Cutting-Edge Methods Shaping Neuroimaging Frontiers

SFIM

Peter A. Bandettini, Ph.D.

FMRIF

Section on Functional Imaging Methods
Functional MRI Core Facility
Center for Multimodal Neuroimaging

National Institute of Mental Health

Impact of fMRI so far?

- fMRI **(dynamic) connectivity** has catalyzed new frameworks and directions in multimodal human brain research.
- fMRI has established a **brain network** perspective: relationships to tasks, behavior, states, and traits, and spontaneous engagement - and pathology*…but not yet sufficient providing mechanistic insights, nor for clinical utility.*
- fMRI has identified **changes** with learning, adaptation, and plasticity, and has clarified processes such as attention, language, sensory integration, emotion, and cognition in general.
- fMRI has catalyzed rethinking of **Individual Differences & Similarities**, and **Categorization of Pathology** (RDoC, DSM-5).
- fMRI has catalyzed research in **Neurovascular coupling** and brain energetics.

Common Criticisms

The signal is slow and coarse, variable over time and space, not fully understood, and not directly informative of neuronal mechanisms.

The images suffer from signal dropout and artifact and are sensitive to motion.

…but it's sensitive, robust, noninvasive, replicable if done well, increasingly confirmed, and the best thing we have.

Why do we want to improve it?

There is more information in the signal.

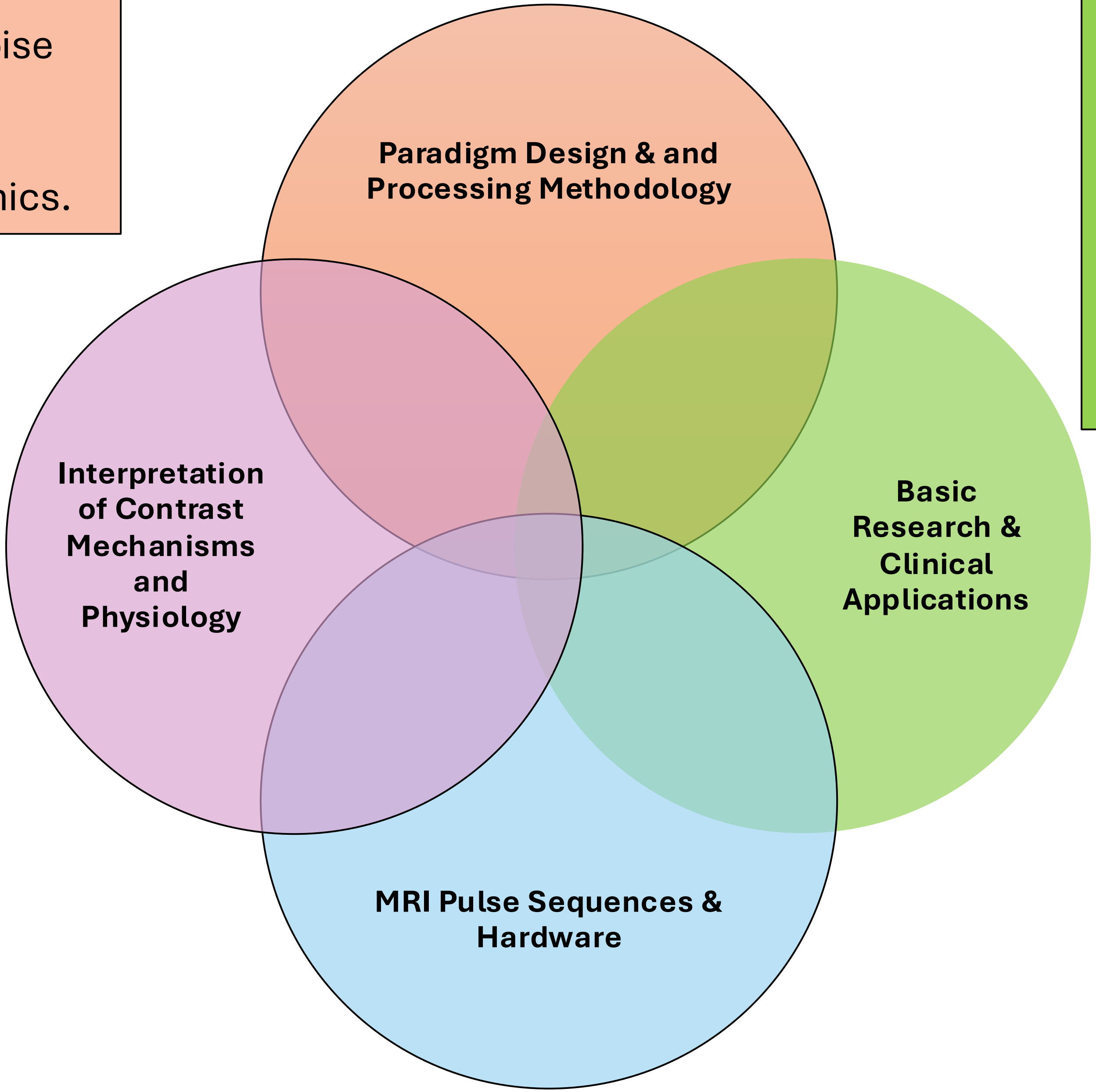
- **Improvements in temporal/spatial resolution, interpretability, and processing methods provide leverage to develop** ***better models of noise and information.***

- **fMRI doesn't suffer from a lack of sensitivity. We just haven't figured out how to fully wrangle the data from the backdrop of variability (in time and across subjects).**

FMRI

- Capture neuronal activity in time and space.
- Separate information from noise and artifact.
- Functional activation, connectivity, patterns, dynamics.

- Biomarker discovery and development
- Targets for neuromodulation
- Presurgical mapping
- Neurofeedback
- Uncover mechanisms and principles of functional brain organization.



- Identify relevant temporal and spatial structure.
- Multi-modal assessment: Relate signal to neuronal activity and non-neuronal measures.

- Speed
- Resolution
- Sensitivity
- Specificity
- Reproducibility
- Quantitation
- New Contrasts

Cutting Edge Methods: Approaches, Challenges, Insights

High resolution

-spatial: layer fMRI

-temporal: rapid decoding

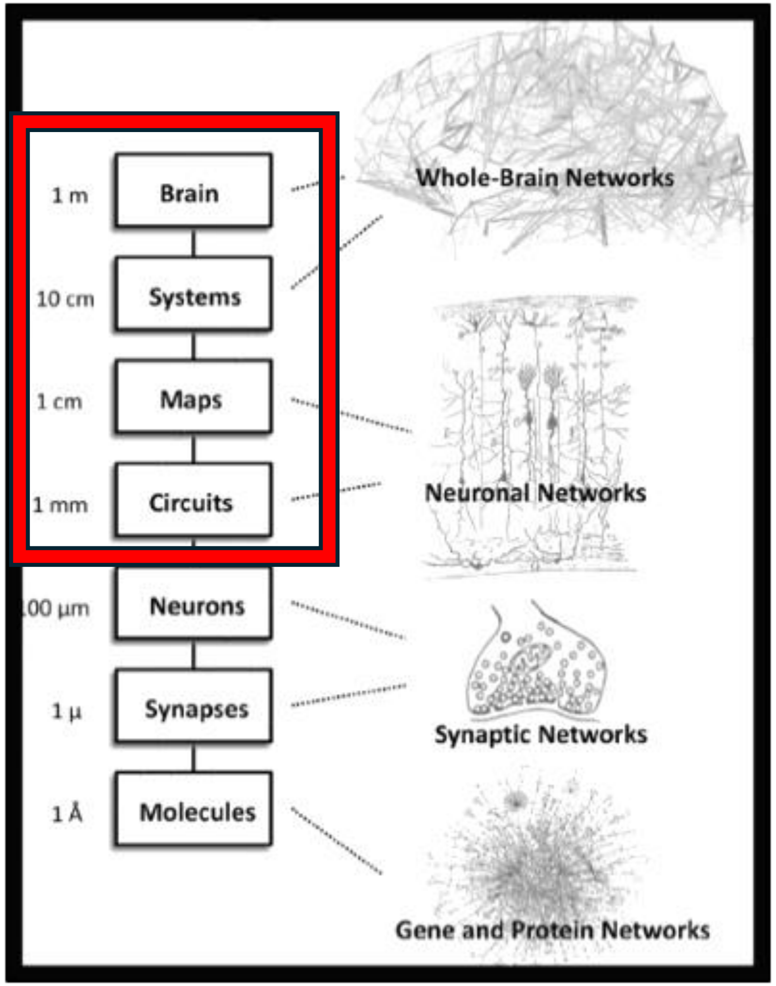
Time Series Information

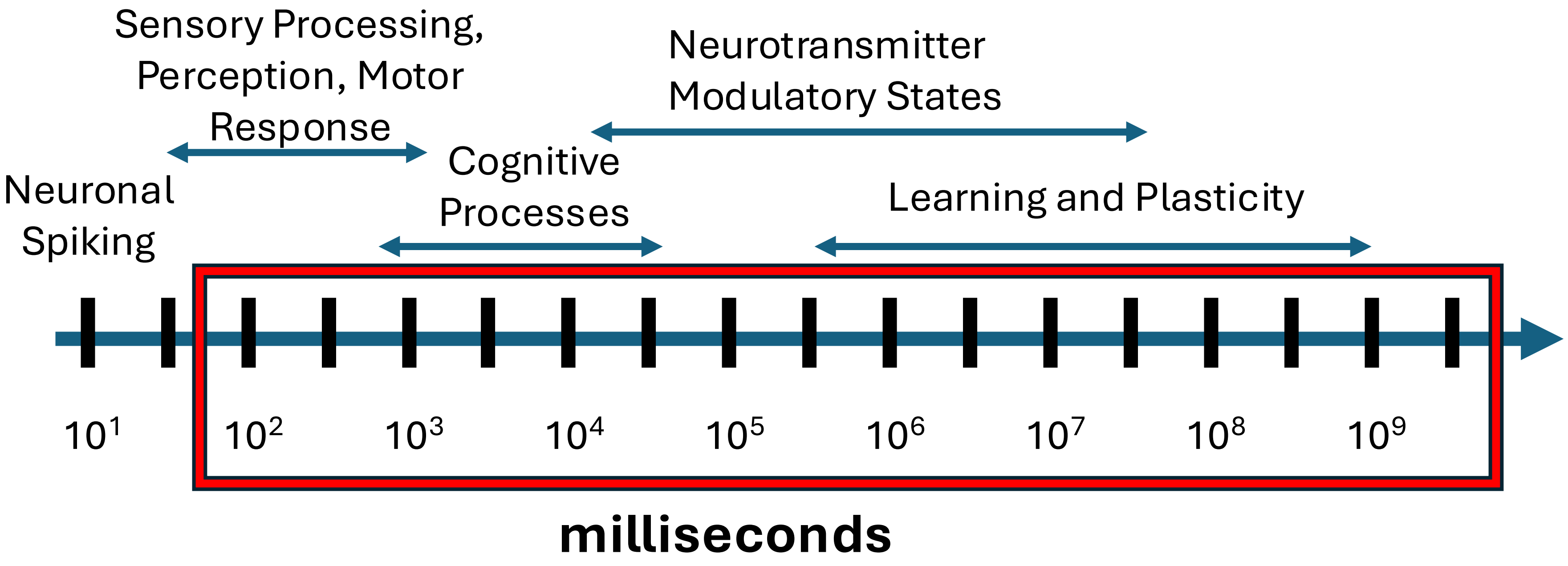
Dense Sampling

Multi-modal Integration

Spatial and Temporal Scales of Brain Function

What ***scale*** provides most leverage to understand human brain organization and mechanistic brain-behavior relationships?





Cutting Edge Methods: Approaches, Challenges, Insights

High resolution

-spatial: layer fMRI

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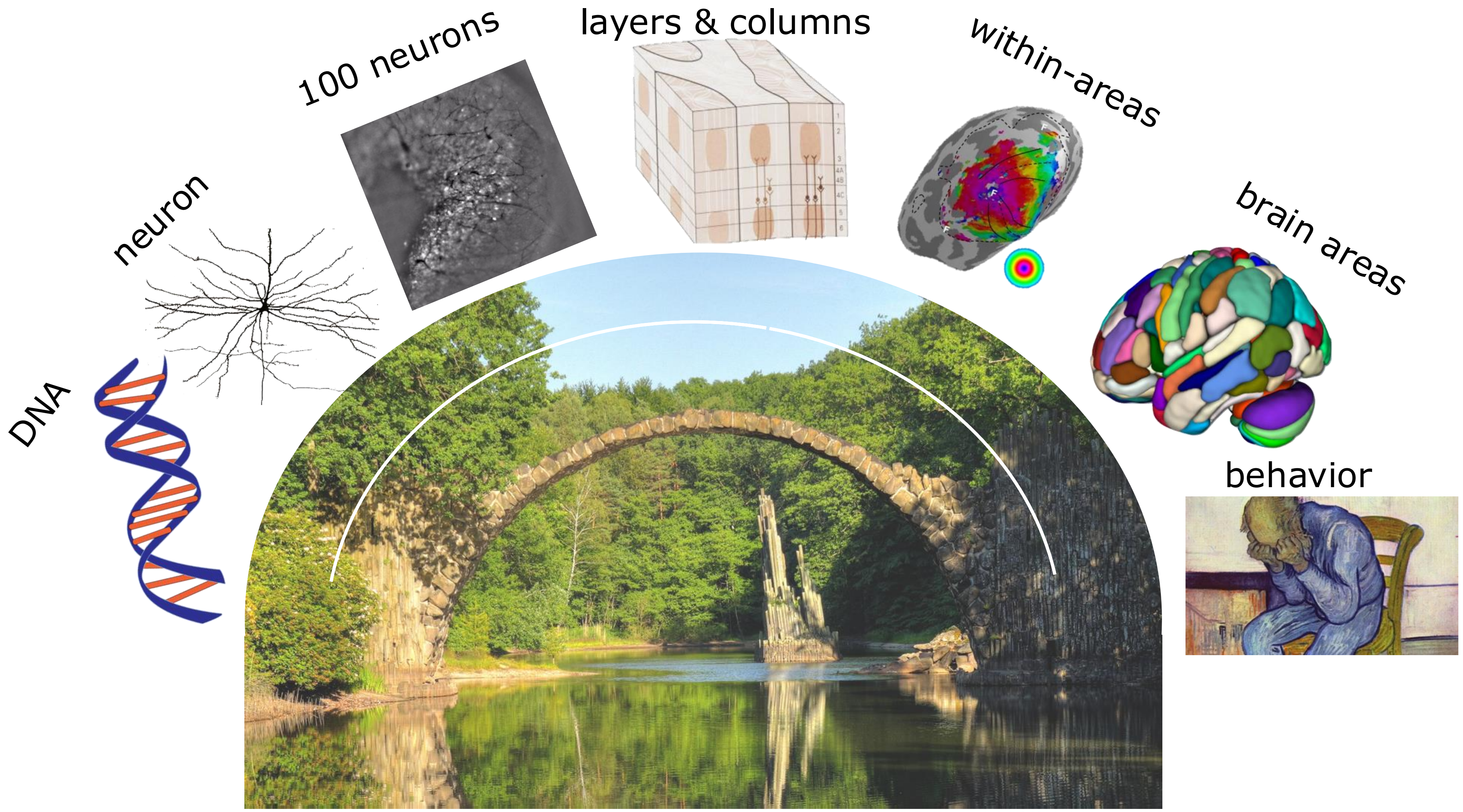
Time Series Information

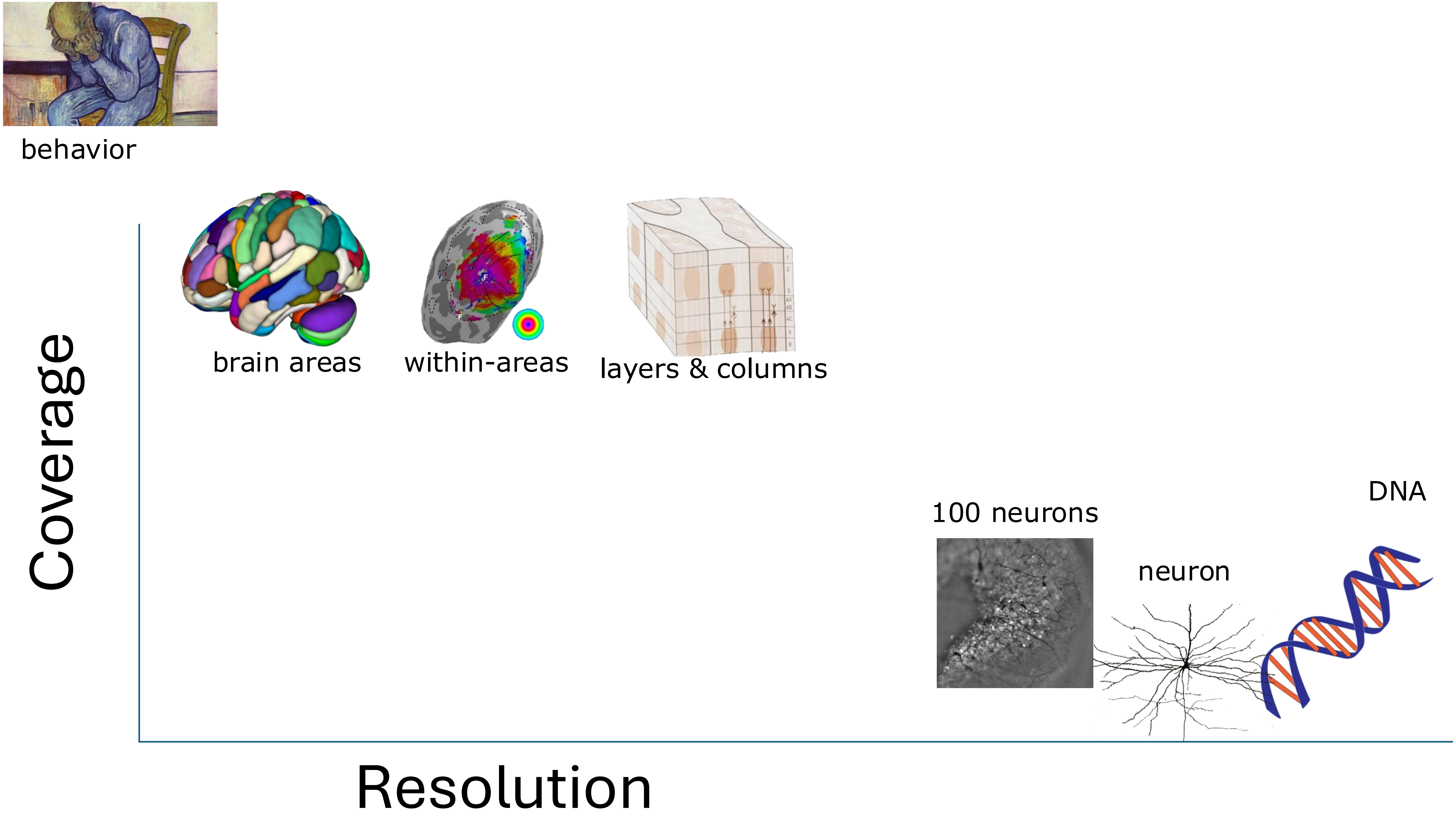
Dense Sampling

Multi-modal Integration

From Genes to Behavior: The Mesoscale Bridge

What ***scale*** provides most leverage to understand human brain organization and mechanistic brain-behavior relationships?




behavior
brain areas
within-areas
layers & columns
100 neurons
neuron
DNA
Coverage
Resolution

What is needed for layer specific fMRI?

Small voxels:

< 0.8 mm
Achieved with Multidimensional Acceleration / Parallel Imaging, multi-shot

High sensitivity:

SNR proportional to Voxel Volume
@ high res, SNR > 40 after averaging (typically working with SNR < 20)
Achieved with local RF coils or high N arrays, high field ($\geq$ 7T)

High specificity:

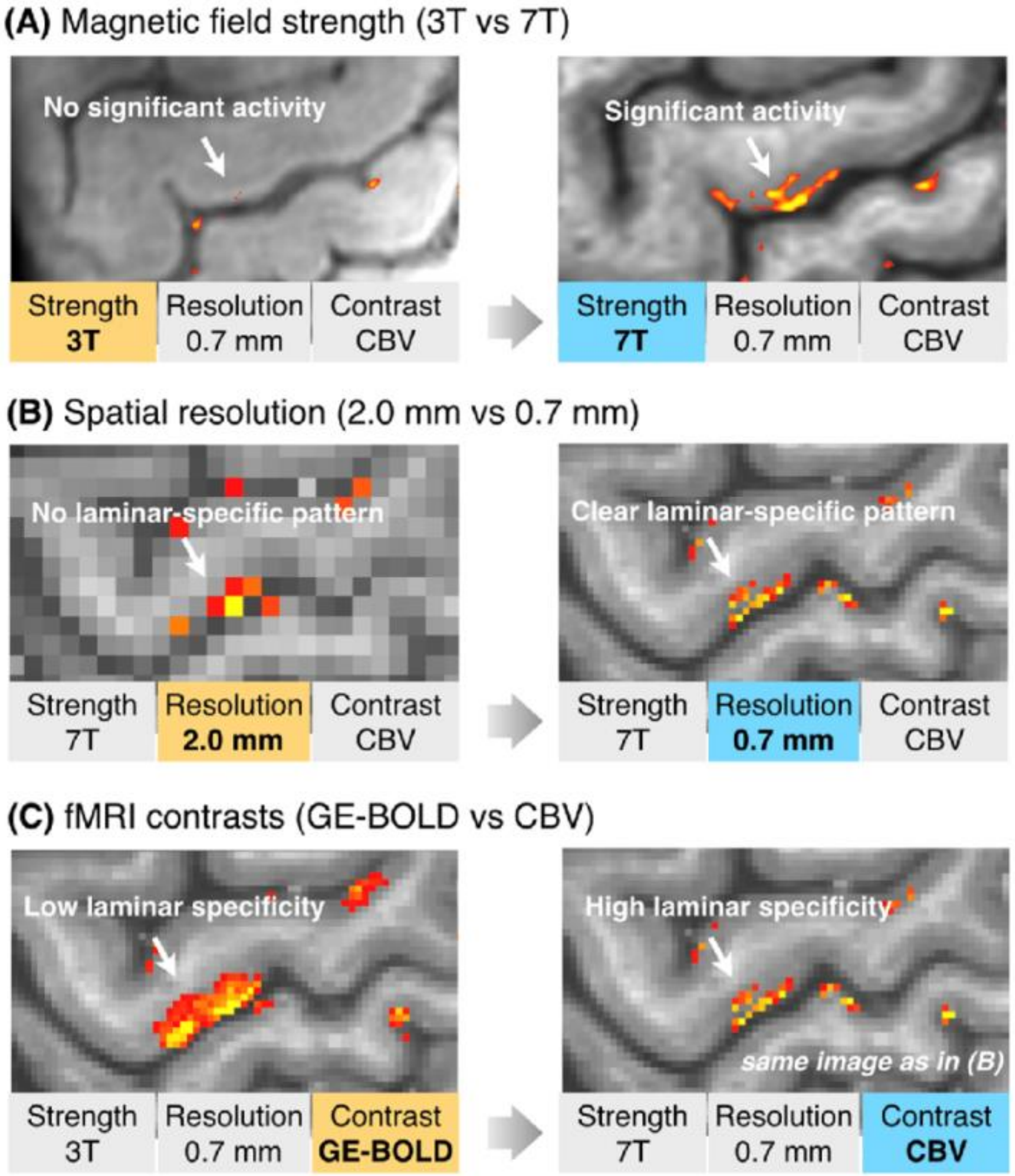
Spin-echo suffers from some intravascular (large vessel) signal
Arterial Spin Labeling has superior specificity but sensitivity too low
VASO and 3D-GRASE seem best, but VASO suffers from low speed/narrow coverage

Layer segmentation and novel processing:

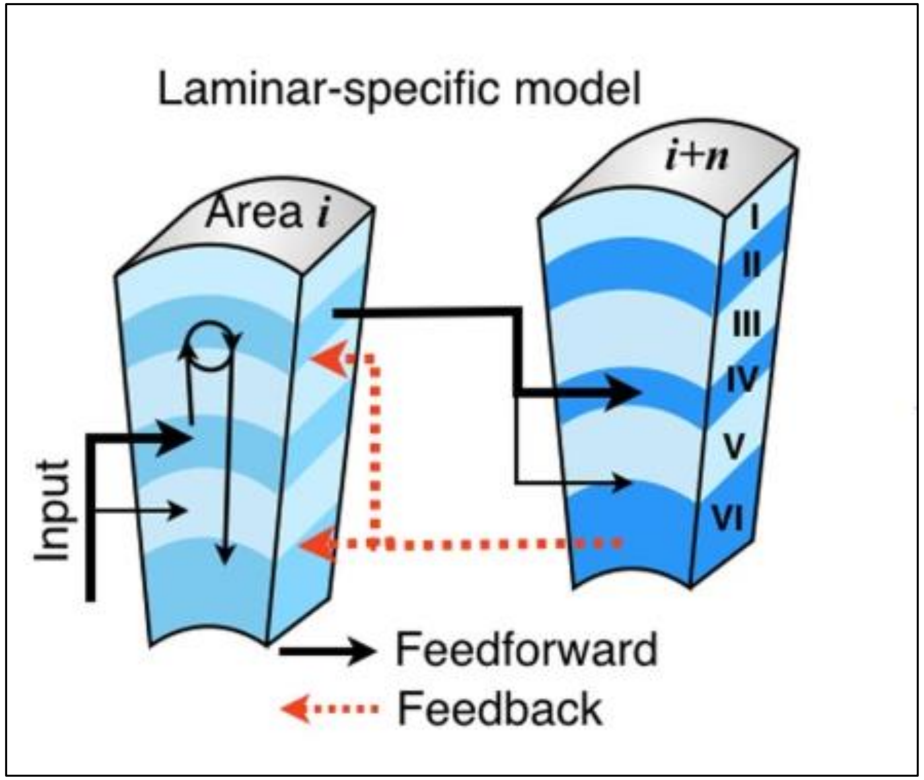
LayNii

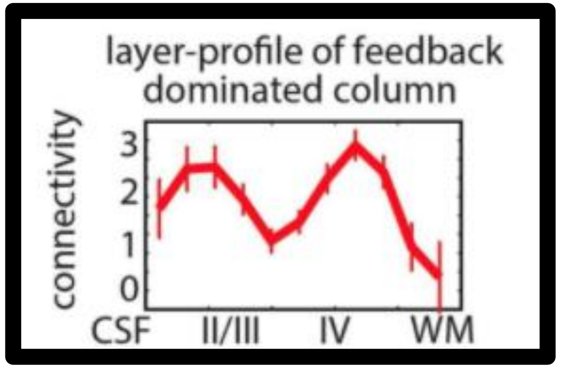
138 Human 7T Scanners Worldwide (Courtesy Renzo Huber)

