Anatomical and Functional NeuroImaging of Animal Models

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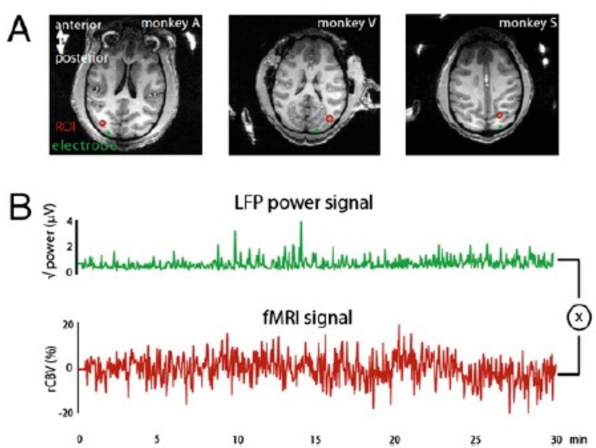
National Institute of Neurological Disorders and Stroke

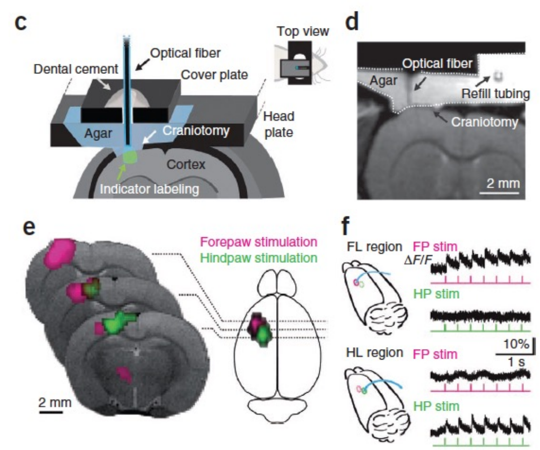
National Institutes of Health

Advantages of Animal Models

- Comprehensive, multi-modal investigations
  - fMRI + Electrophysiology
  - fMRI + Optical Imaging
  - Pharmacological Manipulations
  - Genetics, etc.

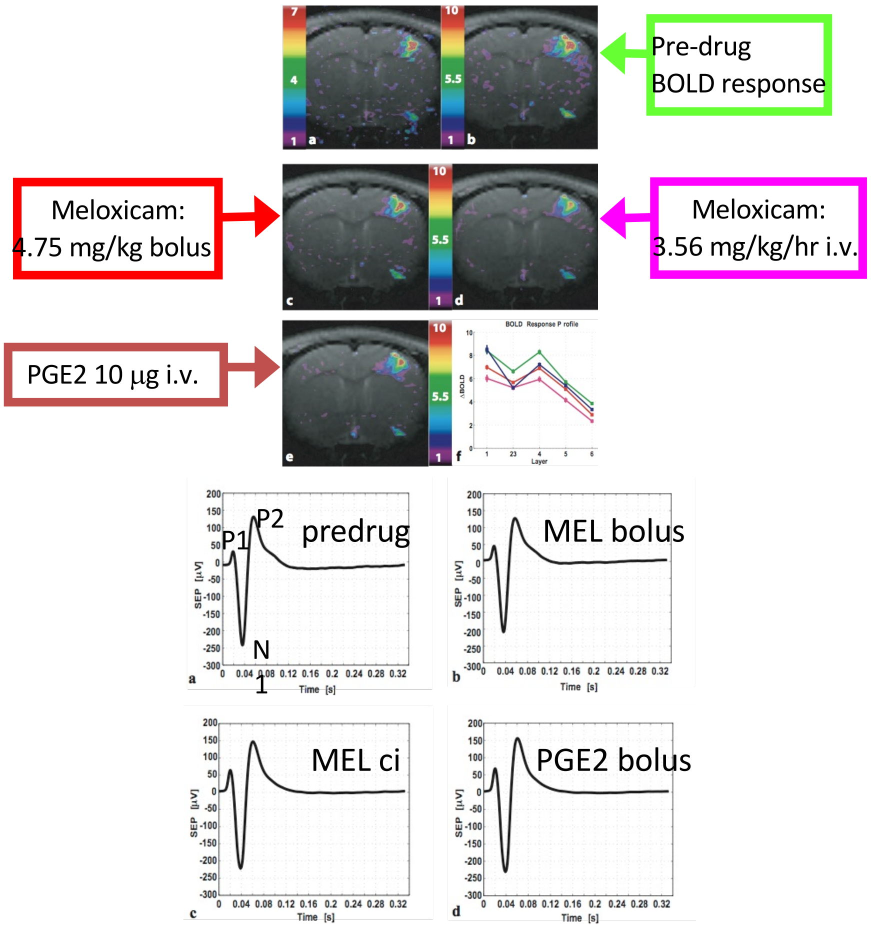


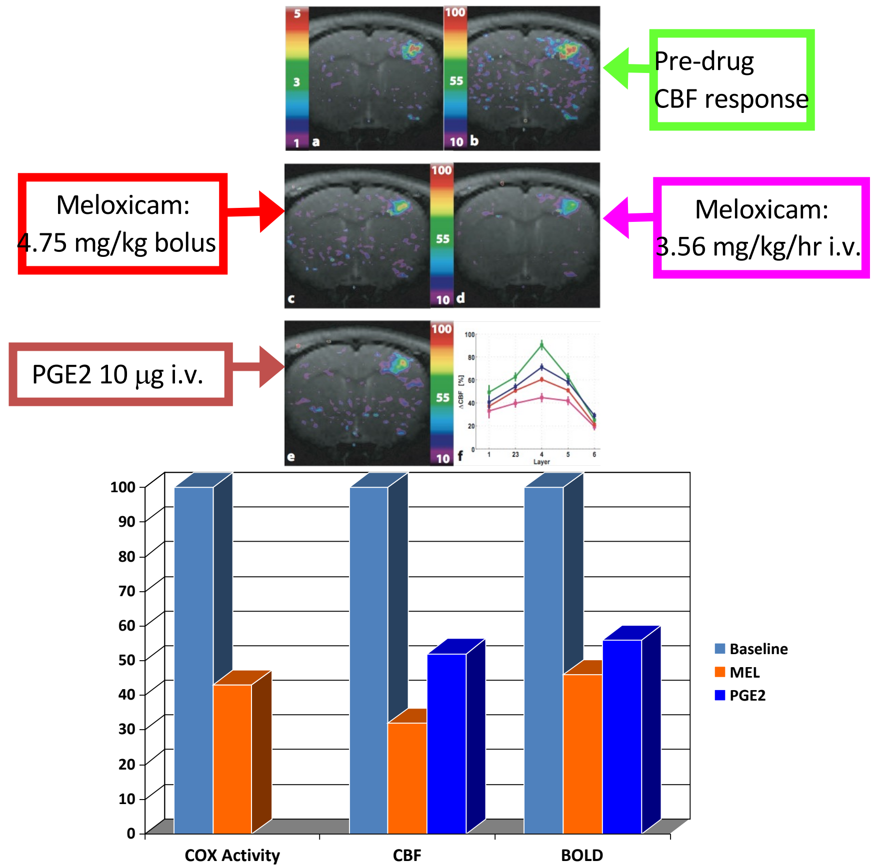
Schölvinck et al. Proc Natl Acad Sci USA. 2010;107(22):10238-43



Schultz et al. Nat Methods. 2012;9(6):597-602

Pharmacological Inhibition of COX-2 Uncouples Hemodynamics from Neural Activity





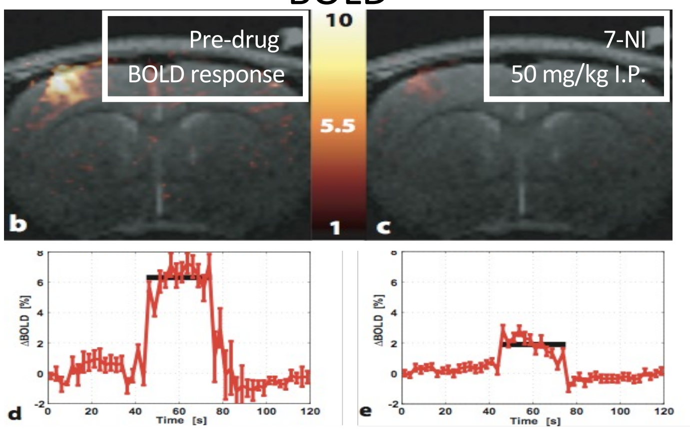
↓ to 43±14 % ↓ to 32±2 % ↓ to 46±1 %

↑ to 52±3 % ↑ to 56±2 %

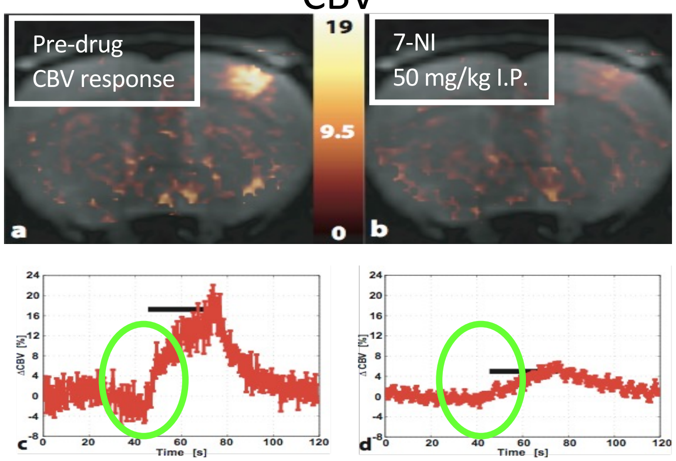
Stefanovic et al, Neuroimage 2006,32(1): 23-32

Pharmacological Inhibition of Nitric Oxide Uncouples BOLD from CBF

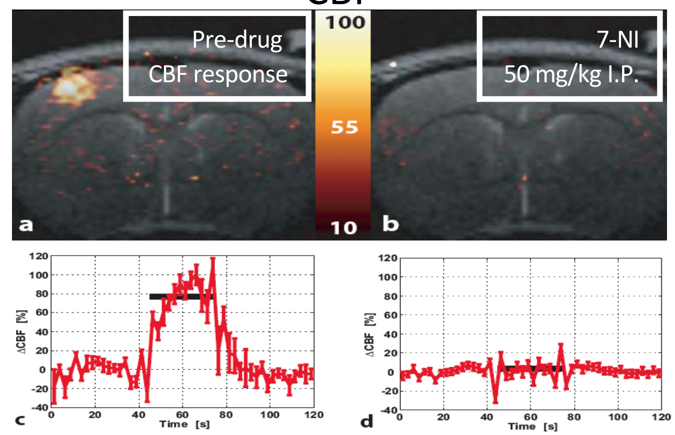
BOLD

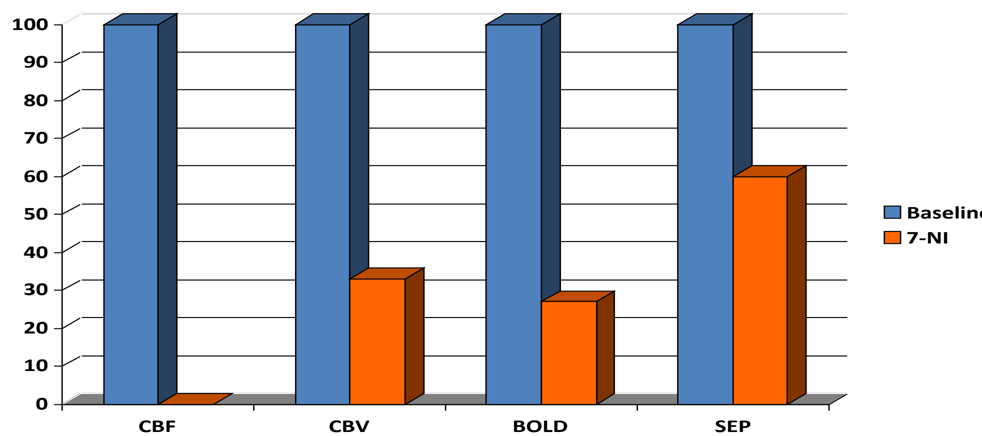


CBV



CBF

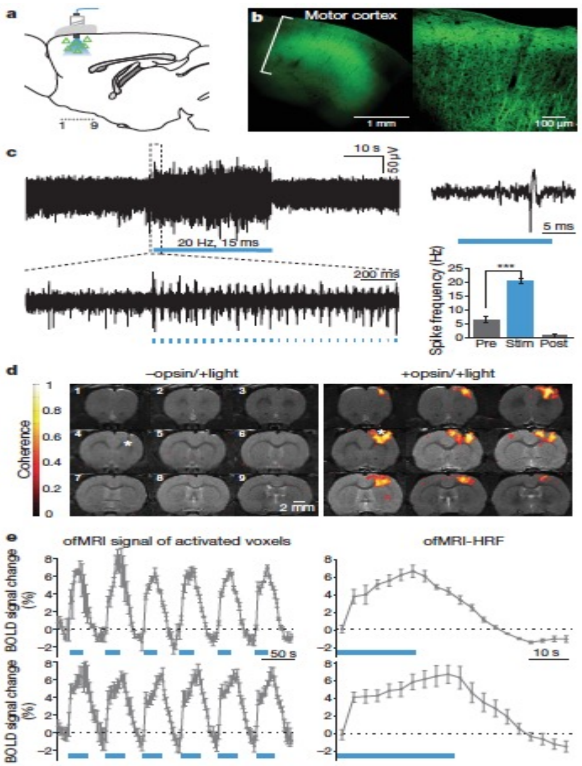




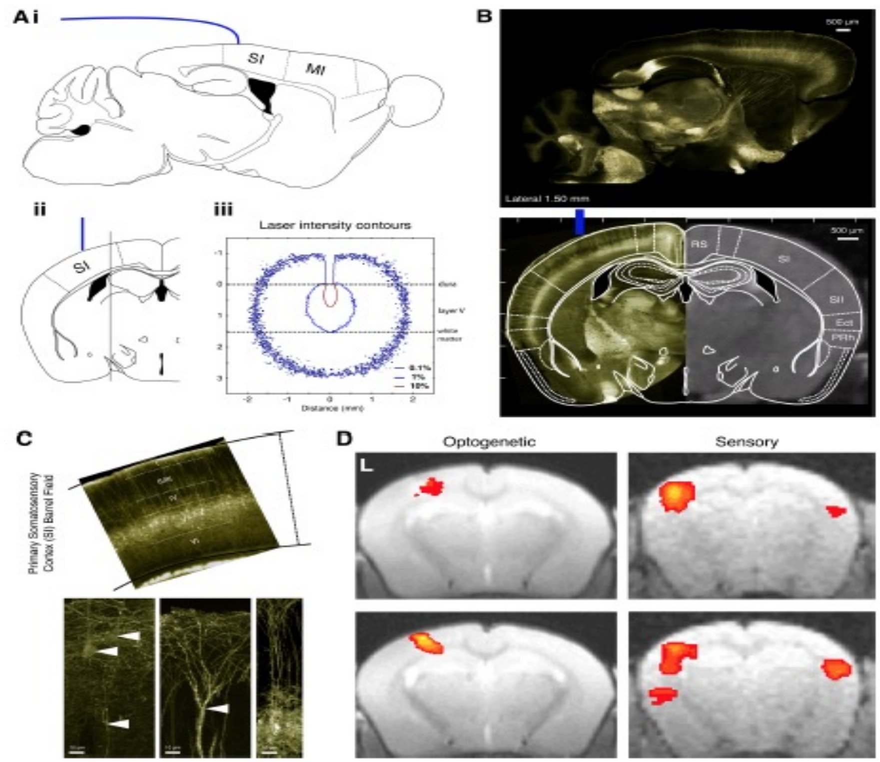
↓ to -1±6 % ↓ to 33±6 % ↓ to 27±13 % ↓ to 60±6 %

Stefanovic et al. J Cereb Blood Flow Metab. 2007;27(4):741-54.

