2ND LEVEL (GROUP) GENERAL LINEAR MODEL

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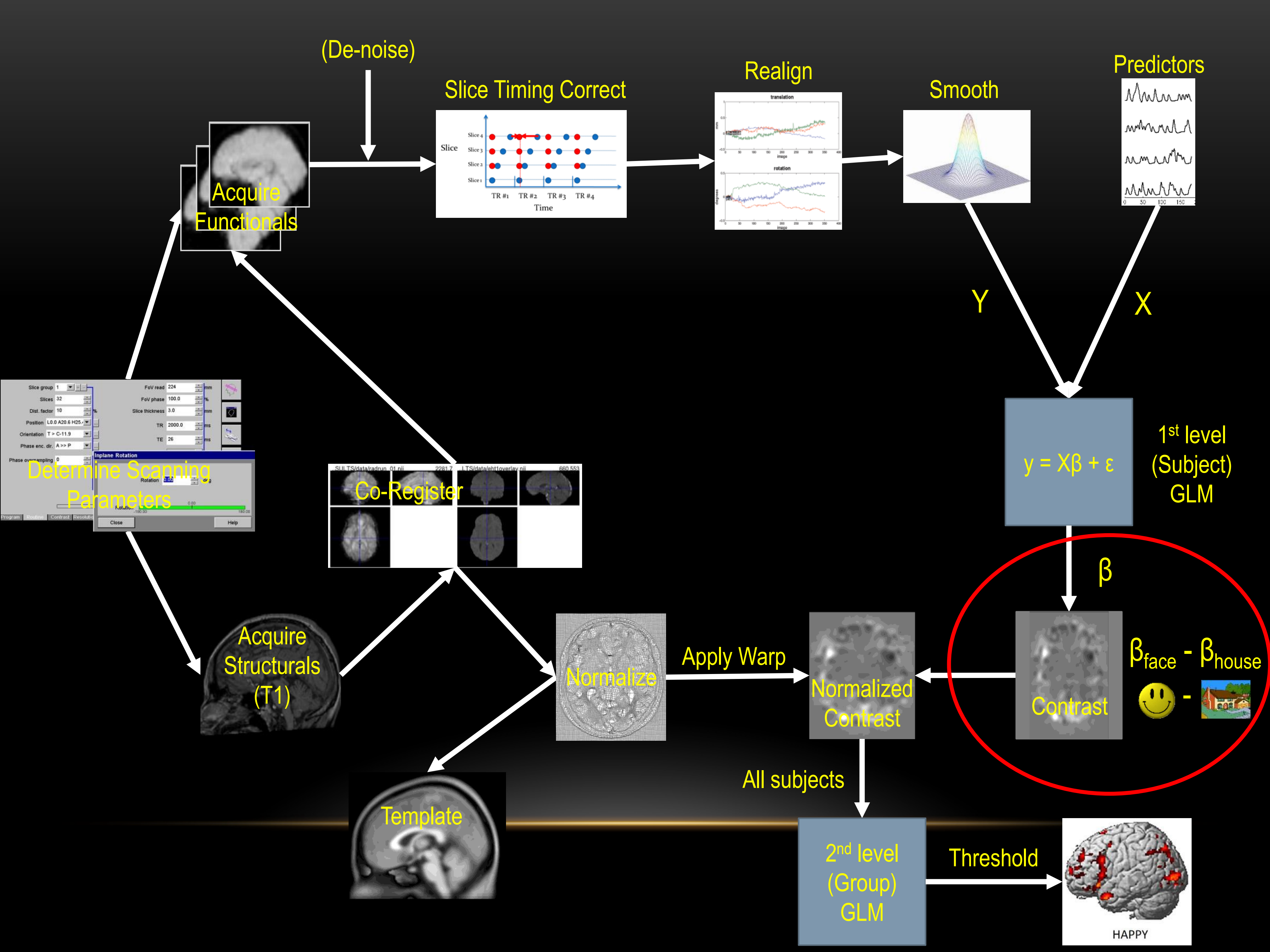
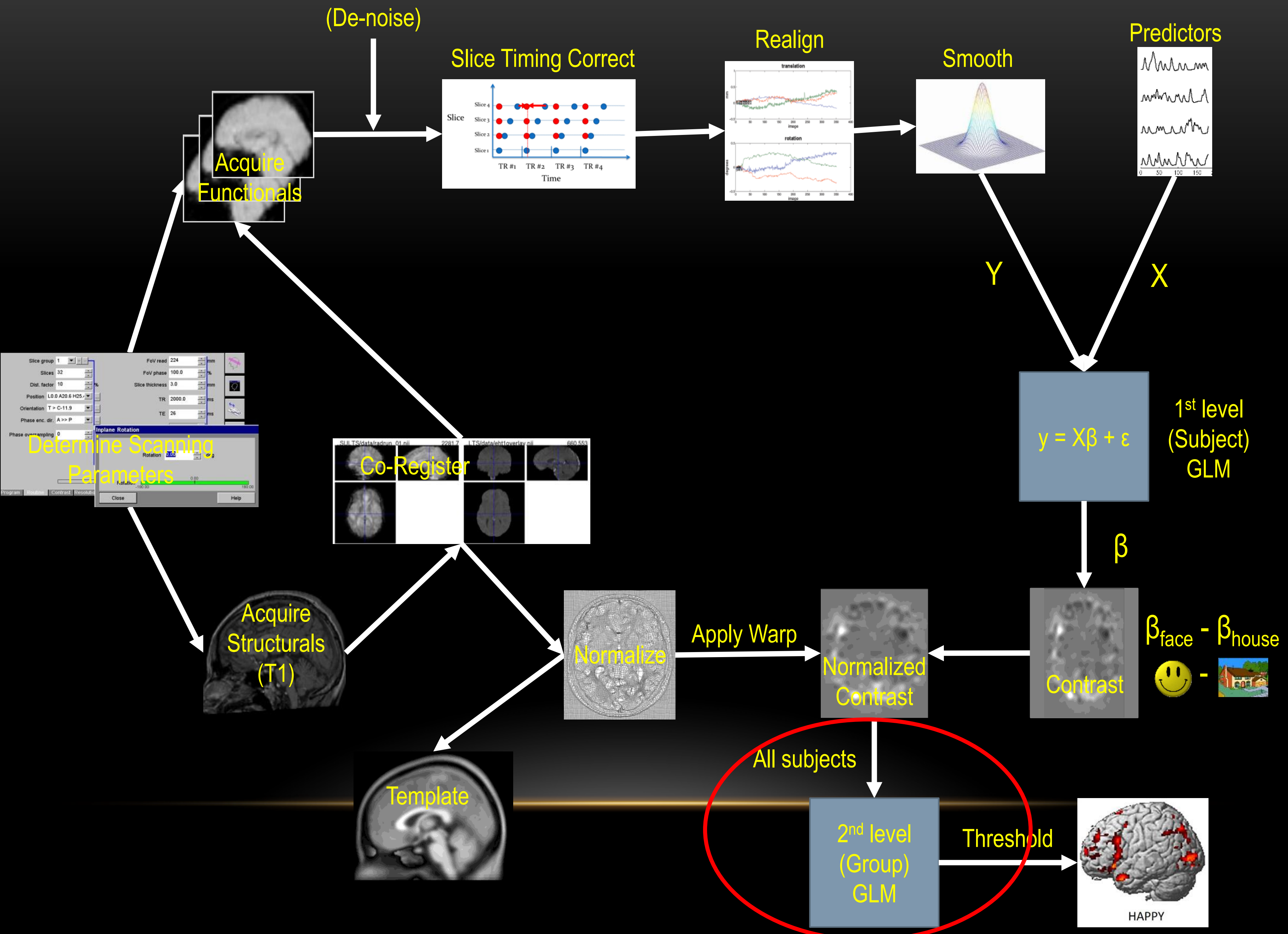

