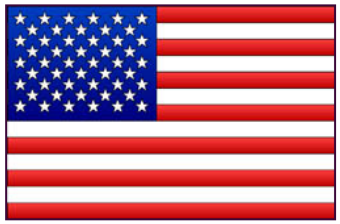
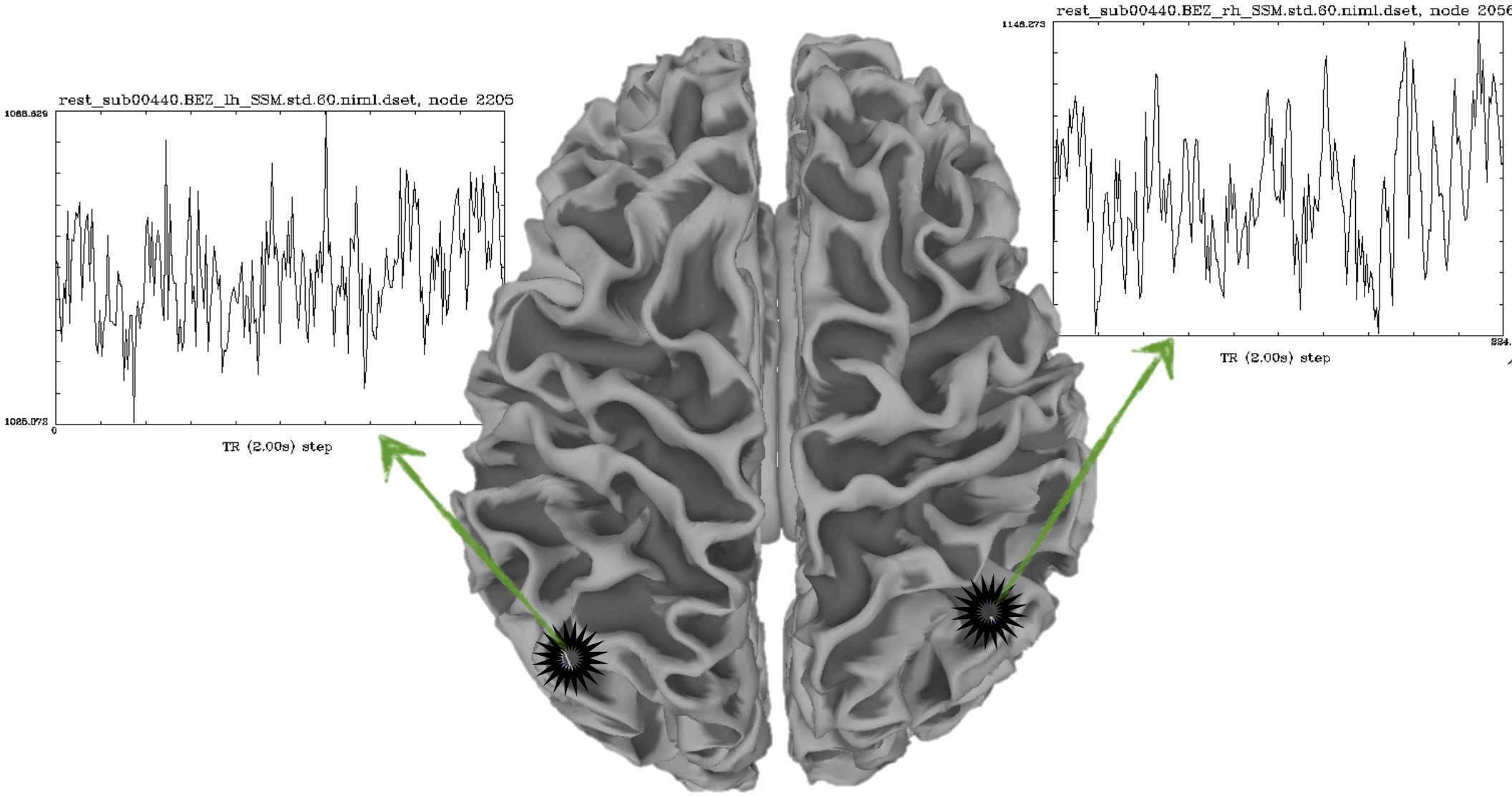
Resting State FMRI:
Analysis Methods
and
Analysis Problems

SSCC / NIMH & NINDS / NIH / DHHS / USA / EARTH



Resting state

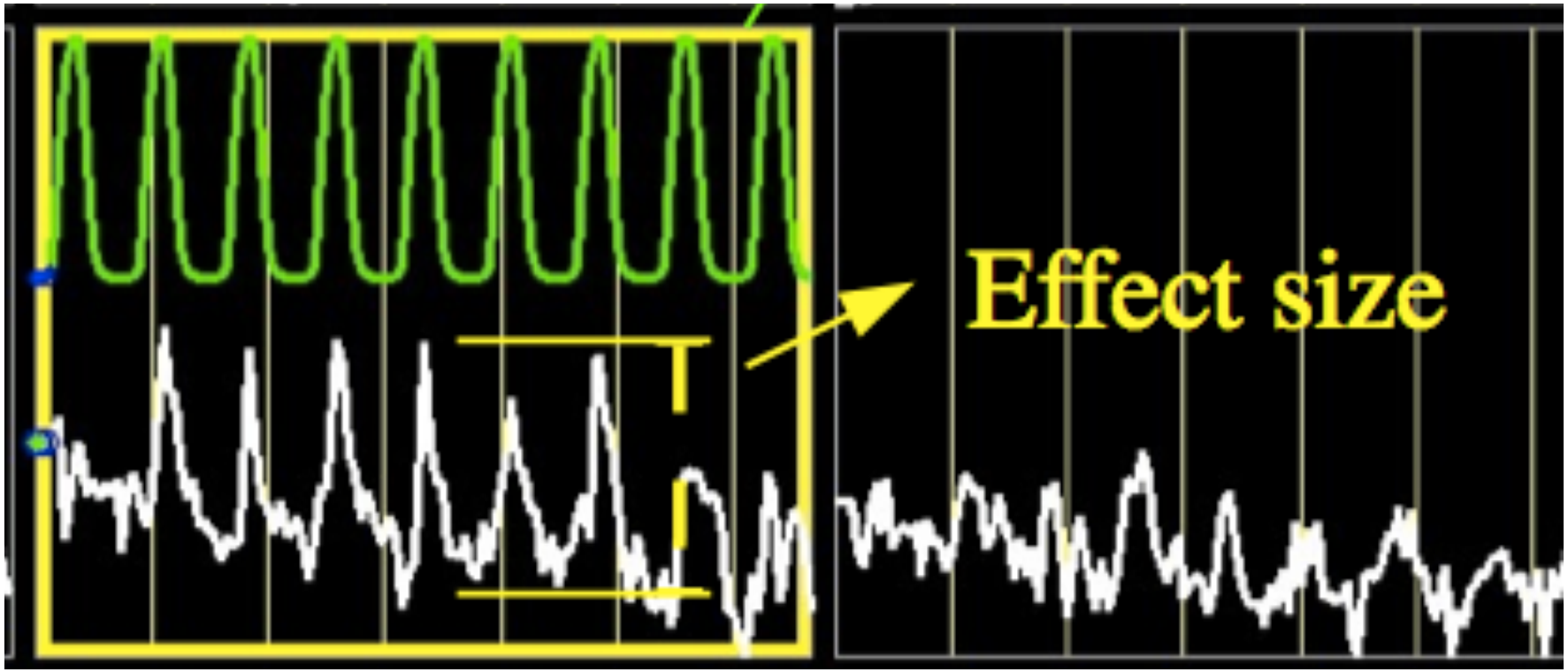
BOLD signal fluctuations during undirected brain activity



Resting state

BOLD signal fluctuations during undirected brain activity

There is no model for signal, such as expected response in task FMRI



Resting state

BOLD signal fluctuations during undirected brain activity

There is no model for signal, such as expected response in task FMRI

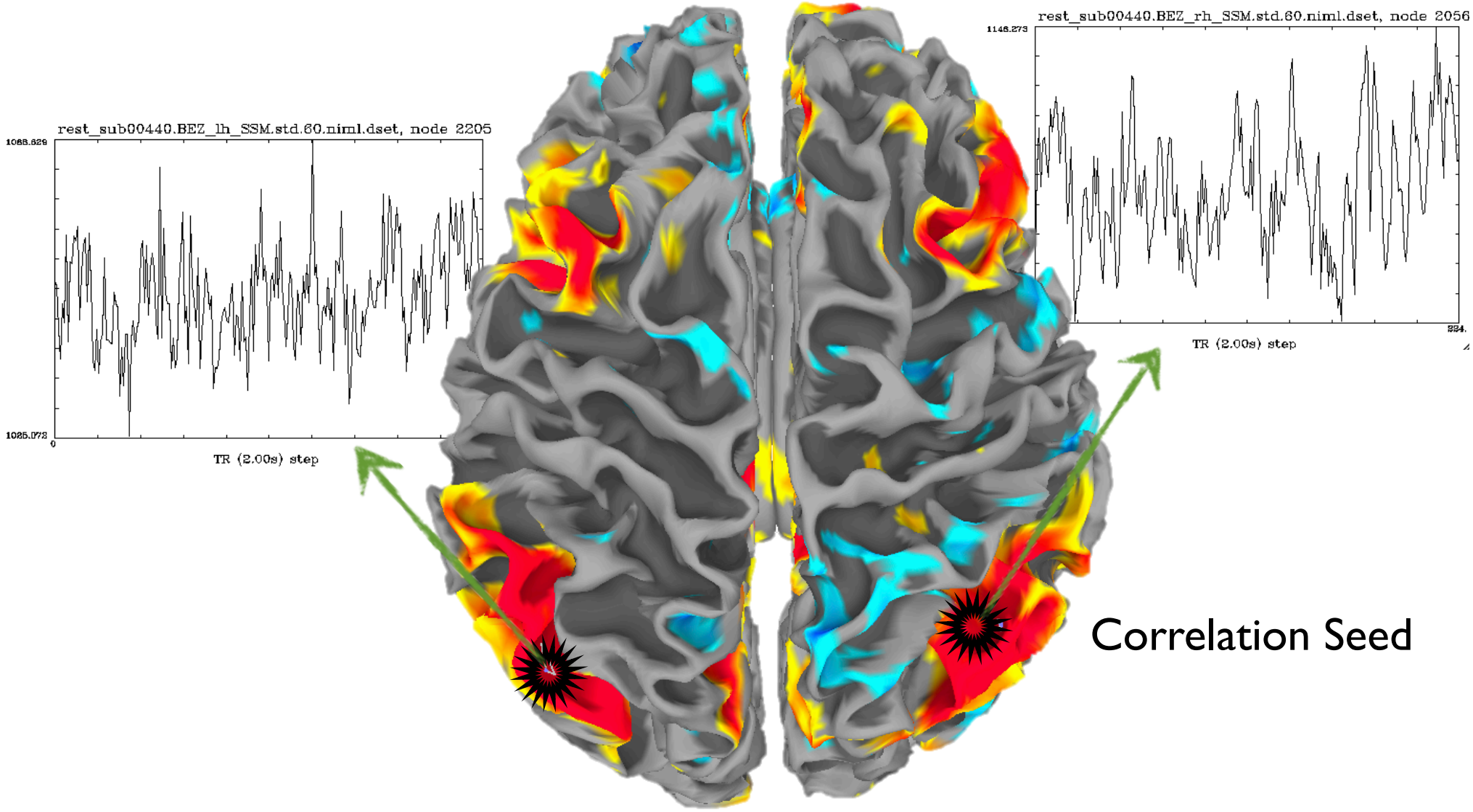
Resort to describing relationships between brain regions

Correlation matrices, graph theory, functional/ effective/??? "connectivity"

Factoring data into space×time components in statistically interesting ways (PCA, ICA)

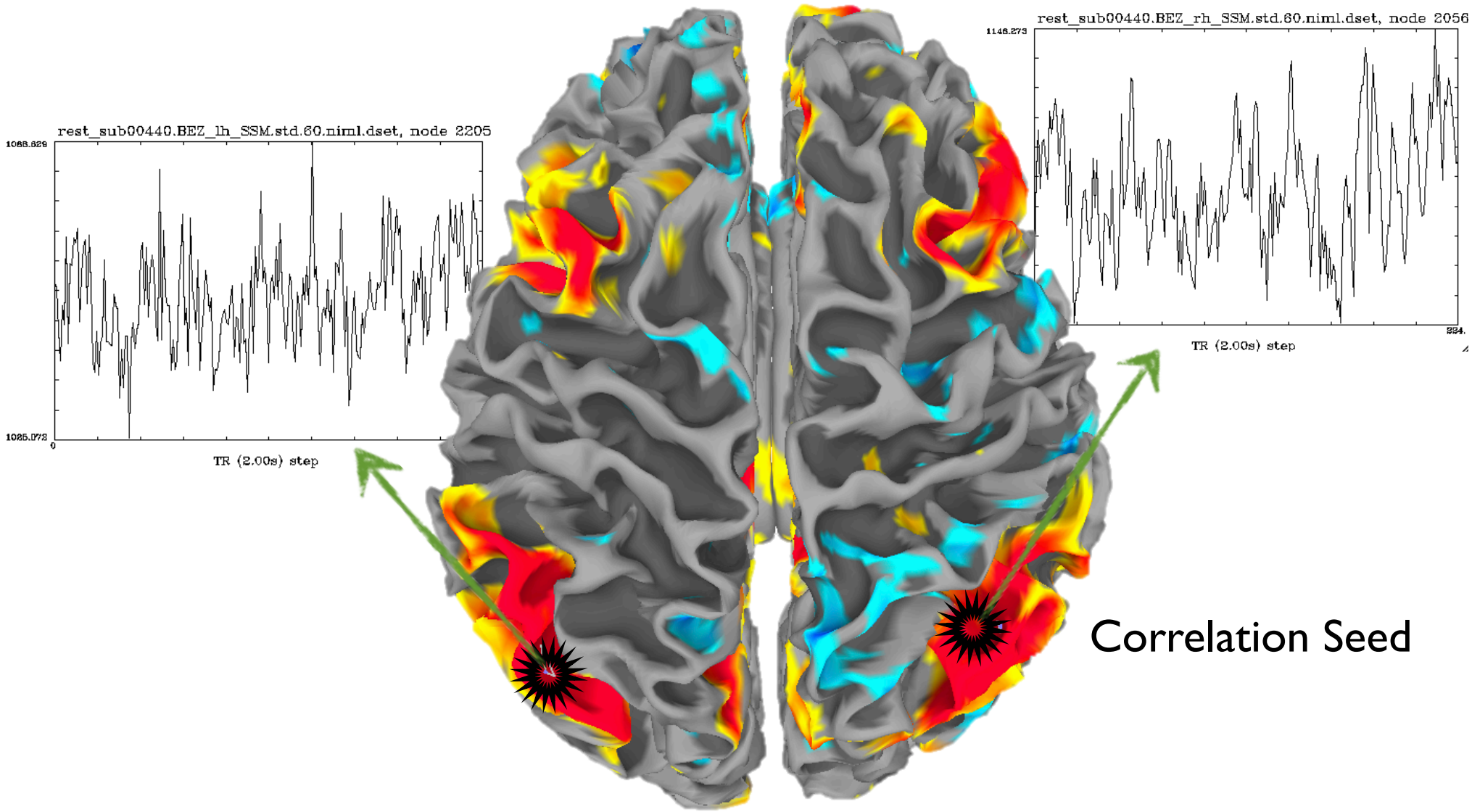
Resting state

Resort to describing relationships between brain regions

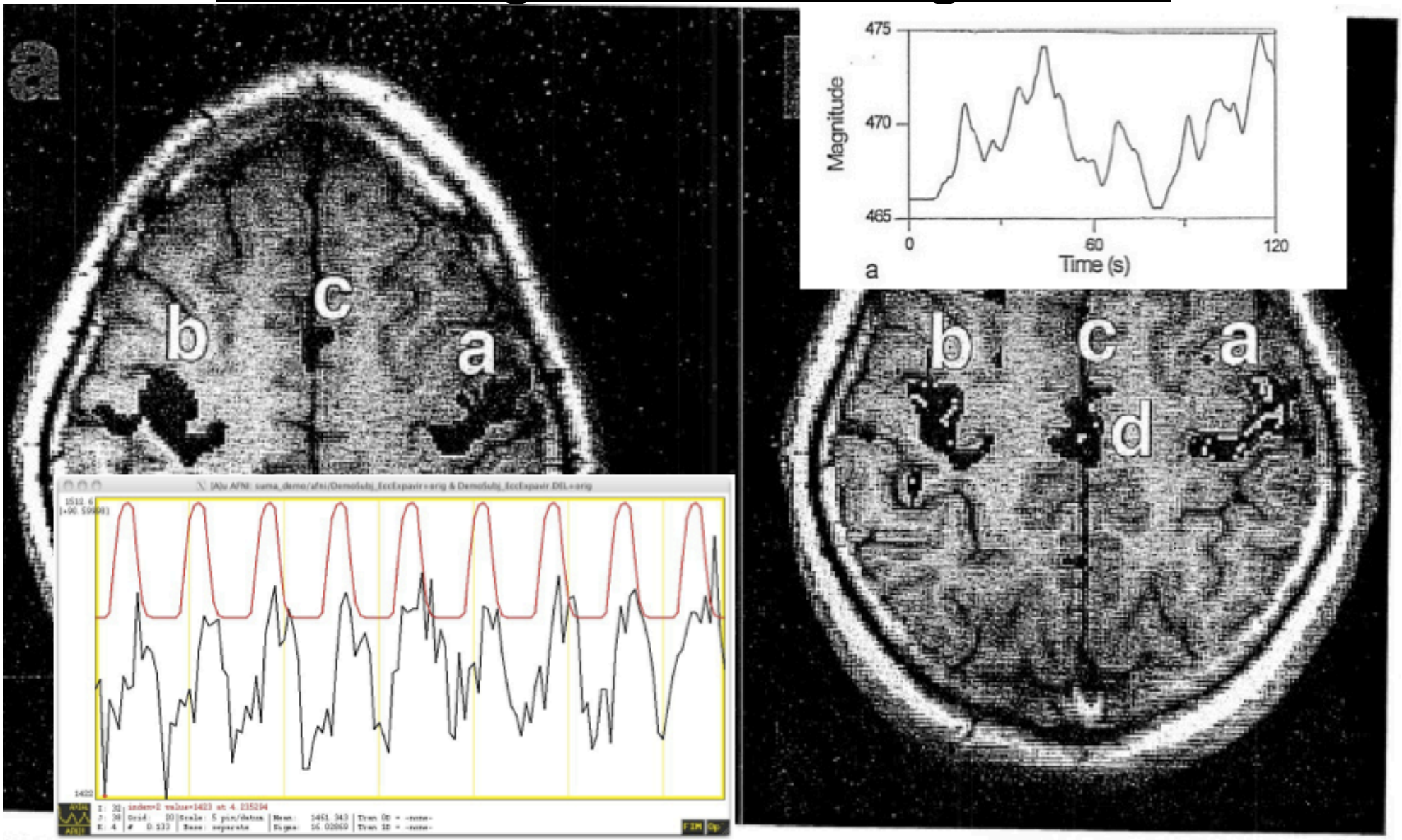


Resting state

Interpret correlation strength as proxy (or stand-in) for brain function coupling between regions



The *magic* of resting state (Biswal 95)



G. 3. (Left) FMRI task-activation response to bilateral left and right finger movement, superimposed on a GRASS anatomic image. (Right) ictuation response using the methods of this paper. See text for assignment of labeled regions. Red is positive correlation, and yellow negative.