Genetic Manipulations: Optogenetics



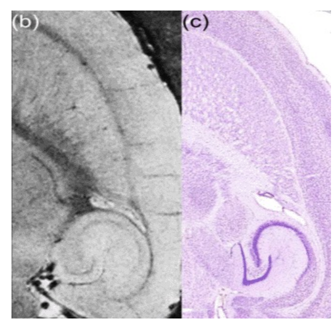
Lee et al. Nature. 2010 Jun 10;465(7299):788-92

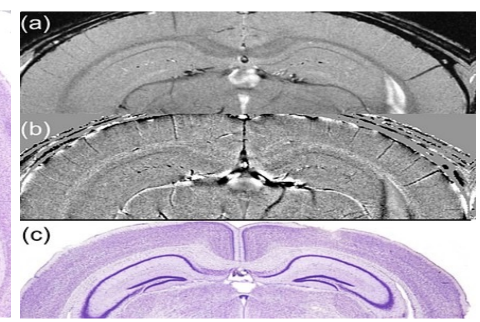


Kahn et al., J Neurosci. 2011 Oct 19;31(42

Advantages of Animal Models

- Technical
  - Ultra-High Field Magnets
    - up to 21T vertical
    - up to 17.6T horizontal
  - Stronger Gradients
    - Up to 1000 mT/m in 12 cm ID
  - Small FOV due to smaller brain size
    - Improved spatial resolution
    - Improved SNR with specialized RF coils



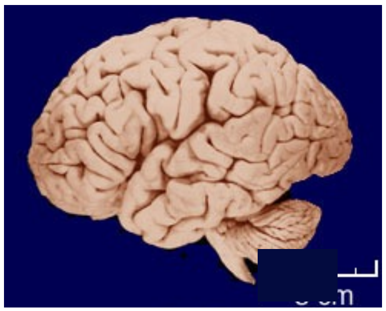


Outline

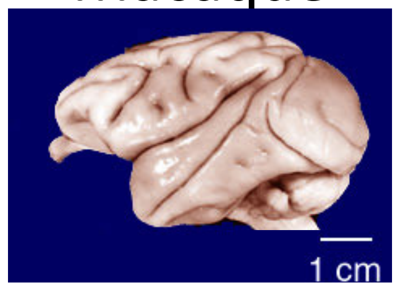
- Marmoset as an animal model of functional brain activation
  - Can be Imaged in Conscious, Awake Conditions
  - In Vivo MRI Reveals Remarkable *Cortical Cytoarchitecture*
    - T1-Weighted MRI
  - *Awake non-human primate multisensory* model of neurovascular coupling
    - Somatosensory Pathway
    - Auditory
    - Resting-State
    - Visual
  - *Direct Visualization* of the Neurovascular Unit in Vivo

Marmosets Retain the Primate Anatomical and Functional Brain Organization

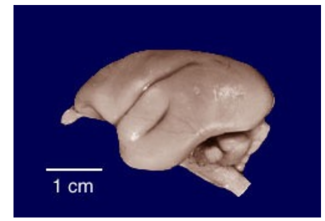
Human



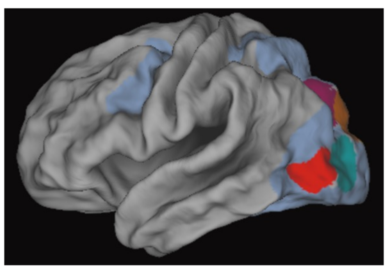
Macaque

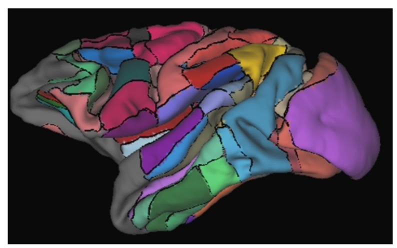


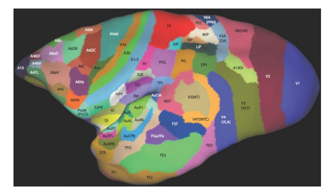
Marmoset



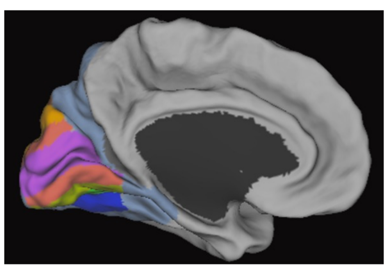
http://www.brainmuseum.org/





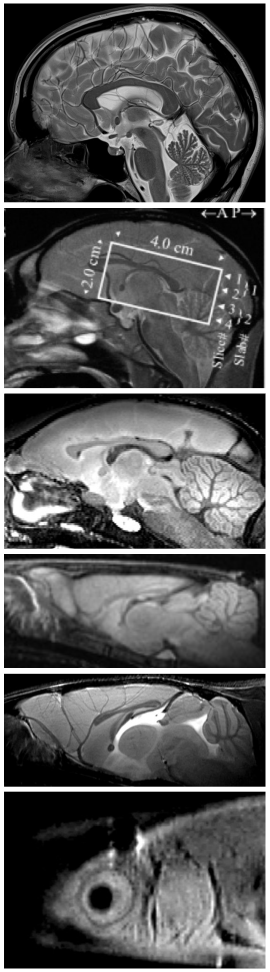


G. Paxinos et al. The Marmoset Brain in Stereotaxic Coordinates, 2011



Web caret, Washington University St. Louis, MO, USA

Marmosets are an Excellent Model in Neuroscience Research



Human

Macaque

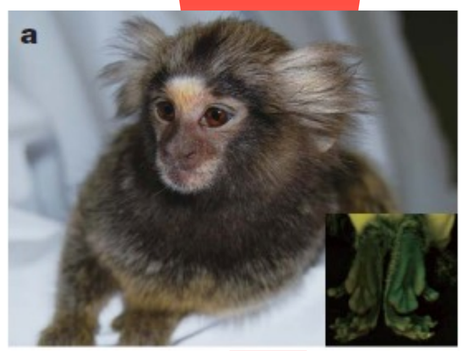
Marmoset

Rat

Mouse

Zebrafish

Neural circuitry
Complexity



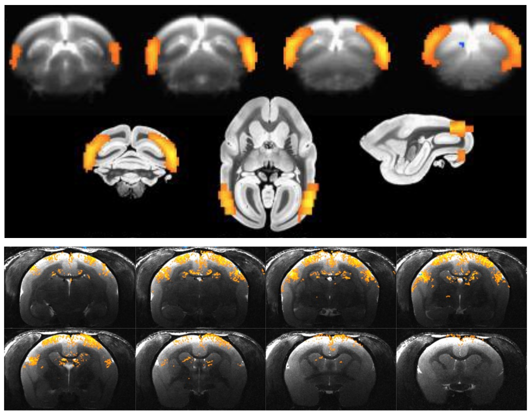
Sasaki et al, Nature. 2009

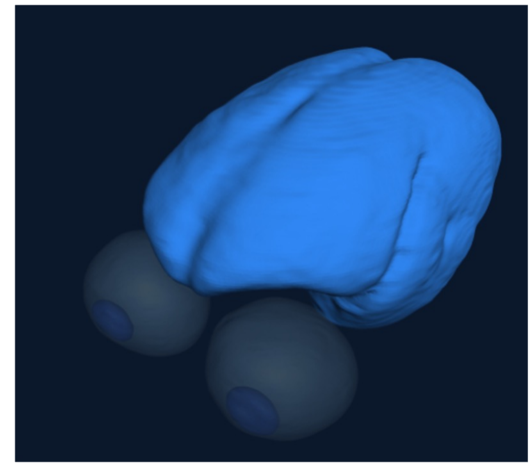
Simpler

Genetic Manipulation

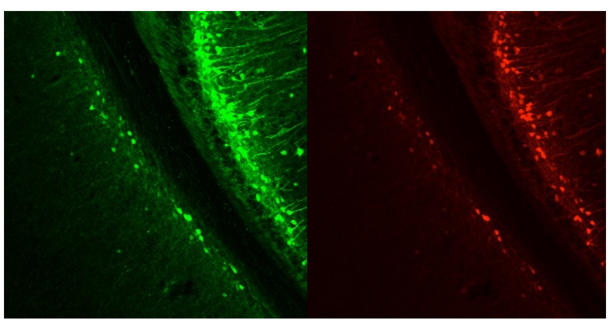
Marmosets are Lissencephalic

fMRI

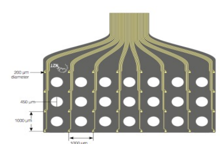


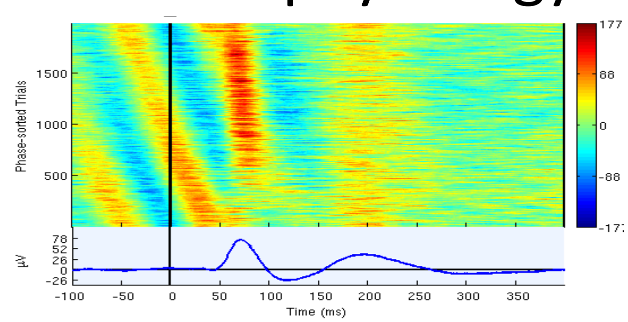


Optical Imaging



Electrophysiology

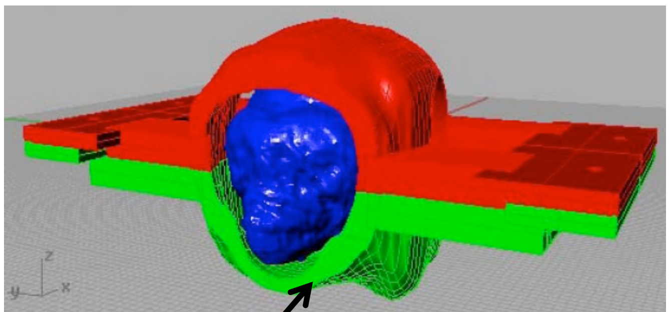




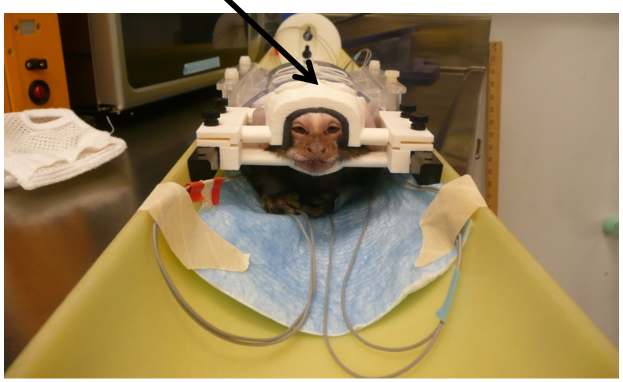
MRI of Marmosets

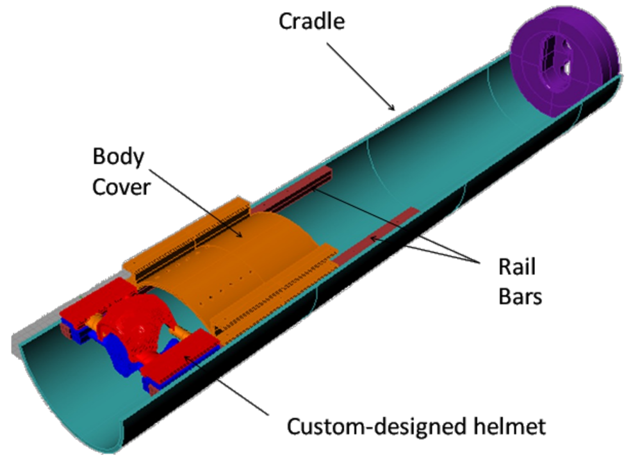
- Two types of setup
  - Anesthetized Animals
    - Isoflurane (anatomic studies)
    - Propofol + Fentanyl (functional studies)
  - Awake
    - Anatomical or functional Studies
- Extensive Physiological Monitoring
  - Temperature
  - Heart Rate
  - Pulse oximetry
  - $ETCO_2$

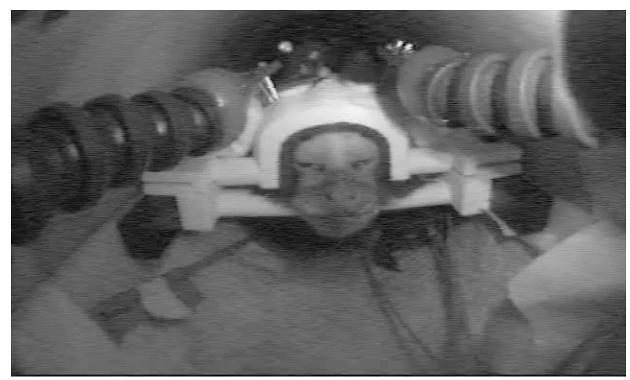
Anatomical and Functional MRI in Conscious, Awake Marmosets



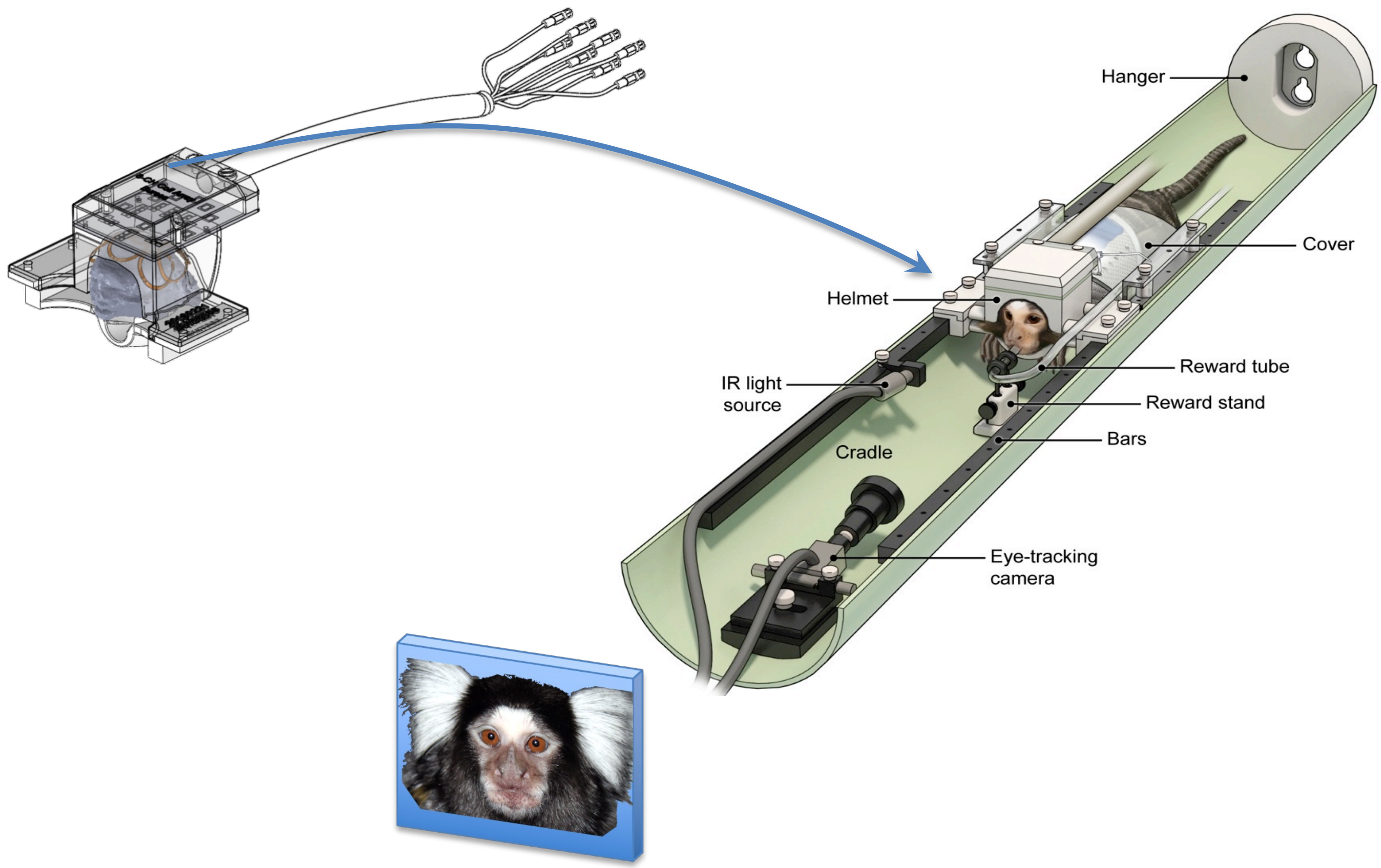
Individual
Helmet



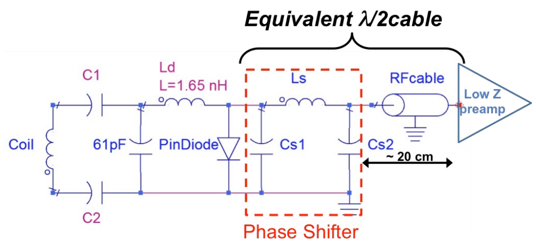




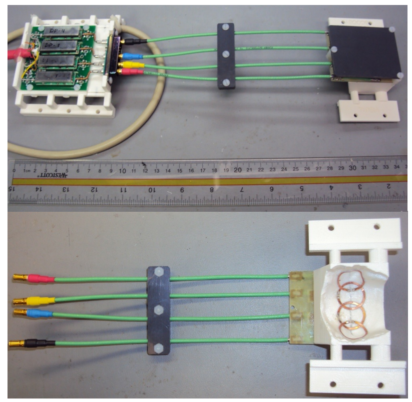
Setup for fMRI of Conscious Awake Marmosets



