Probing the Dynamics of Spontaneous Cortical Activities via Widefield Ca^{+2} Imaging in GCaMP6 Transgenic Mice

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Outline

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- **Introduction**
  - Motivation
  - Problem Statement
- **Experiment**
  - Setup
  - Data Collection
- **Analysis**
  - Framework
- **Results and Conclusions**

Motivation

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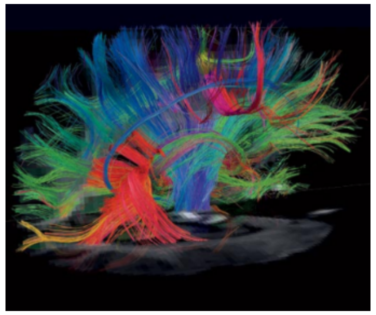
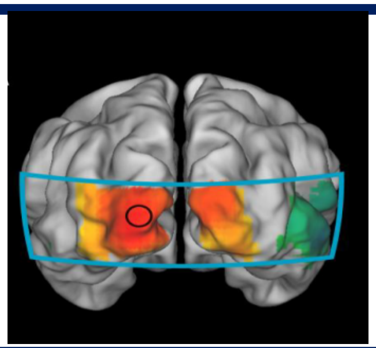
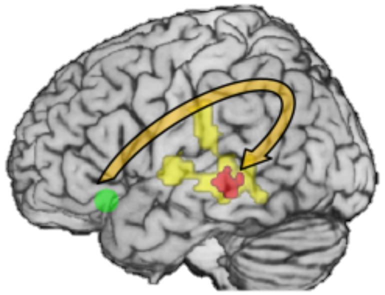
- **Brain Connectivity**
  - anatomical connectivity
    - looks for axonal connections
    - diffusion tensor imaging, tracing techniques



  - functional connectivity (FC)
    - looks for statistical similarities between regional time series
    - functional neuroimaging techniques, seed-based correlation



  - effective connectivity
    - looks for causal influences between regions of brain
    - causal interactions modeling



- **Applications**
  - understanding the brain function at the network level
  - helps in identifying biomarkers of brain-related disorders

Motivation

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- **Dynamic Functional Connectivity**
  - changes in neuronal connections occur at multiple temporal scales (short, long)
  - short term: task execution
  - long term: learning, aging, brain-related diseases

- **Identifying Changes in Functional Connectivity**
  - *forming connections:* correlation, coherence, wavelet transform coherence,…
  - *identifying when there is a significant change in connections*: statistical tools,…

- **Most Studies Have Explored Dynamic FC in Humans**
  - resting-state, task-based
  - patient groups (e.g. schizophrenia) vs healthy
  - wide range of neuroimaging tools: EEG, fMRI, fNIRS

Problem Statement

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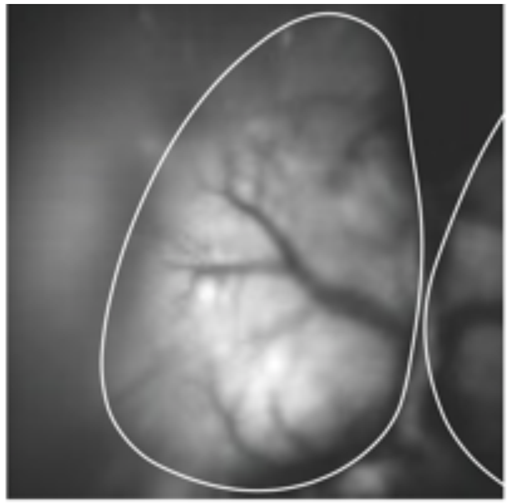
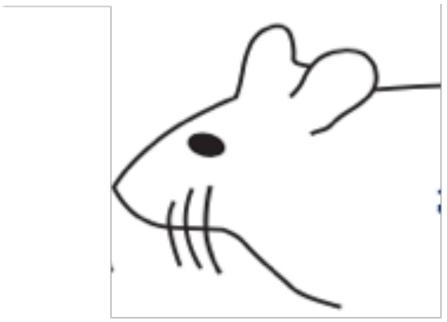
- **Probing Changes in Functional Connectivity Related to Behavior in Mice**



- ***Imaging Tool:* Widefield Calcium Imaging**
  - using mice expressing GCaMP6f
  - enables longitudinal recording of neural activity
  - offers high temporal resolution
  - capable of imaging neural populations over large portions of the cerebral cortex
  - a powerful tool for studying the relationship between brain activity and behavior

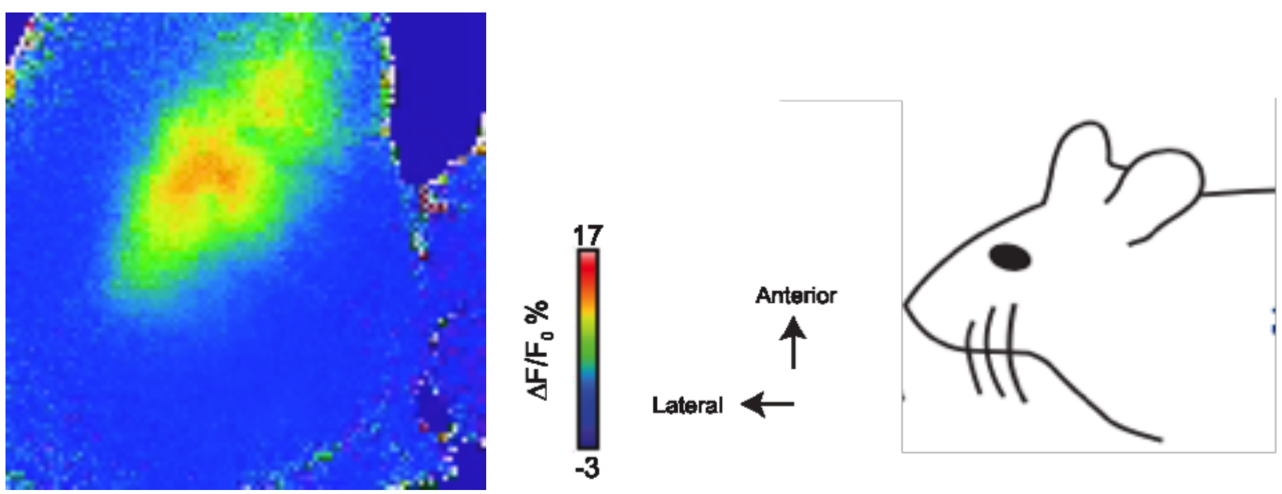
- ***Behavior:* Whisking Conditions**
  - active whisking (AW)
  - no whisking (NW)

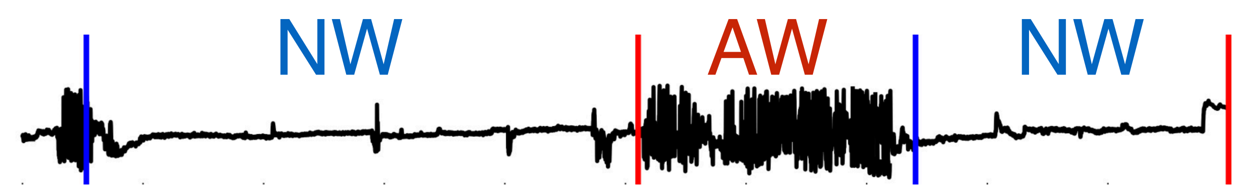


Minderer, Matthias, et al. "Chronic imaging of cortical sensory map dynamics using a genetically encoded calcium indicator." *The Journal of physiology* 590.1 (2012): 99-107.
Madisen, Linda, et al. "Transgenic mice for intersectional targeting of neural sensors and effectors with high specificity and performance." *Neuron* 85.5 (2015): 942-958.

