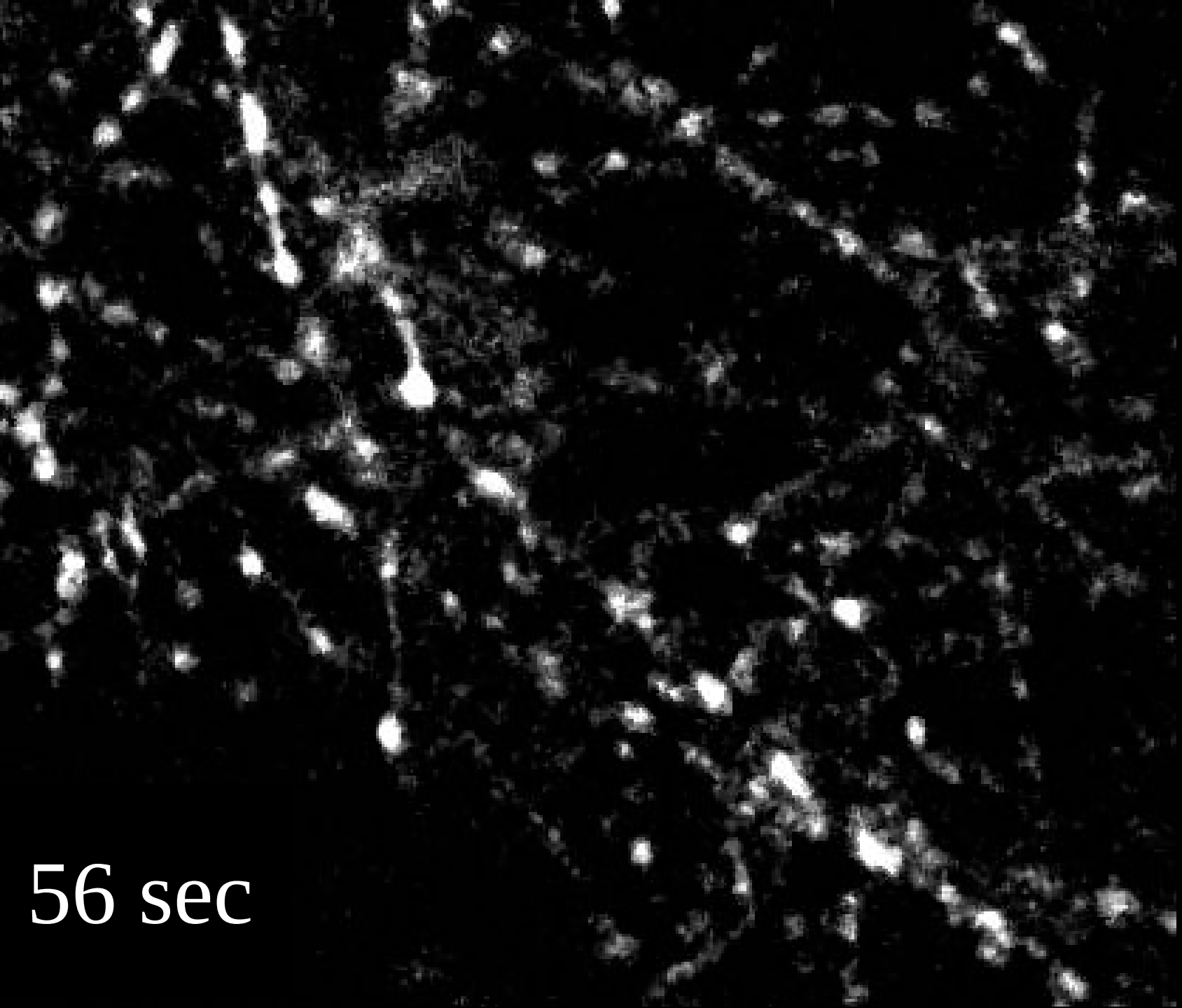


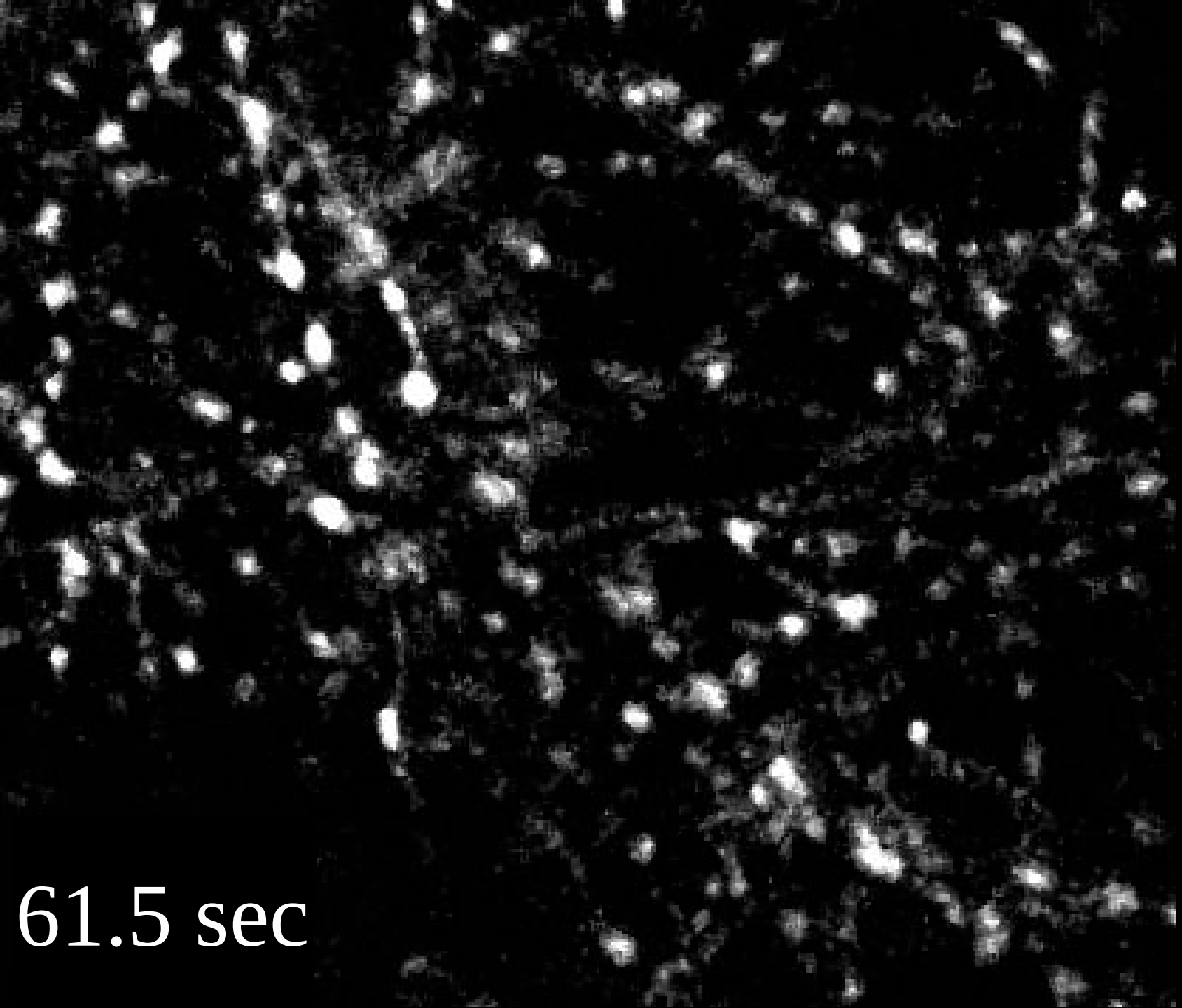
Hasbani et al. 2001

Rapid structural changes during global ischemia.



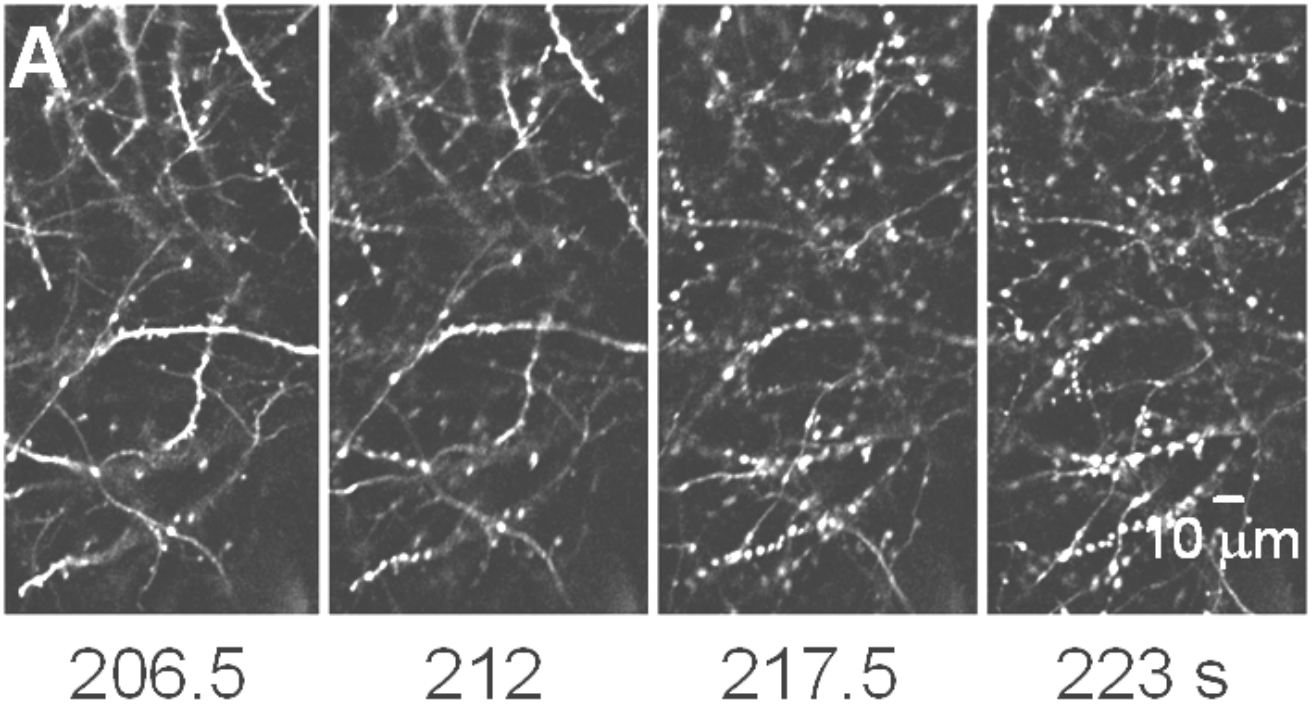

50.5 sec


56 sec

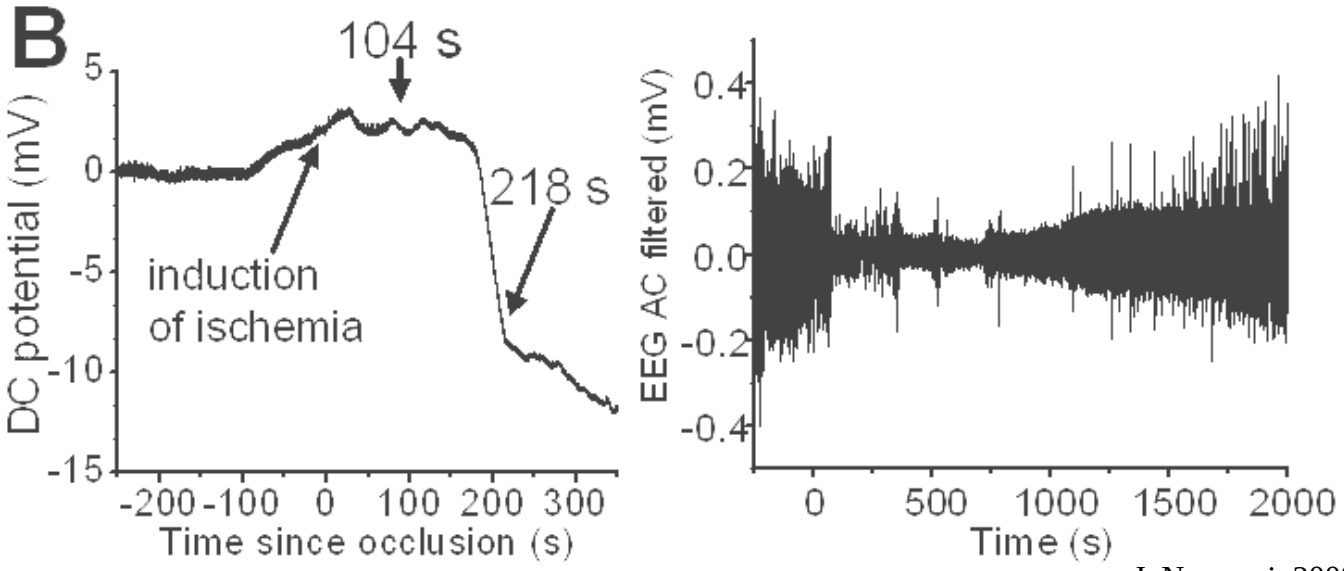

61.5 sec

Changes in structure occur during ischemic depolarization.

Two-photon images.



DC EEG surface.



J. Neurosci. 2008 Murphy et al.

Most spines return after 7 min of global ischemia followed by reperfusion.

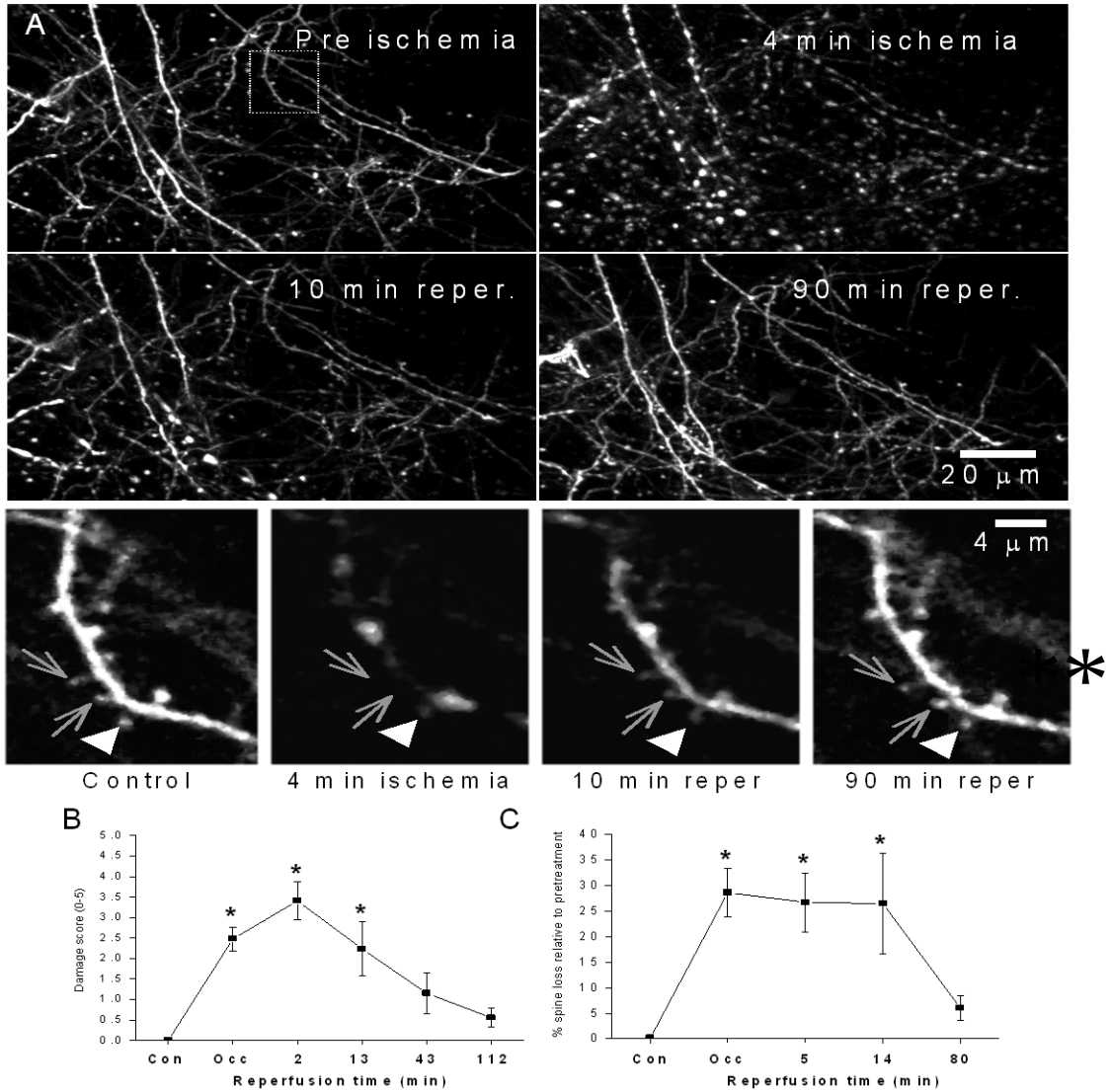
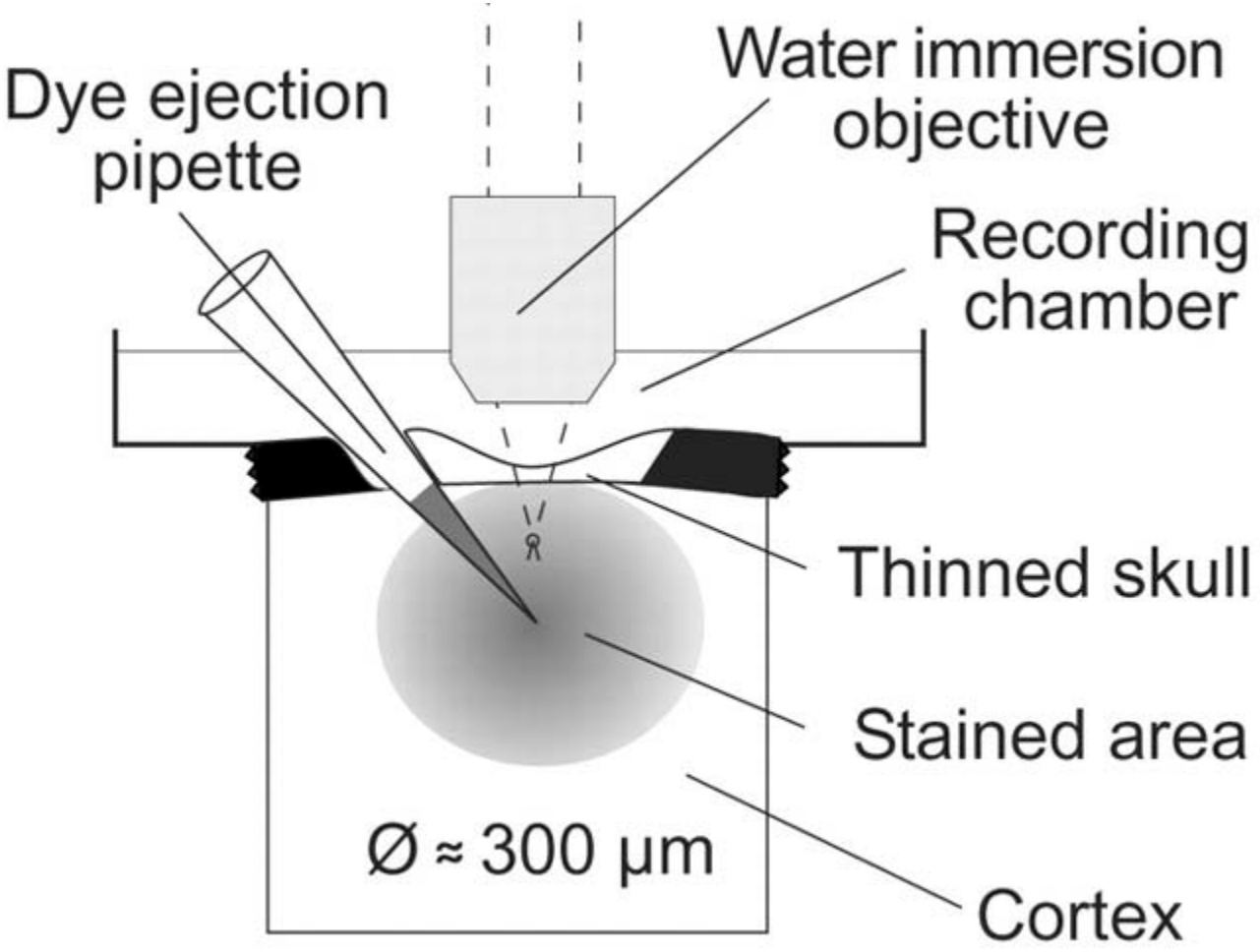


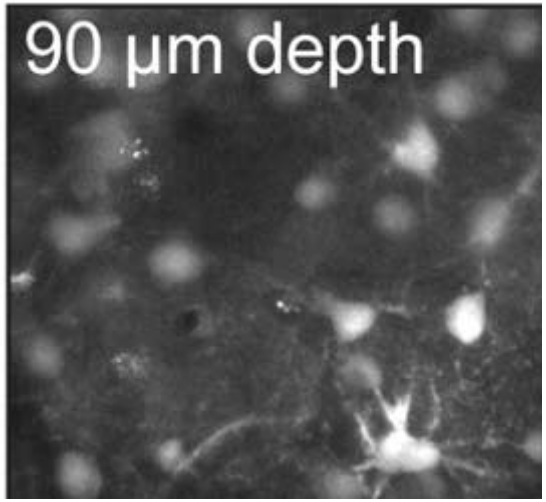
Fig. 2

n=10 animals

Murphy et al. 2008

What happens to $[Ca^{2+}]_i$ during ischemic depolarization?

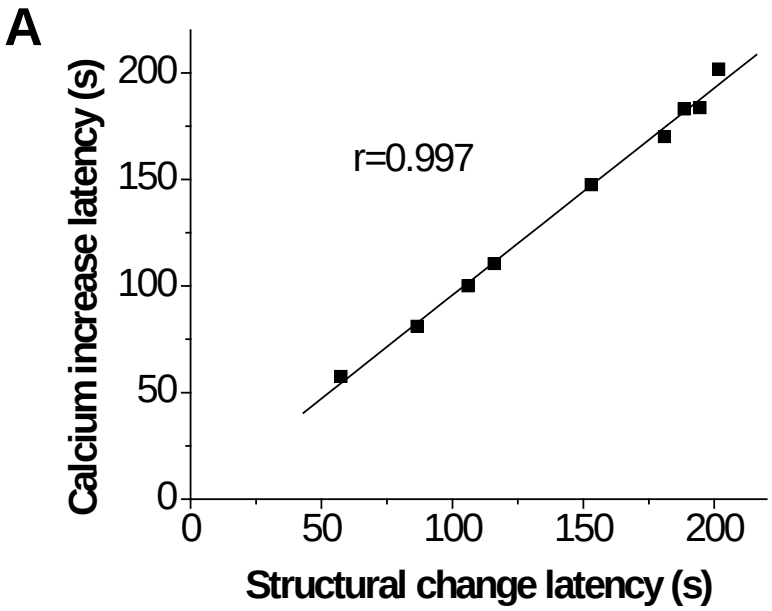




Stosiek et al. 2003

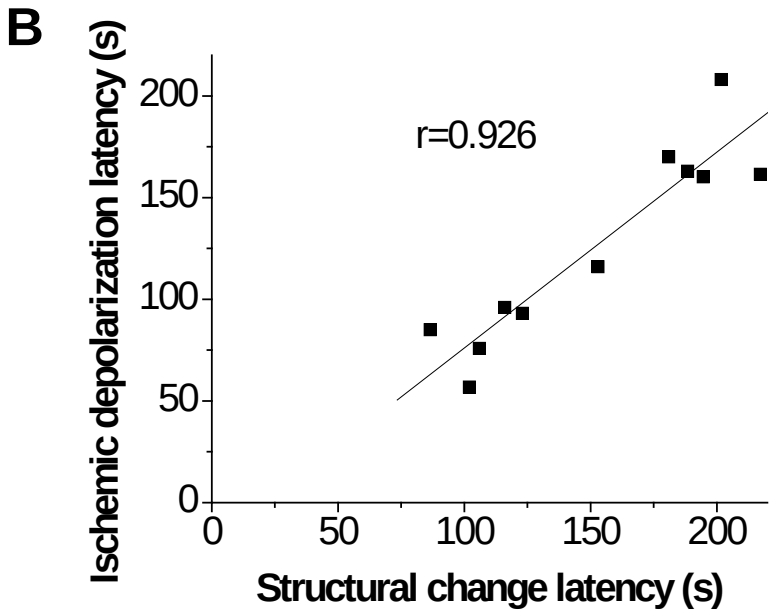
The timing of structural breakdown is correlated with intracellular calcium elevation and ischemic depolarization.

Structure vs $[Ca^{2+}]i$



n=9 animals

Structure vs Depolarization

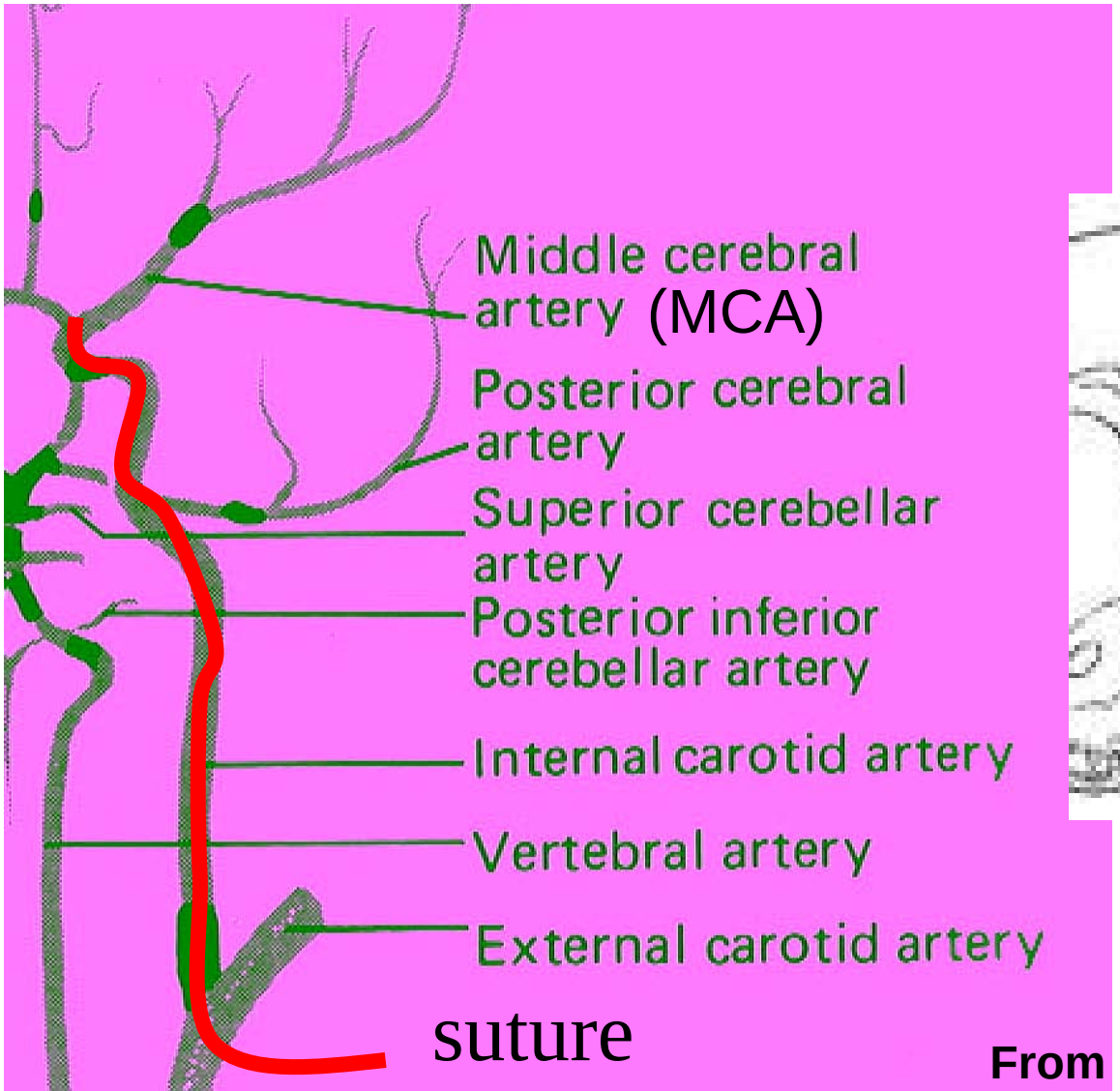


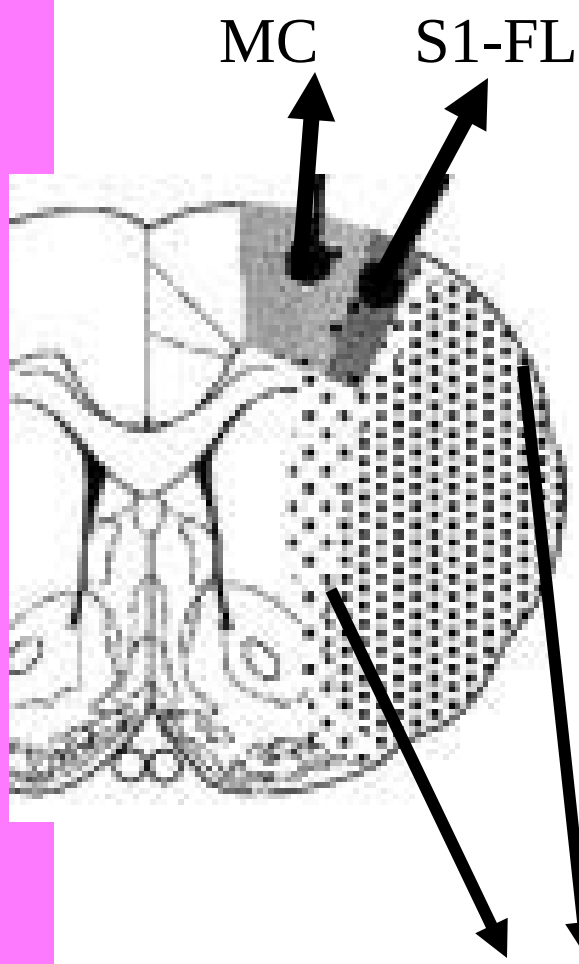
n=11 animals

J. Neurosci. 2008 Murphy et al.

Is reversal of structural damage unique to short-duration global ischemia?

(60 min suture occlusion of the middle cerebral artery)



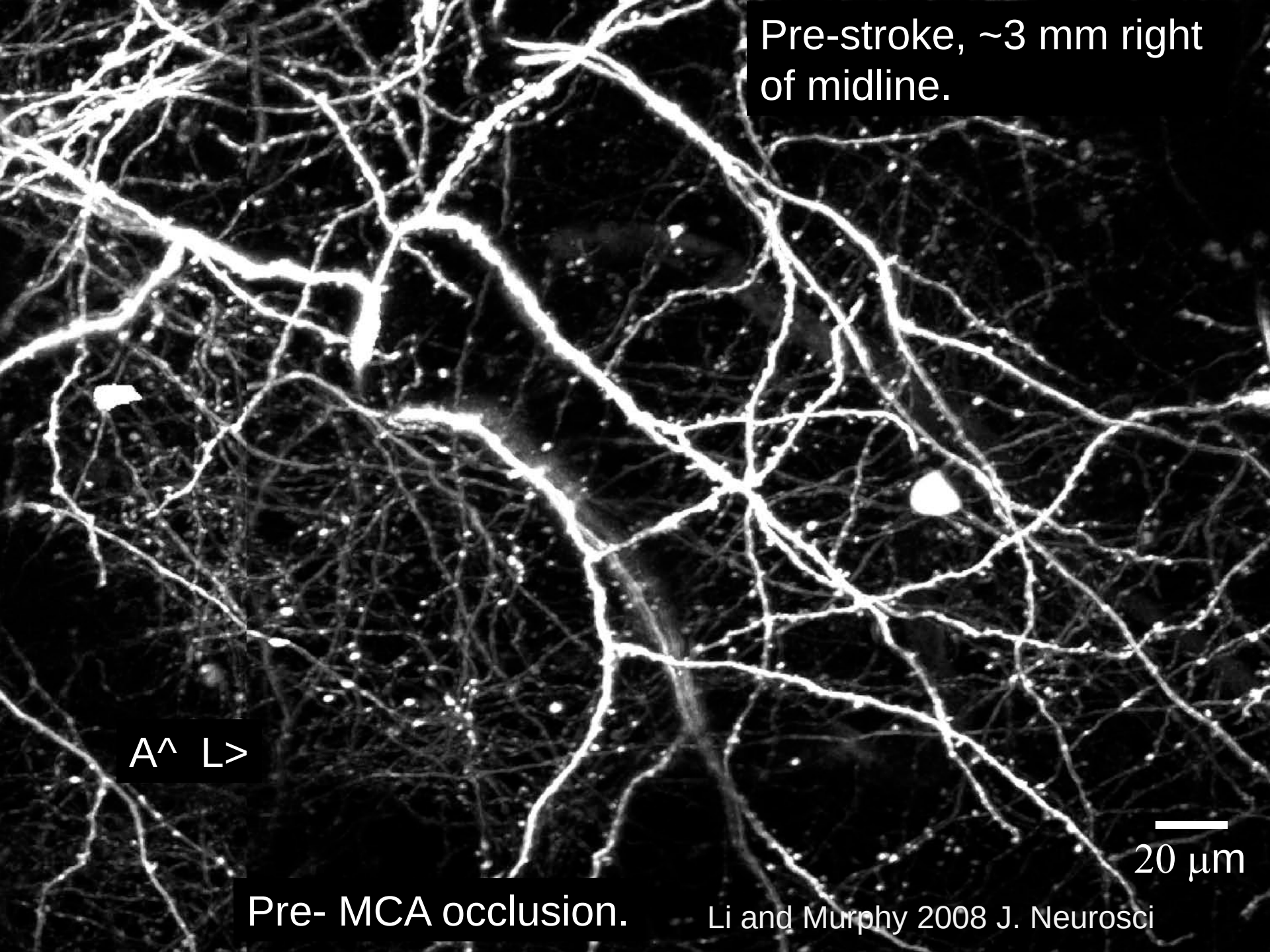


Rat MCA territory

From Dijkhuizen et al. 2001 PNAS

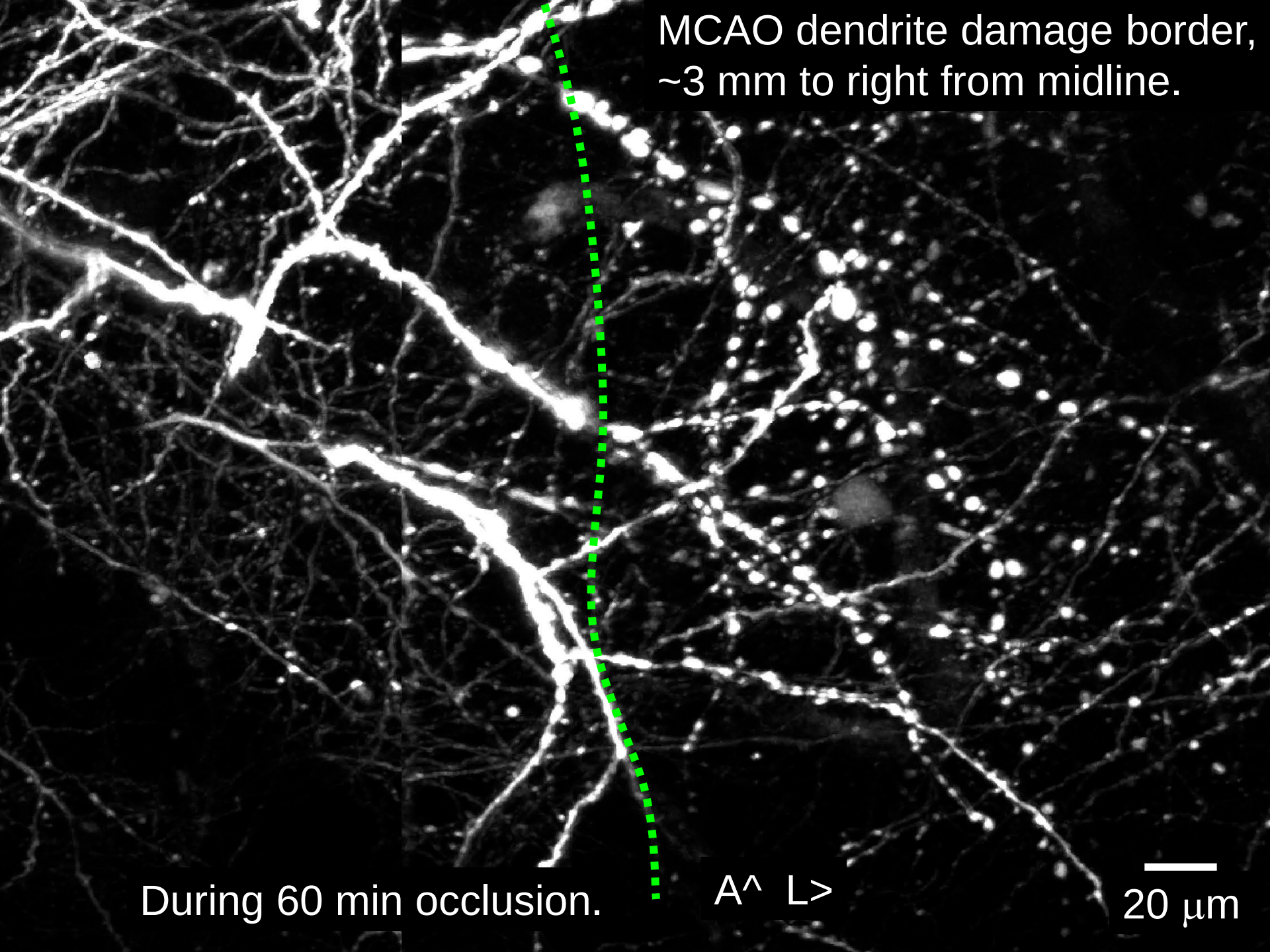
Middle cerebral artery model.