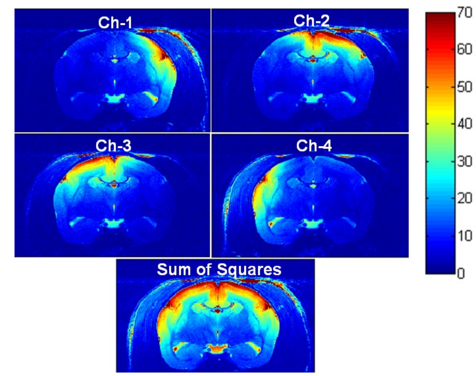
In-Helmet Embedded RF Coil Arrays



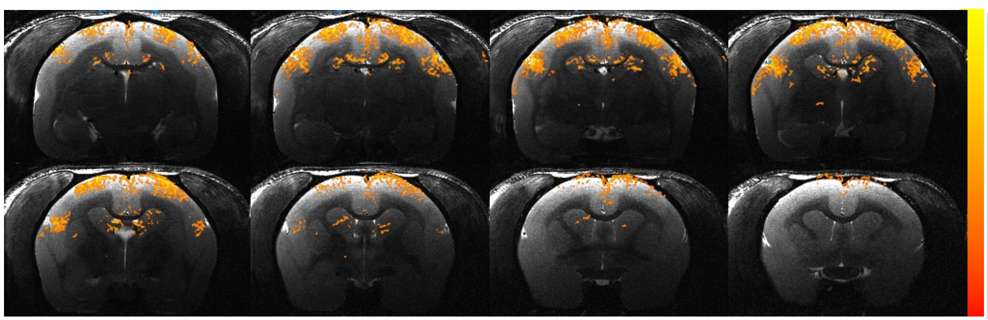
Circuit Diagram

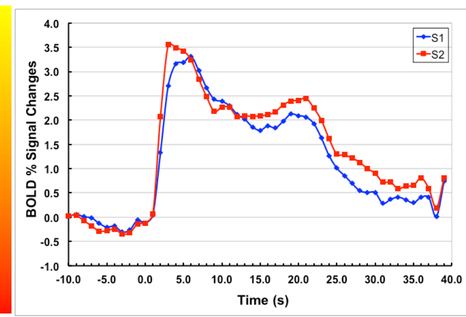


Embedded helmet array and preamps



In vivo SNR Maps



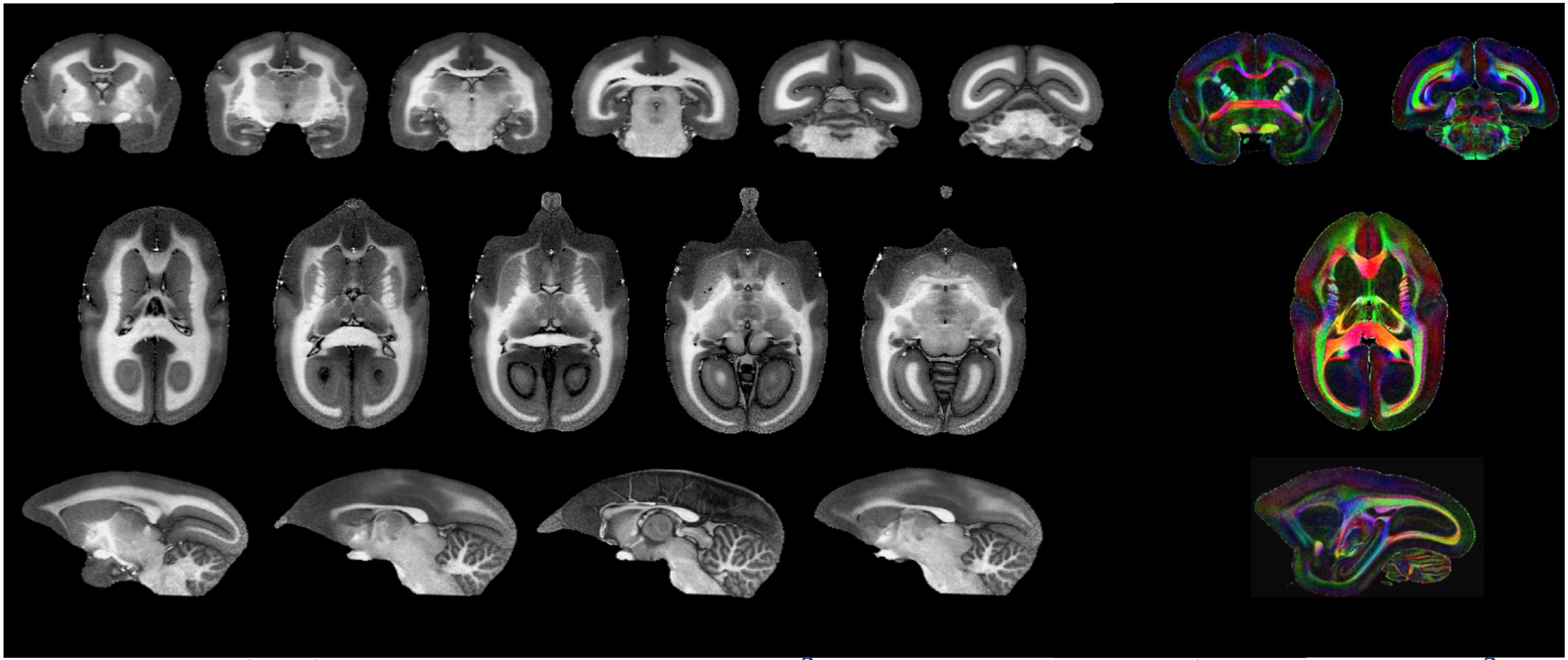


fMRI in Conscious, Awake Marmosets

250 x 250 x 1000 μm^3

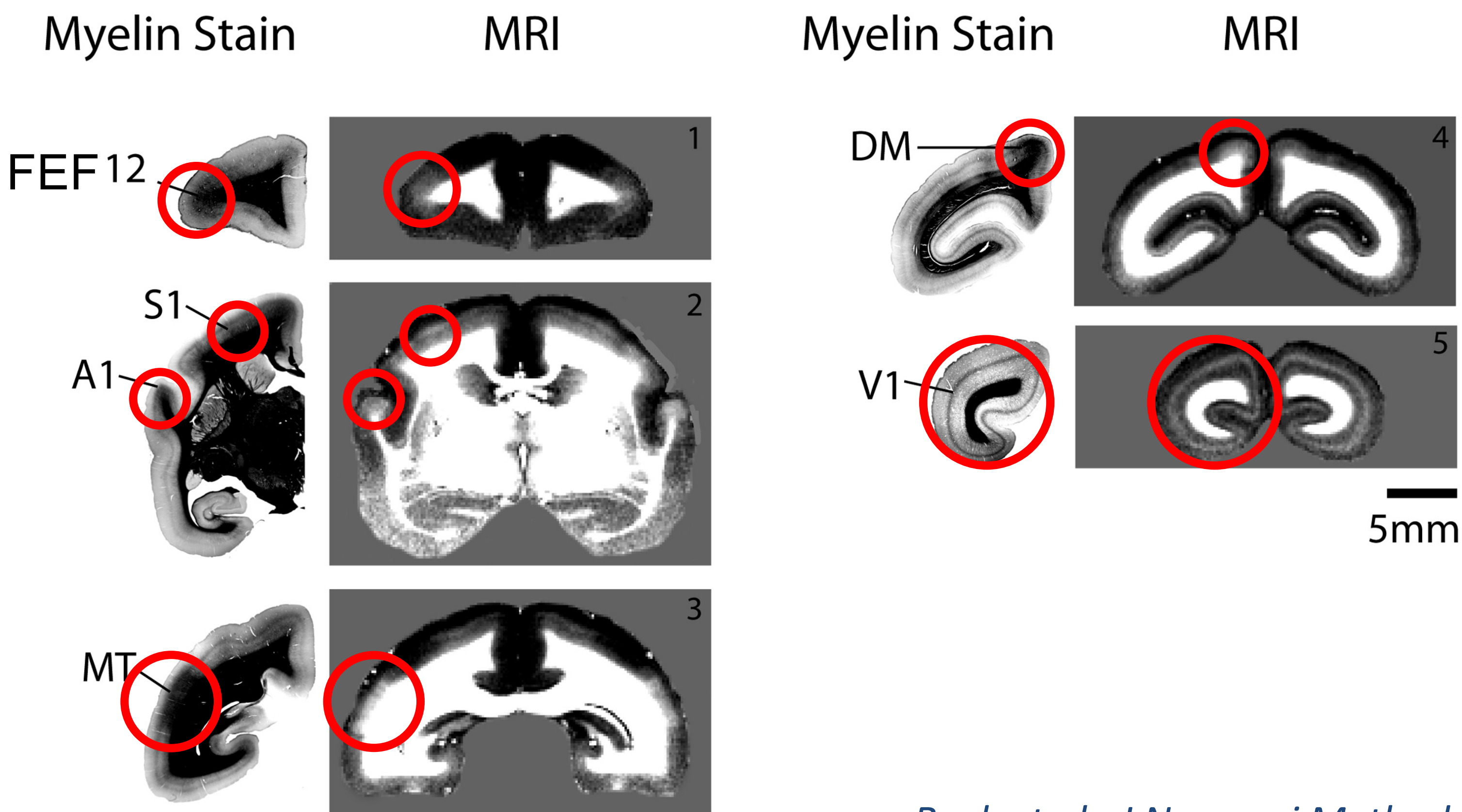
D. Papoti, C.C. Yen, J. Mackel, H.Merkle, A. Silva, NMR in Biomed., 2013

Anatomical MRI of the Marmoset Brain



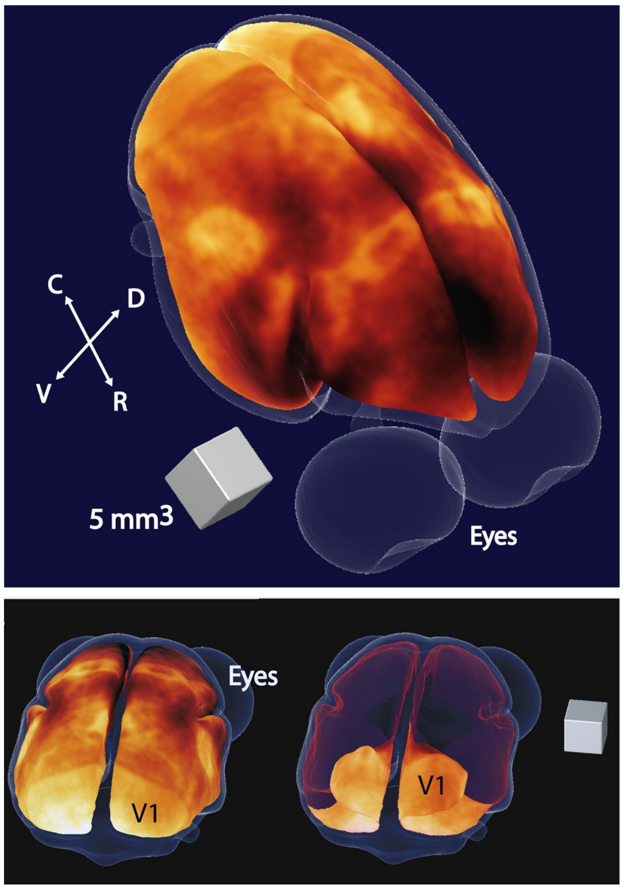
in vivo T1w-MPRAGE 150 μm³ | *ex vivo* DTI 150 μm³

T1-Weighted MRI Reveals Cortical Myeloarchitecture



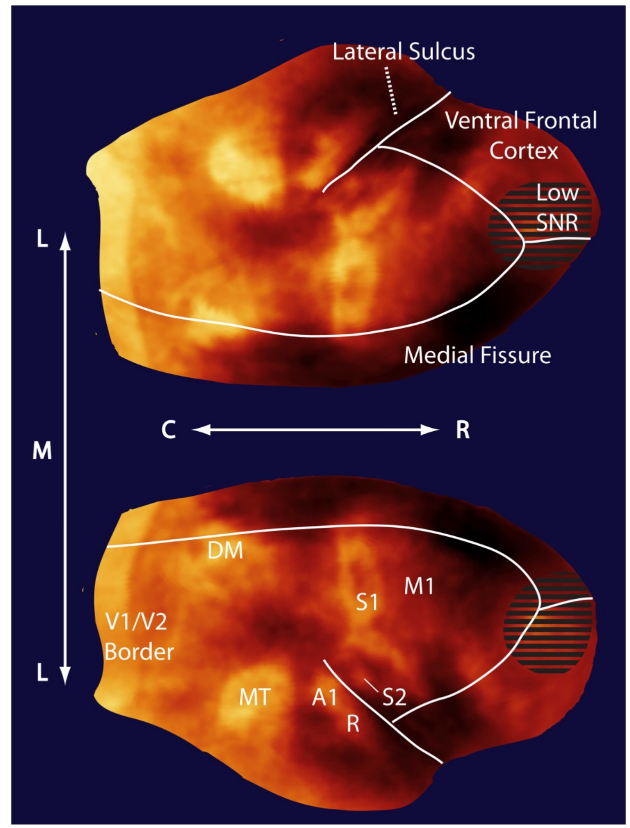
Bock et al., J Neurosci Methods. 2009;185(1):15-22

Cortical Myeloarchitecture Map



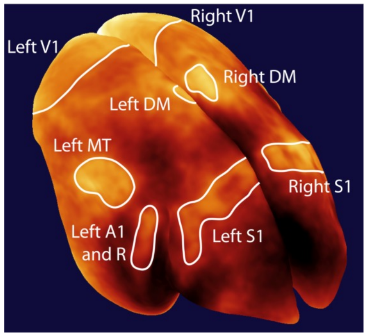
Voxel size 150 µm^3

Flattened View



Bock et al., *J Neurosci Methods. 2009 185(1):15-22*

Reproducible and Quantitative Myeloarchitecture

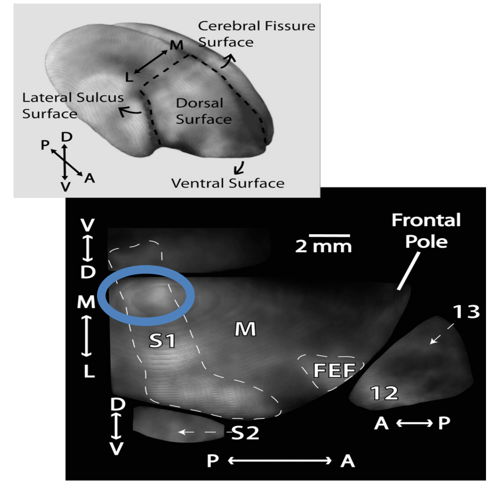


Region	Surface Area (mm^2)		Surface Area (%)
	Left	Right	
Cortex	1005 ± 21	1007 ± 34	100
V1	219 ± 12	222 ± 3	22
S1	28 ± 4	30 ± 4	3
MT	17 ± 3	19 ± 2	2
A1 and R	11 ± 3	11 ± 3	1
DM	8 ± 1	7 ± 1	1

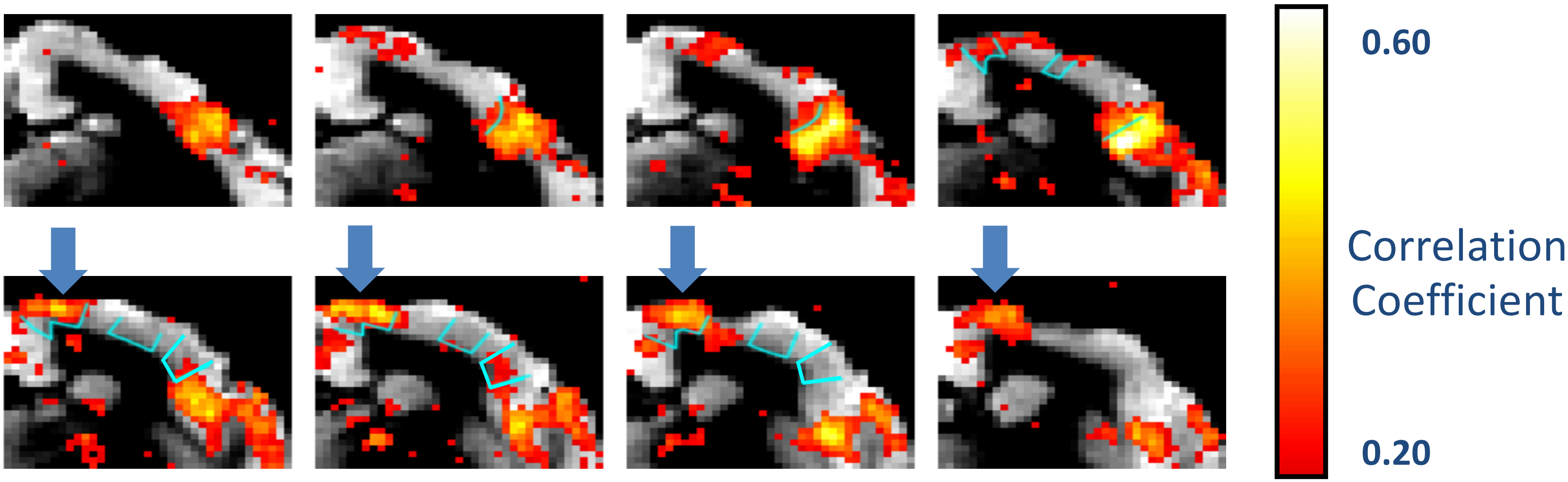
- Agrees well with histological measures of areas:
  - V1: 200-205 mm^2: *Fritsches and Rosa 1996 JCN 372:264-82; Missler, Wolff 1993 JCN 333:53-67*
  - MT: 14 mm^2: *Pessoa et al. 1992 Exp. Brain Res. 2: 459–462.*
  - DM: *no well defined borders.*
- More than ¼ of the marmoset cortex dedicated to processing of visual information

Bock *Ann N Y Acad Sci. 2011 1225 Suppl 1:E171-81*

fMRI Activation Regions Map Well onto Myeloarchitectonic Maps

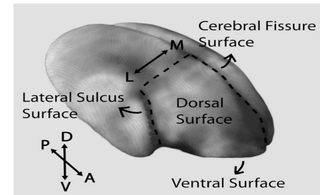


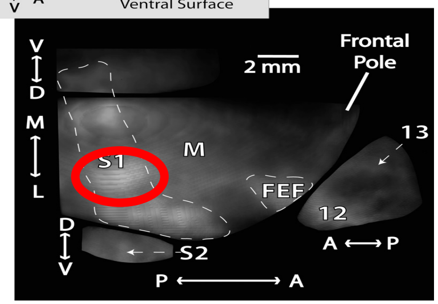
Leg Stimulation: 1.5 mA, 0.3ms, 50 Hz



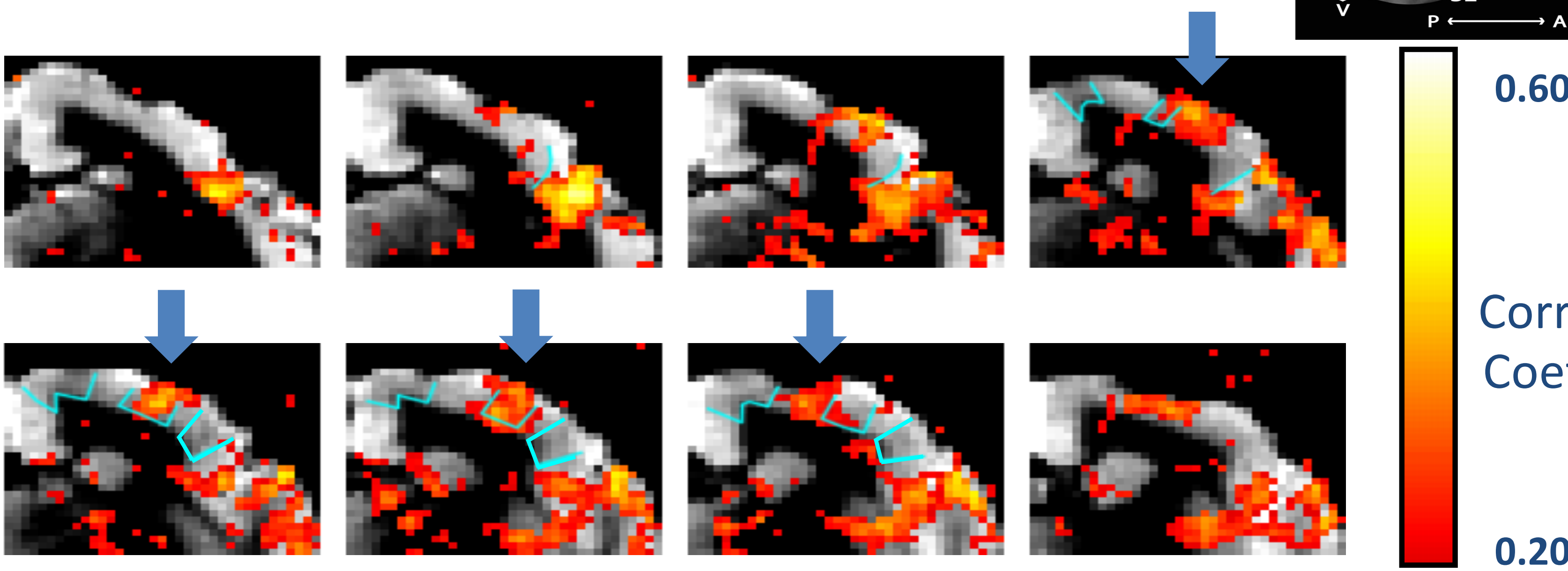
Liu et al . Neuroimage. 2013 78:186-95.