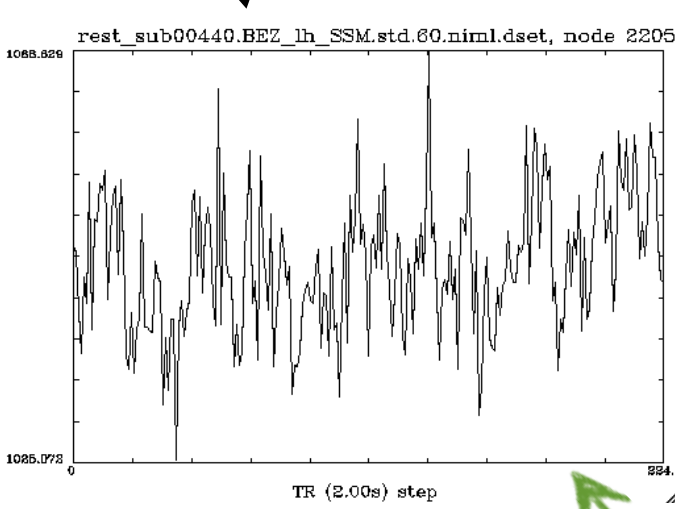
Resting state PROBLEM

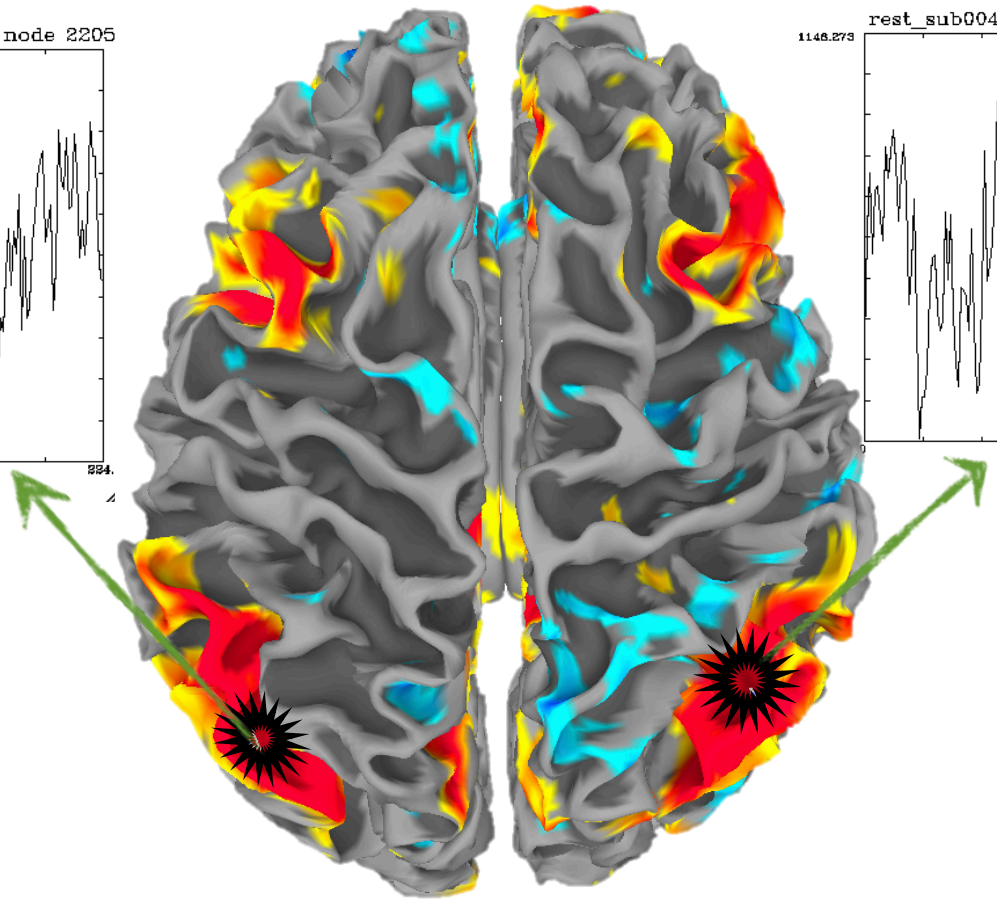
Neuronally driven BOLD fluctuations of interest

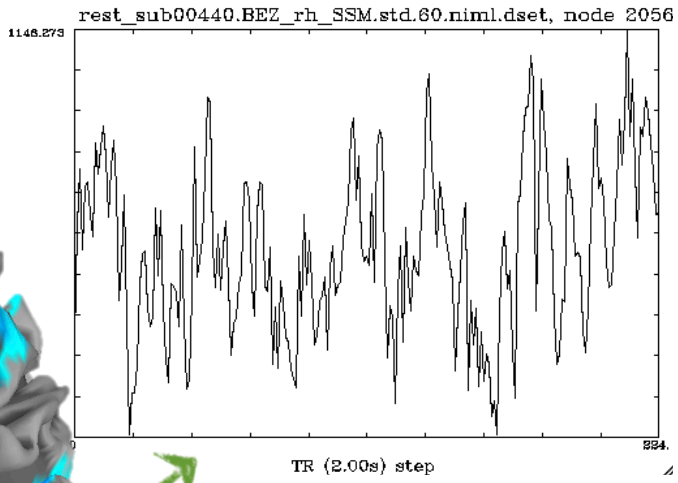
AND

Fluctuations from respiration, heart beat, motion

Are all spatially correlated ☹







The origin of our troubles

We have no model for signal

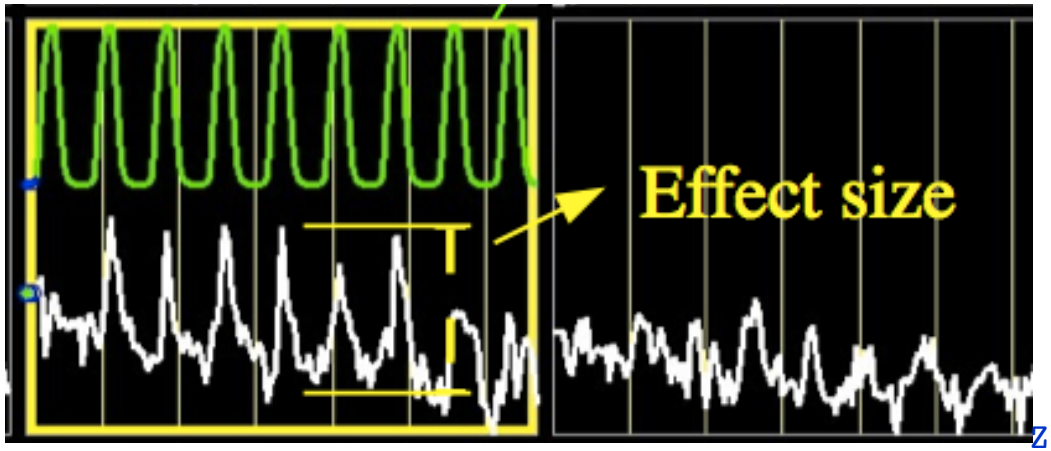
Nothing like the expected response (regressors) of task FMRI

We have no good models for noise

We have some, but they're far from perfect

Effect size (as correlation) is a spatially varying function of noise (fluctuations of no interest)

- Noise can bias correlations **up**, or **down** depending on the noise's spatial covariance
- In task FMRI by contrast, noise affects variance of effect size estimate



The origin of our troubles

Difficult to attach meaning to effect size in RS-FMRI

Effect in RS-FMRI is like an SNR measure, affected by changes in both signal (numerator) and noise (denominator)

For example, if you have 2 groups

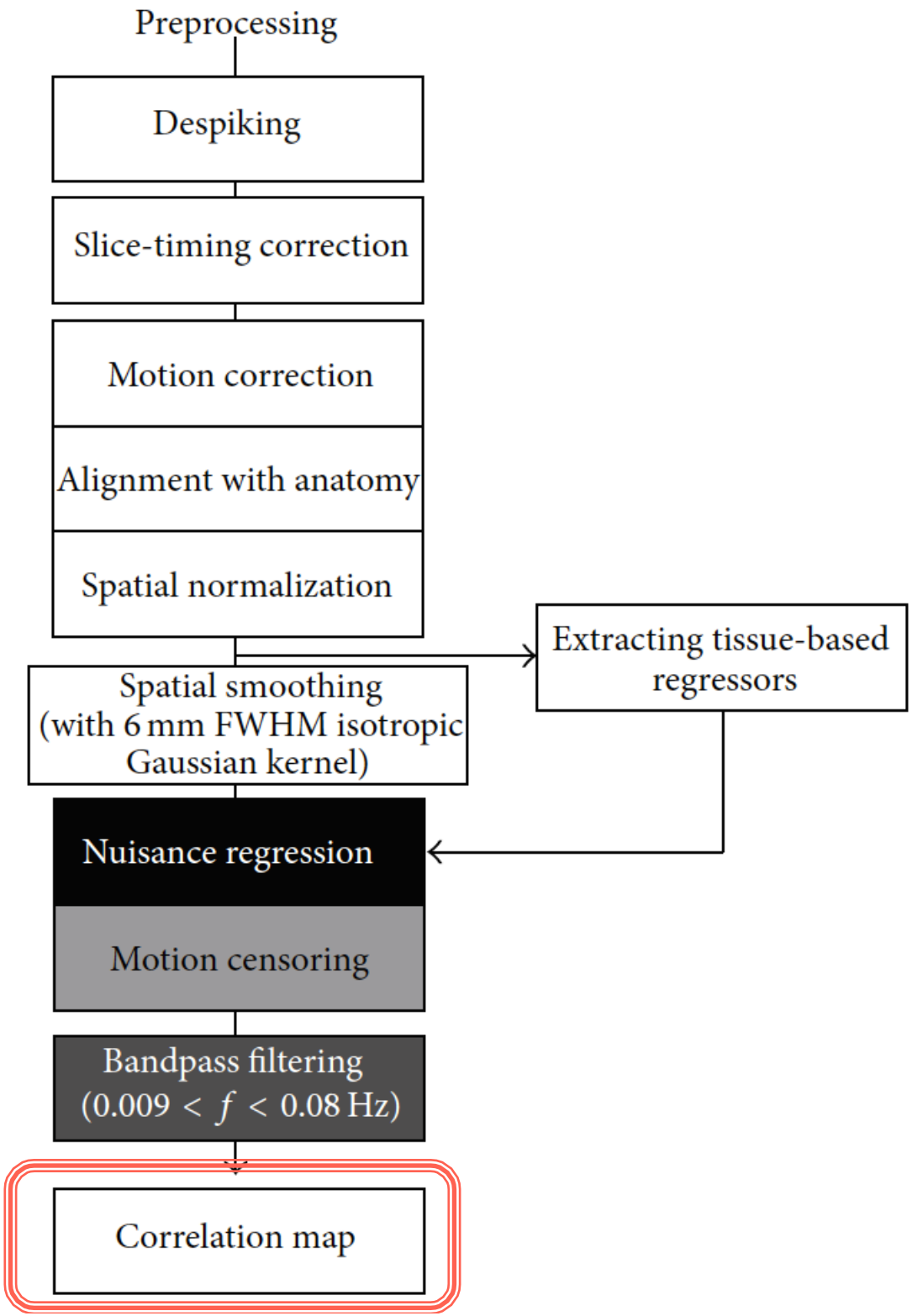
more motion → more noise → more correlation (bias) → group differences

Weak but consistent bias → significant difference

Some sources have brain-wide (global) effects on correlation distribution (e.g. ET-CO2 , motion, etc.)

Sources of bias and error

- Head motion (Van Dijk, 2012) (Power, 2012)
- Physiological "Noise"
  - Respiratory or cardiac cycles (Glover, 2002)
  - Non-stationarity of breathing and cardiac rhythms
    (Birn, 2006) (Shmueli, 2007) (Chang, 2009)
- Hardware instability (Jo, 2010)
- Anatomical bias
- **Pre-processing**

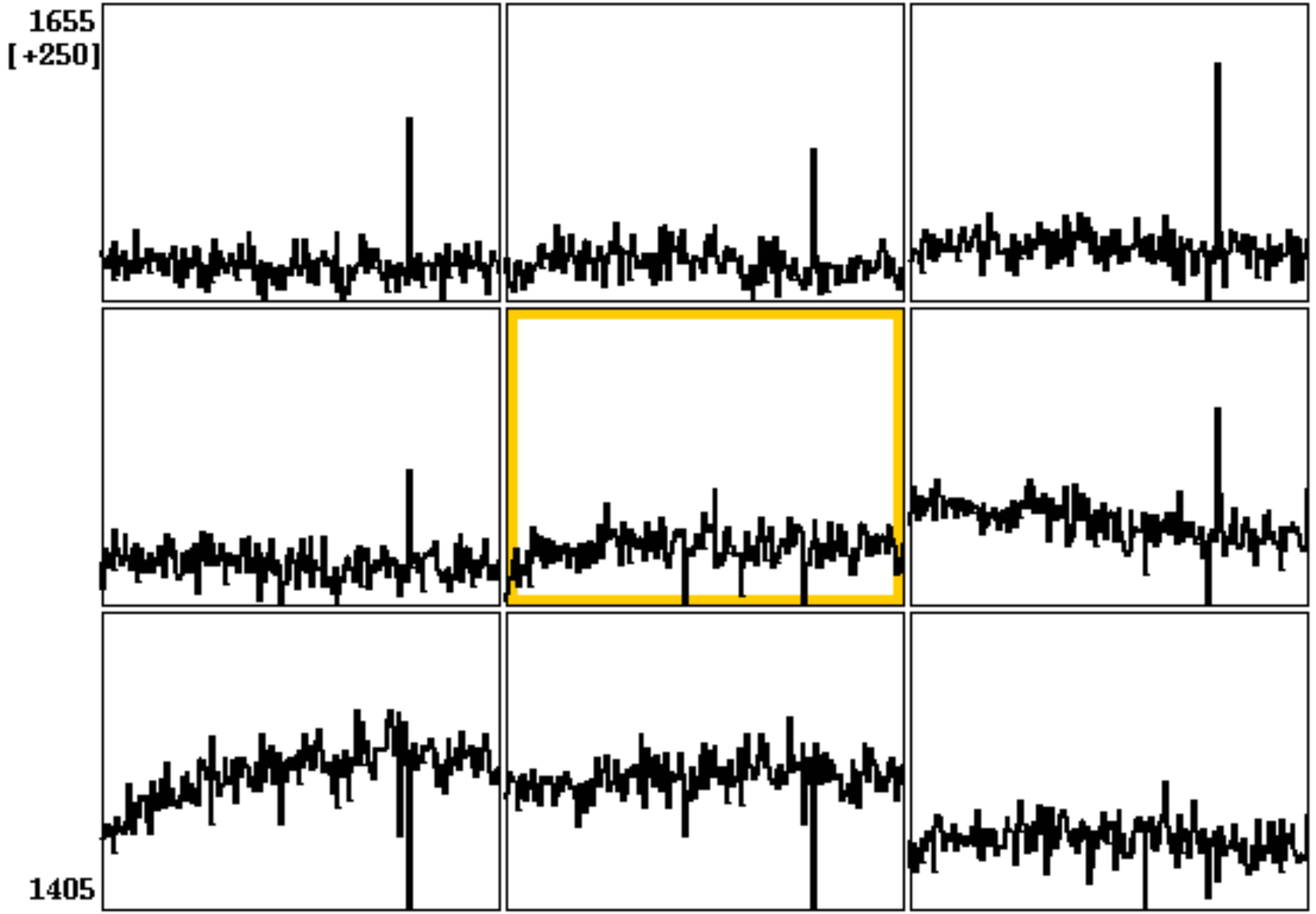


AFNI's recommended RS-FMRI pre-processing steps

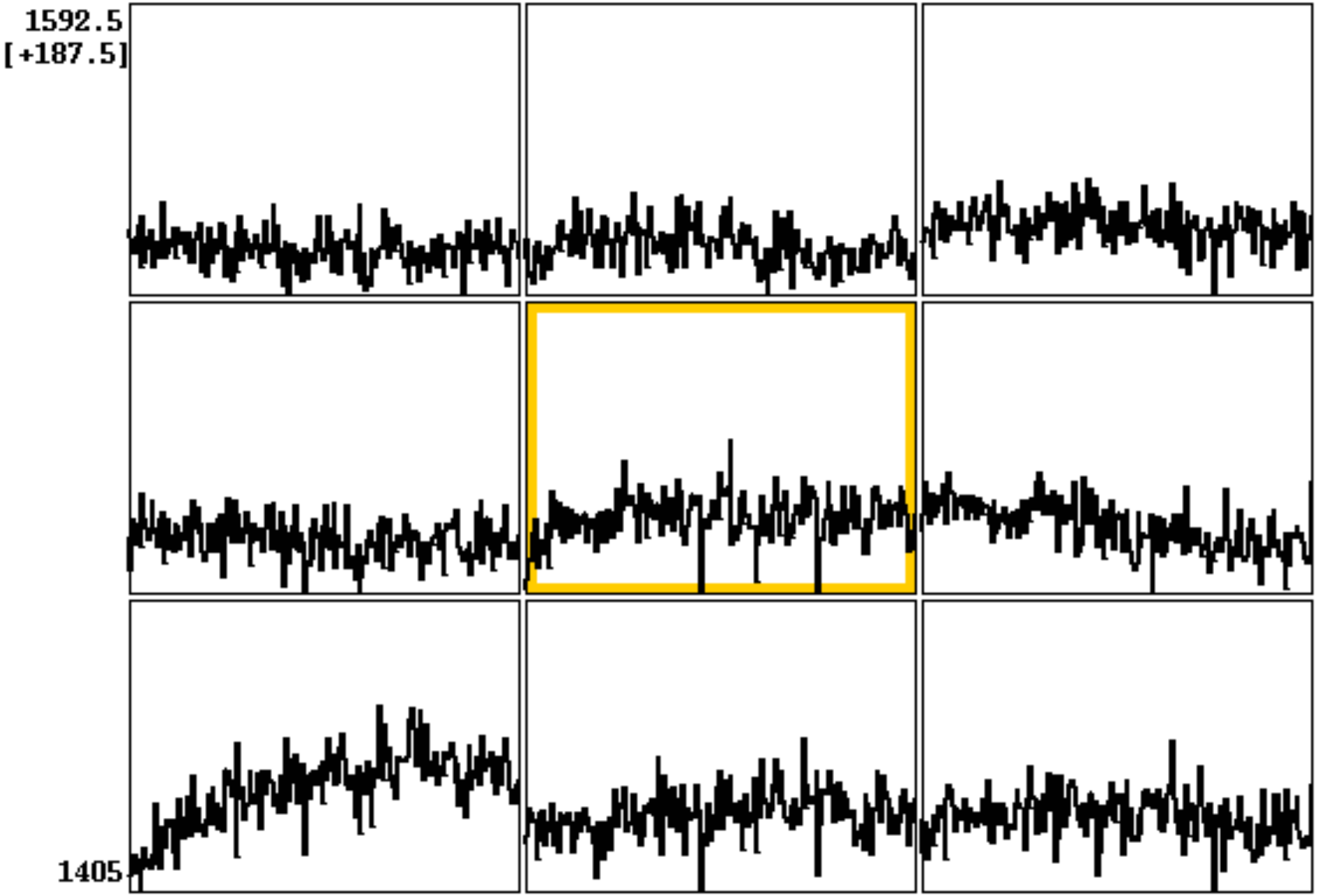
HJ Jo *et al*, 2010 and 2013

Carried out using **afni_proc.py**

Step 1 = Despiking (before)