(De-noise)
Slice Timing Correct
Realign
Smooth
Predictors
Acquire Functionals
Y
X
y = Xβ + ε
1st level (Subject) GLM
Determine Scanning Parameters
Co-Register
β
βface - βhouse
Acquire Structurals (T1)
Normalize
Apply Warp
Normalized Contrast
Contrast
All subjects
Template
2nd level (Group) GLM
Threshold
HAPPY


(De-noise)
Slice Timing Correct
Slice
Slice 4
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TR #1
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TR #3
TR #4
Time
Realign
translation
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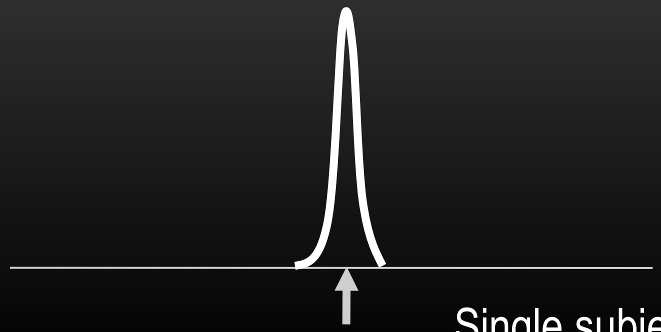
OUTLINE

- Random vs. Fixed effects analysis
- Mixed effects analysis
- Summary Statistic Approach
- ANOVAs
- Correlations

SOURCES OF VARIANCE

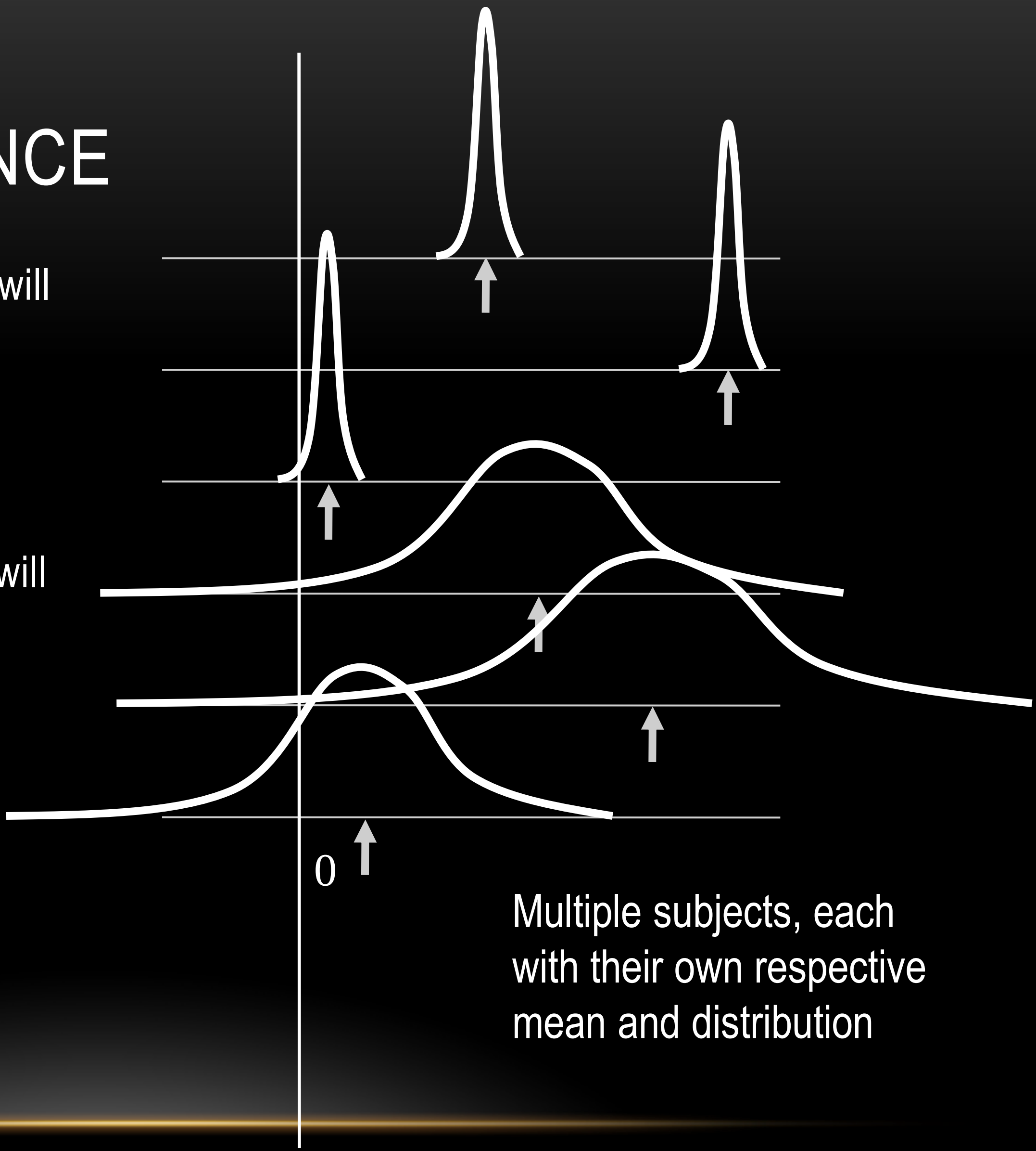
- Repeated sampling of an *individual* will yield different measurements
  - Within-subject variance