fMRI Activation Regions Map Well onto Myeloarchitectonic Maps



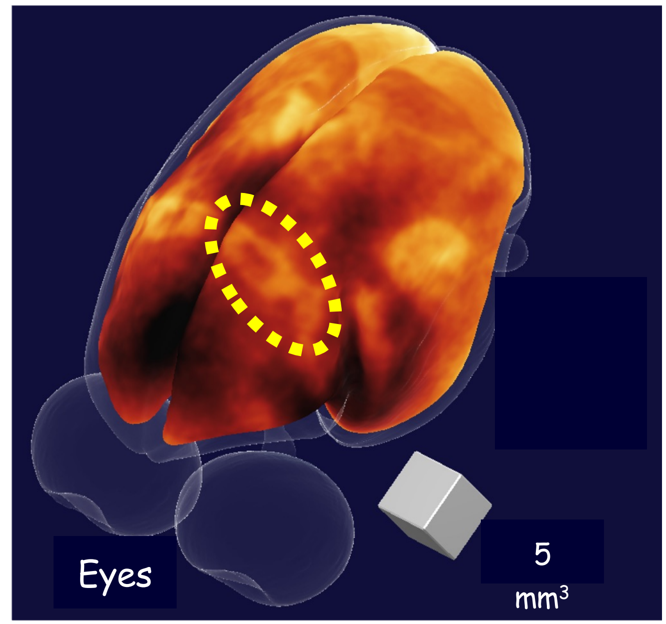


Forearm/Wrist Stimulation: 1.5 mA, 0.3ms, 50 Hz



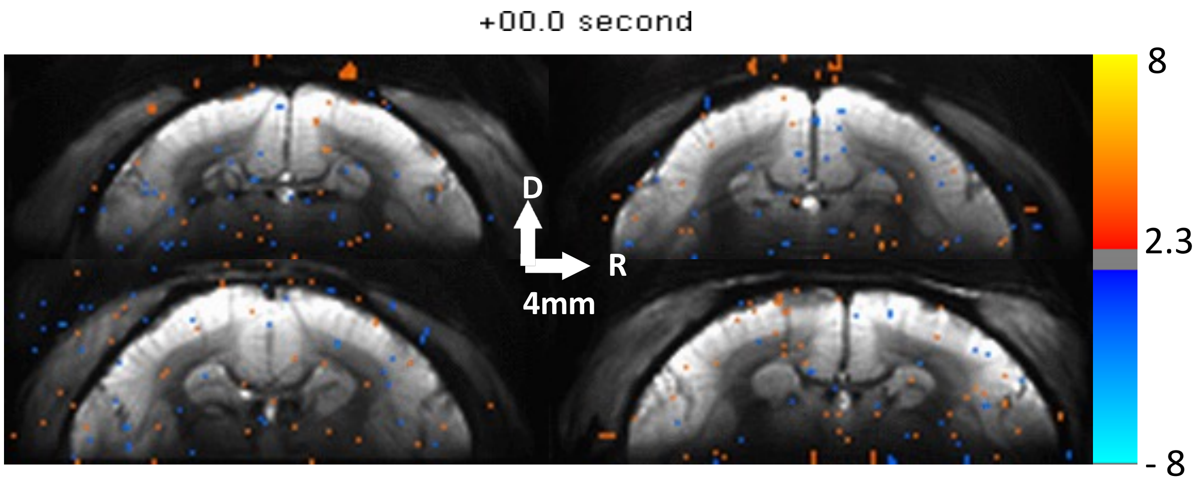
Liu et al . Neuroimage. 2013 78:186-95.

fMRI Response Overlaid on Myeloarchitecture



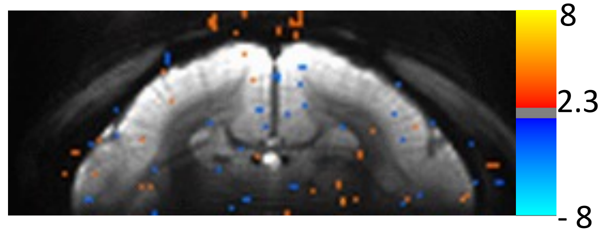
Liu et al . Neuroimage. 2013 78:186-95.

BOLD Response to Hand Stimulation

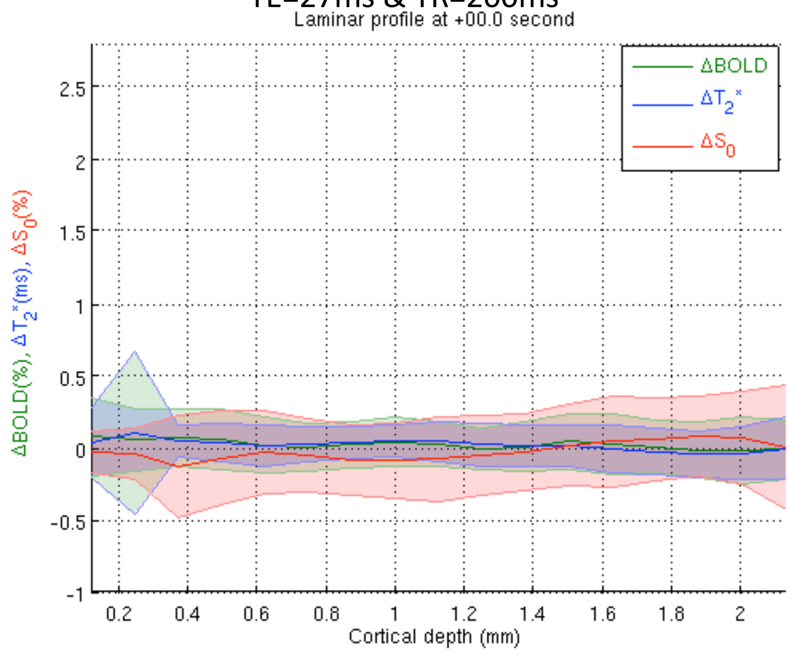


TE = 27 ms & TR = 200 ms

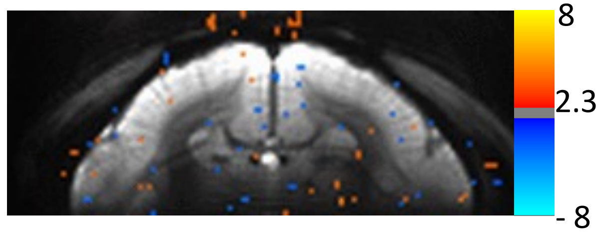
BOLD Response to Hand Stimulation



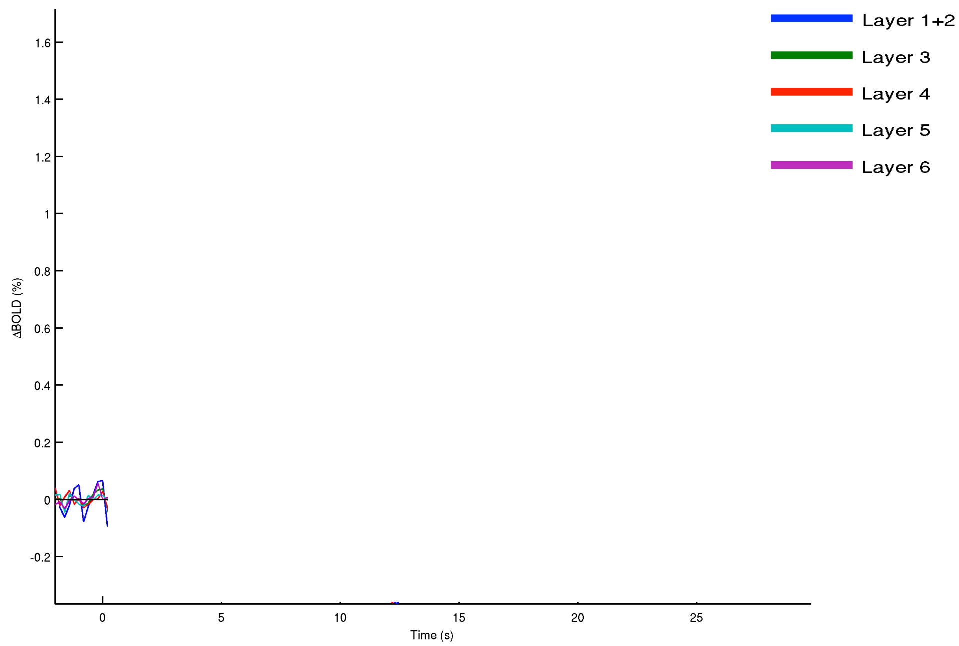
TE=27ms & TR=200ms



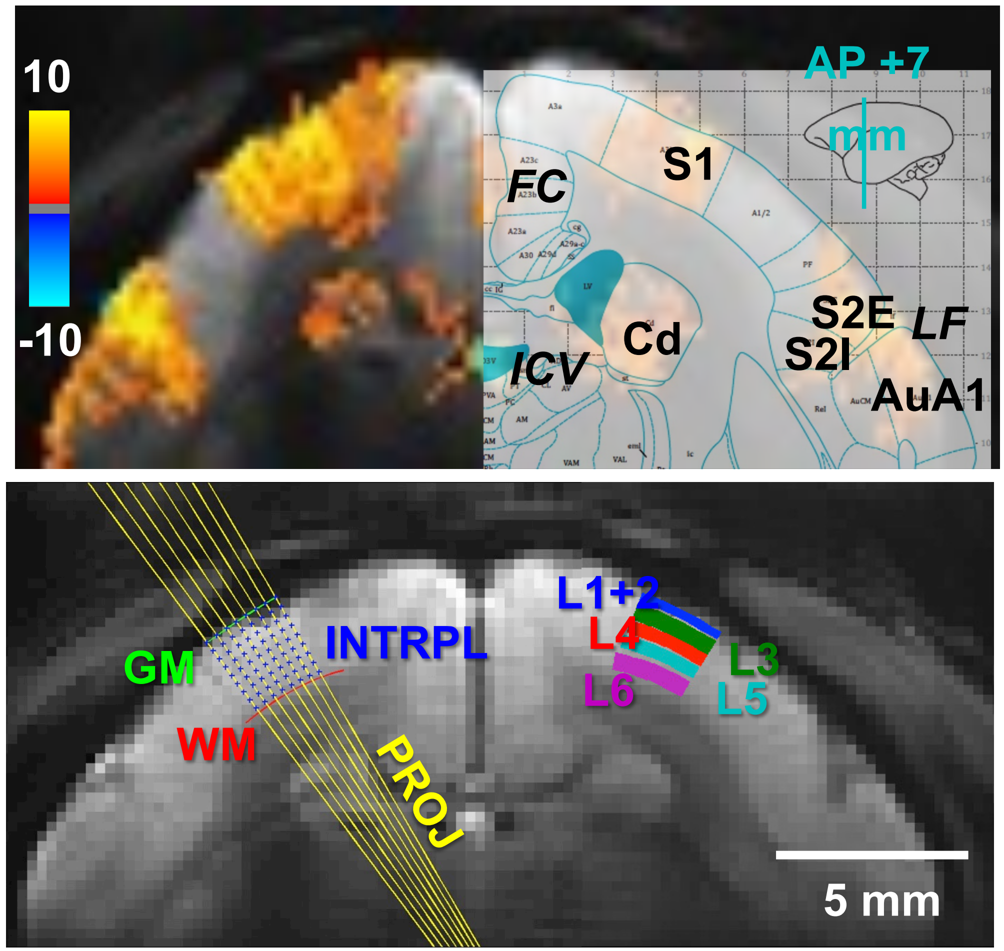
BOLD Response to Hand Stimulation



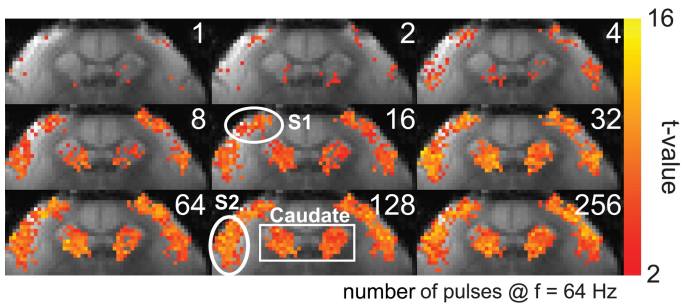
TE=27ms & TR=200ms
+00.0 second

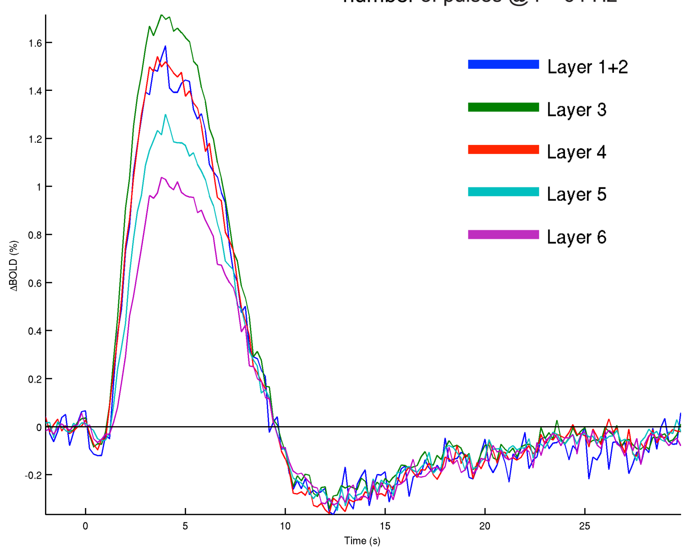


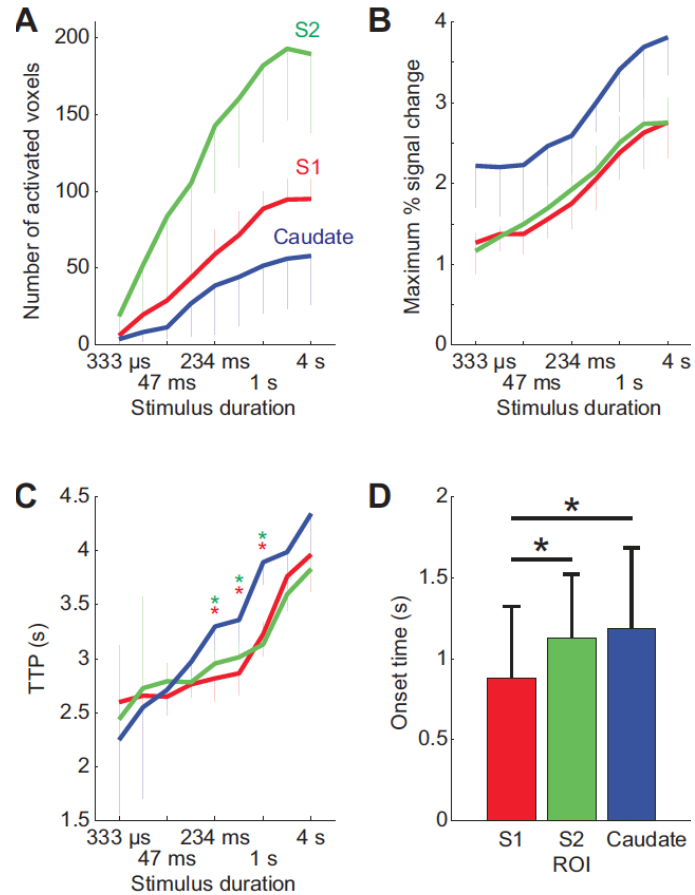
Laminar Profile of T2 Changes*



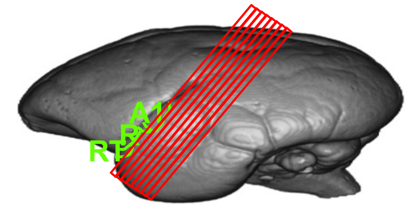
Somatosensory Stimulation Leads to Robust Activation of S1, S2 and Caudate