Step 1 = Despiking (after)



Step 2 = Slice Timing Correction

- 2D Slices acquired at different times within one 3D “volume” TR
- Even the same physiological BOLD effect in 2 different slices will show up differently due to being measured at different times
- And so will be less correlated than they “should be”
- Solution: interpolate in time to some common reference point before calculating correlations
  - Not perfect, because we are also interpolating noise

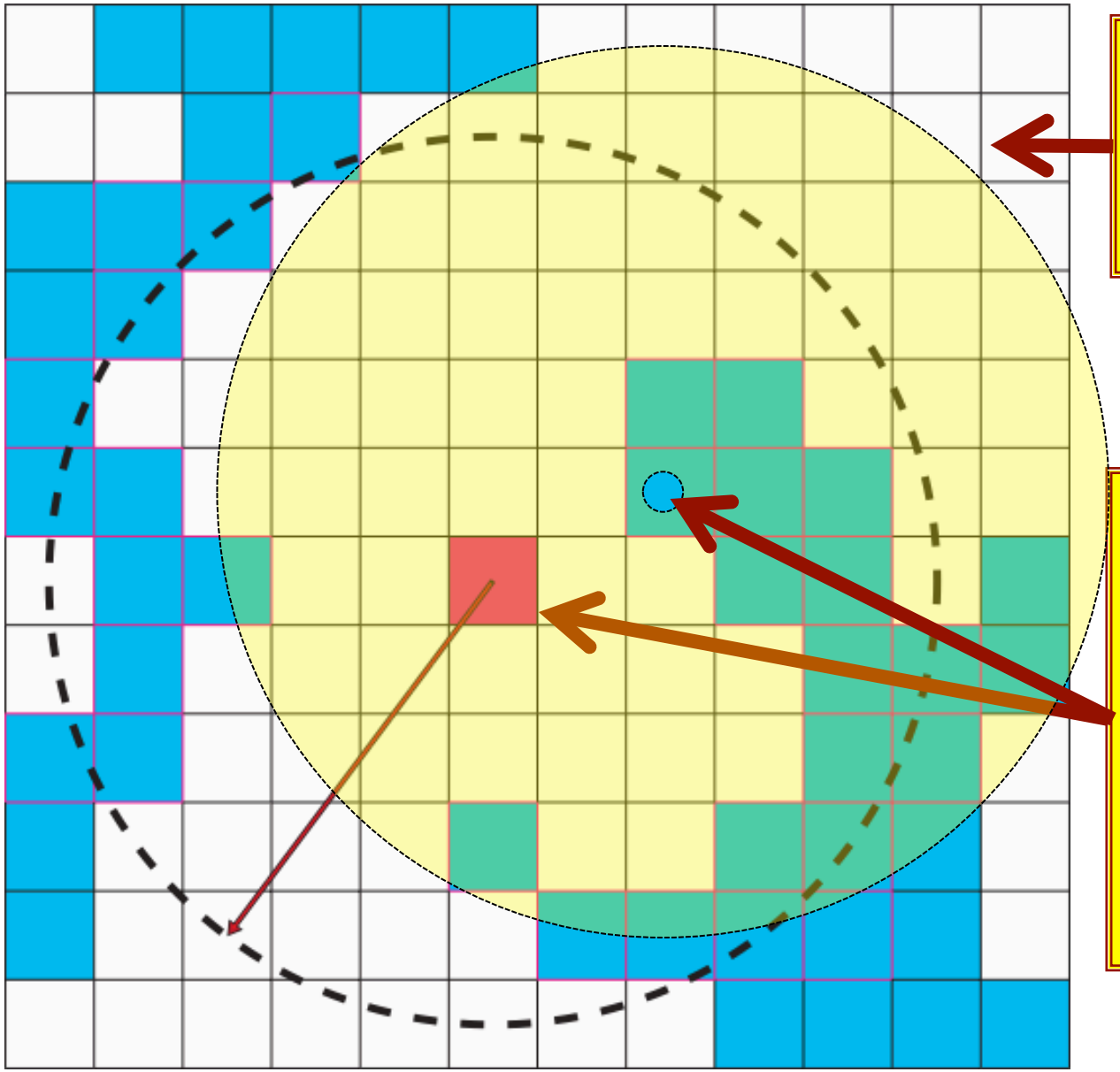
Step 3 = Motion Correction
Step 4 = Alignment with Anatomy
Step 5 = Spatial Normalization

- **Step 3**: Even more important for RS-FMRI, since the BOLD effect is smaller and more spatially diffused than in task-FMRI, so correcting for subject head motion is crucial
- **Step 4**: Needed for step 5, and for assigning RS-FMRI results to brain regions
- **Step 5**: Needed for group studies

Step 6 = Extract Tissue Based Regressors

- The purpose of tissue based regressors is to extract fluctuations that are ***not*** BOLD signal
- So we can regress them out of the data at step 8
- Common choices include:
  - Average white matter (WM) signal time series
  - Several principal components of *all* WM time series (CompCor method)
  - Average global brain signal time series (GS) ☹
  - Average signal from CSF in ventricles
- Less common (only in AFNI): **ANATicor …**

ANATicor – Tissue Based *per voxel*



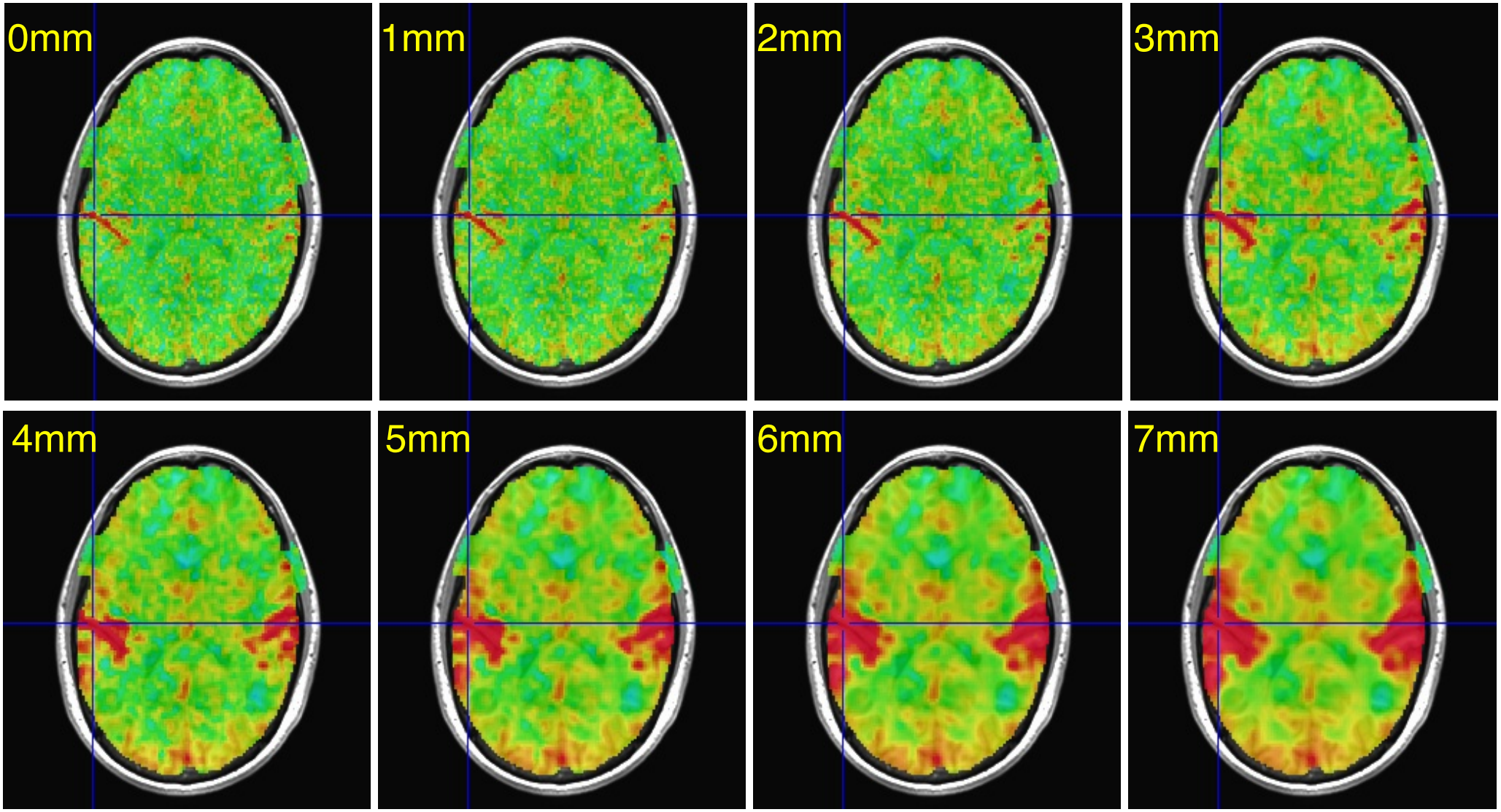
Eroded WM mask (WMe)

Average over WMe voxels inside 25mm radius

Step 7 = Spatial Blurring

- Important for RS-FMRI since the BOLD signal fluctuations are small
- So averaging locally will tend to cancel noise and add up coherent signals
- **Important**: blur ***after*** tissue based signal extraction
- *Otherwise*, will get unintended signals in WM and CSF that were blurred in from nearby GM (gray matter)

Effects of Blurring on Correlation



- Is this a pure vascular/cardiac effect being progressively smeared? Or real neural correlations seen via BOLD? Or some of both?

Step 8 = Nuisance Regression - 1

- In task-FMRI, regression is to find the signal amplitudes of the task model components while at the same time removing the nuisance model components
  - Nuisances: motion parameters, *motion parameter time derivatives*, WM signals, measured respiration signal, *etc*
- In RS-FMRI, there are no task model components to estimate
- All we want is to remove the nuisance components and compute the residuals – these residuals are the output, ready for correlations

Step 8 = Nuisance Regression - 2

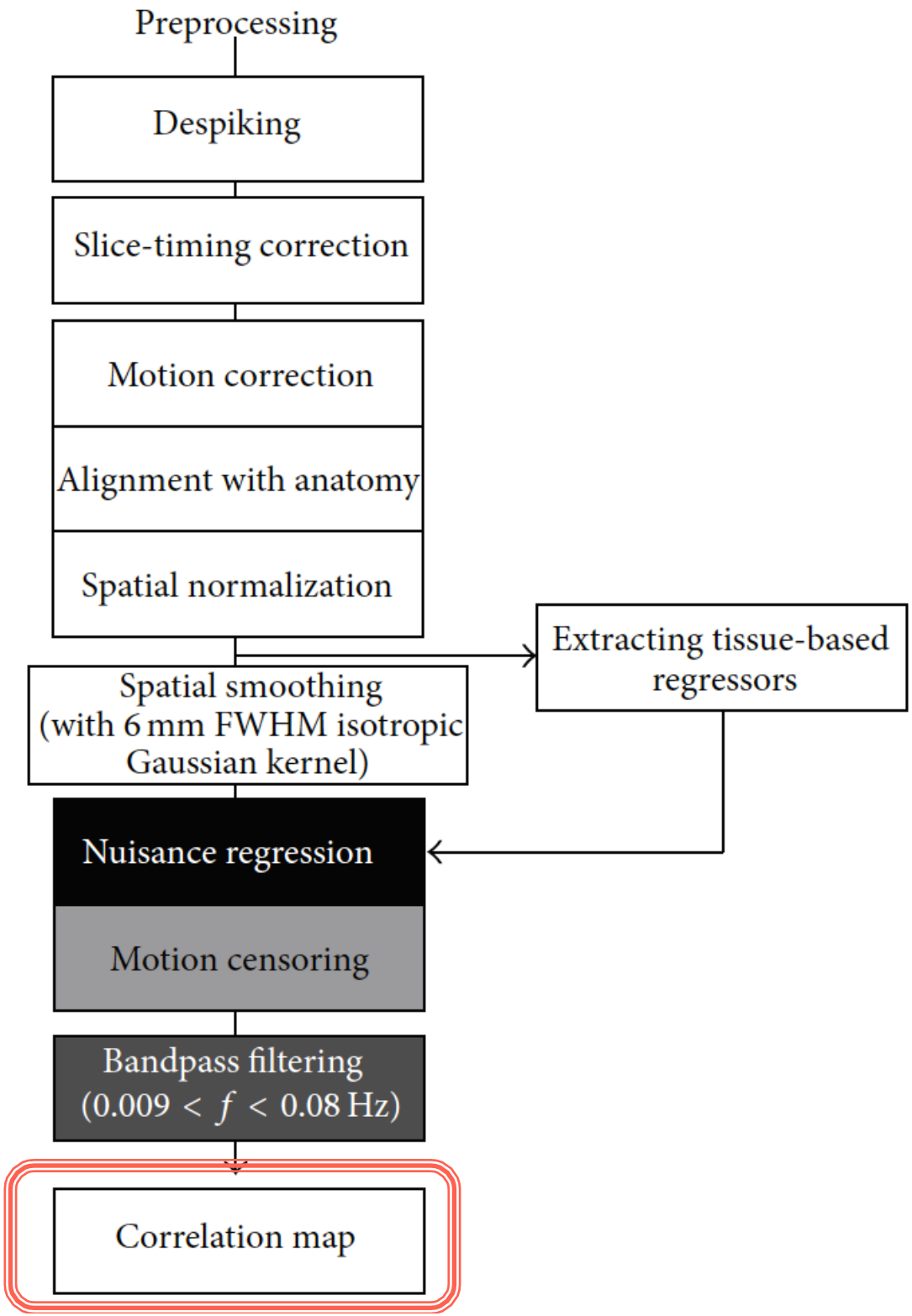
- Another operation usually (but not always) used in RS-FMRI is called **bandpassing**
- It involves removing all frequency components from the data ***except*** those in a specific band
- Frequency: units are Hertz (Hz)
  - 1 Hz = 1 cycle per second
  - 0.01 Hz = 0.01 cycle per second = 1 cycle in 100 seconds
  - 100 Hz = 100 cycles per second = 1 cycle in 0.01 seconds
- In RS-FMRI, it is common to bandpass out all frequencies higher than 0.10 Hz and smaller than 0.01 Hz
  - Keep only 10-100 second cycles; faster or slower = **OUT**
- The idea is that these do not contain BOLD, just noise, so should be removed before correlation

Step 8 = Nuisance Regression - 3

- It is also common to censor out "**bad**" time points, so they aren't used in the correlation
  - "Bad" = too much motion, or that volume has too many "outlier" data points
- It is important to censor bad time points ***before*** the nuisance regression
  - Otherwise, they will affect the regression results and contaminate residuals even at the un-censored times
- In AFNI, nuisance regression, bandpassing, and censoring for RS-FMRI are all done in the same program: **3dTproject**
  - Which allows for voxel-specific regressors (ANATicor)

Step 8 = Nuisance Regression - 4

- Some people did these 2 steps in sequence:
  - Bandpass the data
  - Regress other nuisance components from the bandpassed data
- Doing these operations in 2 steps (instead of one) is not just ***bad***, it is ***WRONG***
- Since the nuisance regressors will contain some of the unwanted frequency components, these unwanted components will "leak" back into the data at the second regression
  - If the nuisance regressors were bandpassed themselves, then the problem would not happen
- The same thing applies to bandpassing and censoring – they should be done together
- These reasons are why **3dTproject** was written



AFNI's recommended RS-FMRI pre-processing steps

HJ Jo *et al*, 2010 and 2013

Carried out using **afni_proc.py**

Preprocess via afni_proc.py

```
## Adapted from Example 9b in afni_proc.py -help
afni_proc.py -subj_id s620                                  \
 -dsets s620_rest_r1+orig.HEAD                              \
 -blocks despike tshift align tlrc volreg                   \
         blur mask regress                                  \
 -tcat_remove_first_trs 2                                   \
 -volreg_align_e2a                                          \
 -blur_size 6                                               \
 -regress_anaticor_fast                                     \
 -regress_censor_motion 0.2                                 \
 -regress_censor_outliers 0.1                               \
 -regress_bandpass 0.01 0.1                                 \
 -regress_apply_mot_types demean deriv                      \
 -regress_run_clustsim no  -regress_est_blur_errts
```