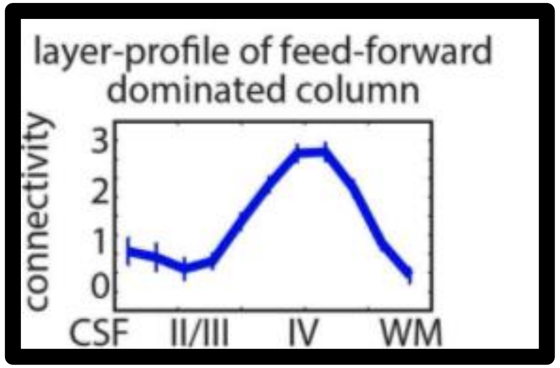
https://www.google.com/maps/d/viewer?mid=1dXG84OZIAOxjsqh3x2tGzWL1bNU&usp=sharing



The Hope: Directional Connectivity and Functional Hierarchy







J. Yang, et al. Neuroscience & Behavioral Reviews 2021

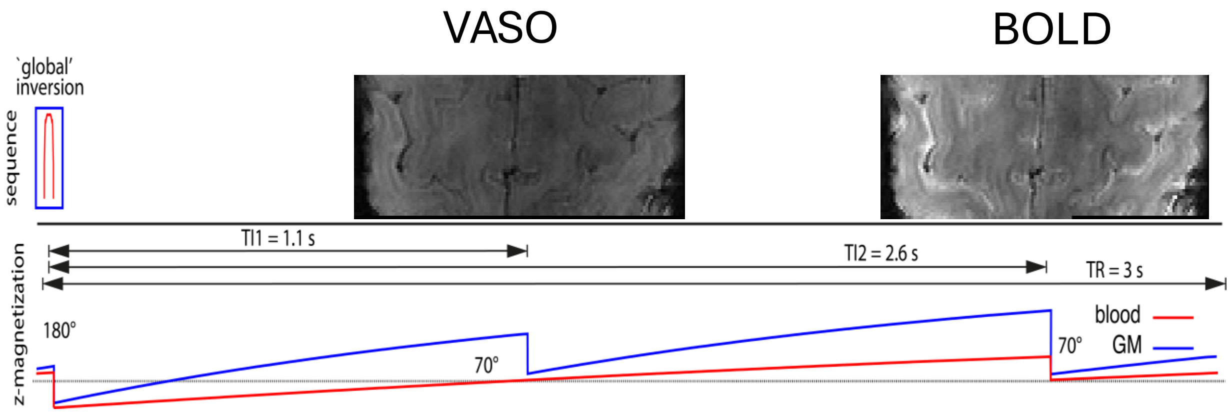
Methods	Pros	Cons
GE-BOLD	High sensitivity, easy to implement, short TR	Poor laminar specificity due to draining and pial veins
SE-BOLD	SE-EPI: reduced macrovascular bias; T2-prep: signal primarily from small vessels	SE-EPI: T2* effects vary with EPI acquisition length; T2-prep: low sensitivity
VASO	High laminar specificity, excellent tissue contrast facilitating layer analysis	Sensitivity ≤50% of BOLD, TR more than twice as long as GE-BOLD
ASL	Best laminar specificity among listed methods, provides quantitative measurements	Sensitivity 10–20% of BOLD, TR longest among listed methods
VAPER	High laminar specificity, dynamic pseudo-continuous blood nulling throughout acquisition thus not sensitive to inflow effects	Sensitivity ≤50% of BOLD, TR more than twice as long as GE-BOLD

Yuhui Chai, Ru-Yuan Zhang, Exploring methodological frontiers in laminar fMRI, *Psychoradiology*, Volume 4, 2024, kkae027, https://doi.org/10.1093/psyrad/kkae027

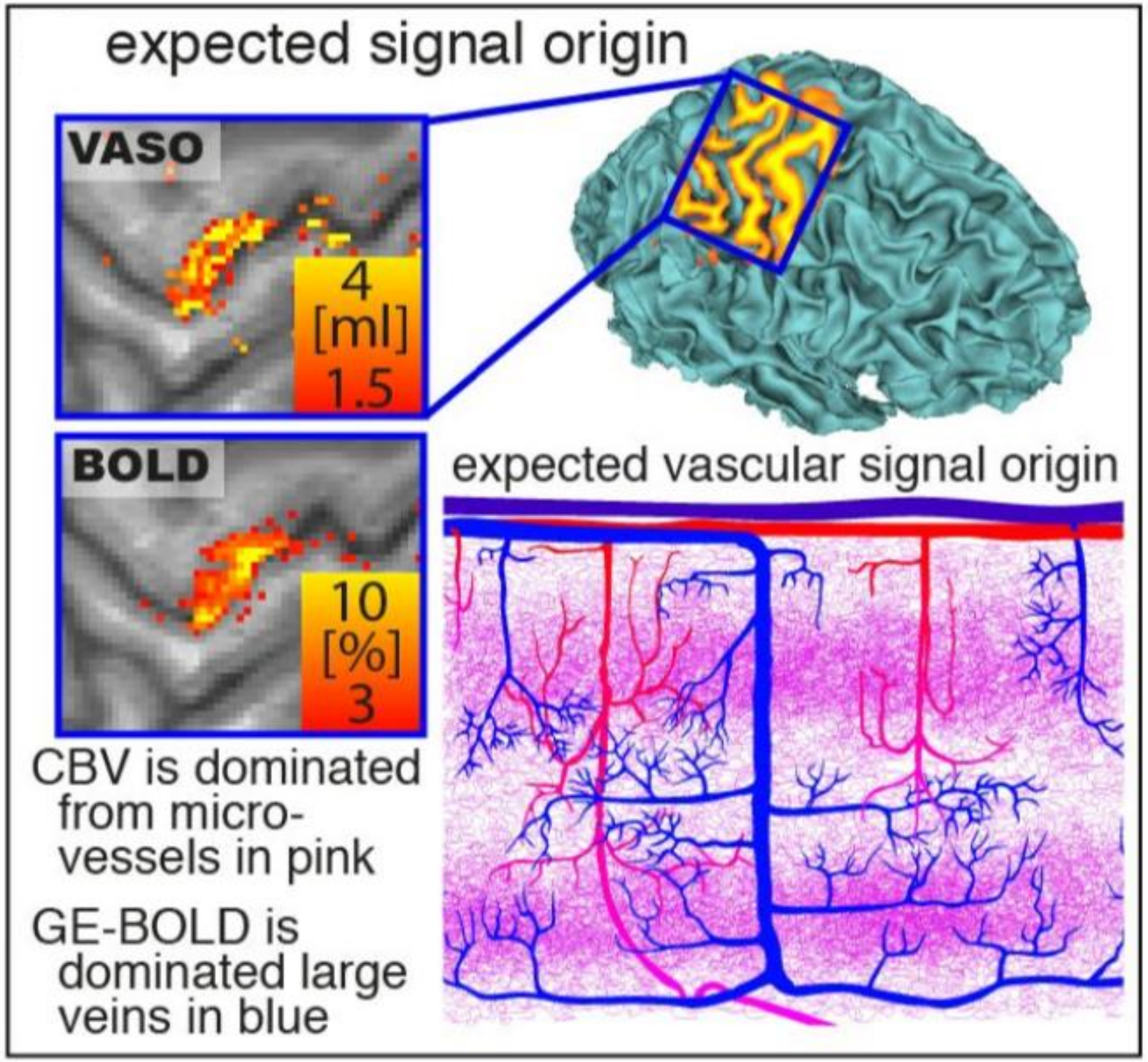
Simultaneous VASO and BOLD

VASO: Vascular space imaging (blood volume changes)
BOLD: blood oxygenation level dependent (amount of doxy-Hb)

Simultaneous VASO & BOLD:
Our primary pulse sequence for high-res fMRI:

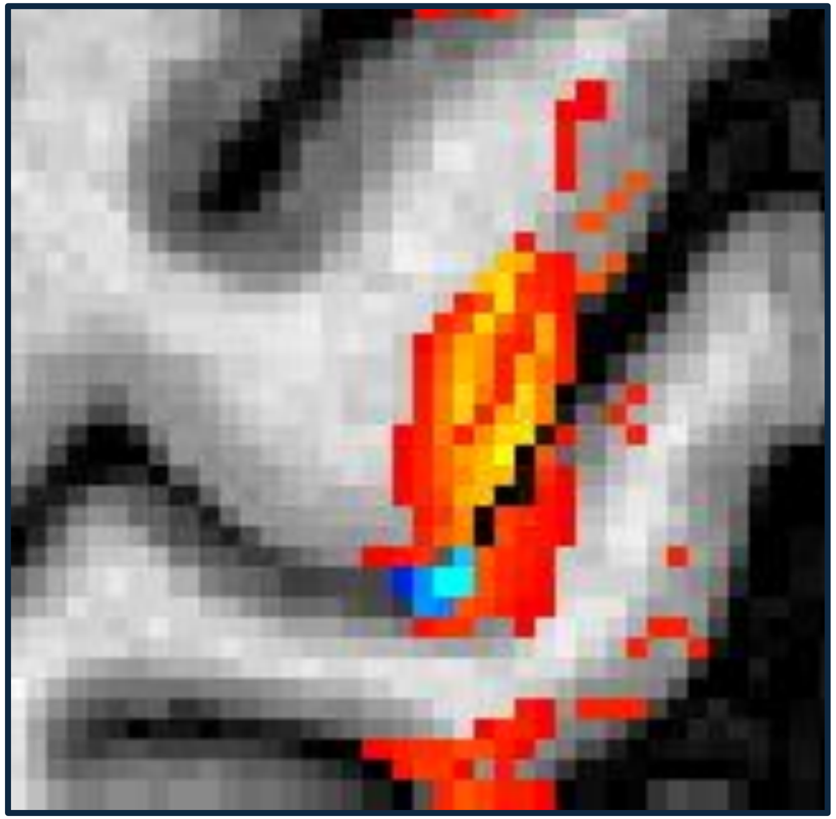


[Lu et al. MRM 2003], [Huber et al., MRM, 2014]



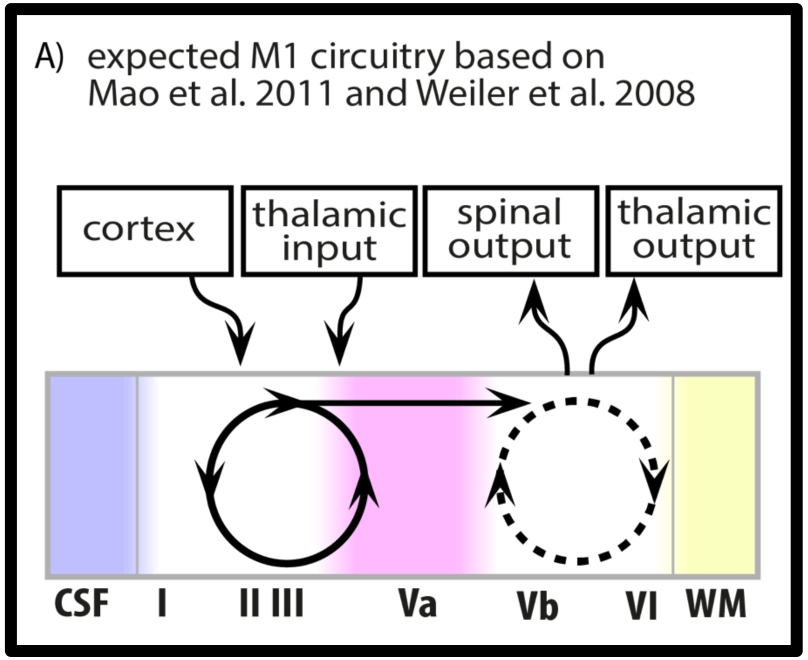
Functional Structure Changes < 0.8 mm

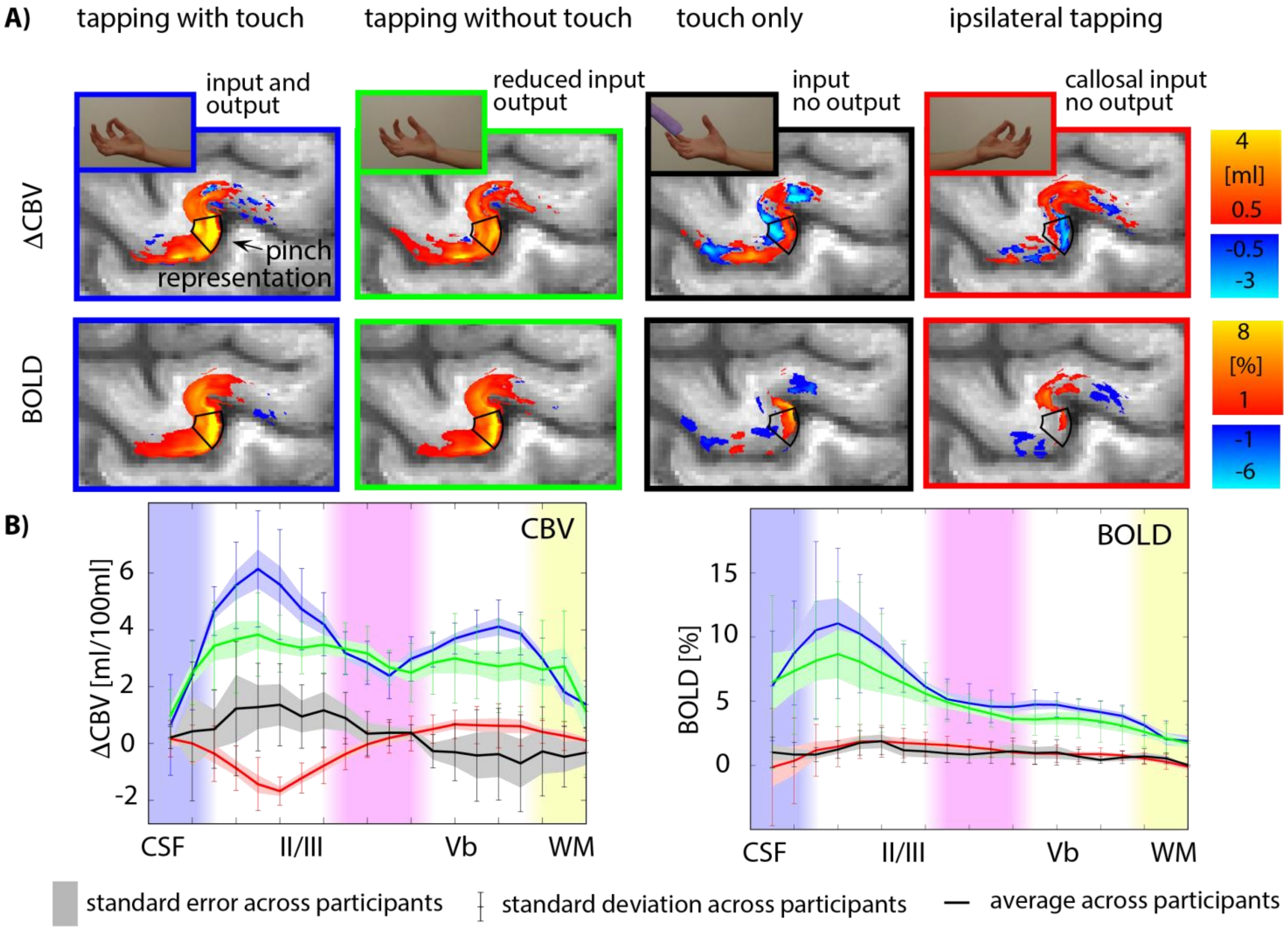
right tapping



resol. 0.75mm

Layer Activity Modulation and Mapping with VASO





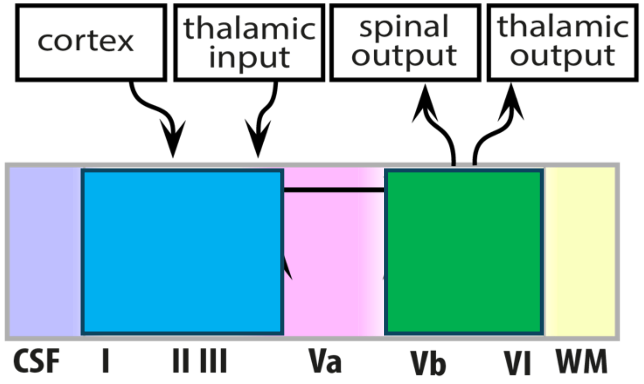
3D-EPI, TR=2.5s
FLASH GRAPPA 3
0.8 x 0.8 x 1

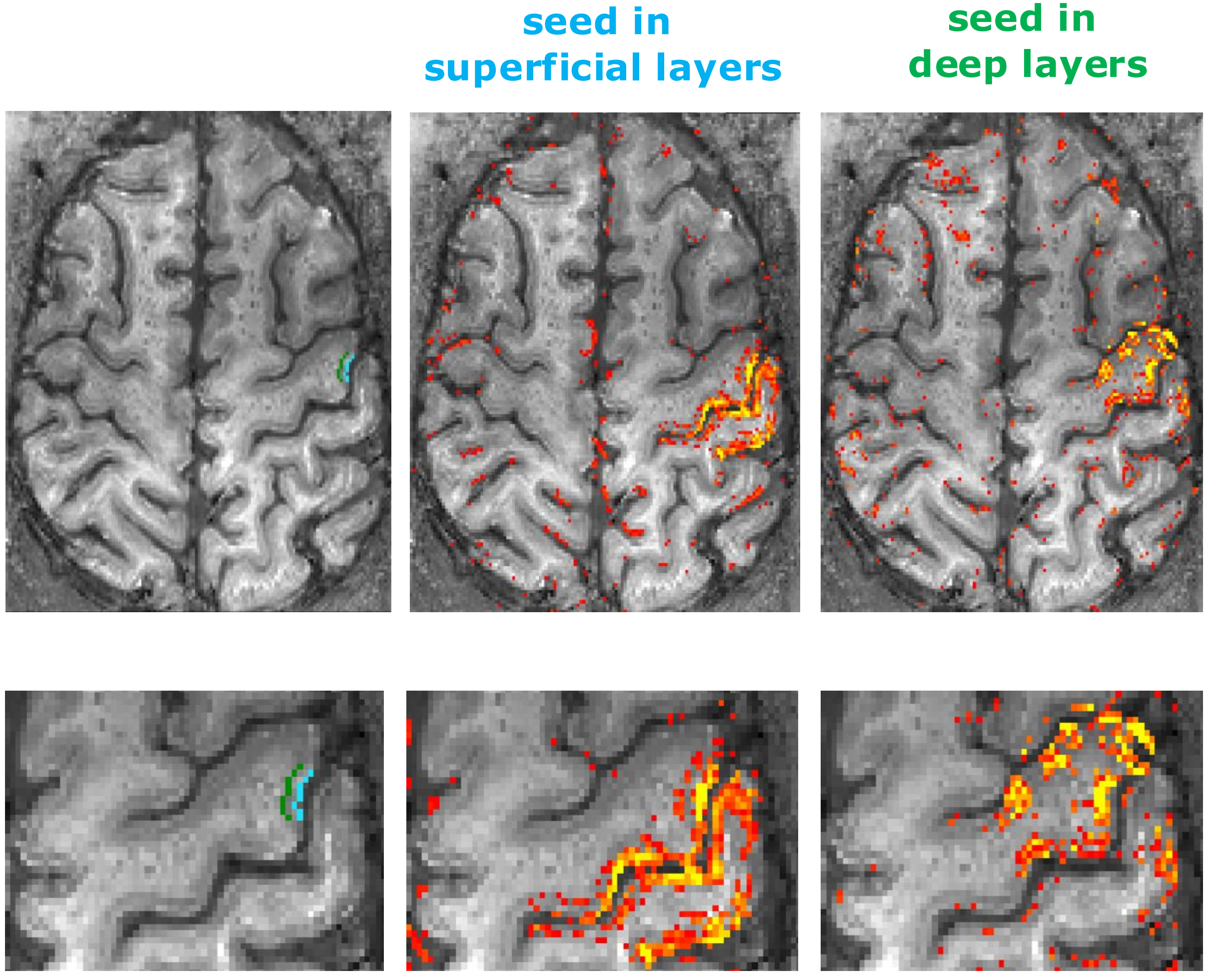
High-resolution CBV-fMRI allows mapping of laminar activity and connectivity of cortical input and output in human M1 **L. Huber, et al. Neuron, 96(6), pp. 1253-1267 (2017)**

Resting State:

Seeds in Layers of M1: Mapping of Areas Connected to Superficial and Deep Layers

A) expected M1 circuitry based on Mao et al. 2011 and Weiler et al. 2008





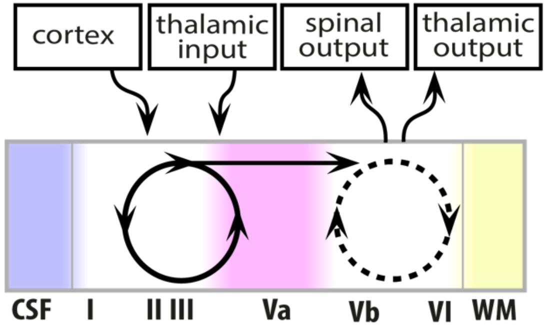
Resting State:

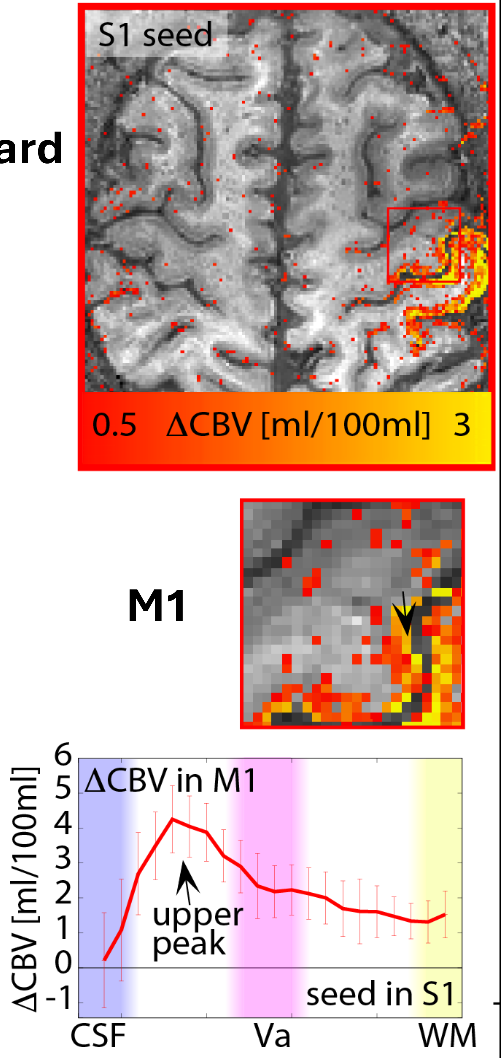
Seeds in S1 and Pre-motor cortex: Assessing Layer Connectivity Profiles in M1