Problem Statement

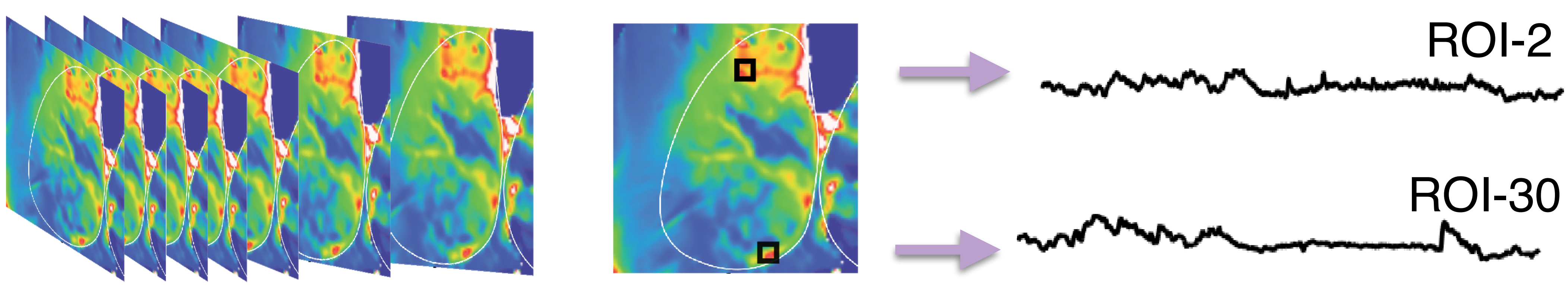
- **Probing Changes in FC during Different Whisking Conditions**
  - identify significant changes in FC during AW vs NW





Spontaneous Whisking Status (AW and NW)

  - exploring two questions
    - where changes in FC occur (i.e. which ROI pairs)?
    - at which frequency bands changes in FC occur?



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Experimental Setup

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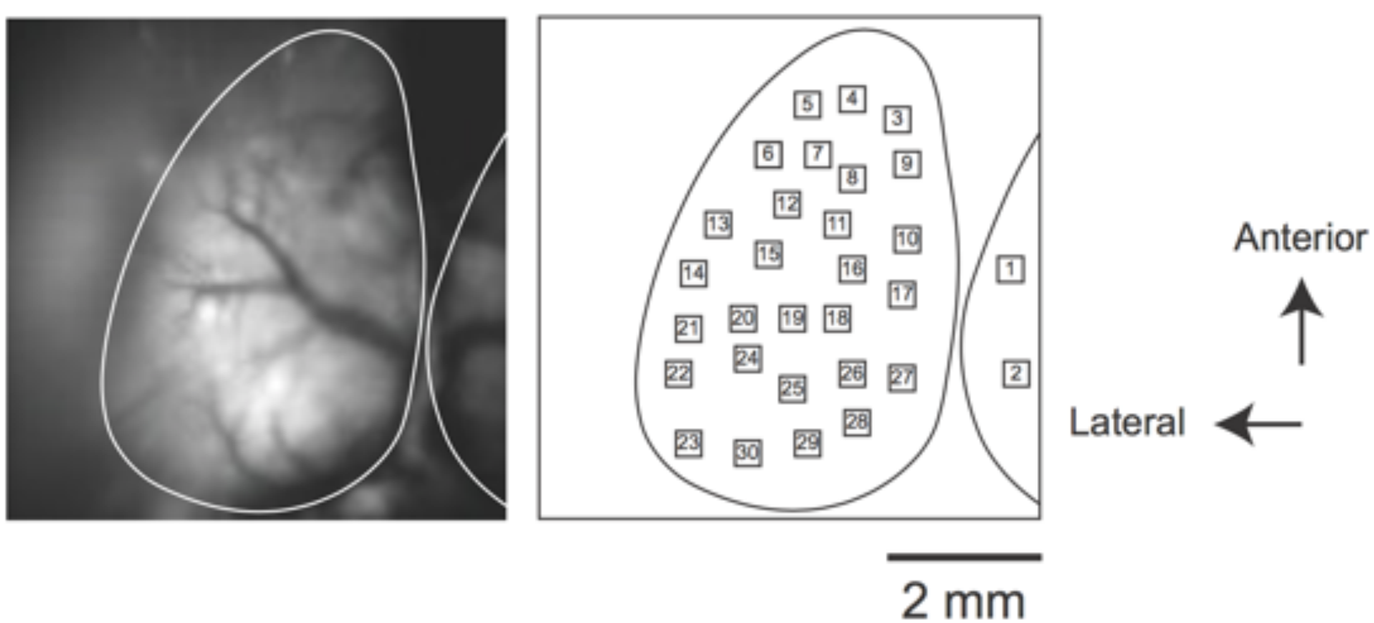
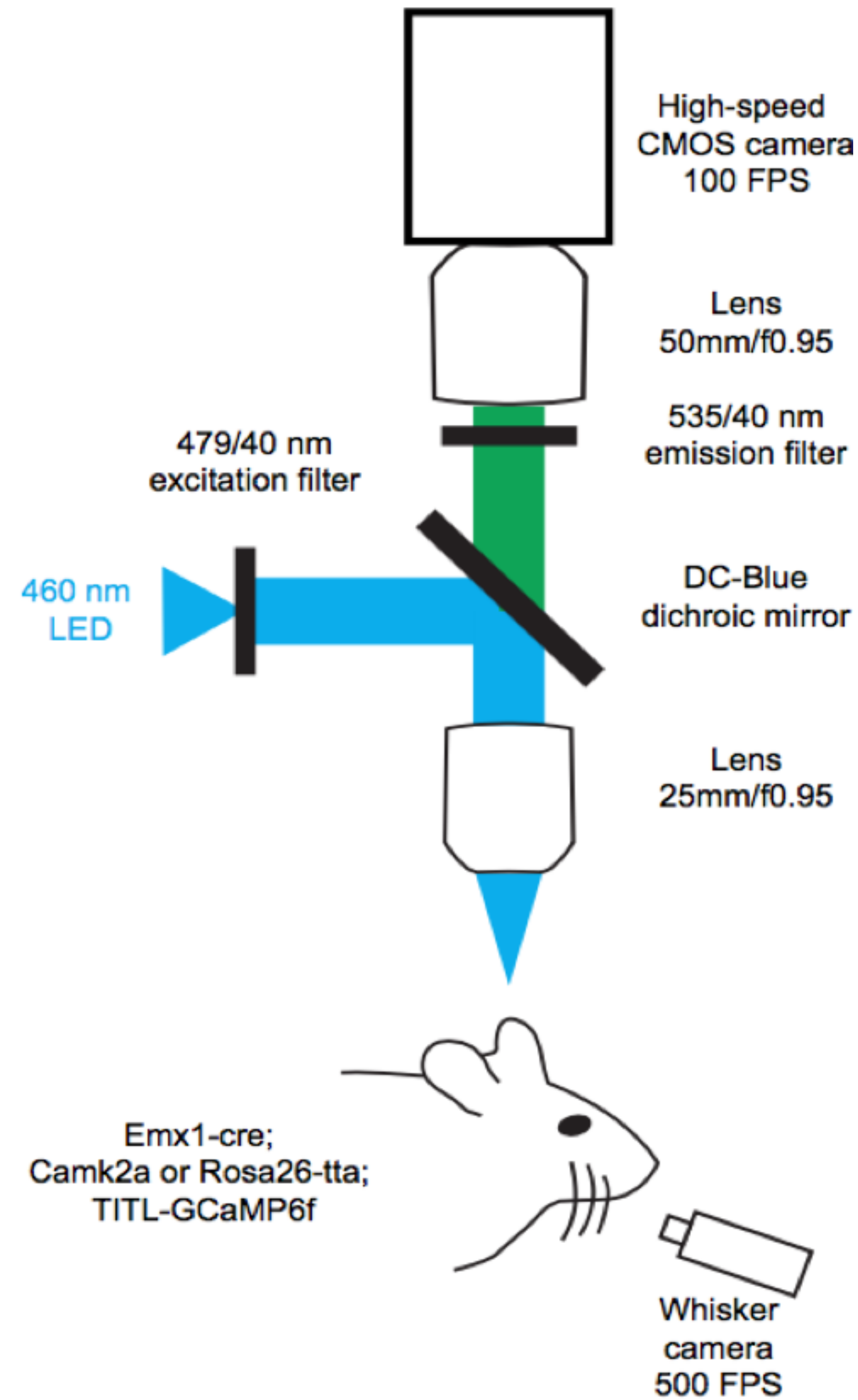
- **Widefield Calcium Imaging**
  - six head-fixed GCaMP6f mice
  - two recording sessions for each mouse
  - entire left and mediate right hemisphere
  - 100 x 100 pixels per frame
  - sampling rate at 100 frames per second
  - 30 ROI locations (5x5 pixels) were selected