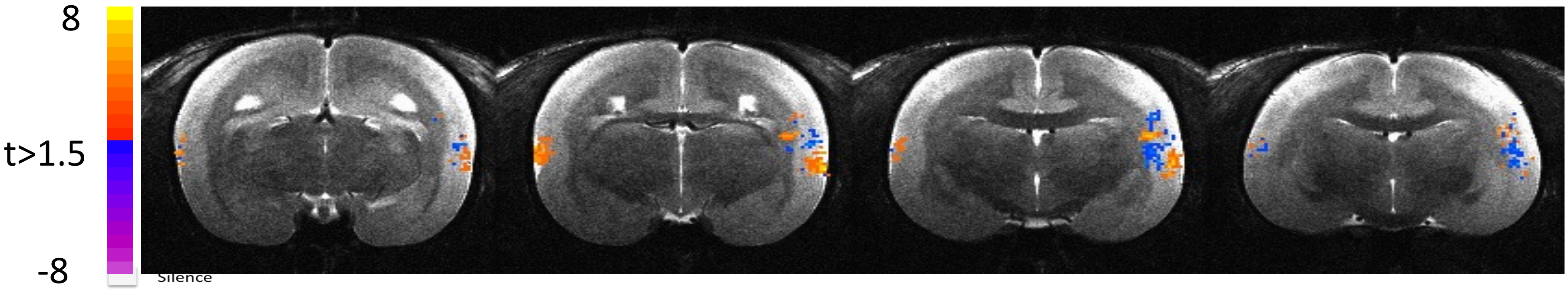


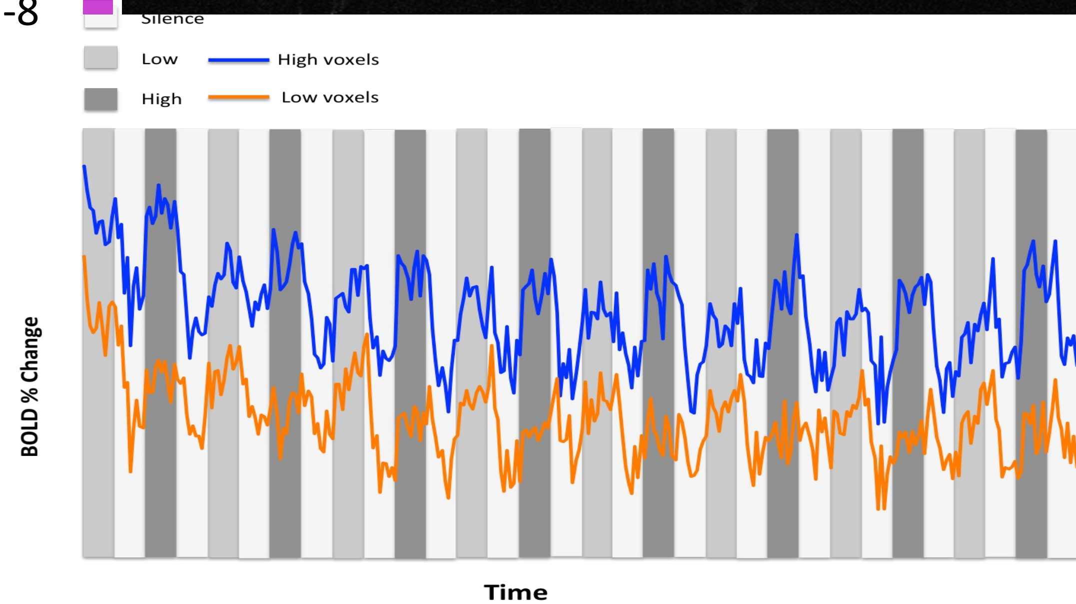




Tonotopic Mapping in Marmoset Auditory Cortex







In collaboration with Cory Miller (UCSD) and David Leopold (NIMH)

Resting State Networks In Conscious Marmosets

High Order Visual Cortex

V3, V4, A19, A19DI

Basal Ganglia

Primary Visual Cortex

Dorsomedial

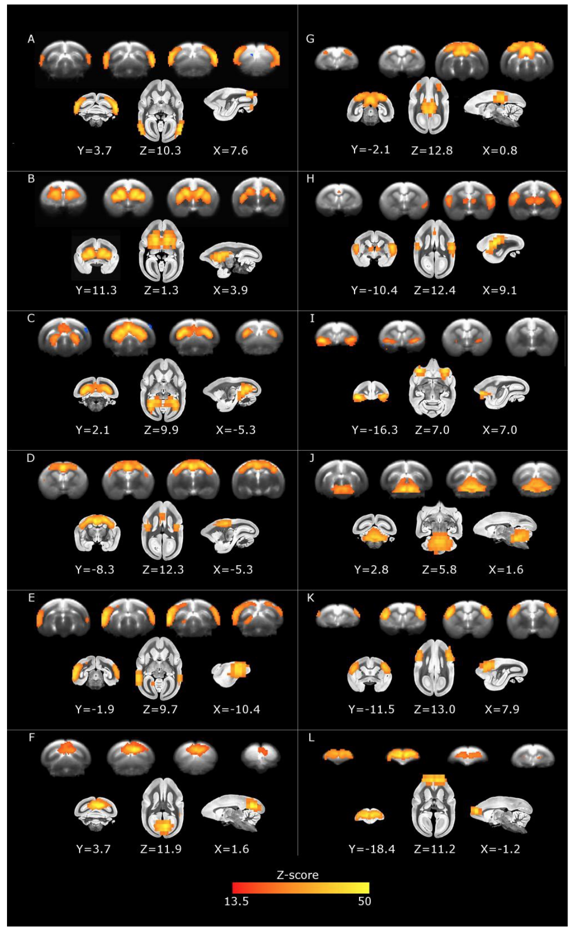
Somatomotor

High Order Visual Cortex

V4, V5, V6, FST, TE3

High Order Visual Cortex

V2, A19M, V6(DM)



Default Mode Network

Salience

Orbitofrontal Cortex

Cerebellum

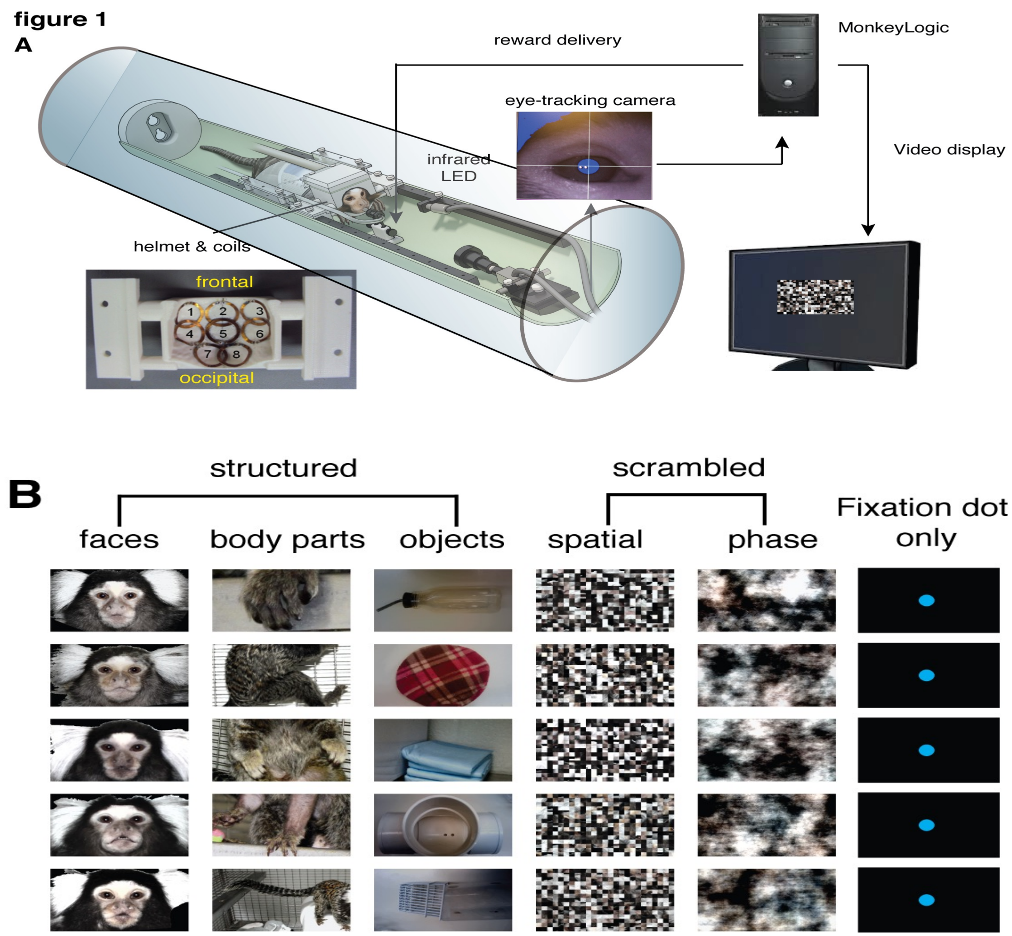
Ventrolateral

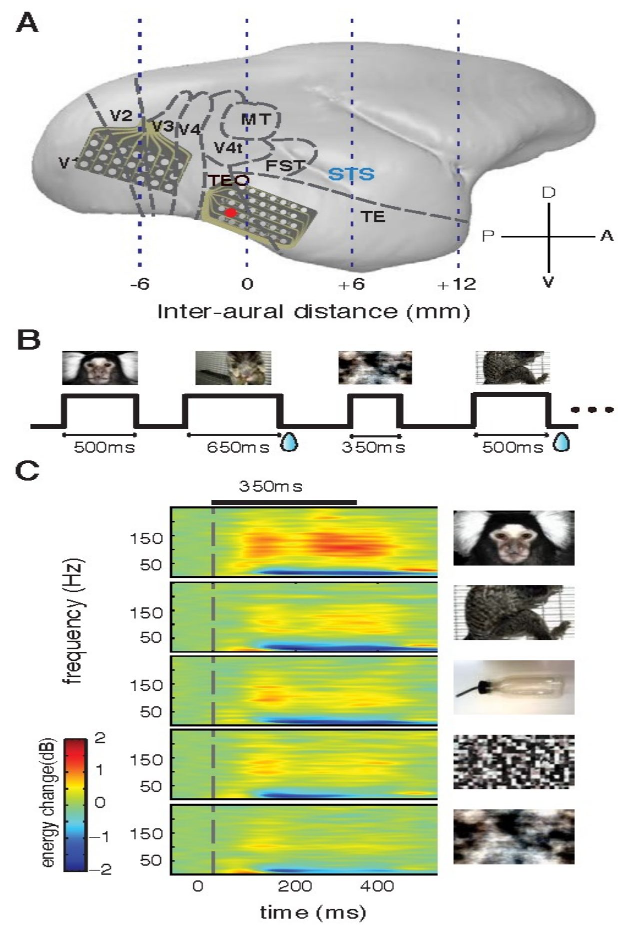
Somatomotor

Frontal Pole

Belcher J Neurosci. 2013 33(42):16796 –16804

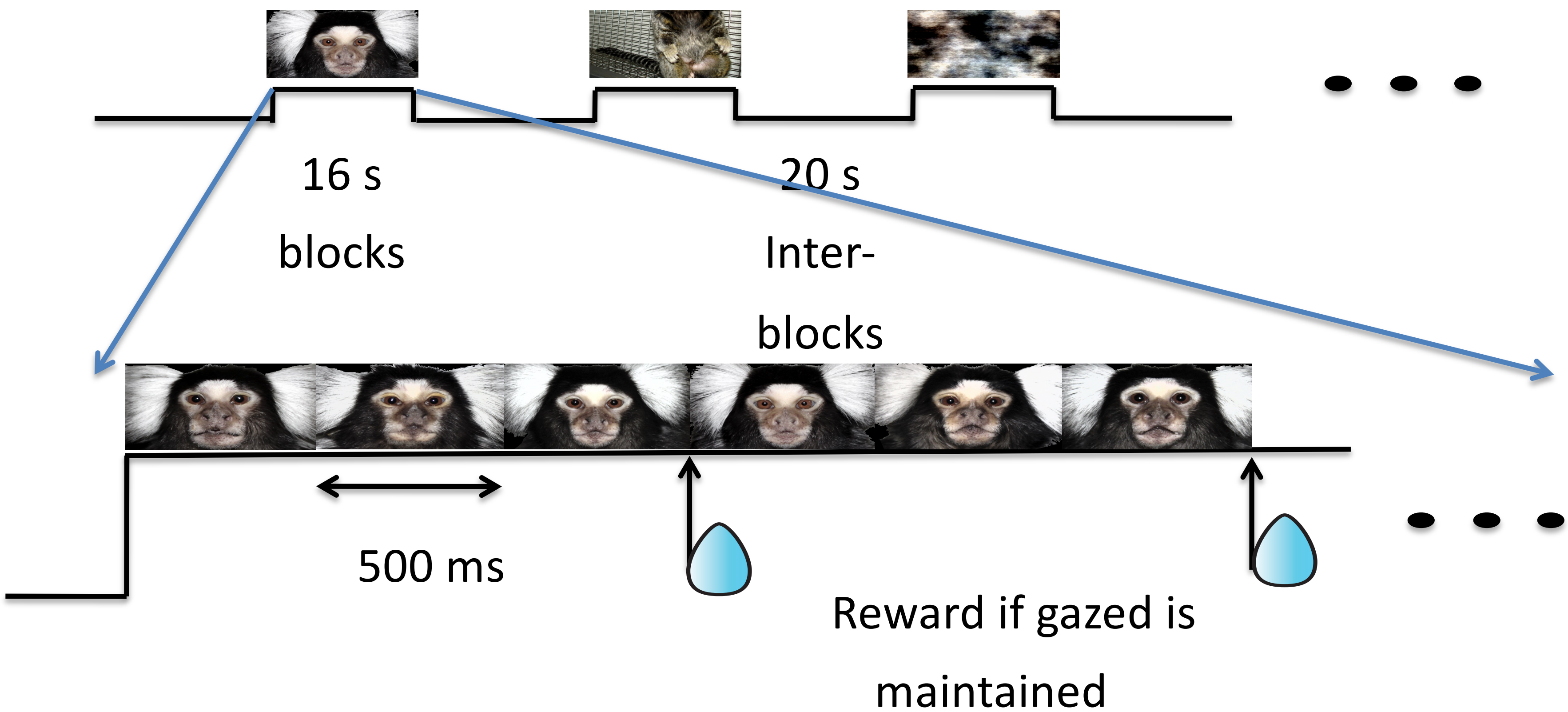
fMRI/ECoG during visual stimulation





C.-C. Hung et al Neuroimage 2015;120(10):1-11. C.-C. Hung et al J Neurosci 2015 35(3):1160-72.

Experimental paradigm for fMRI of Visual System

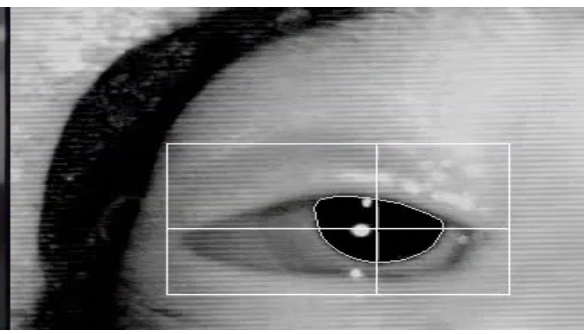


C.-C. Hung et al J Neurosci 2015 35(3):1160-72.