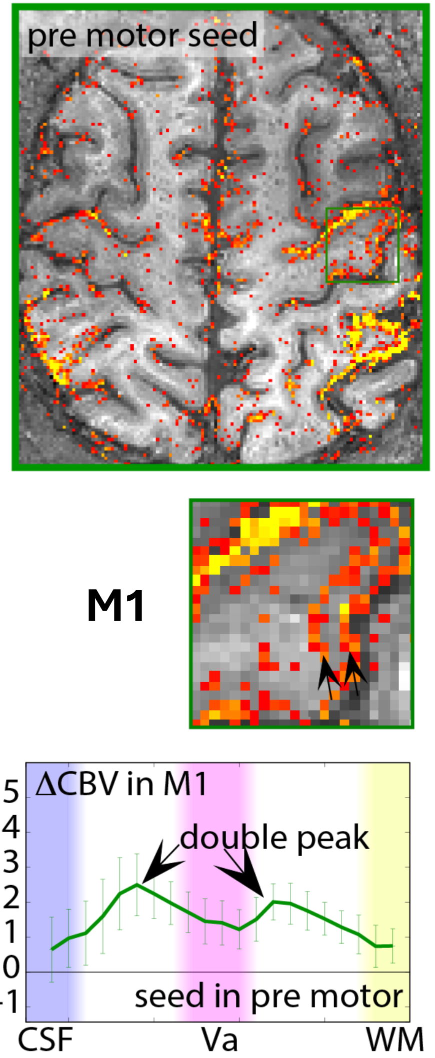
Receives Feedforward Input from S1

Receives Feedback Input from Pre-motor

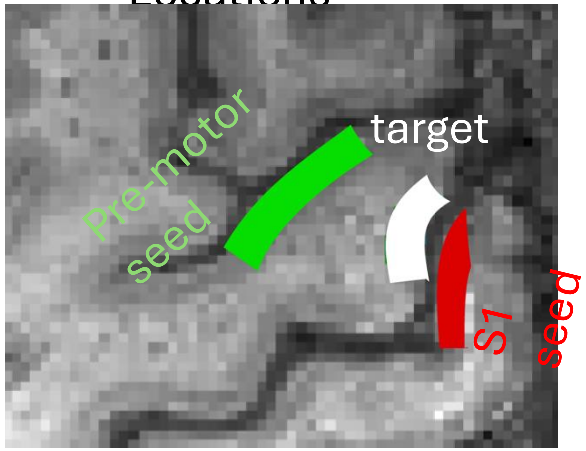
A) expected M1 circuitry based on Mao et al. 2011 and Weiler et al. 2008







Seed Locations

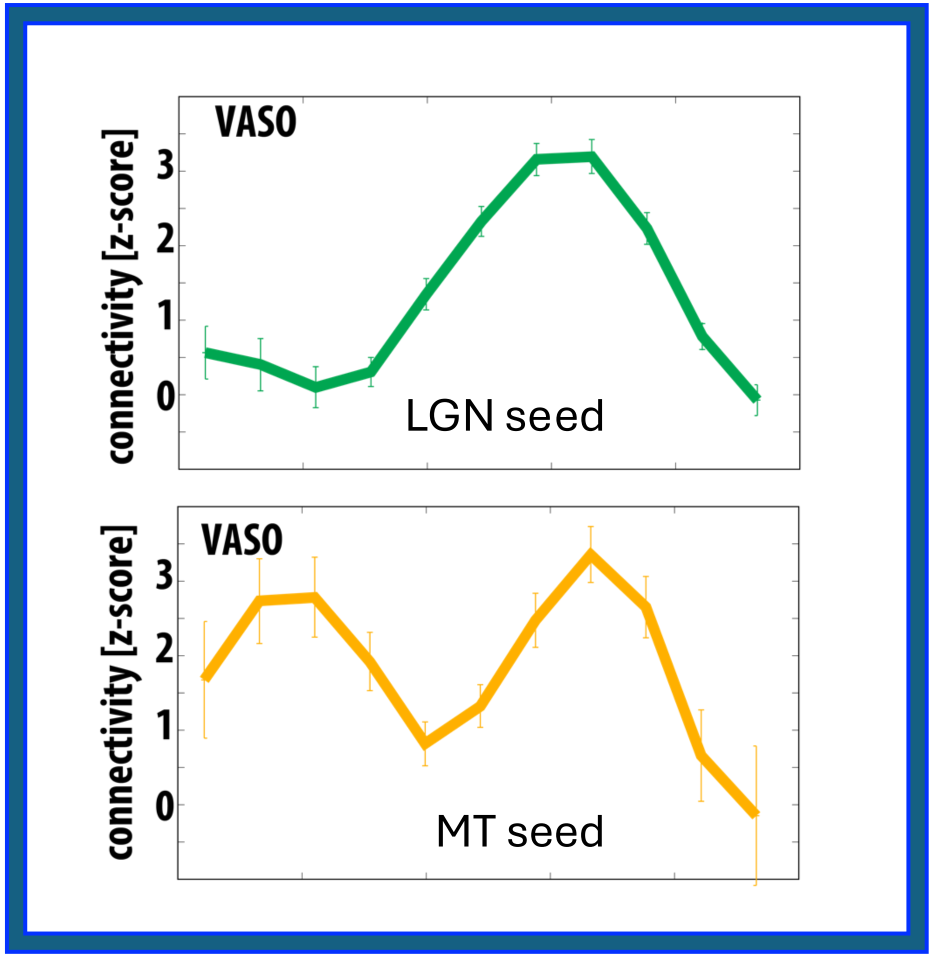


Directional connectivity from Resting State Signal

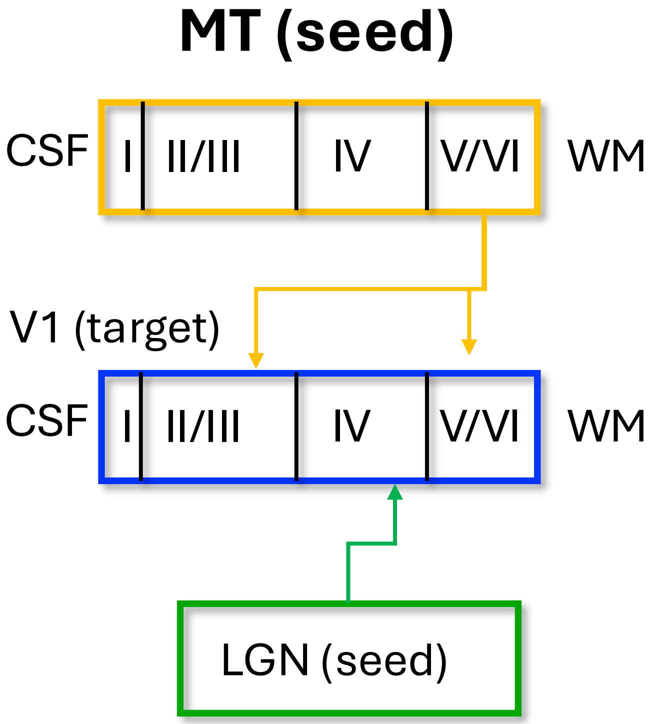
V1 Layer Profile

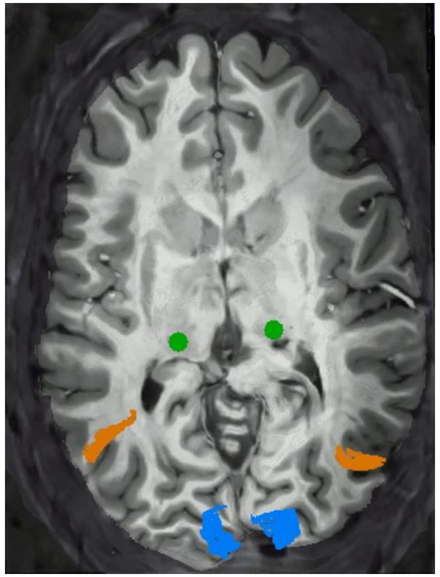
Receives Feed-forward Input from LGN

Receives Feedback Input from MT



CSF WM

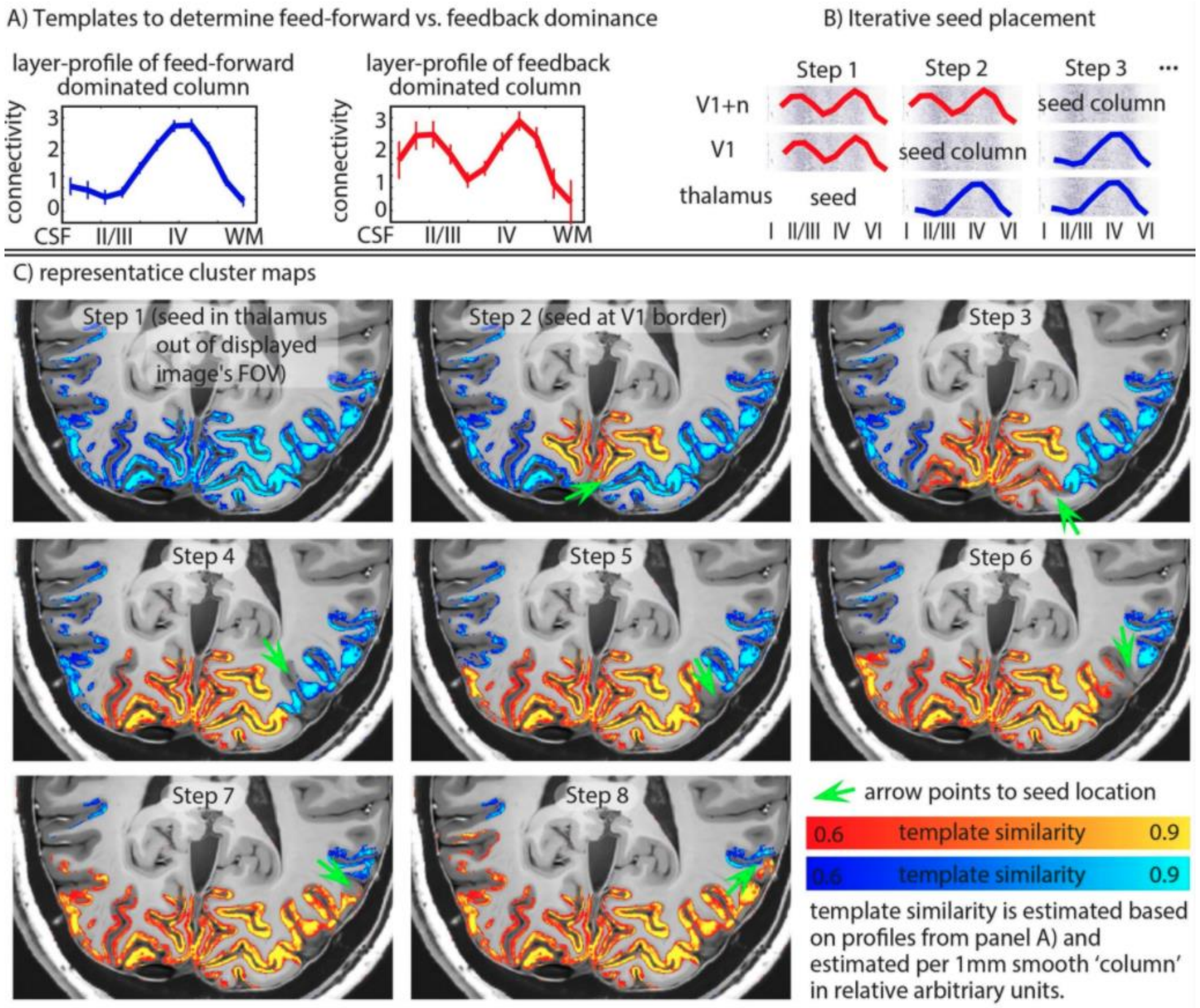




Res. 0.8 x 0.8 x 0.8 mm^3
FLASH GRAPPA 3

3D-EPI
TR = 2.5 s

Huber L, et al.: **Layer-dependent functional connectivity methods**. *Prog Neurobiol* 2020, doi:10.1016/j.pneurobio.2020.101835.



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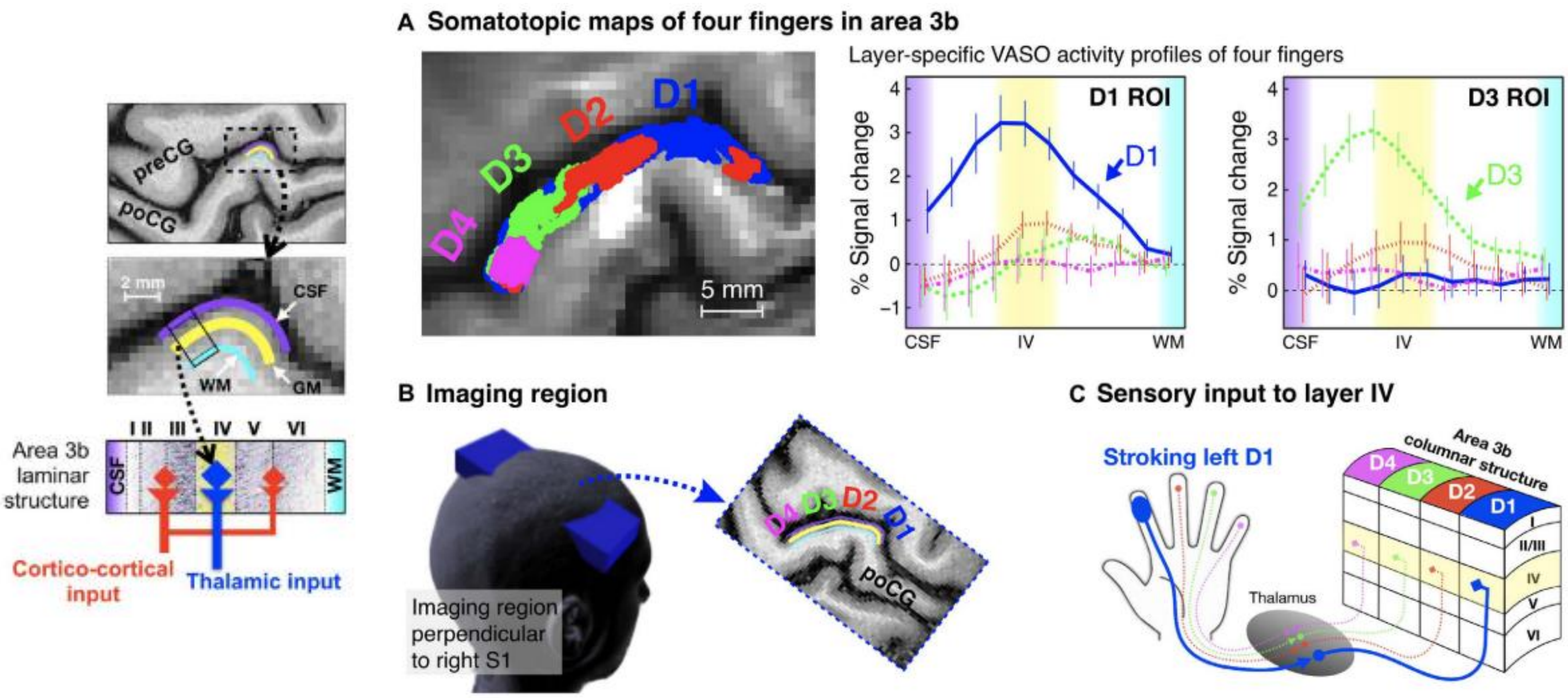
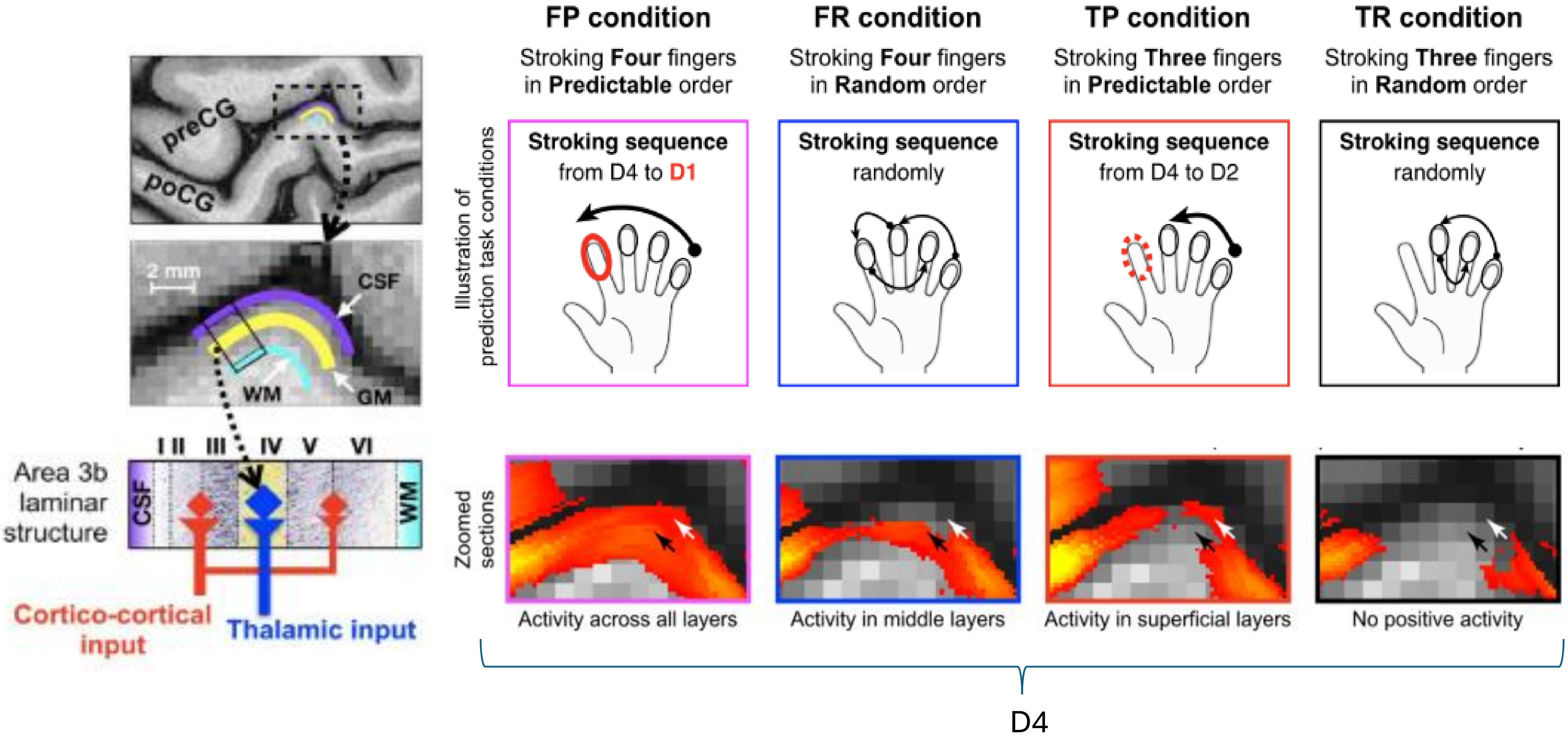


Yu Y, Huber L, Yang J, Jangraw DC, Handwerker DA, Molfese PJ, Chen G, Ejima Y, Wu J, Bandettini PA: **Layer-specific activation of sensory input and predictive feedback in the human primary somatosensory cortex**. *Sci Adv* 2019, **5**:eaav9053.

Strong prediction -> strong upper/lower layer activation, **Weak prediction** -> weak upper/lower layer activation



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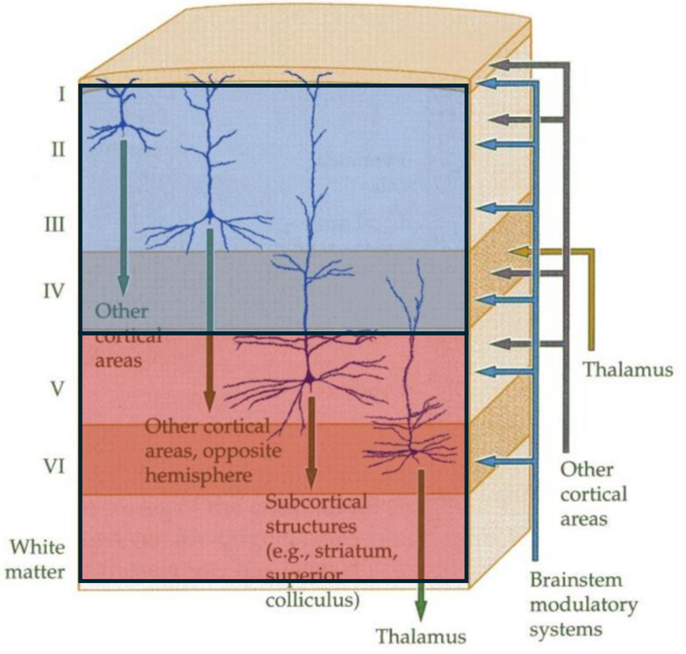
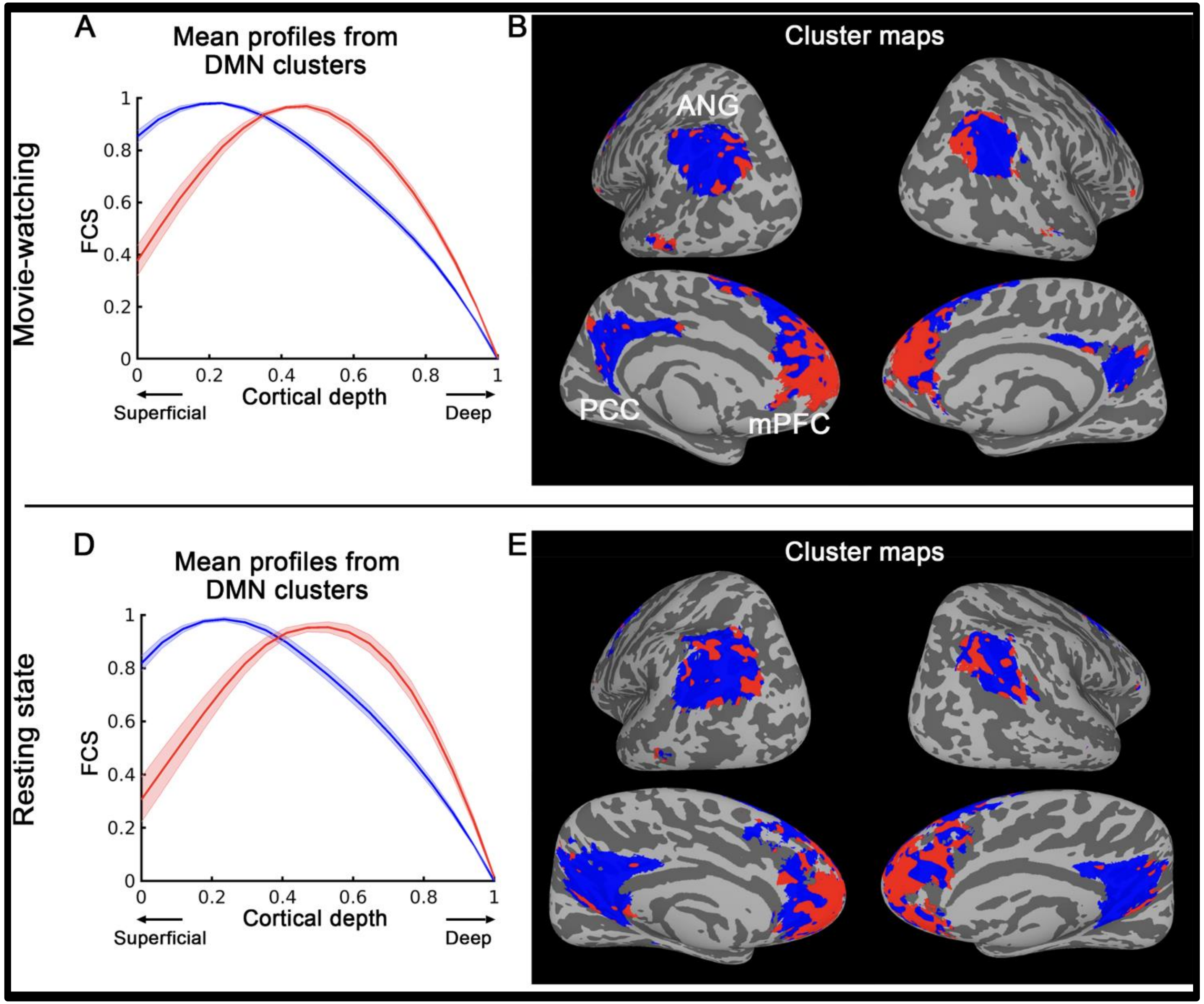
K-means clustering across depth. *(K=2 differentiating "upper vs lower" layer activity)*