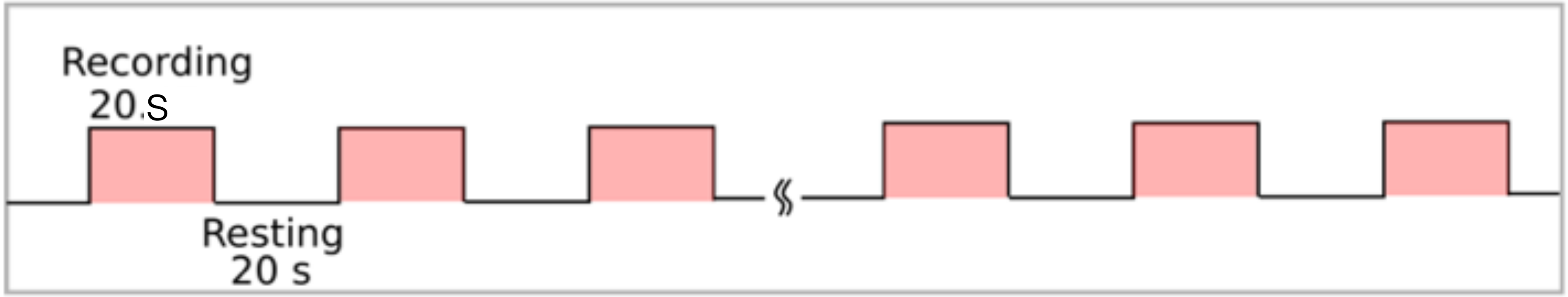


- **Whisker Movement Recoding**
  - simultaneously recorded at 500 frames per second