Adjusting brain-wide nuisances

- Model noise effect on time series and project
  - Motion estimates
  - Retroicor/RVT/etc requires simultaneous recordings of cardiac and respiratory cycles

    (Glover 2002; Birn 2006; Shmueli 2007; Chang 2009)
  - Nuisance signals estimates from dataset
  - Tissue-based nuisance regressors

    (Beckmann 2004; Fox 2009; Behzadi 2007; Beall 2007, 2010; Jo 2010, 2013; Kundu 2012; Bright 2013; Boubela 2013)
- Group level adjustments
  - Covariates for motion, brainwide levels of correlation

    (Van Dijk 2012; Satterthwaite 2012; Saad 2013; Yan 2013)

AFNI Progams for Correlating - 1

- **3dTcorr1D** = correlate all time series in a dataset with time series in a text 1D file
- **3dTcorrMap** = correlate each voxel time series in the input with *every other voxel*, combine these correlations in some way (linear, nonlinear), save that combined correlation as a measure of how "connected" each voxel is with the rest of the brain
- **3dAutoTcorrelate** = correlate each voxel time series with *every other voxel*, and save all of these correlations
  - Output dataset will be HUGE unless you are careful and use a gray matter only mask (*e.g.,* program **3dSeg**)

AFNI Progams for Correlating - 2

- **AFNI** GUI InstaCorr – single subject seed based correlation by pointing and clicking
  - Subject of another talk
- **3dGroupInCorr** – group analysis of seed based correlations, also by pointing and clicking
  - Also in the InstaCorr presentation
- **AFNI** does *not* contain a program for doing ICA for network parcellation or identification from RS-FMRI data
  - **GIFT** software from Vince Calhoun lab, for example
  - **http://mialab.mrn.org/software/gift/**

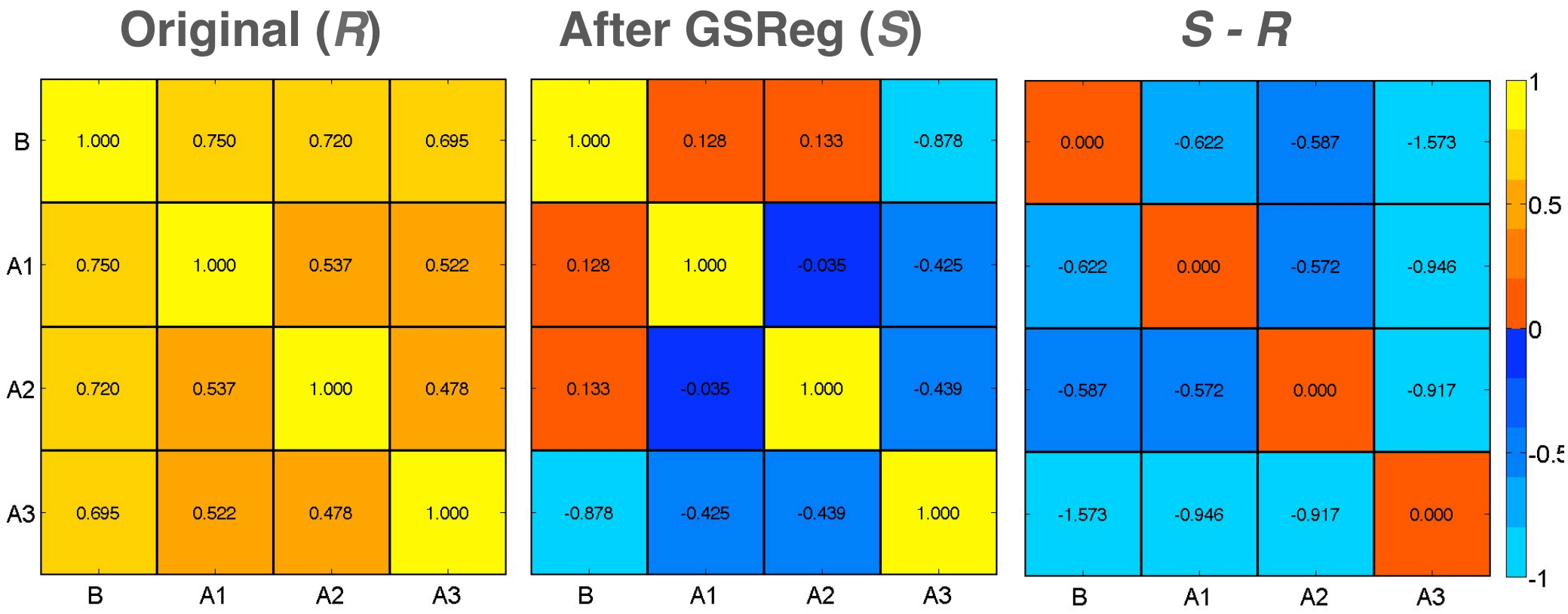
Tissue-based nuisance regressors

- **Avoid Projecting Fluctuations of Interest**
- OK to sample nuisance signals from regions whose fluctuations are not correlated with the *fluctuations of interest* in the regions of interest
- Should not project time series containing aggregates of fluctuations of interest, even if they contain contribution from noise
  - Sagittal sinus voxels might allow sampling of aliased heart rate, HOWEVER they also exhibit BOLD fluctuations of interest from the regions being modeled (Jo, 2010)

And why not?

- Because you will end up **differentially biasing** the correlation matrices of your groups, and considerably distorting group differences
- Best explained with GSReg (using the Global Signal as a nuisance Regressor) because math is straight forward.
  - What follows applies whether or not noise exists or differs between groups

Why not GSReg ?



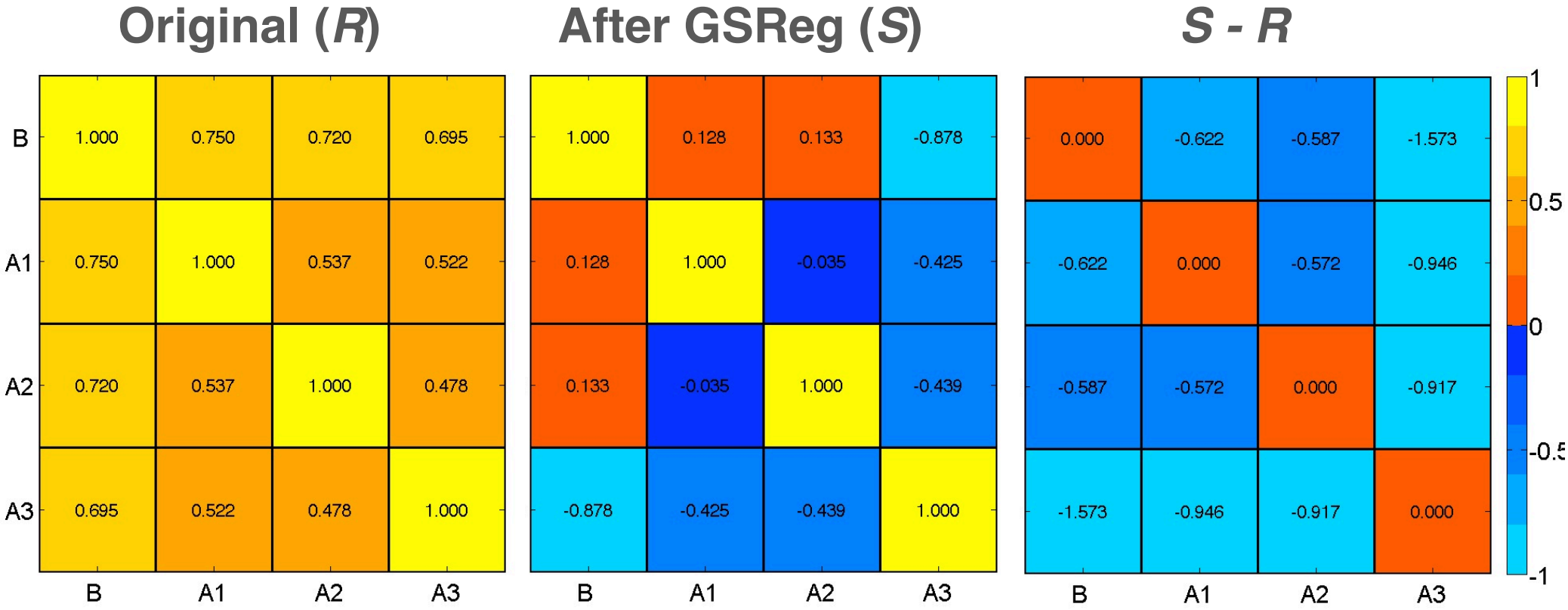
Bias will vary by region pair

AND will be

Entirely dependent on ***true*** covariance matrix ***P***

(therefore your grouping variable)

Why not GSReg ?



For any FMRI time series (not just simulations)

$$S - R = (P - (P11^{T}P)/(1^{T}P1)) * \sigma_Q \sigma_Q^{T} - P * \sigma_P \sigma_P^{T}$$

S-R is constant for group with same cov. matrix ***P***

(*Q* is also a sole function of *P*) **(Saad, 2013)**

Are biased estimates useful?

- Region pair dependent biasing is OK if:
  - Not interpreting correlations between regions as those between the sampled BOLD signals and by extension neuronal signals
    - Not just about interpretability of negative correlations (Murphy, 2008; Weissenbacher, 2009; Cole, 2010)
    - Two strongly correlated regions after GSReg DOES NOT imply regions were strongly correlated before GSReg
  - Using correlations after GSReg as some feature space for parcellation, classification, etc

Are biased estimates useful?

Region pair dependent biasing is OK if:

- Not interpreting correlations between regions as those between the sampled BOLD signals and by extension neuronal signals
  - Not just about interpretability of negative correlations (Murphy, 2008; Weissenbacher, 2009; Cole, 2010)
  - Two strongly correlated regions after GSReg DOES NOT imply regions were strongly correlated before GSReg
- Using correlations after GSReg as some feature space for parcellation, classification, etc

Region pair dependent biasing is problematic if:

- **Comparing two groups** with possibly different signal covariance

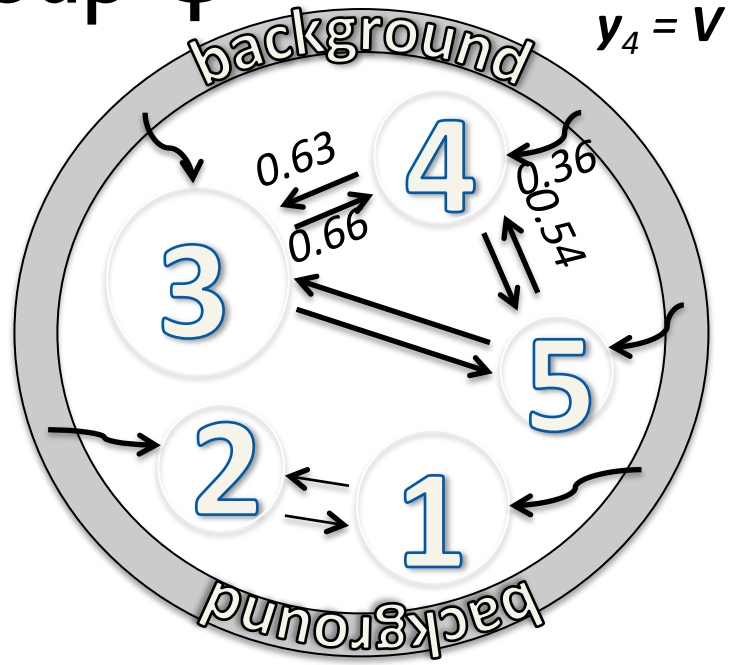
$$S - R = (P - (P11^{T}P)/(1^{T}P1)) * \sigma_Q \sigma_Q^{T} - P * \sigma_P \sigma_P^{T}$$

S-R is constant for group with same cov. matrix ***P***

S-R will differ between groups with different ***P***

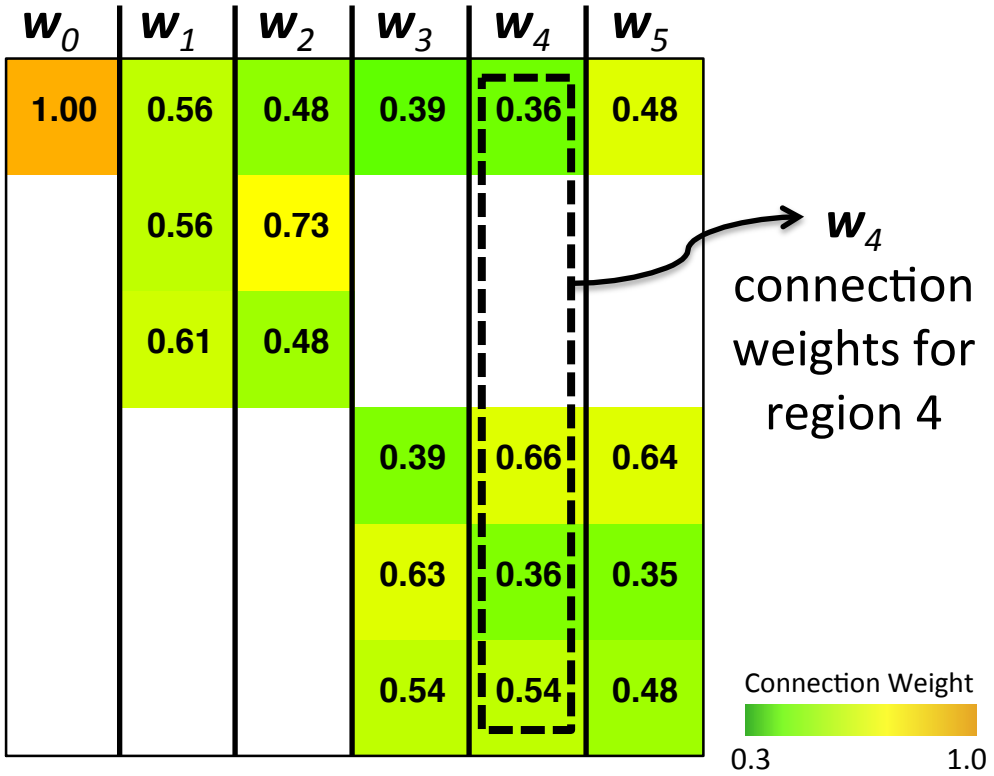
An illustrative model

Group ψ



Observed signal from region 4:

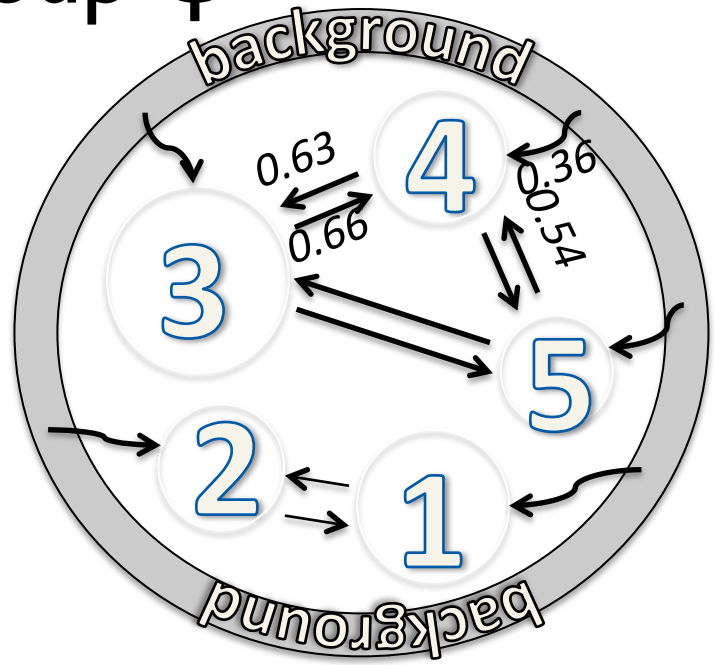
$$y_4 = V w_4 + e = 0.36\, v_0 + 0.66\, v_3 + 0.36\, v_4 + 0.54\, v_5 + e$$



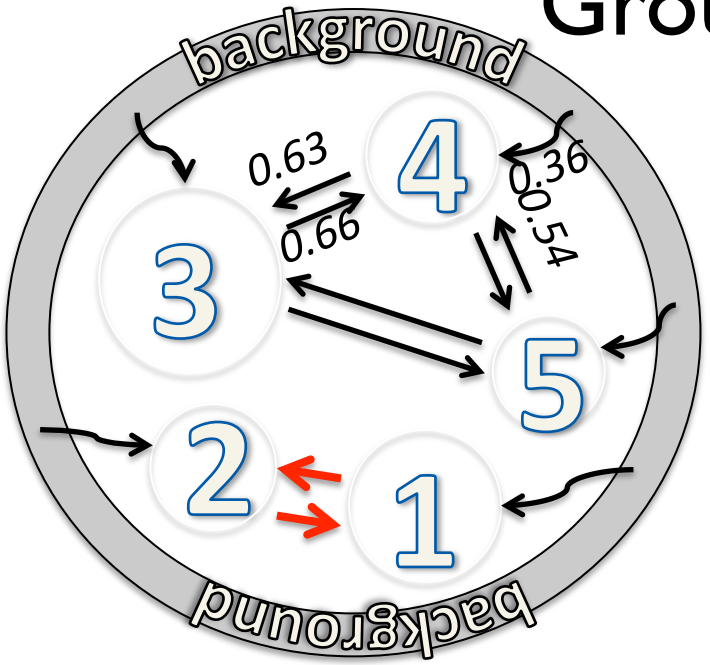
In simulations 9 regions + background were used