Single subject distribution
Mean indicated by arrow

SOURCES OF VARIANCE

- Repeated sampling of an individual will yield different measurements
    - Within-subject variance
- Repeated sampling of a *population* will yield different measurements
    - Between-subject variance



Multiple subjects, each with their own respective mean and distribution

RANDOM EFFECTS ANALYSIS

- Subjects treated as a “random” effect
  - Randomly sampled from population of interest

- Sample is used to make estimates of population effects

- Results lead to inferences on the population

SOURCES OF VARIANCE

- Repeated sampling of an individual will yield different measurements
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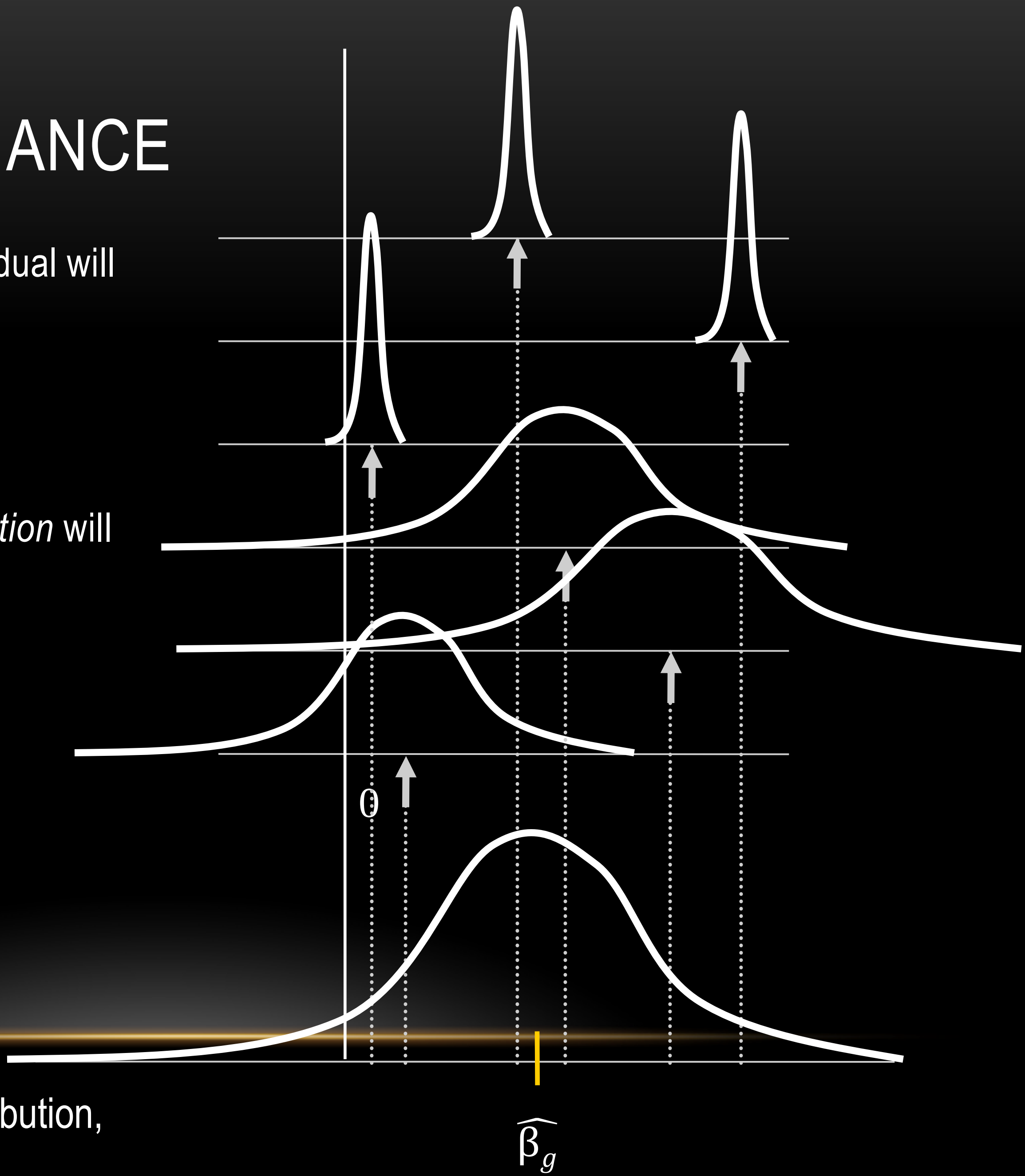
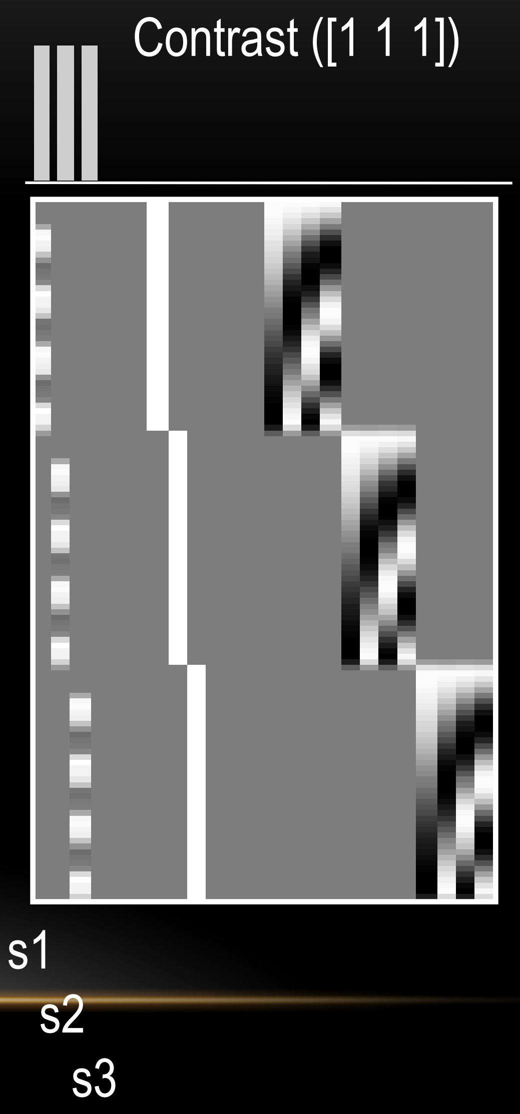