Data Collection

- **Paradigm**
  - 16 blocks with 20 second rest in between for each session



Data Collection

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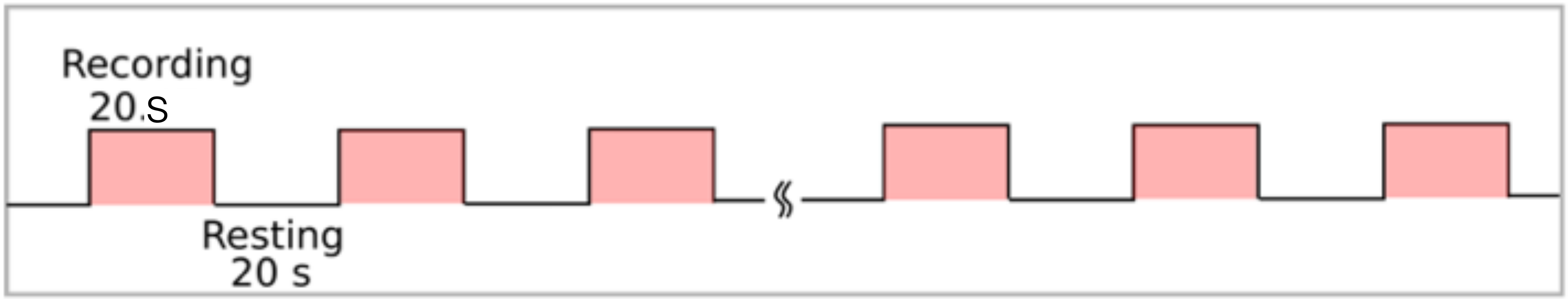
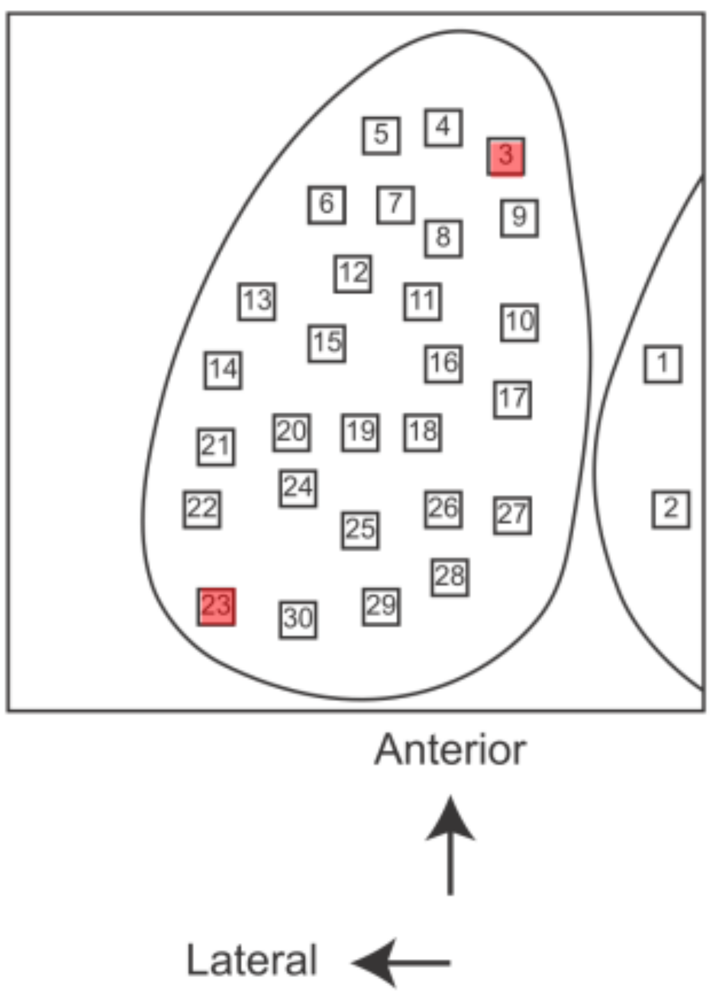
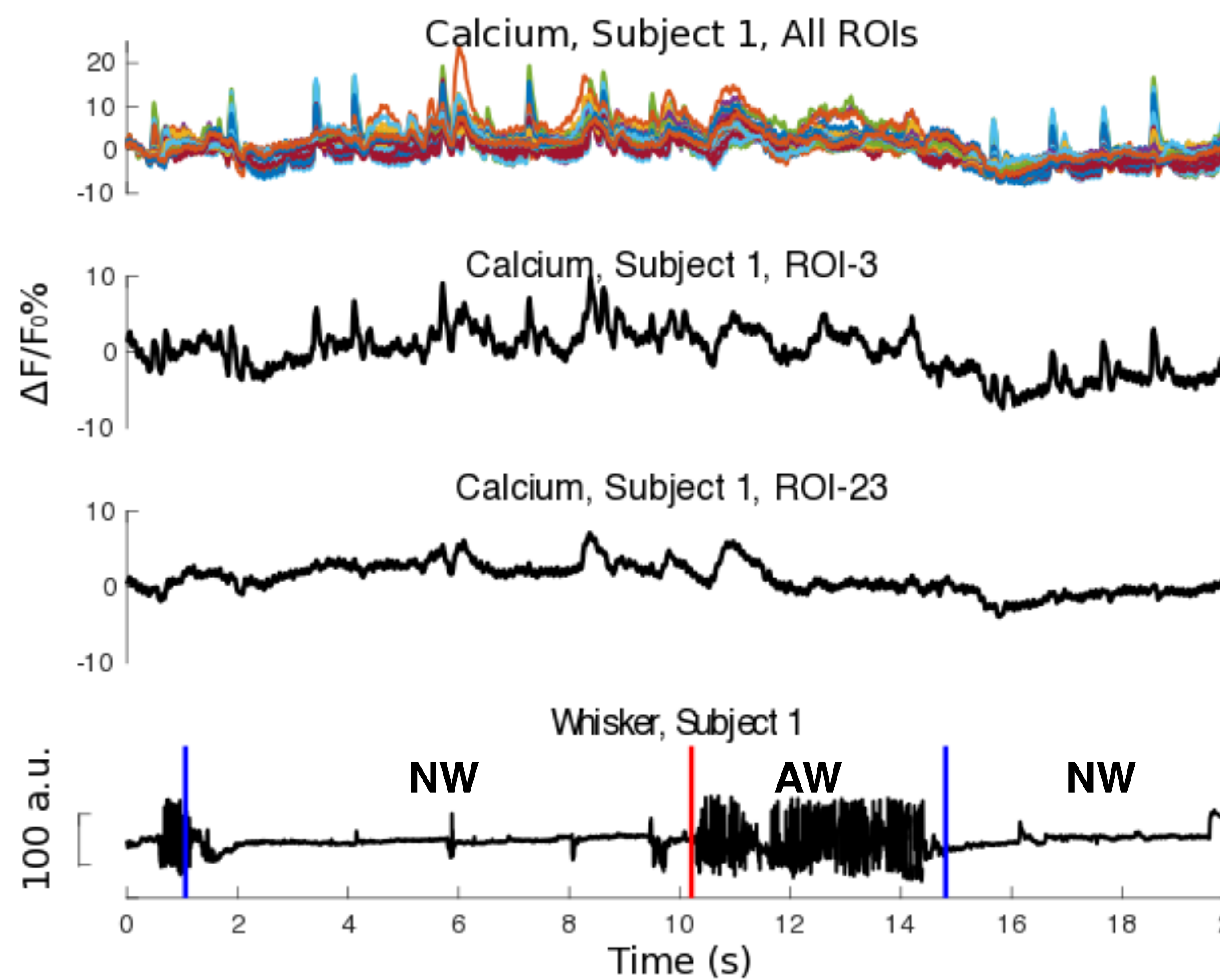
- **Paradigm**
  - 16 blocks with 20 second rest in between