Comparing Groups

Group Ψ



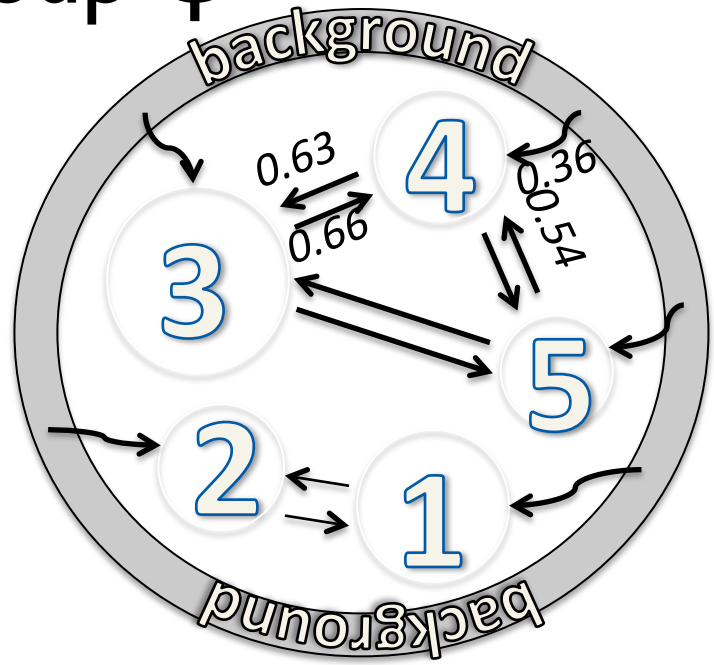
Group Ψ_L



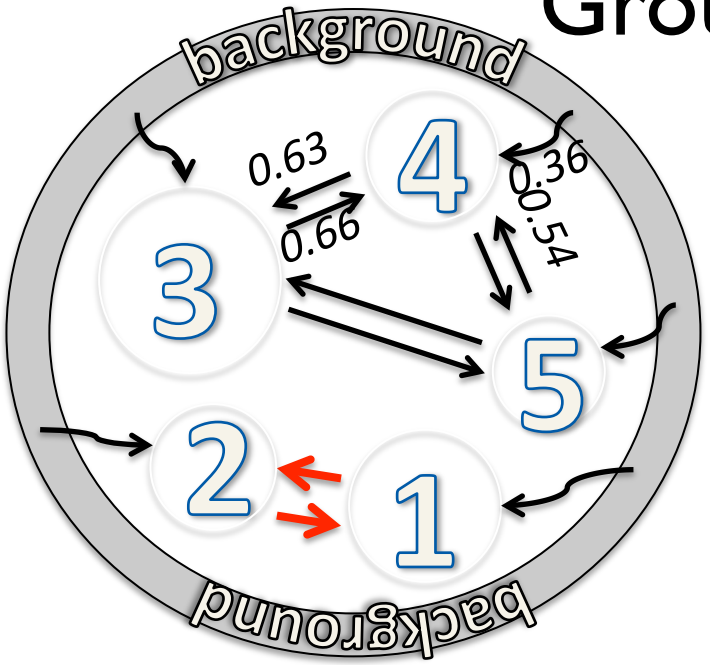
Increased connection
between regions 1 and 2 only

Comparing Groups

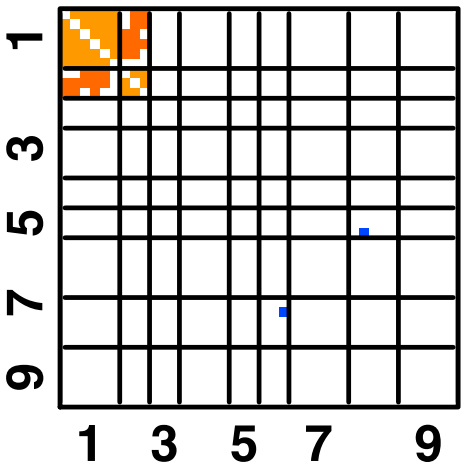
Group ψ



Group ψ_L



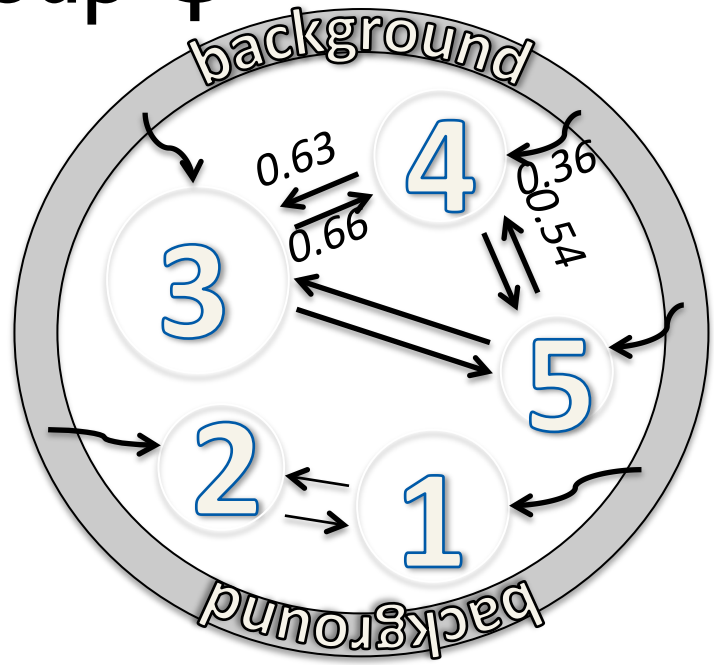
$\psi_L - \psi$ Base



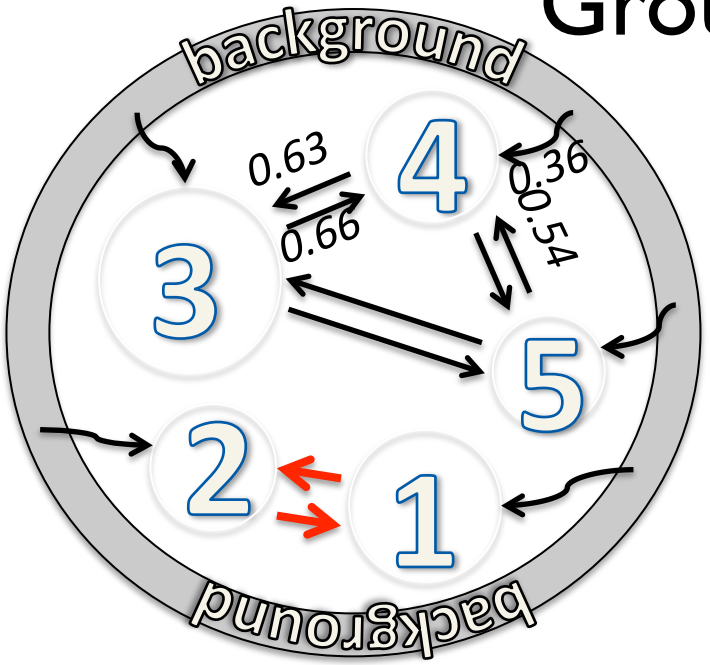
Difference confined to two regions

Comparing Groups

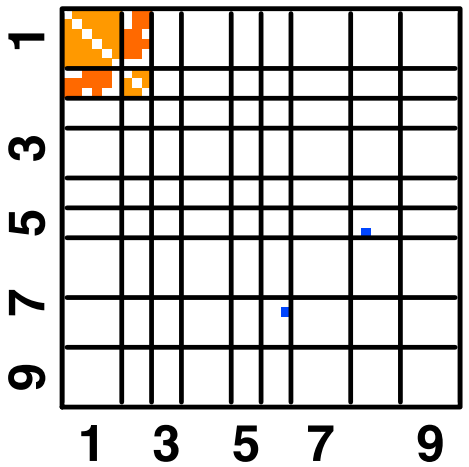
Group Ψ



Group Ψ_L



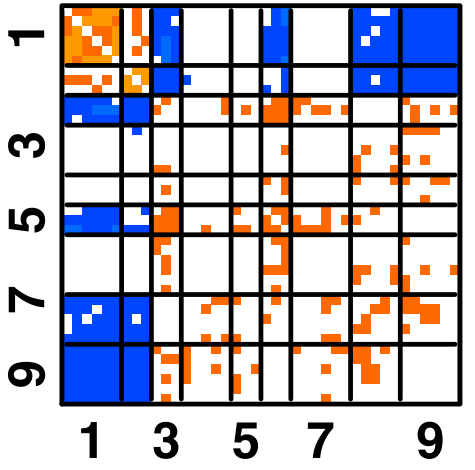
$\psi_L - \psi$ Base



Difference confined to two regions

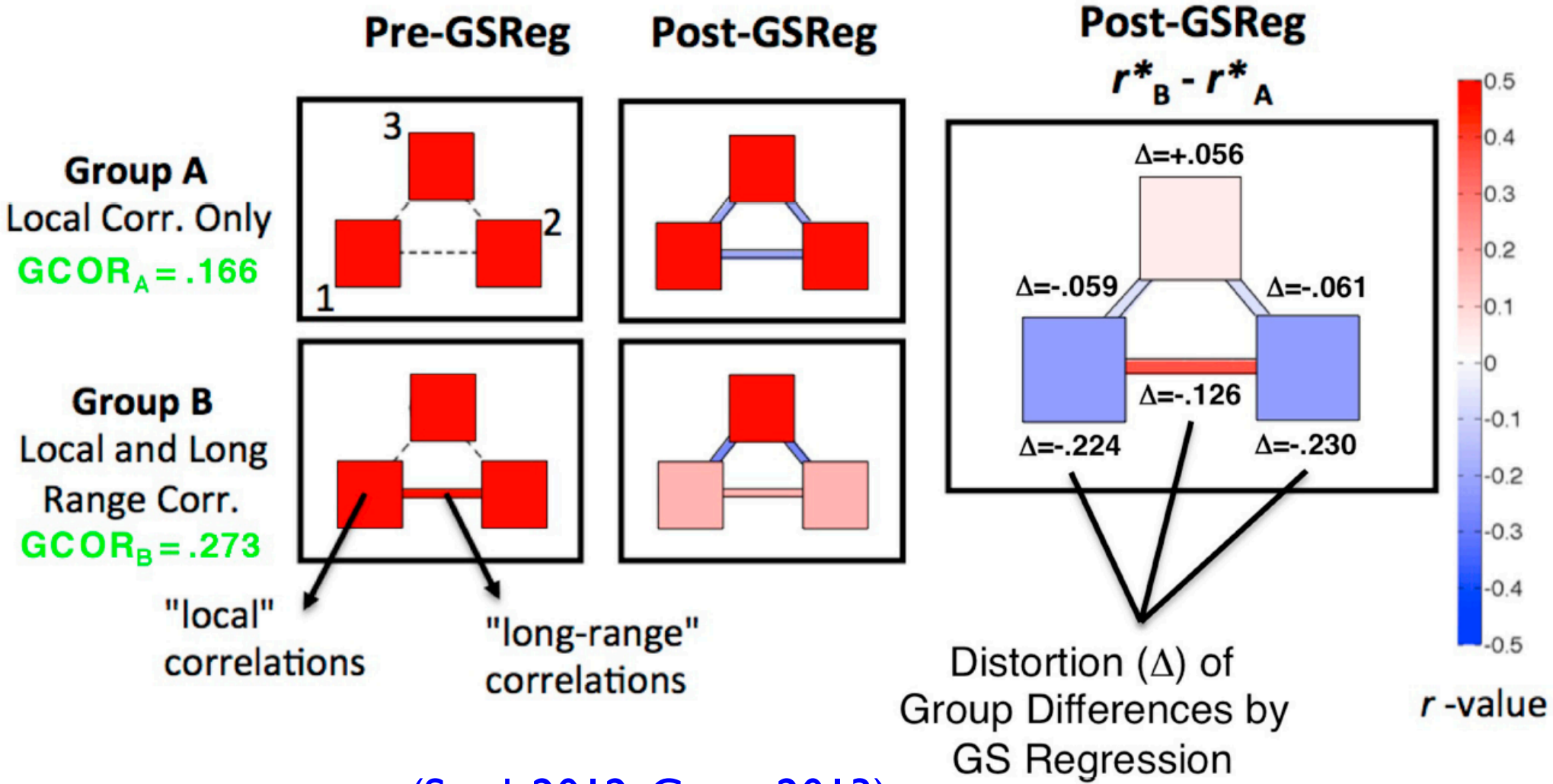
Ends up all over the place

$\psi_L - \psi$ GSReg



Distortion of long/short range correlations

Contrast of correlations between groups A and B
'long-range' correlations in Group B only



(Saad, 2012, Gotts 2013)

Comparing Groups with GSReg

One seeks and hopes for differences in covariance/correlation structures between groups.

Using GSReg means each group will be biased DIFFERENTLY for different region pairs.

→ Even in the absence of noise difference, you can find group correlation differences in places where none existed before.

→OK if you're teaching a classifier to differentiate between the two groups.

→NOT OK if interpreting correlation differences to evoke correlation differences of neuronally induced BOLD signal between these regions.

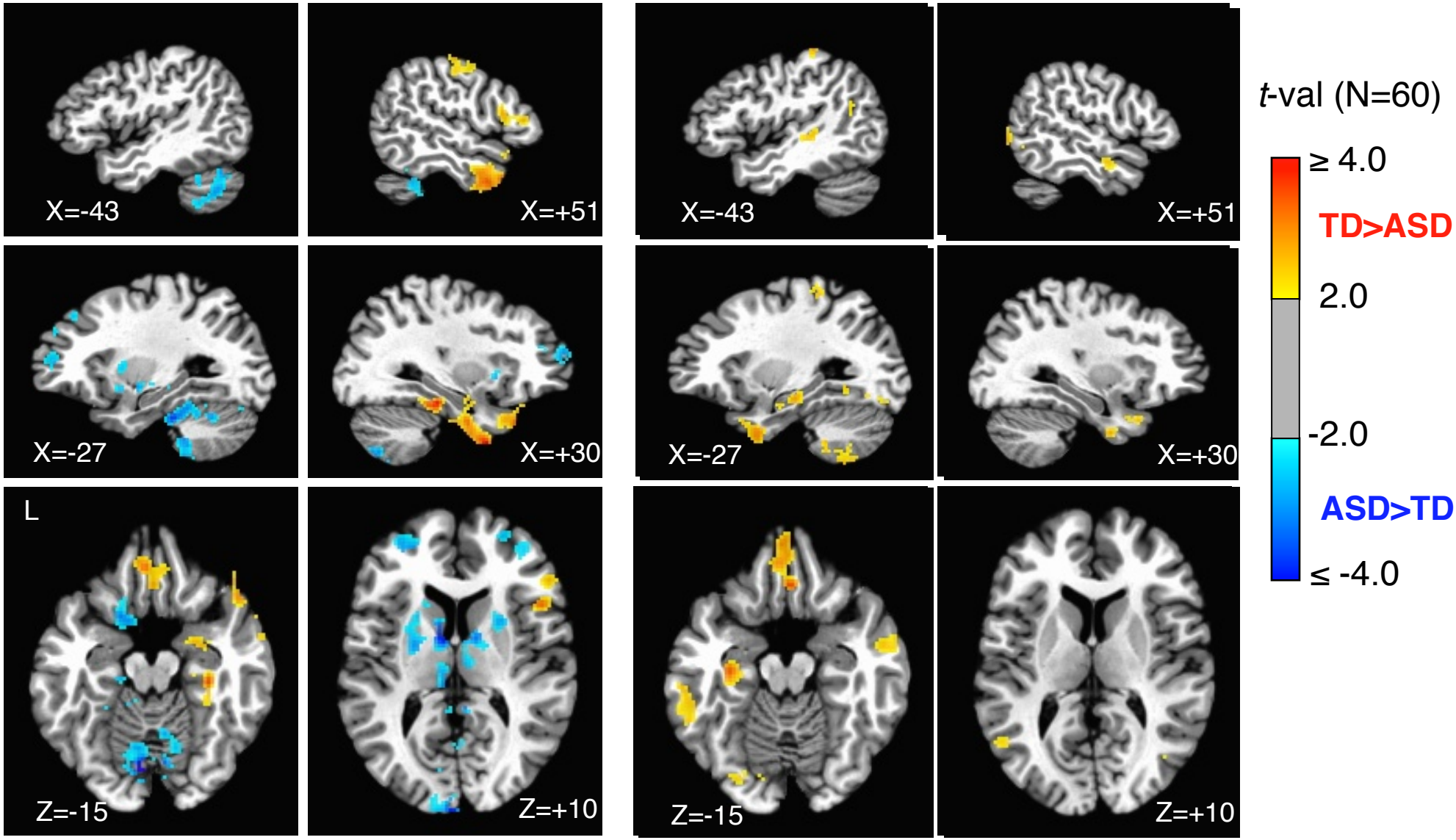
With noise previous problems remain

→ However bias now depends on the covariance structures of noise and signals of interest though we can't tell them apart.

→ Interaction between GSReg projection effects and grouping variable remains

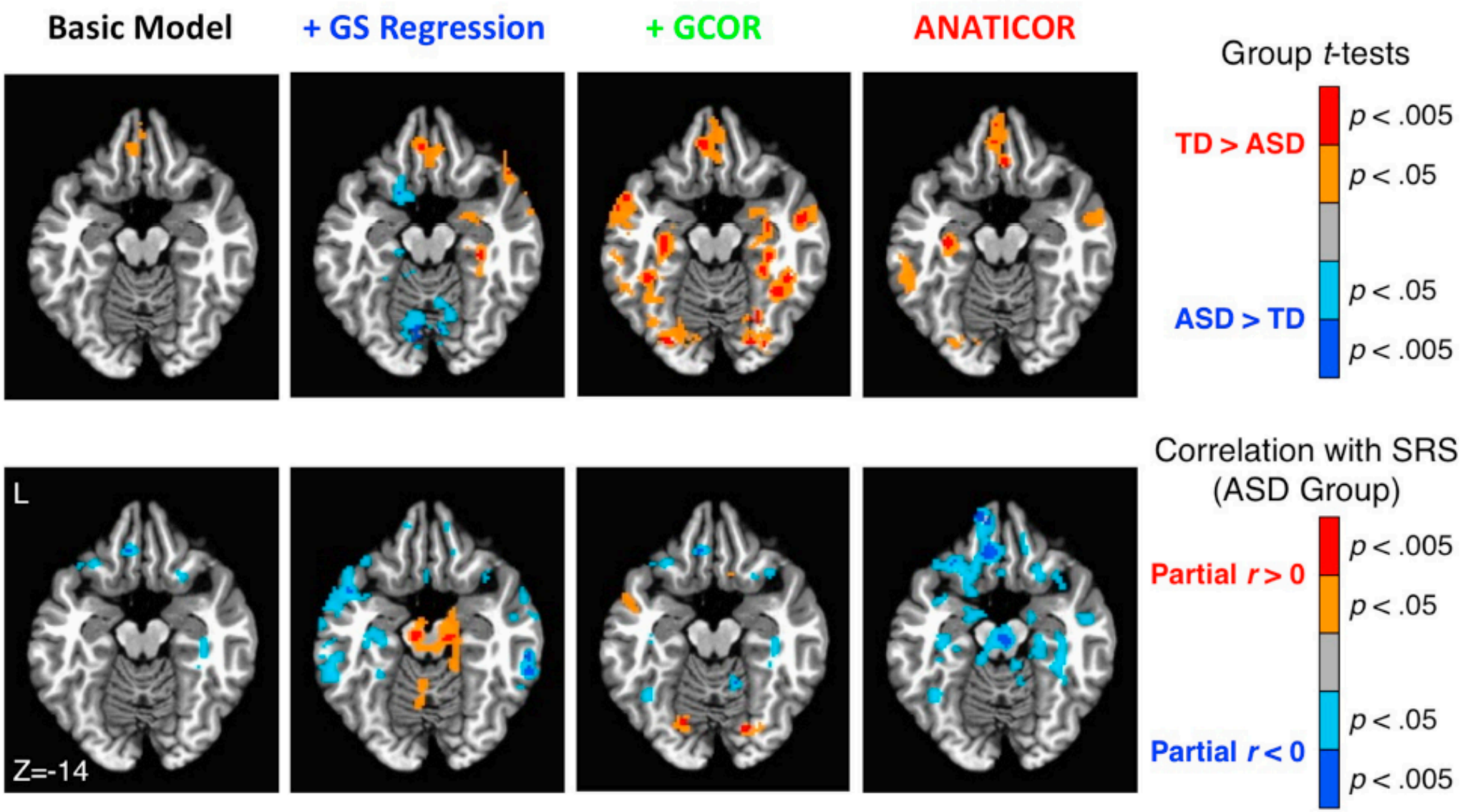
SAME holds with empirical data

+ GS Regression **ANATICOR** (Jo, 2010)



(Gotts, 2013)

SAME holds with empirical data



(Gotts, 2013)

It is not just GSR

- Nuisance regressors correlated with fluctuations of interest in regions of interest (not the noise) will cause the same problems.