Estimated population distribution, with mean $\widehat{\beta_g}$ using RFX

FIXED EFFECTS ANALYSIS

- Treats subject as a "fixed" effect
  - Can only make inferences on the subjects themselves
  - Cannot make group inferences
- One grand GLM
  - 1st level model with each subject concatenated
  - Between-subject variability not considered
- Used in some early fMRI studies
  - Often inappropriate inferences to population



RANDOM VS FIXED EFFECTS

- Whereas some early studies used fixed effects models, virtually all current studies use random effects models
- Know fixed effects and understand the inferential limits
- Use random effects
- All analyses that follow treat subject as a random effect

MIXED EFFECTS ANALYSIS

- There are two major sources of variability in group analysis
  - Within-subject variance: how variable a given parameter estimate is upon repeated samplings of the same subject (also called measurement error)
  - Between-subject variance: how variable a given parameter estimate is across different individuals of the same population (also called individual differences)

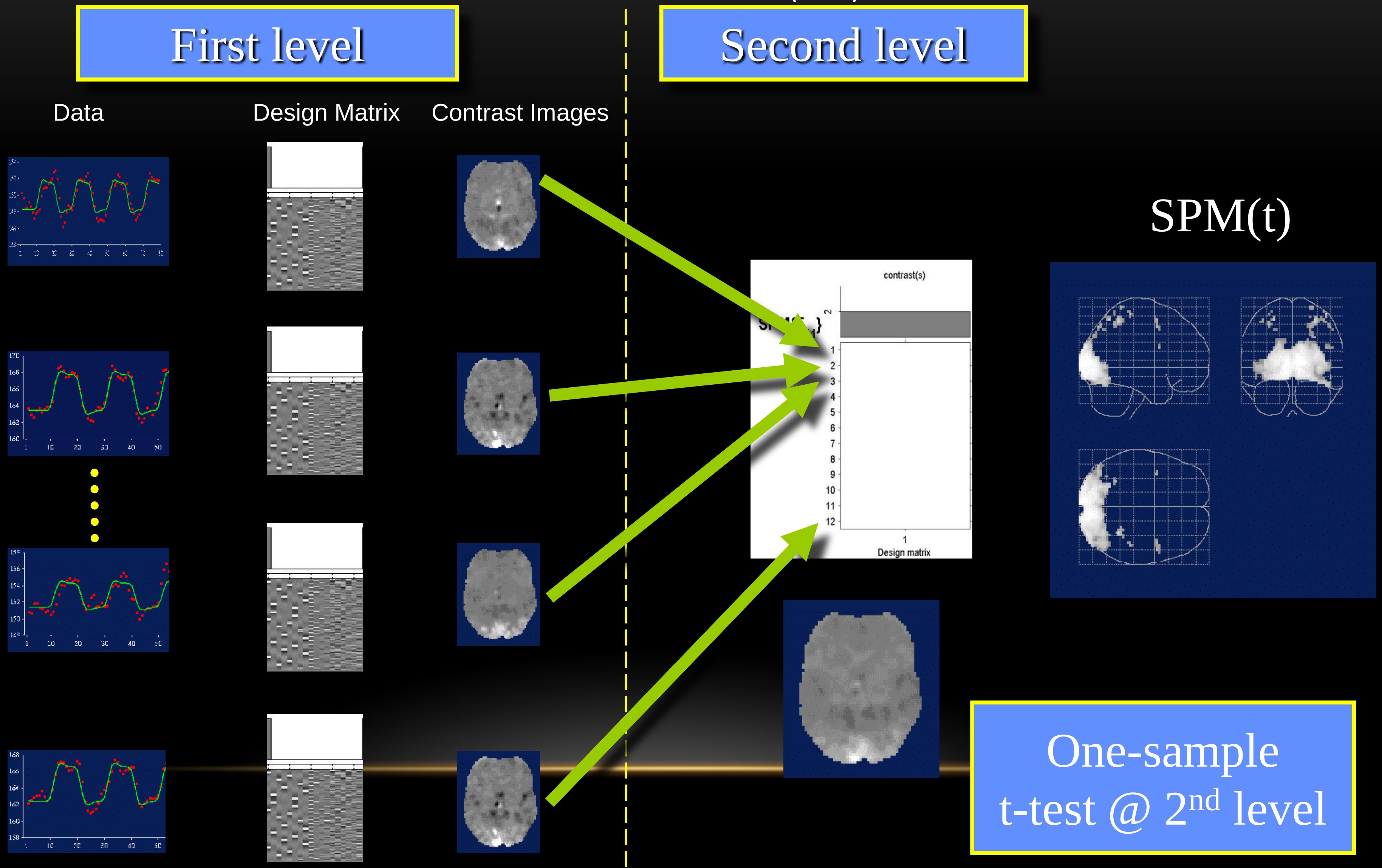
- Different analysis methods vary with regard to how these different sources of variance are estimated

- Simplest method is a 2nd (group) level t-test where within-subject variance is assumed to be homogenous

SUMMARY STATISTIC APPROACH: 1 SAMPLE T-TEST

- Contrasts are computed at 1st (subject) level
- Each subject contributes a single contrast estimate
  - Measures magnitude of effect of interest
- A simple GLM is fit to the group data
  - Only 1 predictor: intercept (i.e. mean)
  - $Y_g = \beta_g X_g + \varepsilon_g$
- Contrast is simply “1” (i.e. mean)

GROUP ANALYSIS USING SUMMARY STATISTICS: A SIMPLE KIND OF ‘RANDOM EFFECTS’ MODEL THE “HOLMES AND FRISTON” APPROACH (HF)