Profiles and K=2 maps

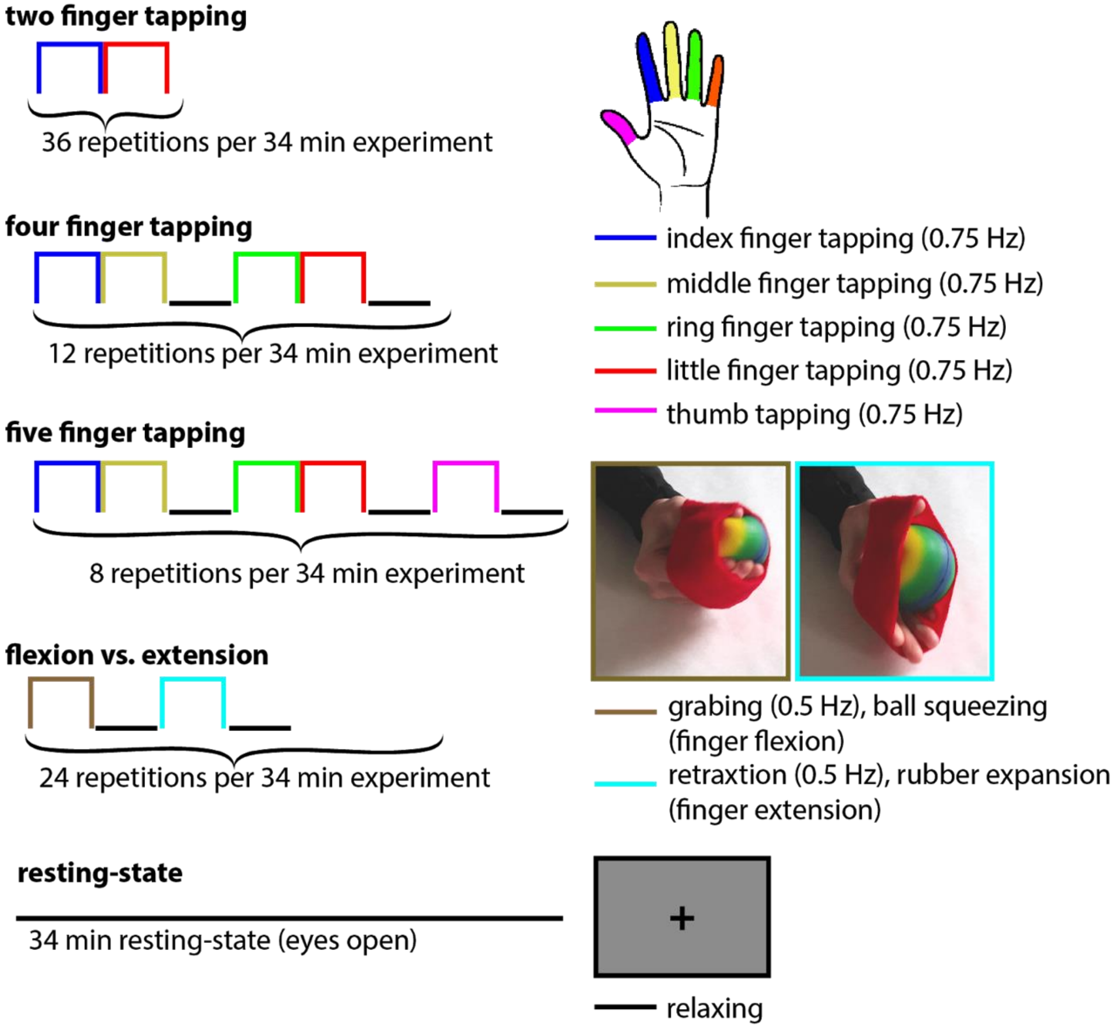
Default Mode Network

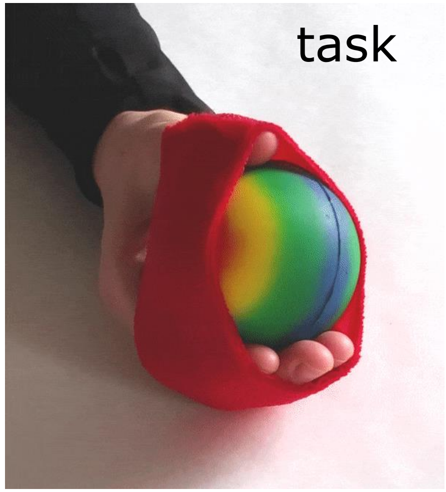




Y. Chai, et al. NeuroImage 2020

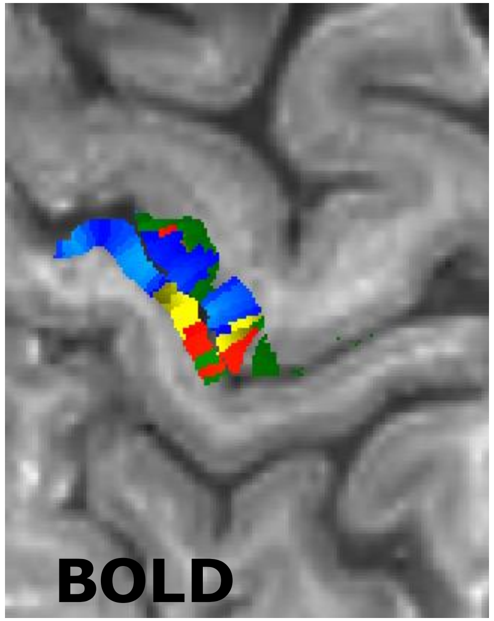
Finger Representation in M1: tapping fingers and flexion vs extension

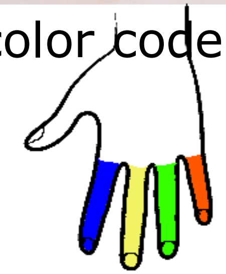


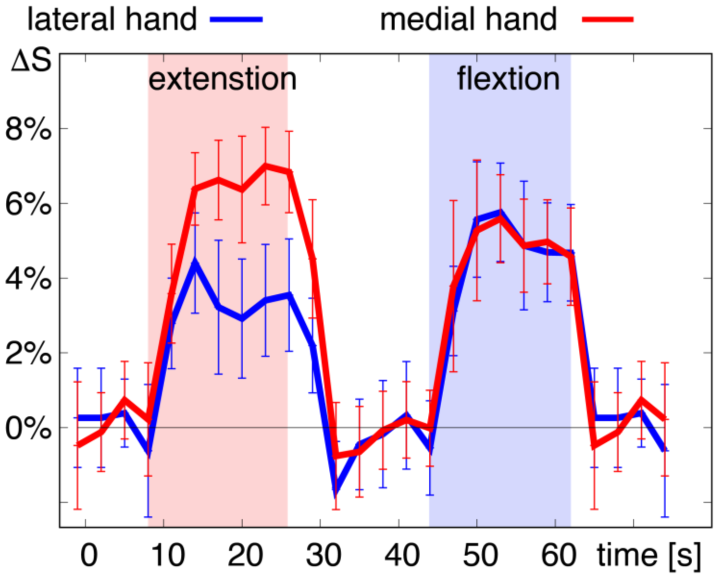


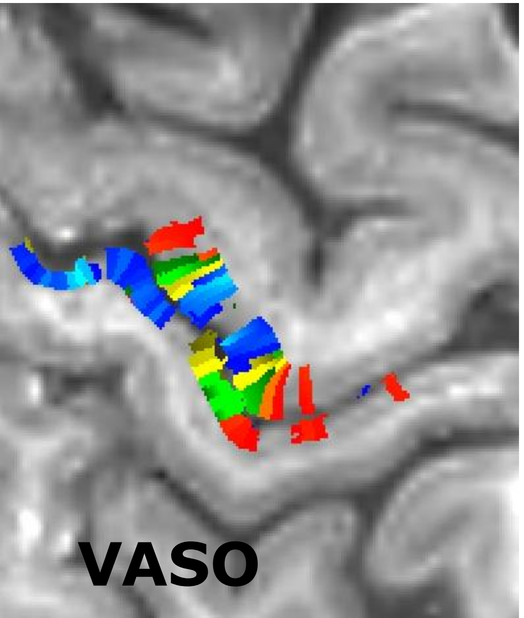
Huber, L., et al . (2020). Sub-millimeter fMRI reveals multiple topographical digit representations that form action maps in human motor cortex. *NeuroImage*

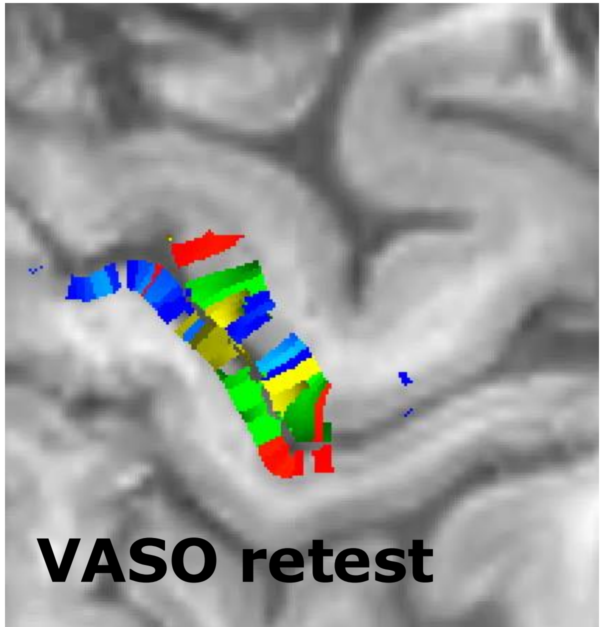
Digit Representation in M1

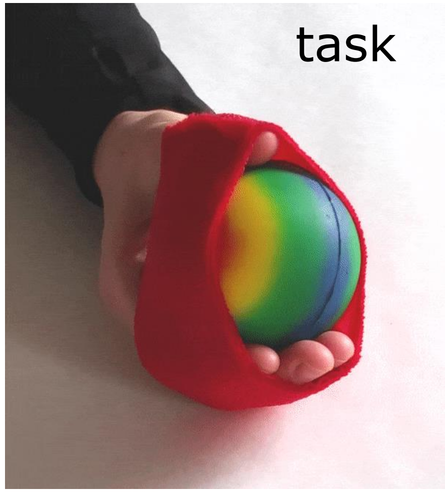






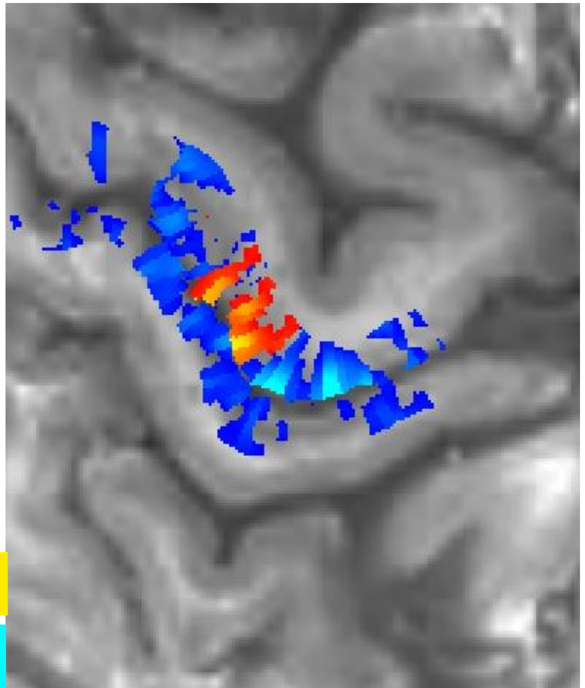






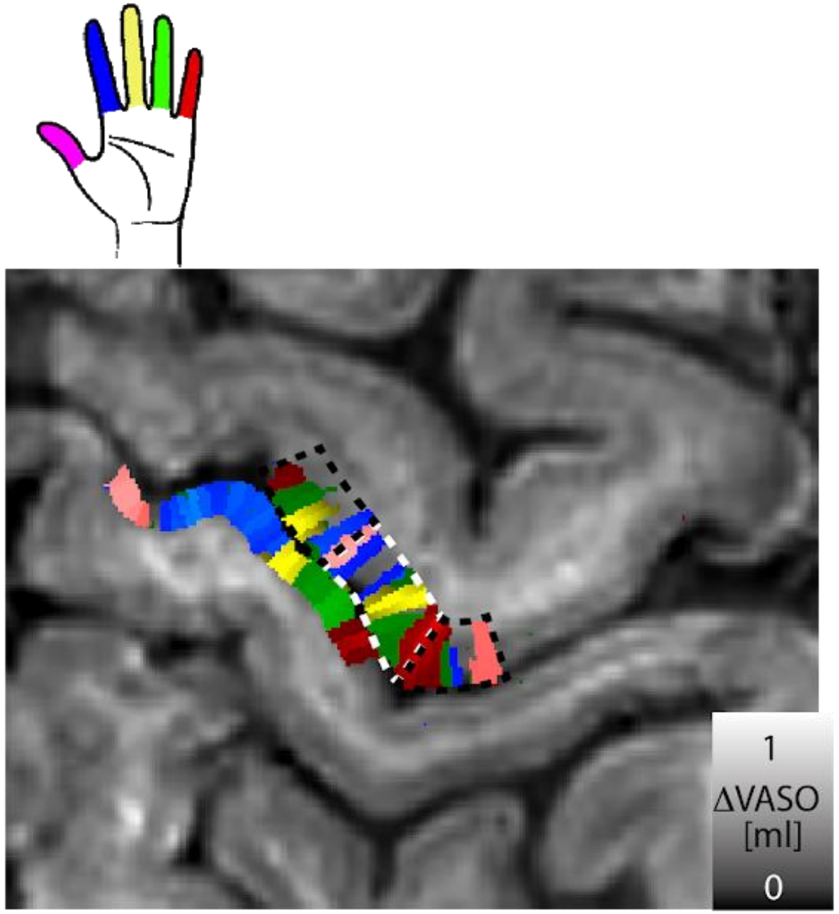
Extension (spreading)

Flexion (compressing)

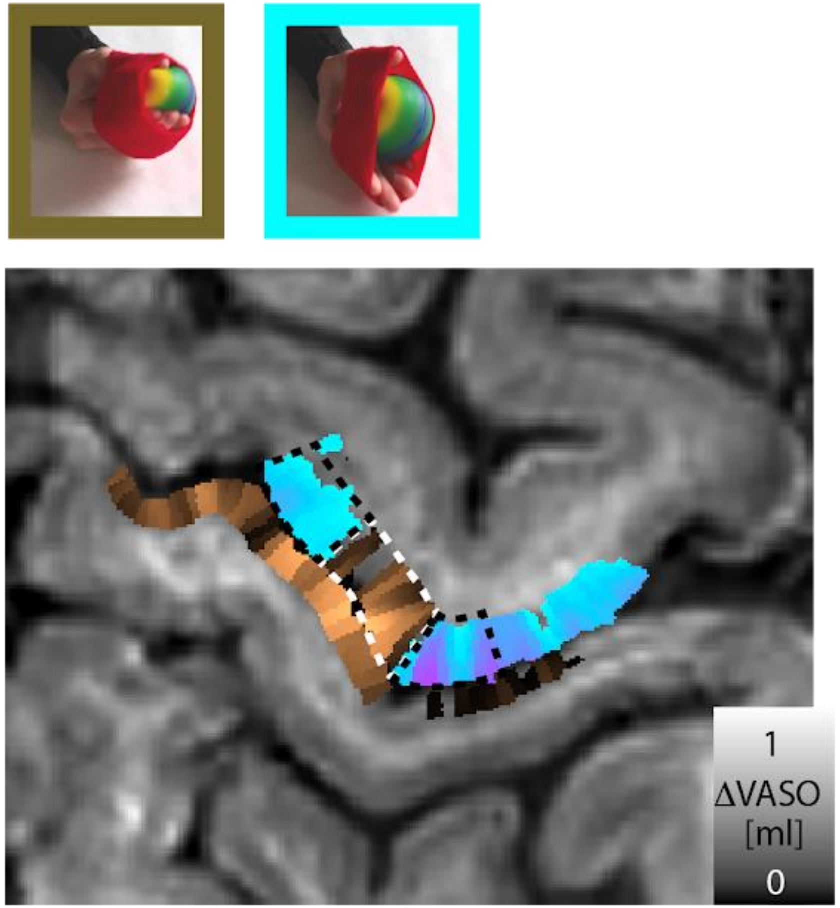


Digit representations appear to be organized by opposing movements

five-finger tapping

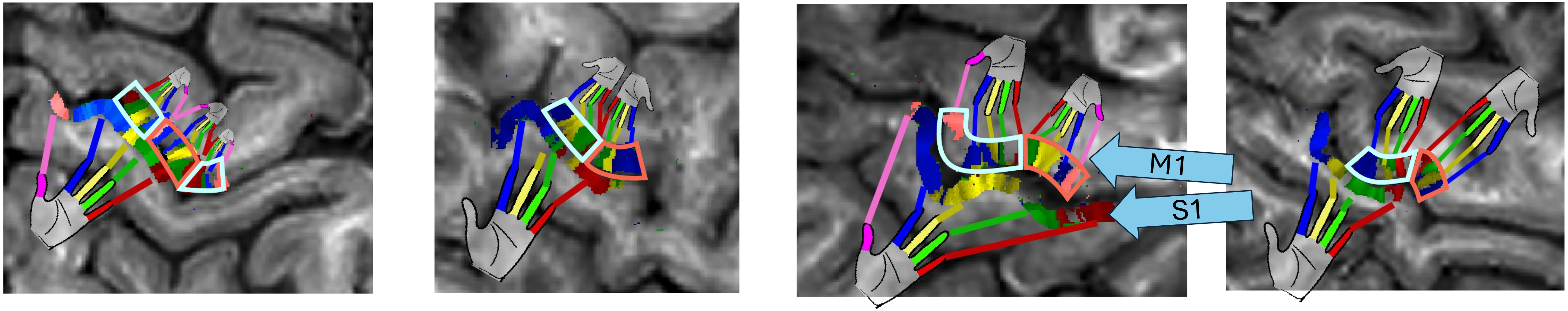


finger flexion vs. finger extension



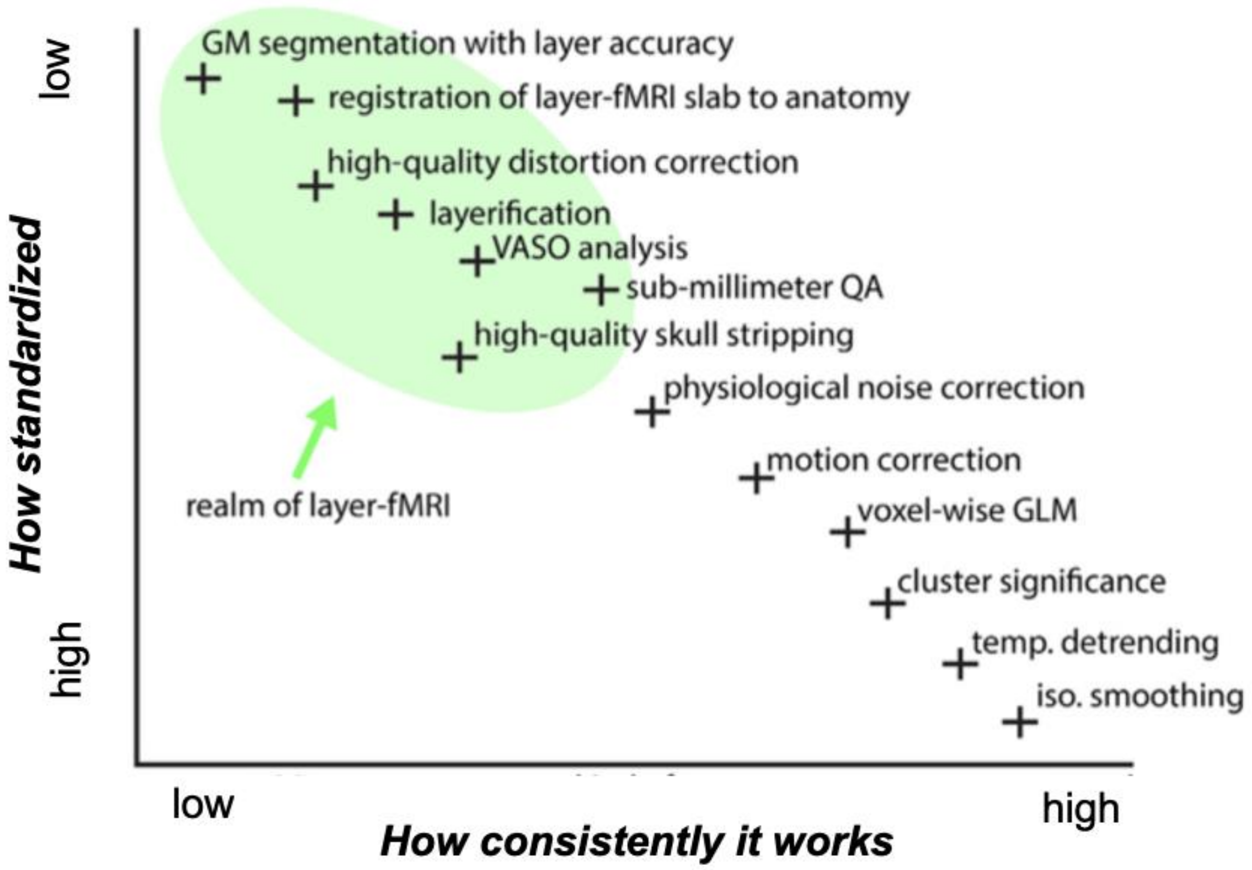
Consistency across subjects

participant 1 participant 2 participant 3 participant4

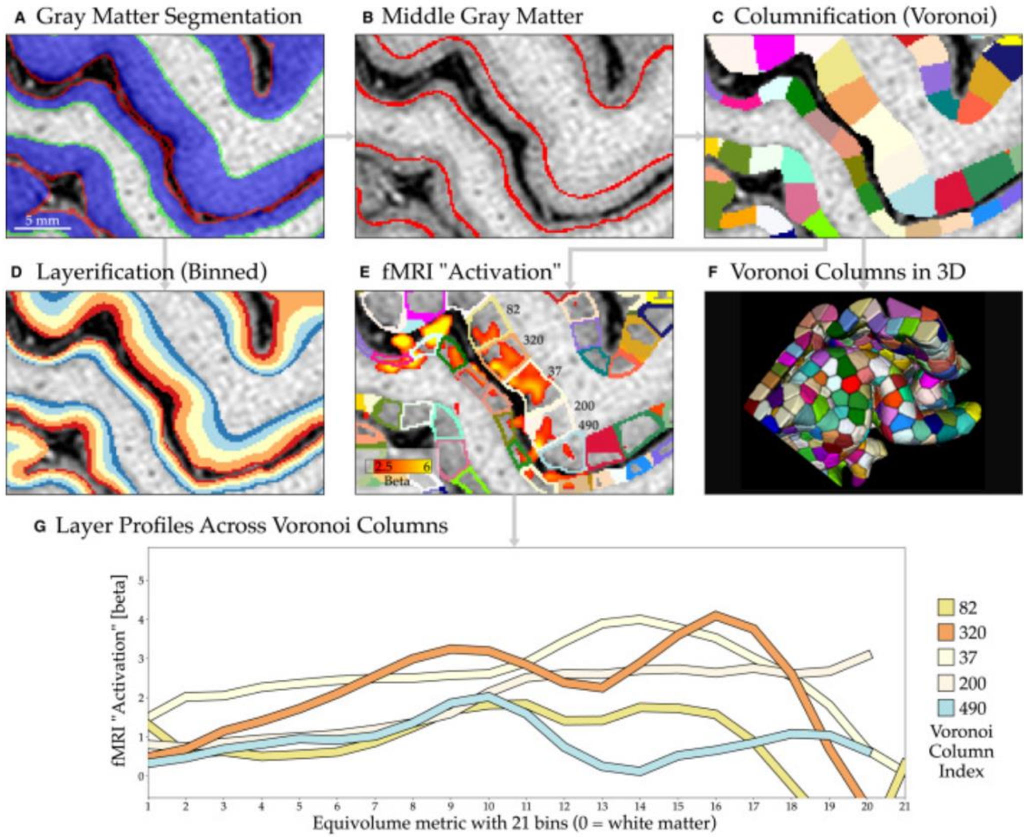


hand representations match “action maps”

Analysis of Layer fMRI Data



Typical Pipeline

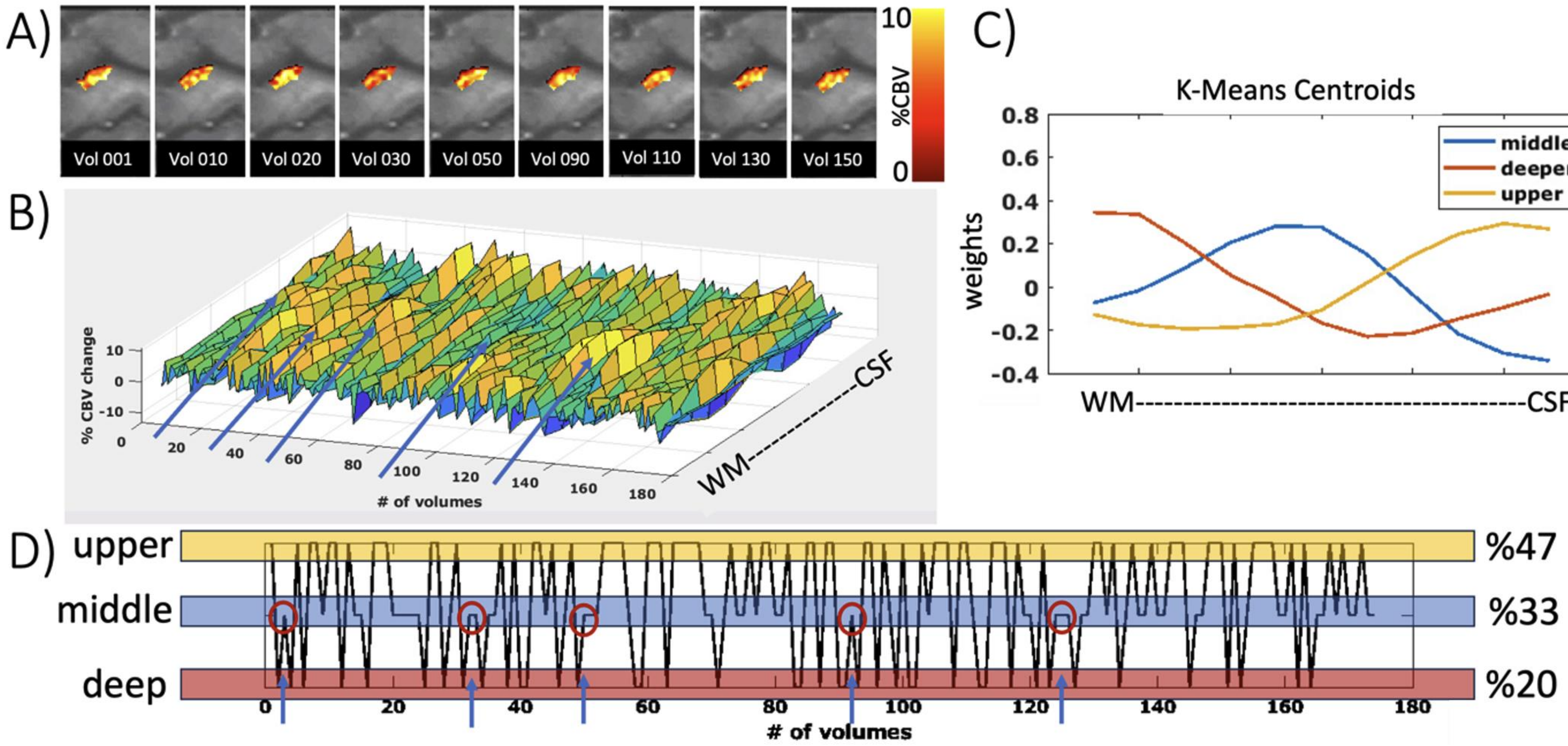


Multi-subject Averaging?