Typical behavior of awake marmoset to a stimulus block

Positive reinforcement

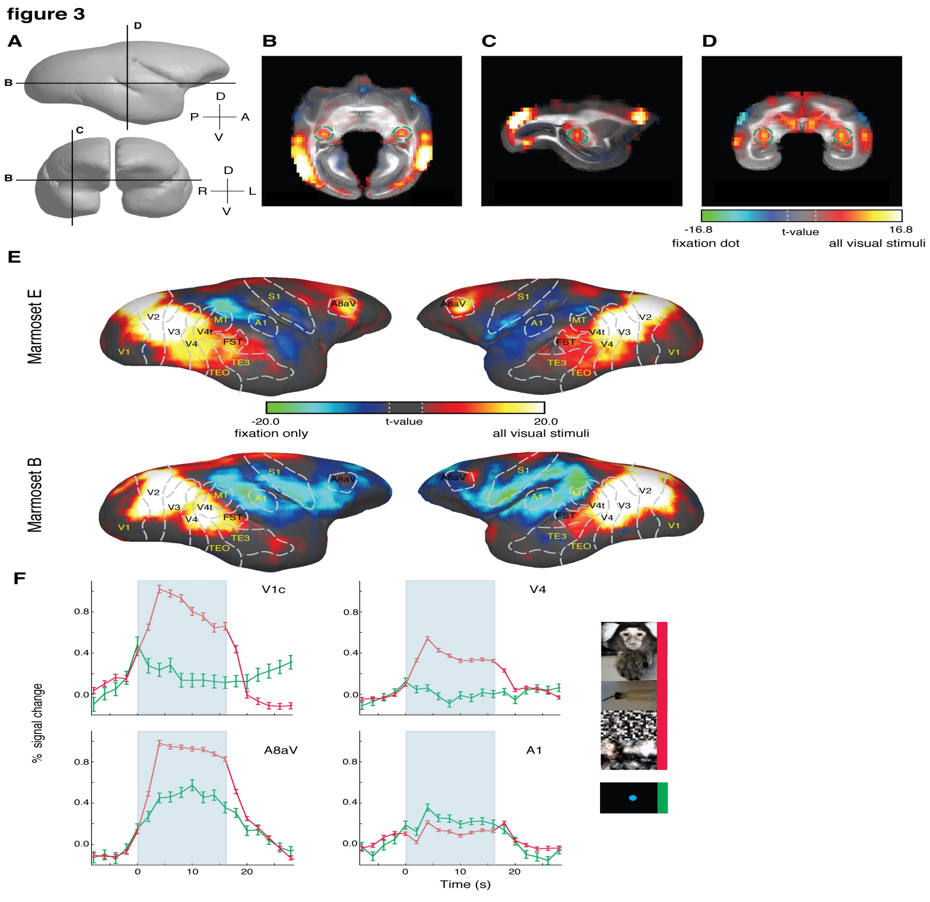
Infra-red eye-tracking



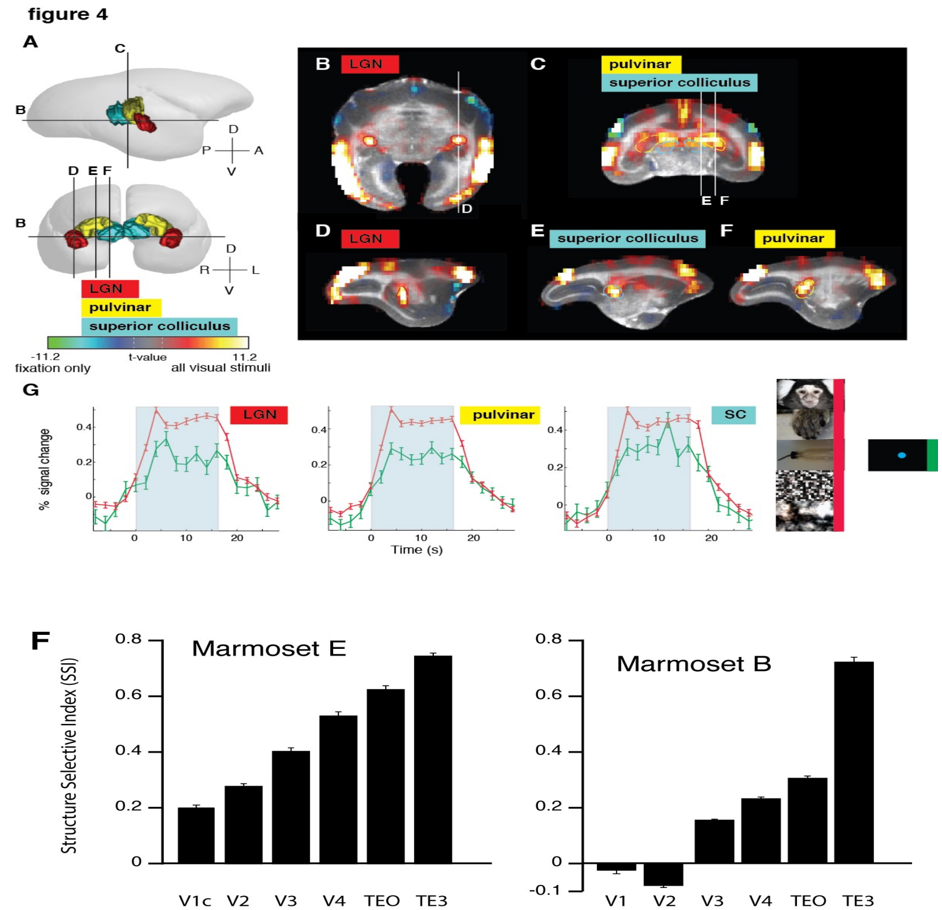
Visual Stimulus

C.-C. Hung et al J Neurosci 2015 35(3):1160-72.

Visual responses in cortical and sub-cortical areas

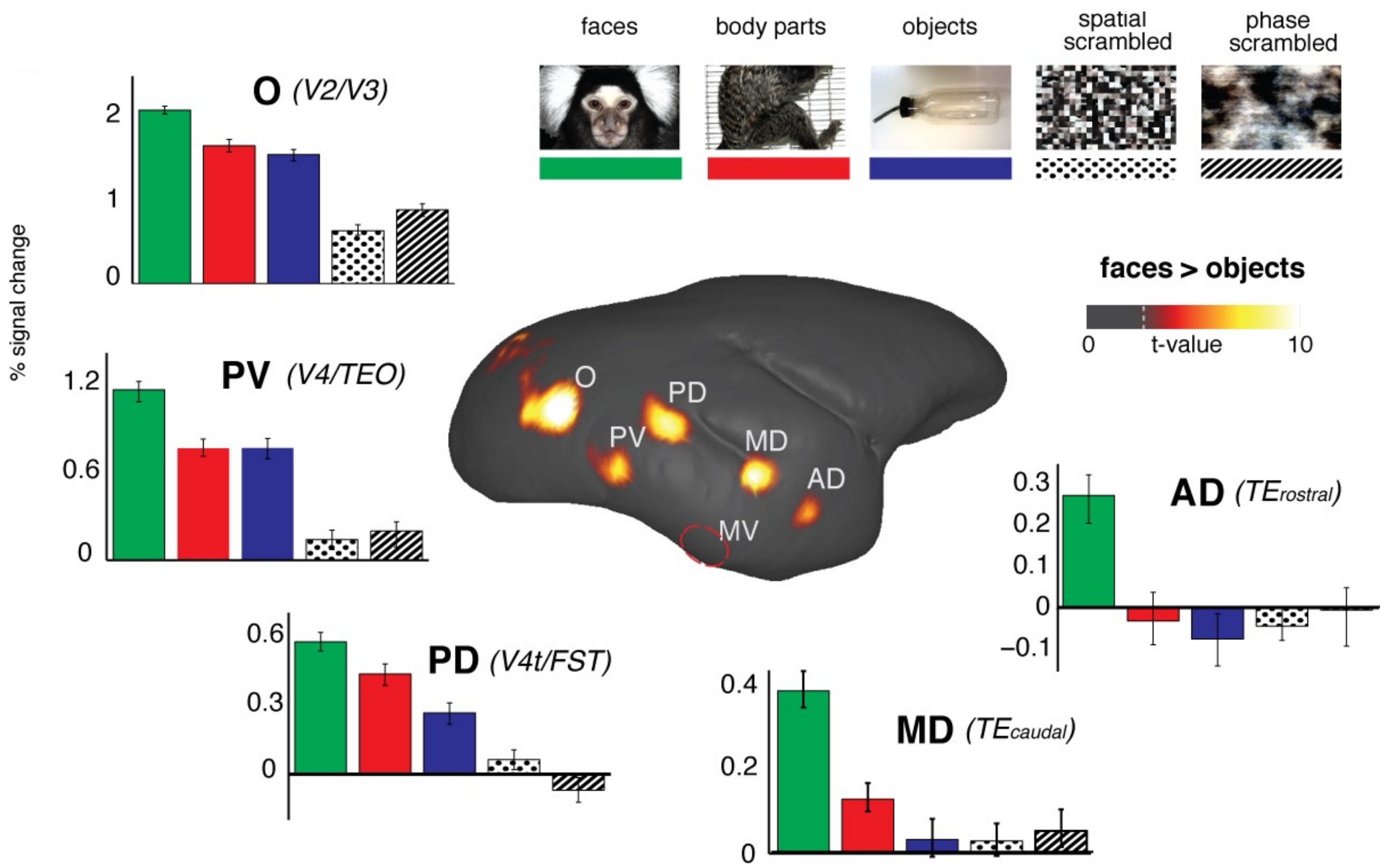


C.-C. Hung et al J Neurosci 2015 35(3):1160-72.



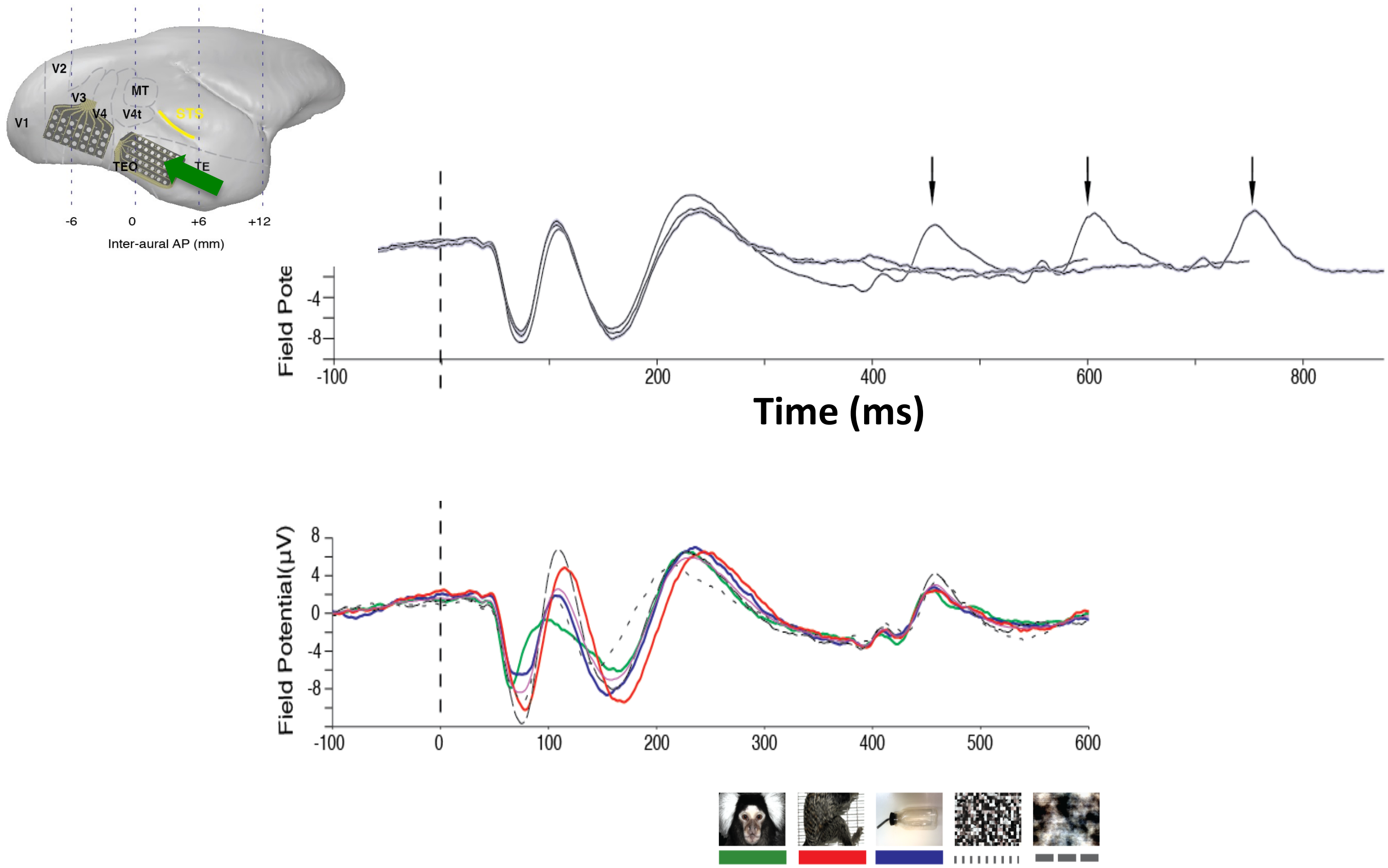
C.-C. Hung et al Neuroimage 2015 120(10):1-11

Face-selective patches along ventral visual pathway



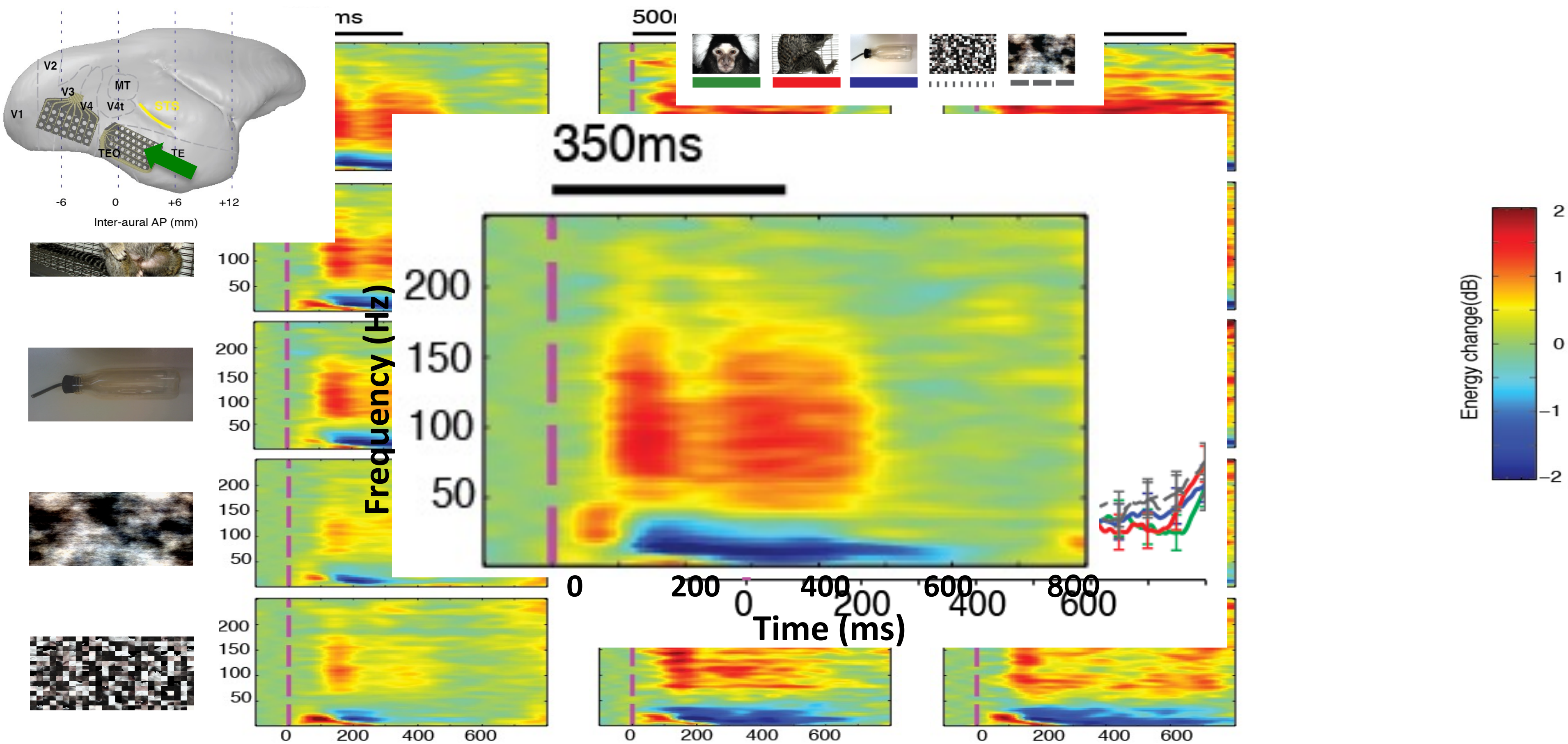
C.-C. Hung et al J Neurosci 2015 35(3):1160-72.

ECoG measures event-related potentials



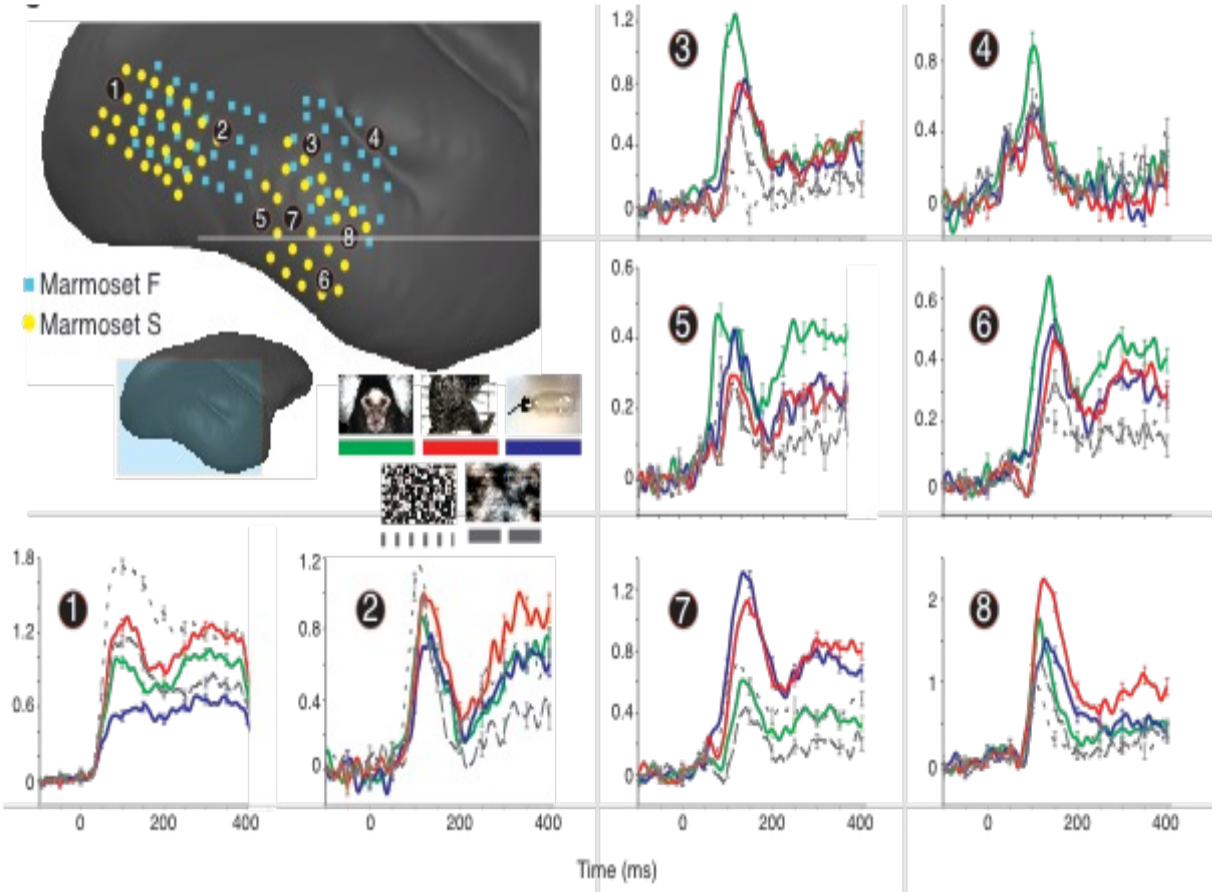
C.-C. Hung et al J Neurosci 2015 35(3):1160-72.