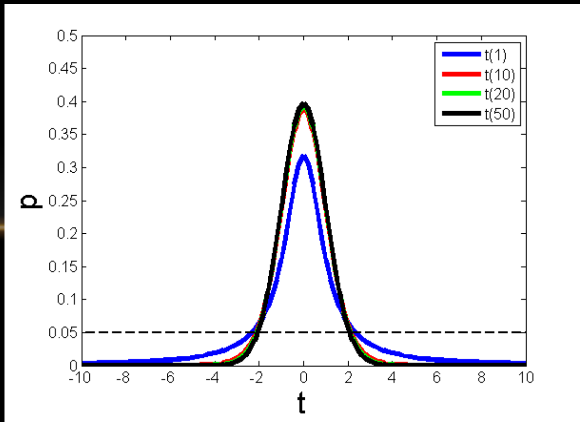
Courtesy of Tor Wager

SUMMARY STATISTIC APPROACH: INFERENCE

- In a 1-sample t-test, the contrast C = 1 derives the group mean
  - If images taken to second level represent the contrast A – B, then
    - C = 1 is the mean difference (A > B)
    - C = -1 is the mean difference (B > A)

- Dividing by the standard error of the mean yields a t-statistic
  - Degrees of freedom is *N* – 1, where *N* is the number of subjects
  - $\hat{\sigma}_g^2 = \frac{\sum \varepsilon_g^2}{df_g}$
  - $T = \frac{\hat{\beta}_g}{\sqrt{\hat{\sigma}_g^2 / N}}$

- Comparison of the t-statistic with the t-distribution yields a p-value
  - P(Data|Null)



SUMMARY STATISTIC APPROACH: 2 SAMPLE - TEST

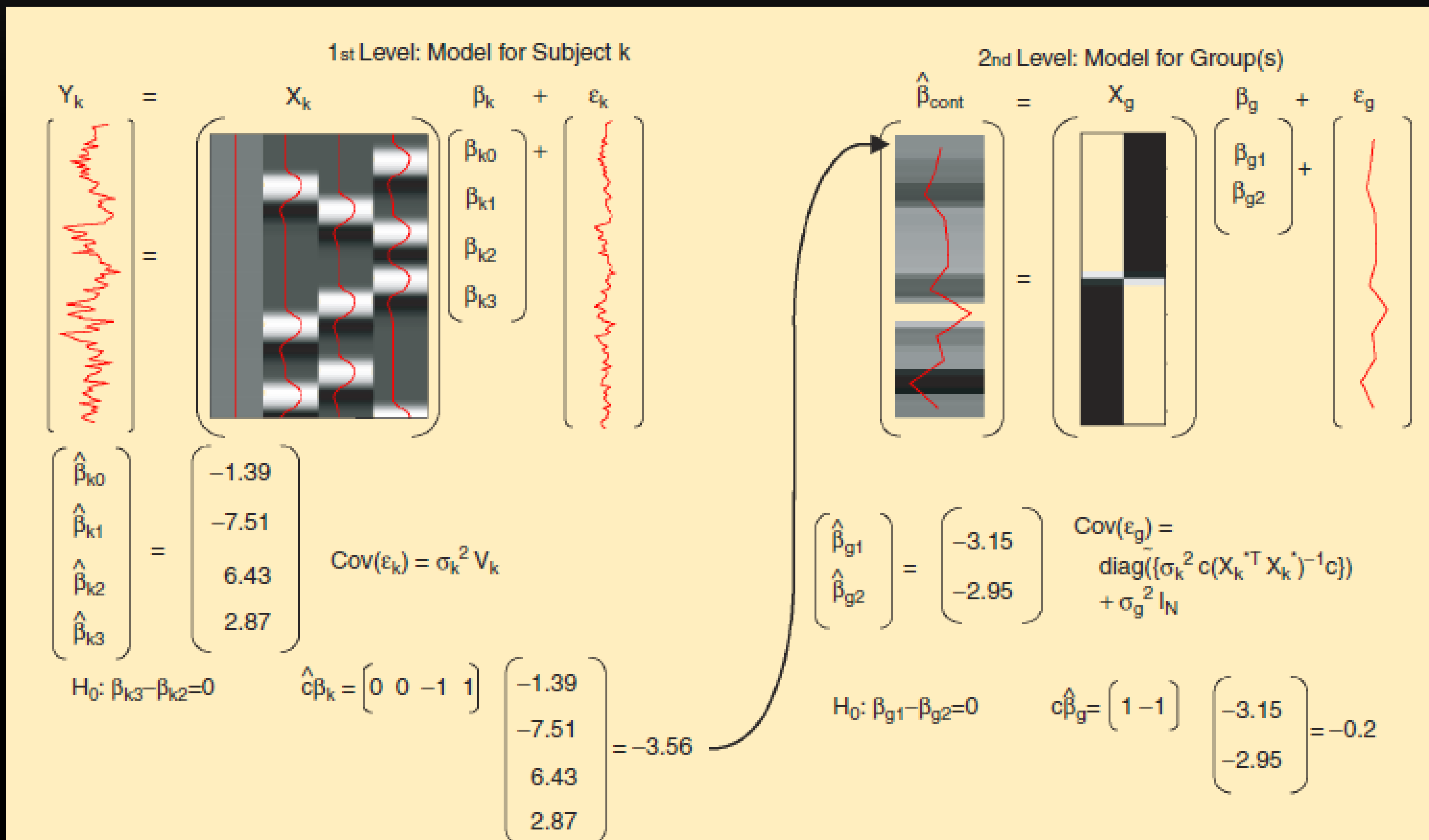
- Minor differences from 1 sample t-test
  - 1) 2 predictors, 1 for each group
    - 1 denotes group membership, 0 otherwise

$$
\begin{array}{cc}
 & \begin{array}{cc} G1 & G2 \end{array} \\
X_g = & \begin{bmatrix} 1 & 0 \\ 1 & 0 \\ 1 & 0 \\ 1 & 0 \\ 0 & 1 \\ 0 & 1 \\ 0 & 1 \\ 0 & 1 \end{bmatrix}
\end{array}
$$

  - 2) Separate variance estimates for each group, if appropriate
  - 3) Contrasts can compare groups, average groups, or consider just one group