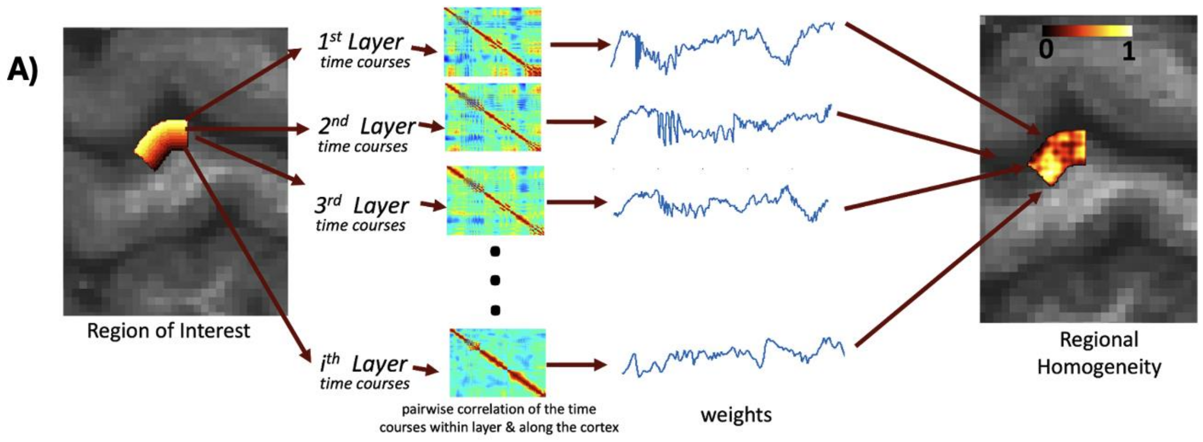
L. Huber, et al. NeuroImage 2021

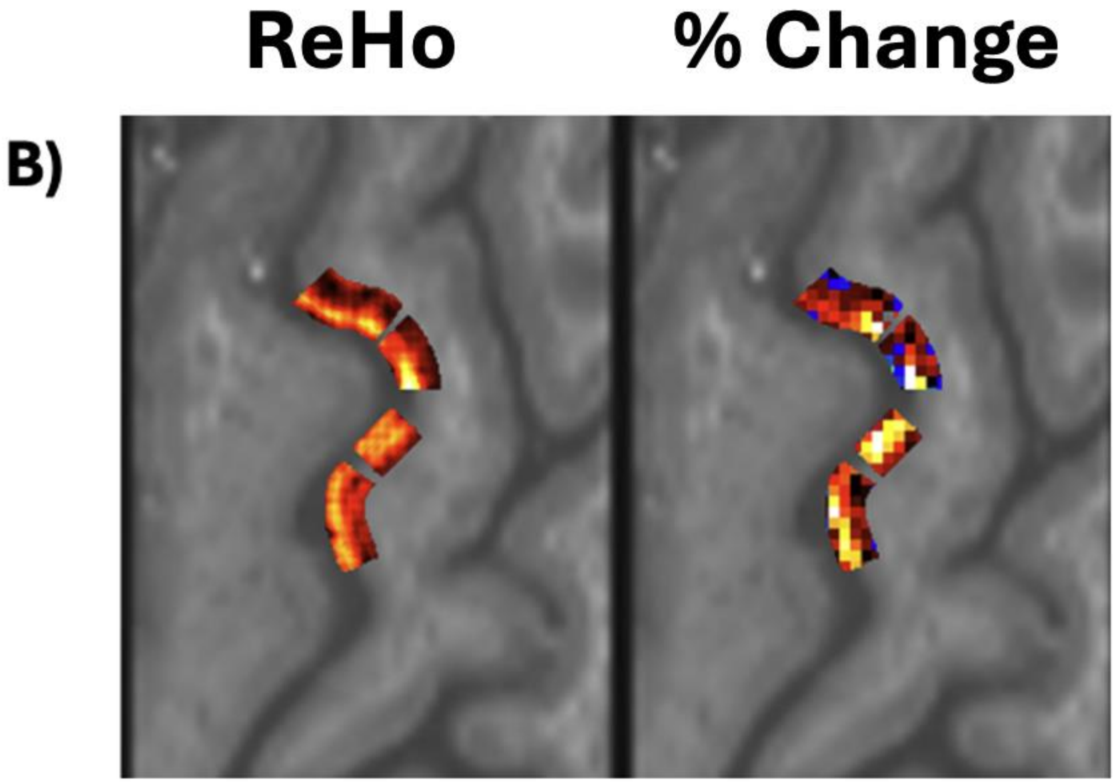
O. F. Gulban, Encyclopedia of the Human Brain (Second Edition), 2025

In development: ”Patch Analysis” of layers

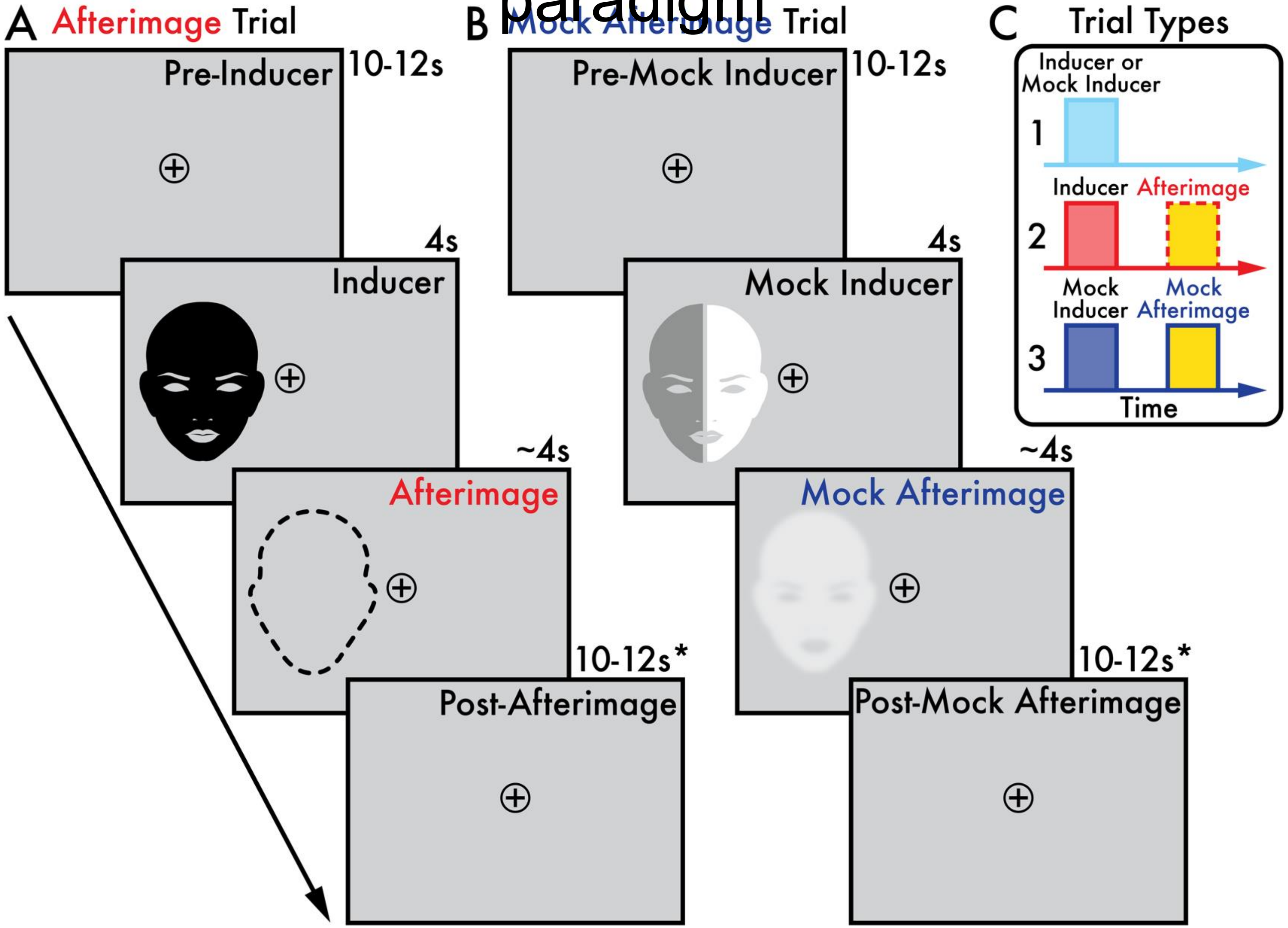


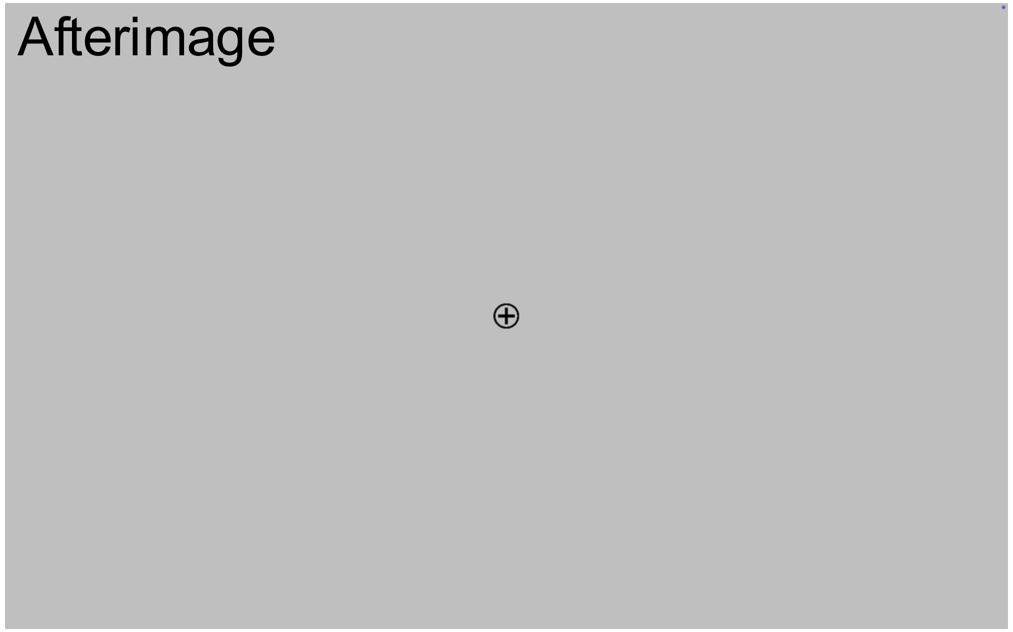
In development: Regional Homogeneity (ReHo) of Layers

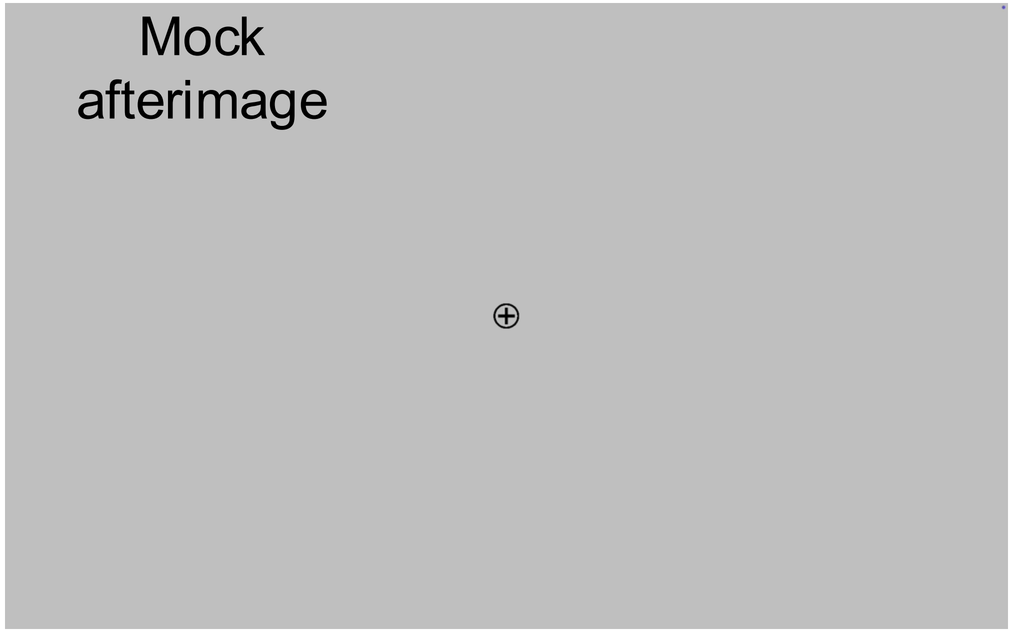




Afterimage and image perception paradigm

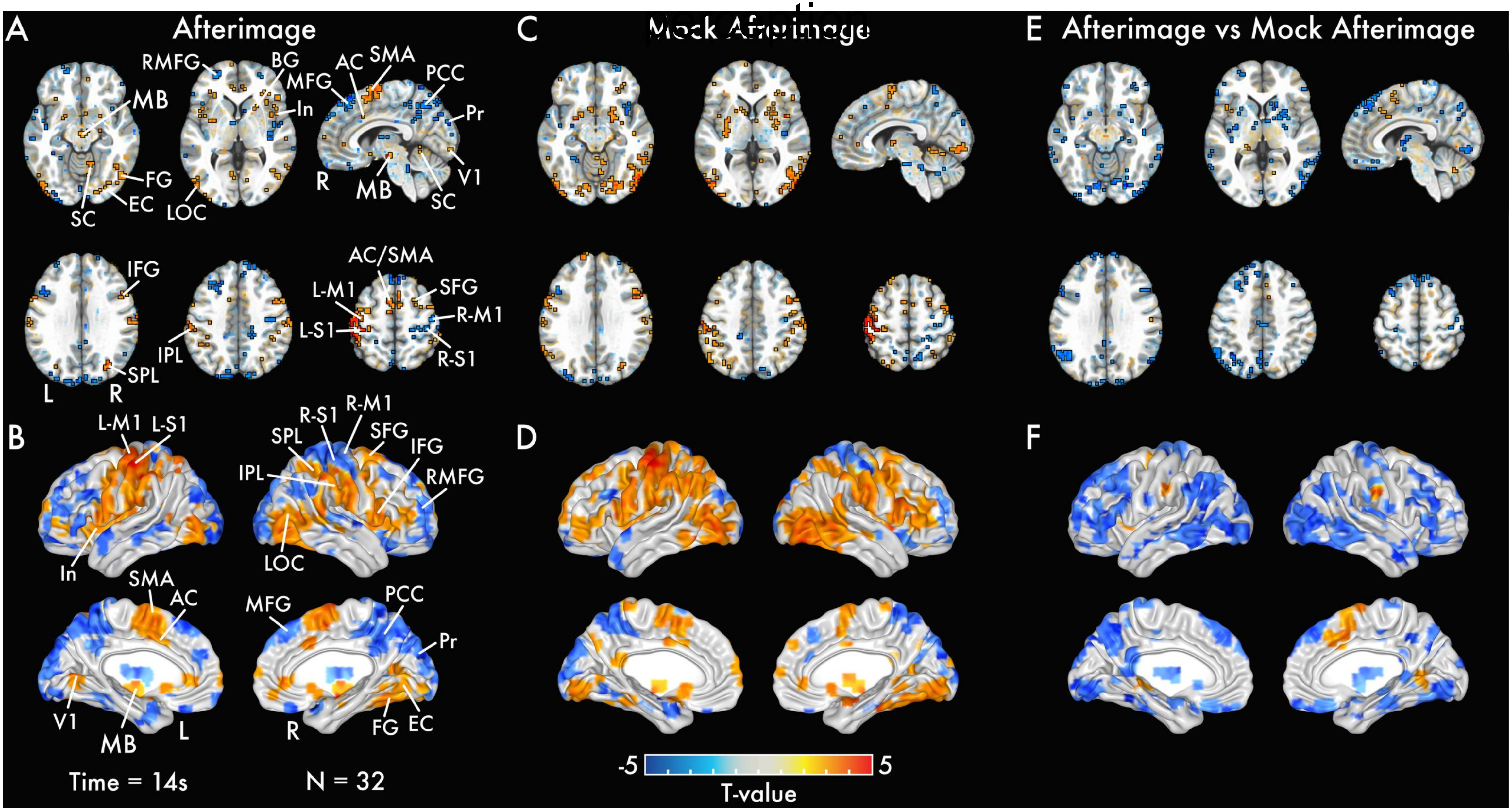






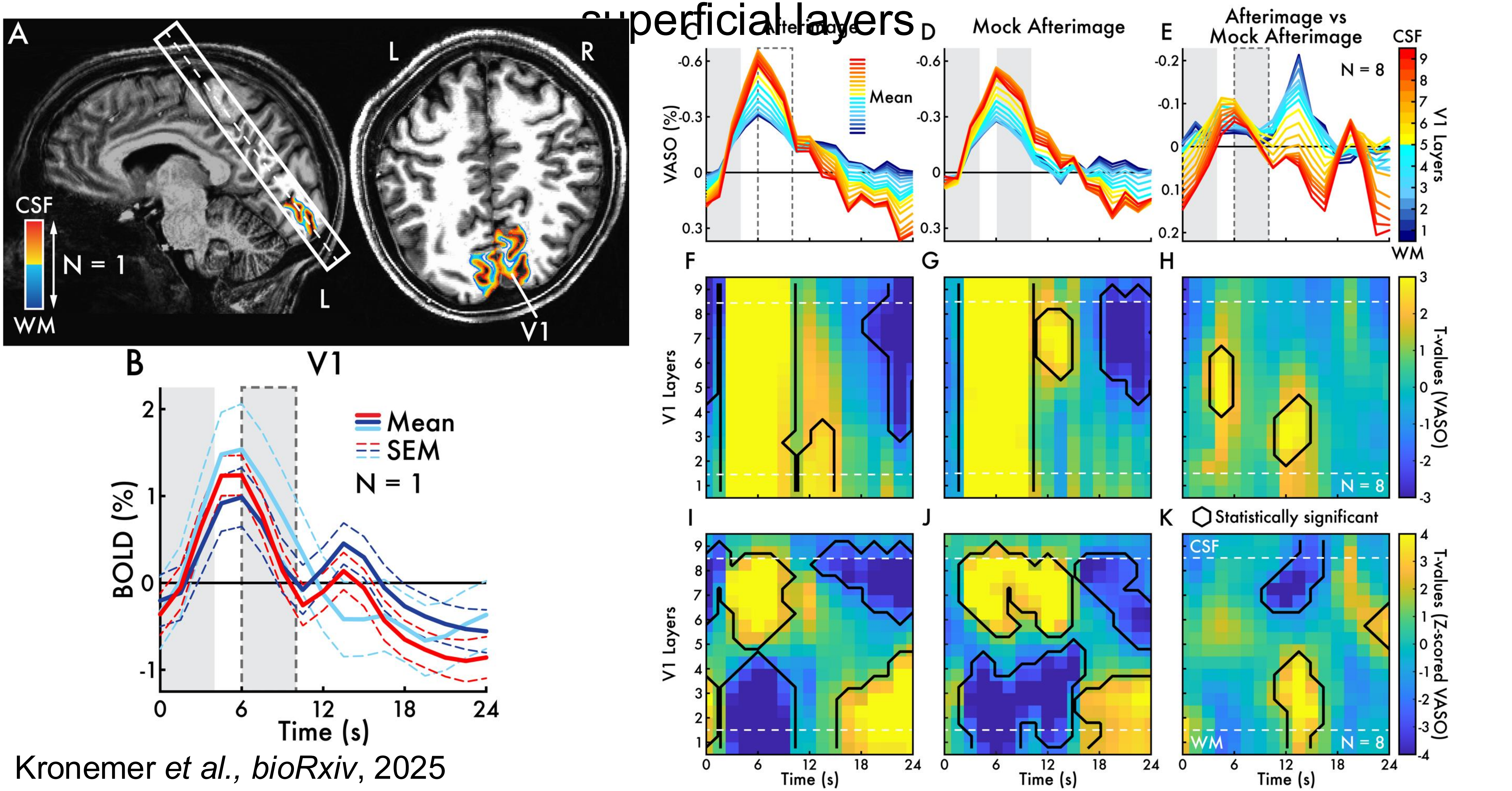
Kronemer *et al., bioRxiv*, 2025

Shared brain network activity for afterimage and image perception



Kronemer *et al.*, *bioRxiv*, 2025

Afterimages involves deep layers of V1; images middle and superficial layers



Kronemer *et al.*, *bioRxiv*, 2025

Cutting Edge Methods: Approaches, Challenges, Insights

High resolution

-spatial: layer fMRI

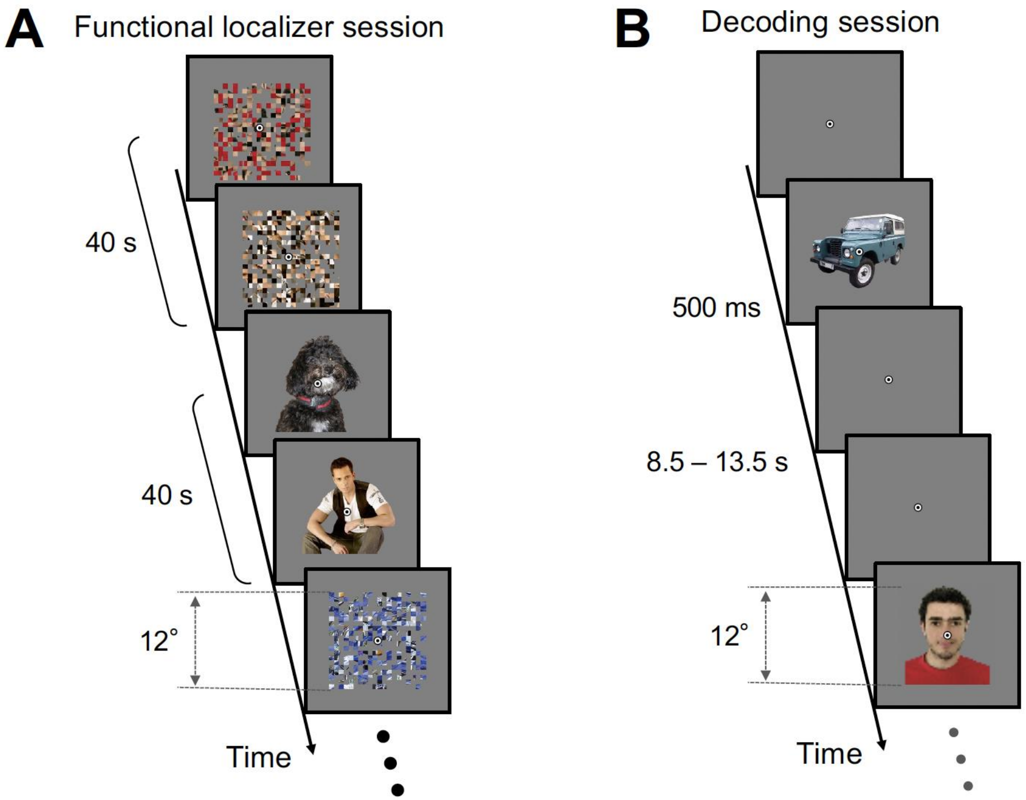
-temporal: rapid decoding

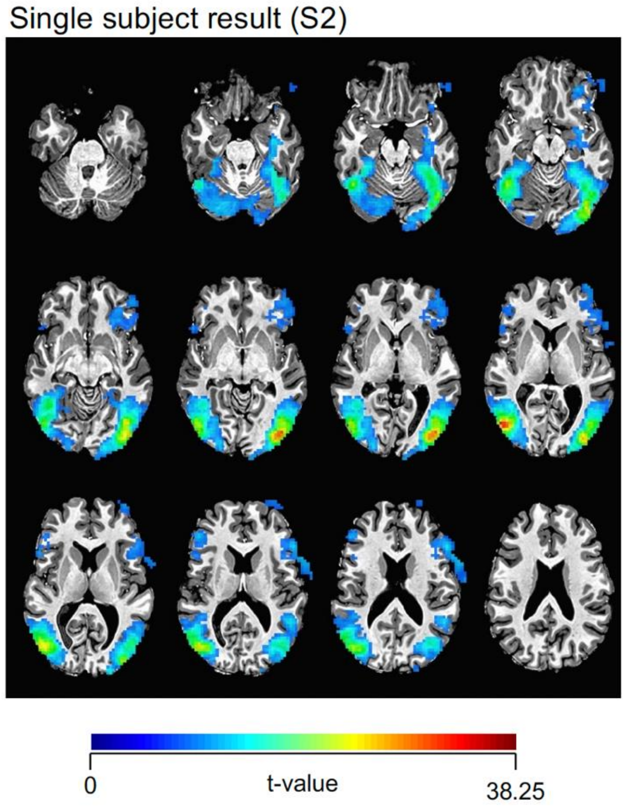
Time Series Information

Dense Sampling

Multi-modal Integration

Event-Related Decoding
Focusing on Information to filter the hemodynamic response