- **Sample Recoding**





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Forming Connections: WTC

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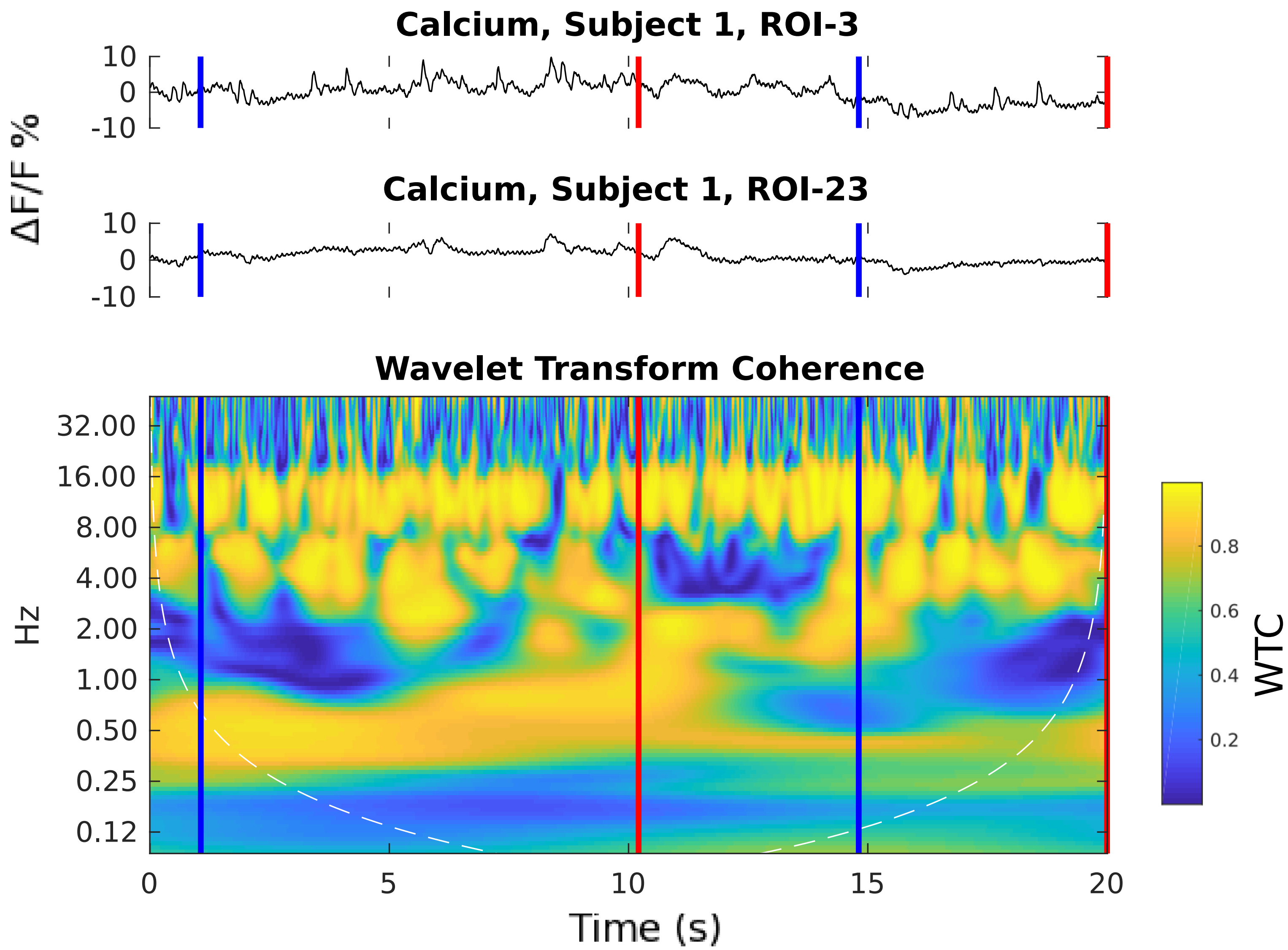
- **Wavelet Transform**

$$W_{x_n}(n,s) = \sqrt{\frac{\Delta t}{s}} \sum_{m=1}^{N} x_m \psi_0[(m-n)\frac{\Delta t}{s}]$$

- **Wavelet Transform Coherence (WTC)**

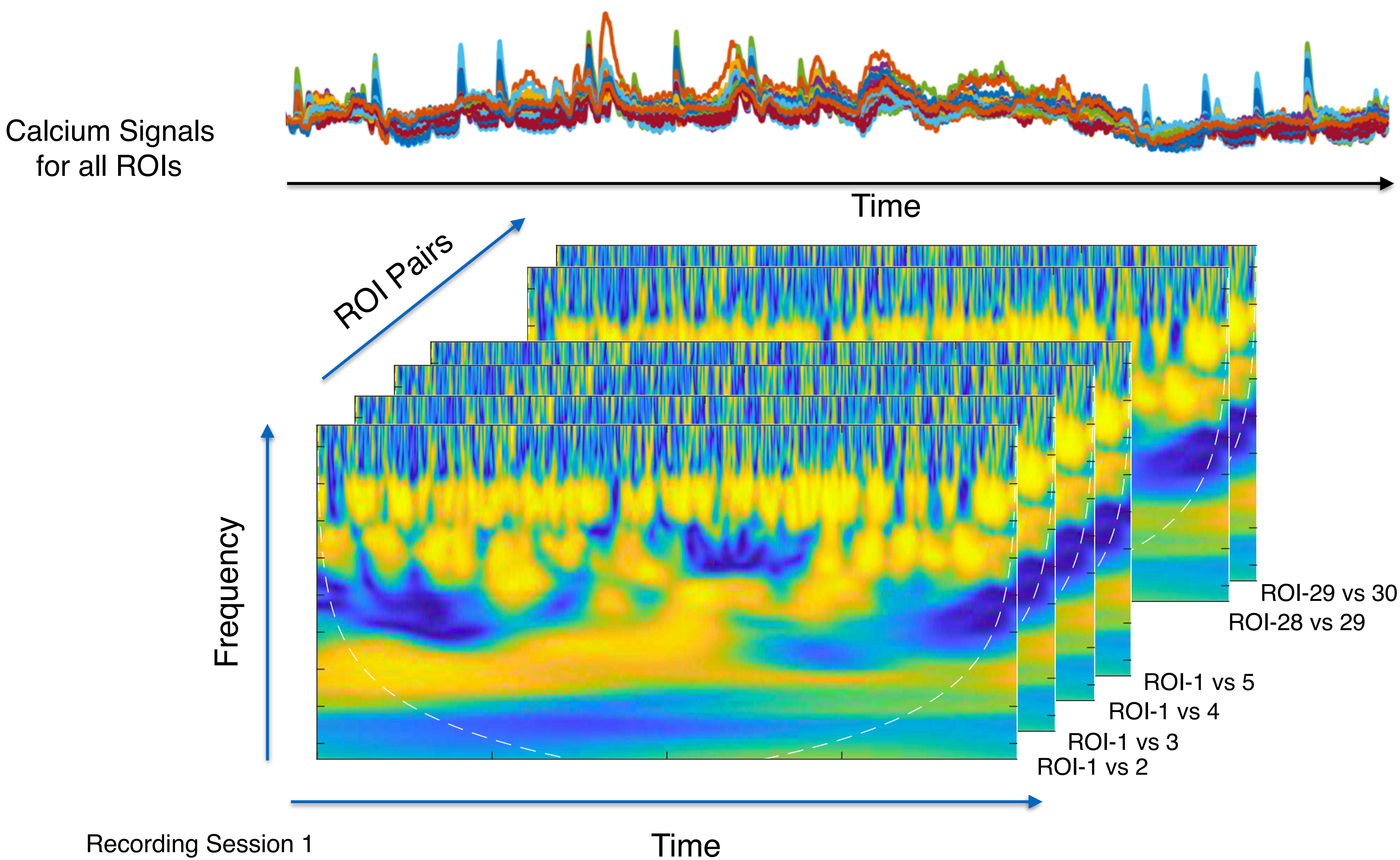
$$R^2_{x_n,y_n}(n,s) = \frac{|S(s^{-1}W_{x_n,y_n}(n,s))|^2}{S(s^{-1}|W_{x_n}(n,s)|^2) \cdot S(s^{-1}|W_{y_n}(n,s)|^2)}$$