G1 > G2	G1 + G2	G1
$C^T = [1 \; -1]$	$C^T = [1 \; 1]$	$C^T = [1 \; 0]$

SUMMARY STATISTIC APPROACH: 2 SAMPLE T-TEST



from Mumford & Nichols, 2006

SUFFICIENCY OF SUMMARY STATISTIC APPROACH

- With simple t-tests under the summary statistic approach, within-subject variance is assumed to be homogenous (within a group)
  - SPM's approach, but other packages can act differently

- If all subjects (within a group) have equal within-subject variance (homoscedastic), this is ok

- If within-subject variance differs among subjects (heteroscedastic), this may lead to a loss of precision
  - May want to weight individuals as a function of within-subject variability

- Practically speaking, the simple approach is good enough (Mumford & Nichols, 2009, NeuroImage)
  - Inferences are valid under heteroscedasticity
  - Slightly conservative under heteroscedasticity
  - Near optimal sensitivity under heteroscedasticity
  - Computationally efficient

FACTORIAL DESIGNS: 2 FACTORS/LEVELS

- Preferred approach in SPM is to estimate contrasts at the 1st level and perform t-tests at the 2nd level
  - Avoids need to estimate non-sphericity to account for within-subject correlations across repeated measures (more in a moment)
  - Generally more accurate estimation of error

- T-test approach works well for 2 x 2 factorial designs

Main effect of A

	B1	B2
A2	-1	-1
A1	1	1

Main effect of B

	B1	B2
A2	1	-1
A1	1	-1

Interaction A x B

	B1	B2
A2	1	-1
A1	-1	1

FACTORIAL DESIGNS: 2+ FACTORS/LEVELS

- If more than 2 factors or levels exist, a single t-contrast cannot capture main effects and interactions
- 2^{nd} level ANOVA will be necessary

ONE-WAY ANOVA (WITHIN SUBJECTS)

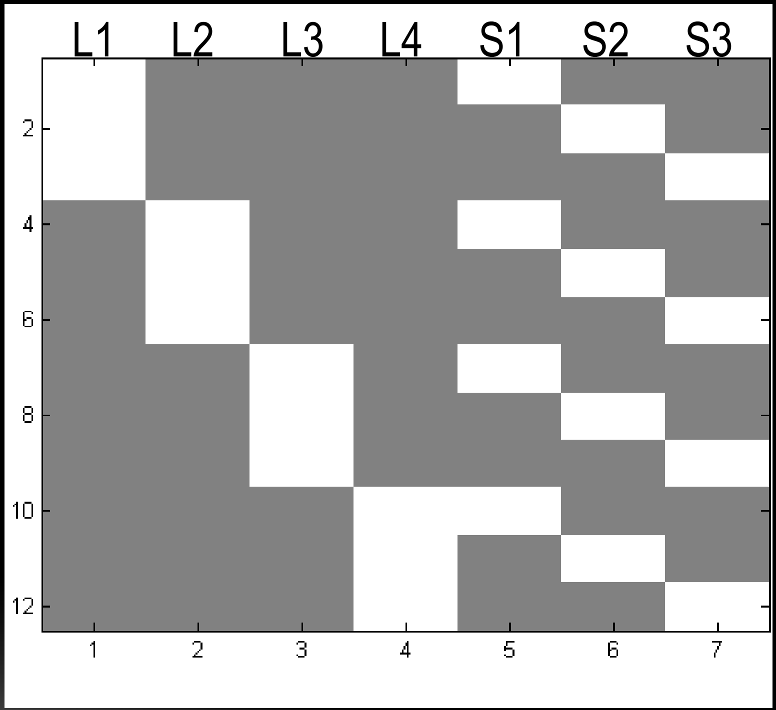
- Suppose single factor with 4 levels and 3 subjects
  - 4 1st level contrasts: L1, L2, L3, L4
  - 4 images per subject taken to 2nd level

Main Effect F-Contrast

$$C^T = \begin{bmatrix} 1 & -1 & 0 & 0 & 0 & 0 & 0 \\ 0 & 1 & -1 & 0 & 0 & 0 & 0 \\ 0 & 0 & 1 & -1 & 0 & 0 & 0 \end{bmatrix}$$

$$Y = \begin{bmatrix} L1S1 \\ L1S2 \\ L1S3 \\ L2S1 \\ L2S2 \\ L2S3 \\ L3S1 \\ L3S2 \\ L3S3 \\ L4S1 \\ L4S2 \\ L4S3 \end{bmatrix}$$

$$X = \begin{array}{c} \begin{matrix} L1 & L2 & L3 & L4 & S1 & S2 & S3 \end{matrix} \\ \begin{bmatrix} 1 & 0 & 0 & 0 & 1 & 0 & 0 \\ 1 & 0 & 0 & 0 & 0 & 1 & 0 \\ 1 & 0 & 0 & 0 & 0 & 0 & 1 \\ 0 & 1 & 0 & 0 & 1 & 0 & 0 \\ 0 & 1 & 0 & 0 & 0 & 1 & 0 \\ 0 & 1 & 0 & 0 & 0 & 0 & 1 \\ 0 & 0 & 1 & 0 & 1 & 0 & 0 \\ 0 & 0 & 1 & 0 & 0 & 1 & 0 \\ 0 & 0 & 1 & 0 & 0 & 0 & 1 \\ 0 & 0 & 0 & 1 & 1 & 0 & 0 \\ 0 & 0 & 0 & 1 & 0 & 1 & 0 \\ 0 & 0 & 0 & 1 & 0 & 0 & 1 \end{bmatrix} \end{array}$$