- Non-gray matter averages may be comparable to GSReg (partial voluming with grey matter)
  - Averaging over small regions of **eroded** non-gray matter tissue are advantageous (Jo, 2010, 2013)

- Decomposition methods that cannot separate BOLD (fluctuations of interest) from noise also problematic.

The Siren's Song

What of results being more stable after GSR?

There is a denoising component to the approach and bias is consistent for consistent covariance structure

- However, interpretation of correlations is now difficult (Cole, 2010)
- Interaction effect with grouping variable completely ignored
- Differences can get spread in unknown ways
- Tests of processing methods should always consider group comparisons

What of GSReg for motion compensation?

Some denoising effect → reducing residual variance and motion-based group differences

However, caveats from above remain

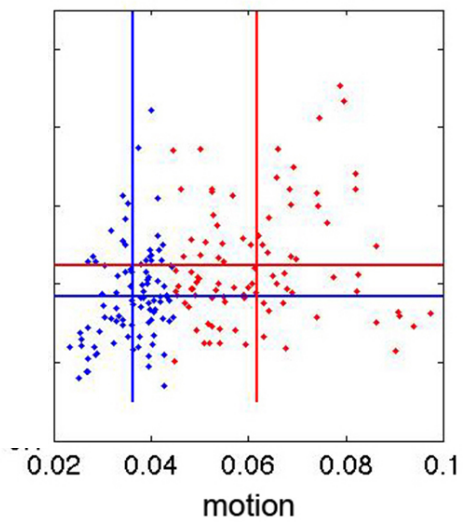
AND are we actually compensating for motion?

Grouping Based on Motion

FCON 1000: Cambridge_Buckner

Mean Motion
Small Movers

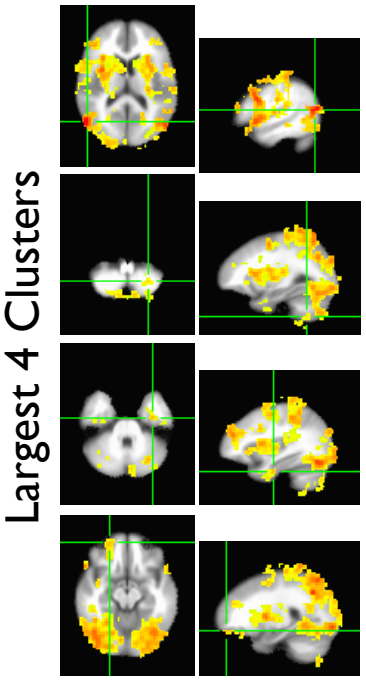
Mean Motion
Big Movers



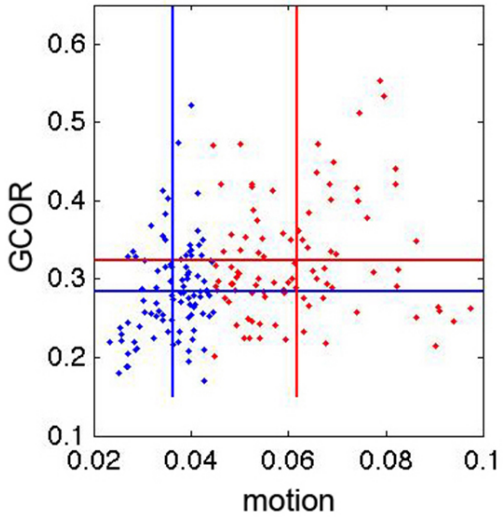
Grouping Based on Motion

FCON 1000: Cambridge_Buckner

β_1 Base

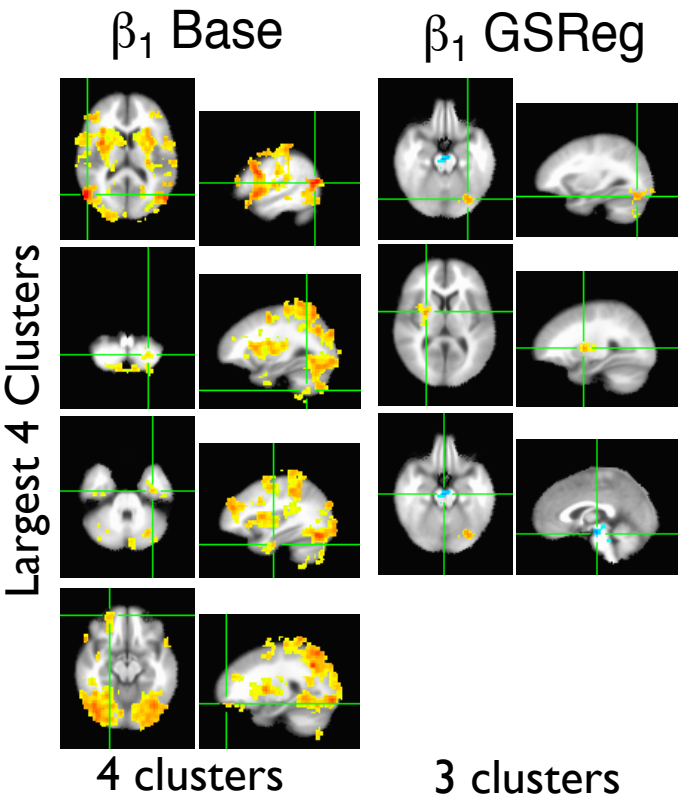


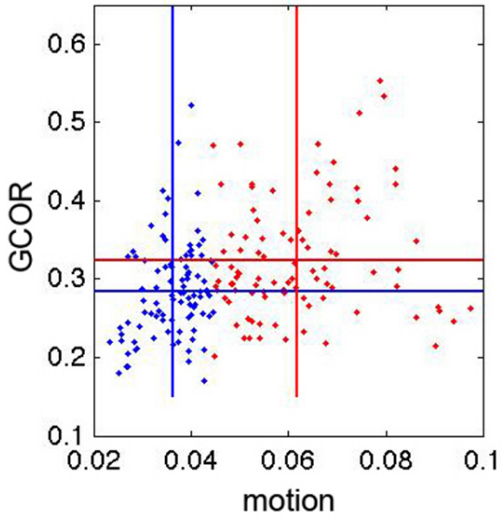
4 clusters



Grouping Based on Motion

FCON 1000: Cambridge_Buckner



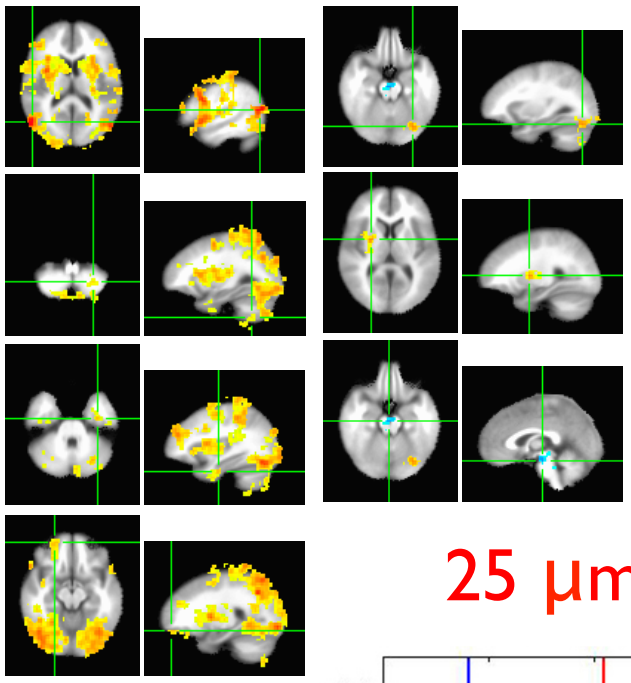


Grouping Based on Motion

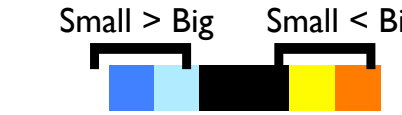
FCON 1000: Cambridge_Buckner

β_1 Base β_1 GSReg

Largest 4 Clusters



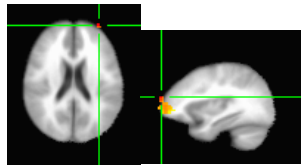
4 clusters



Group Difference, p<0.01, α=0.05

FCON 1000: Beijing_Zang

β_1 Base β_1 GSReg



1 cluster

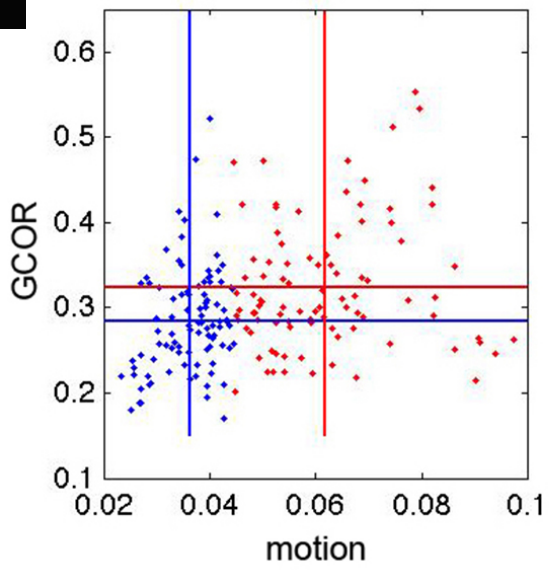
0 clusters

More Motion

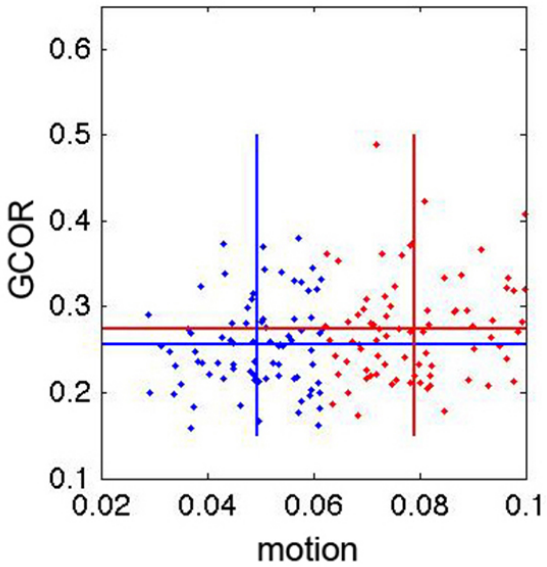
More Motion Difference

Much less group difference!

25 µm/TR



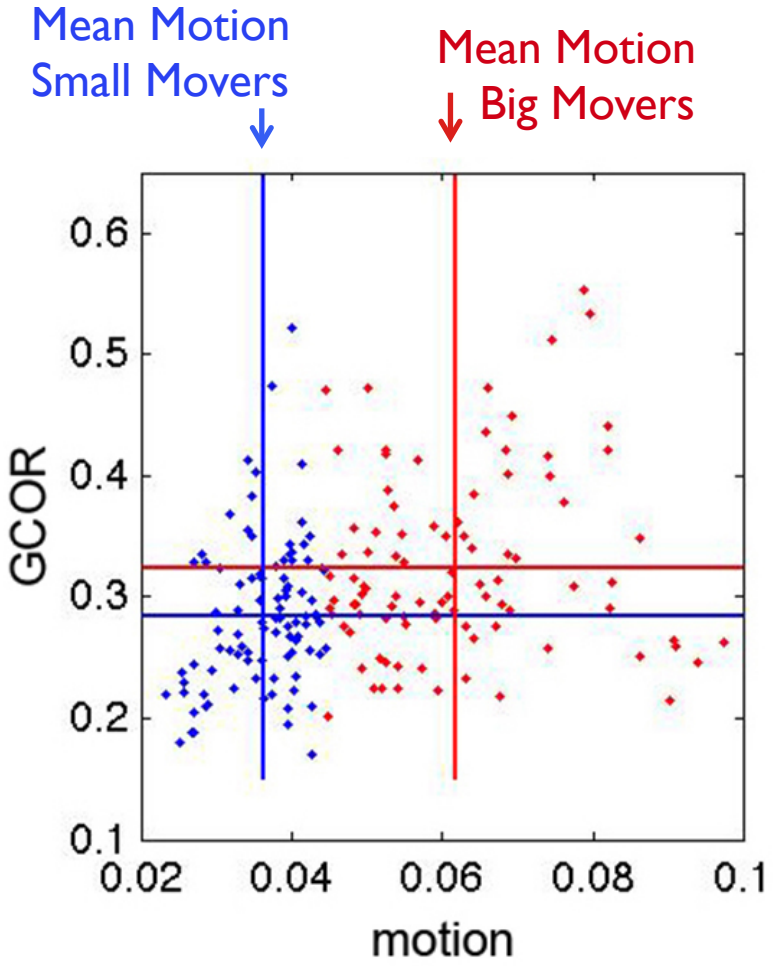
29 µm/TR



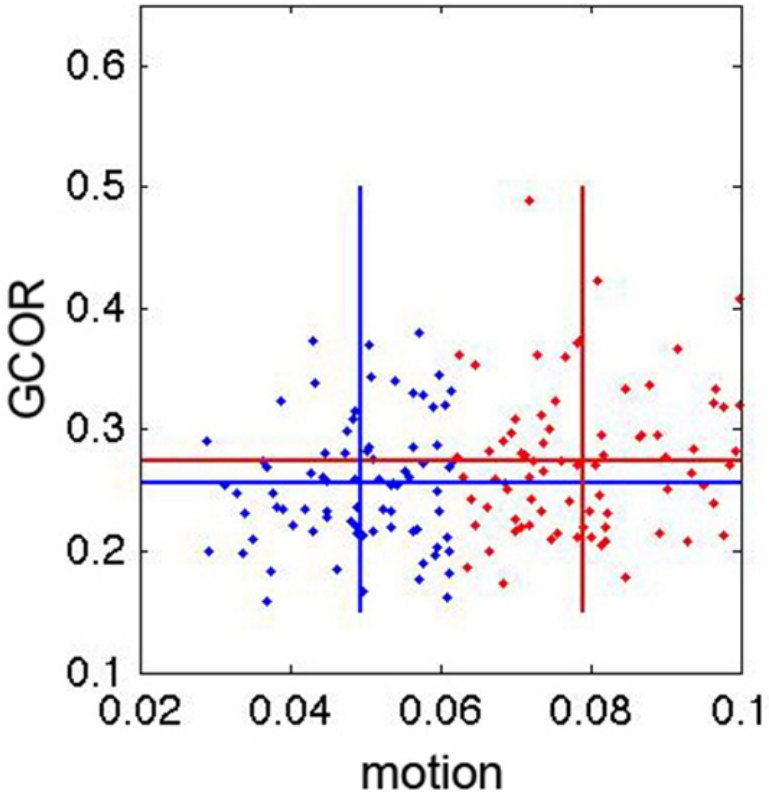
(Saad, 2013)

Grouping Based on Motion

The average correlation of every voxel with every other voxel in the brain



FCON 1000: Cambridge_Buckner



FCON 1000: Beijing_Zang

Note weak correlation between motion and GCOR (R^2=11% Cambridge, 4.3% Beijing)

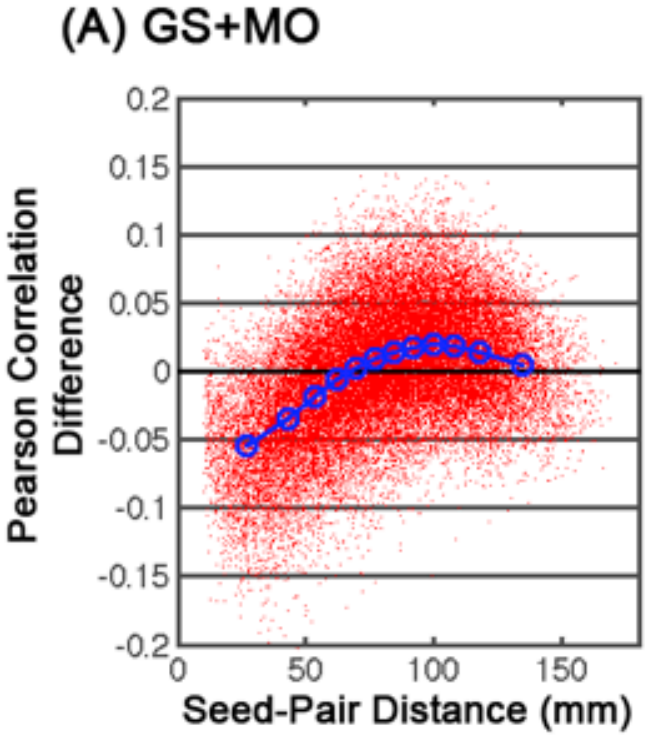
Can GSReg help with motion?