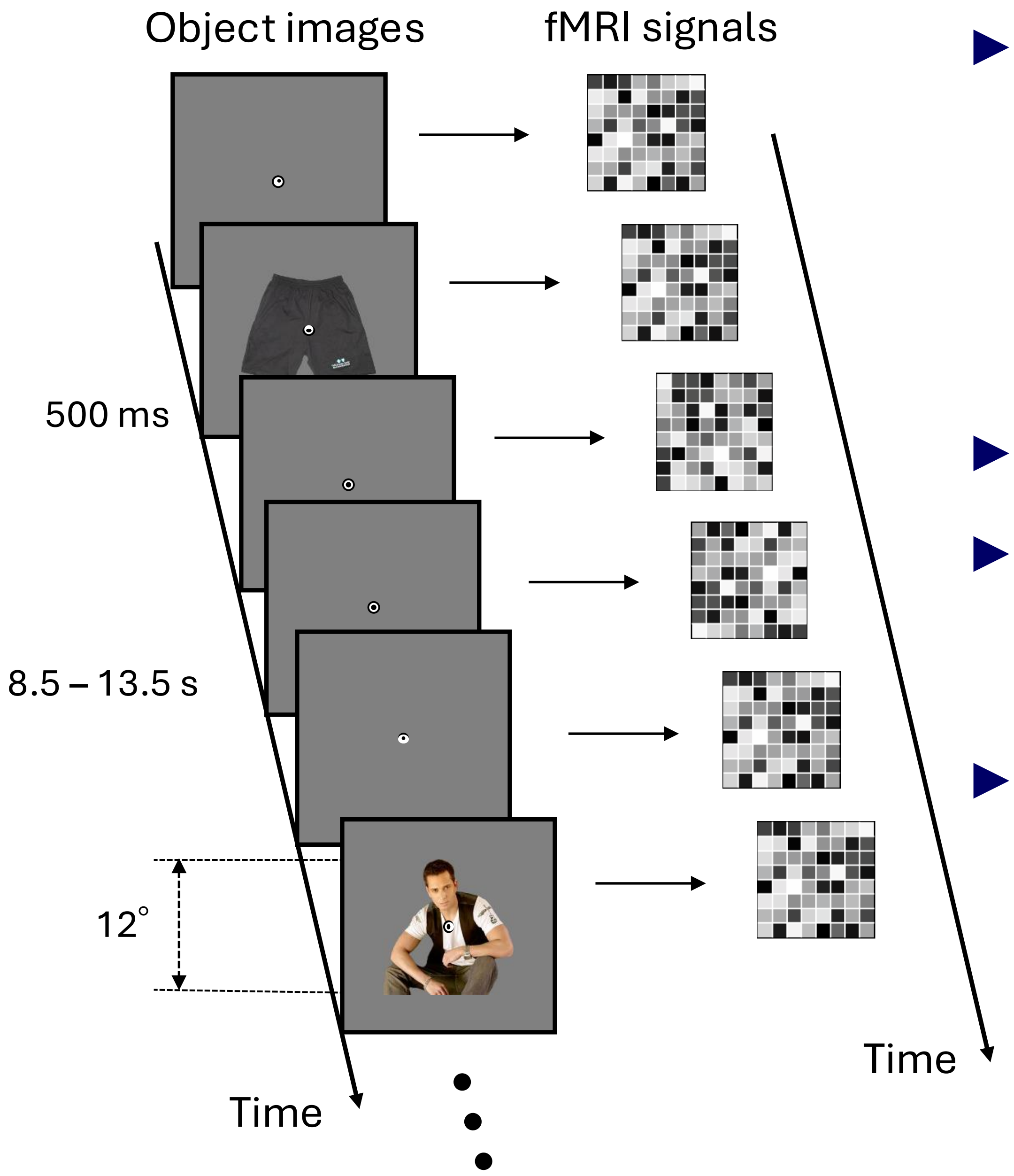




[Rapid decoding of neural information representation from ultra-fast functional magnetic resonance imaging signals]

Yoichi Miyawaki, Kenshu Koiso, Daniel A Handwerker, Javier Gonzalez-Castillo, Laurentius Huber, Arman Khojandi, Yuhui Chai, Daniel Glen, Peter A Bandettini bioRxiv 2025.07.21.665938; doi: https://doi.org/10.1101/2025.07.21.665938

Experimental design: object image presentation



- Acquisition parameters:

  TR = 125 ms, TE = 20.8 ms,
  flip angle 15 deg,
  voxel size = 3 x 3 x 5 mm,
  9 slices, SMS = 3,
  3040 vols/run (~6 min)

- Subjects: N = 12

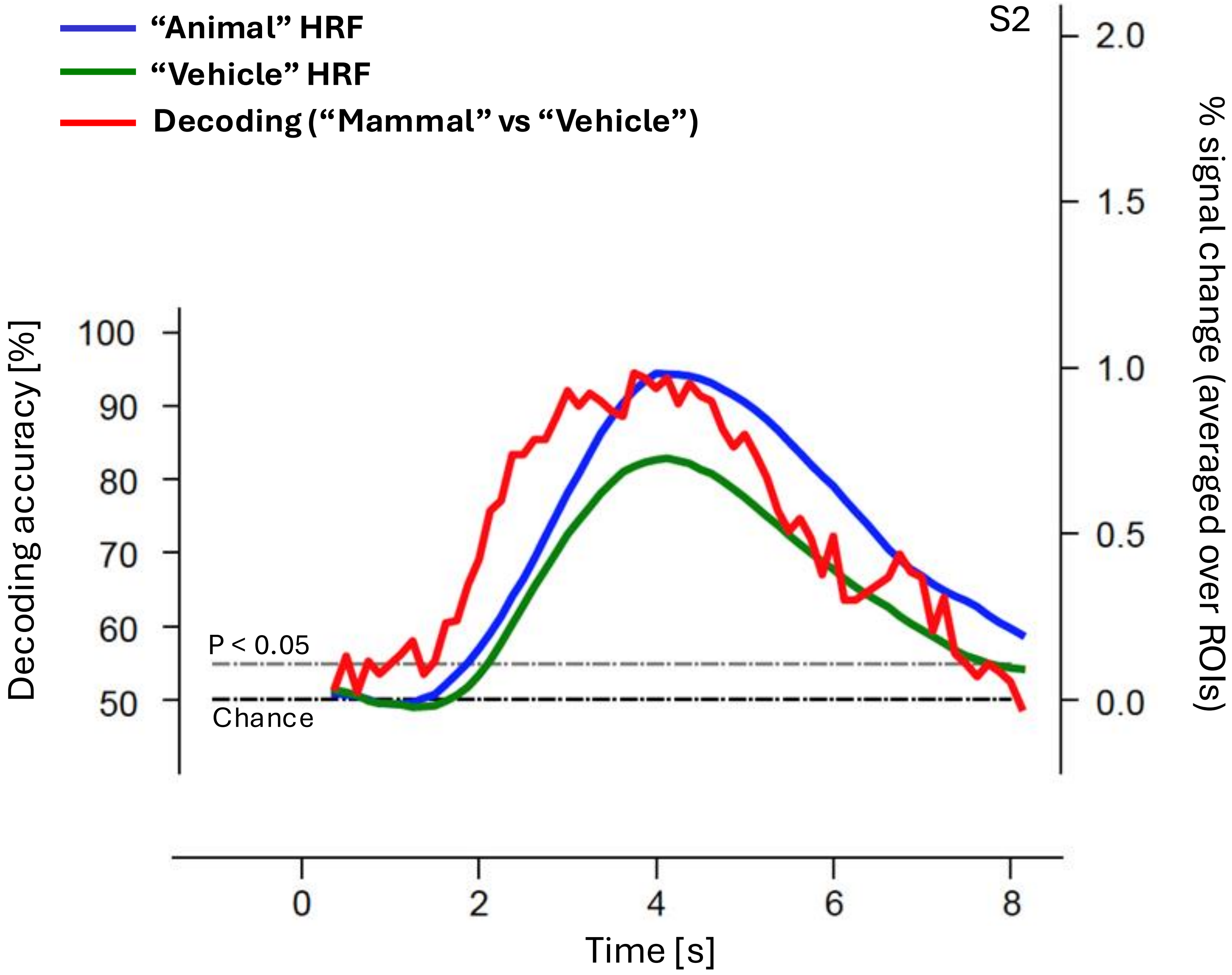
- Subject task:

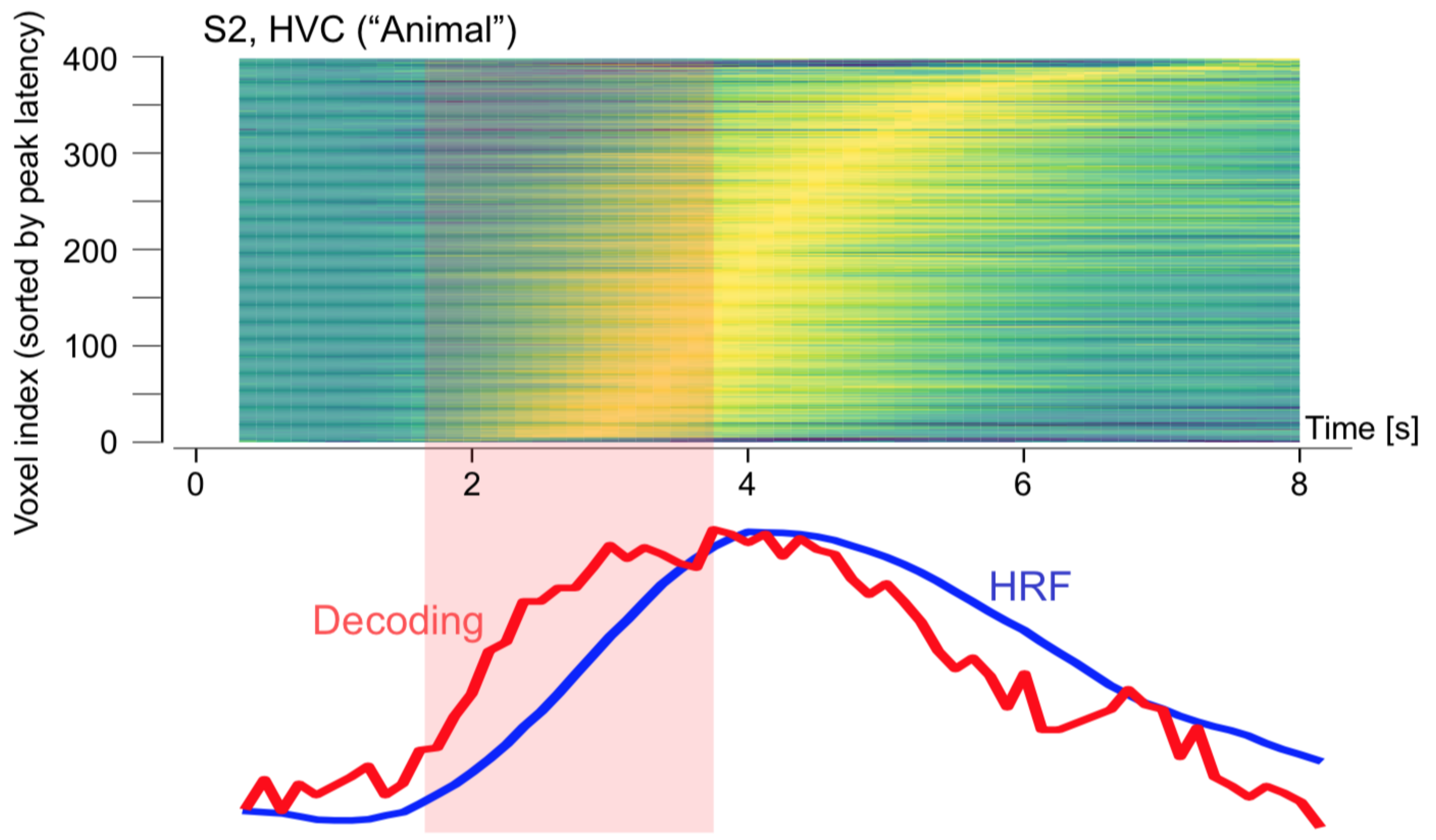
  Detection of fixation color change (orthogonal task)

- Images:

  Animal (person, dog)
  Vehicle (car, airplane)

Decoding precedes the hemodynamic peak





Only information-rich voxel-wise information from spatially precise "fast" voxels presumably capillaries contributed to deco

Cutting Edge Methods: Approaches, Challenges, Insights

High resolution

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Time Series Information

Dense Sampling

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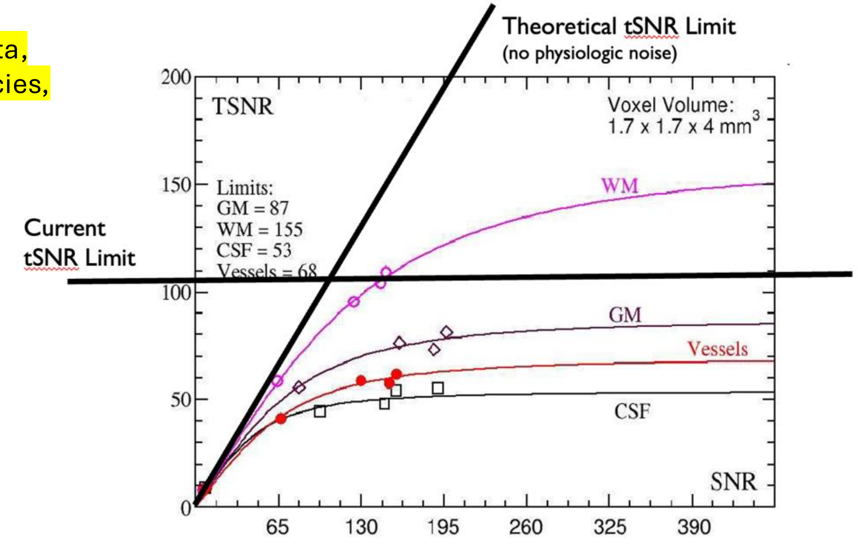
What contributes to spontaneous fluctuations?

Anastasia Dimakou, Giovanni Pezzulo, Andrea Zangrossi, Maurizio Corbetta,
The predictive nature of spontaneous brain activity across scales and species,
Neuron, Volume 113, Issue 9, 2025, Pages 1310-1332,



J. Bodurka et al. NeuroImage 2007

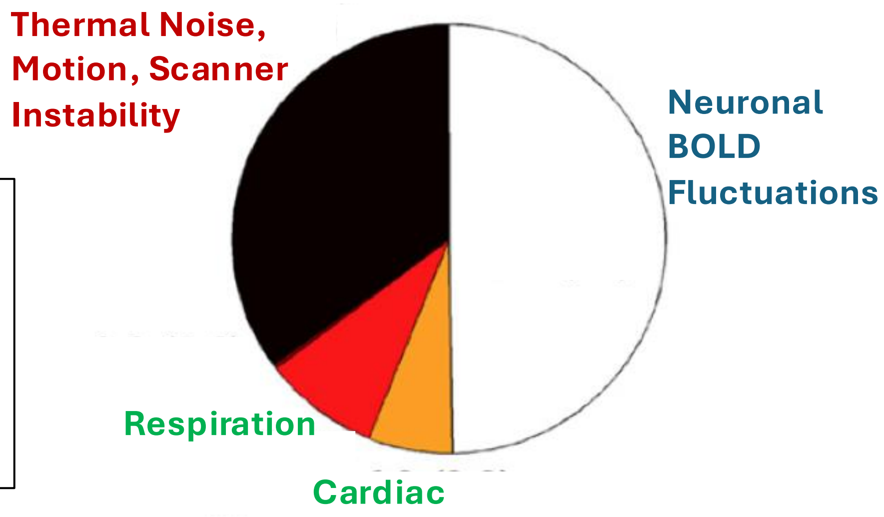
Spontaneous Neuronal Activity
-arousal
-conscious thought
-unconscious processes

Respiration
-chest wall B_0
-variable end tidal CO_2 related flow/BOLD changes
Cardiac Pulsation

Thermal Noise
Motion
Scanner Instability

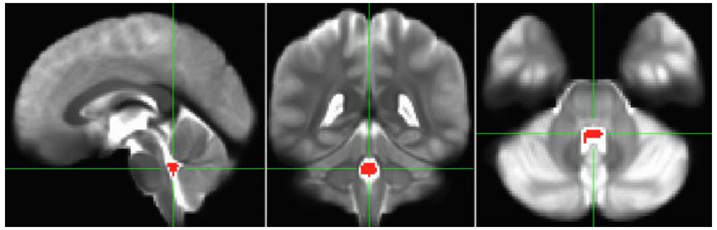
We don’t fully understand most of these.

We also don’t understand the noise well enough to fully remove it.



Bianciardi et al. MRI 27: 1019-1029, 2009

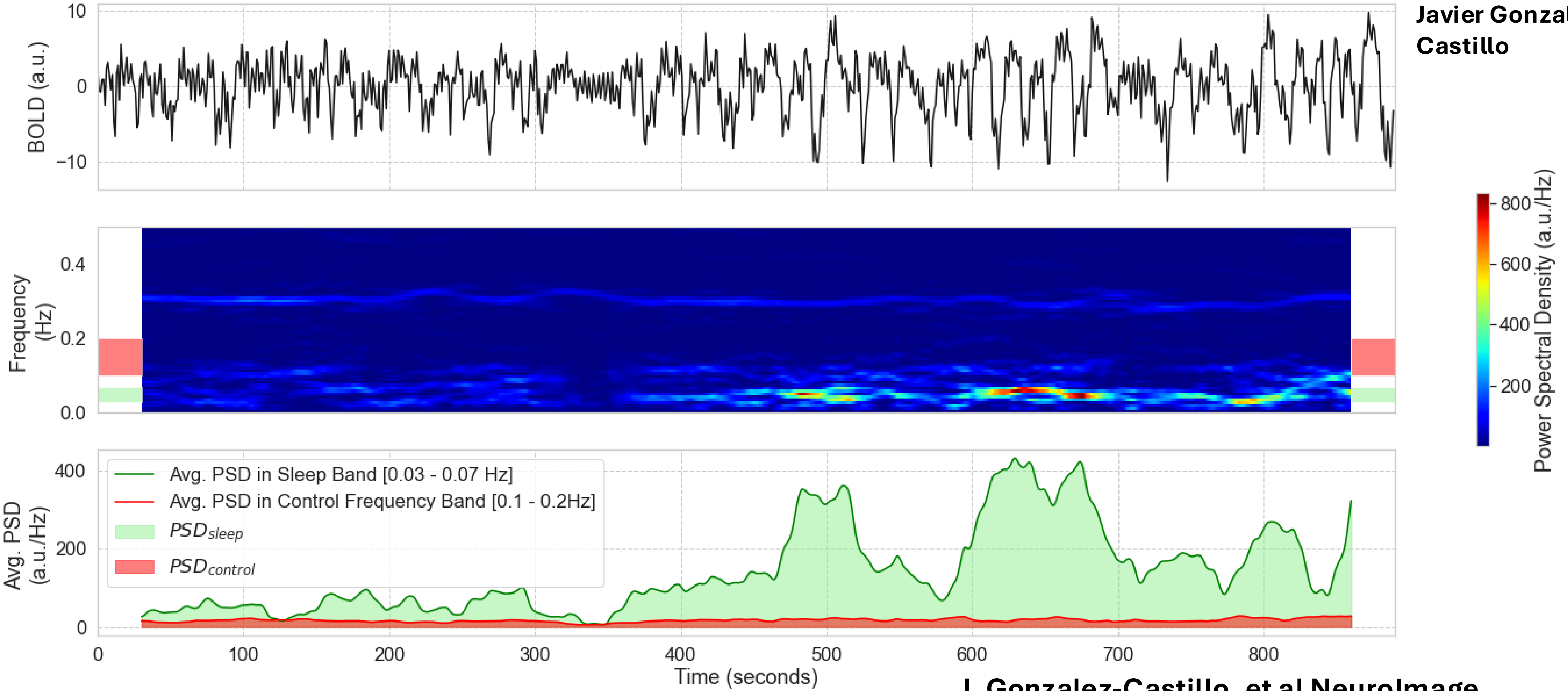
Arousal



Power at 0.05 Hz in 4th Ventricle

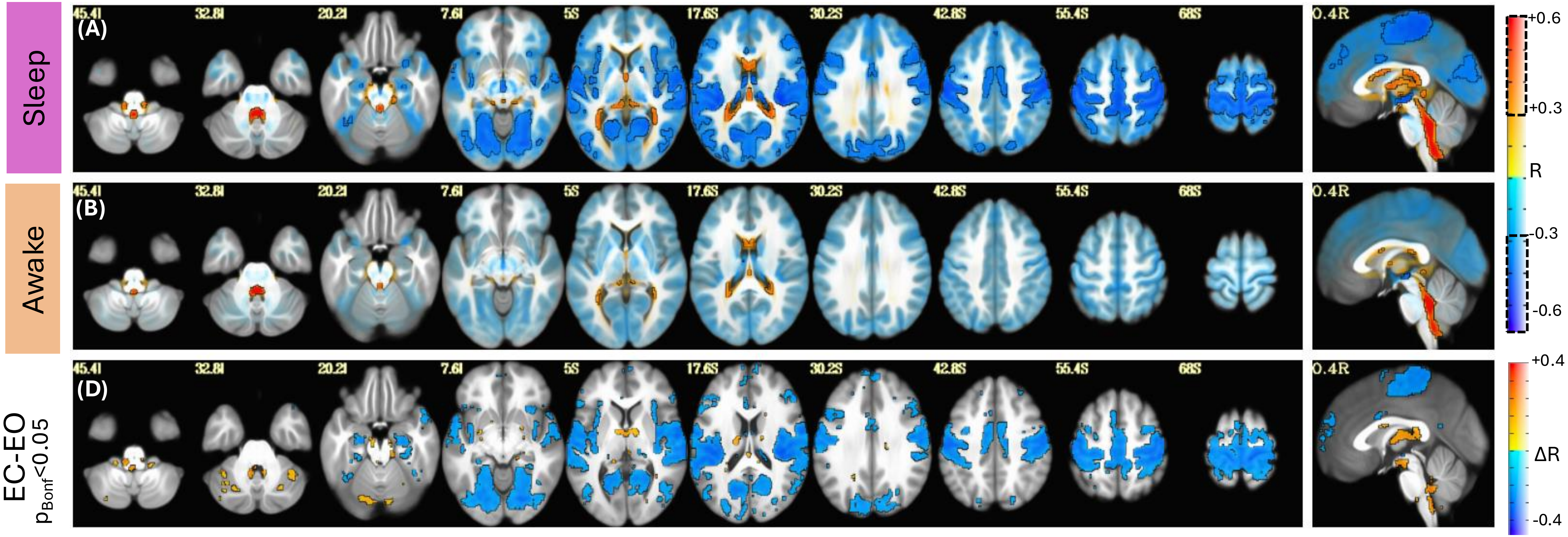
- 404 scans HCP 7T Resting-state sample
- Concurrent Eye Tracking
- Label scans as awake or drowsy
- Categorization based on eyes closed/open