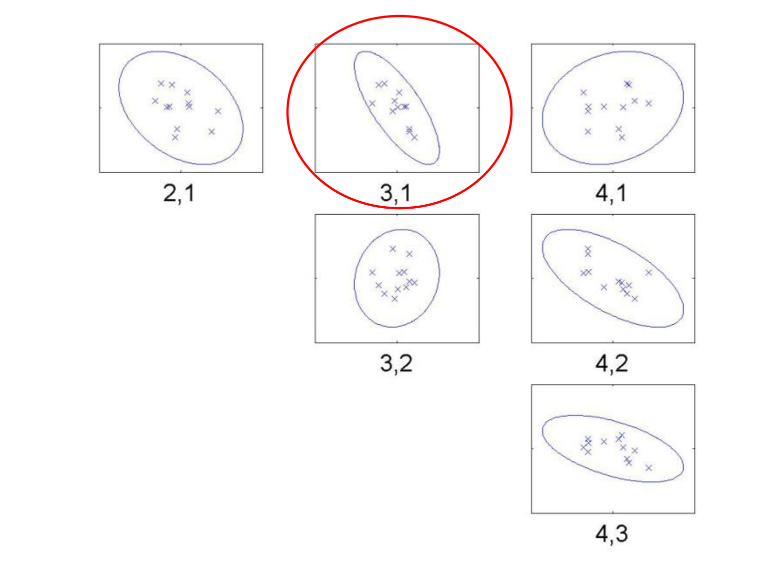


L1-4 represent 4 levels of the factor

S1-3 represent 3 subjects

REPEATED MEASURES AND NON-SPHERICITY

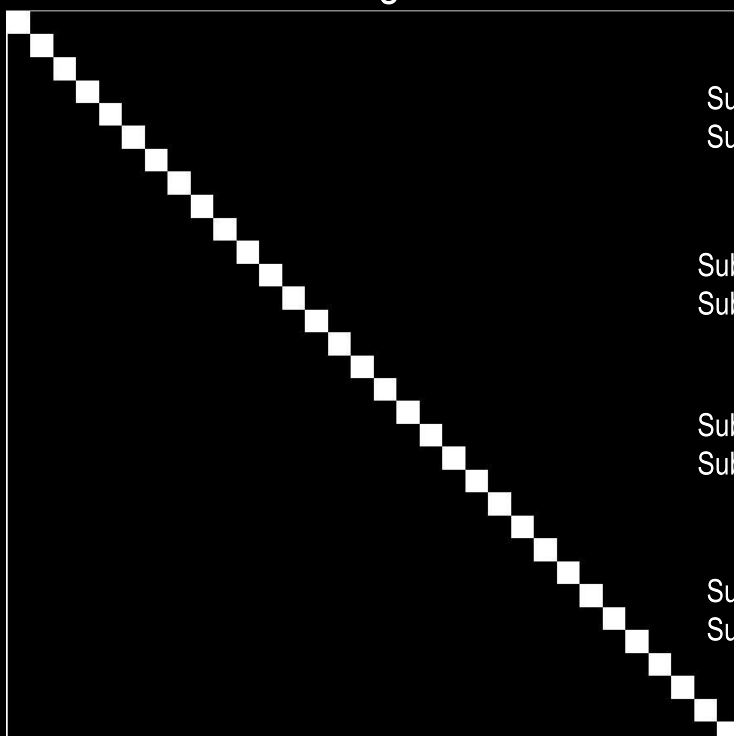
- GLM assumes that errors are independent and identically distributed (i.i.d.)
- At the 1st level, we've seen this is not the case and must be corrected
  - Temporal autocorrelation
- At the 2nd level, the i.i.d. error assumption is often violated when measures are repeated across a subject
  - Repeated measures are typically correlated within a subject
  - Referred to as non-sphericity
    - i.i.d. errors plotted in 2D space form a spherical cloud of points
    - Correlated errors form an ellipse
- In SPM, if you indicate that measures are not independent
  - Co-variance will be estimated through restricted maximum likelihood estimation (ReML)
  - Corrections will be applied



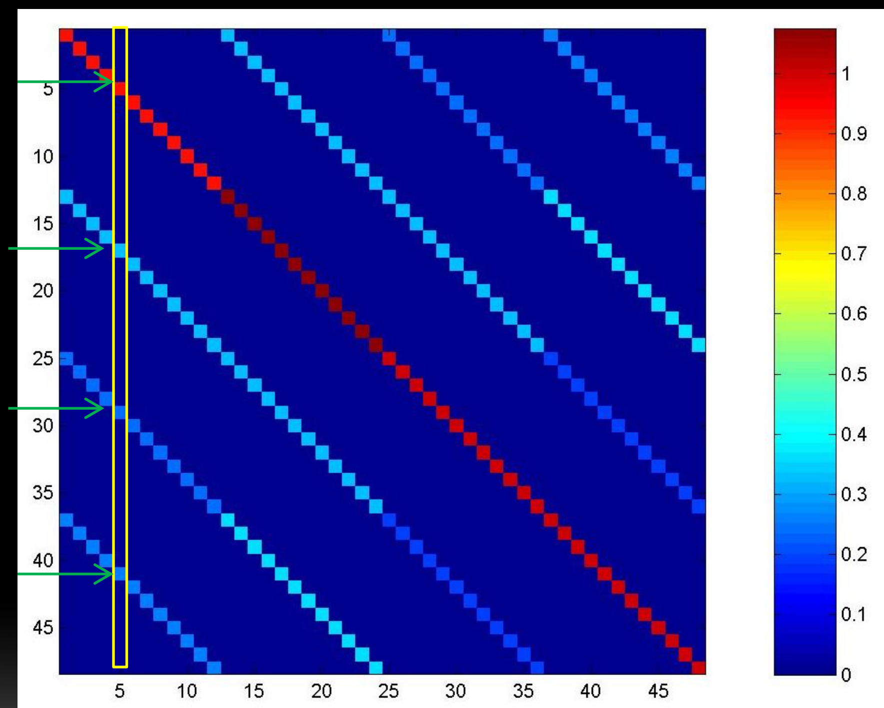
Responses to condition i vs condition j
Each "x" is a subject
Conditions 3 and 1 are highly correlated