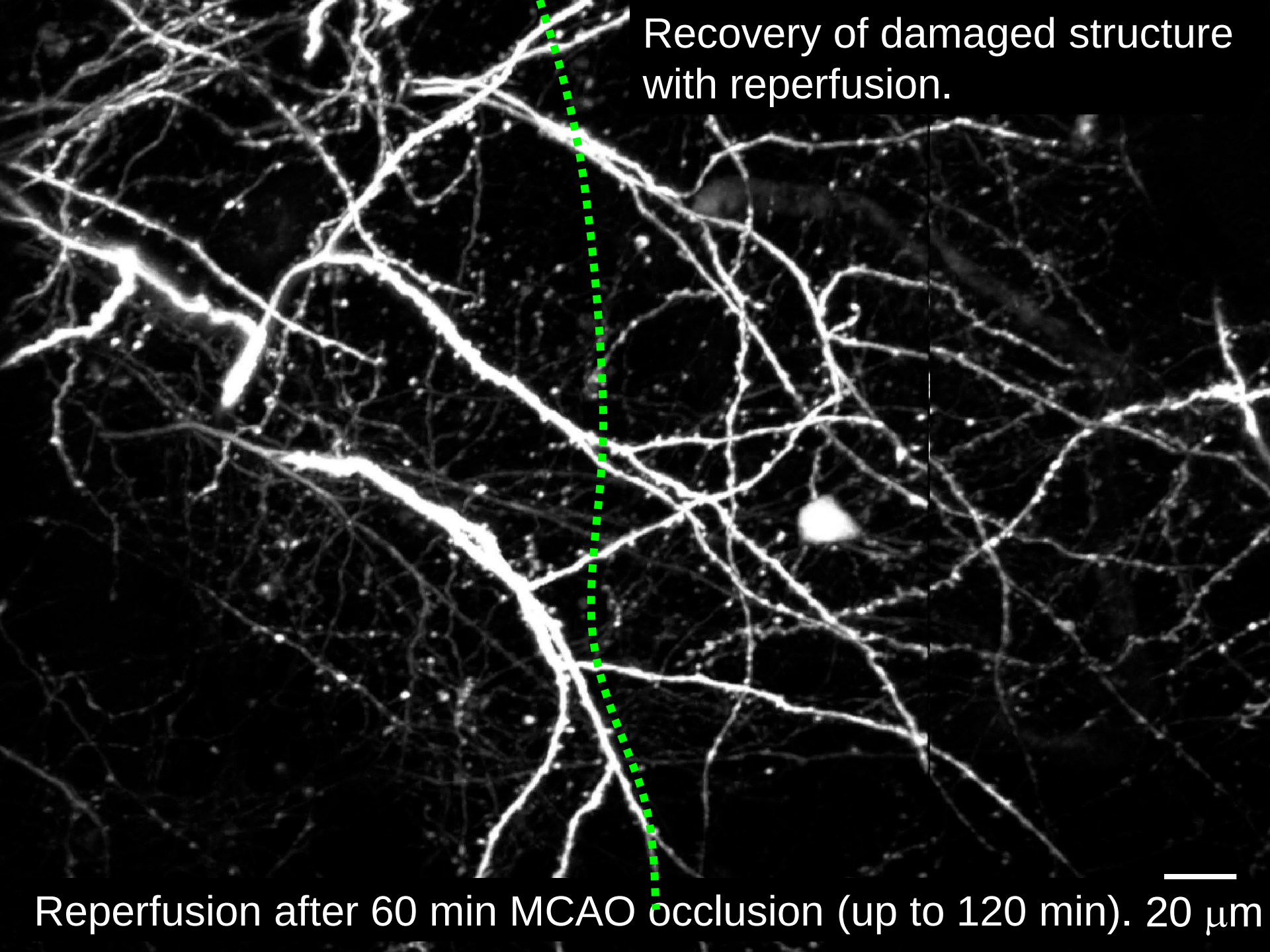
- A focal ischemia model that affects that lateral side of the forebrain and striatum on the side where the artery is blocked.
- Technically demanding in the mouse requires creation of sutures with club like ending that plug the middle cerebral artery without rupturing it.
- Clinically relevant since it results in a large penumbra (area with partial blood flow).
- Mouse usually inverted during insertion and retraction of suture so the animal needs to move in and out of the microscope.



Pre- MCA occlusion.

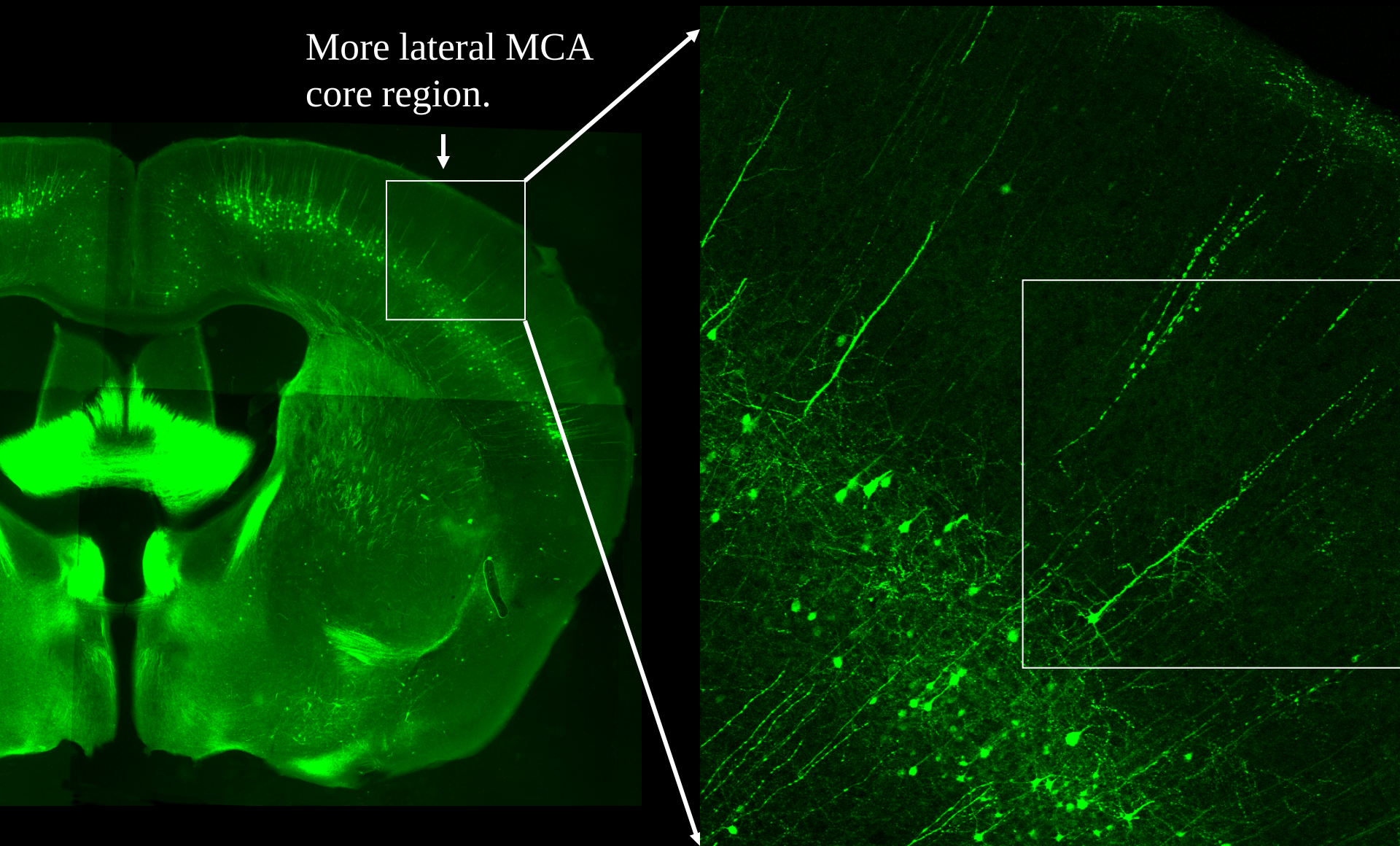
Li and Murphy 2008 J. Neurosci


MCAO dendrite damage border,
~3 mm to right from midline.
During 60 min occlusion.
A^ L>
20 μm

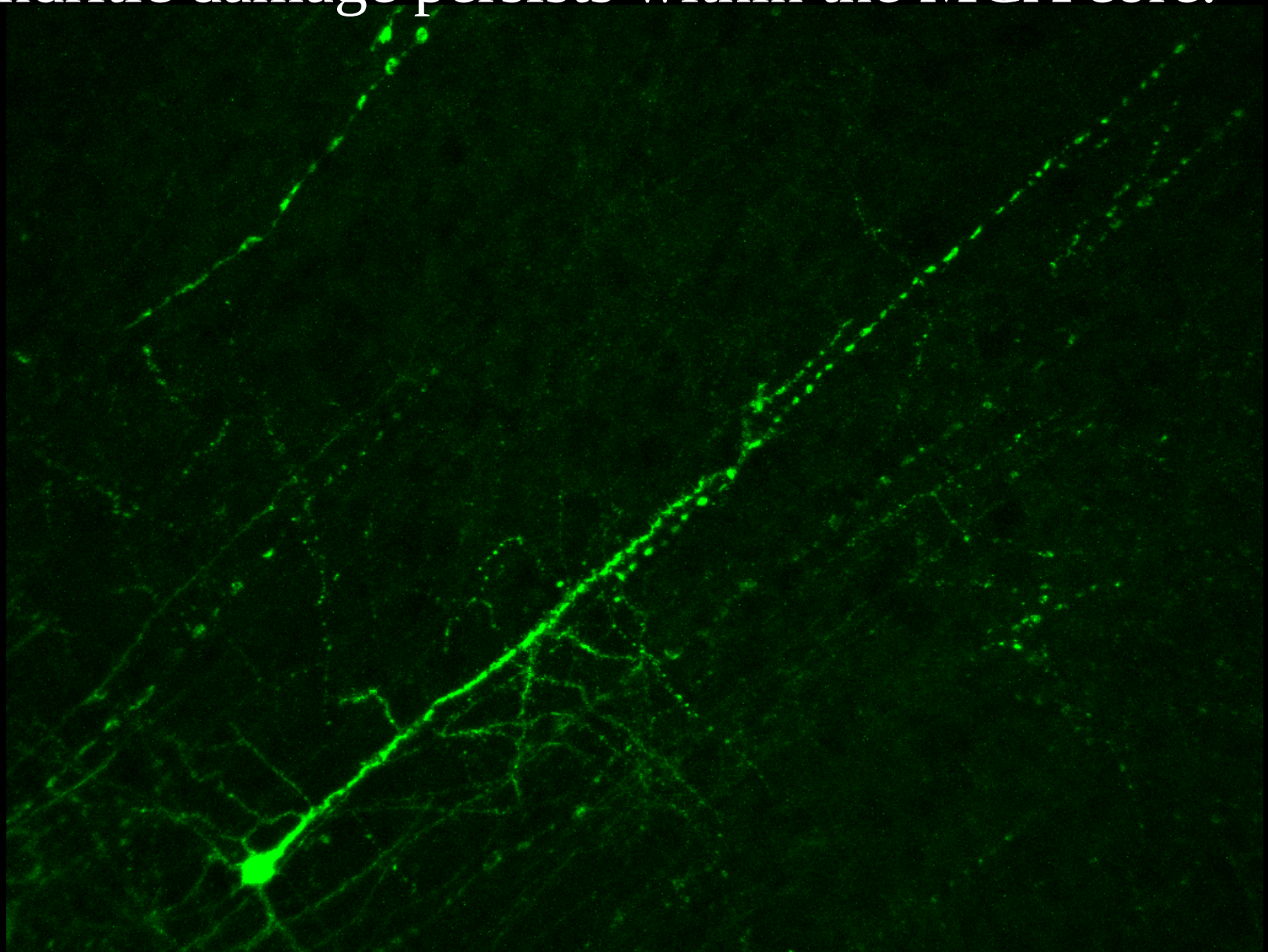


Reperfusion after 60 min MCAO occlusion (up to 120 min). 20 μm

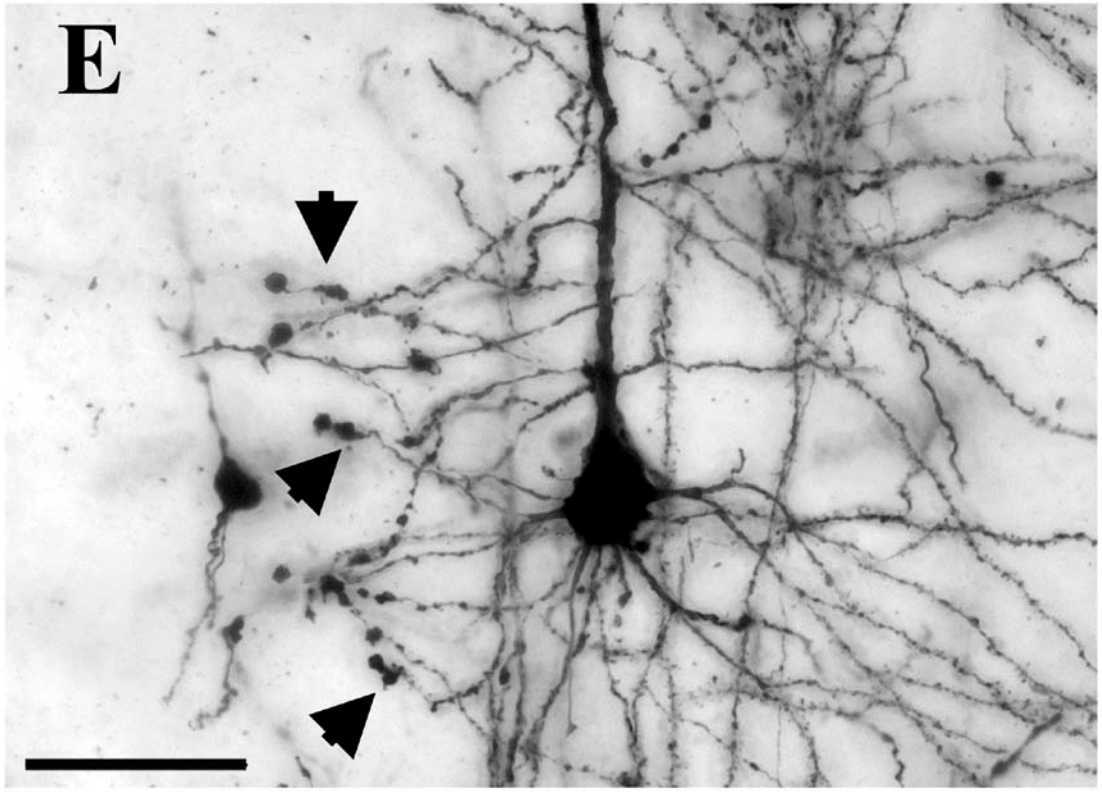
Histology 3 h after stroke induction (60 min) and reperfusion, dendritic damage persists within the MCA core.

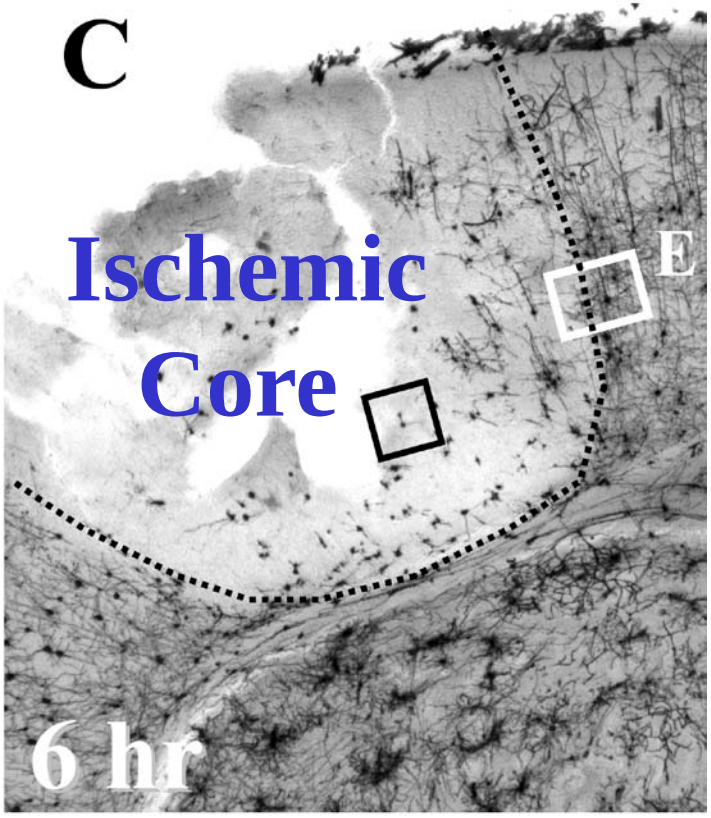


Histology 3 h after stroke induction and reperfusion, dendritic damage persists within the MCA core.



Blebs form locally within single neurons, golgi staining.

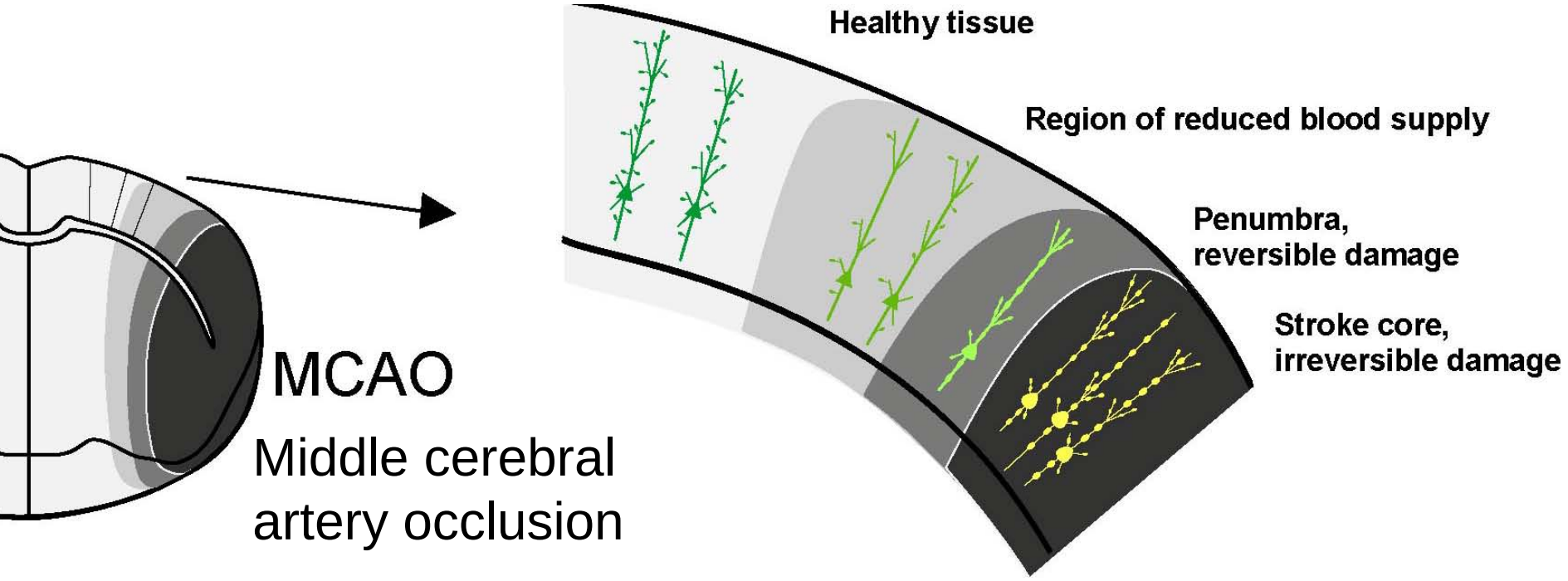




Ischemic ⟷ **Flowing**

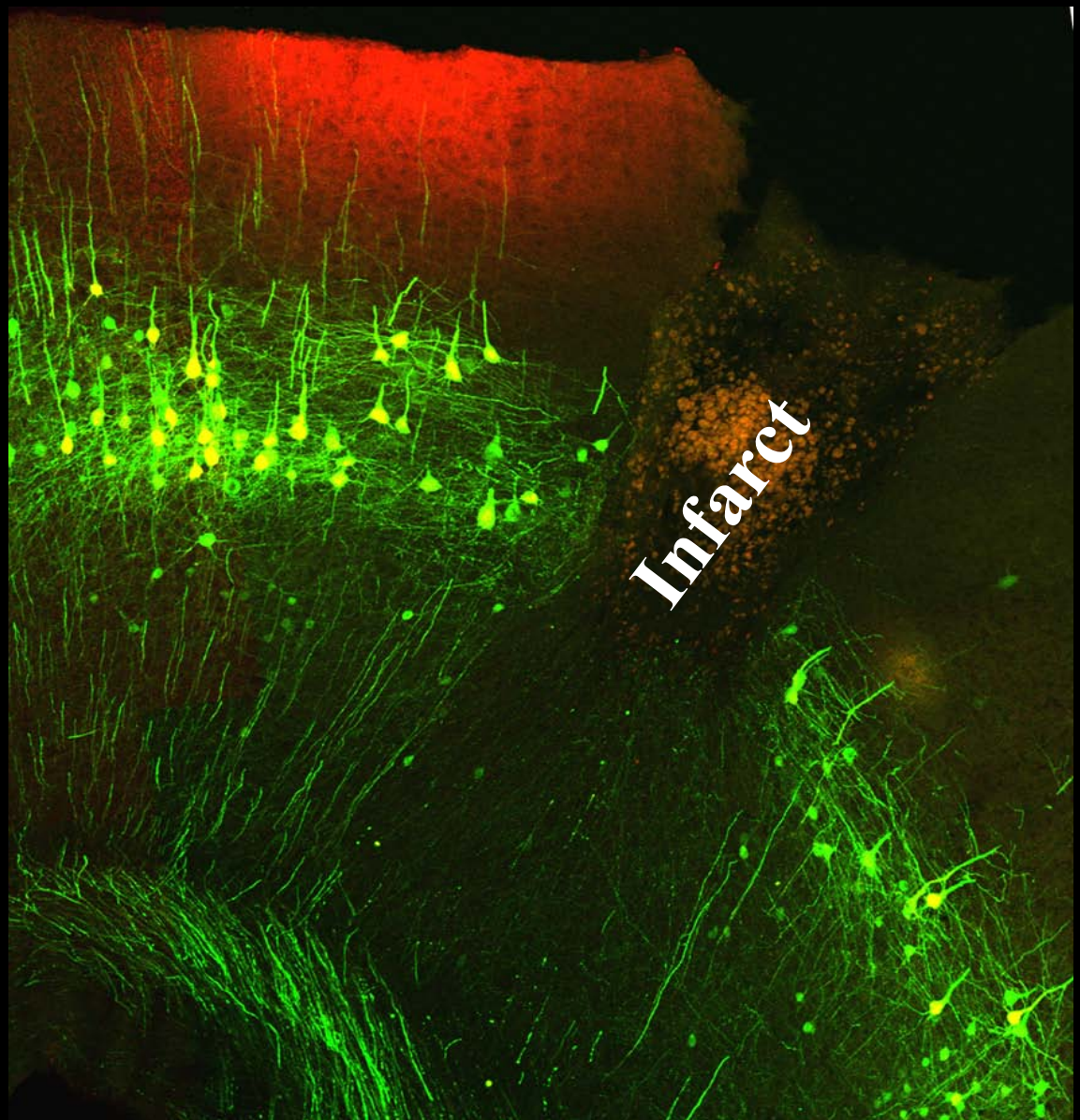
Brown et al. 2007 Stroke

Stroke has rapid effects on synaptic circuitry, some of which can reverse with reperfusion.

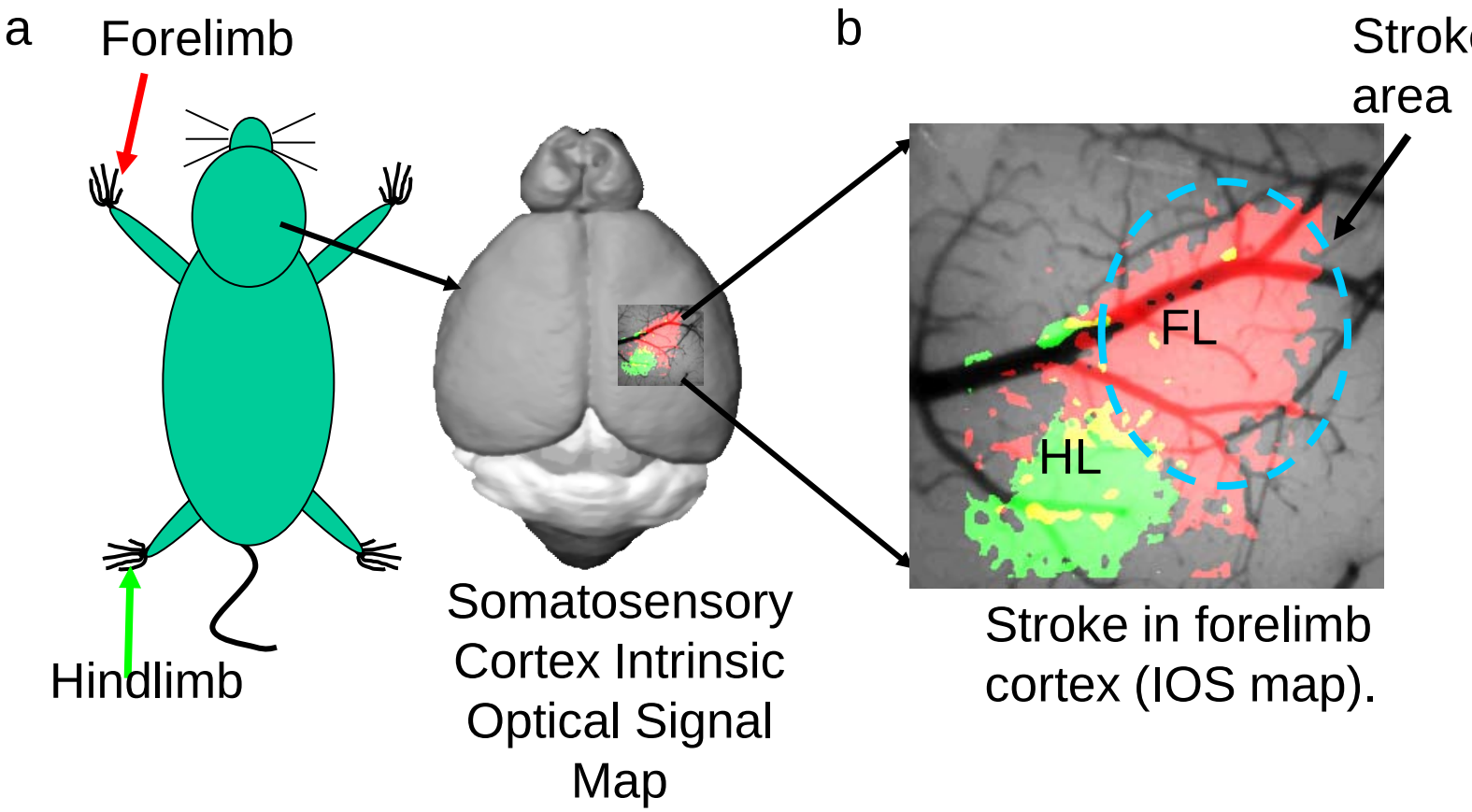


Zhang et al. 2005, 2007
Li et al. 2008
Murphy et al. 2008

How do sensory and motor circuits change weeks after stroke?



Selective reductions in blood flow using photothrombosis allow the study of re-mapping of function after stroke.

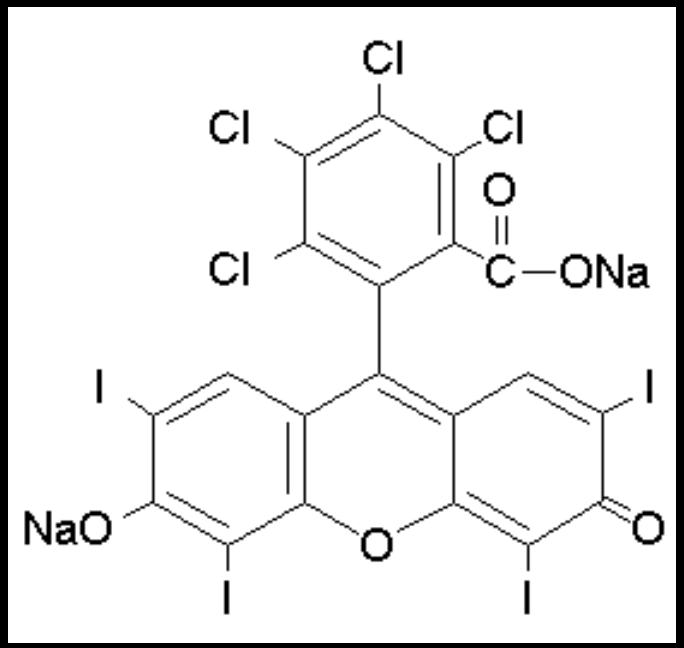


Move limbs at 100 Hz for 1 s using a piezoelectric translator image changes in red scattered light signal.

Zhang and Murphy
PLoS Biol. 2007.

Targeted strokes, produced by photo-activation of Rose Bengal

Watson, B. D., W. D. Dietrich, et al. (1985). Ann Neurol **17**(5): 497-504



Rose bengal injected into the tail vein (30 mg/kg **body weight)**

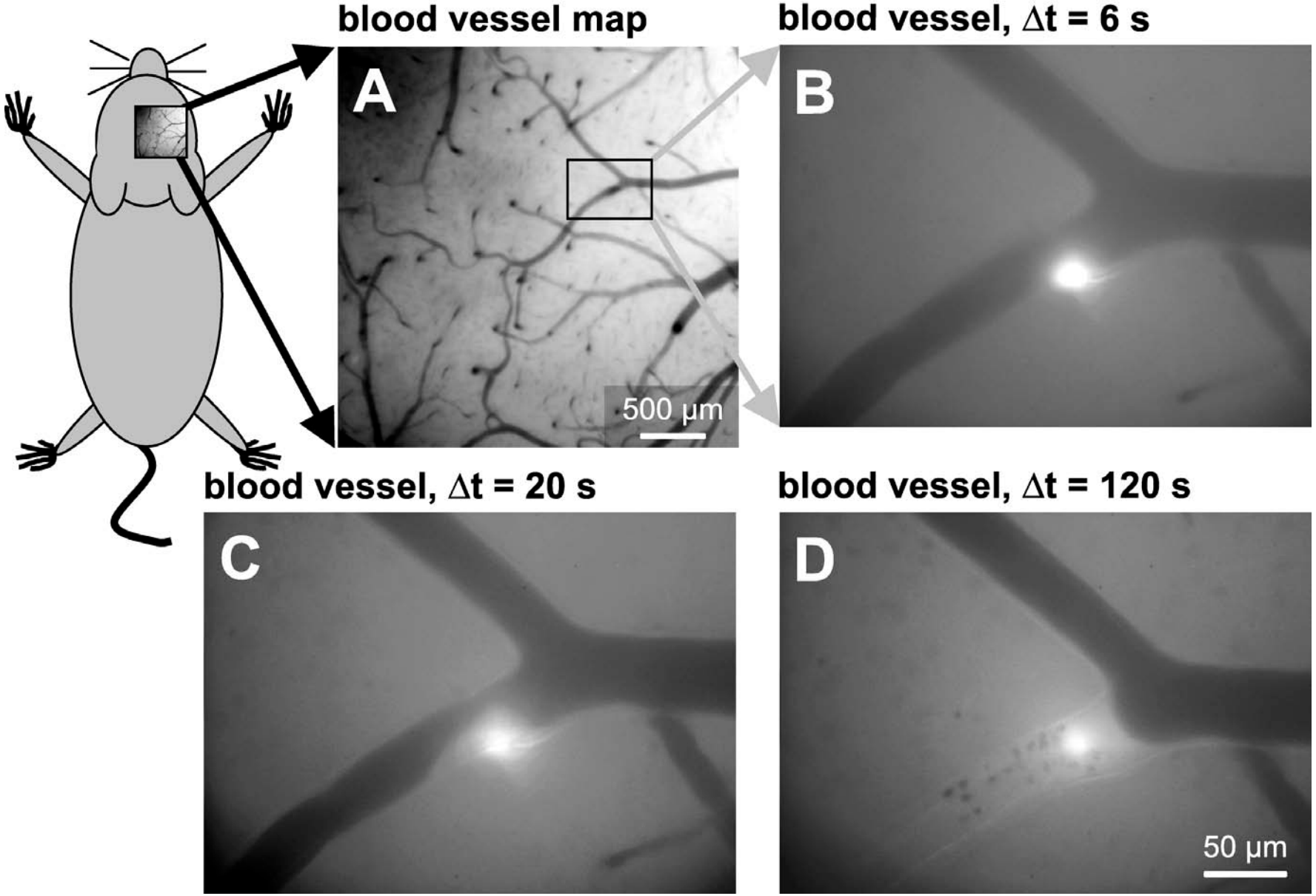
Illumination of the brain with green light (535/50) for 1 –3 min.

Reactive singlet O_2

Irritation of endothelial cells lining vessel walls, leading to platelet aggregation and fibrin-mediated coagulation cascades.

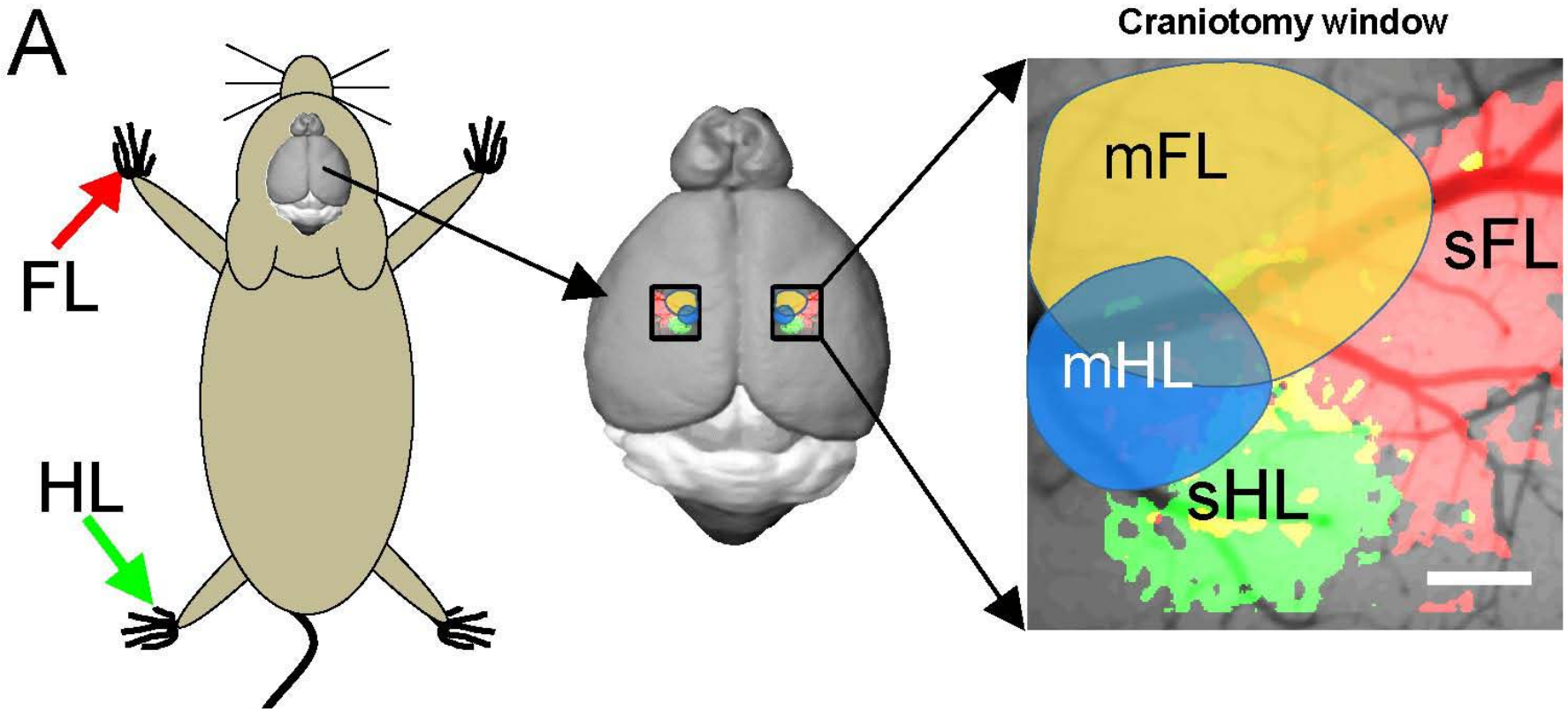
photothrombotic ischemia

Targeting individual brain arterioles for photoactivation, CCD camera imaging shown.