Javier Gonzalez-Castillo



J. Gonzalez-Castillo, et al NeuroImage,

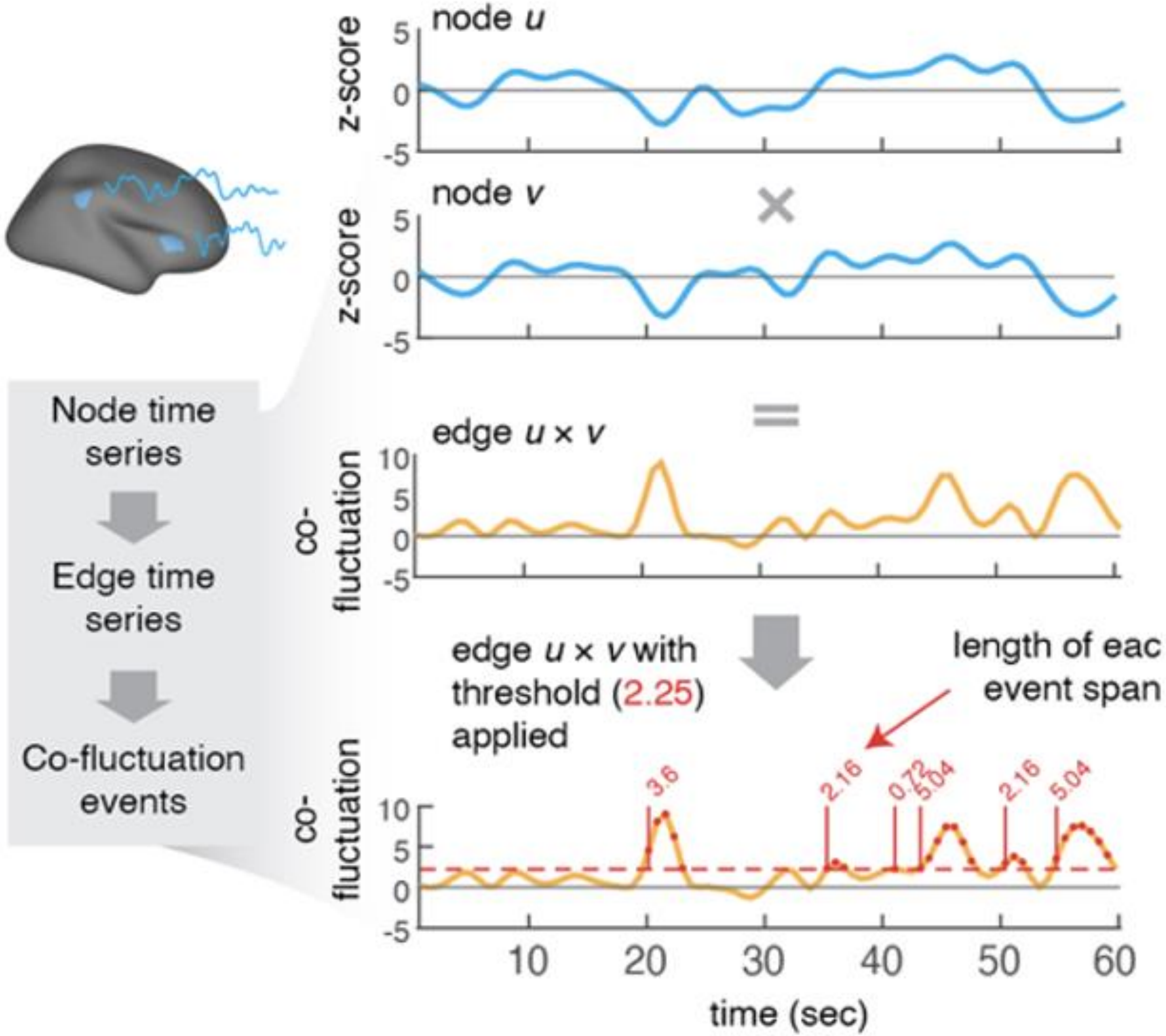
Fluctuations: Awake vs Sleep



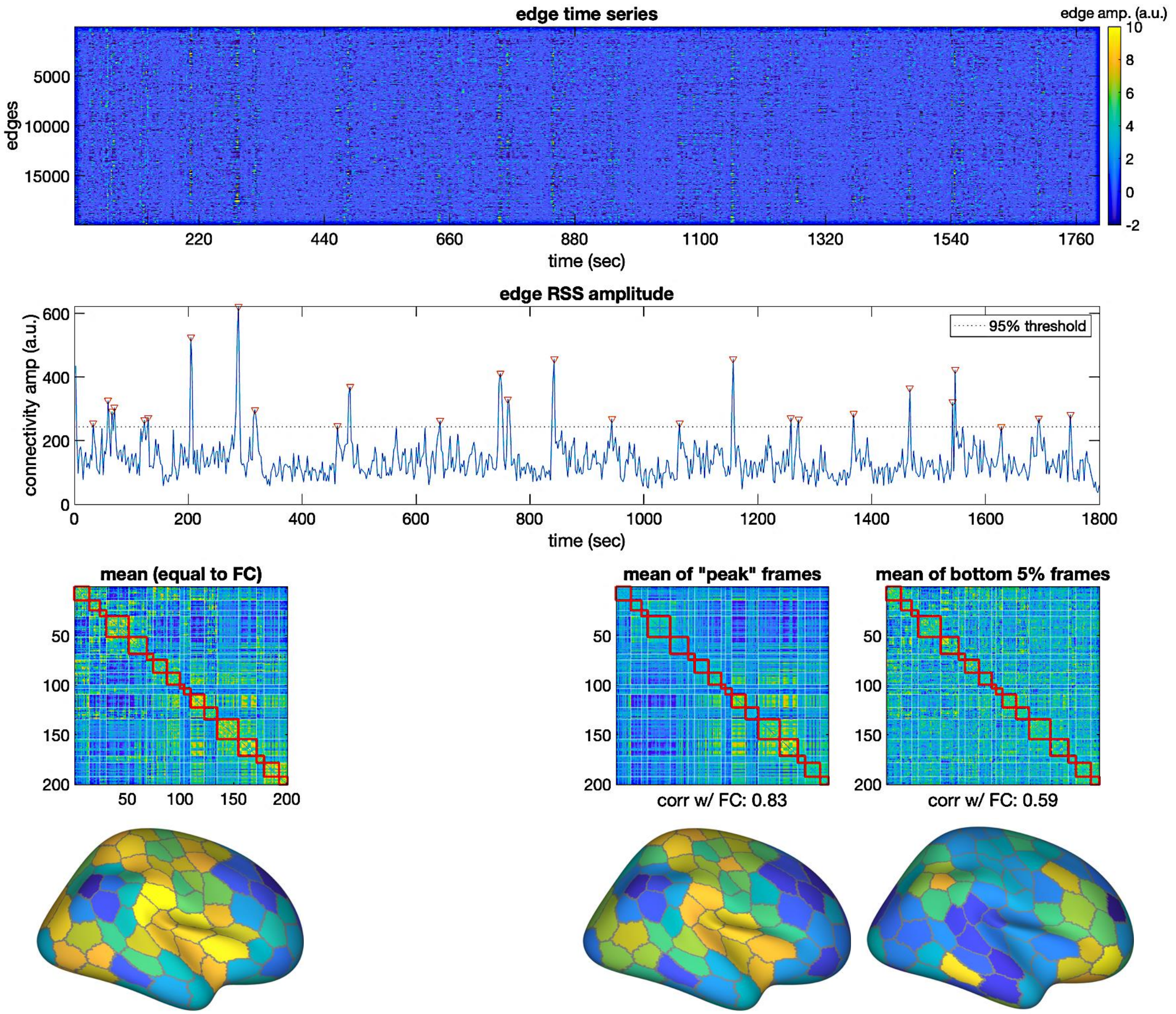
J. Gonzalez-Castillo, et al NeuroImage, 2022

Edge Analysis and Event Duration Mapping

- **Resting state fMRI (0.2 Hz).** ***However, coactivation events that drive resting state connectivity are not oscillatory.***
- **These events have different durations.**
- **The predominance of specific event durations shows spatial structure.**



Faskowitz, J.; Bandettini, P. A.; Gonzalez-Castillo, J. Mapping high-amplitude fMRI edge time series across space and time, *Imaging Neuroscience* 2026.



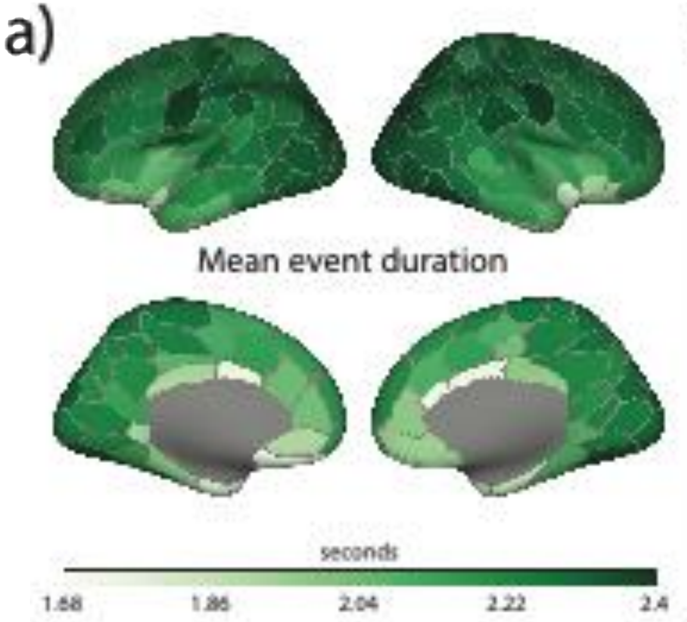
By taking the instantaneous similarity in a small fraction of the timepoints **(about 3%),** we can reconstruct broad-scale FC organization

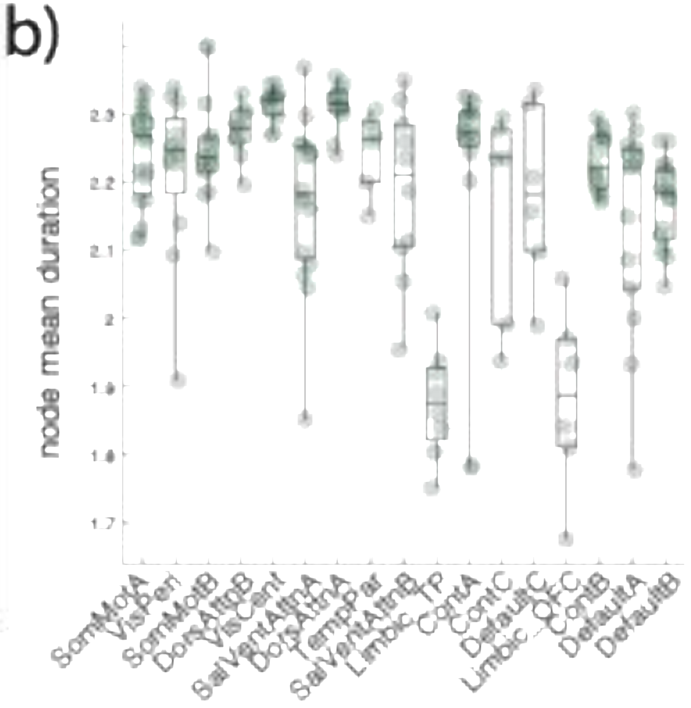
Suggesting that FC organization is expressed in punctate events

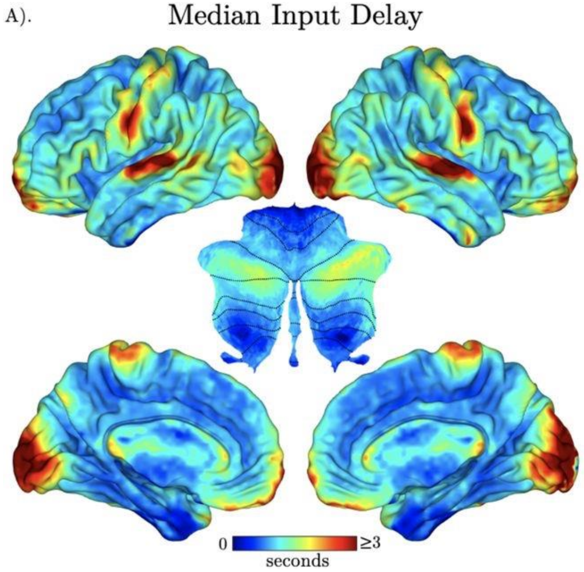
Faskowitz, J et al, *Imaging Neuroscience* 2026.

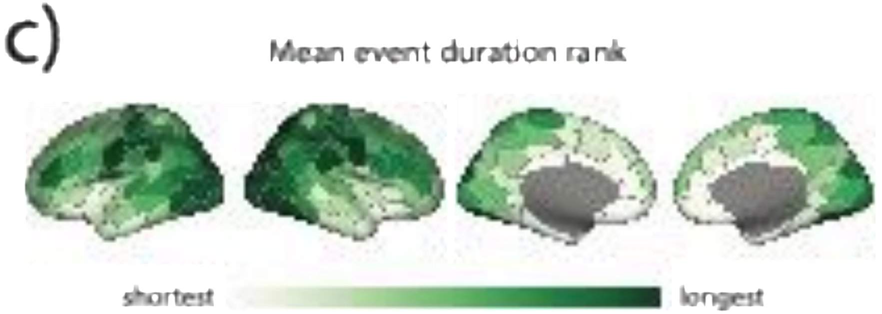
Spatial Pattern of Event Duration

Takeaways:
- Event Duration is an additional feature we can read from edge time series.
- Longest edge events emanate from primary sensory cortices – forming a similar map to the median input delay map
- Long durations in the input delay map are hypothesized to reflect increased contextual signals (i.e., feedback) which shape ongoing sensory-processing.









G. Cooper et al. "Where the present gets remembered: Sensory regions communicate with the brain over the longest timescales." bioRxiv 2023

Faskowitz, J et al, *Imaging Neuroscience* 2026.

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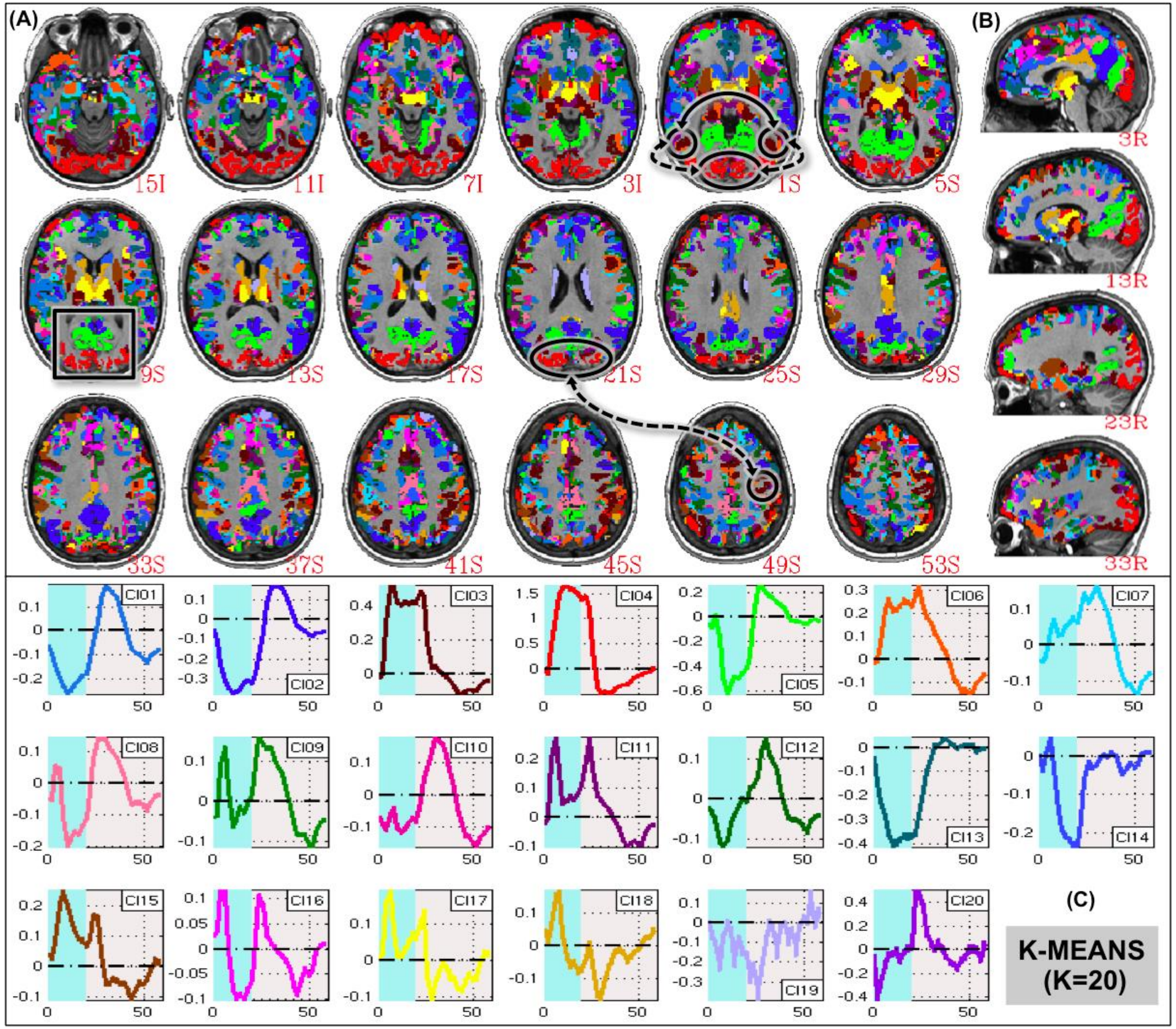
Dense Sampling of Individual Subjects: Detecting more subtle activity



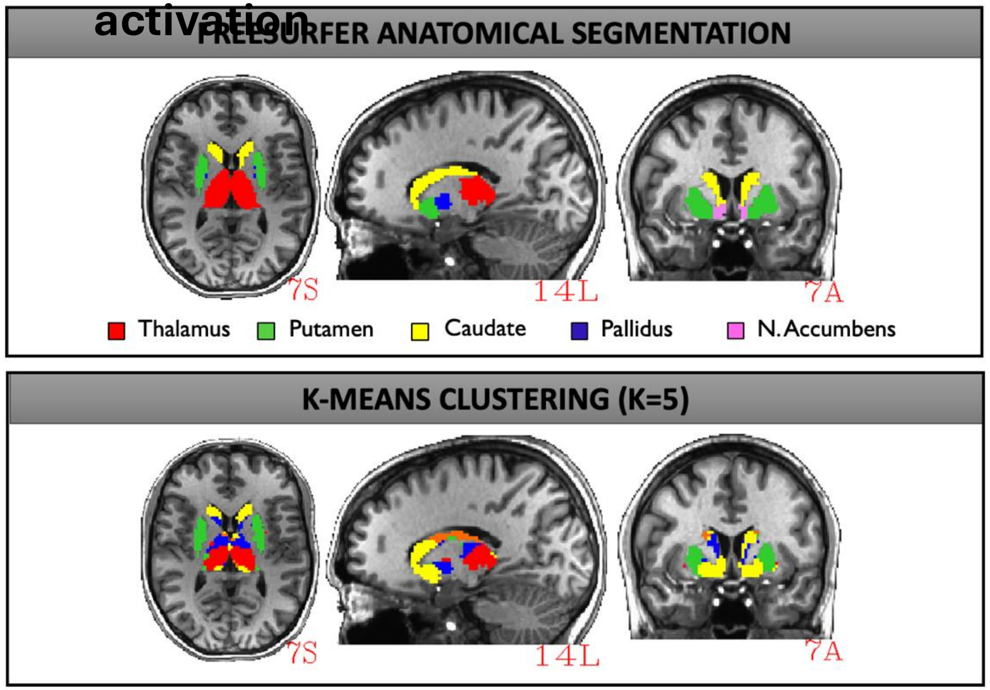
9 hours of averaging layer fMRI data with single subject and simple task

K-means clustering of all time-locked signal

Simple full field checkerboard with word/letter discrimination task

Javier Gonzalez-Castill

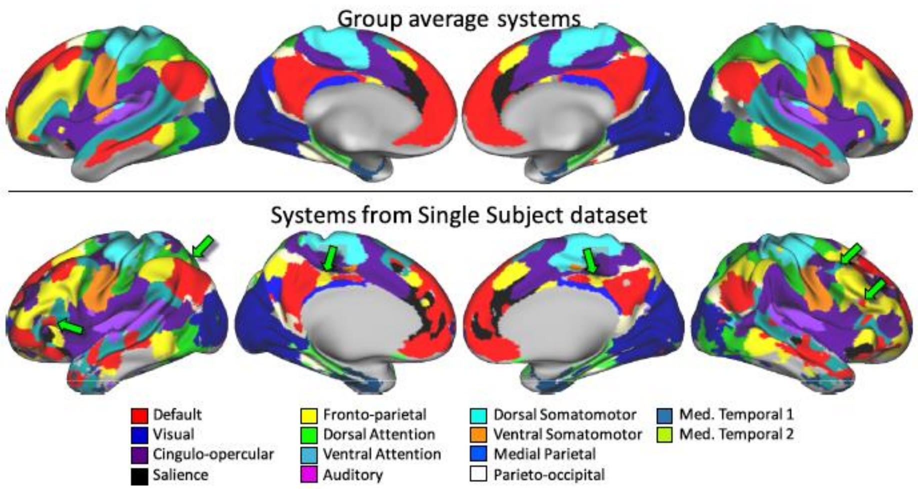
Subcortical activation

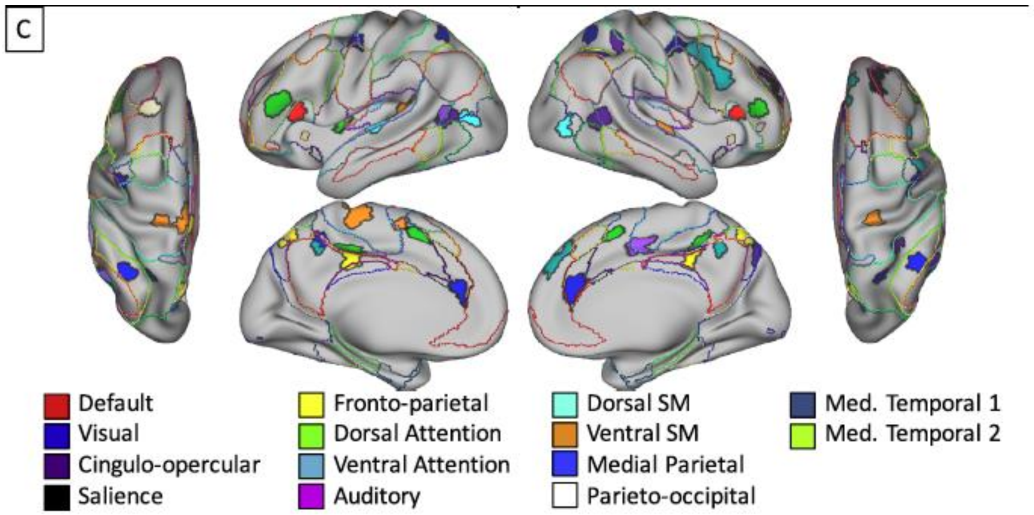


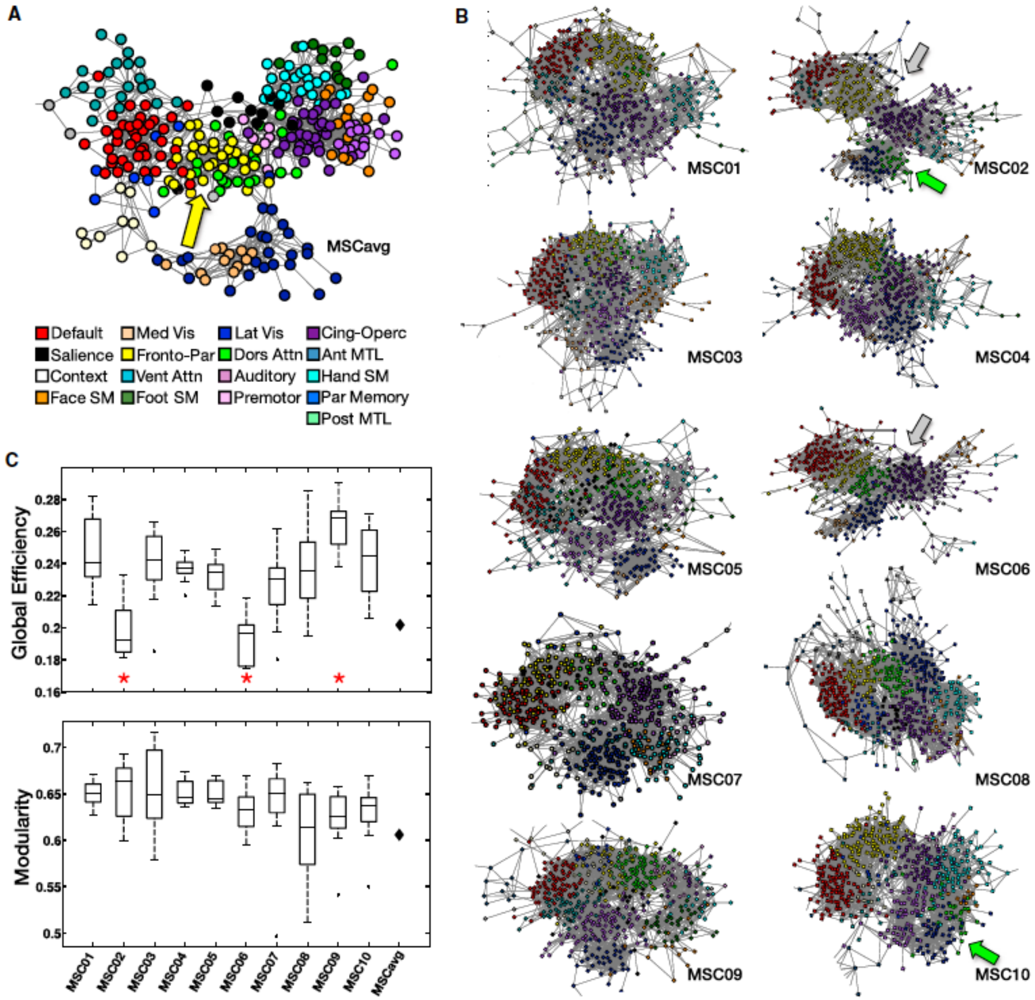
J. Gonzalez-Castillo, et al. PNAS 109, 5487-5492 (2012)

Dense sampling Features:

Individual Differences in parcellation and global efficiency







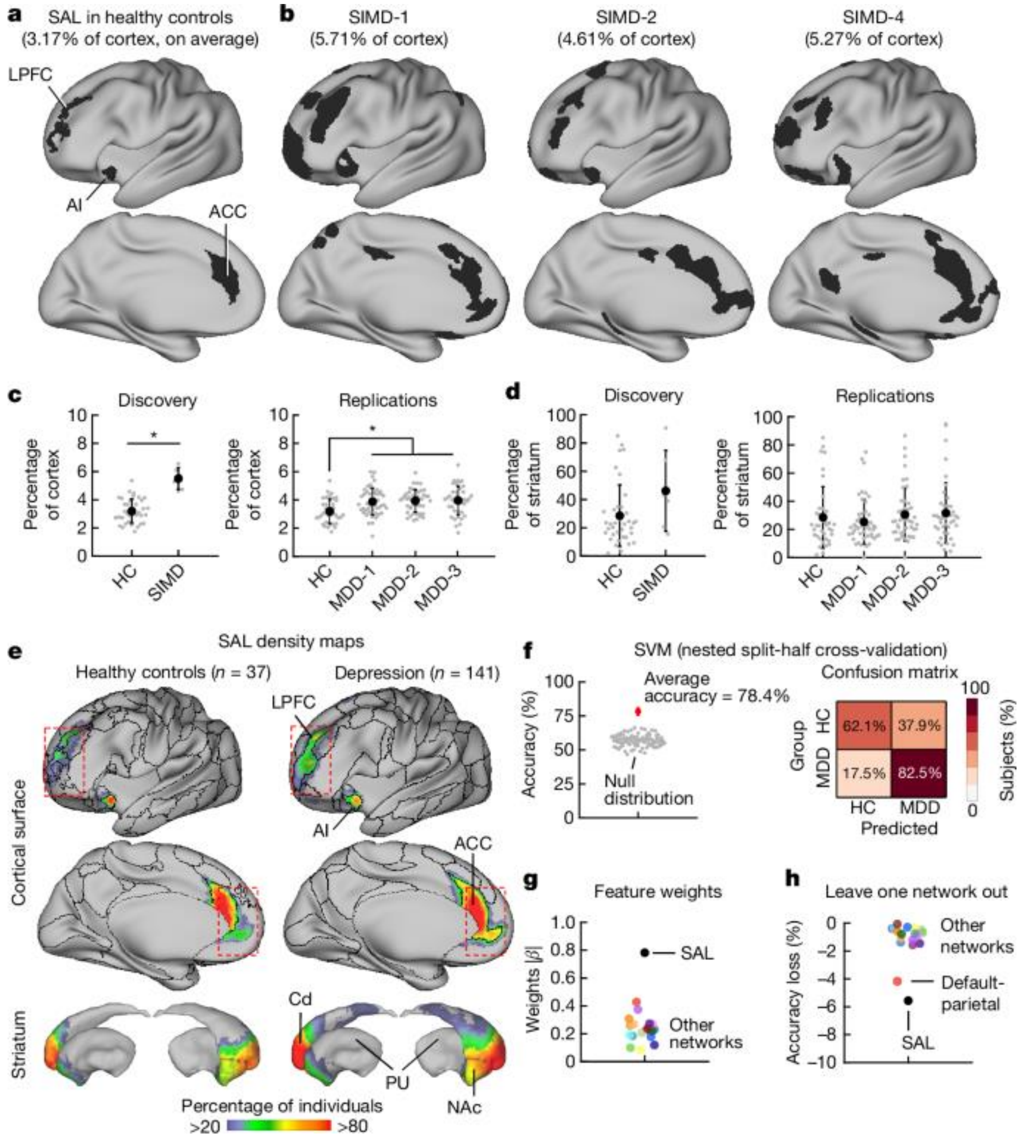
Gordon, EM, et al Neuron 2017 95 (4). Precision Functional Mapping of Individual Human Brains.