Chang, Catie, and Gary H. Glover. "Time–frequency dynamics of resting-state brain connectivity measured with fMRI." Neuroimage 50.1 (2010): 81-98.

Forming Connections: WTC

- **WTC Is Computed for All Possible ROI Pairs**



Recording Session 1

Relating to Behavior

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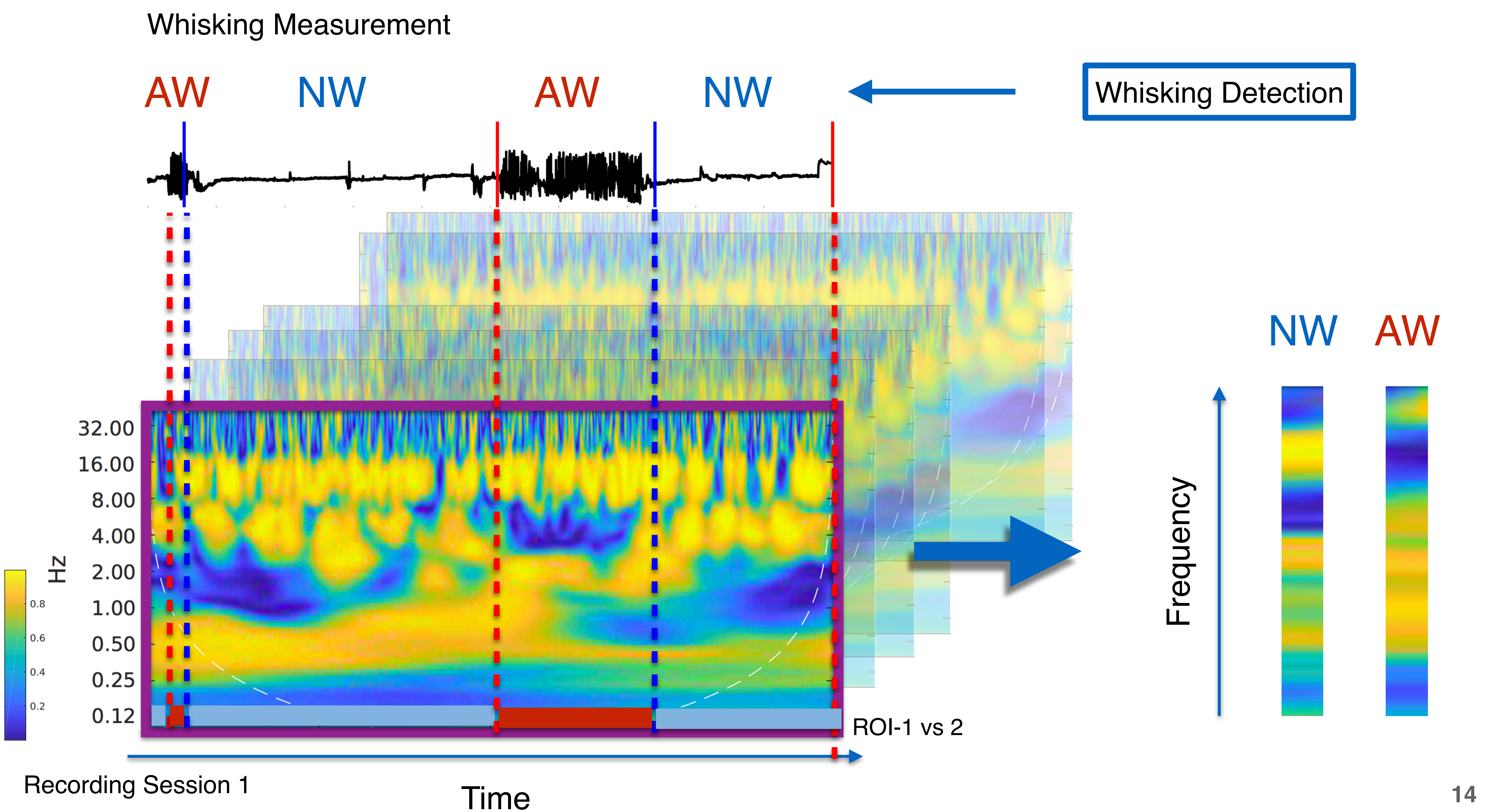
- **WTC Labeling According to Whisking Condition**
  - WTC columns are labeled as NW and AW
  - WTC columns related to same whisking condition are averaged