Sigler et al. J. of Neurosci Meth 2008

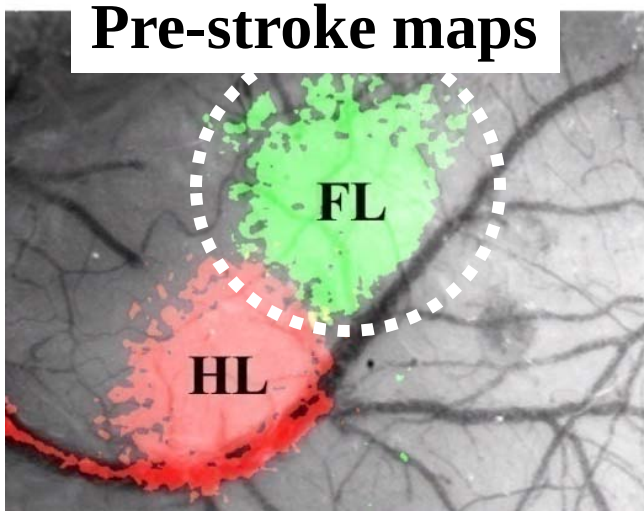
Nearby regions with similar function aid remapping of stroke-affected tissues.

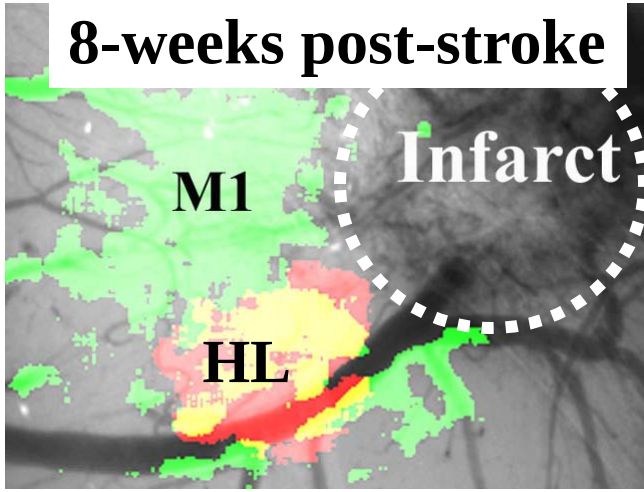


Sensory Forelimb (sFL), Motor Forelimb (mFL)
Sensory Hindlimb (sHL), Motor Hindlimb (mHL)

Factors that contribute to re-mapping of function in adult animals after stroke.

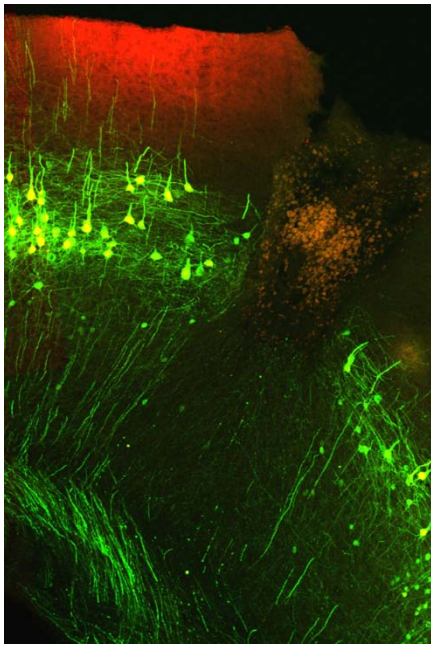
- Circuit redundancy, most areas are connected (EG Jones/ M. Merzenich).
- Adult sensory plasticity deafferentation experiments (Merzenich).
- Structural plasticity in the adult through axonal sprouting and spinogenesis. (Svoboda, Gan, others).
- Stroke leads to a permissive environment for plasticity (Carmichael, Cramer, Kleim, T. Jones).



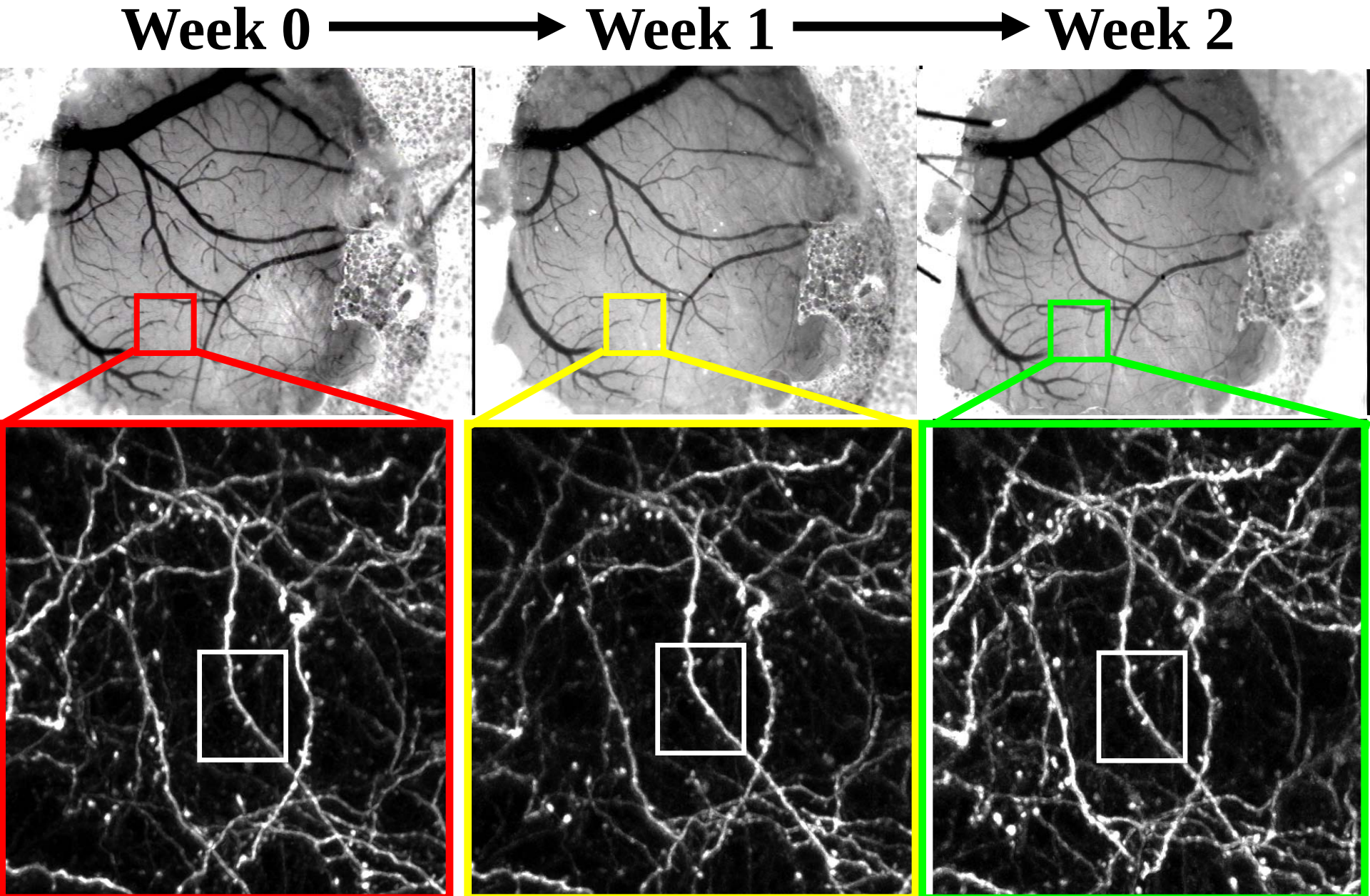


Replacement of synapses that are lost acutely after stroke?

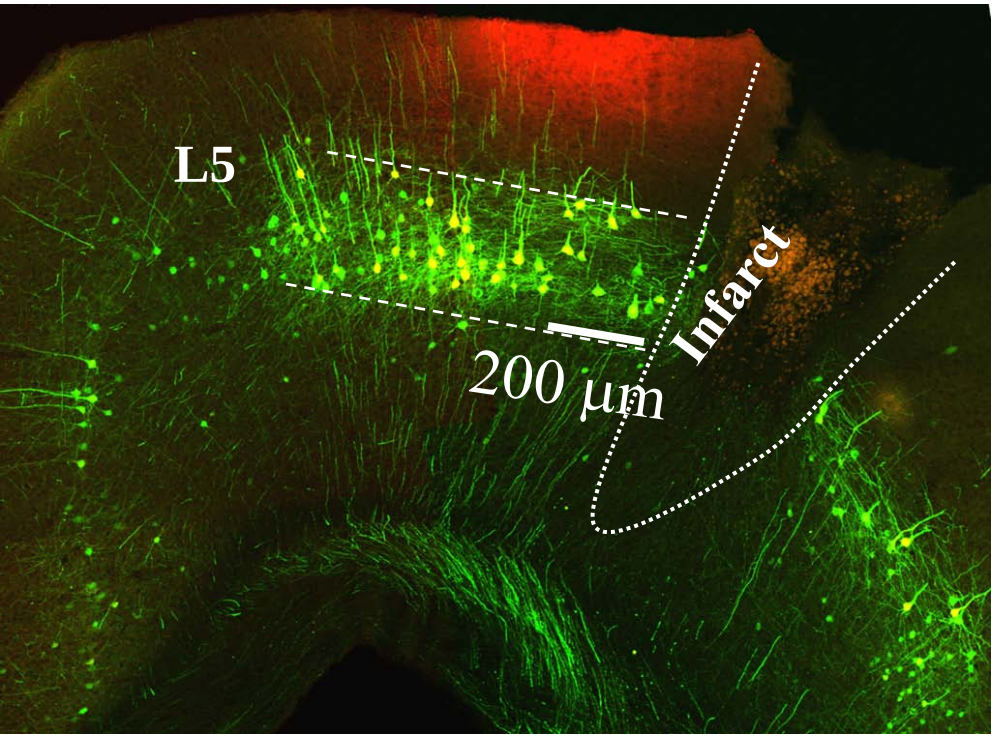
- Predicted that neurons will add more synapses based on homeostatic plasticity (synaptic scaling).
- Sites of synapse production and re-wiring are likely close to damaged areas to make to best use of remaining circuits and to take advantage of stroke-associated plastic factors.



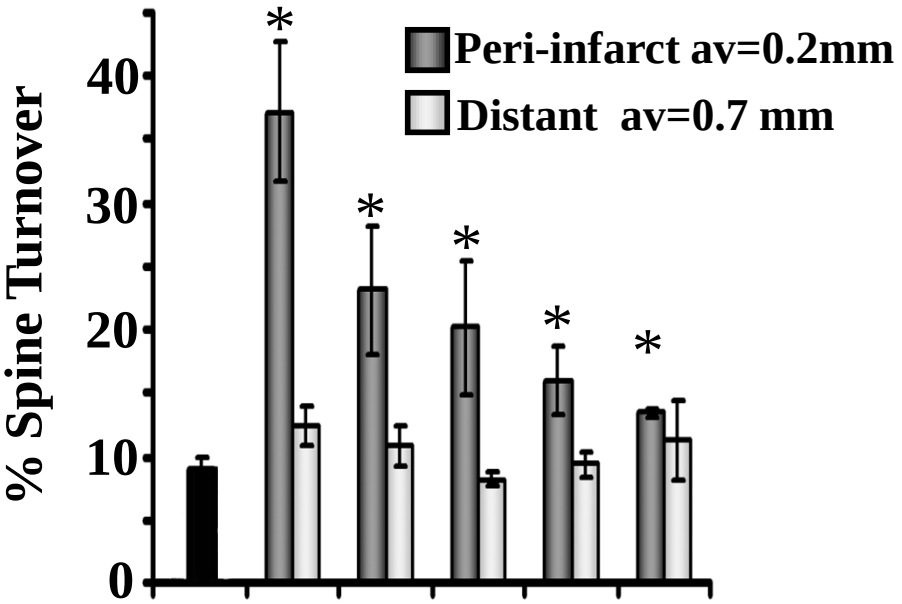
Chronic *In Vivo* 2-photon Imaging of Cortical Dendrites



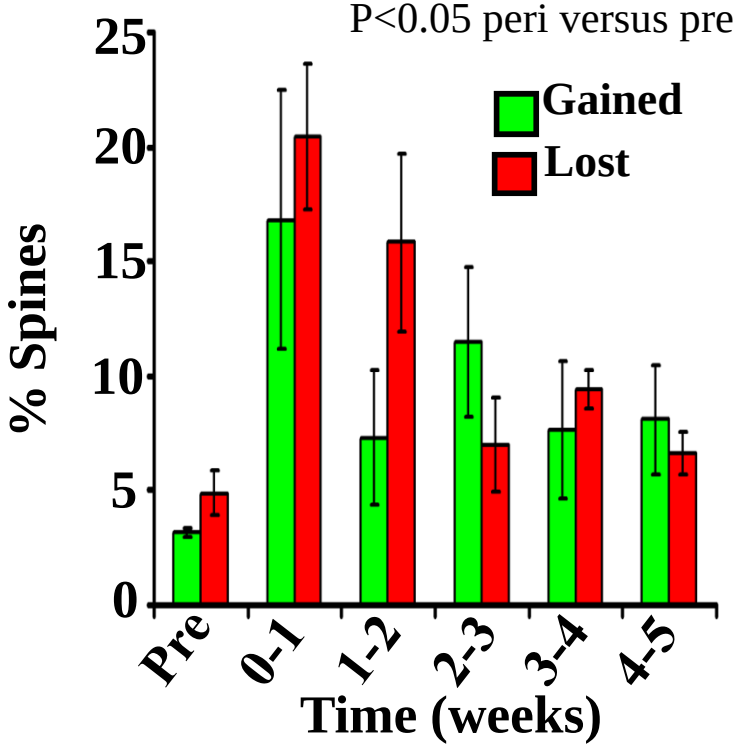
Increased dendritic spine turnover after stroke, weekly time points, greatest in peri-infarct zone.



Brown et al. 2009



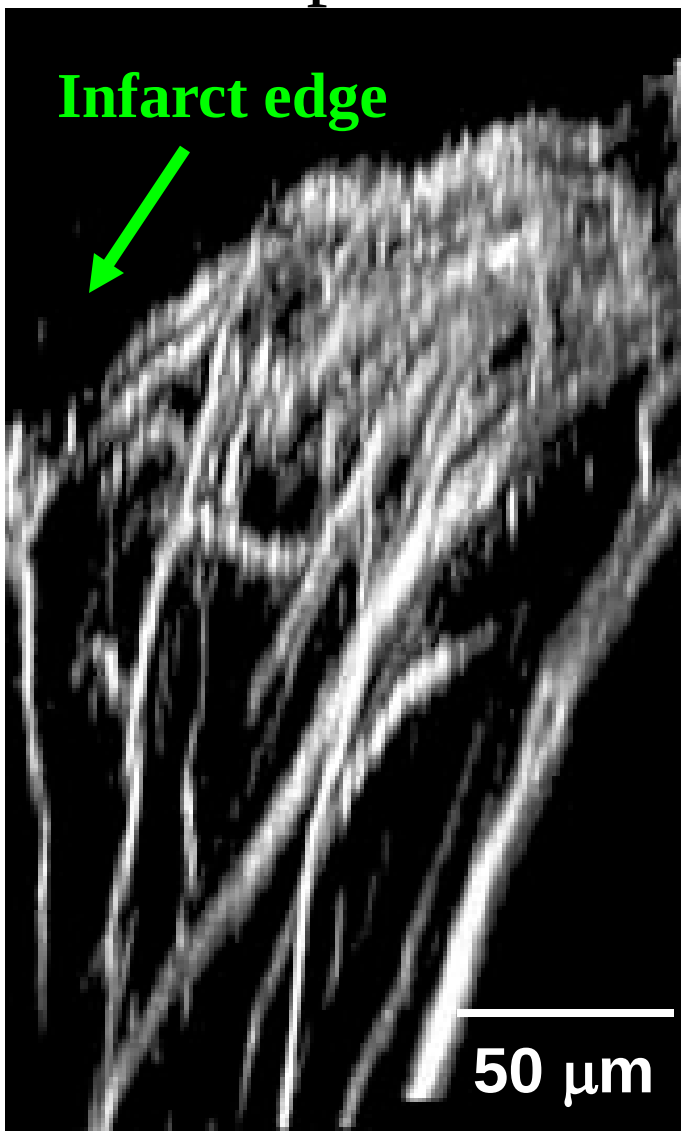
P<0.05 peri versus pre

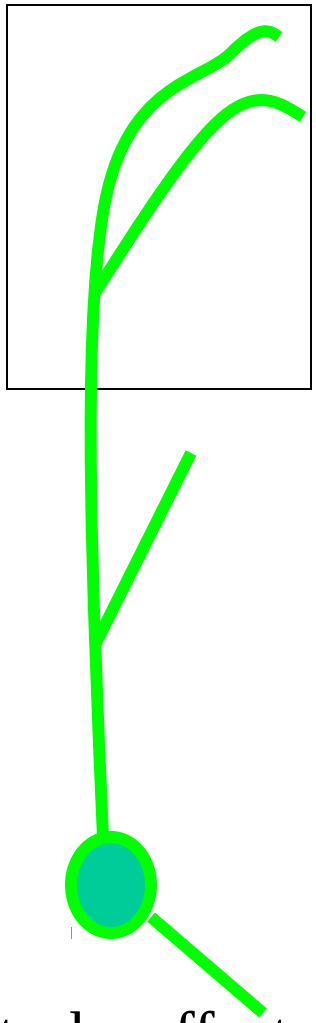


Pruning of peri-infarct dendrites after stroke.

sham

6 weeks post stroke

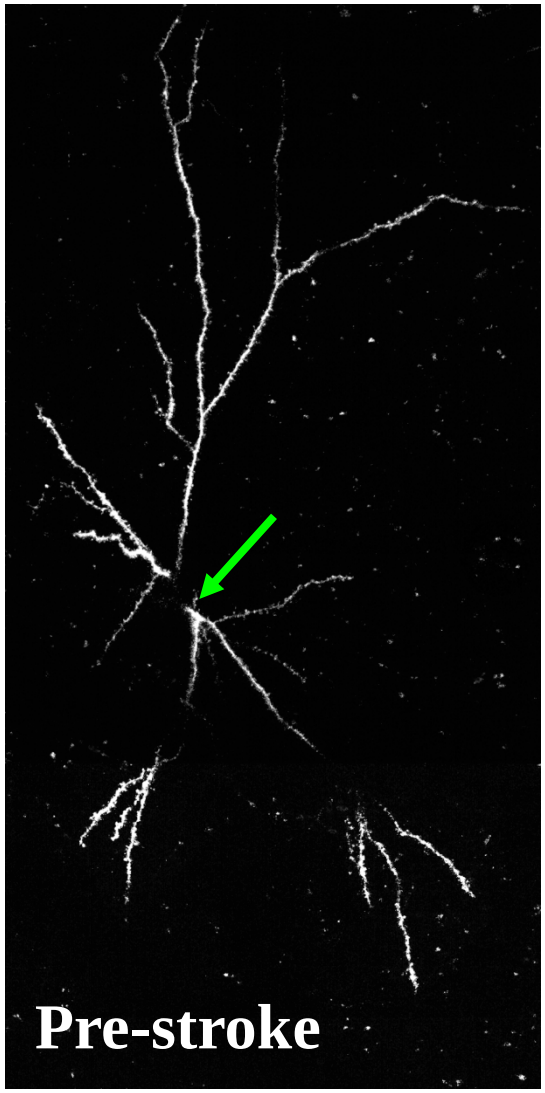


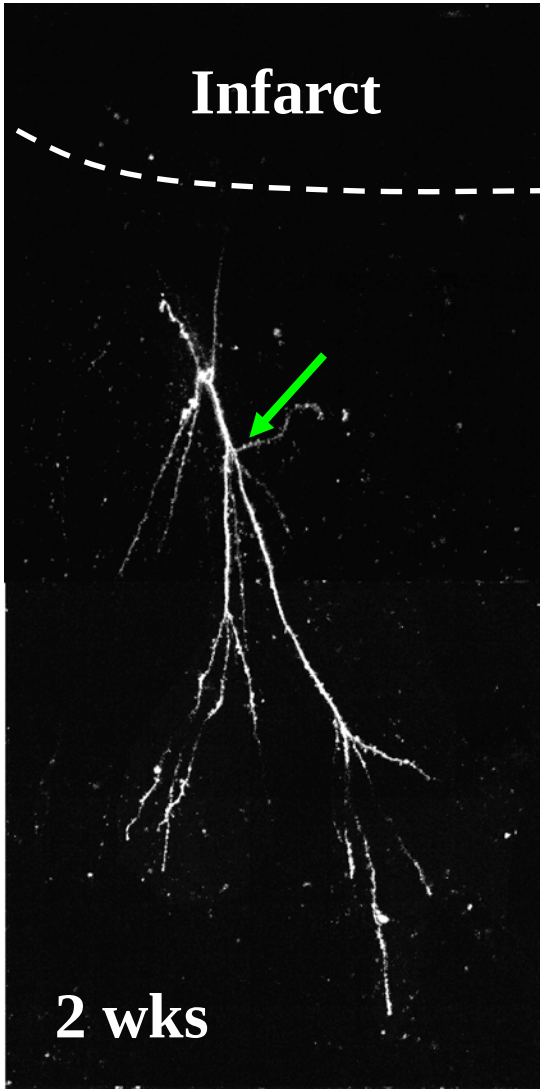


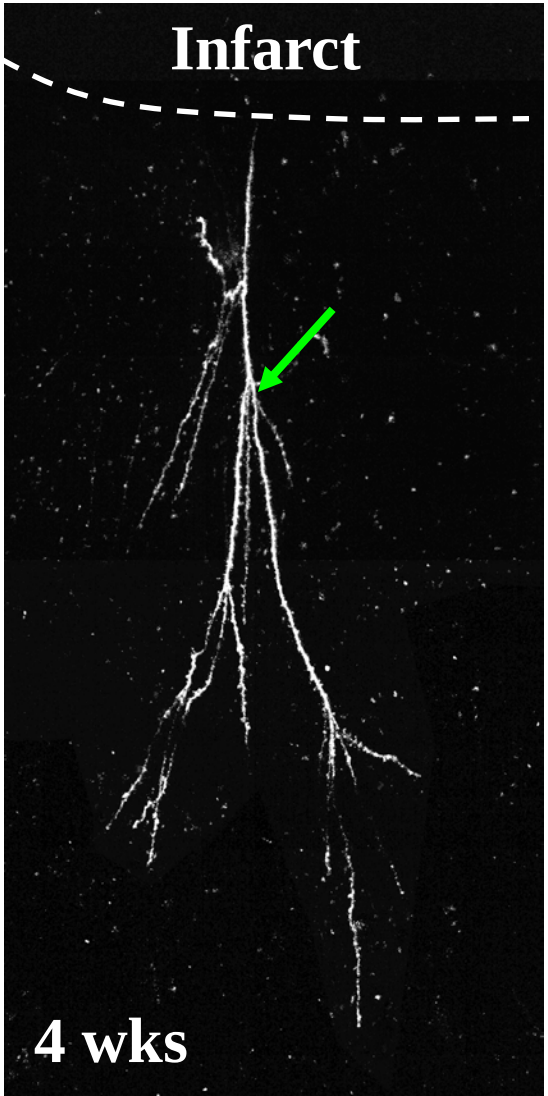
Stroke affected neuron.

Brown et al. 2007 J. Neurosci.

Neuron with severed dendrites survives for weeks at stroke border.

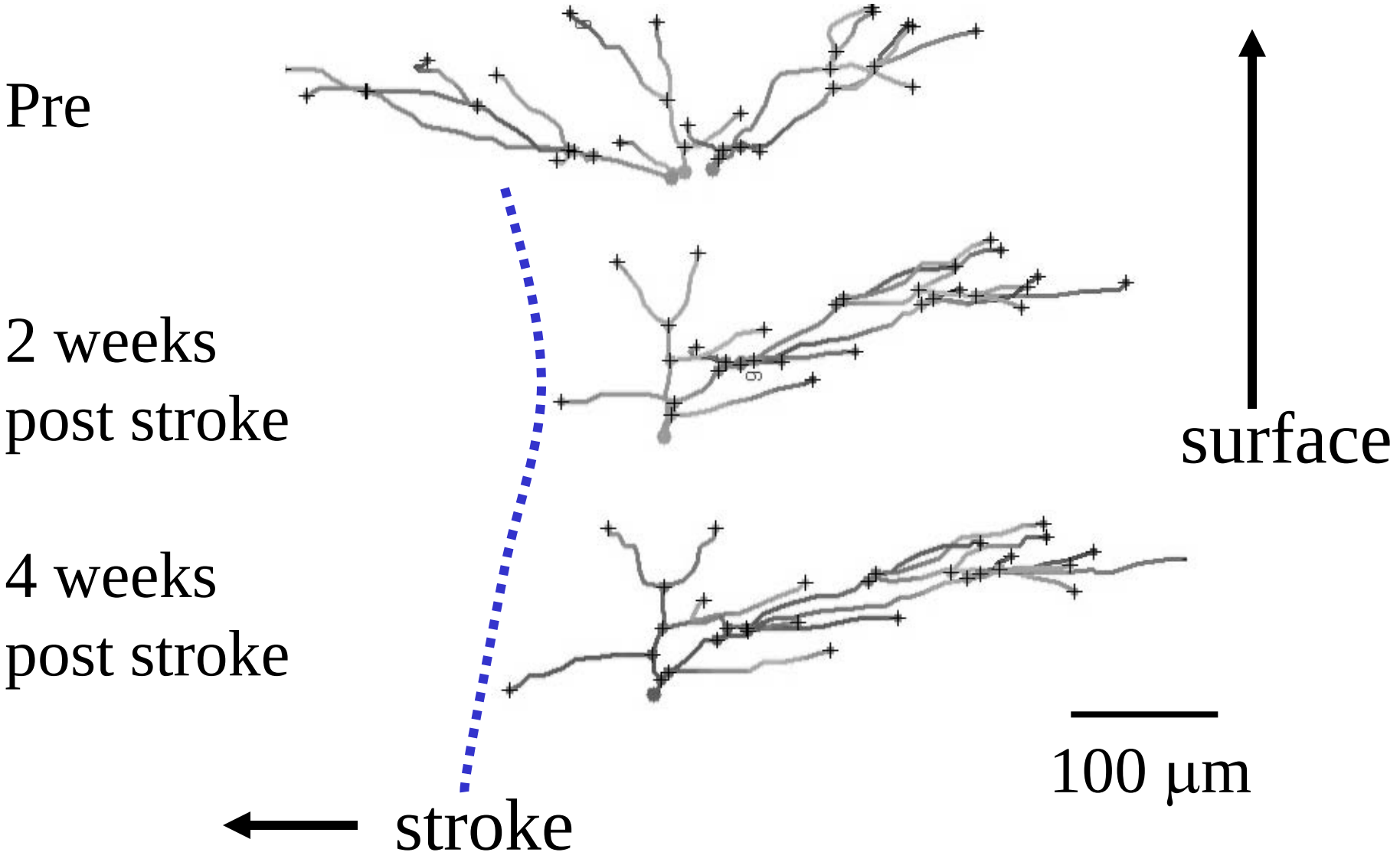




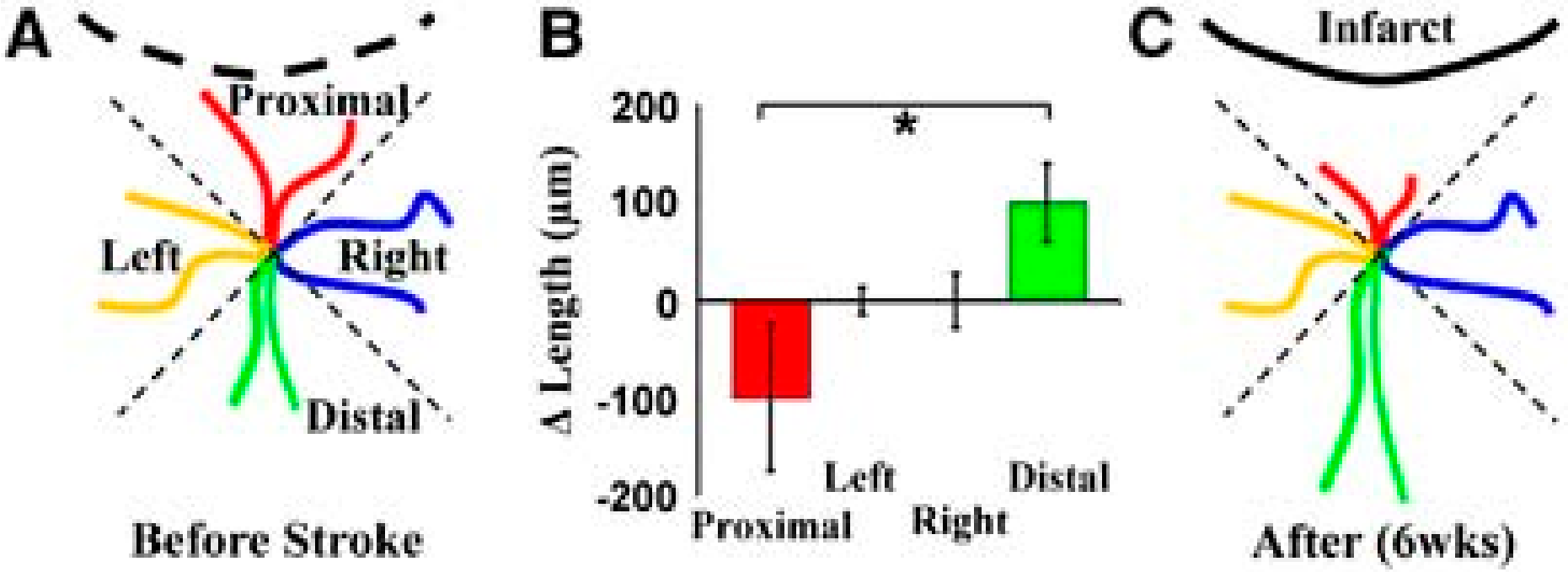


Brown et al. submitted 2009

Side view tuft dendrite arbors before and weeks after stroke (layer II neuron).

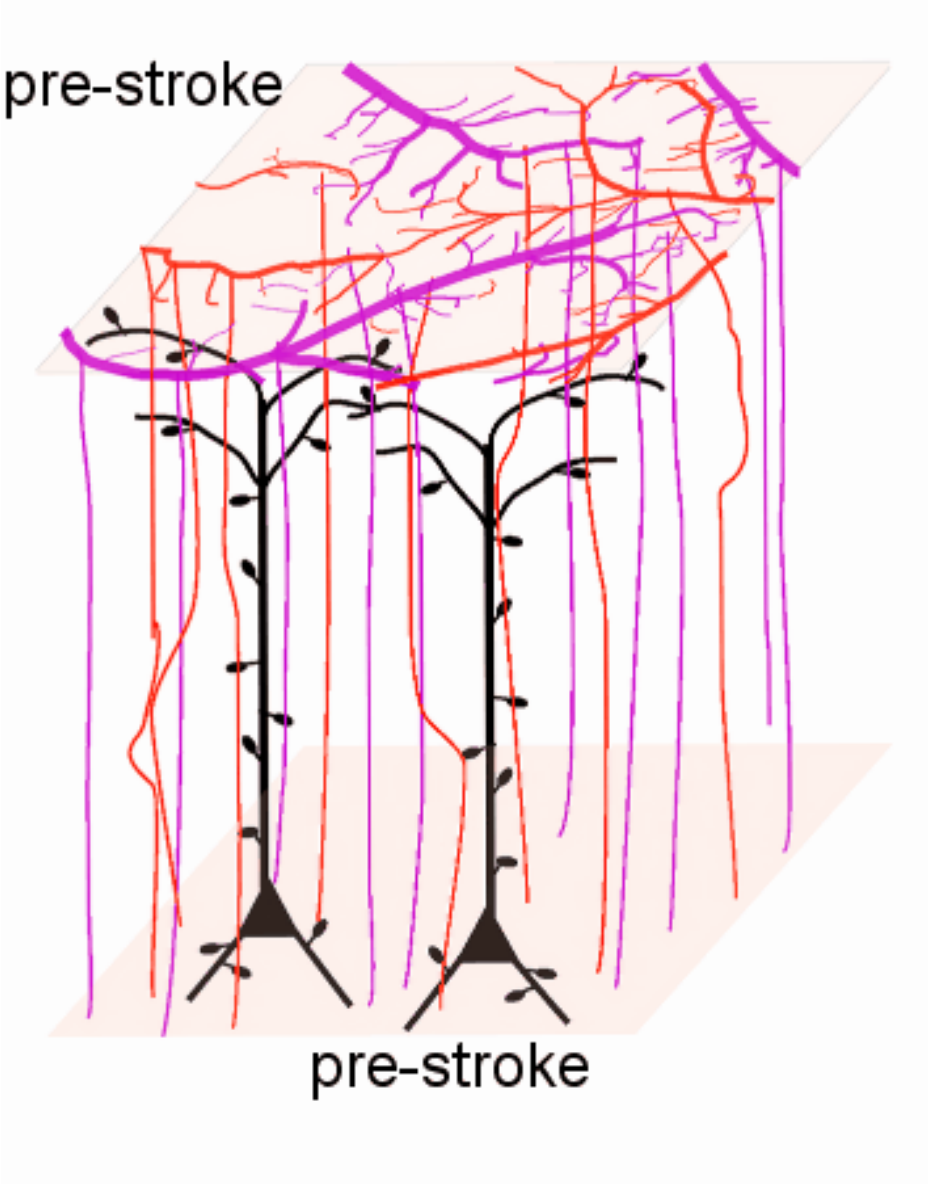


Peri-infarct dendrites that project towards the infarct retract and those away grow, however no new branches were found up to 6 weeks post.



Data from n=6 neurons, 1 L-2, 4 L-5, and 1 L-6

Neurons near
the stroke core can
lose parts of their
dendrites (within min),
but can survive
for weeks and
undergo structural
plasticity at
remaining sites.

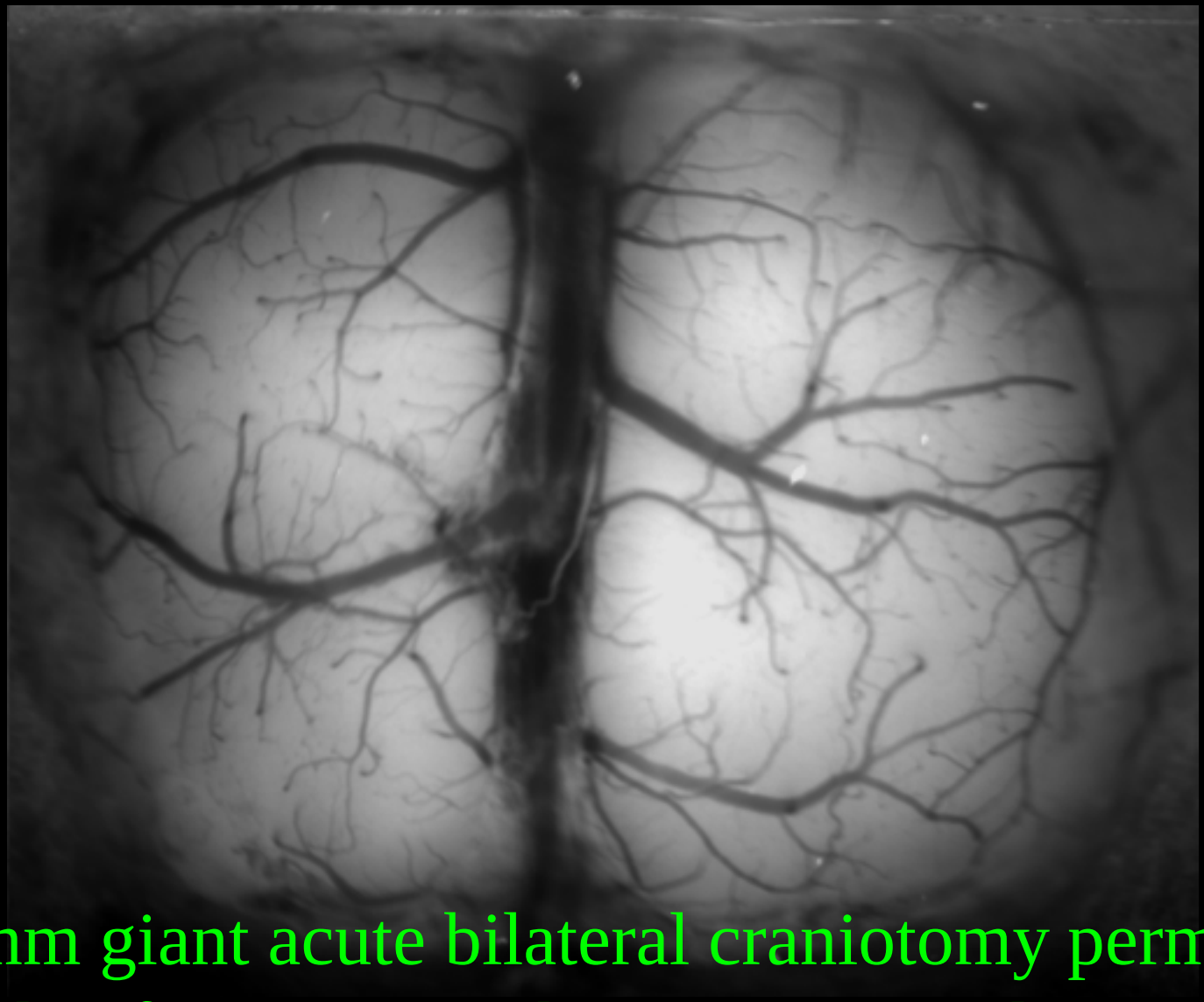


Do new structural circuits that form over weeks support new patterns of function?