Time-frequency Analysis (An example site)



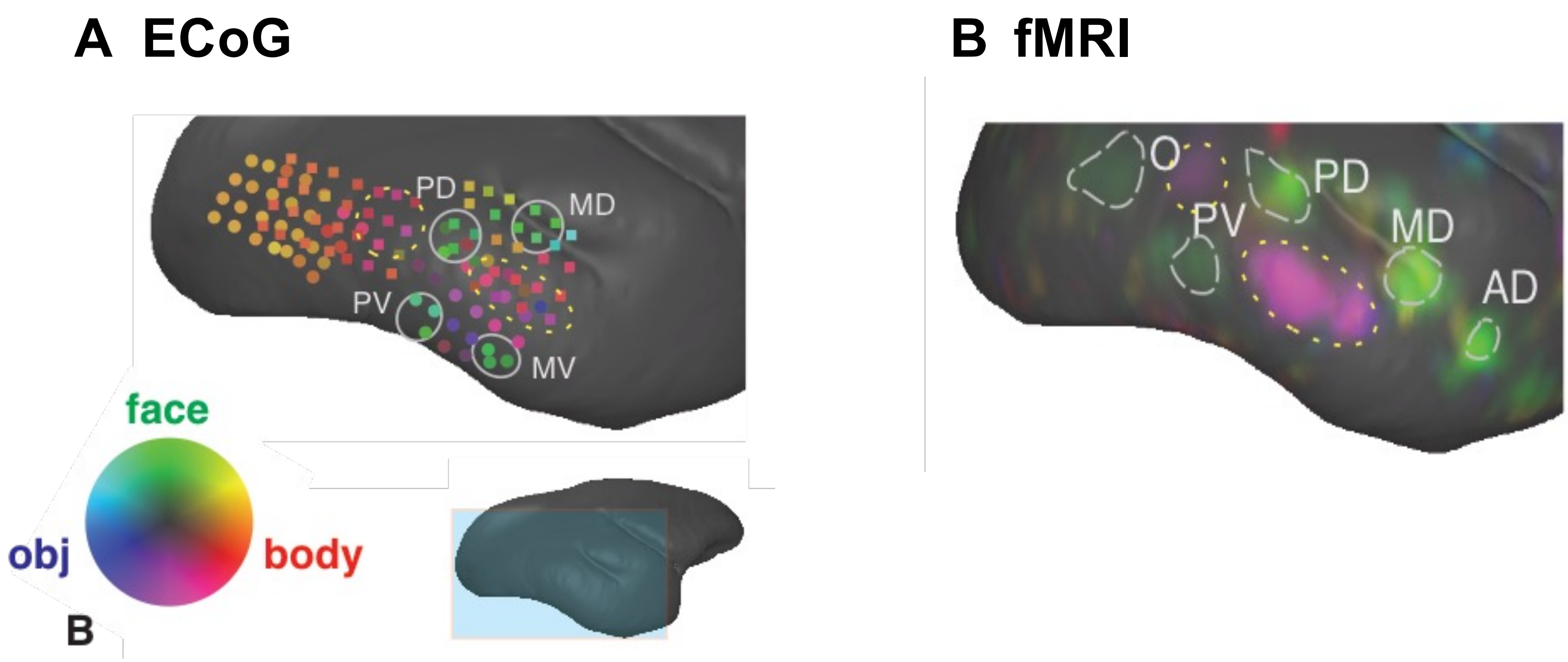
C.-C. Hung et al J Neurosci 2015 35(3):1160-72.

Spatial layout of the high-gamma responses



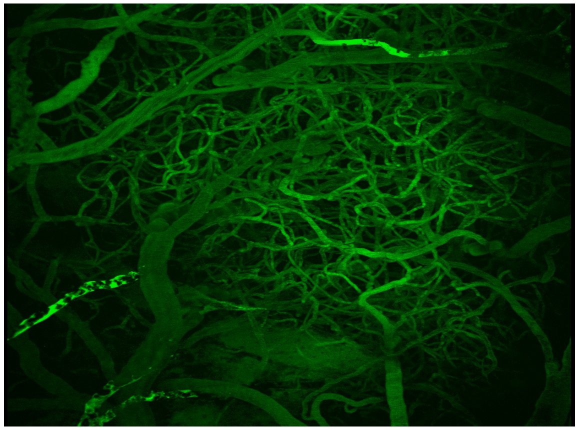
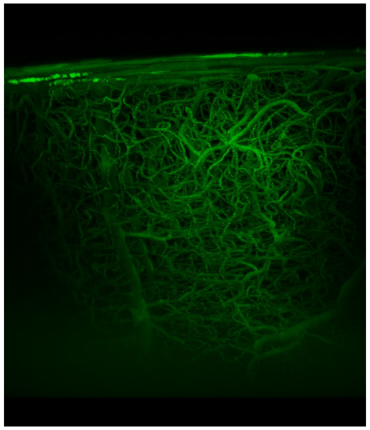
C.-C. Hung et al J Neurosci 2015 35(3):1160-72.

Good spatial correspondence between fMRI and ECoG in marmoset extrastriate visual pathway



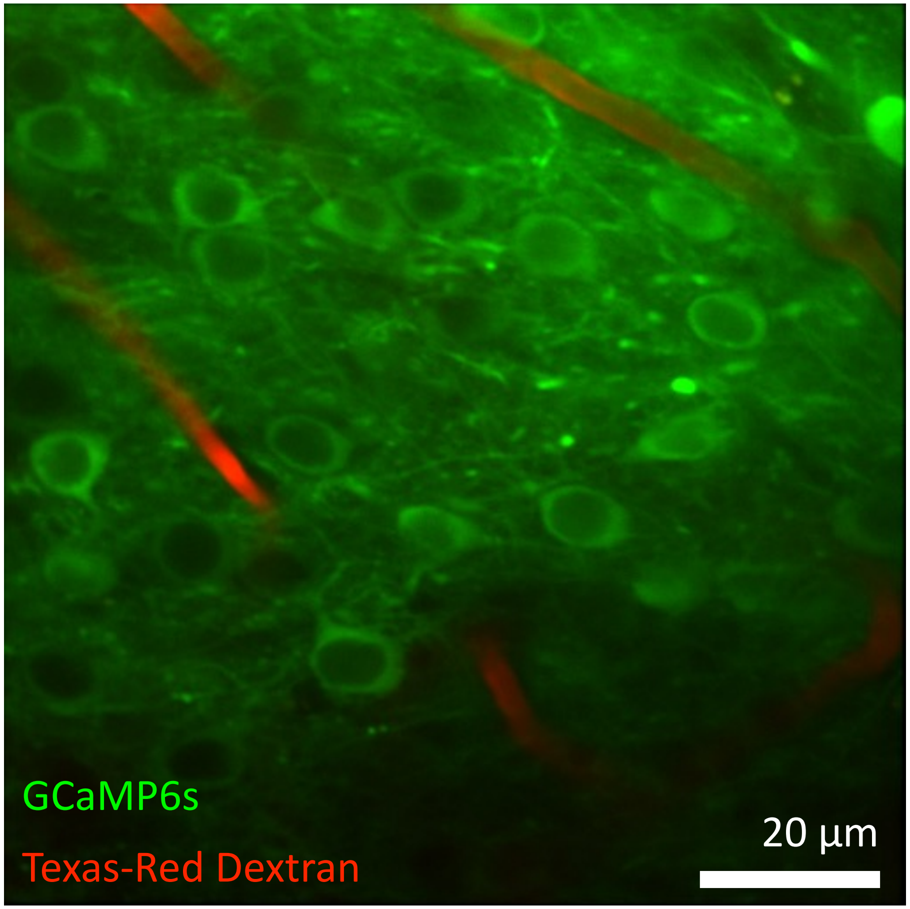
C.-C. Hung et al J Neurosci 2015 35(3):1160-72

Using Two-Photon Microscopy to Directly Visualize the Neurovascular Unit in Vivo

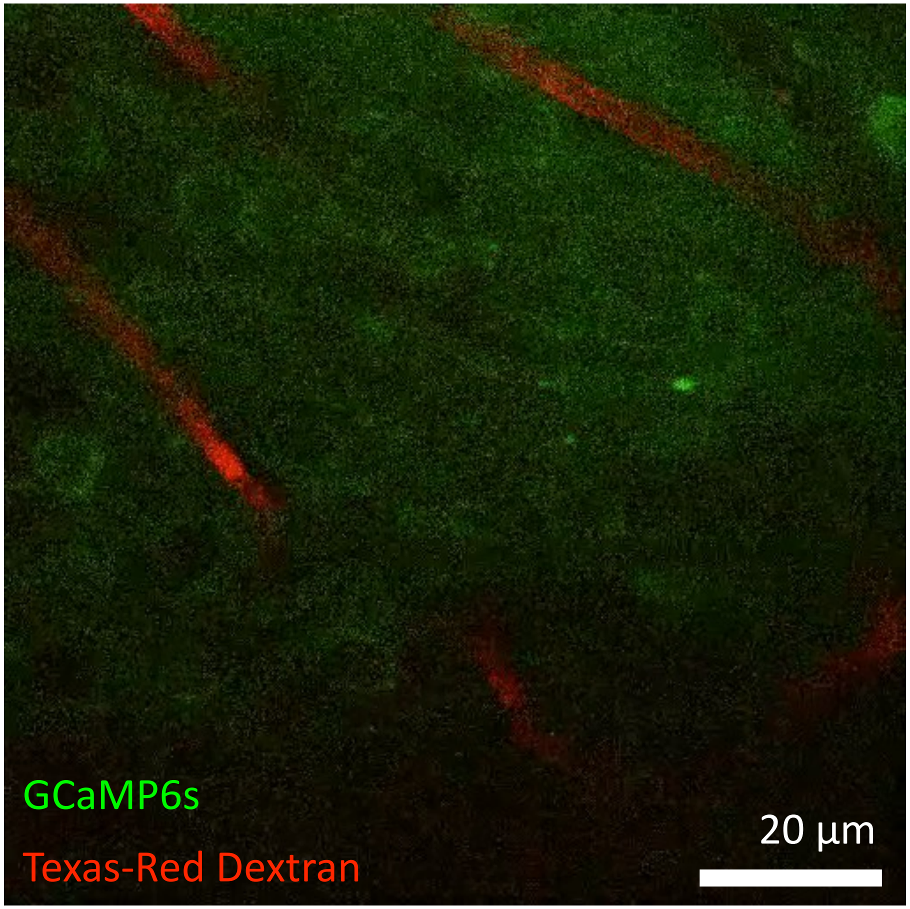




- FITC- labeled cerebral microcirculation of mouse cortex
- FOV: 600 x 600 μm^2
- Depth: 725 µm

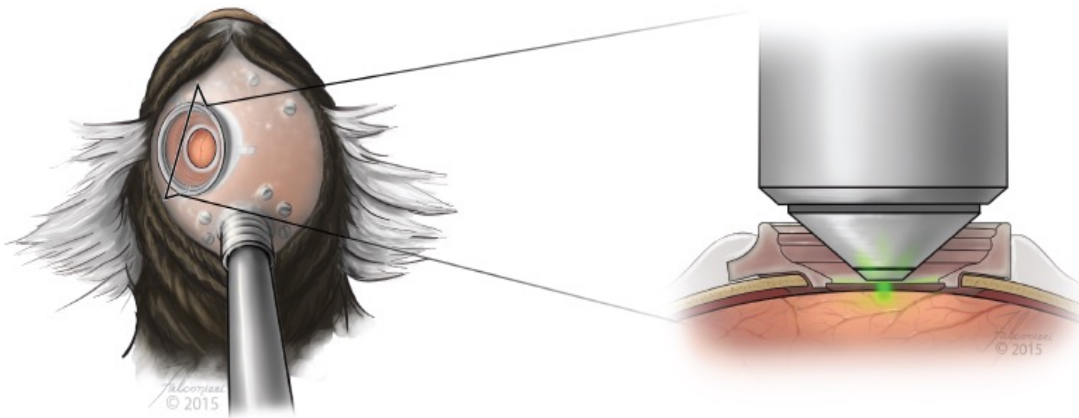
GCaMP6s Labeled Neurons and Cortical Microvasculature in Mouse Cortex

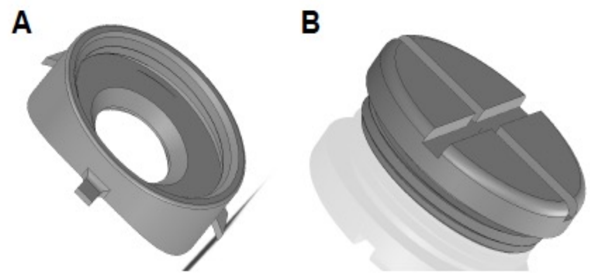


GCaMP6s Labeled Neurons and Cortical Microvasculature in Mouse Cortex

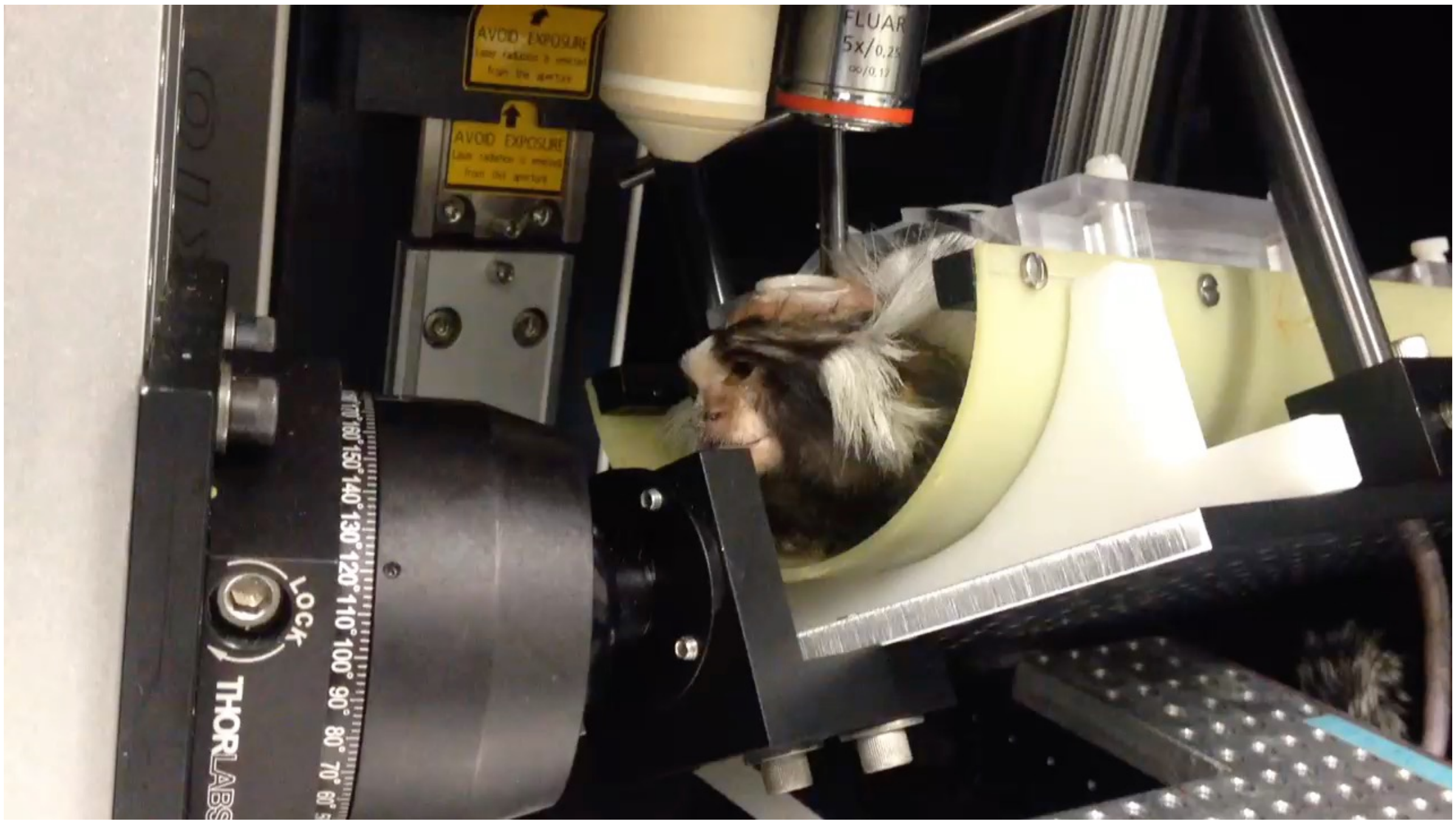


Glass-cover craniotomy provides optical access to image the brain

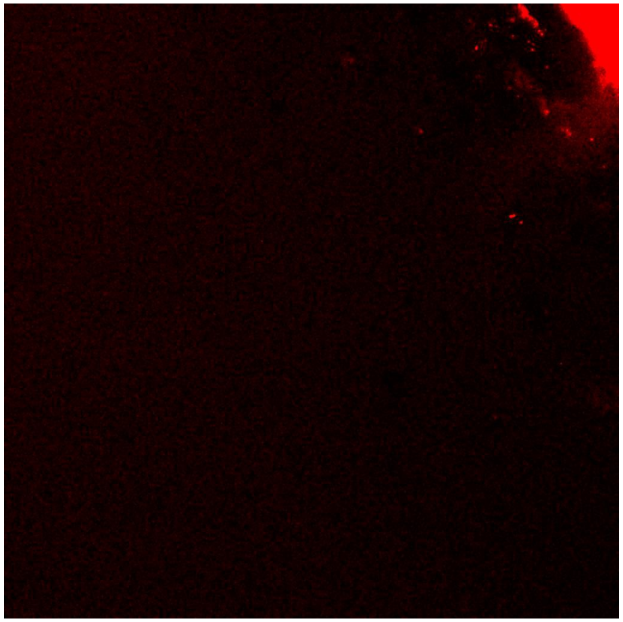




Rotatable+translatable stereotax positions marmoset with adequate stability for two-photon imaging