Censoring (scrubbing) high motion samples changes inter-regional correlations in distance dependent manner.

→ suggests effect of motion on correlations depends on distance between regions (Power et al. 2012)

→ importance of censoring high motion

Data generously made public by Power & coauthors 2012



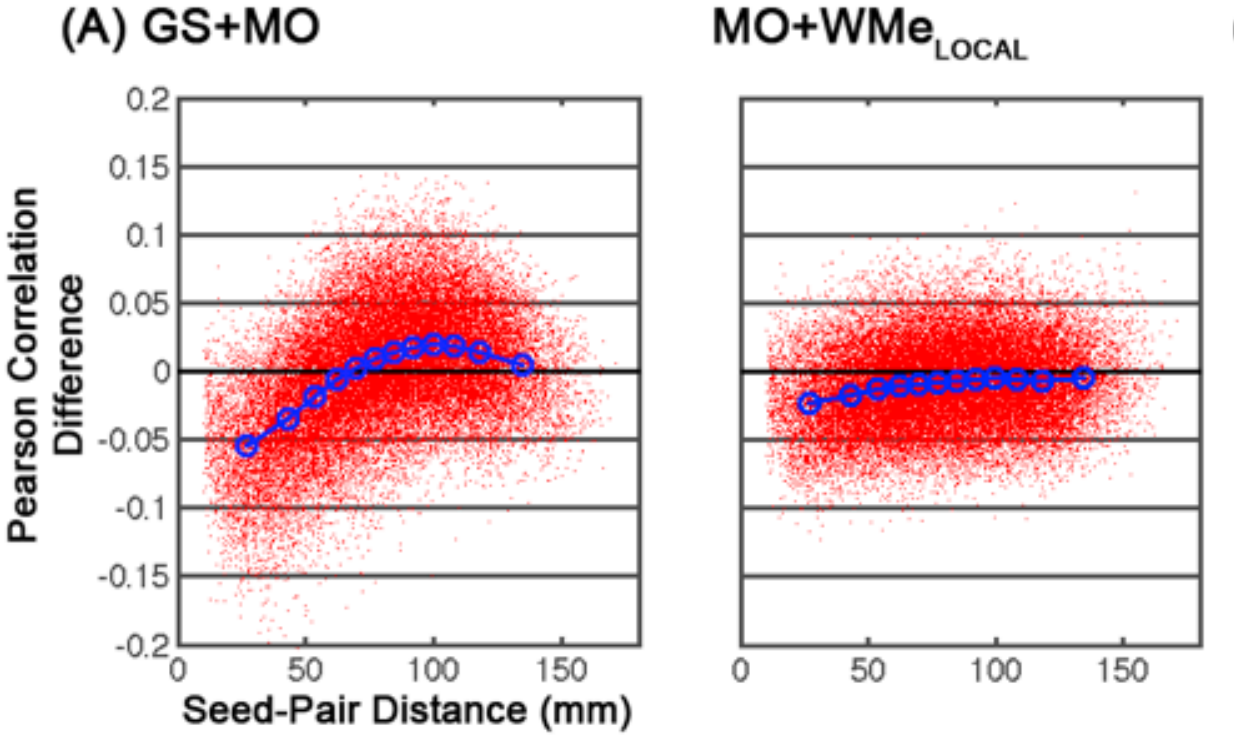
Can GSReg help with motion?

Censoring (scrubbing) samples of high motion changes inter-regional correlations in a distance manner.

→ suggests effect of motion on correlations depends on distance between regions (Power et al. 2012)

→ importance of censoring high motion

Less dependence without GSReg



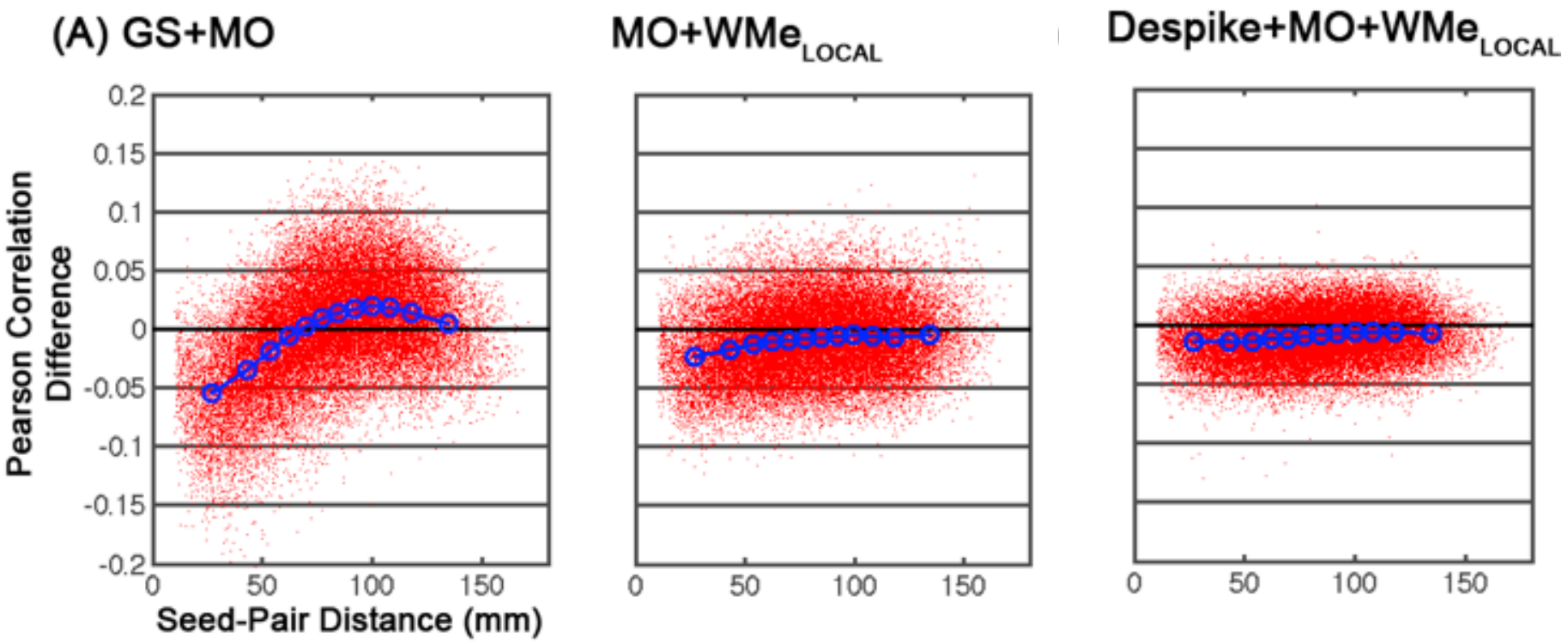
Can GSReg help with motion?

Censoring (scrubbing) samples of high motion changes inter-regional correlations in a distance manner.

→ suggests effect of motion on correlations depends on distance between regions (Power et al. 2012)

→ importance of censoring high motion

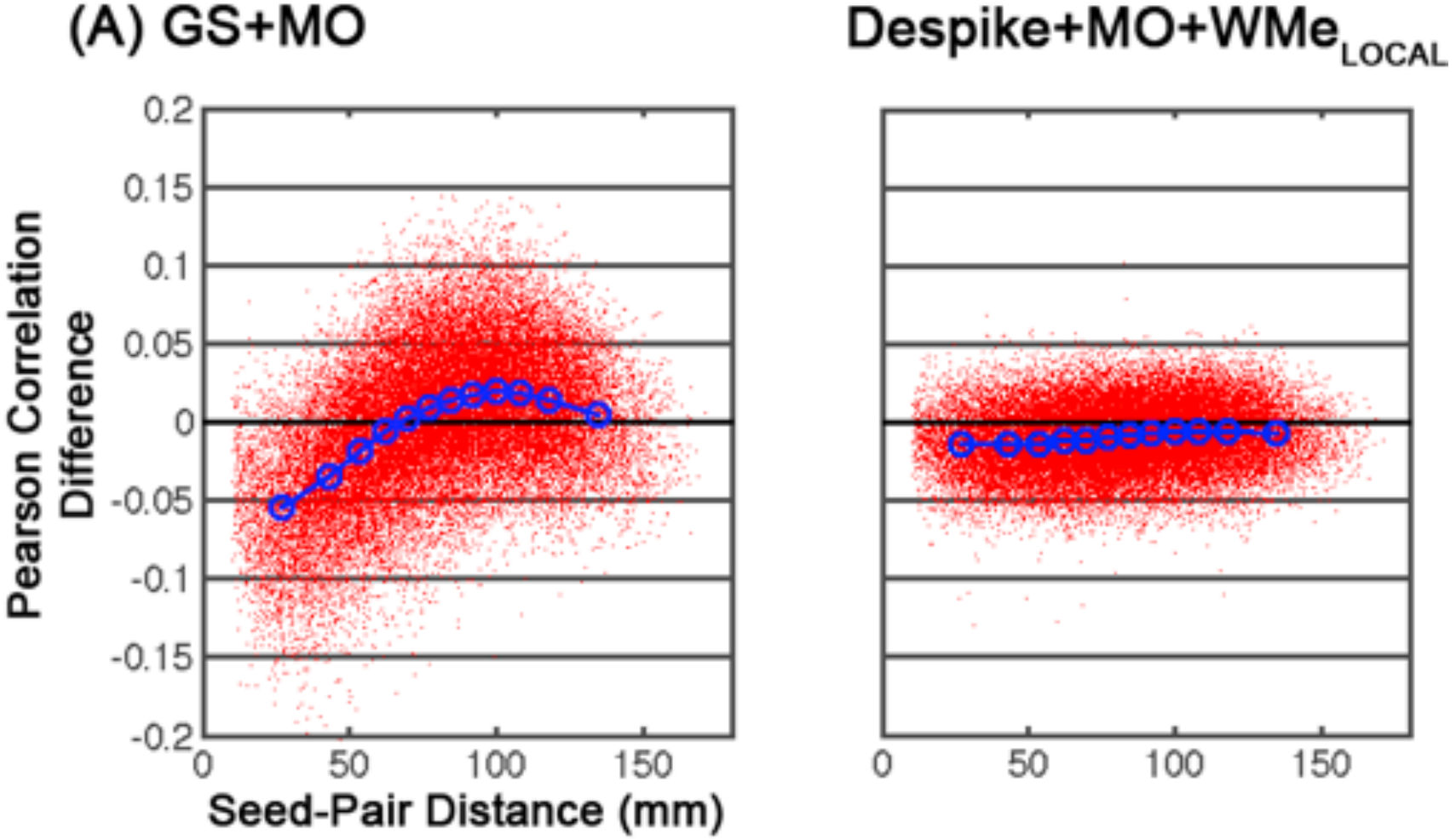
Least dependence



Can GSReg help with motion?

GSReg → Correlation more sensitive to motion
→ Correlation more sensitive to censoring (Jo, 2013)

Improved denoising largely eliminates distance dependent bias



Sampling nuisance TS regressors

- Sample noise without aggregating over regions with fluctuations of interest
  - Erode white matter masks to avoid partial voluming
  - Avoiding regions with fluctuations of interest (Anderson 2011)
  - Local eroded white matter masks improve denoising without increasing DOFs (Jo, 2010, 2013)

- Use decomposition methods that can separate BOLD from non BOLD fluctuations of interest (Kundu, 2012, Bright, 2013)

  or attempt to identify noise components (Beckmann 2004, Beall 2010, Boubela, 2013)

- Use noise models RICOR/RVT/etc. (Glover 2000; Shmueli 2007; Birn 2008; Chang 2009)

Brain-wide correlation adjustments?

- If subject to subject variations in brain-wide correlations exist, why not correct for them?
- Consider GCOR, the average over the entire correlation matrix of every voxel with every other voxel (Saad, 2013)
  - Measure would be costly to compute if one had to estimate the entire correlation matrix first.
  - However estimating GCOR is trivial:

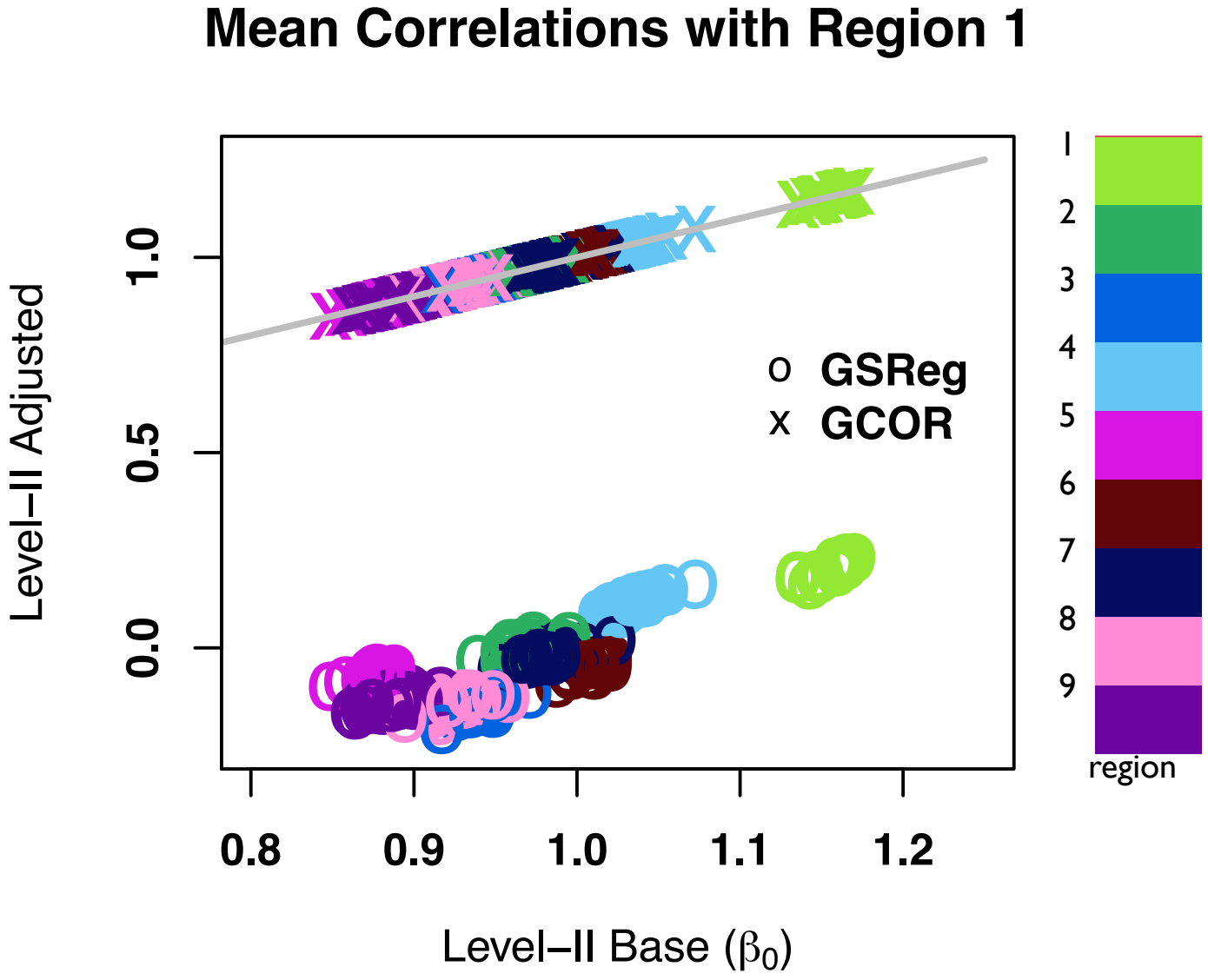
$$\gamma = 1/(M^2 N)\ \mathbf{1}^{\mathrm{T}} \boldsymbol{U}^{\mathrm{T}} \boldsymbol{U}\ \mathbf{1}$$
$$= 1/N\ \boldsymbol{g}_u{}^{\mathrm{T}}\ \boldsymbol{g}_u,$$

$\boldsymbol{g}_u$ is the average of all (M) unit variance time series of length N in matrix $\boldsymbol{U}$

GCOR as group level covariate

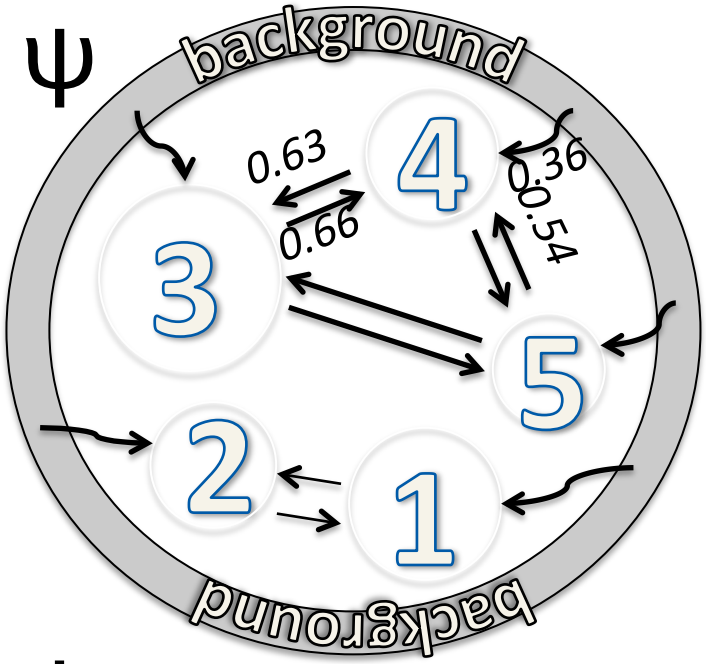
- Using models described earlier, we consider group level correlation (differences) from three models:
  - No adjustment: $\boldsymbol{r}_{i,j} = \beta_0 + \beta_1 \boldsymbol{x}$
  - GSReg at level I: $\boldsymbol{s}_{i,j} = \beta_0 + \beta_1 \boldsymbol{x}$
  - GCOR as covariate: $\boldsymbol{r}_{i,j} = \beta_0 + \beta_1 \boldsymbol{x} + \beta_2 \boldsymbol{\gamma} + \beta_3 \boldsymbol{x}\,\boldsymbol{\gamma}$