Tracking post-stroke circuits using voltage sensitive dye (VSD) imaging. Allows direct visualization of changes in membrane potential in cortical areas with millisecond resolution using fluorescent probes such as RH-1692 developed by Grinvald. IOS imaging is potentially an indirect measure of sensory evoked activity.

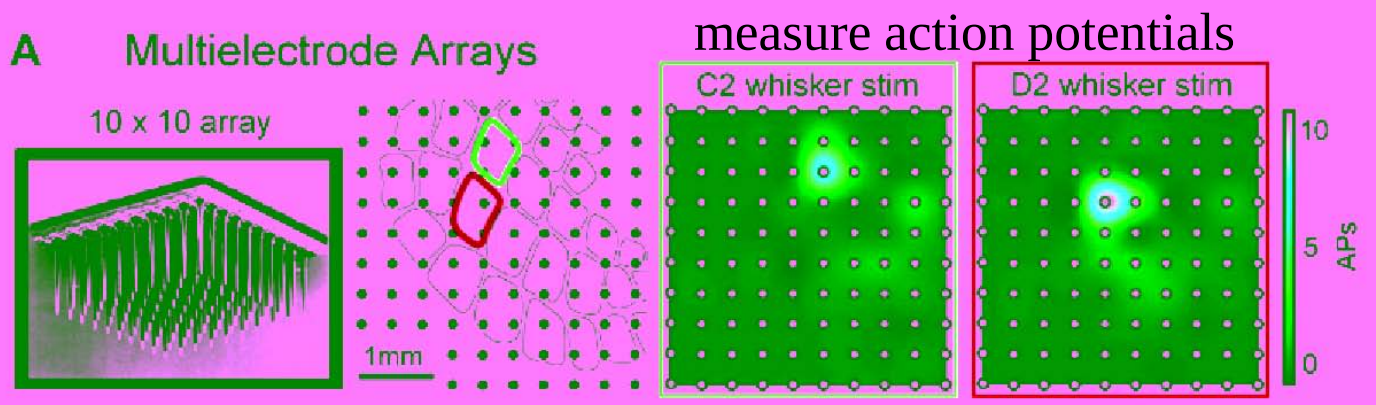
- Used on in vivo preparations lacking dura. Signals tend to be small (0.2% change or less), but are resolved with large numbers of photons and stable LED-based excitation.

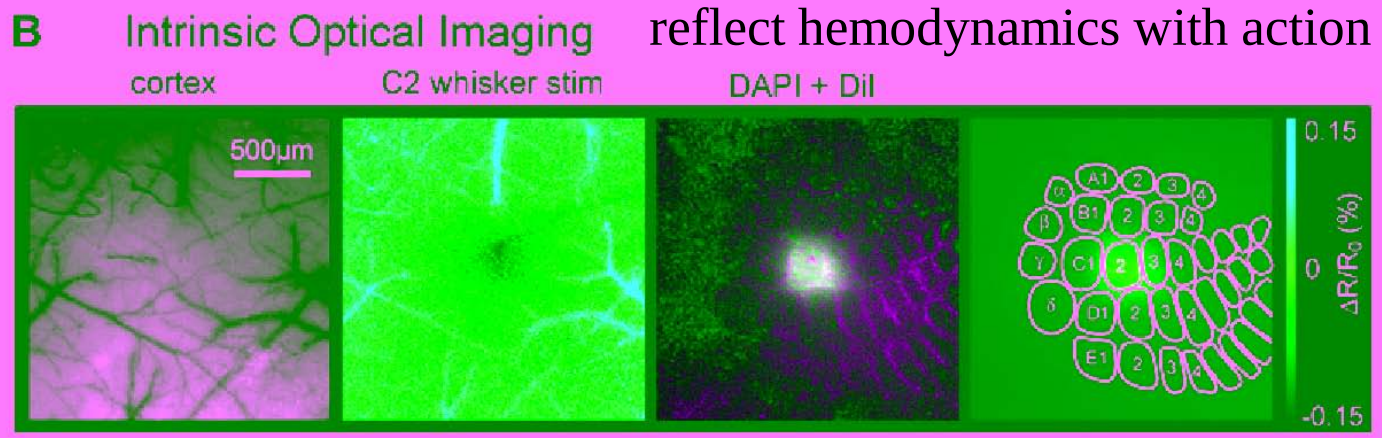
Need techniques to assess function over millimeter scales with millisecond resolution.

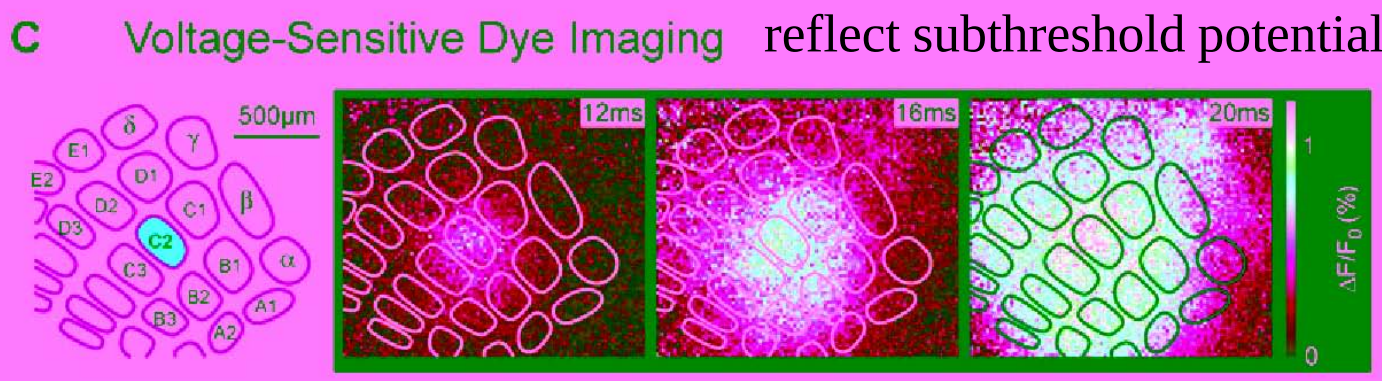


7 mm giant acute bilateral craniotomy permits study of interactions between cortical regions.

Assays for assessment of sensory evoked activity in vivo.

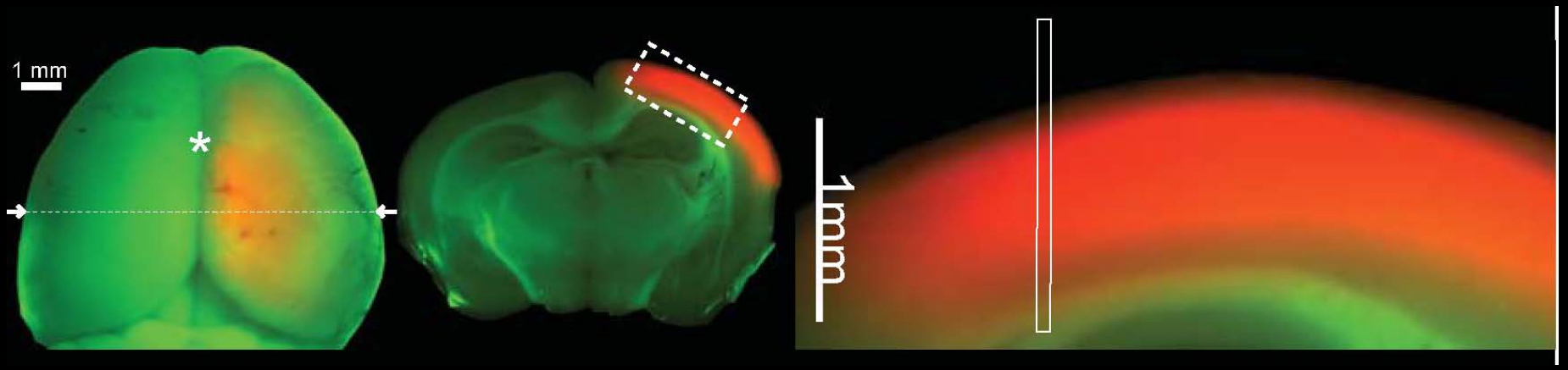




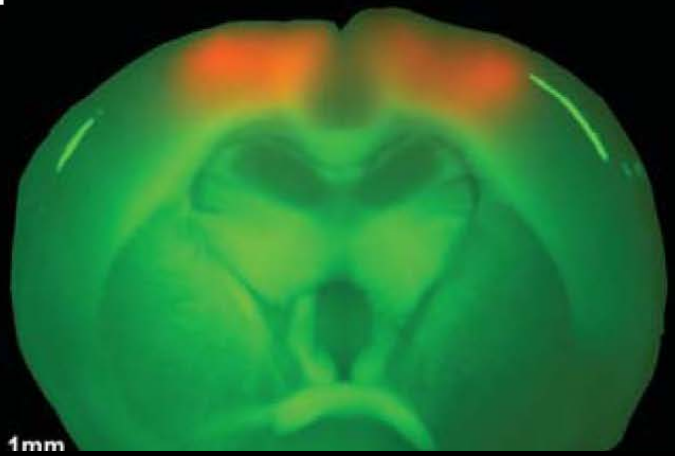


From C. Petersen 2007 Neuron.

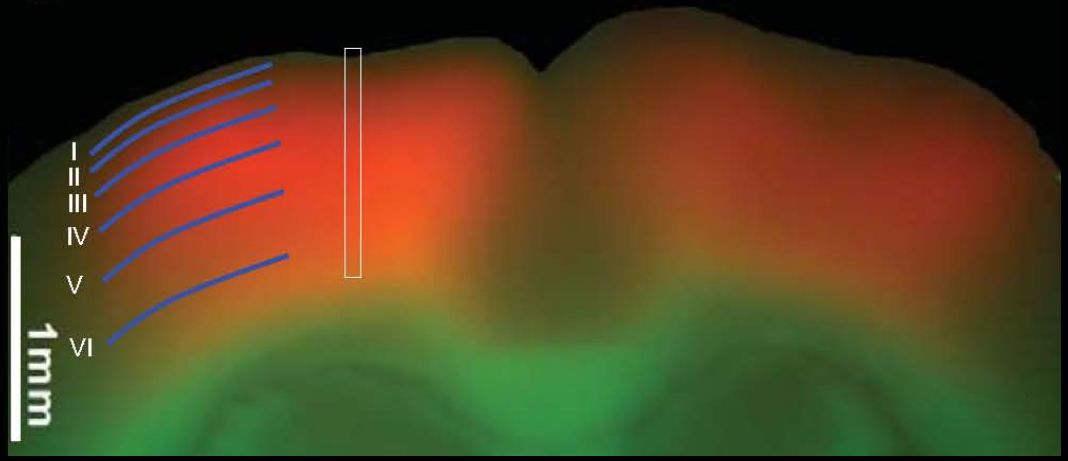
Voltage sensitive dye labelling (red), histology.



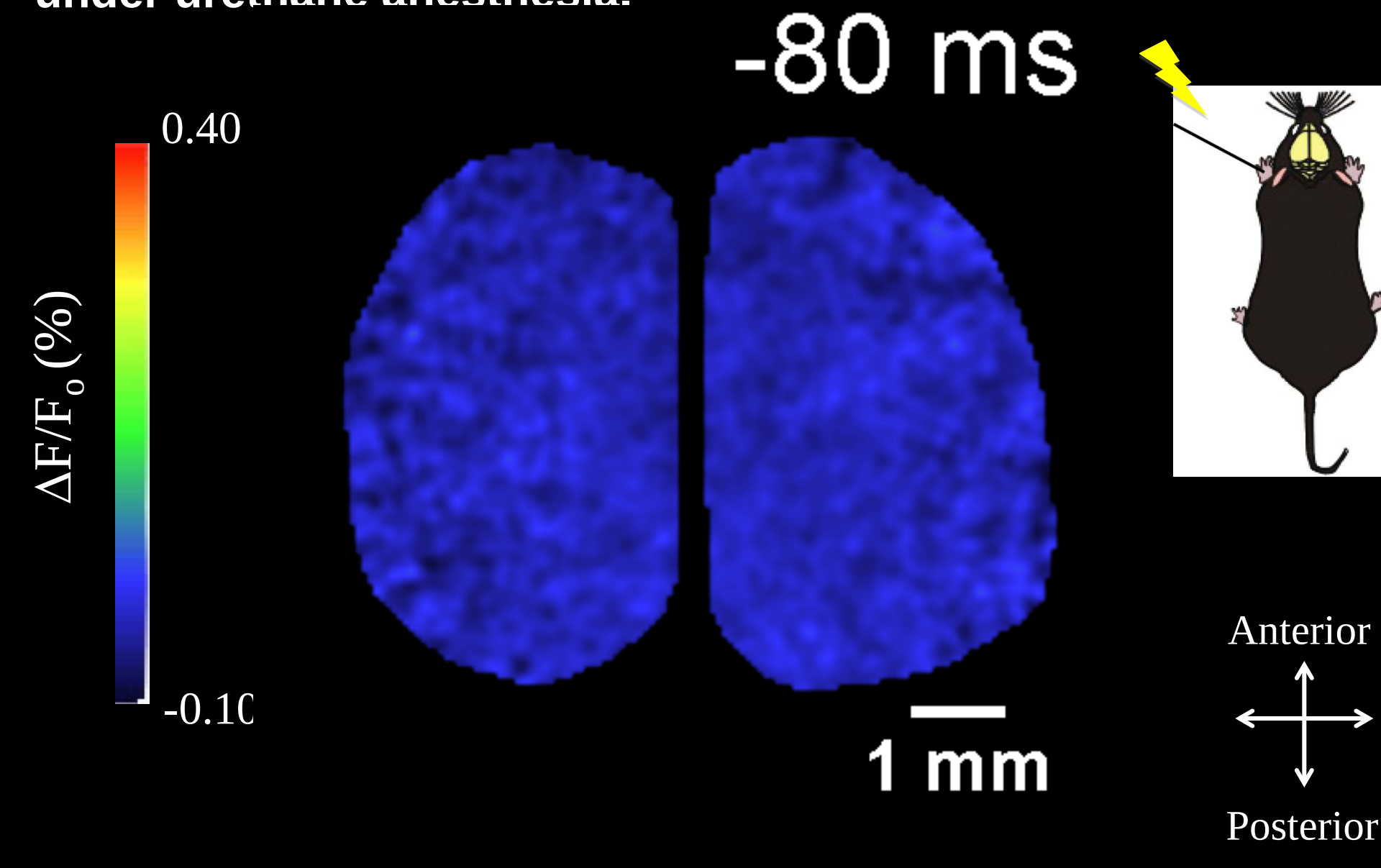
i.



ii.



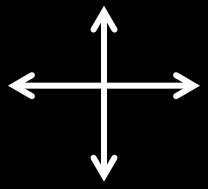
Pattern of depolarization in response to left forelimb stimulation (5 ms tap) in a naïve animal average response under urethane anesthesia.



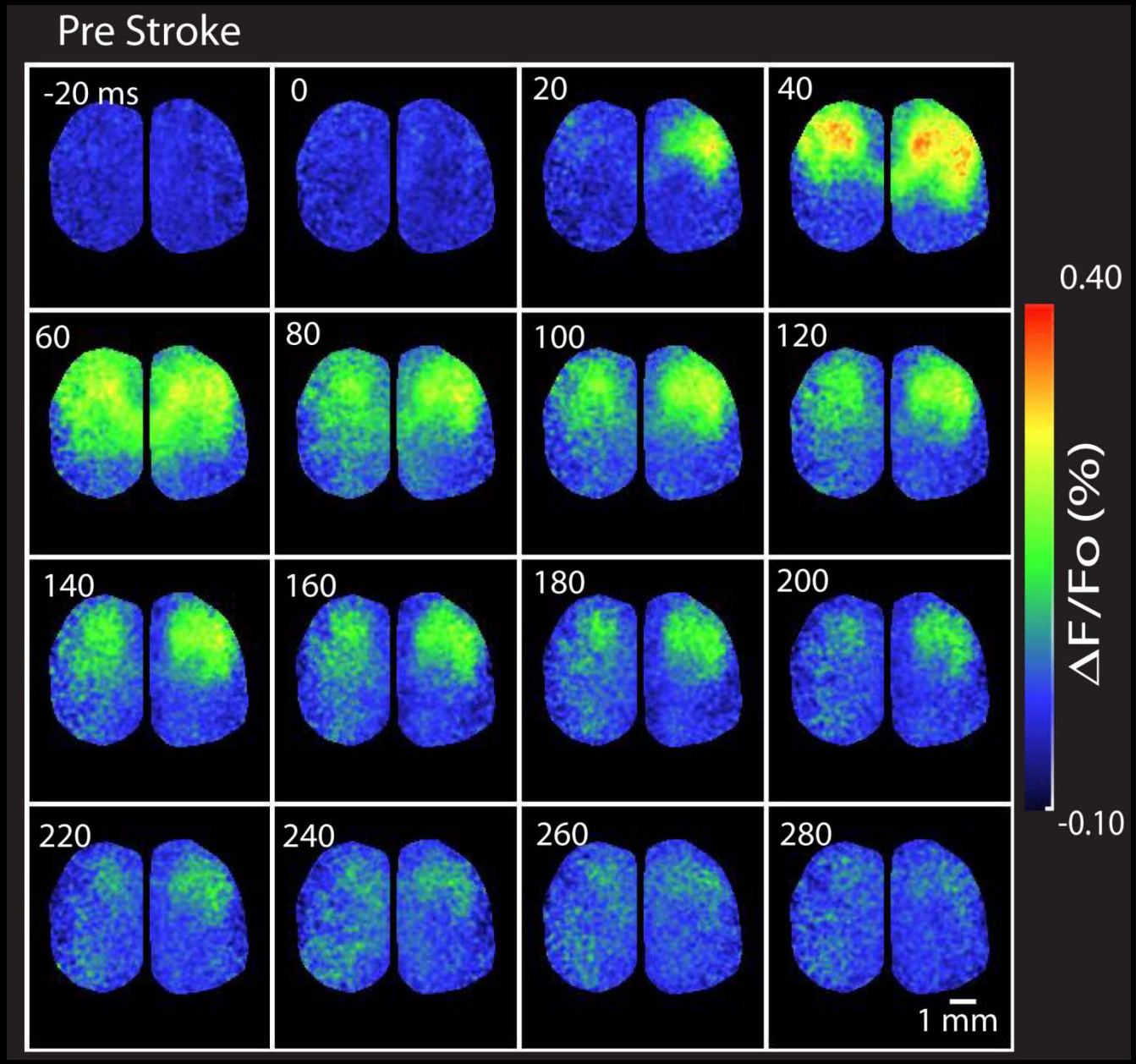
VSD Response to Left Forelimb Stimulation Pre Stroke

Left Forelimb Stimulation

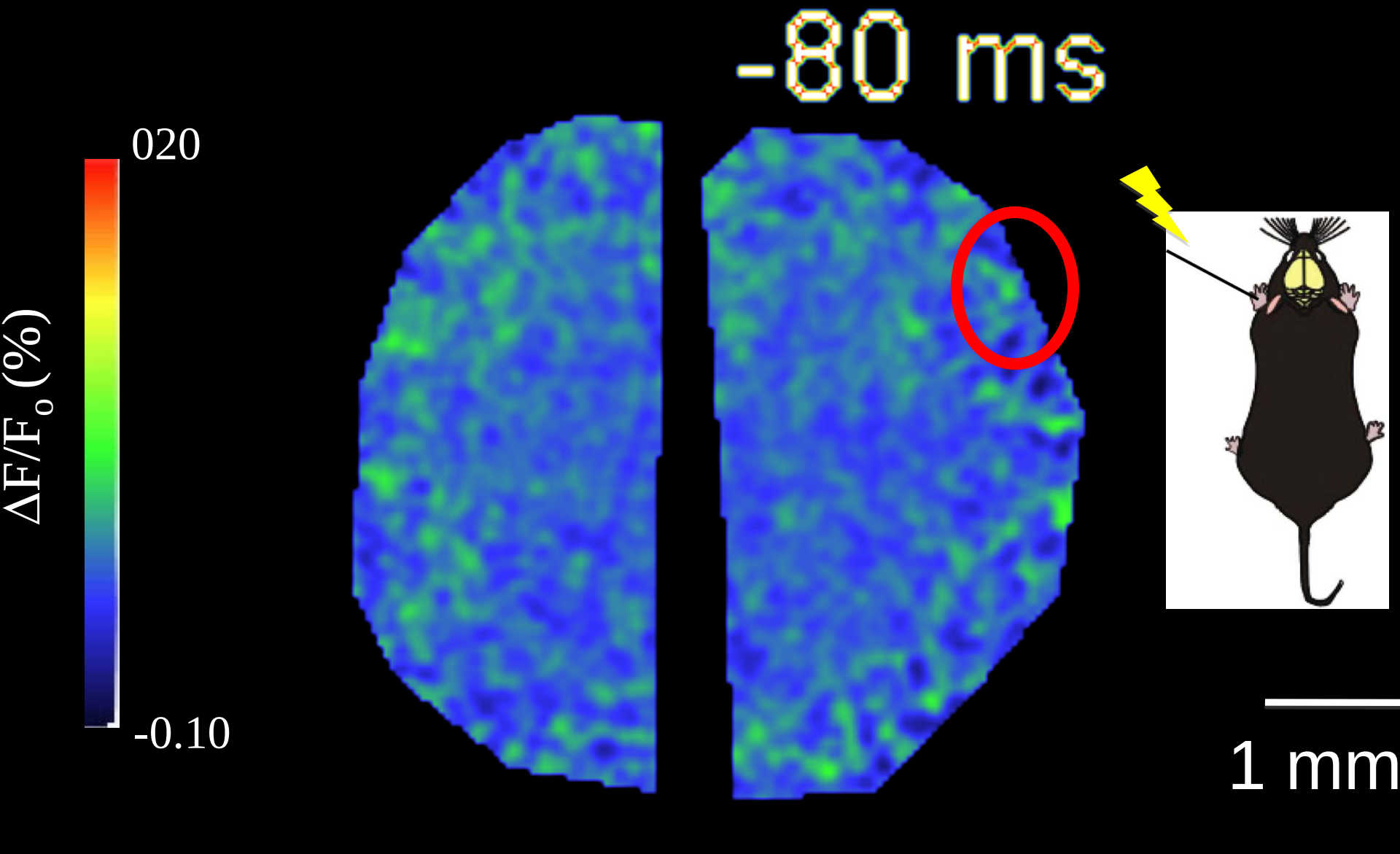
Anterior



Posterior



Pattern of depolarization in response to forelimb stimulation after 8 weeks of recovery from small stroke to the forelimb somatosensory cortex, average response.



VSD response to left forelimb 8 weeks post sm stroke.

Anterior

Posterior

-20 ms | 0 ms | 20 ms | 40 ms

stroke

60 ms | 80 ms | 100 ms | 120 ms

140 ms | 160 ms | 180 ms | 200 ms

220 ms | 240 ms | 260 ms | 280 ms

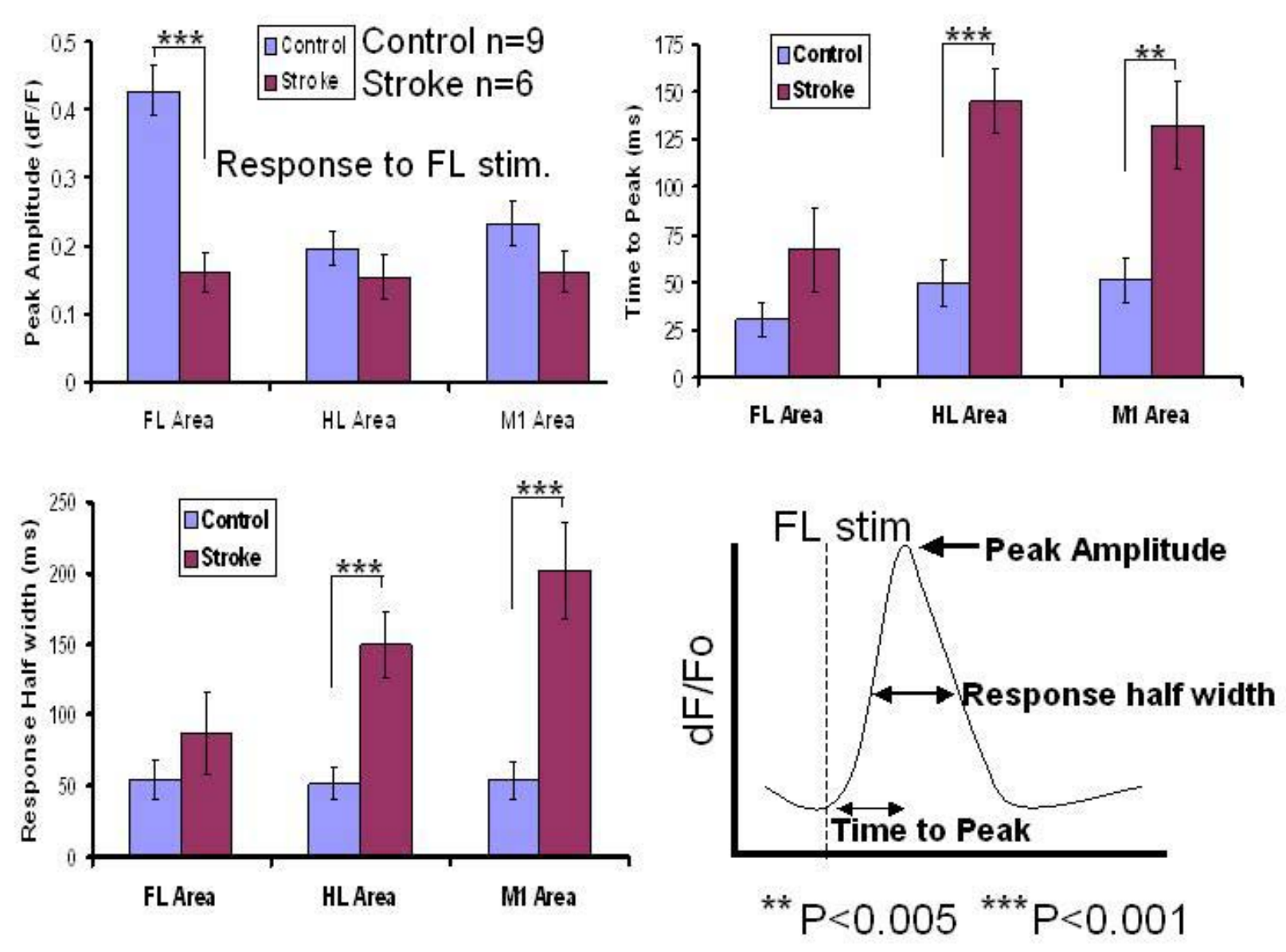
0.2

-0.1

Prolonged posterior medial responses

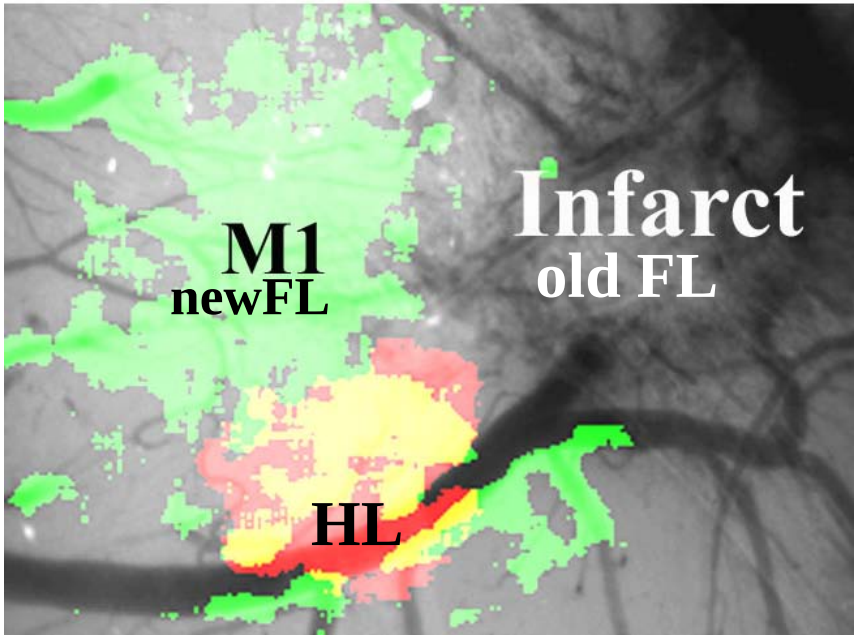
1 mm

Prolonged remapped sensory responses after 8 weeks of recovery from stroke in mice.



How do individual neurons process information in re-mapped areas recovering from stroke?

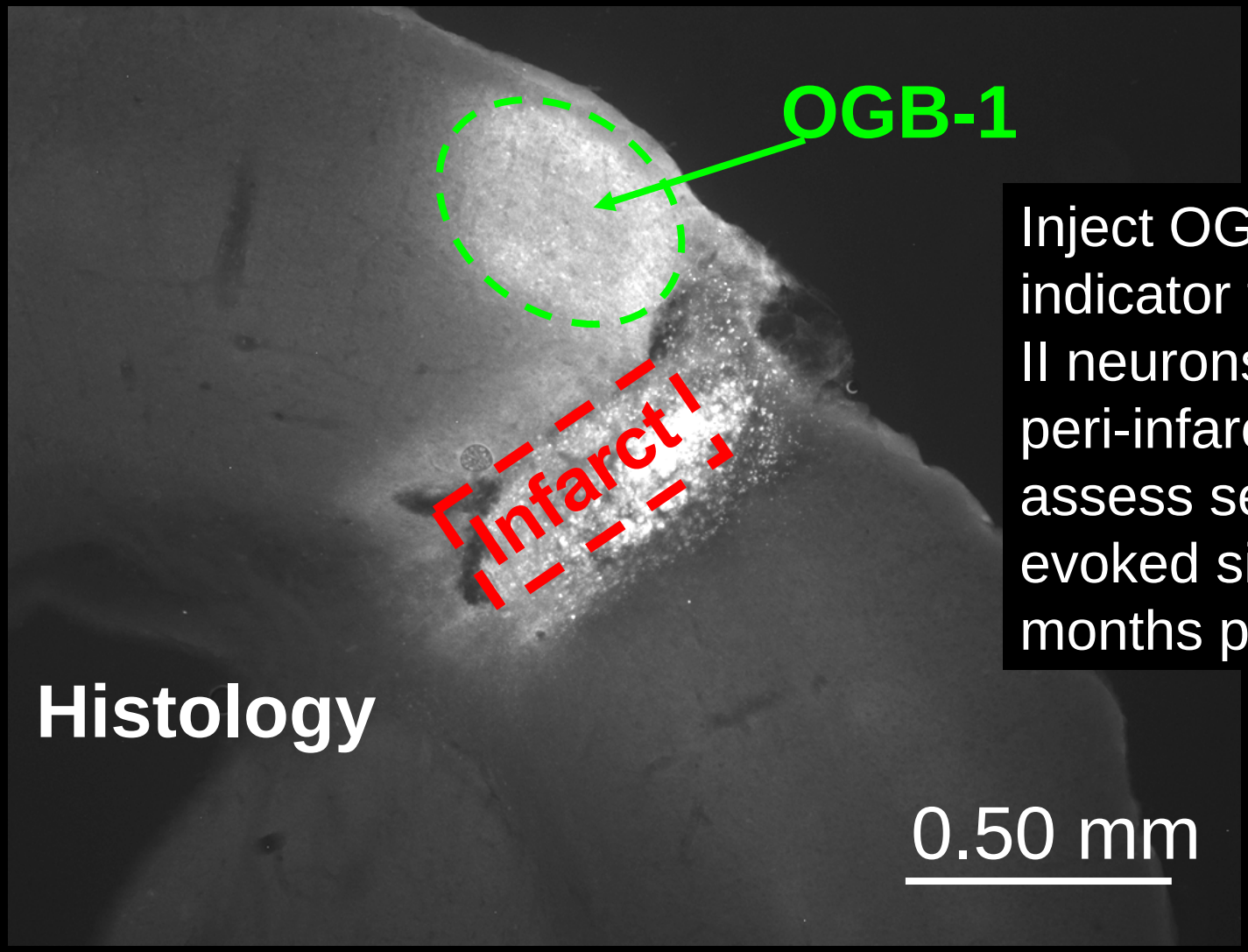
- Monitor sensory evoked calcium signals from layer II neurons in vivo.
- In novel map representations with mixed responses (to both fore and hindlimbs), do single neurons process information from multiple limbs?



IOS maps 8 weeks post-stroke

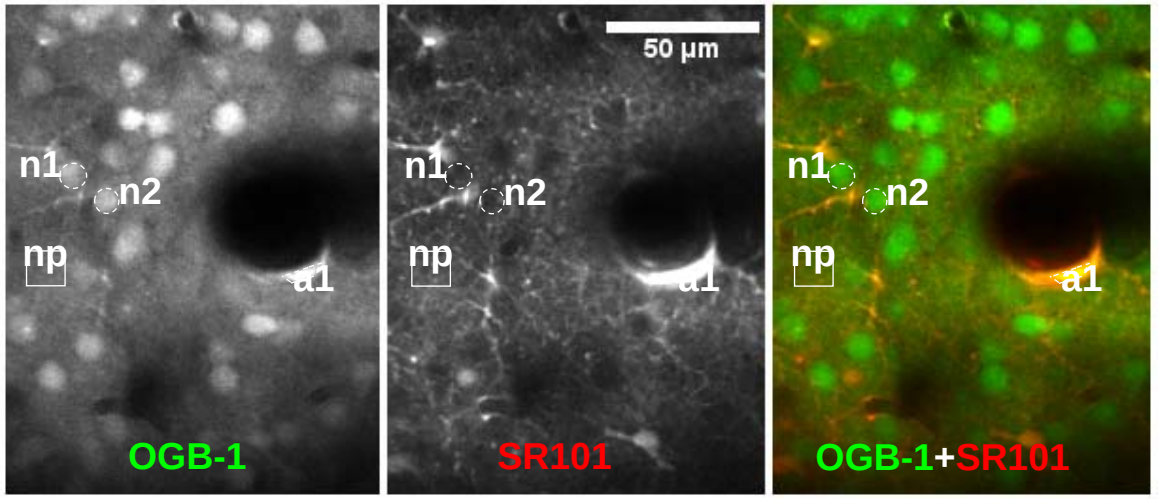
Winship and Murphy 2008 J. Neurosci.

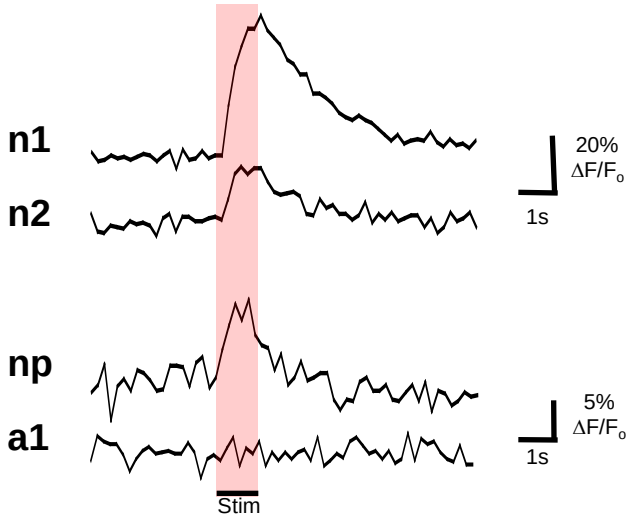
In vivo imaging of single cell response properties in animals recovering from focal stroke damage over weeks.