Gordon EM, et al . Neuroimage 2017. Individual-specific features of brain systems identified with resting state functional correlations.

Dense Sampling Reveals other Features:

Salience network size

(expansion with depression)



Lynch, C.J., Elbau, I.G., Ng, T. *et al.* Frontostriatal salience network expansion in individuals in depression. *Nature* 633, 624–633 (2024).

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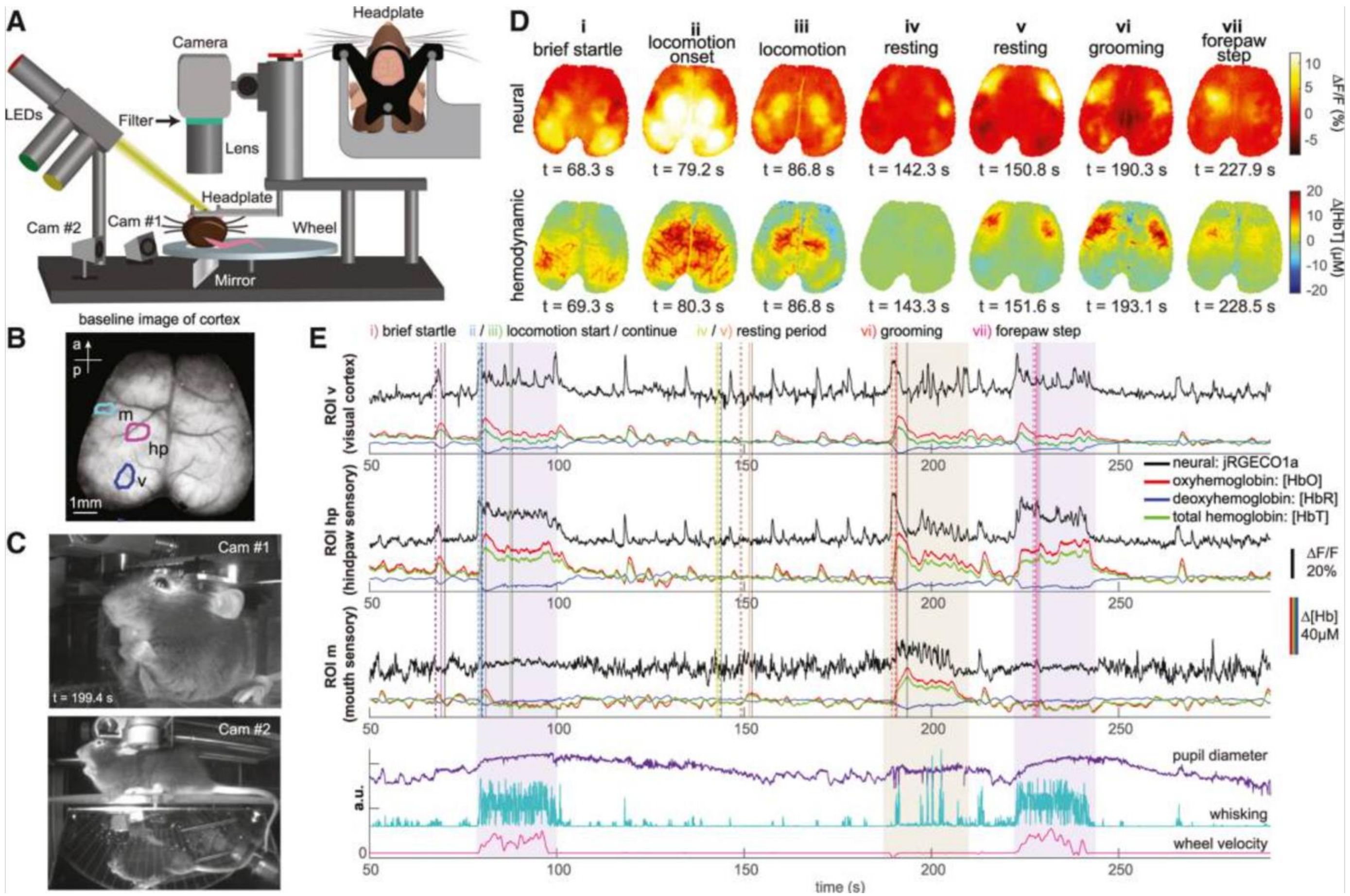
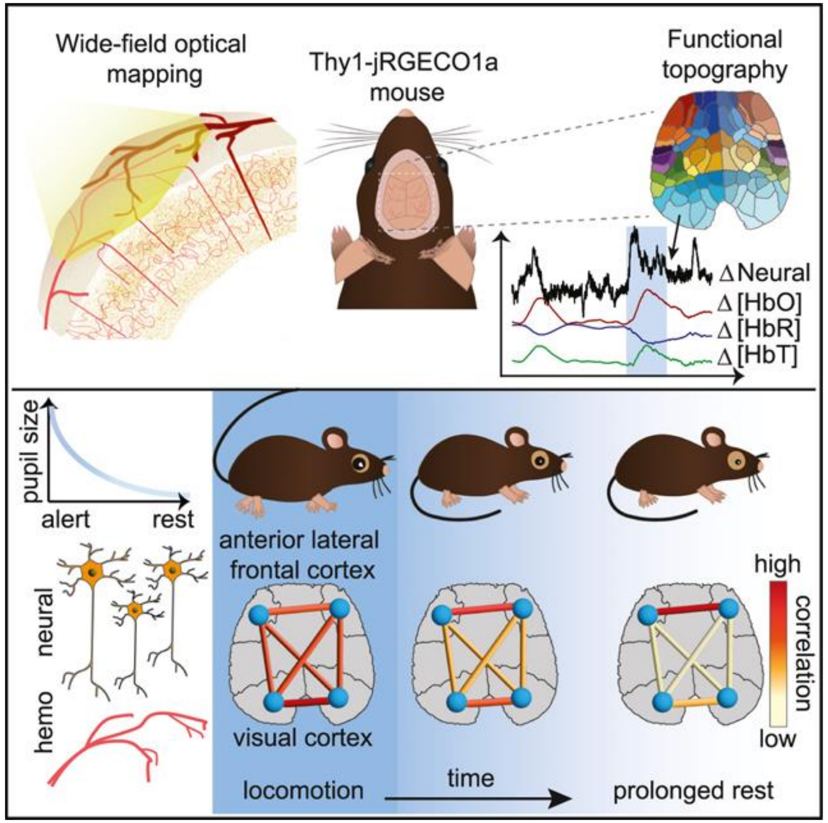
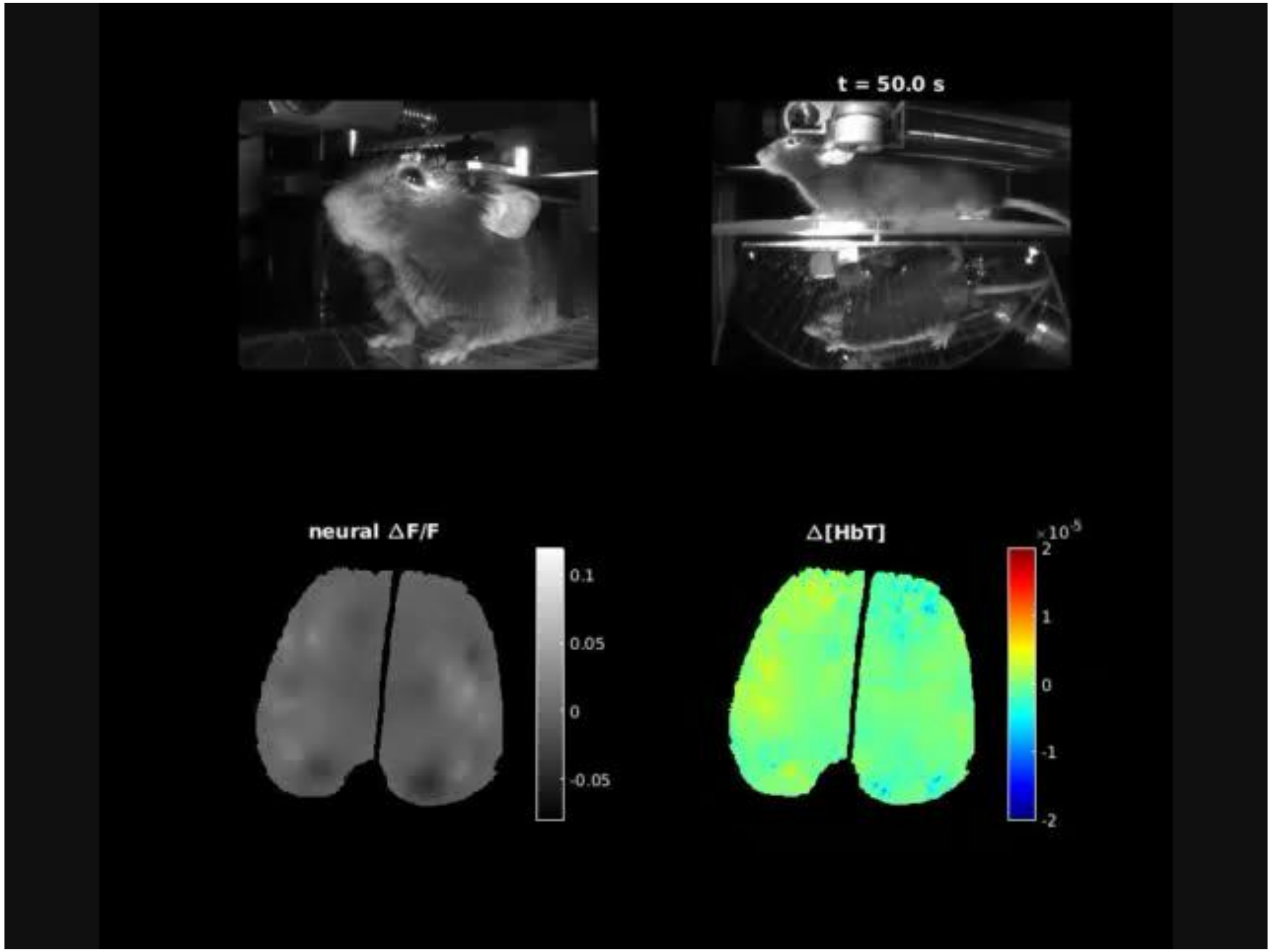
Simultaneous Wide Field Calcium and Hemodynamic Imaging





Shahsavarani et al., 2023, Cell Reports 42, 112527

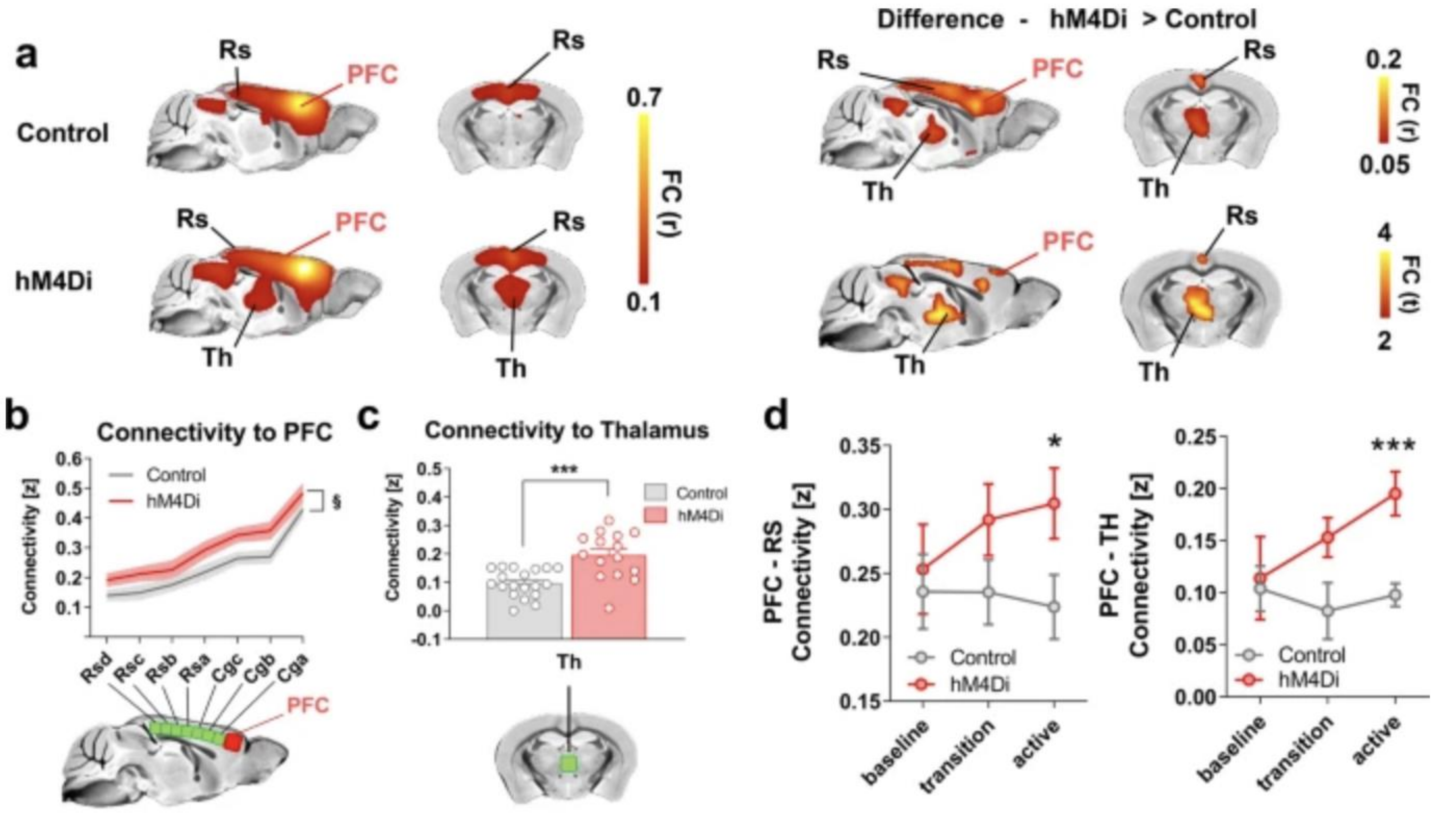
neural, jRGECO1a: % fluorescence change after hemodynamic correction ΔF/F
hemodynamic, Δ[HbT]: Δ[HbO] + Δ[HbR]



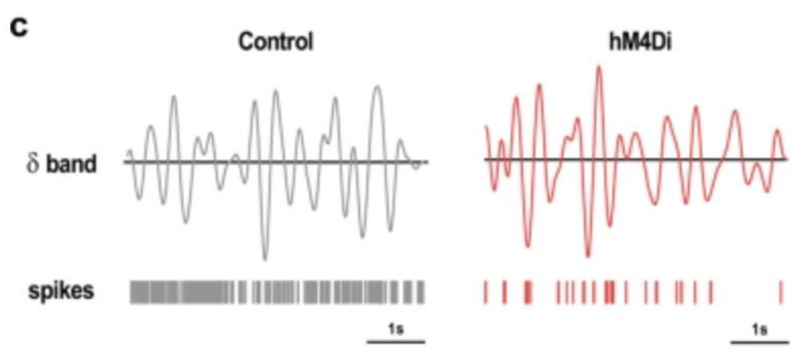
Shahsavarani et al., 2023, Cell Reports 42, 112527

Paradoxical Increase in fMRI connectivity with Chemogenetic inhibition

Fig. 3: Chemogenetic inhibition of the mouse PFC results in rsfMRI overconnectivity.



Spiking decreases but low frequencies increase.



Rocchi, F., Canella, C., Noei, S. *et al.* Increased fMRI connectivity upon chemogenetic inhibition of the mouse prefrontal cortex. *Nat Commun* 13, 1056 (2022).

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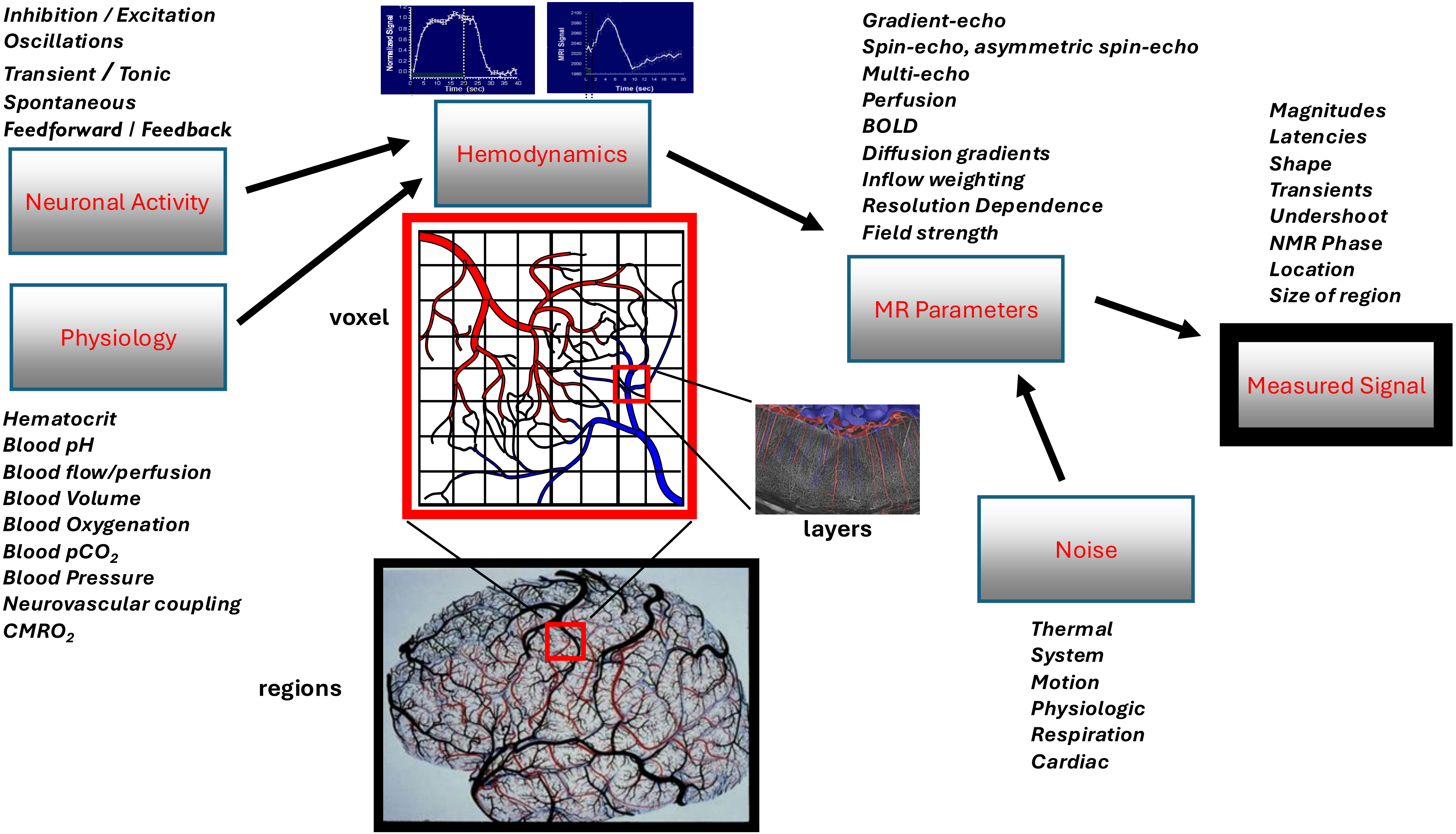
Inhibition / Excitation
Oscillations
Transient / Tonic
Spontaneous
Feedforward / Feedback
Neuronal Activity
Physiology
Hematocrit
Blood pH
Blood flow/perfusion
Blood Volume
Blood Oxygenation
Blood pCO_2
Blood Pressure
Neurovascular coupling
$CMRO_2$
Normalized Signal
Time (sec)
MRI Signal
Time (sec)
Hemodynamics
voxel
layers
regions
Gradient-echo
Spin-echo, asymmetric spin-echo
Multi-echo
Perfusion
BOLD
Diffusion gradients
Inflow weighting
Resolution Dependence
Field strength
MR Parameters
Noise
Thermal
System
Motion
Physiologic
Respiration
Cardiac
Magnitudes
Latencies
Shape
Transients
Undershoot
NMR Phase
Location
Size of region
Measured Signal

Possible Future Impact of fMRI

- Clinical: Guide for Neuromodulation (TMS, focused ultrasound, infrared laser)
- Clinical: Assessment of individual changes over time (recovery, therapy....)
- Clinical: Neurofeedback for therapy.
- Clinical: Neurovascular health assessment.
- Clinical: Useful information on Excitatory/Inhibitory Balance.
- *Big scanner vendors will likely not drive clinical fMRI. Small third-party vendors will push clinical applications, and eventually (20 years?) it will be manifest in some way in daily clinical use.*

- Resting state is still not understood and might be fundamental to understanding states and traits.
- There may still be surprise contrasts in the vast pulse sequence parameter space…
- Multi-modal fusion, novel processing methods & AI are just getting started....there's more in the time series waiting to be revealed.

- Insights into similarities and differences through dense sampling.
- Hierarchy and causality across the whole brain (layer fMRI).

Section on Functional Imaging Methods

Staff Scientists:

Javier Gonzalez-Castillo

Dan Handwerker

Peter Molfese

Programmer/IT:

Catherine Walsh

Visiting Professor:

Yoichi Miwawaki

Post Docs:

Burak Akin

Sharif Kronemer

Bahar Shahsavarani

Josh Faskowitz

Post Bac IRTAs:

Cassie Levesque

Marly Rubin

Marlene Smith

Plyfaa Suwanamalik-Murphy

SFIM

Functional MRI Facility

Staff Scientists:

Renzo Huber

Tyler Morgan

Andy Derbyshire

Vinai Roopchansingh

Linqing Li

Programmer/IT:

Hoan Le

Technologists:

Chung Kan

Marcela Montequin

Alda Ottley

Paula Rowser

Jennifer Yeager

Jayne Zolfaghari

Sarah Ohman

Center for Multimodal Imaging:

Pete Molfese

Program Assistant:

Dorian Van Tassell

Post Bac IRTAs

Esther Lin

Stephanie Swegle

FMRIF