Identifying Changes: Multivariate Permutation Test

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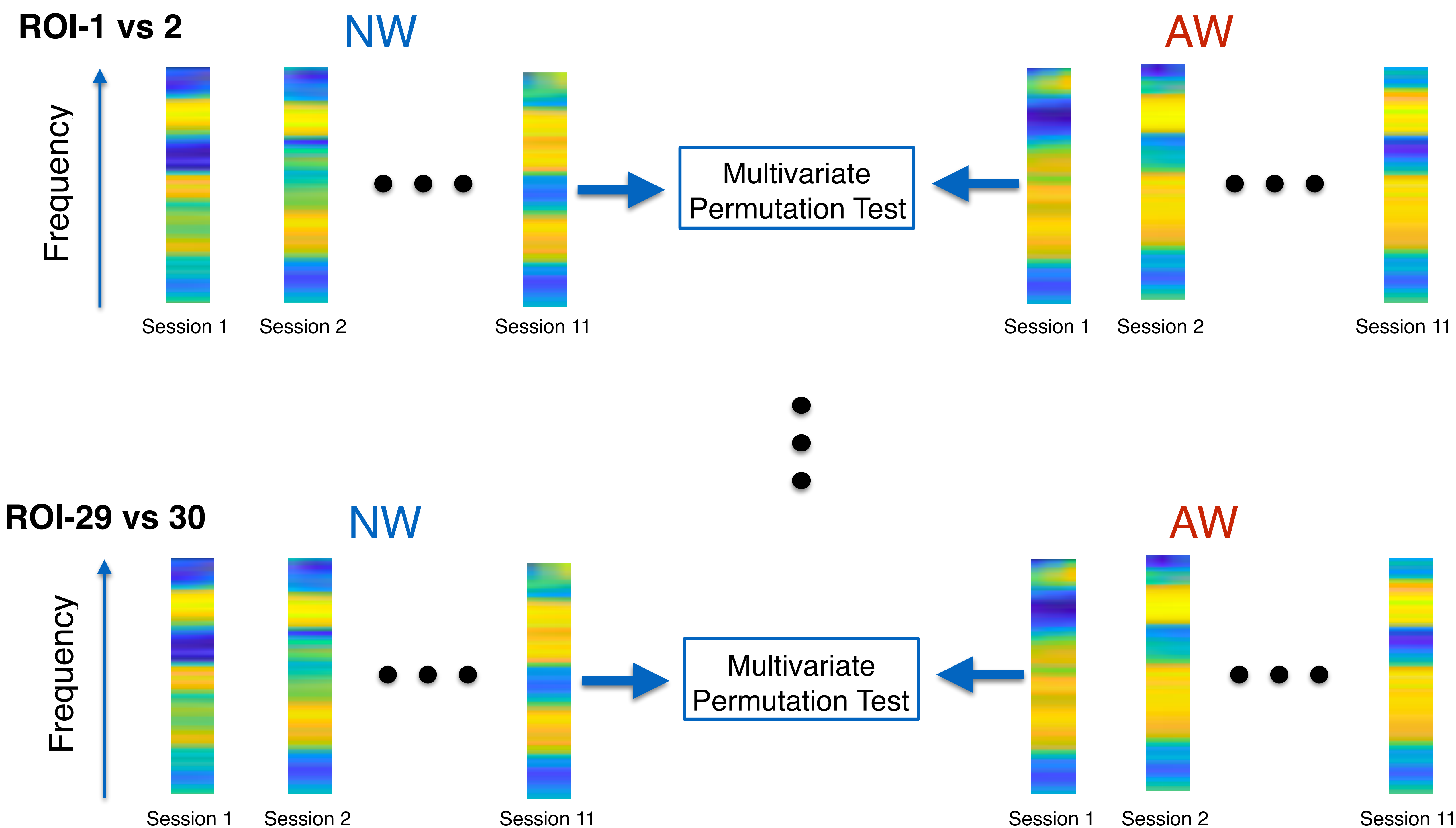
- **Organizing Data**
  - averaged WTC columns per condition are subjected to multivariate permutation test



Identifying Changes: Multivariate Permutation Test

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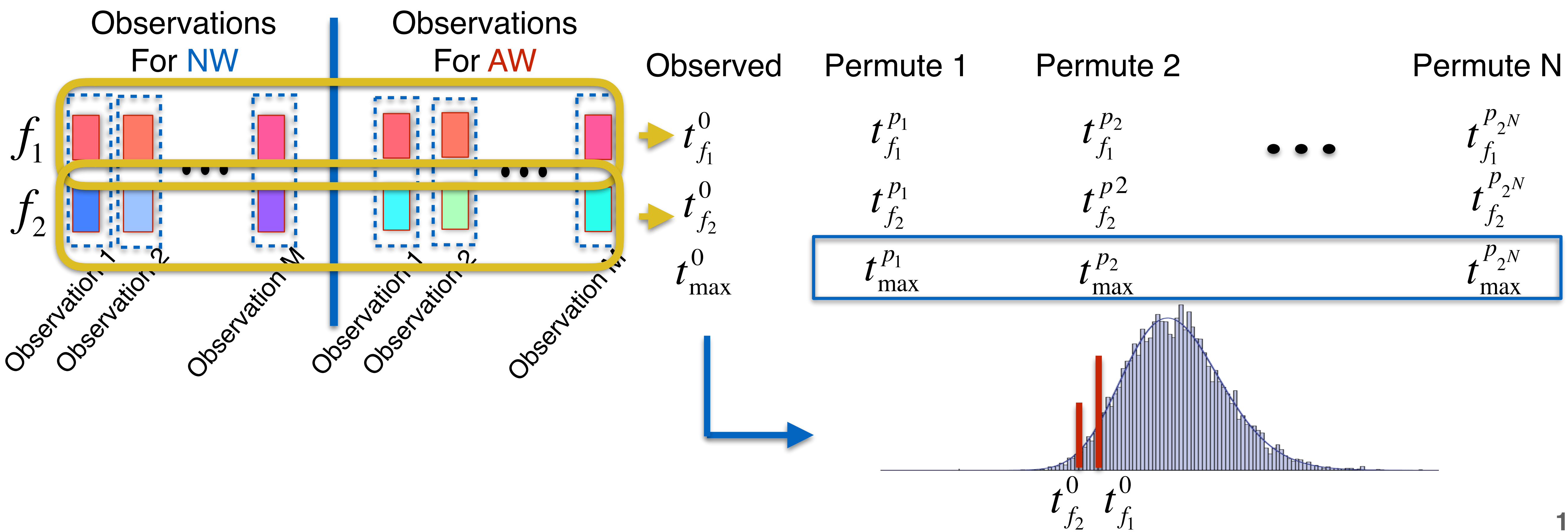
- **MPT Approach**
  - two frequency bins, two conditions, *M* observations, *N* permutations
  - procedure:
    - compute *t*-value t_0 for each frequency bin from the original observations
    - shuffle observations across conditions, compute *t*-value for each frequency bin
    - find t_{max} for each frequency bin
    - repeat shuffling *N* times, obtain null hypothesis distribution
    - compute statistical significant by referring t_0 to the constructed distribution