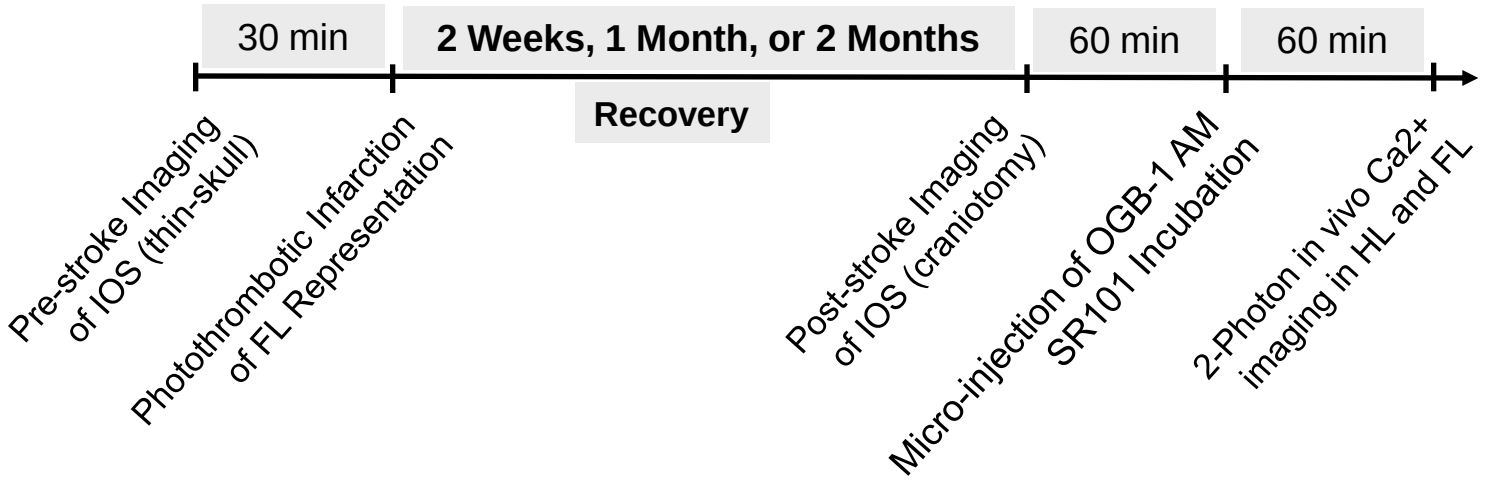
Inject OGB-1 Ca indicator to label layer II neurons within the peri-infarct zone, assess sensory-evoked signals up to 2 months post-stroke.

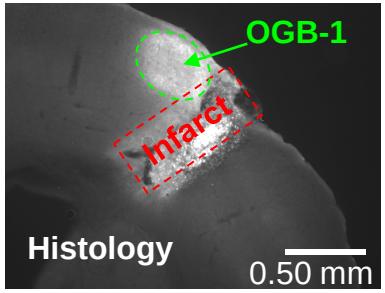
A Two-Photon *In Vivo* Calcium Imaging layer II. **B** cHL-evoked Ca^{2+} Transients

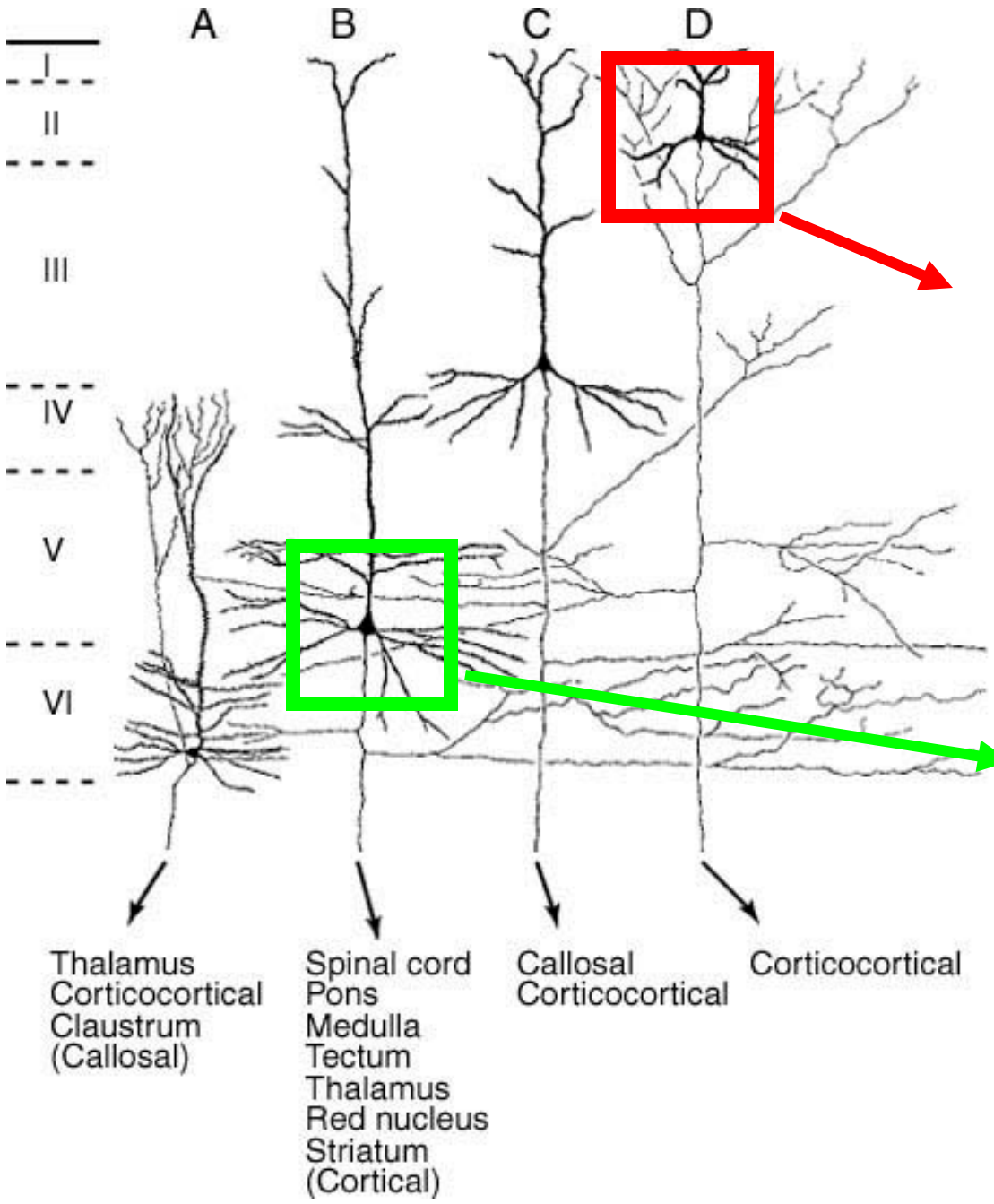




C Experimental Timeline



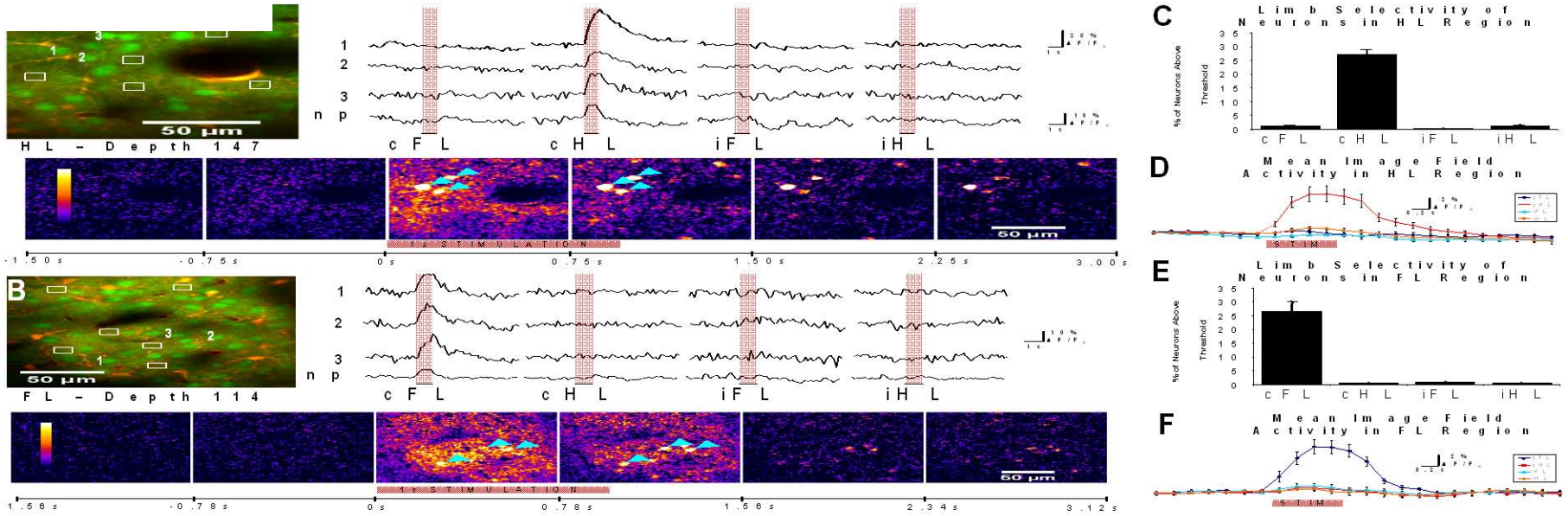




Layer II intracortical processing

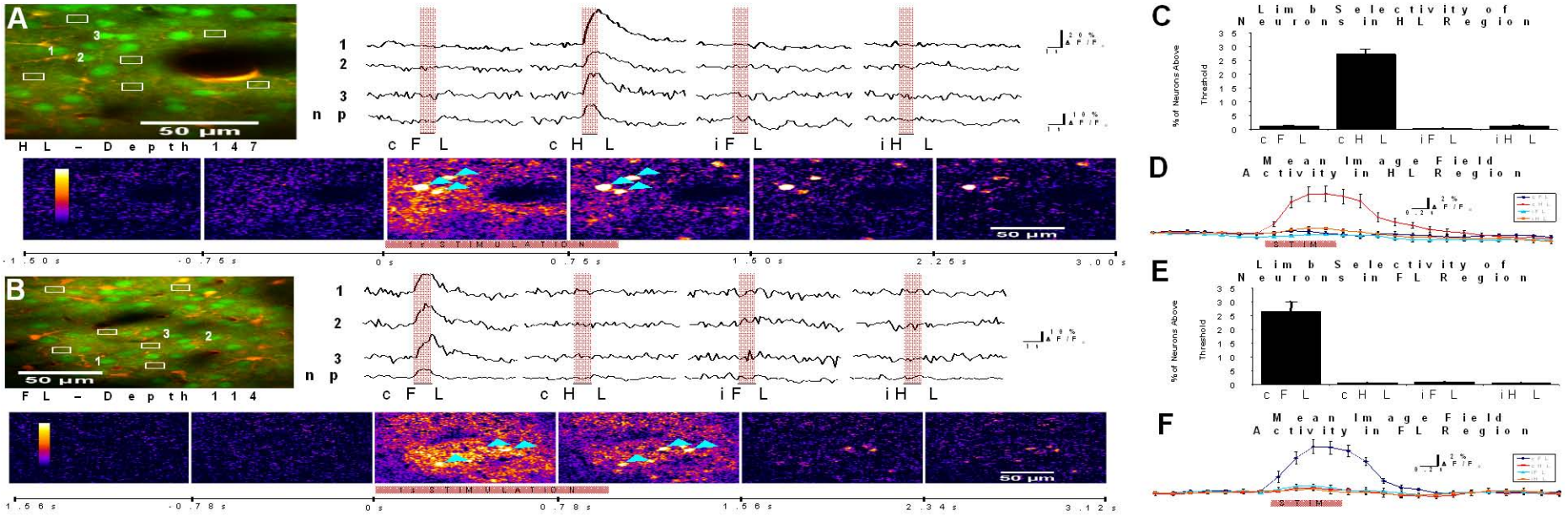
Layer V cortical output

In sham or control mice, layer II neurons in forelimb area are selective to contralateral forelimb stimuli.

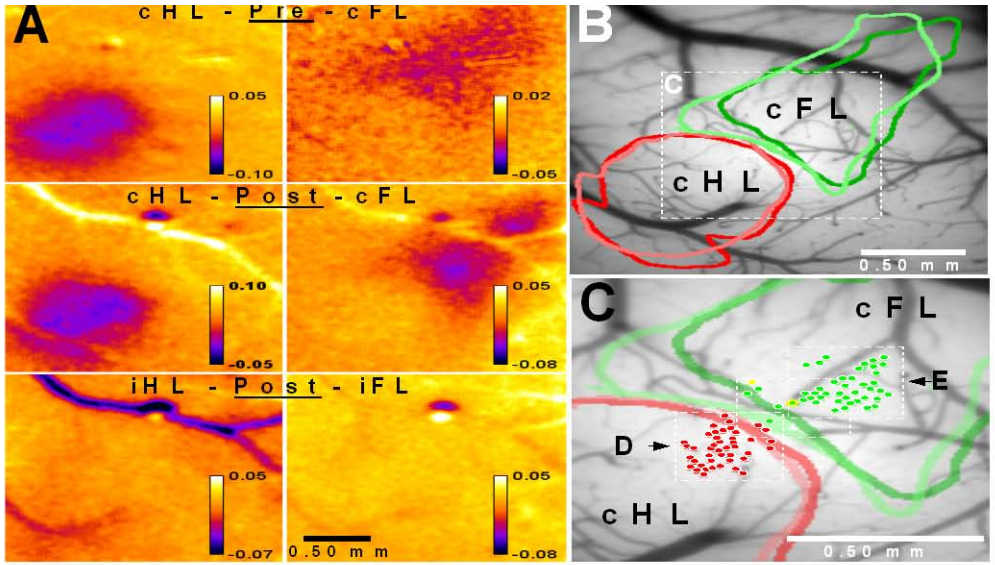


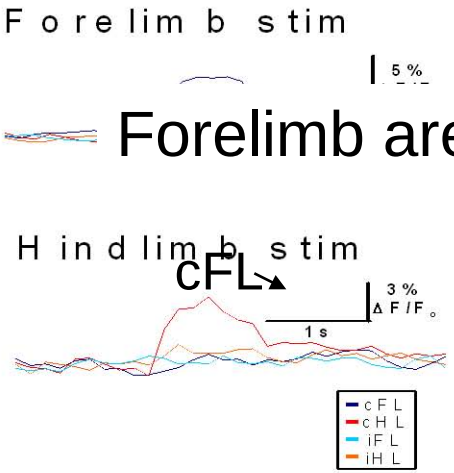
Winship and Murphy 2008 J. Neurosci.

In sham or control mice, layer II neurons in hindlimb area are selective to contralateral hindlimb stimuli.



Highly selective map borders in sham stroke animals.





Forelimb area

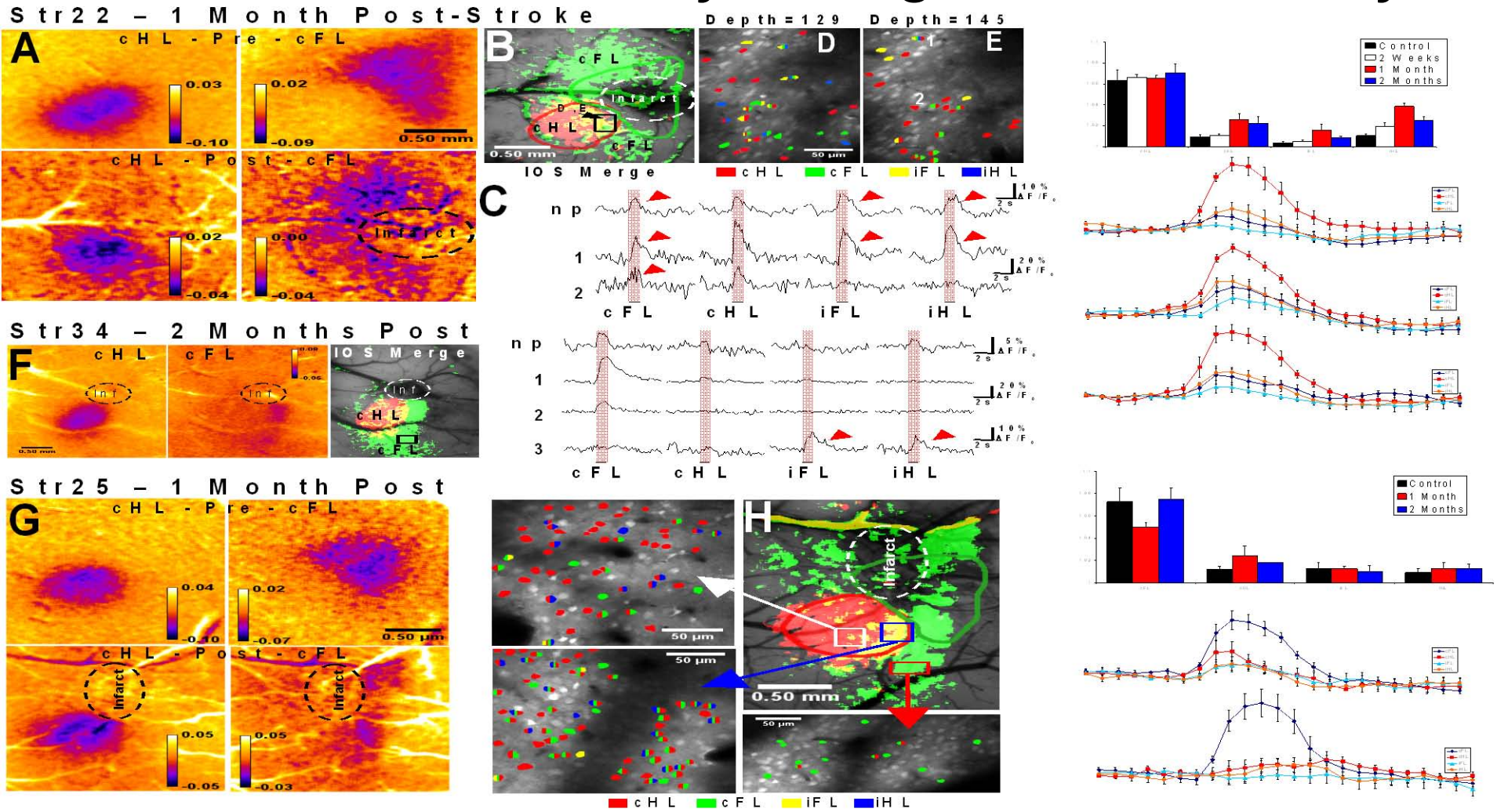
Hindlimb area

cHL

Red dot for HL selective cell
Green dot for FL cells

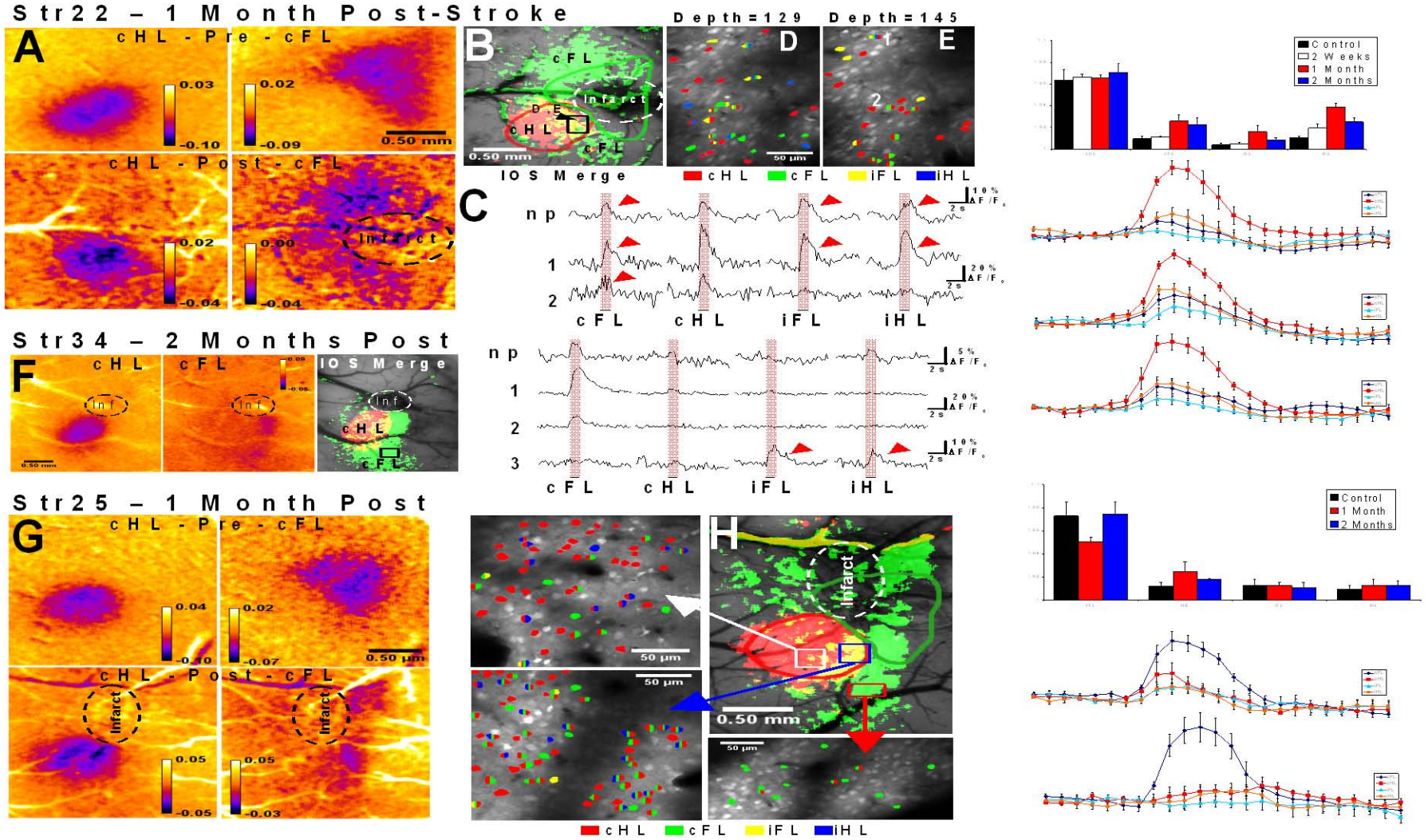
IOS maps

Altered limb selectivity during stroke recovery.

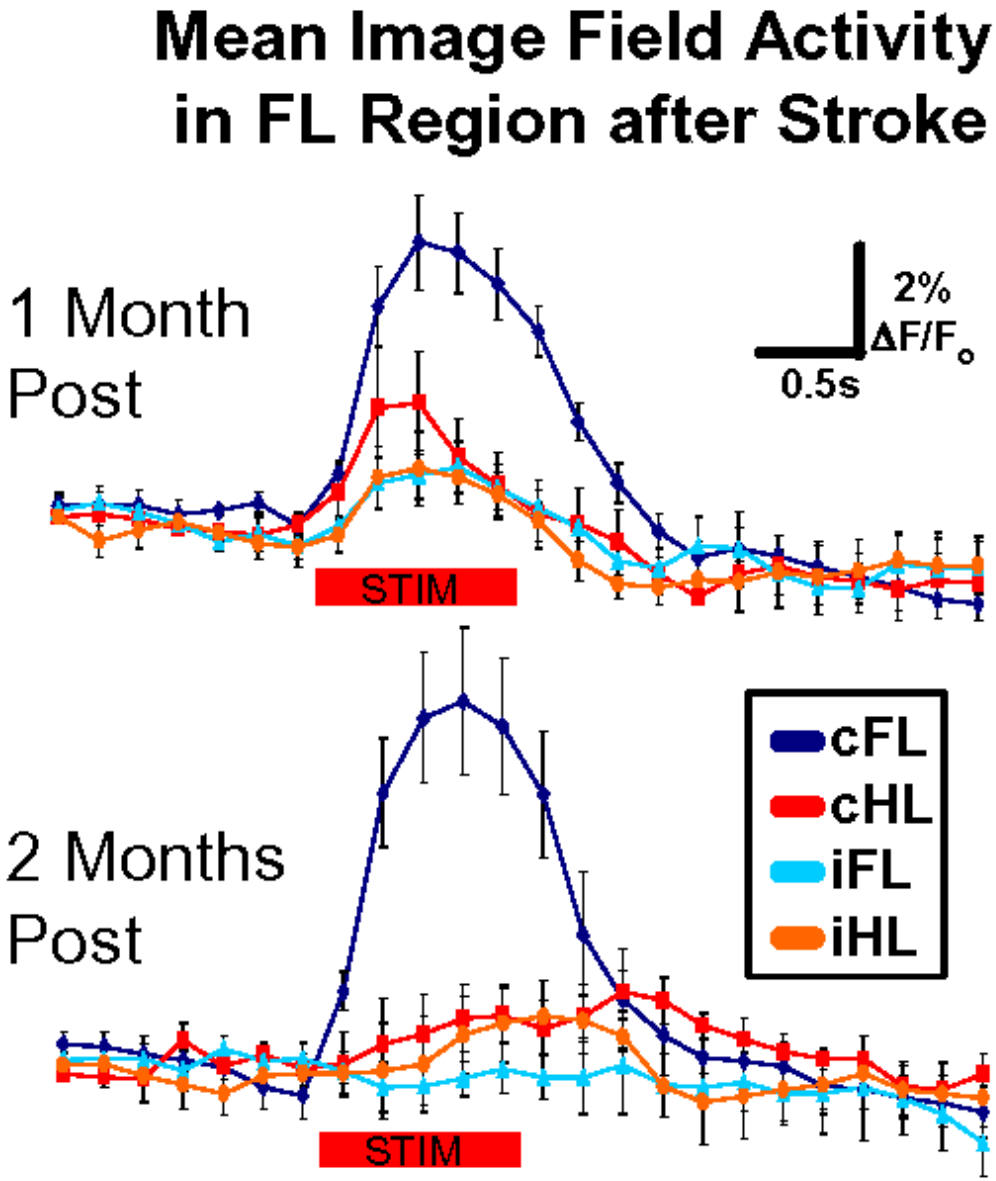


IOS maps

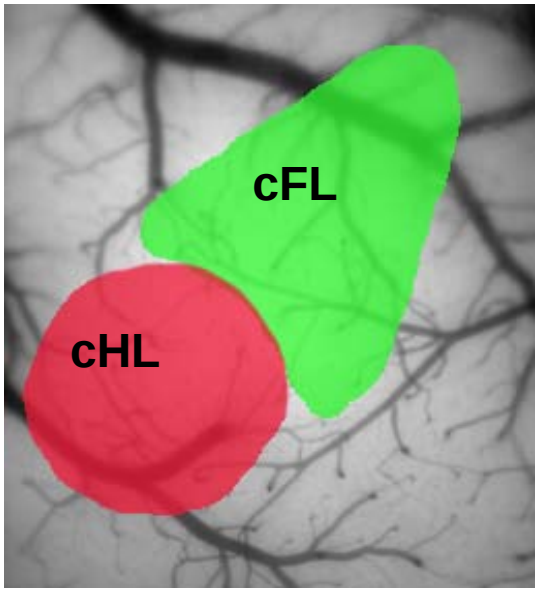
single cell calcium responses


Str22 – 1 Month Post-Stroke
A
cHL - Pre - cFL
0.03
-0.10
0.02
-0.09
0.50 mm
cHL - Post - cFL
0.02
-0.04
0.00
-0.04
Infarct
B
cFL
Infarct
D E
cHL
cFL
0.50 mm
Depth = 129
D
Depth = 145
E
1
2
50 µm
IOS Merge
cHL cFL iFL iHL
C
np
1
2
cFL cHL iFL iHL
10 % ΔF/F
2 s
20 % ΔF/F
5 % ΔF/F
np
1
2
3
cFL cHL iFL iHL
Control
2 Weeks
1 Month
2 Months
Str34 – 2 Months Post
F
cHL
cFL
IOS Merge
Inf
cHL
cFL
Str25 – 1 Month Post
G
cHL - Pre - cFL
0.04
-0.10
0.02
-0.07
0.50 µm
cHL - Post - cFL
Infarct
Infarct
0.05
-0.05
0.05
-0.03
H
50 µm
50 µm
Infarct
0.50 mm
50 µm
cHL cFL iFL iHL
Control
1 Month
2 Months

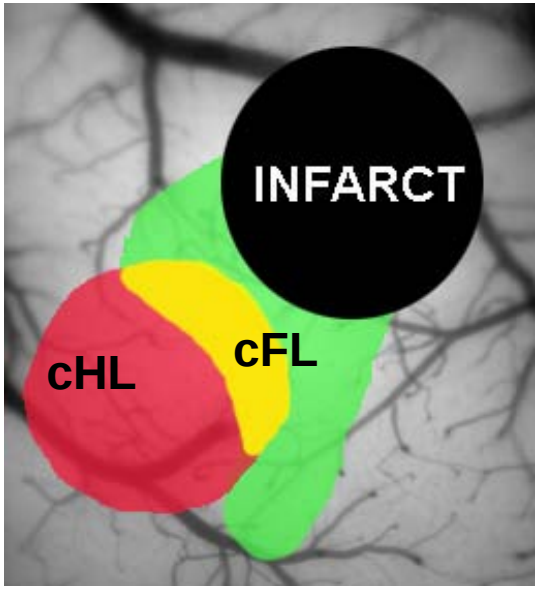
Overtime, neurons in reorganized areas become more selective.



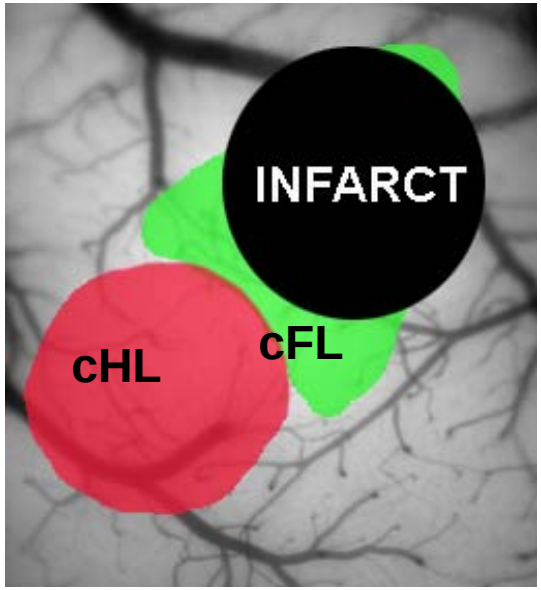
Remapping sensory cortex after stroke.



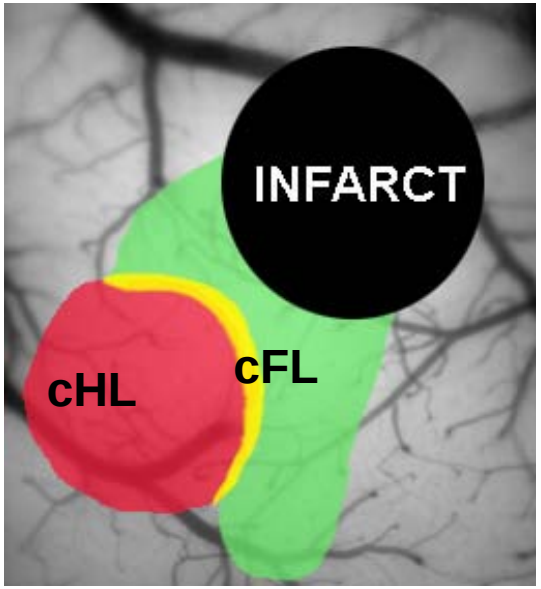
Pre-Stroke
Functional segregation



1 Month Post-Stroke
Re-mapping, increased receptive fields and reduced limb specificity

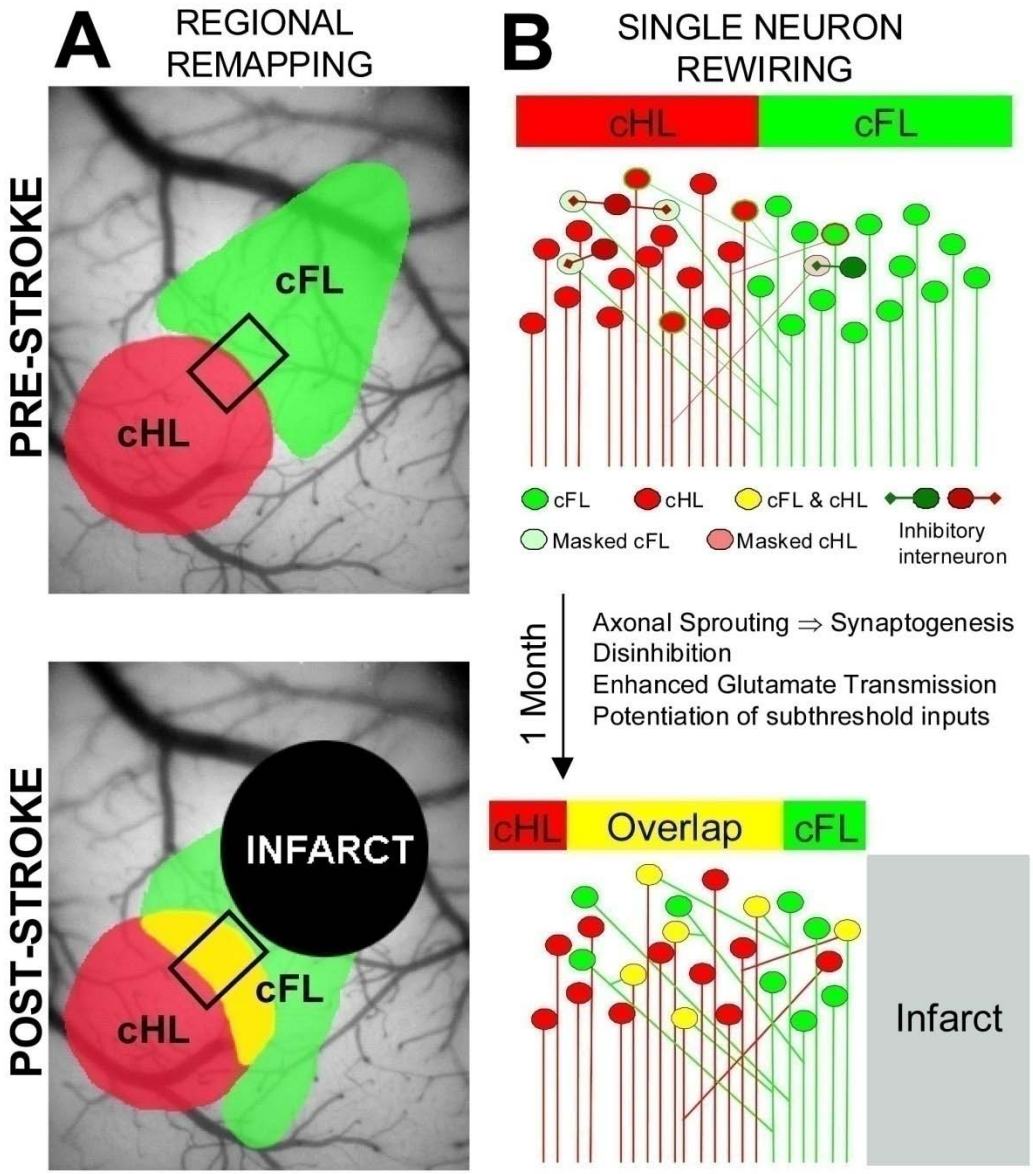


1-6 h Post-Stroke
Partial function, circuits available for later plasticity



2 Months Post-Stroke
Increasing specificity, encroachment on other maps

Single neuron re-mapping model after stroke, use of subthreshold connections and dis-inhibition.



Individual neurons process information from multiple limbs during stroke recovery.

- Mixed sensory areas (FL and HL responsive) in regions recovering from stroke are contributed by individual neurons that respond to multiple limbs.
- Limb selectivity is reduced during the first month after stroke, possibly reflecting increased plasticity after stroke.
- Overtime (at 2 months) new limb representations become more selective.

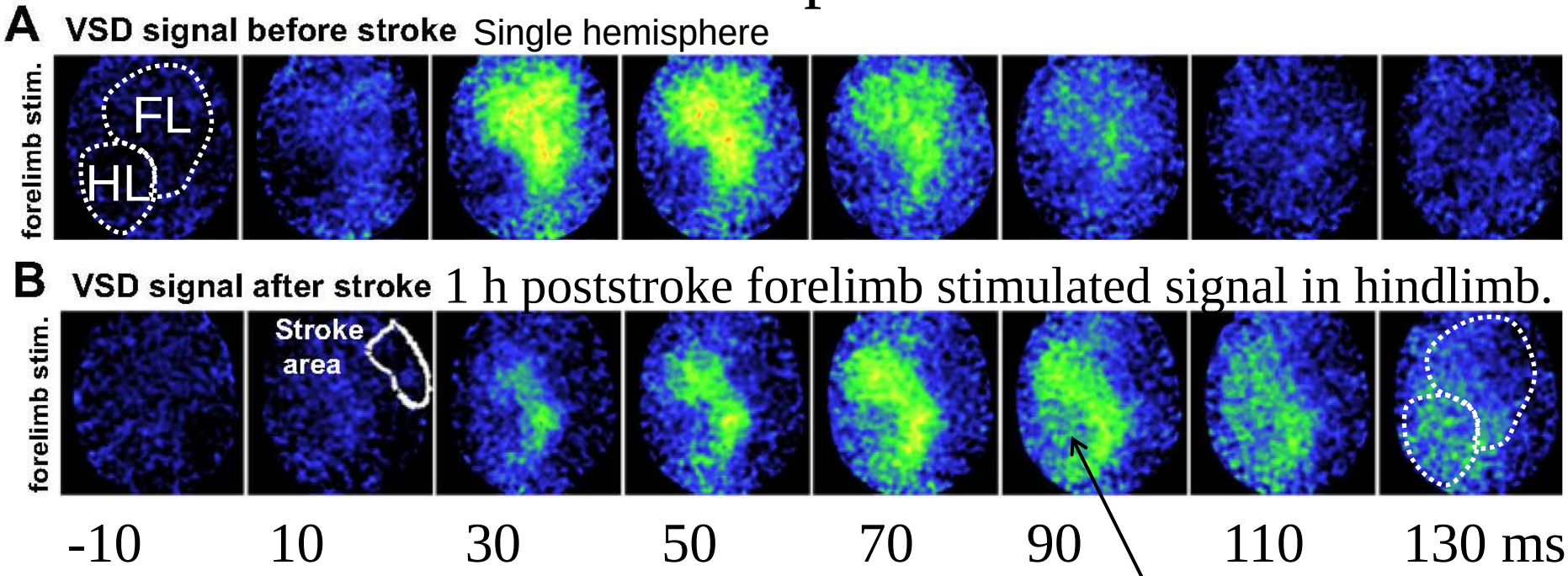
Re-mapping of forelimb sensory responses after stroke.