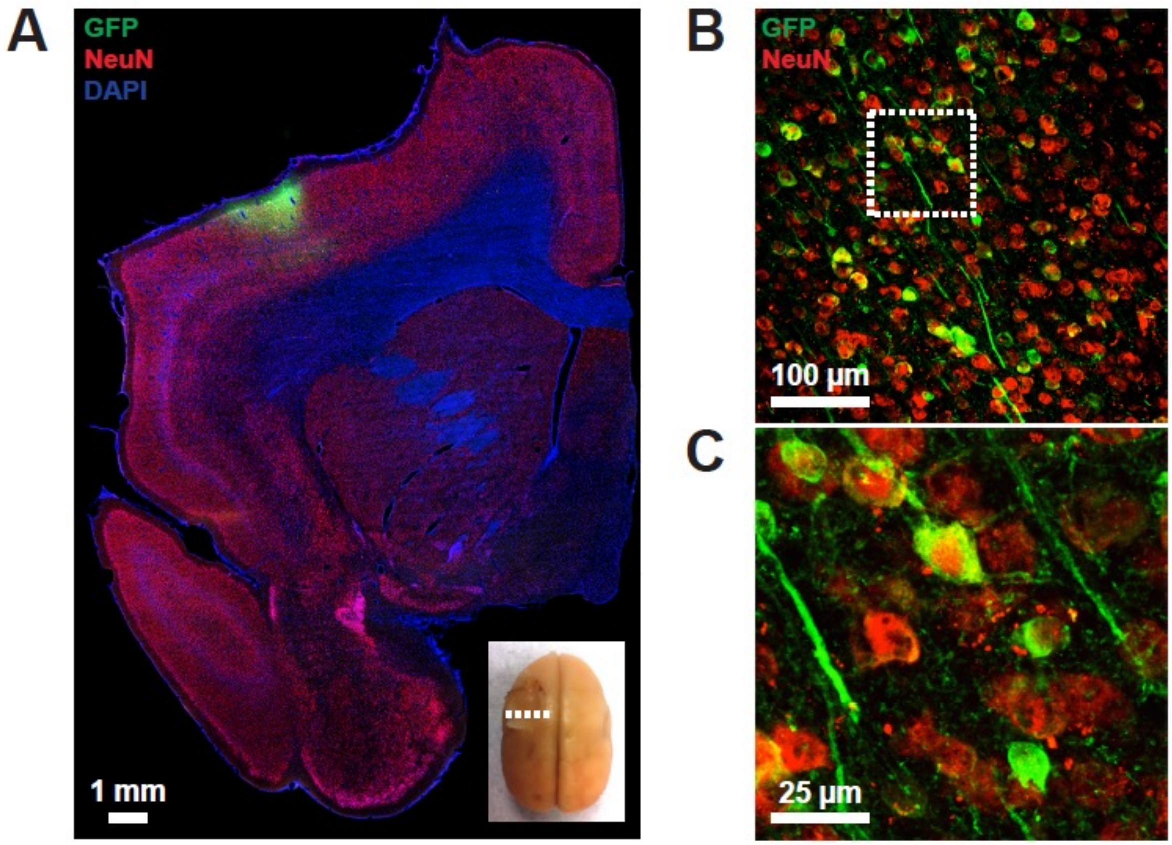


Using Two-Photon Microscopy to Directly Visualize the Neurovascular Unit in Vivo

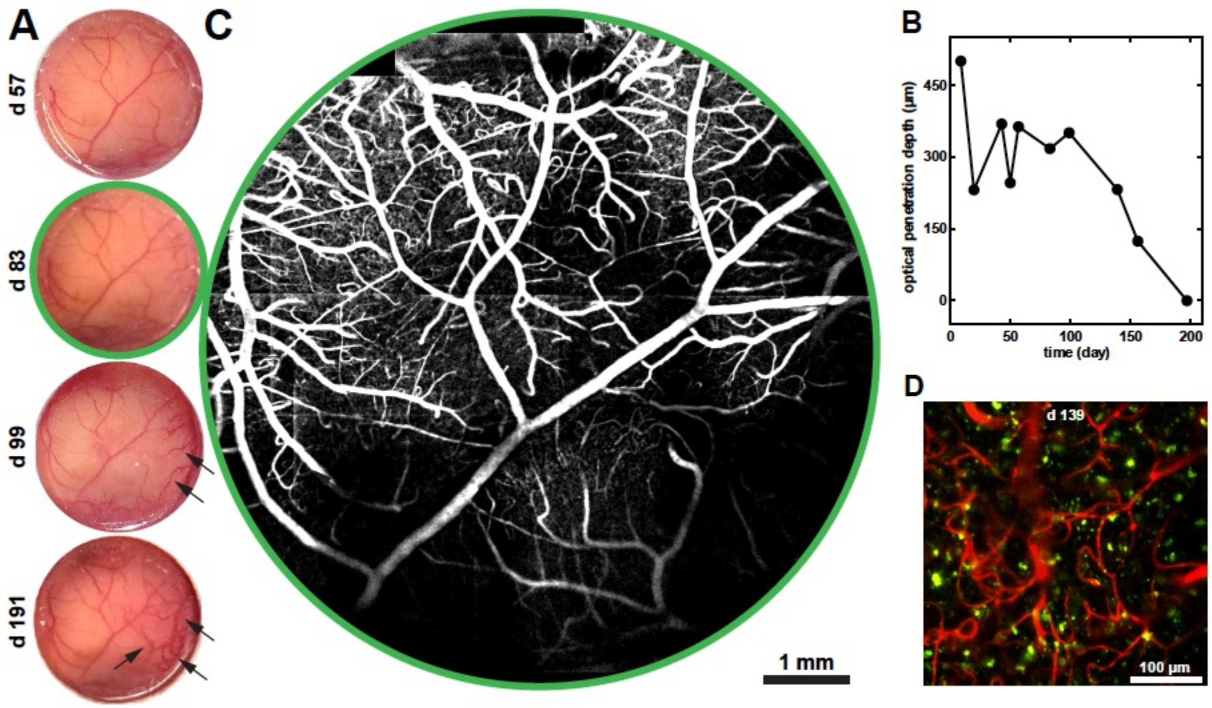


- Texas-Red labeled cerebral microcirculation of awake marmoset cortex
- FOV: 1200 x 1200 µm^2
- Depth: 500 µm
- Capillary density = 5,601 capillaries/mm3

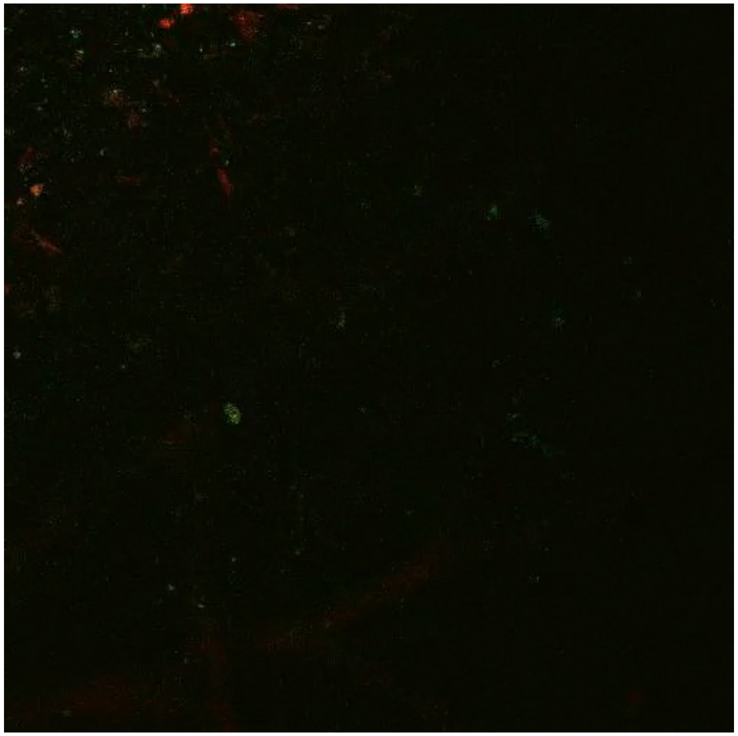
Robust expression in neurons 3 weeks after AAV1-hSyn-GCaMP5G intracranial delivery



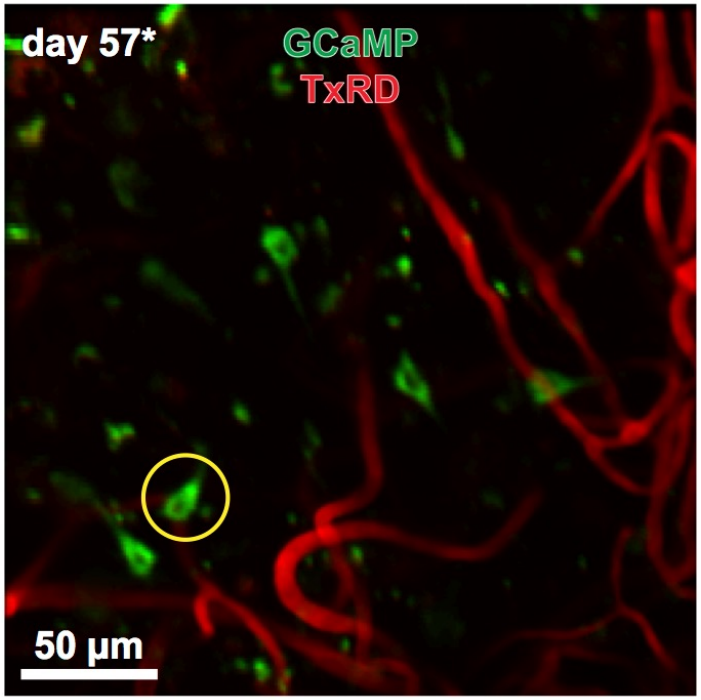
Custom-designed cranial chamber provides optical access to somatosensory cortex for over 6 months



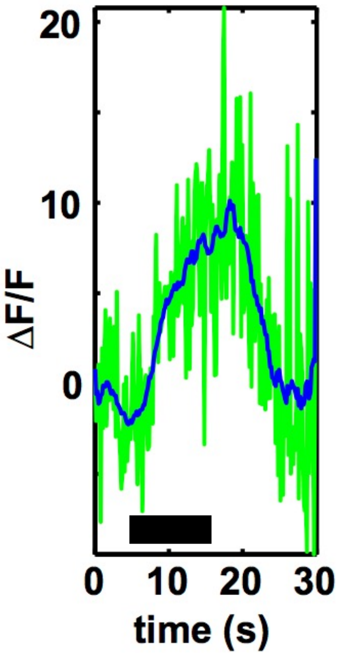
Two-Photon Microscopy of Neurovascular Coupling in Awake Marmoset



GCaMP5-expressing neurons firing during somatosensory stimulation



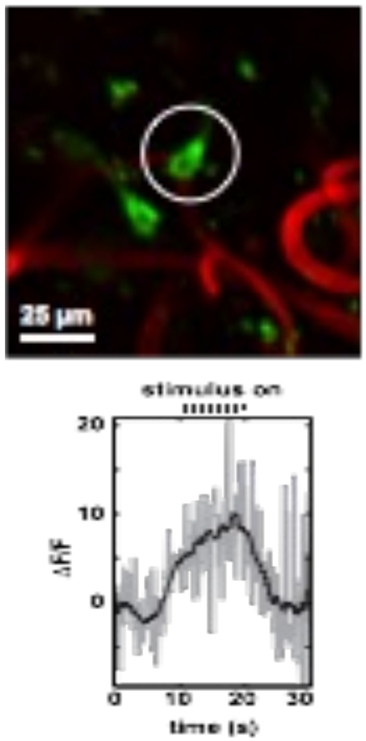
* 135 days after AAV1-GCaMP5 intracranial injection



16% of GCaMP-labled neurons responsive

17% average fluorescence increase

Robust Neurovascular Coupling in Primary Somatosensory Cortex of Awake Marmosets



16% of GCaMP-labled neurons responsive

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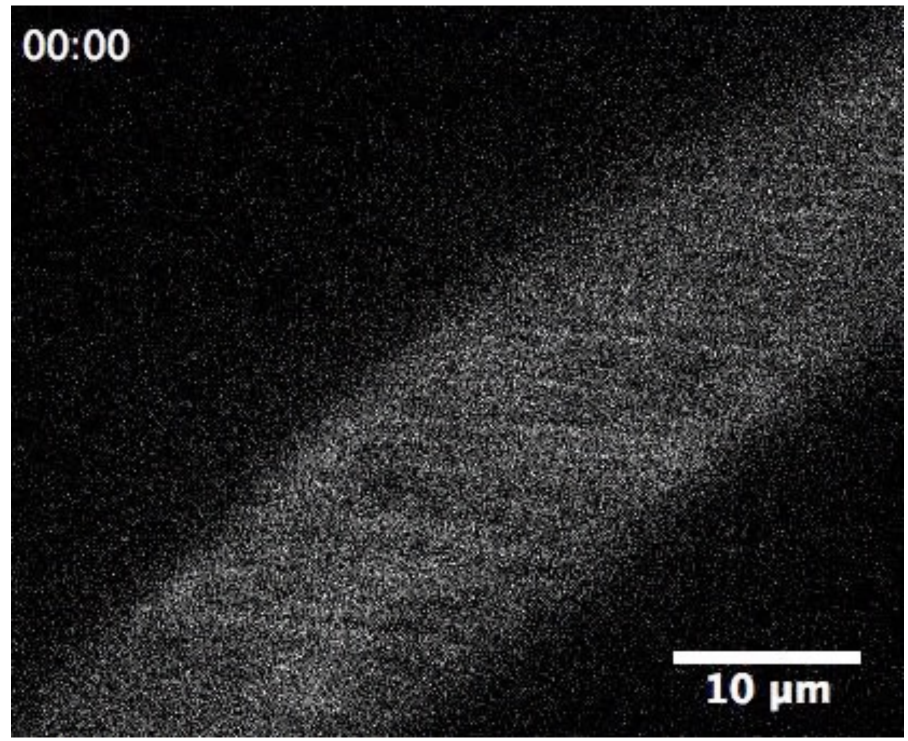
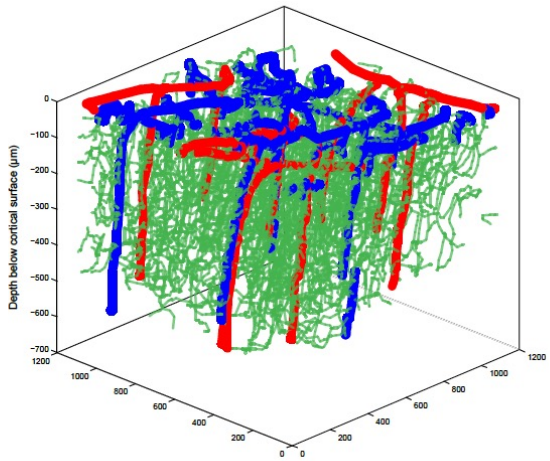
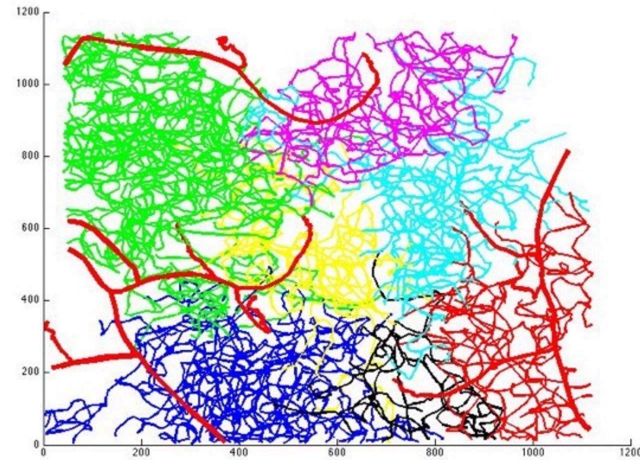


32% of arterioles responsive

14% average peak dilation at 5 s following 2s stimulation

Using Two-Photon Microscopy to Directly Visualize the Neurovascular Unit in Vivo





capillary length = 79.4±1.9 µm

capillary density = 6,695 capillaries/mm^3

capillary tortuosity (arc-chord ratio) = 1.2

Conclusions

- Advantages of Animal Models
  - Allow comprehensive, multi-modal investigations
  - Can be Performed in State of the Art MRI Systems
  - High SNR, Spatial and Temporal Resolution
- Challenges
  - Use of anesthesia is a major confound for fMRI studies
  - Training of animals to perform specialized
- Marmoset is an important experimental animal model for basic science and translational research
- High resolution MRI of the marmoset brain can be obtained with remarkable cytoarchitectonic detail
- Functional MRI can used to study various sensory system including somatosensory, auditory and visual areas.
- Lissencephalic cortex facilitates study of neuronal circuits with optical imaging techniques

Cerebral Microcirculation Section

https://www.lfmi.ninds.nih.gov/CMSWeb/cms-main.html

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  - ***Sang-Ho Choi*** *(Molecular Biology, Neuroinflammation)*
  - ***Cecil Yen*** *(fMRI, Stroke)*
- Postdoctoral Fellows:
  - ***Jungeun Park*** *(Transgenic Marmosets)*
  - ***Soo Hyun Park*** *(David Leopold – fMRI, Electrophysiology, Behavior)*
- Predoctoral Fellow
  - ***Wen-Yang Chiang*** *(RF Hardware)*
- Post-bac IRTAS
  - ***Brandon Chen*** *(2PLSM, Awake Mice)*
  - ***Joseph Choi*** *(Molecular Biology, CRISPER/cas9)*
- Lab Technician
  - ***Lisa Zhang*** *(Surgeries, Organization, Supplies, Marmoset Colony Management, Transgenic Marmoset Procedures, Training)*