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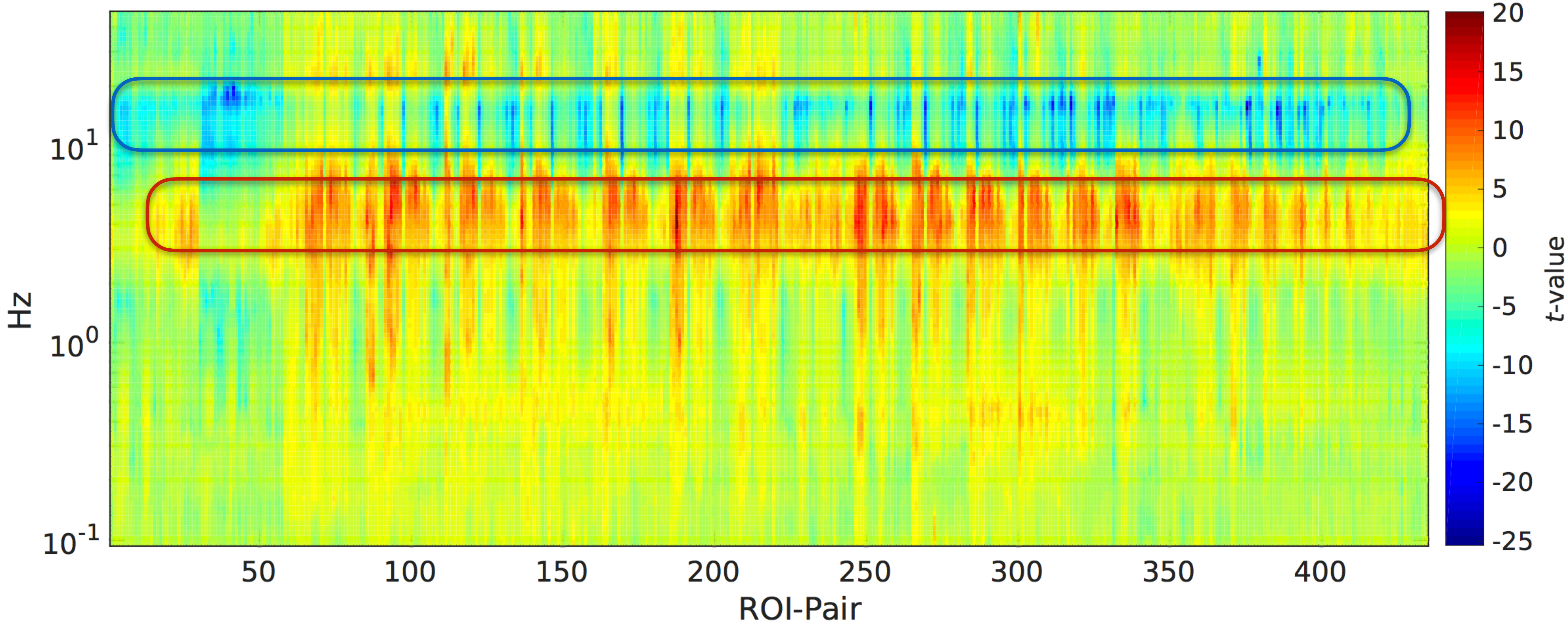
Results

- **Observed *t*-values**
  - raster diagram illustrating *t*-values for all frequency bins and ROI pairs
  - two groups of ROI pairs can be clustered in different frequency bands
    - ROI pairs with positive *t*-values: ROIs are more “connected” during NW
    - ROI pairs with negative *t*-values: ROIs are less “connected” during NW



Results

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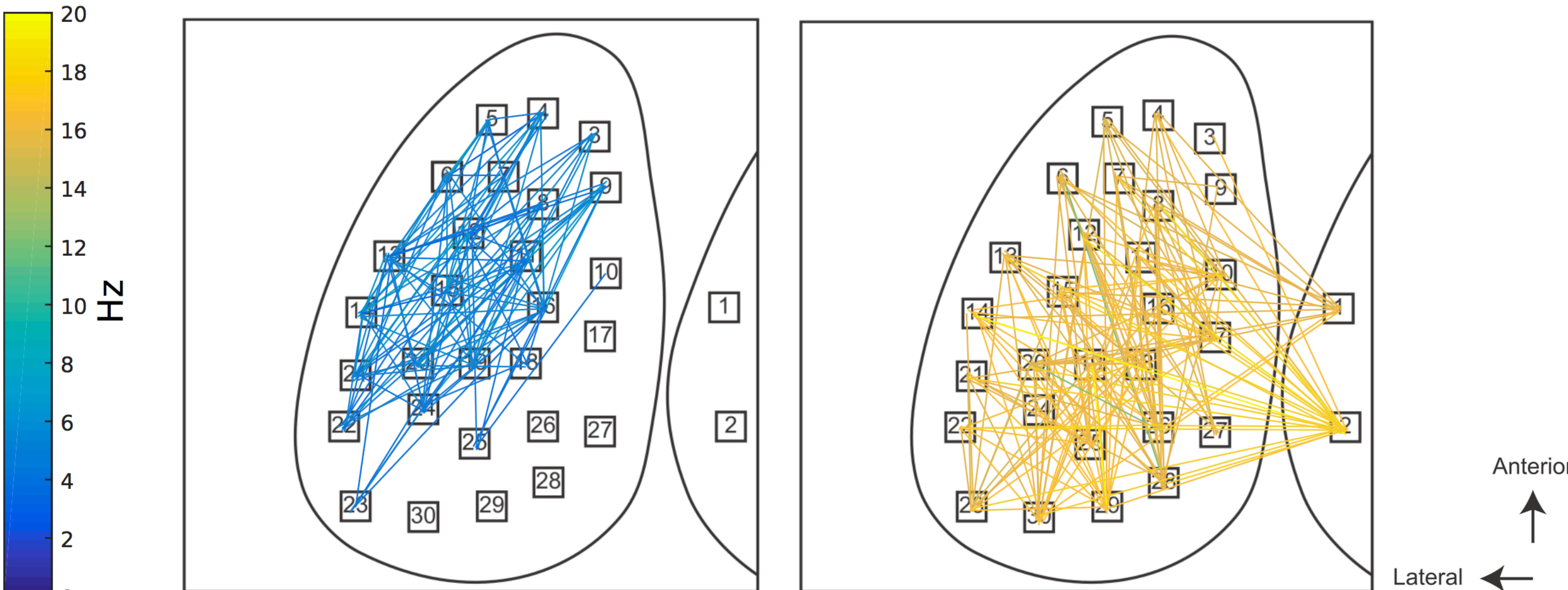
- **ROI Pairs and Their Dominant Frequencies with Significant Dynamics in FC are Detected**
  - ROI pairs are detected with significant changes in FC across conditions ($p < 0.001$)
  - detected ROI pairs can be clustered into two groups
  - NW condition: slower oscillation, neighboring neurons are synchronized
  - AW condition: higher oscillation, distributed neurons are synchronized



$WTC_{NW} > WTC_{AW}$ $WTC_{NW} < WTC_{AW}$

Conclusions

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- Widefield Imaging Was Used to Record Cortical Activity in GCaMP6f Mice during Active Whisking and No Whisking
- An Analysis Framework Combining Wavelet Transform Coherence and Multivariate Permutation Test Was Presented to Study Changes in Functional Connectivity
- It Was Demonstrated that Neighboring ROI Pairs Are More Synchronized During NW with Slower Oscillation and Distributed ROI Pairs Are More Synchronized During AW with Faster Oscillation
- Future Work Include Applying the Method to Task-Based Data to Identify Changes in FC

Thank You!

- **MPT Approach - Overview**
  - generalized from univariate permutation test: If H_0 is true, shuffling the data won't affect the test statistics
  - corrects multiple comparisons

Review of the univariate permutation test