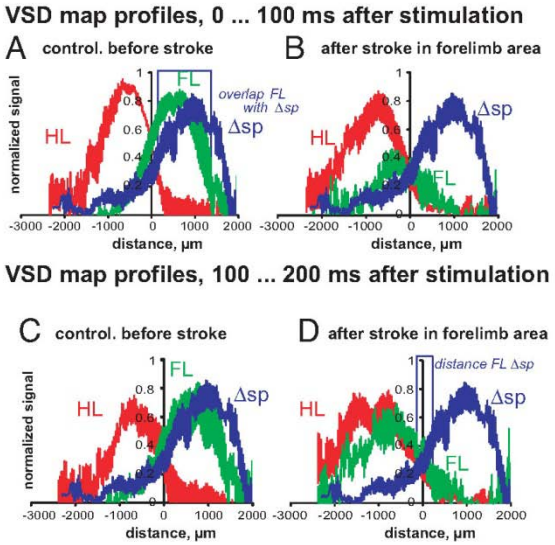
- Re-mapped FL circuits, preferentially observed in areas with related function, i.e., HL and M1 and have slow kinetics, however novel activations can spread over much of the cortex after stroke.
- Widespread activation after stroke may be associated with dis-inhibition.
- Individual neurons in re-mapped areas process information in a less specific manner (data from Ca2+ imaging Winship and Murphy 2008).

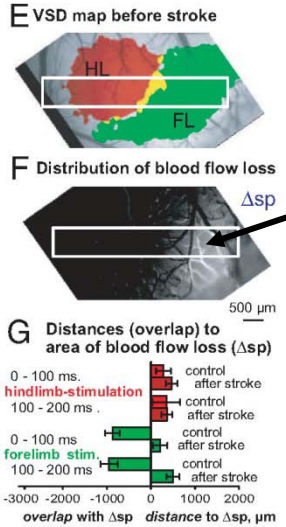
Does new or existing hard wiring support novel maps after stroke?

- The only way to know this is to control the activity of new formed circuits directly.
- We have tested whether latent circuits are available by asking whether some re-distribution of function can occur over short timescales (hours) that are inconsistent with re-wiring.

Off target responses to forelimb stimulation in non-forelimb areas are preserved 1h after stroke.

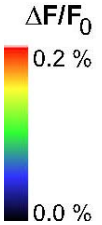






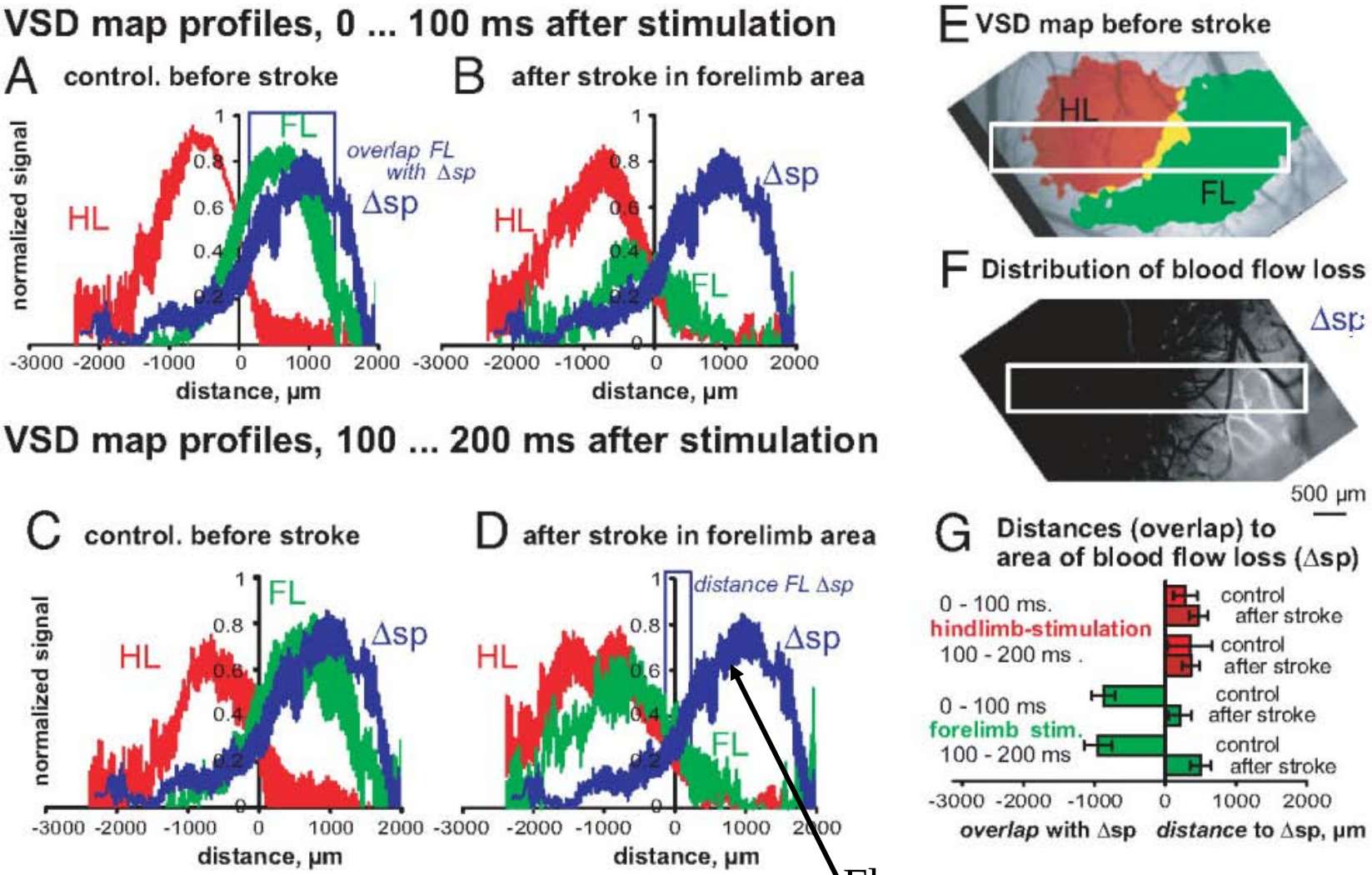
Flow lost here

FL response measured in HL area (right hemi shown).



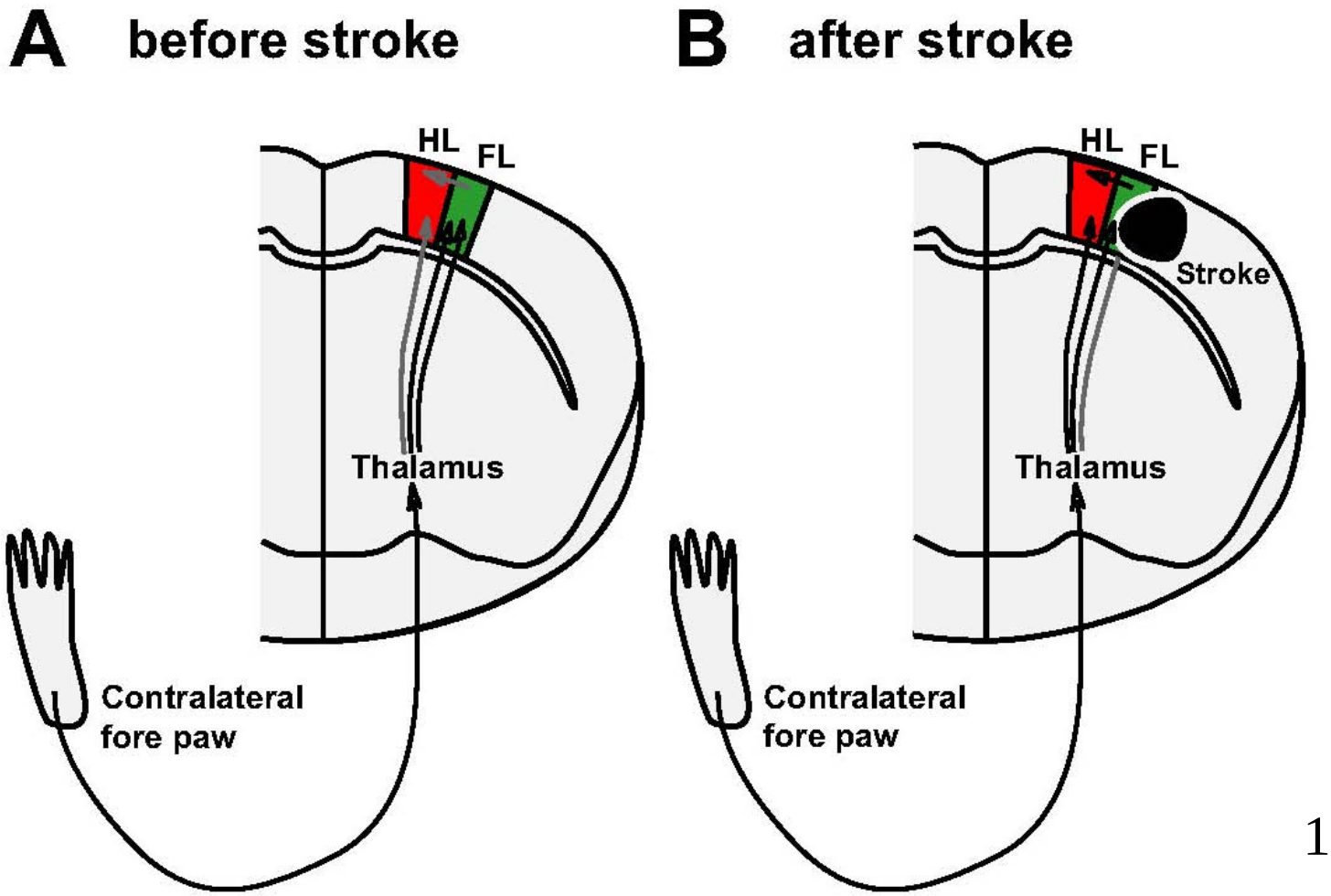
Group data from n=8 animals

Off target responses to forelimb stimulation in non-forelimb areas are preserved 1h after stroke.



Group data from n=8 animals

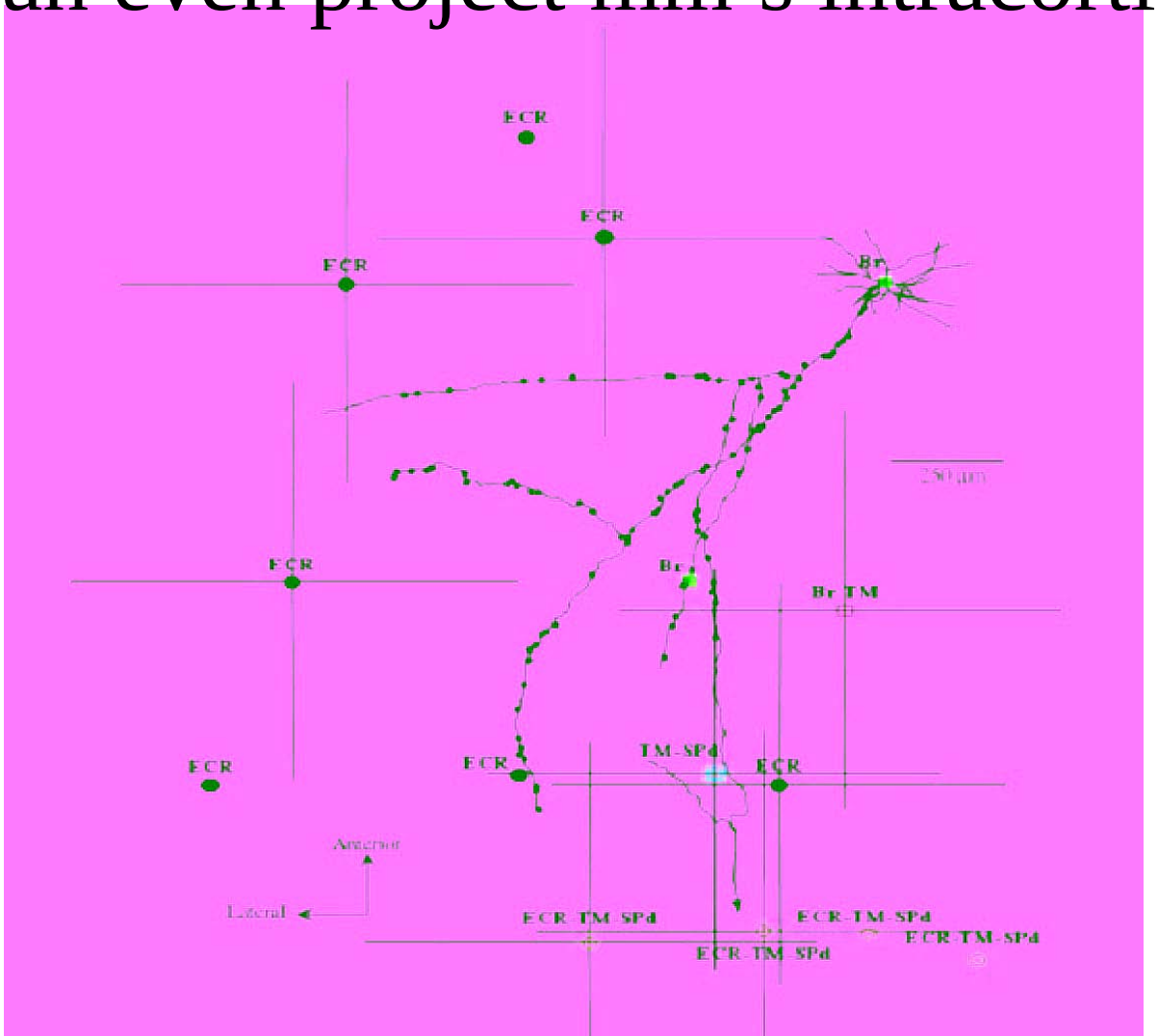
Off target responses to forelimb stimulation in non-forelimb areas are preserved 1h after stroke.



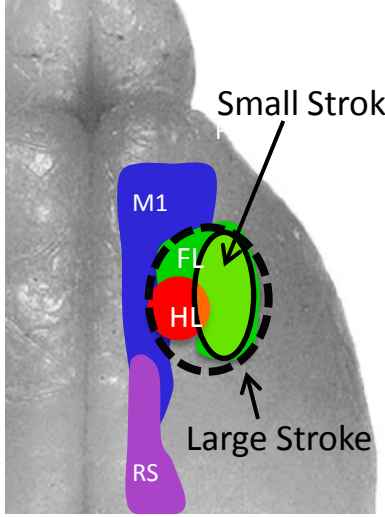
1 h poststroke.

Sigler et al. 2009 PNAS

Many intra-hemispheric and inter-hemispeheric connections already exist, layer 5 neuron axons can even project mm's intracortically.

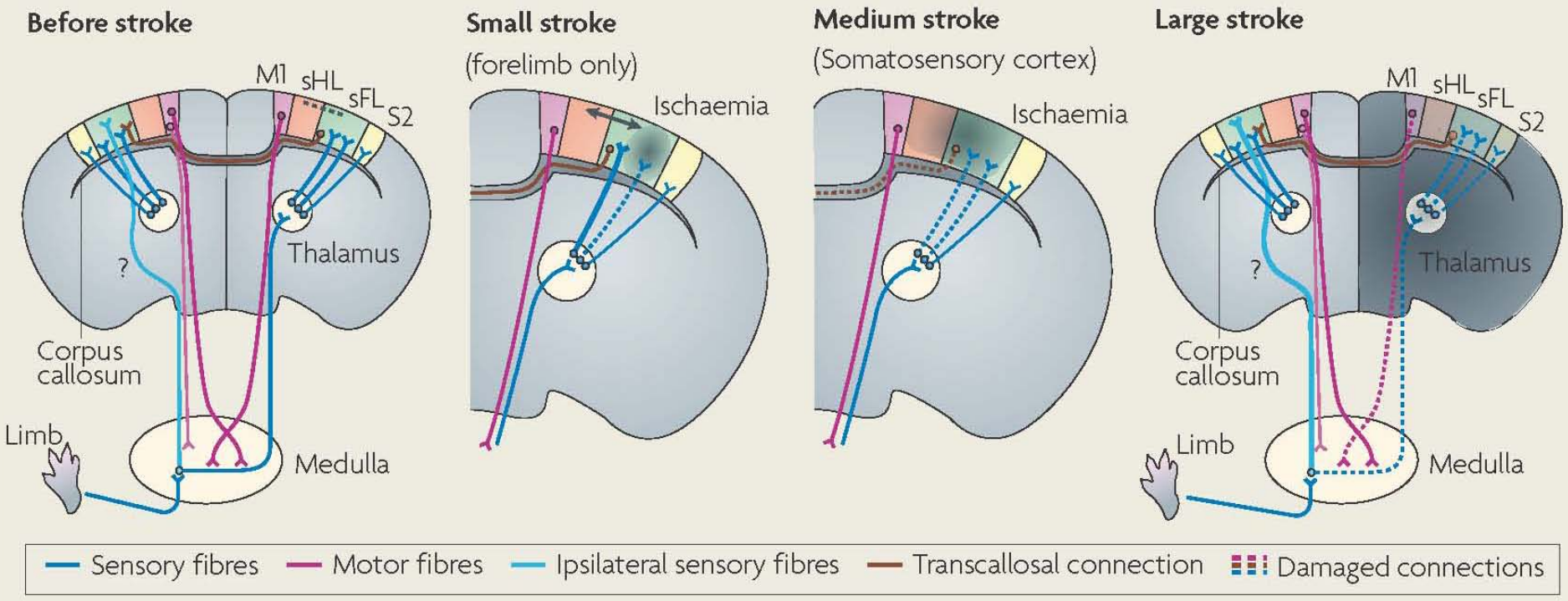


Capaday 2004
the Neuroscientist



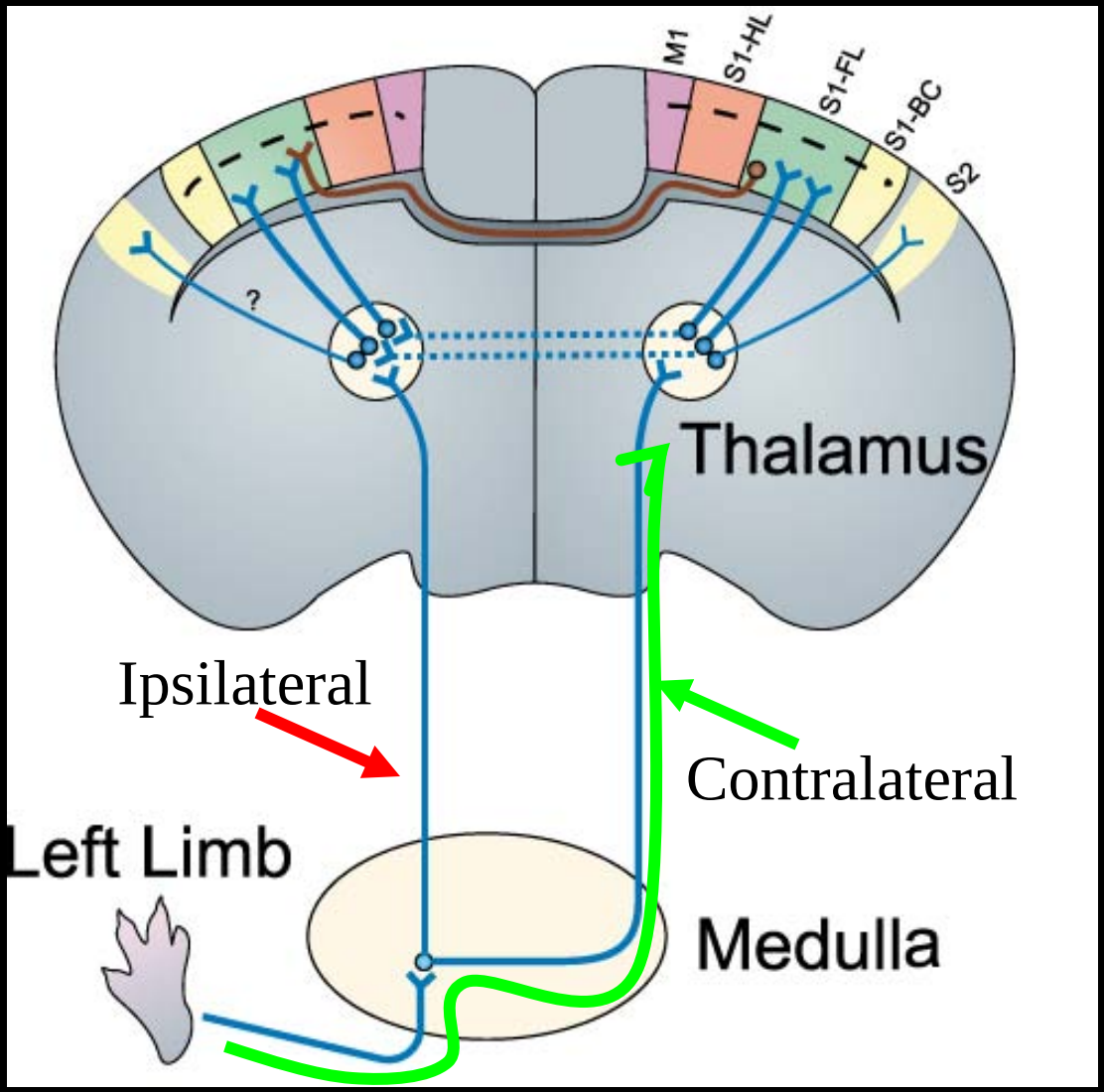
Large strokes may recruit ipsilateral pathways during recovery.

Box 4 | **Brain locations predicted to mediate stroke recovery**

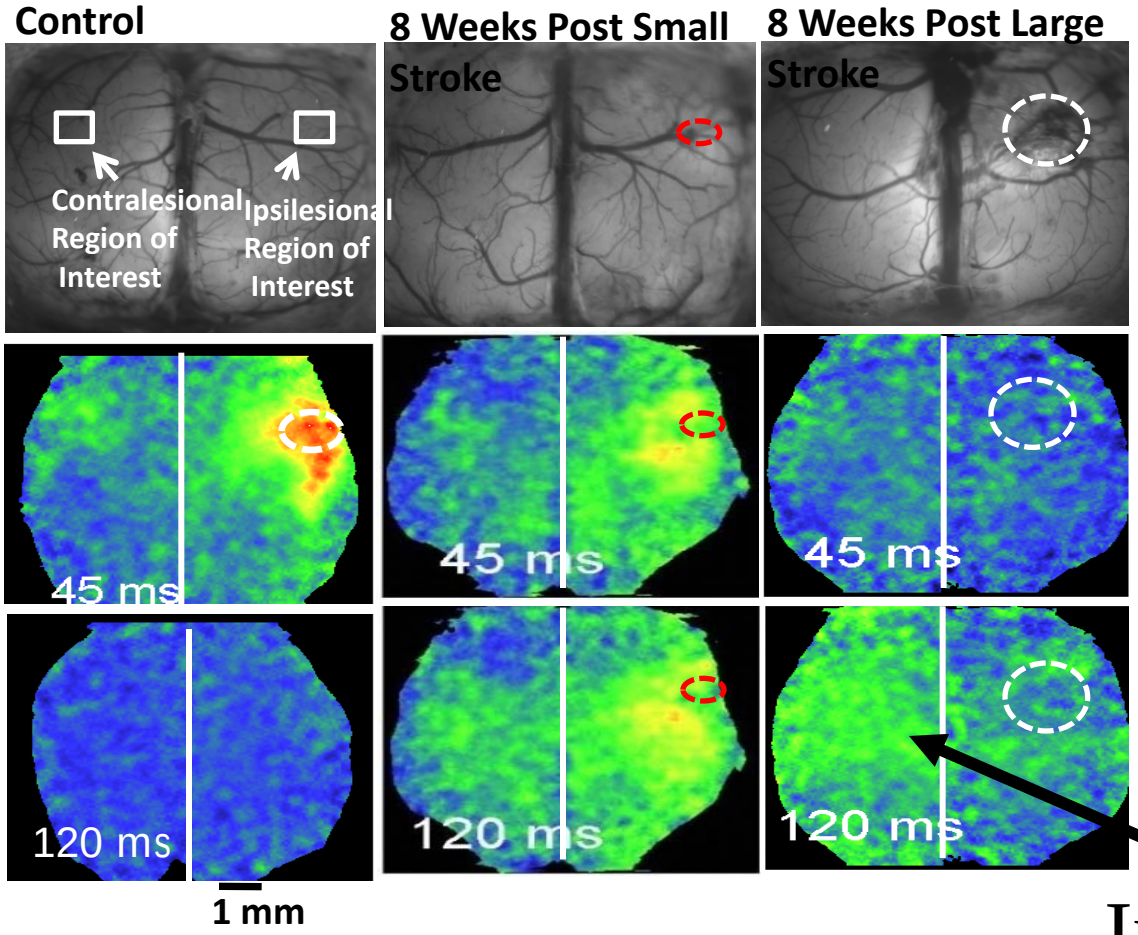


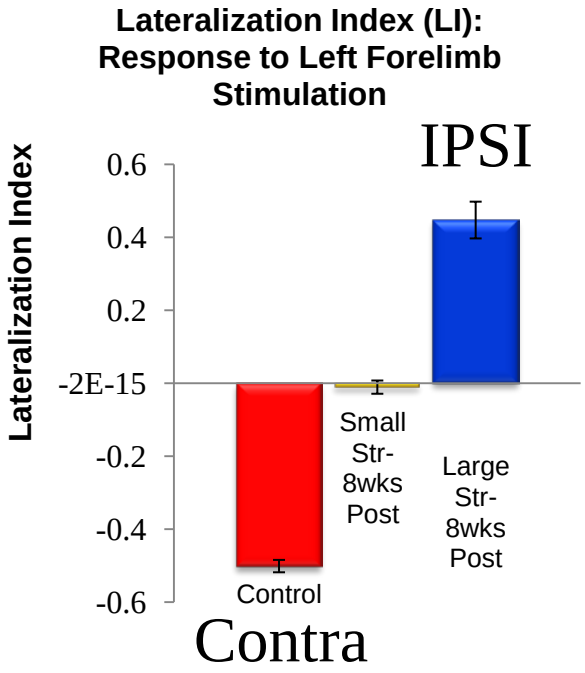
Murphy and Corbett Nat. Rev. Neurosci. 2009

Most sensory stimuli are processed contralaterally



Ipsilateral pathways are involved 8 wks post large stroke, left forelimb VSD responses shown.





$$LI = \frac{\sum \text{Activation}_{left} - \sum \text{Activation}_{right}}{\sum \text{Activation}_{left} + \sum \text{Activation}_{right}}$$

Ipsilateral FL response, lg. stroke.

Control n= 5

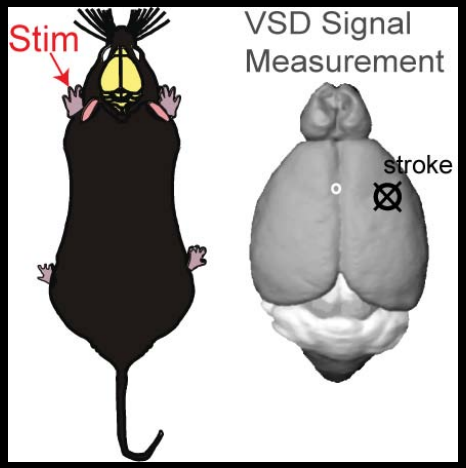
8 wks post small Str n= 6

8 wks post large str n= 6

Can inter-hemispheric changes in signal processing also occur over short timescales?

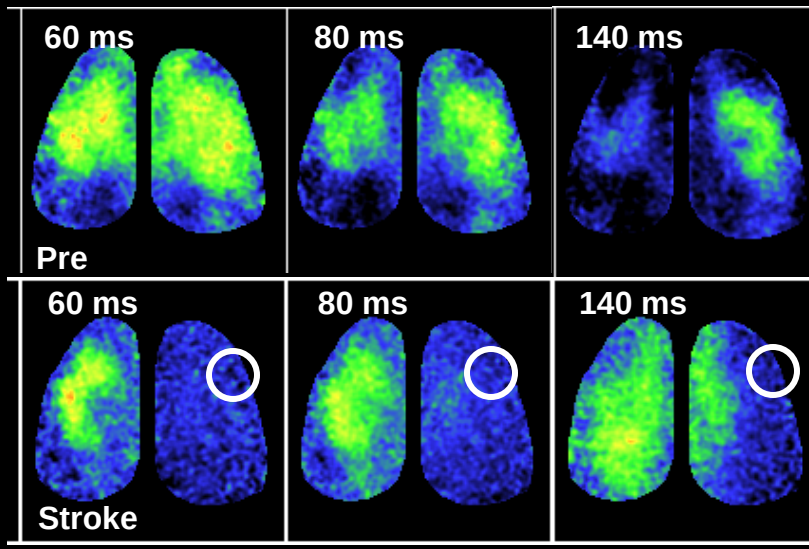
- Examine bilateral sensory processing within the first hours after a stroke targeted to a subset of one hemisphere using voltage sensitive dye (VSD) imaging.

VSD evoked response to stimulation of left (affected) forelimb before and <2h after stroke.

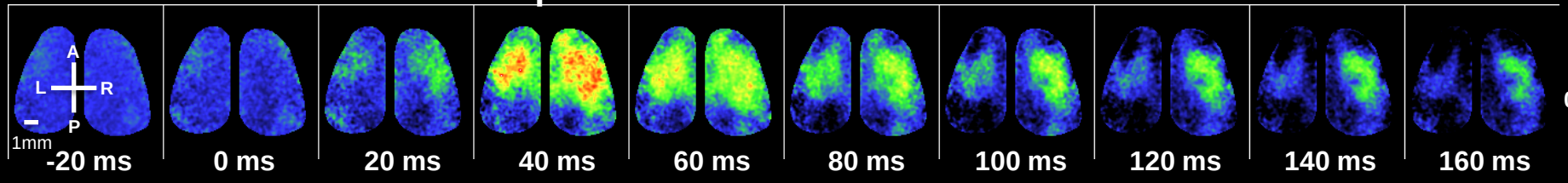


VSD evoked response to stimulation of left FL before stroke, 60, 80, 140 ms

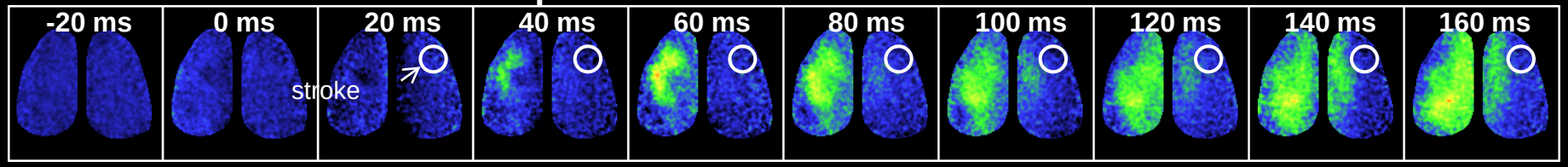
after stroke 60, 80, 140 ms



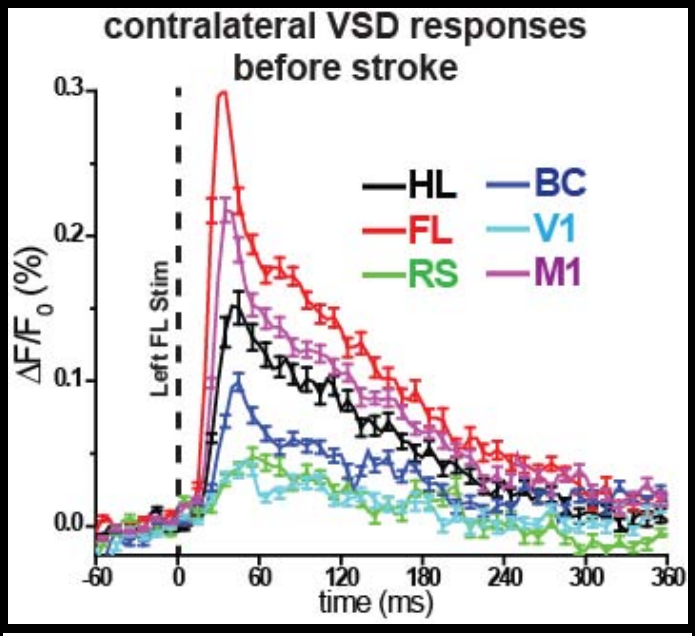
VSD evoked response to stimulation of left FL before stroke

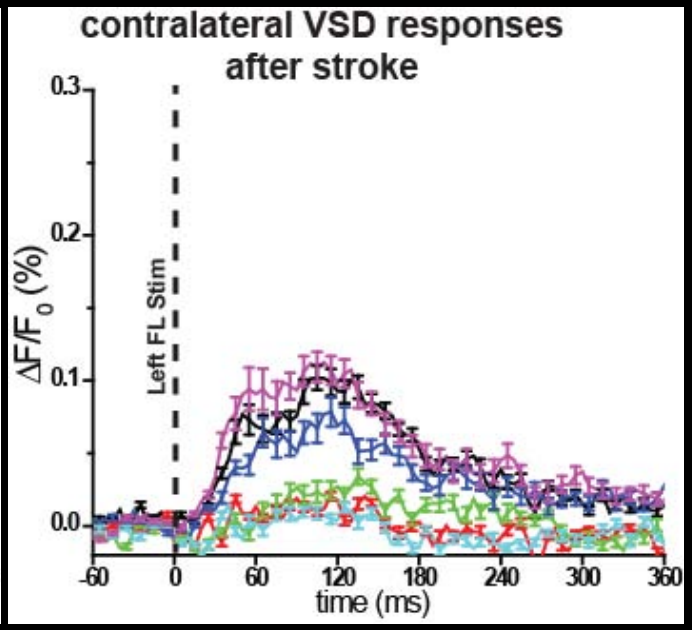


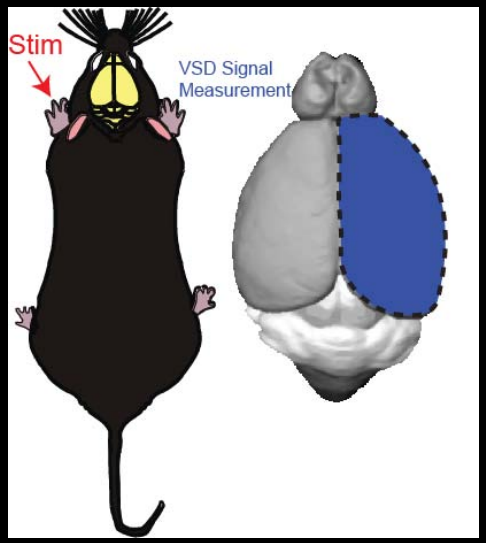
VSD evoked response to stimulation of left FL after stroke

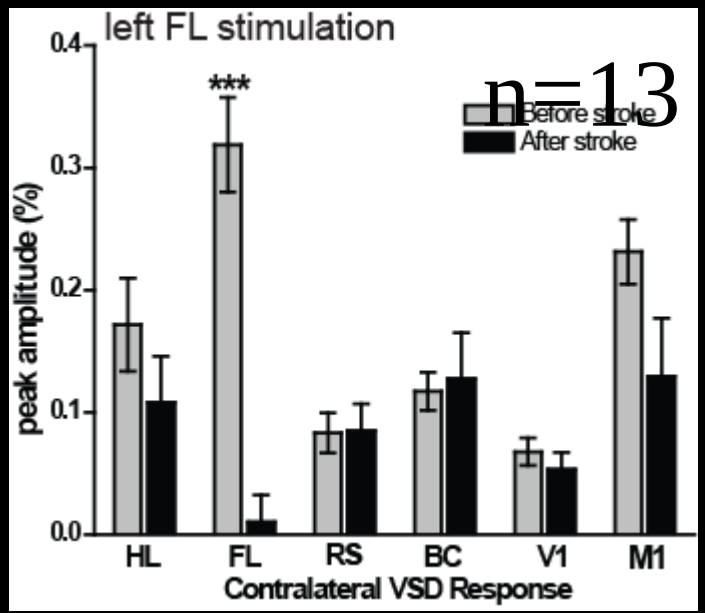


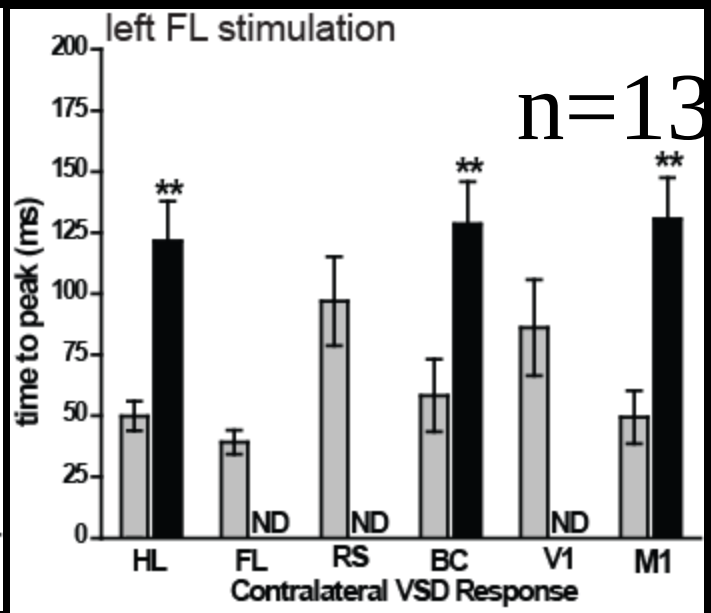
Responses due to stimulation of the affected forelimb are preserved within nearby cortical regions (<2 h) post stroke.