Less bias than with GSReg for 1 sample tests

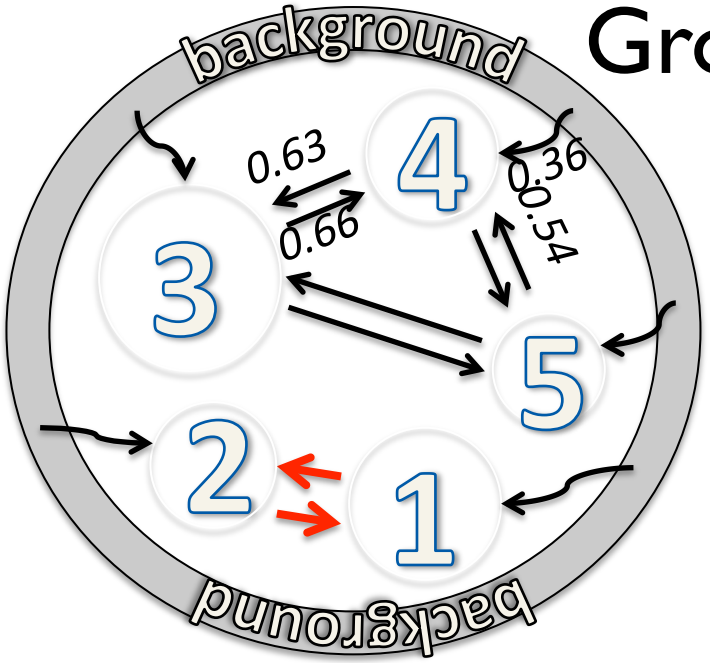


Comparing Groups

Group ψ



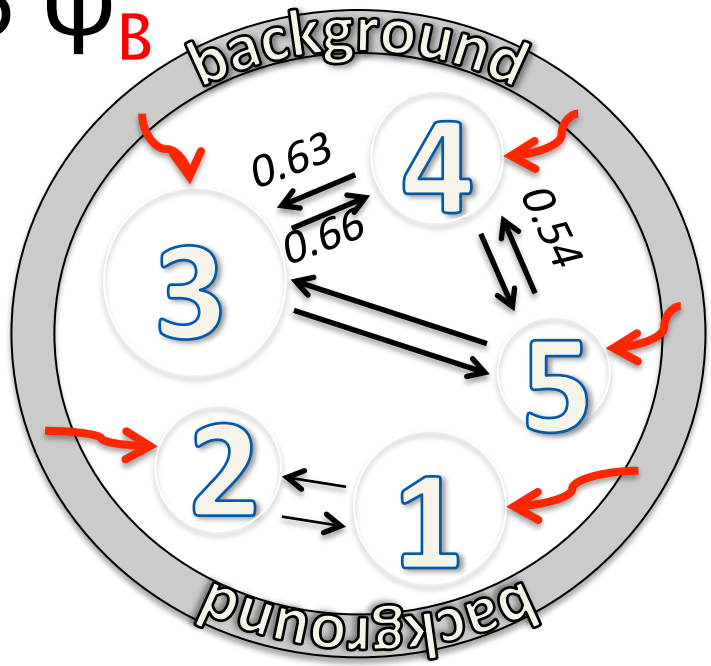
Group ψ_L



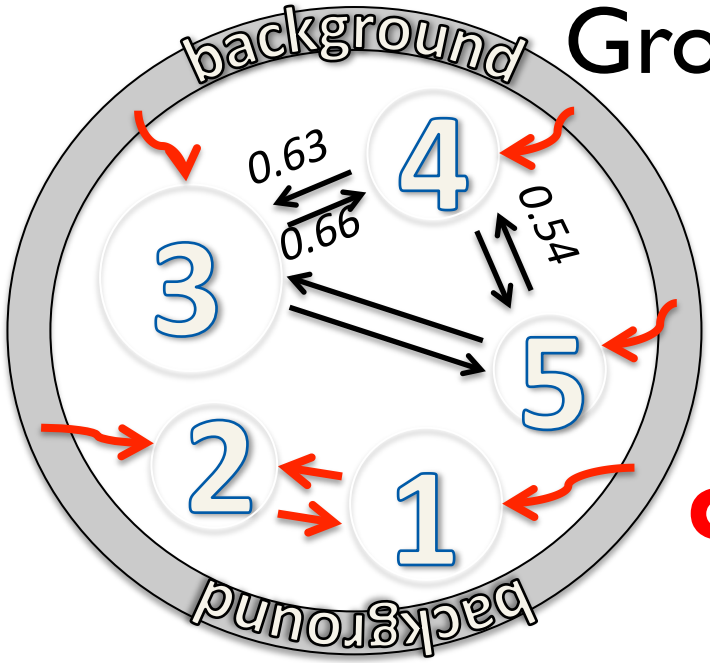
More Local

Group ψ_B

More Backg.

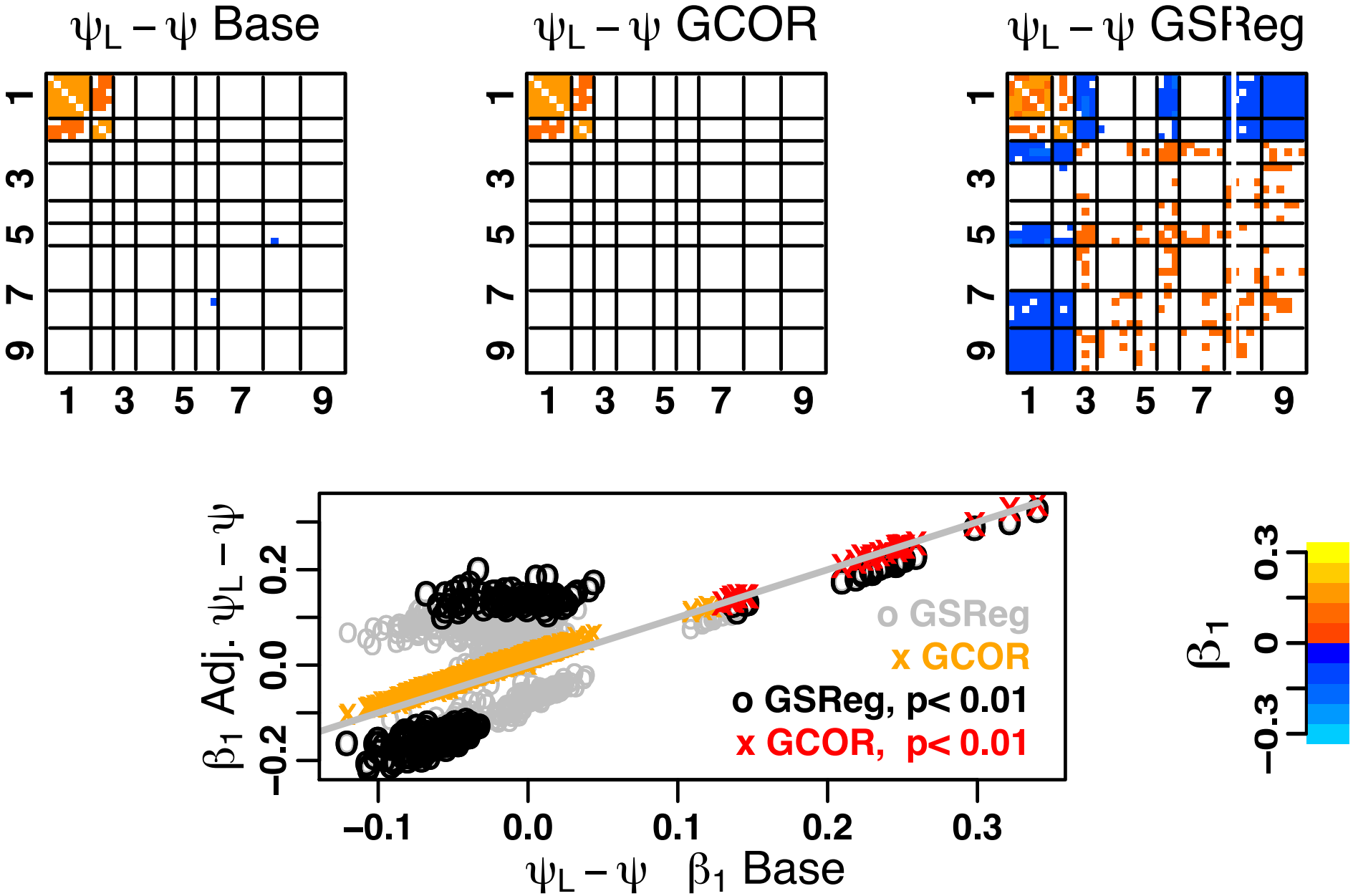


Group ψ_{BL}

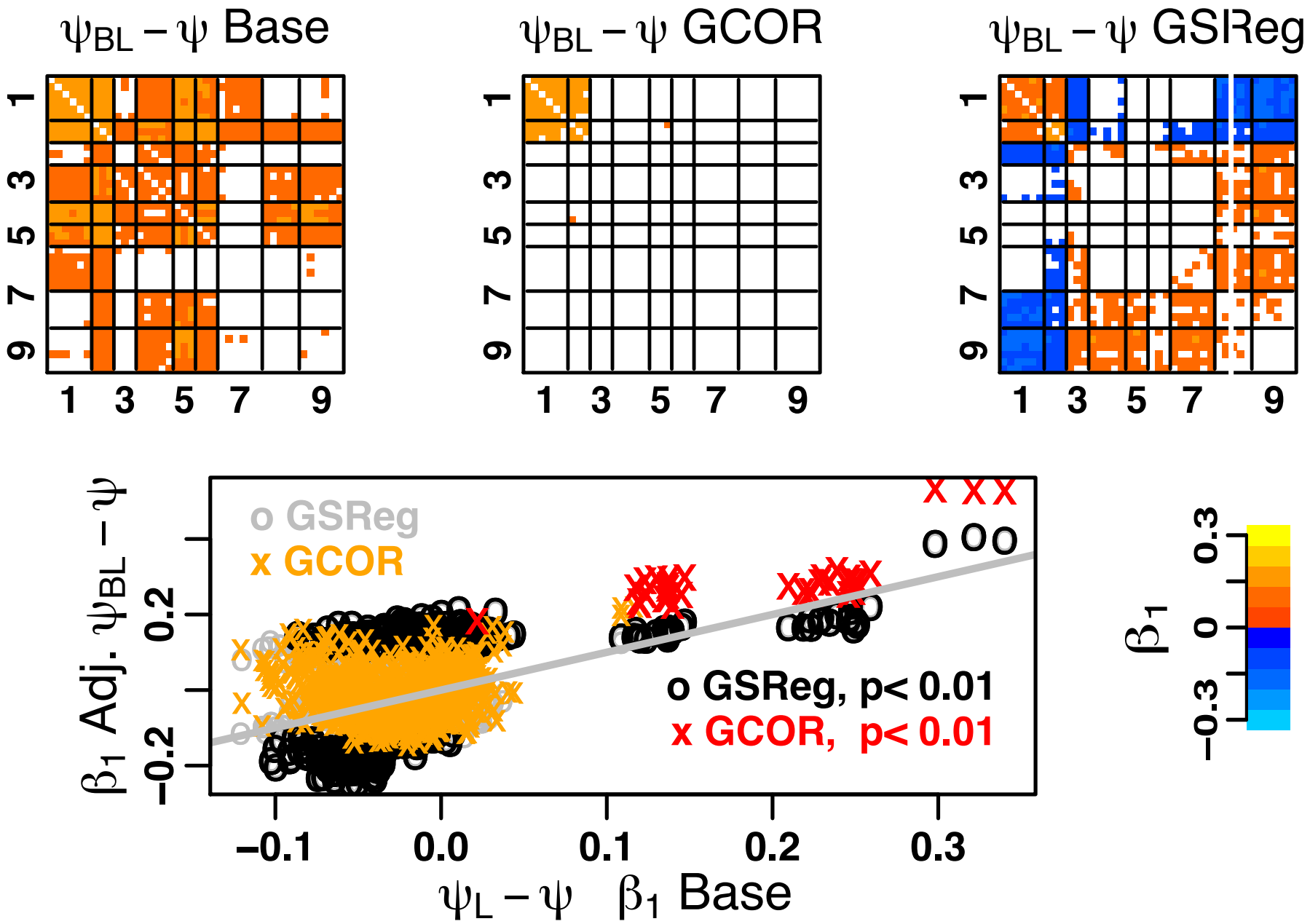


More Cowbell

Group Contrast, Only Local Change



Group Contrast, Local & Backg. Change

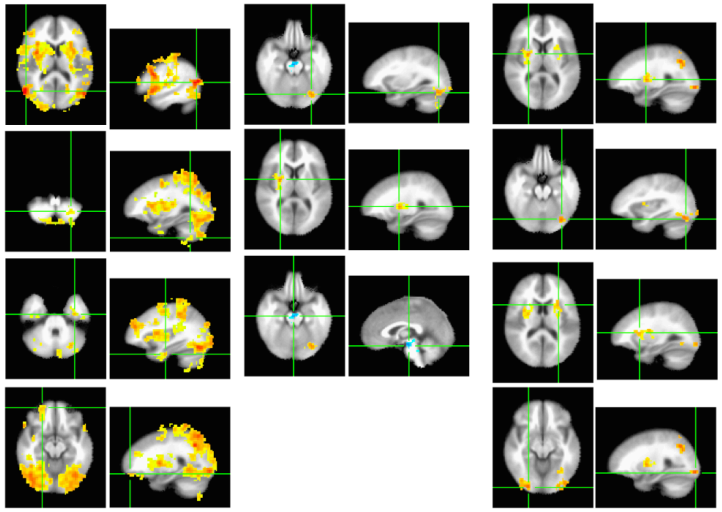


GCOR and *Motion* Grouping

FCON 1000: Cambridge_Buckner

β_1 Base β_1 GSReg β_1 GCOR

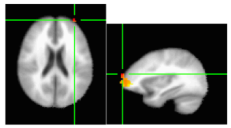
Largest 4 Clusters



4 clusters 3 clusters 6 clusters

FCON 1000: Beijing_Zang

β_1 Base β_1 GSReg β_1 GCOR

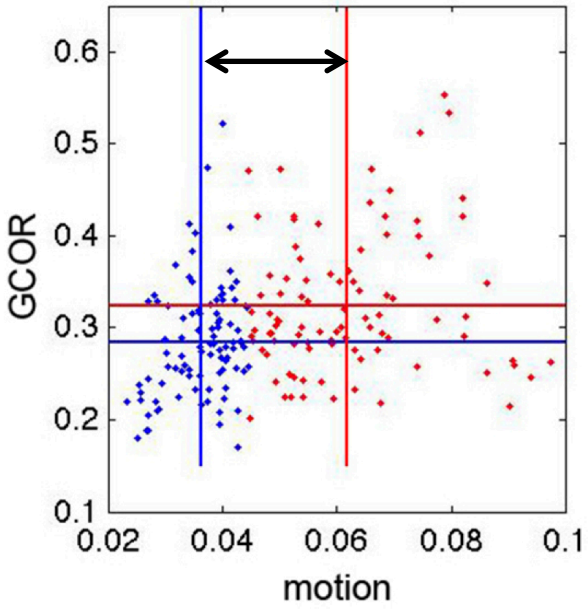


1 cluster 0 cluster 0 cluster

Small > Big Small < Big

Group Difference, p<0.01, α=0.05

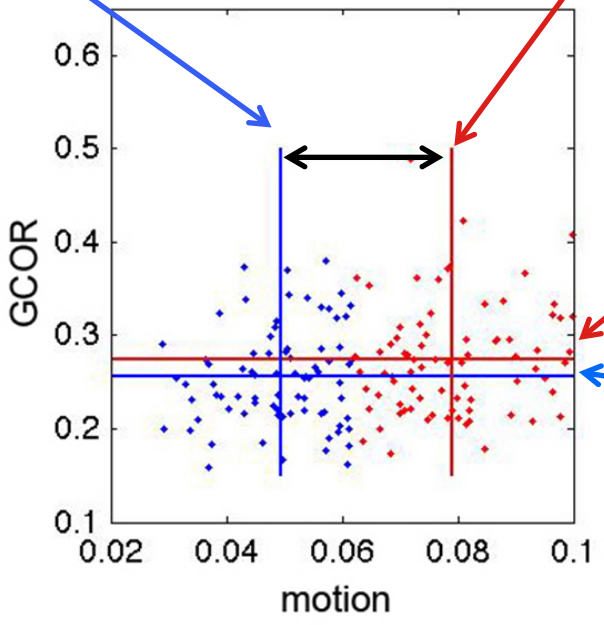
Δ=25 μm/TR



Mean Motion Small Movers

Mean Motion Big Movers

Δ=29 μm/TR



Mean GCOR Big Movers

Mean GCOR Small Movers

GCOR as Group Level Covariate

Correlations less biased with GCOR, than GSReg.

- ***when*** GCOR has low correlation with grouping variable

Level-II tests conservative

- Less likely to detect difference as grouping variable and covariate correlation increases

Adjustment outside of level II test is NOT recommended

- There is always potential for interaction effect with group
- GCOR (and other params. (Yan 2013)) depend on noise AND/ OR inter-regional correlations of interest

→contrast results very likely depend on covariate centering

•Centering at overall mean makes sense if GCOR is driven by noise.

•What if it is also driven by correlations of interest?

→ contrast sign might even get reversed

Conclusions

- Stay away from using regions with Fluctuations of Interest to calculate regressors of No Interest
- GSReg and its variants are bad for inter-group comparisons
- One MUST consider interactions of method with grouping variable
  - Generative models clarify matters since there is no base truth
- GCOR is very simple to compute and is useful to assess global correlation levels
- Use of GCOR and comparable measures is better than GSReg
  - However, their interaction with grouping variable can confound interpretation

Use should be as last resort

- Use them as covariates and consider interaction terms
- Separate covariate modeling prior to level-II not recommended
- Risks of false negatives
- Centering issues

Conclusions

The best approach remains with careful denoising

- motion parameter estimates
- physiological measurements (chest belt = plethysmograph, pulse oximeter, end tidal CO_2 = ET-CO2)
- local estimates of nuisance signals from eroded white matter
  - ANATicor, CompCor
- denoising decompositions in as far as they can dissociate nuisance estimates from signal fluctuations of interest

Look at your data

Acknowledgments

Robert Cox
Gang Chen
Steve Gotts
Hang Joon Jo
Alex Martin
Rick Reynolds

Kelly Barnes
Catie Chang

Carlton Chu

Jonathan Power
and coauthors
for releasing data

NIMH
National Institute
of Mental Health

National Institute of
NEUROLOGICAL
Disorders and Stroke