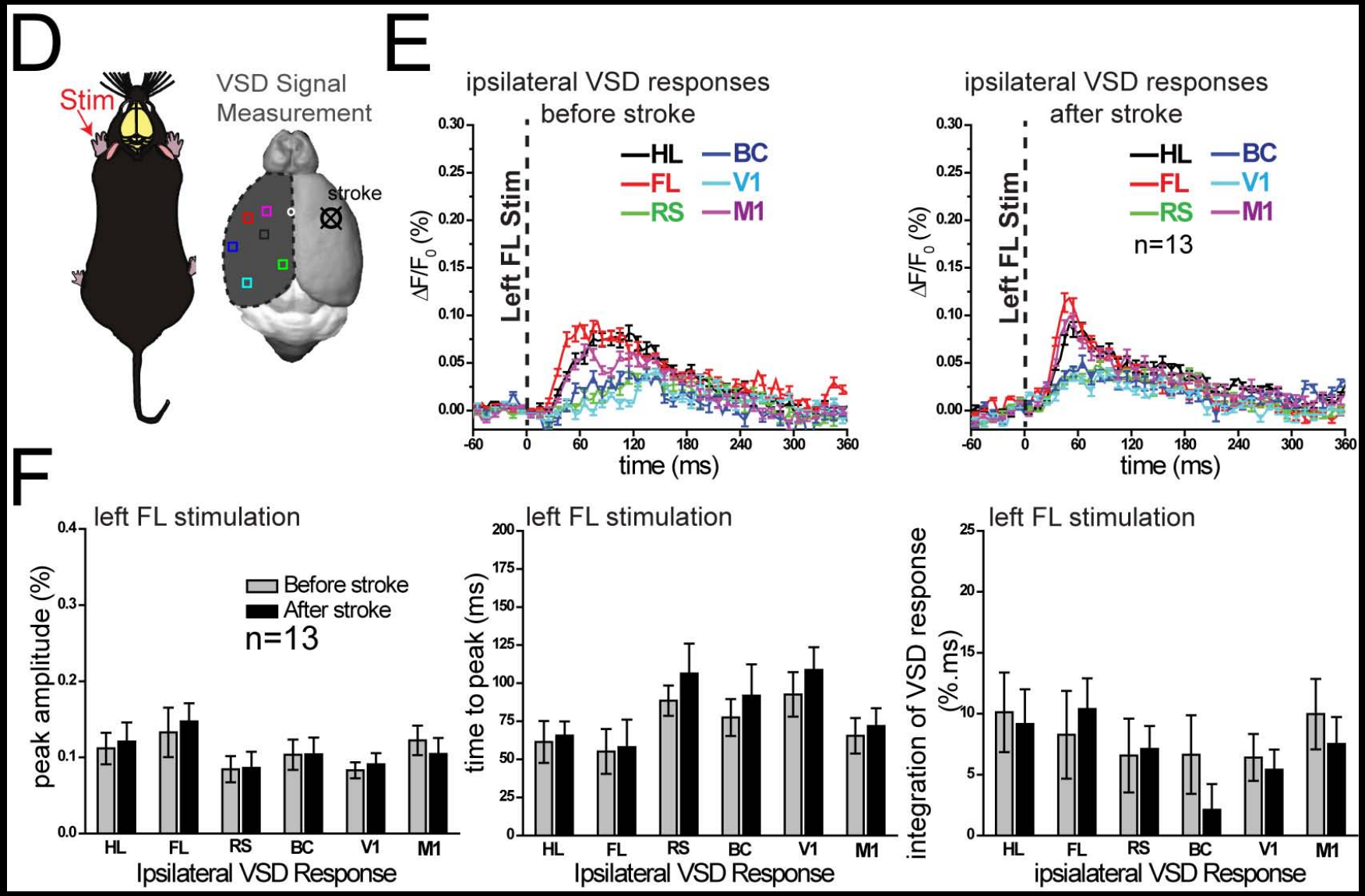




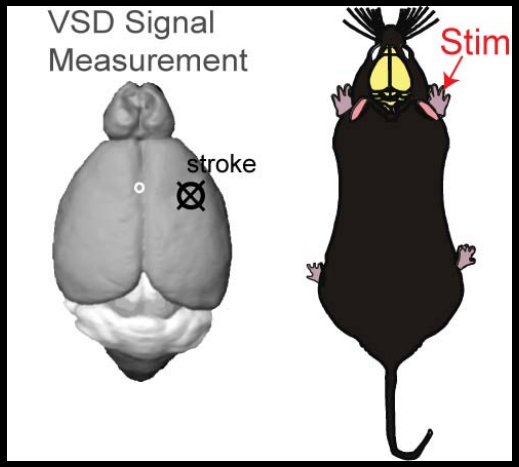




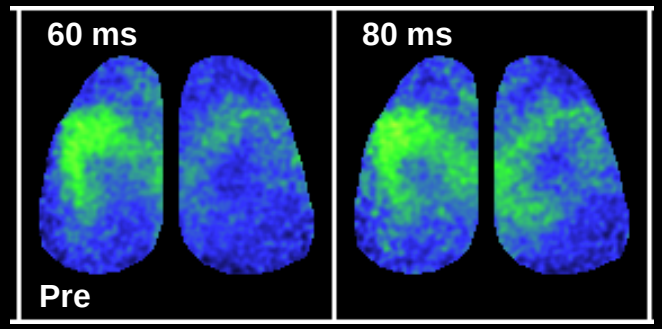
Ipsilateral responses due to stimulation of the affected limb are relatively preserved.



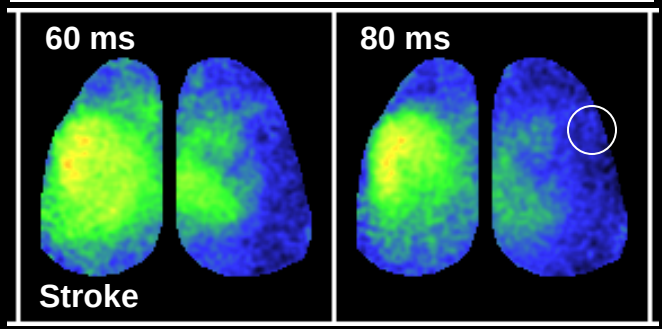
Enhancement of responses within the un-affected hemisphere produced by stimulation of right forelimb (un-affected limb).



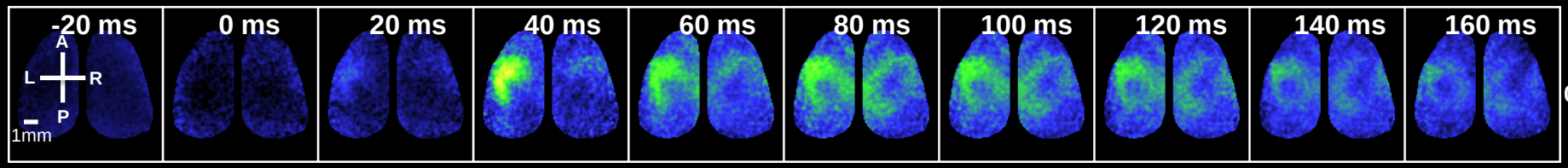
VSD evoked response to stimulation of right FL **before stroke, 60, 80 ms**



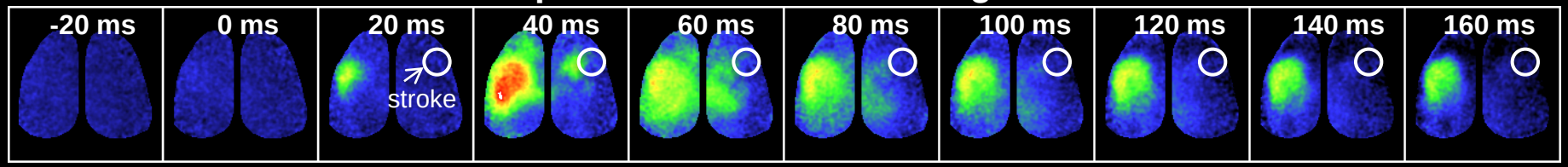
after stroke 60, 80 ms



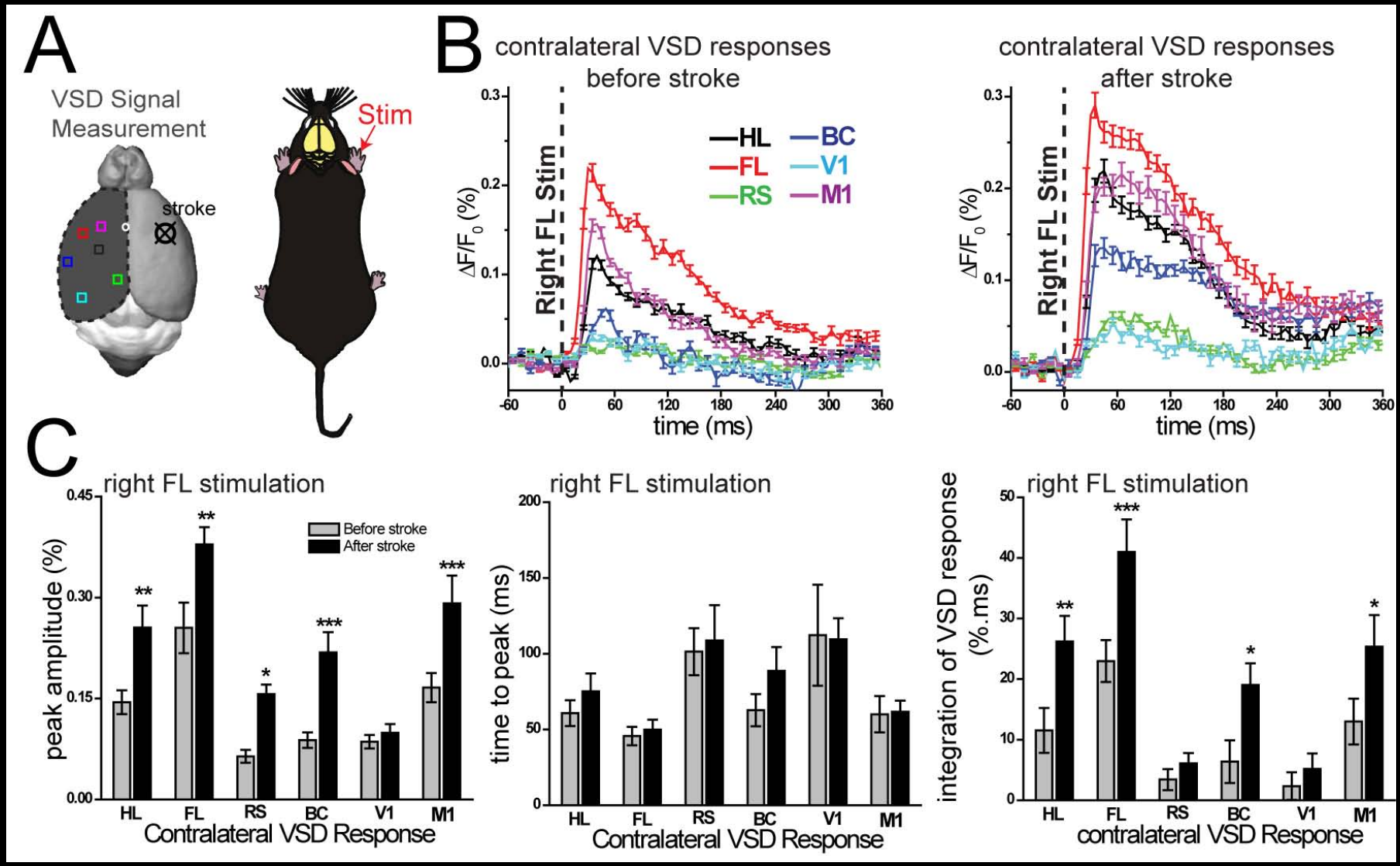
VSD evoked response to stimulation of right FL **before stroke**



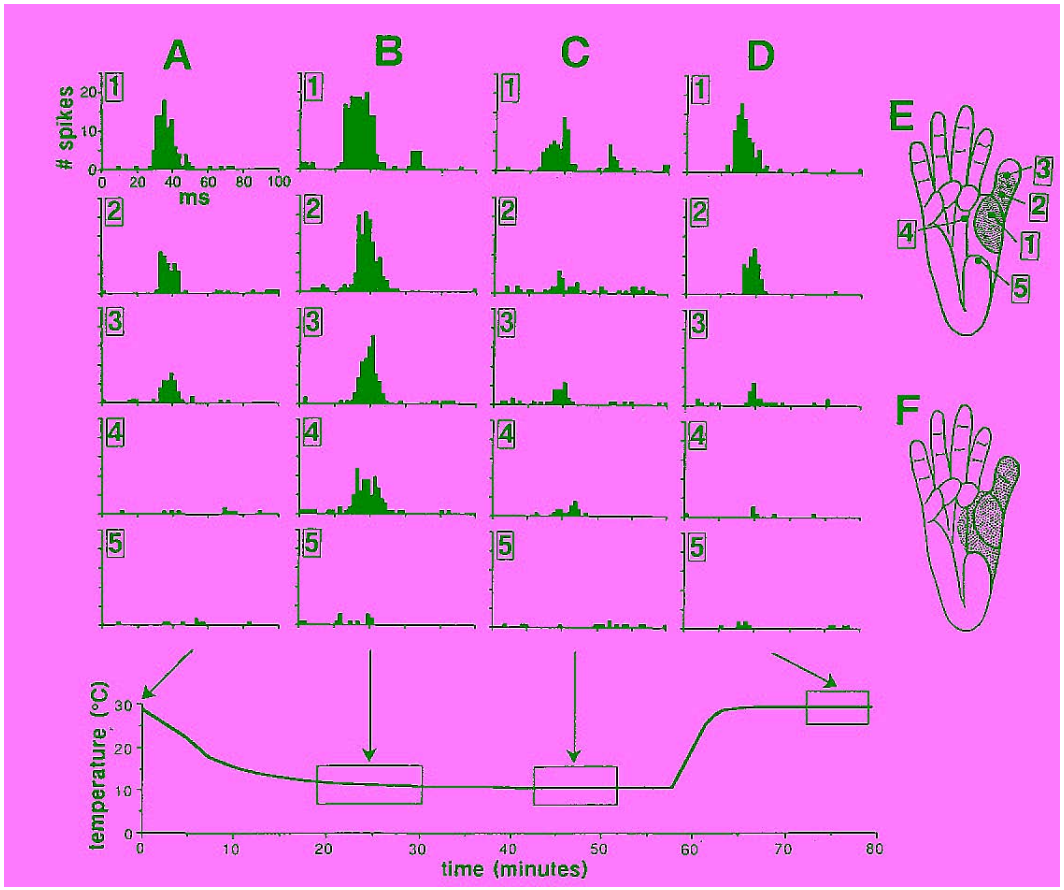
VSD evoked response to stimulation of right FL **after stroke**



Contralateral VSD responses due to stimulation of the un-affected limb are significantly enhanced <2 h after stroke.



Intracortical dis-inhibition has been observed with focal cooling in primate models.

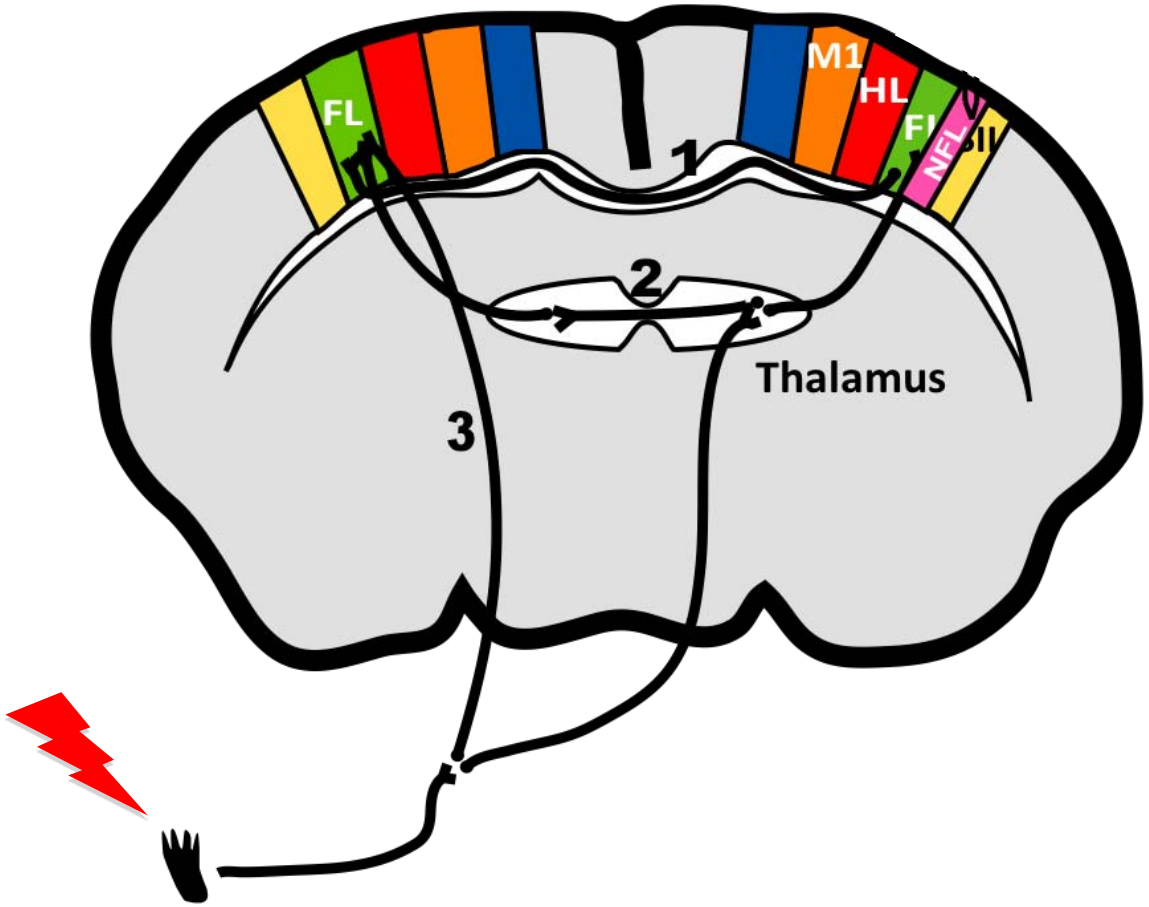


Calford et al. 1996 J Cereb. Cortex

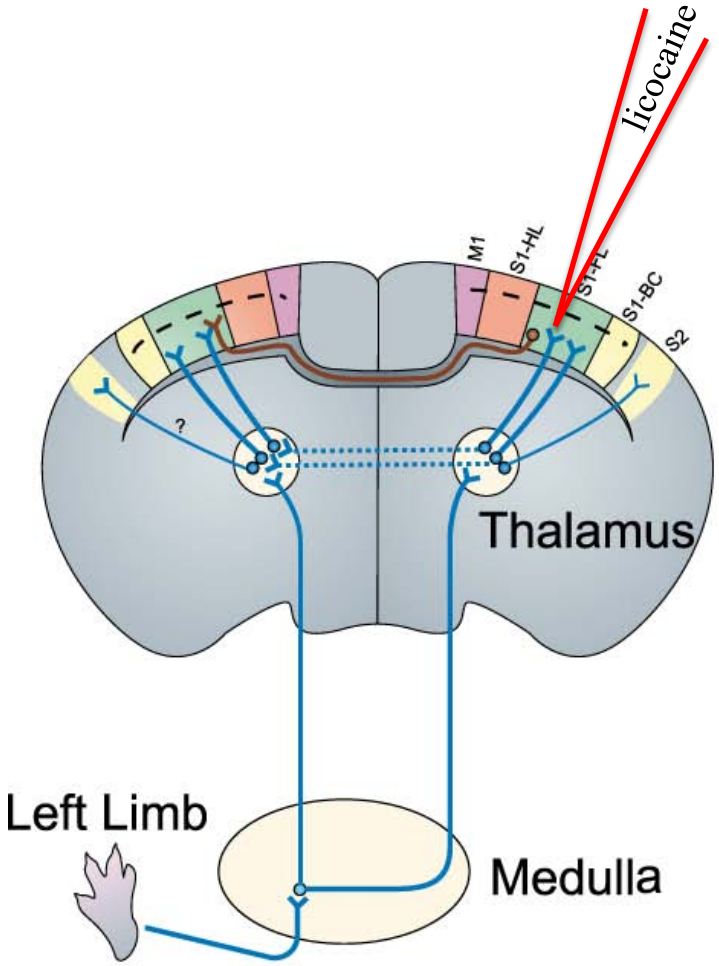
Hypothesis:

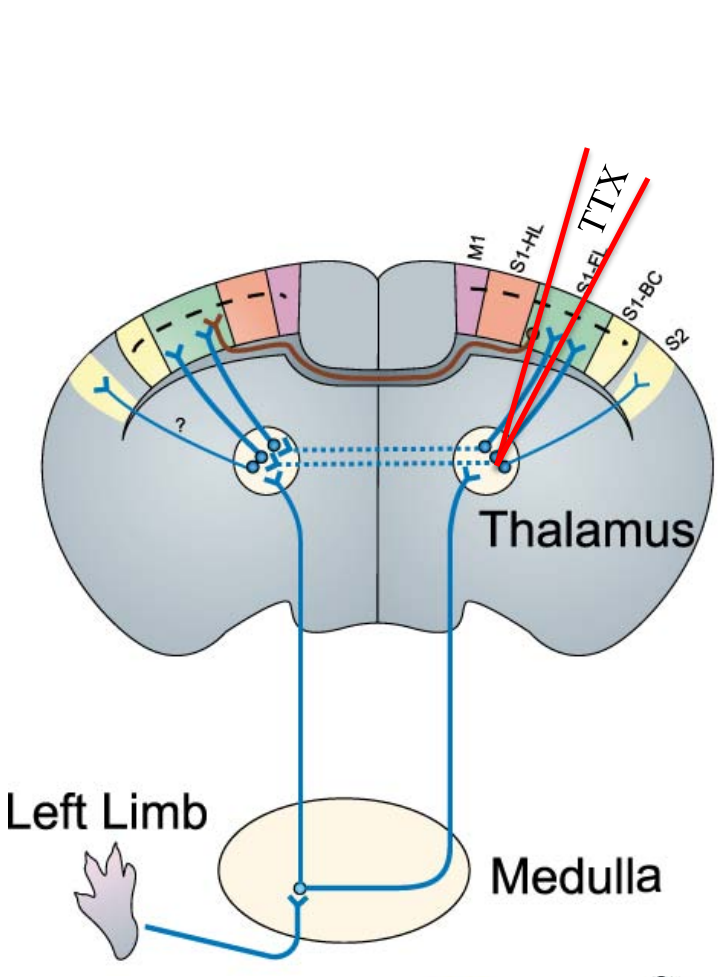
After a large focal ischemic insult to the forelimb and hindlimb somatosensory cortex,

1) transcallosal, 2) sub-cortical or **3) un-crossed circuits are responsible for ipsilateral activation.**

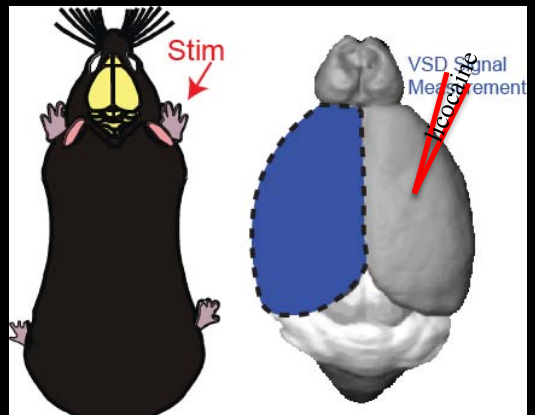


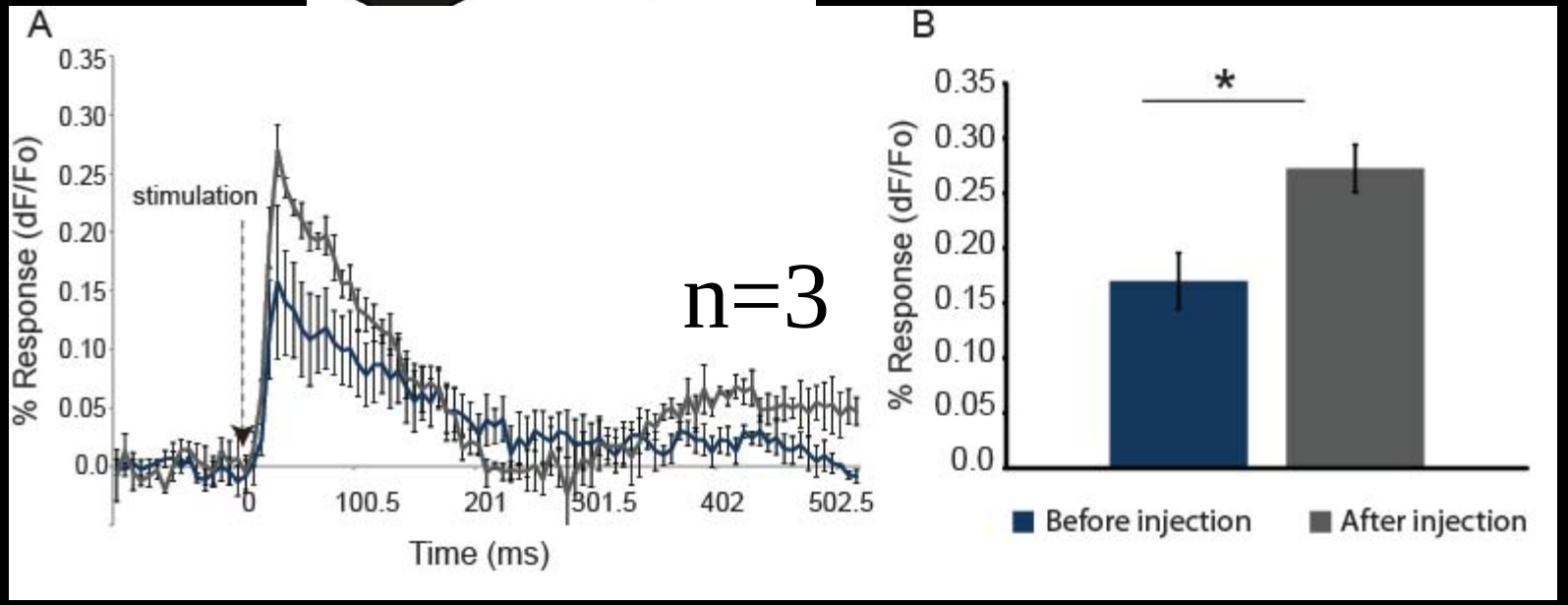
Silencing versus Stroke?



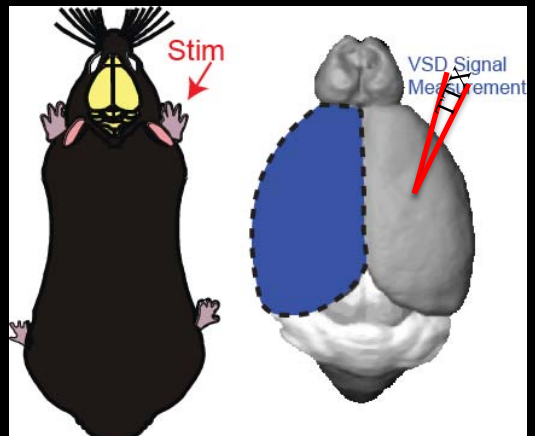


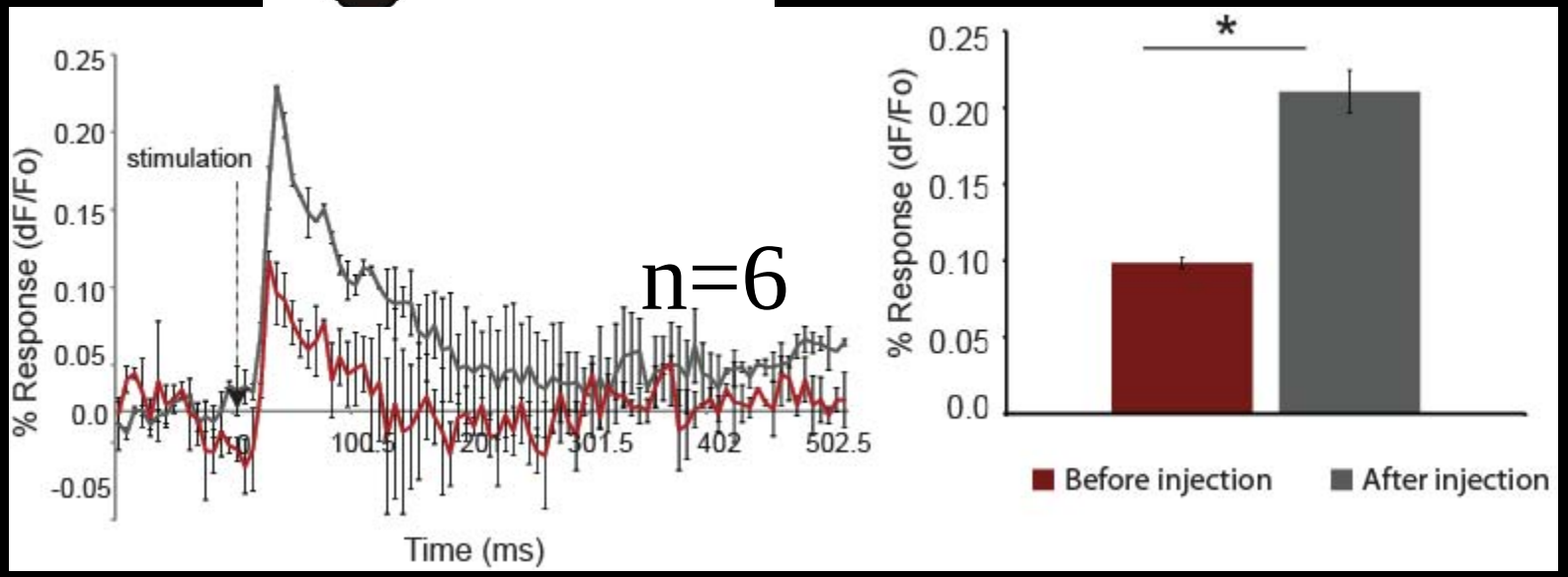
Contralateral VSD evoked response to stimulation of right FL before and after silencing the right cortex.



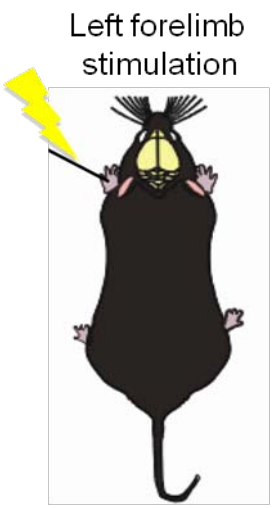


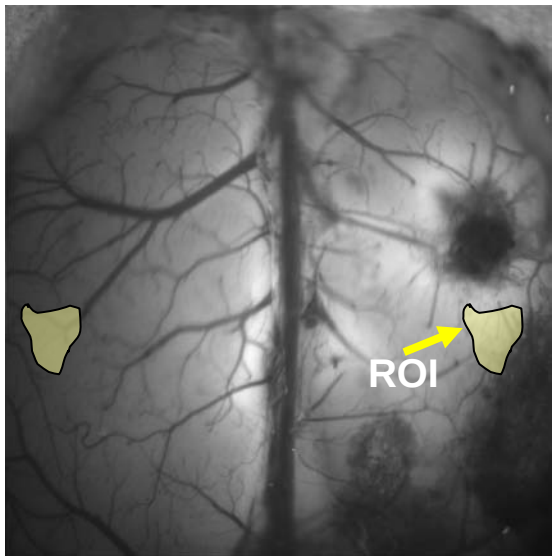
Enhancement of contralateral VSD evoked response to stimulation of right FL before and after acute (< 2h) silencing of right thalamus.

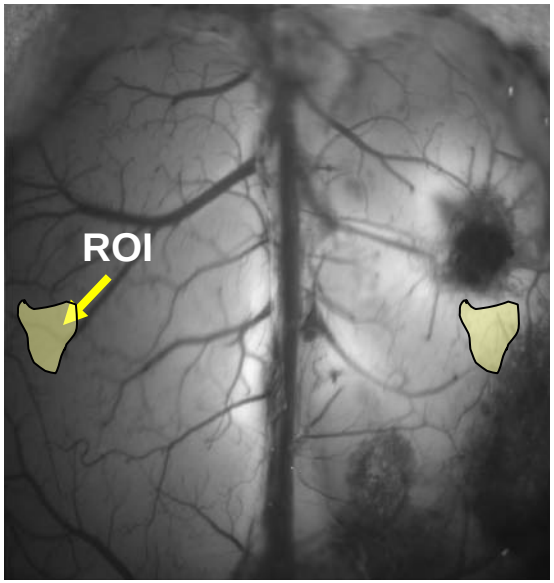


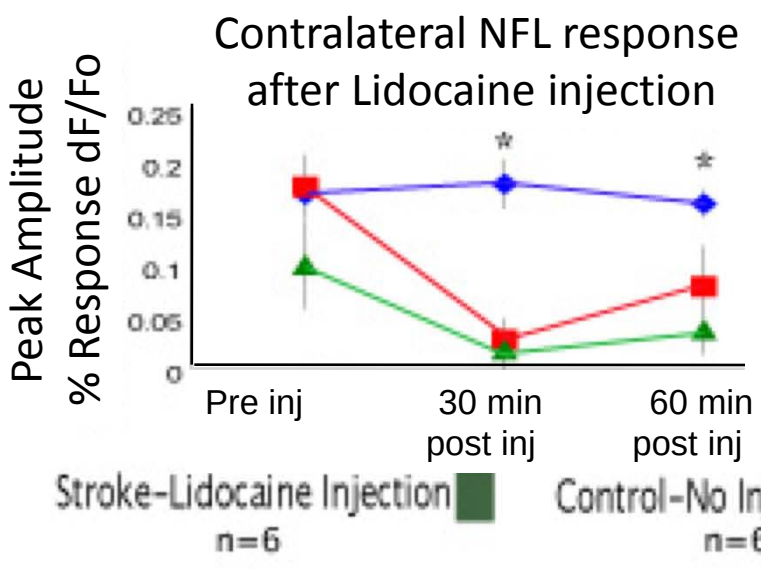


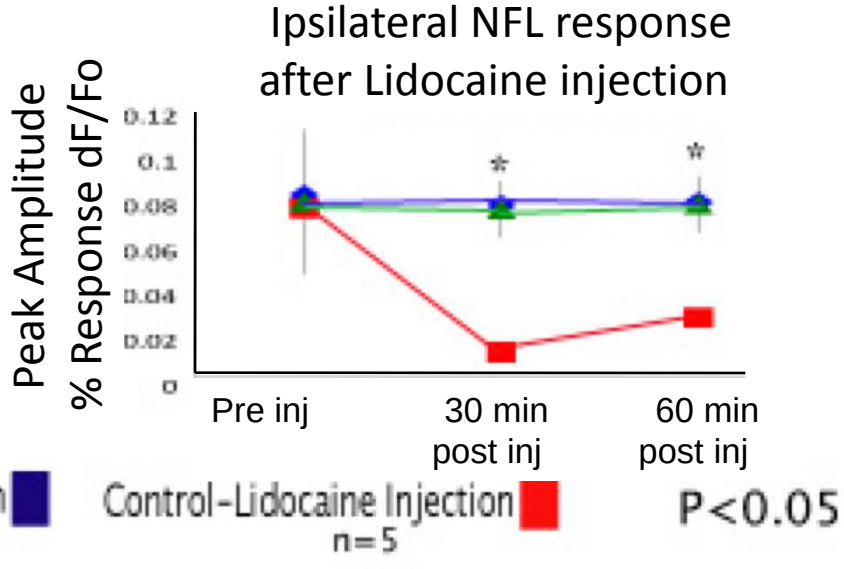
Cortical lidocaine blocks sensory responses in control but not stroke recovering animals.





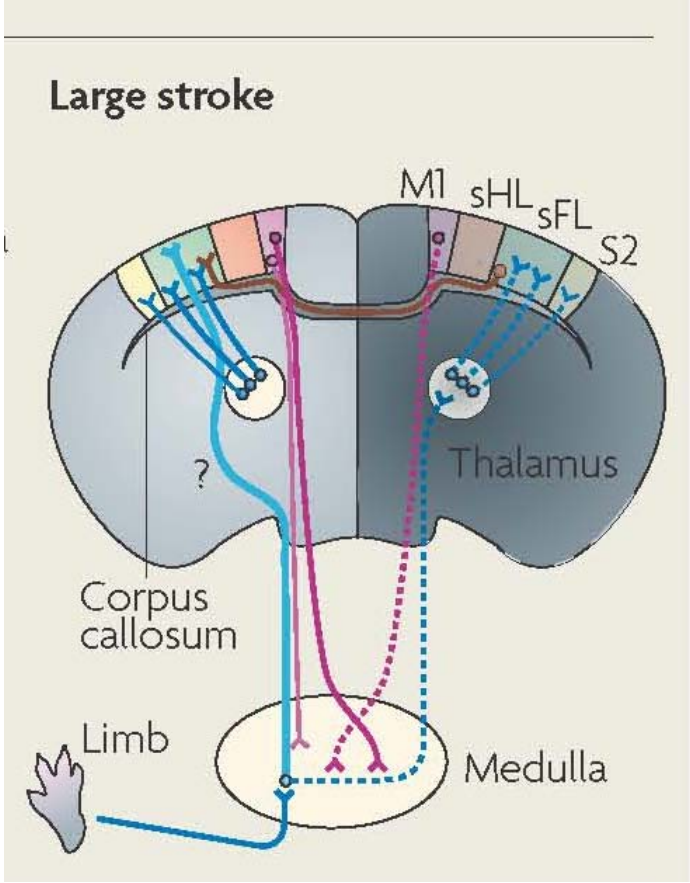






What is the difference between acute and chronic stroke for cortical circuits?

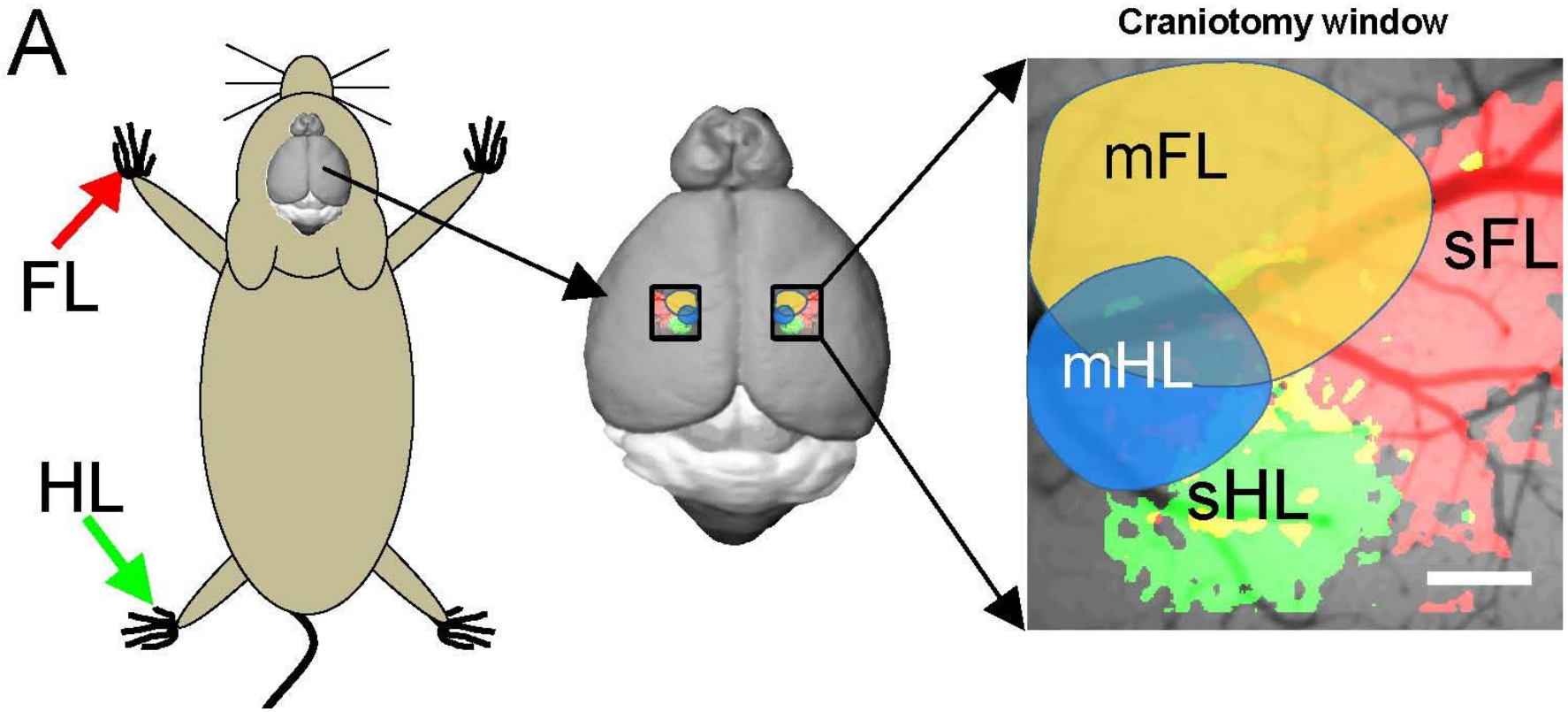
- Ipsilateral responses in 8 weeks post-stroke animals were found to not be dependent on the contralateral hemisphere, while in unlesioned animals the response was silenced by lidocaine injection.



Intra and interhemispheric redistribution of sensory processing is observed both hours and weeks after stroke.

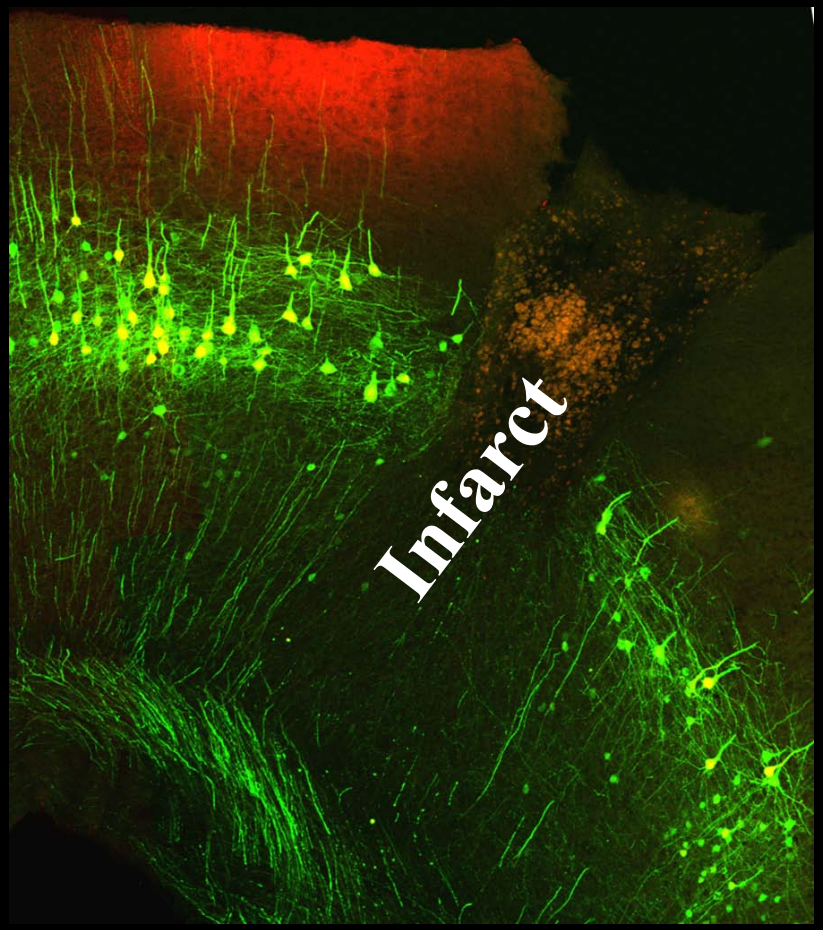
- Similar changes in intra and interhemispheric processing are seen at 2 hr time points too early to be attributed to new circuitry.
- Local pharmacological silencing of activity in either thalamus or cortex can also lead to re-distribution of sensory processing suggesting the effect of stroke is mainly due to a loss of local activity that could lead to intra or interhemispheric dis-inhibition.
- Although changes in activity patterns can be observed at early time points whether the effects observed at 8 weeks are entirely explained by this mechanism is still unclear.

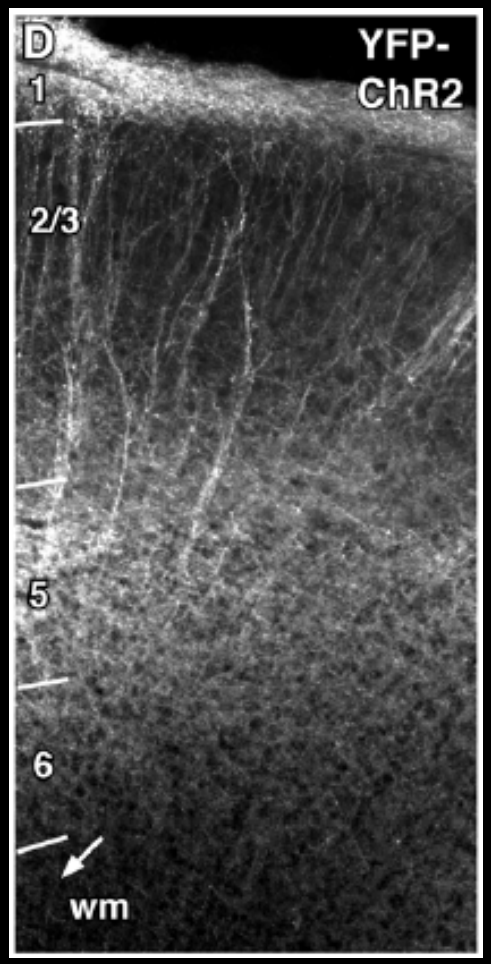
Nearby regions with similar function aid remapping of stroke-affected tissues.



Sensory Forelimb (sFL), Motor Forelimb (mFL)
Sensory Hindlimb (sHL), Motor Hindlimb (mHL)

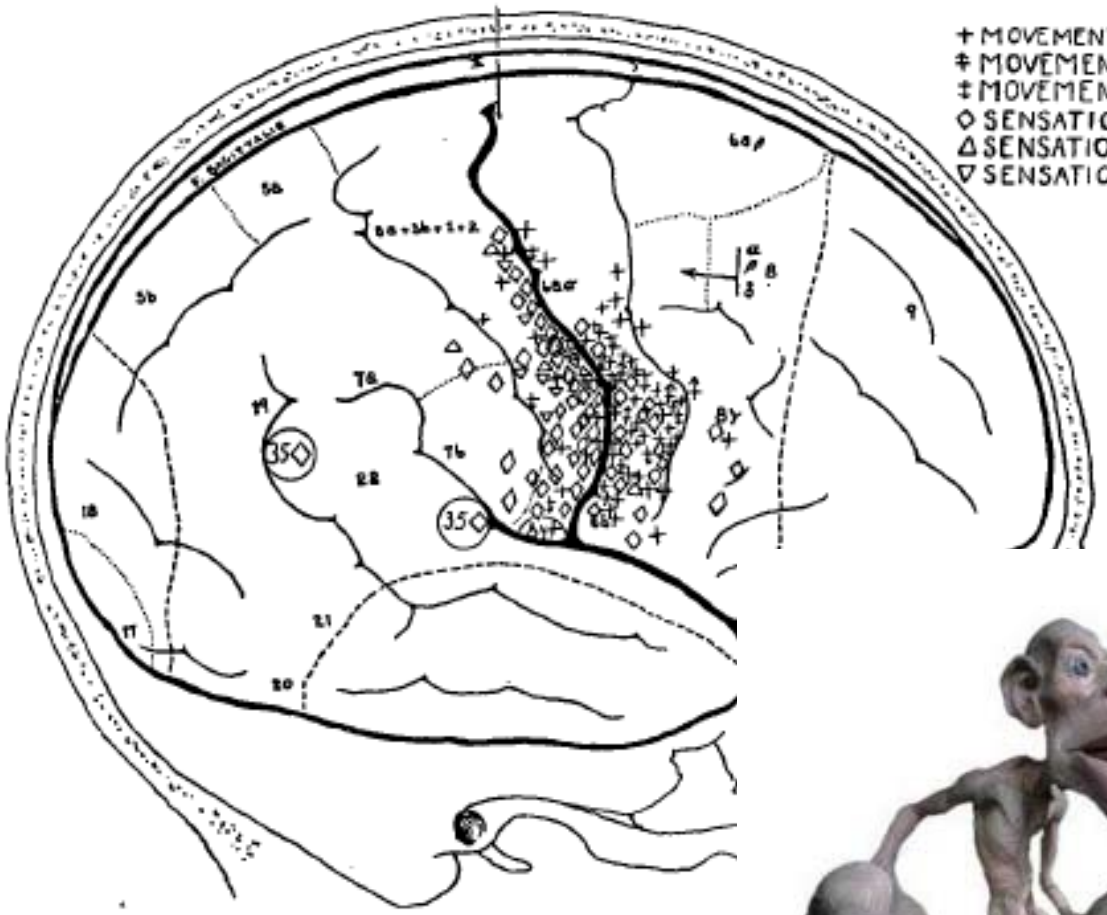
We needed a method to study motor cortex function after stroke?

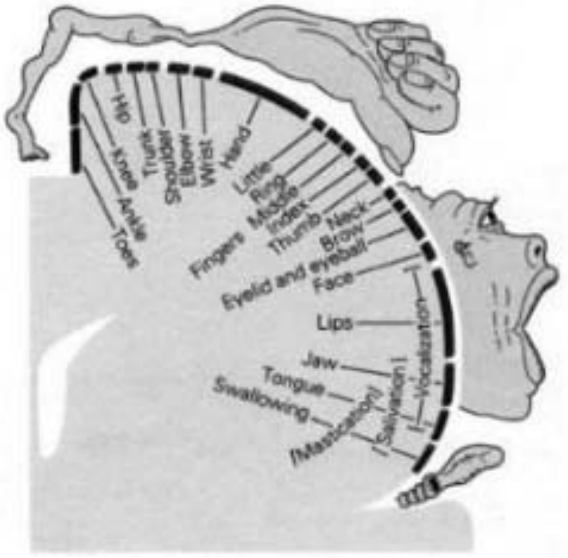




ChR2 mouse from Arenkiel, *Neuron* **54** (2), 205 (2007).

The motor cortex has a crude somatotopic organization based on cortical electrode stimulation.





PENFIELD AND BOLDREY 1937

Intracranial microstimulation motor mapping using electrodes.

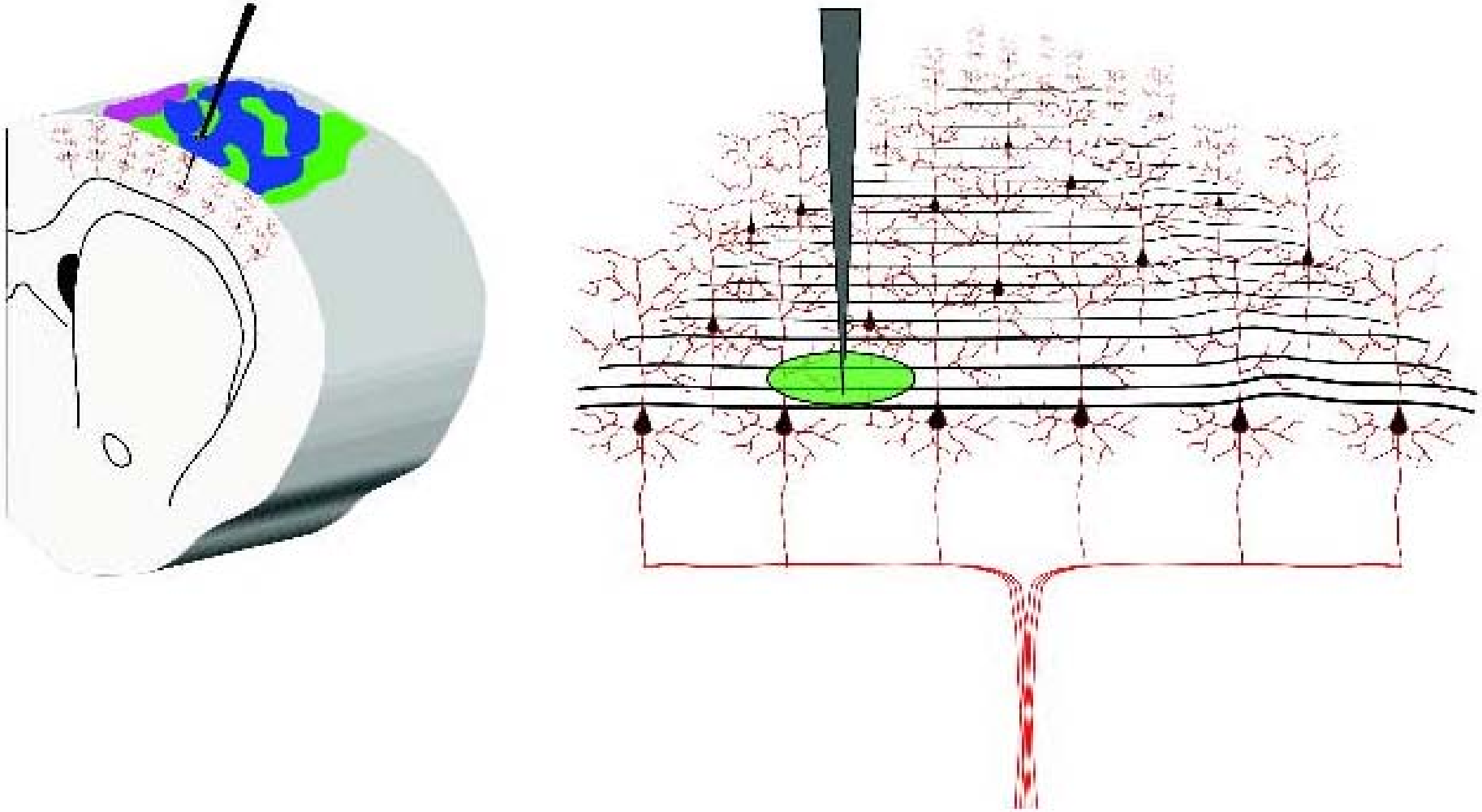
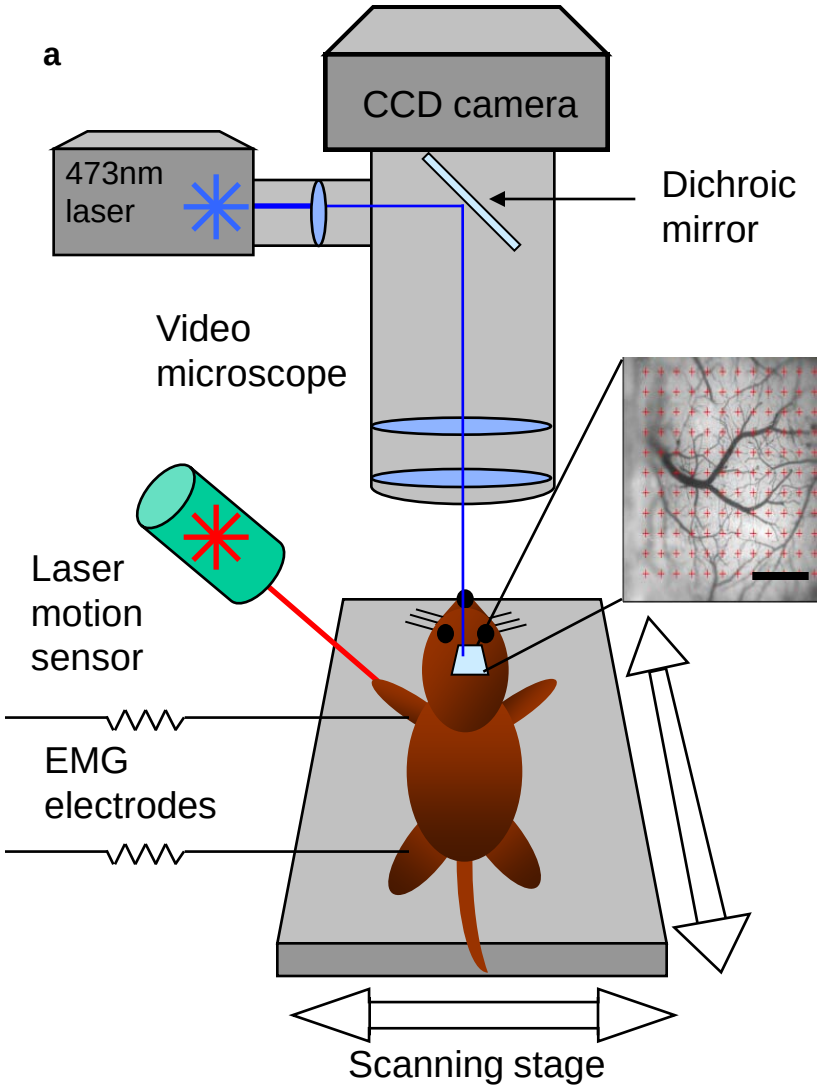


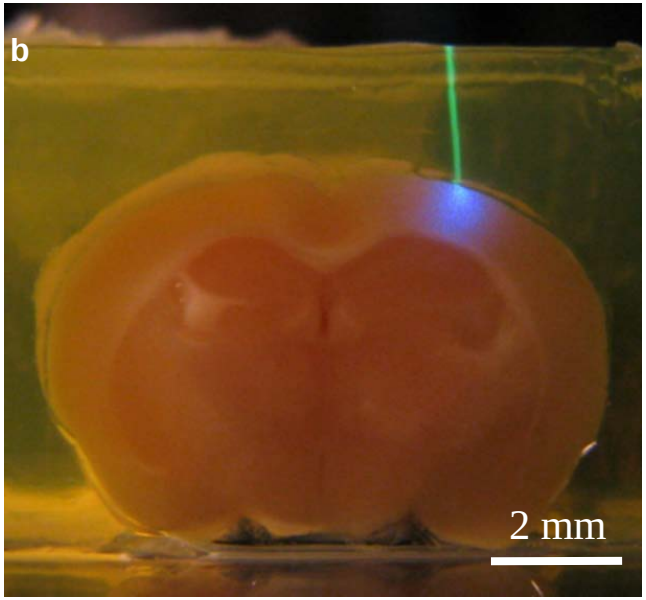
Fig. 3. Diagram illustrating how microstimulation evokes movement within the rat motor cortex. A microelectrode is lowered into cortical layer V, and small amounts of current are passed that stimulate corticospinal neurons transsynaptically via intracortical afferents.

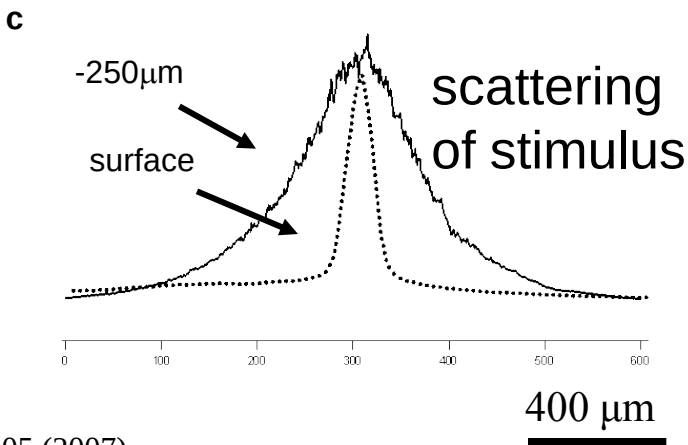
from Monfils et al. 2005

Automated motor mapping using ChR2 mice.

(ketamine/xylazine or ketamine diazepam anest.)

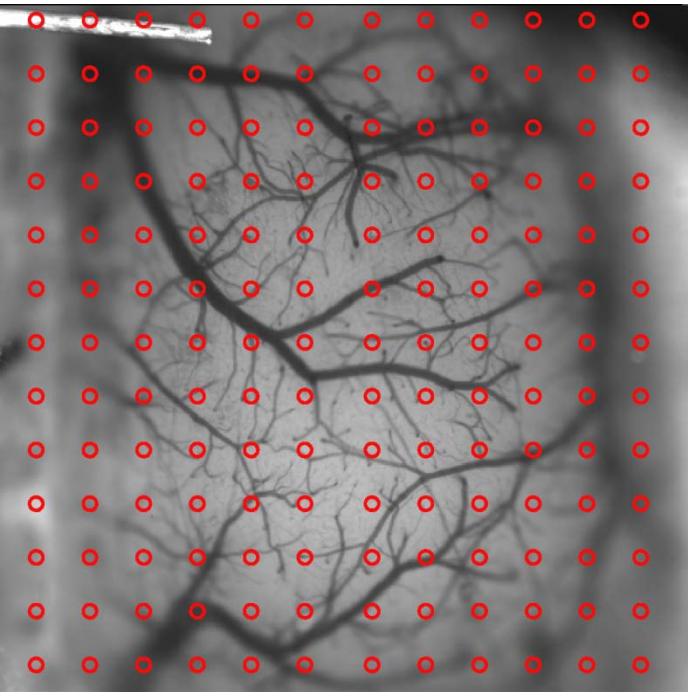




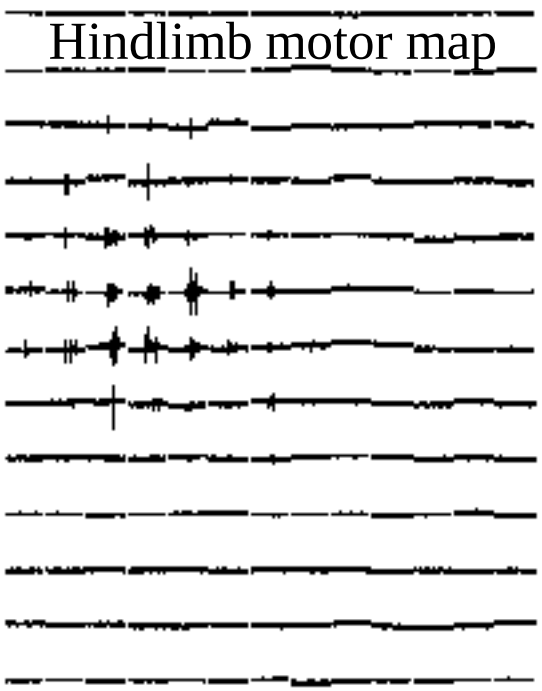


ChR2 mouse from B. R. Arenkiel, J. Peca, I. G. Davison et al., *Neuron* **54** (2), 205 (2007).

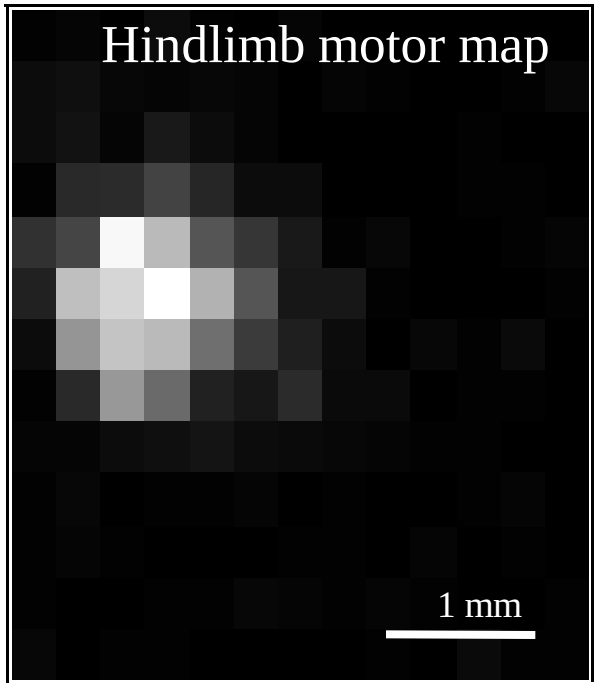
Automated mapping and laser deliver system Ayling et al. 2009 Nat. Meth.

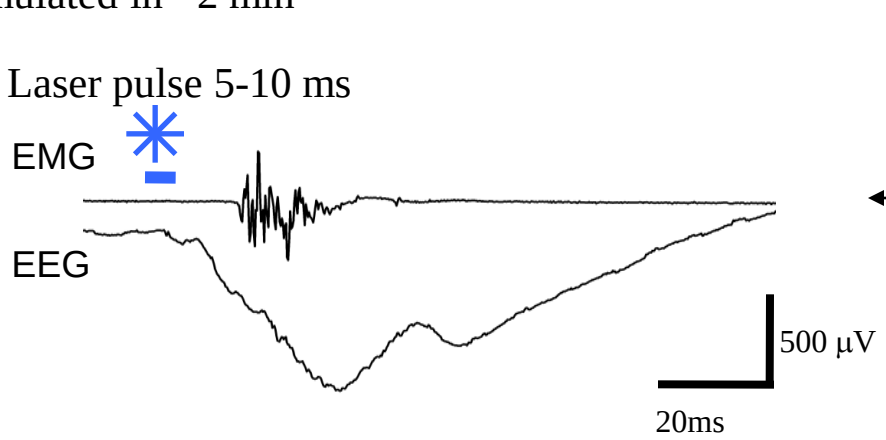


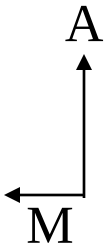
Stimulation points: 13x13, 300 μm spacing
169 points stimulated in ~2 min

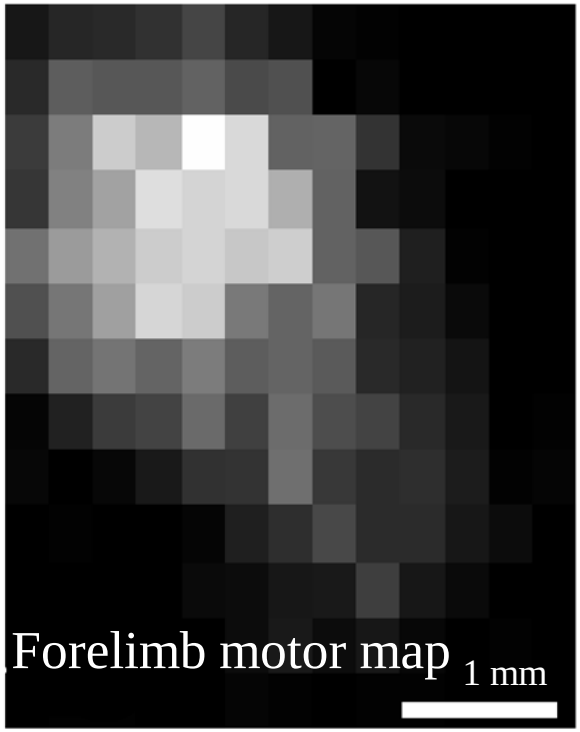


Raw EMGs 13x13



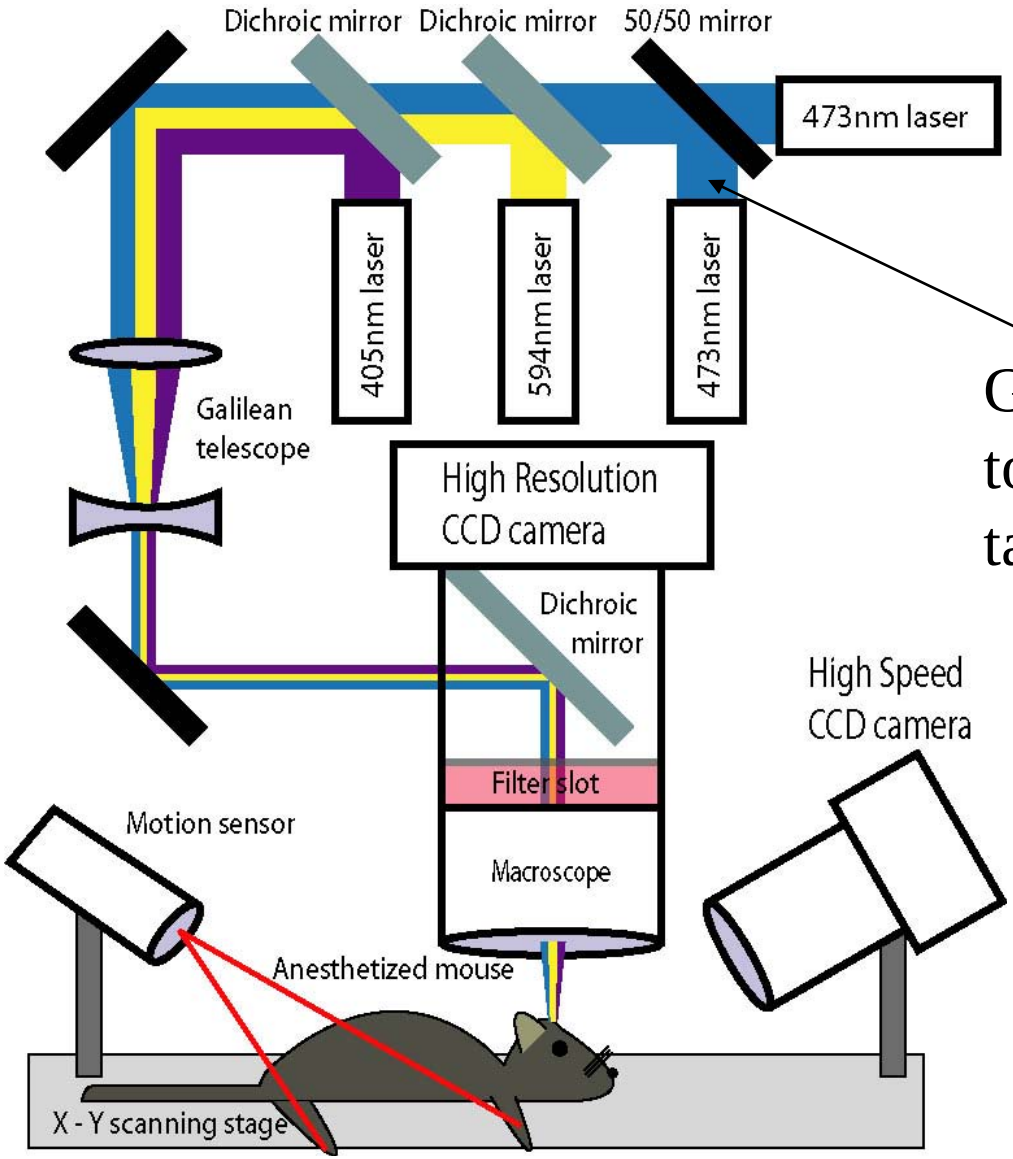






EMG-based maps from photoactivation

Multi-laser optogenetic brain manipulation.



Galvos added to independently target lasers.

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- **Majid Mohajerani**
- **Khatereh Aminoltejari**
- **Craig Brown**
- **Ian Winship**
- **Ping Li**
- **Albrecht Sigler**
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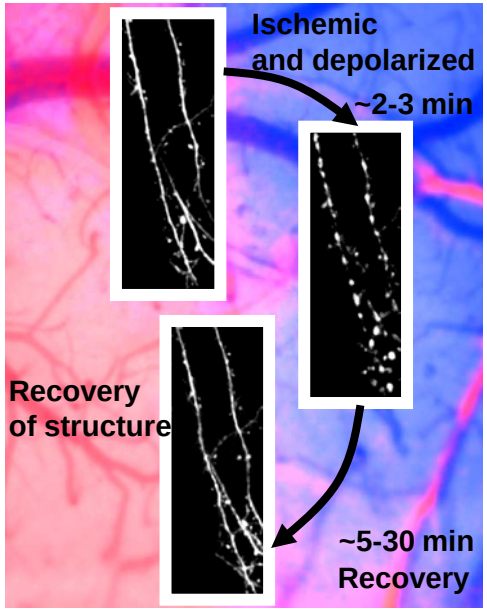
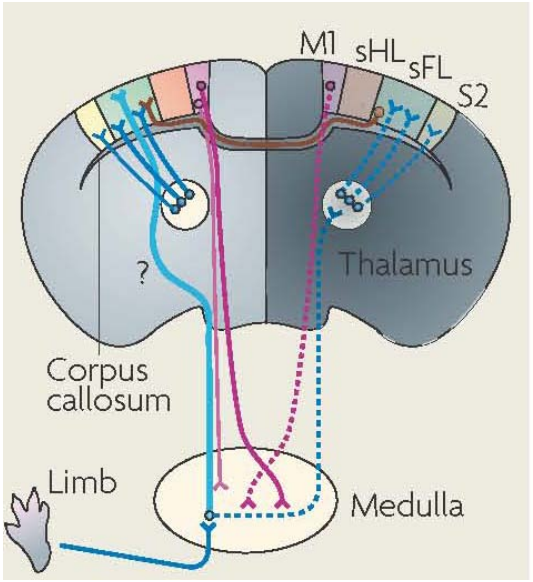
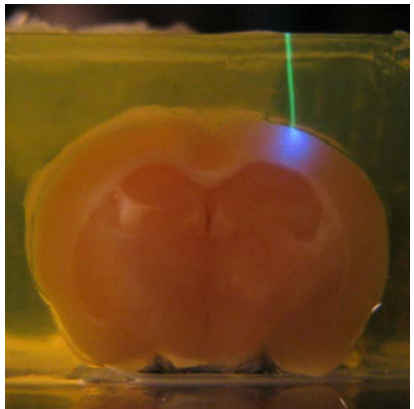
- Kerry Delaney/Jamie Boyd
- **Alexander Goroshkov**
- Cindy Jiang, Heidi Erb, Pumin Wang
- Supported by HSF of BC and Yukon, CIHR, and the CSN.



Stroke time
Minutes.



Stroke time
weeks.



Novel mapping
paradigms.