NON-SPHERICITY IN SPM

Spherical co-variance matrix



Estimated non-sphericity due to repeated measures



Each image is correlated only with itself and not other images

Repeated measures from same subject are correlated

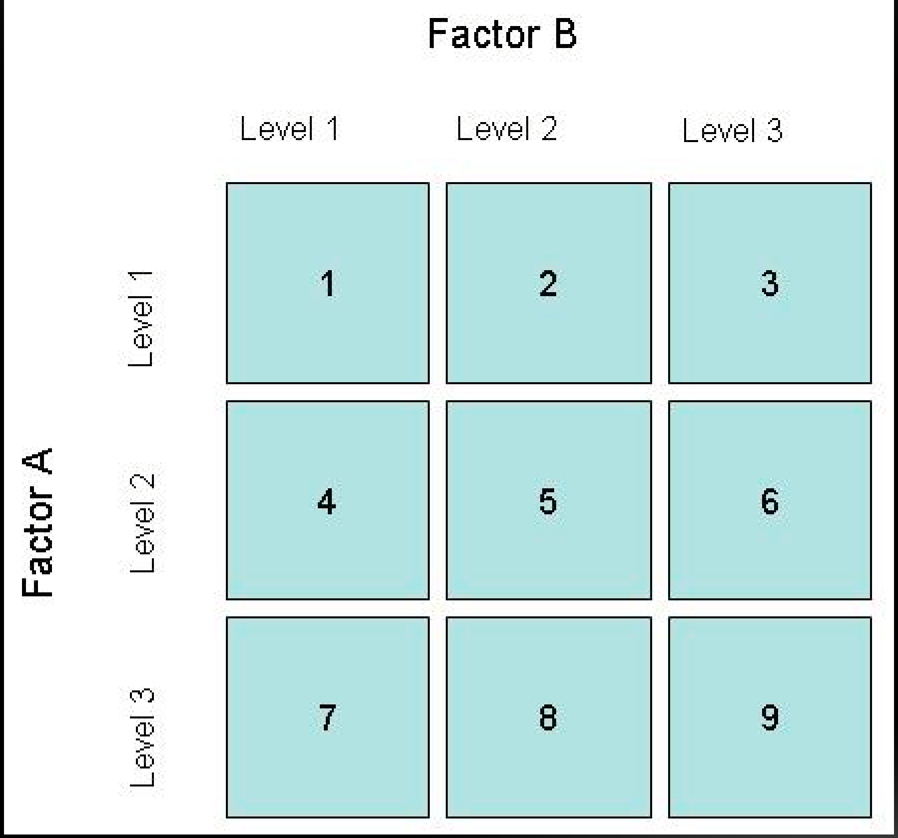
NON-SPHERICITY IN SPM: LIMITATIONS

- For computational efficiency, SPM pools across (important) voxels to calculate non-sphericity
- In reality, non-sphericity is likely not homogenous across the brain
- So, test statistics will not be exact
  - Better to use t-tests where possible

M-WAY ANOVAS

- Higher dimensional ANOVAs add further complications regarding pooled vs partitioning errors
- Appropriate designs and contrasts in SPM become very complex and confusing
- If you must perform a high dimensional ANOVA, generally advisable to condense it through contrasts at the 1st level

3 X 3 ANOVA EXAMPLE



Main effect A

(1+2+3) – (4+5+6)
(4+5+6) – (7+8+9)

Send two contrasts to 2^{nd} level one-way ANOVA

Main effect B

(1+4+7) – (2+5+8)
(2+5+8) – (3+6+9)

Send two contrasts to 2^{nd} level one-way ANOVA

A x B

(1–4) – (2–5)
(2–5) – (3–6)
(4–7) – (5–8)
(5–8) – (6–9)

Send four contrasts to 2^{nd} level one-way ANOVA

At 2^{nd} level, test is F-contrast of the form I (identity matrix)

SPM RECIPE

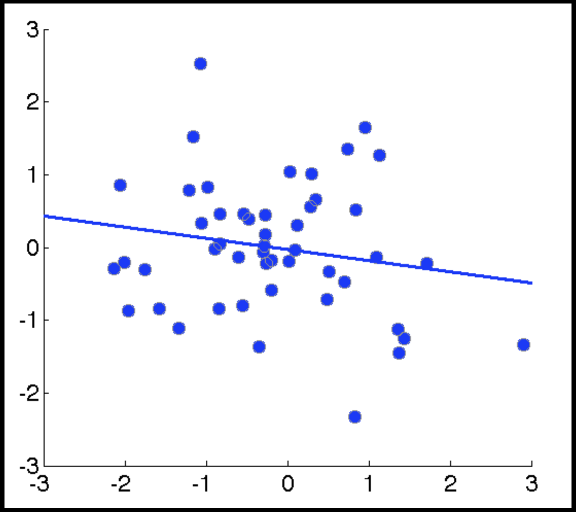
Design	1^{st} Level	2^{nd} Level
1 group, 1 factor, 2 levels	A1 – A2	One-sample t-test
1 group, 1 factor, 2+ levels	A1,A2, …, A_n	One-way ANOVA (within-subjects)
1 group, 2 factors, 2 levels each	(A1B1+A1B2)-(A2B1+A2B2): ME A (A1B1+A2B1)-(A1B2+A2B2): ME B (A1B1+A2B2)-(A1B2+A2B1): A x B	One-sample t-tests
1 group, 2+ factors/2+ levels	Multiple contrasts for each ME and interaction	One-way ANOVA
2 groups, 1 factor, 2 levels	A1 – A2	Two-sample t-test
2 groups, 1 factors, 2+ levels	A1, A2, …, An	Two-way ANOVA (mixed)
2 groups, 2 factors, 2 levels each	(A1B1+A1B2)-(A2B1+A2B2): ME A (A1B1+A2B1)-(A1B2+A2B2): ME B (A1B1+A2B2)-(A1B2+A2B1): A x B	Two-sample t-tests
2 groups, 2+ factors/2+ levels	Multiple contrasts for each ME and interaction	Two-way ANOVA

CORRELATIONS

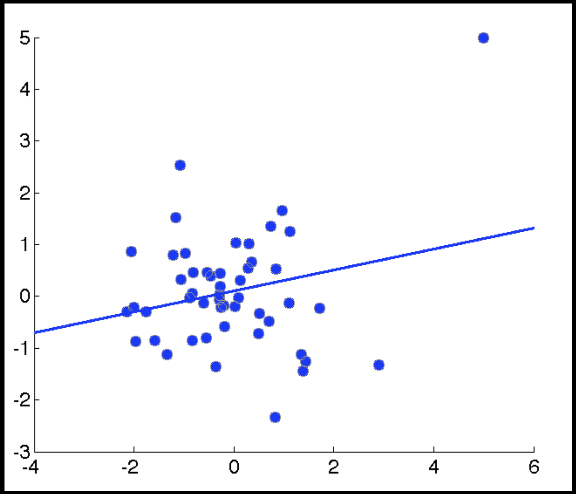
- To perform mass bi-variate correlations, use SPM's "Multiple Regression" option with a single co-variate
    - Can also specify multiple co-variates and perform true multiple regression
        - Be cautious of multi-collinearity!
- Correlations are done voxel-wise
- % of explained variance necessary to reach significance with appropriate correction for multiple comparisons may be unrealistically high
    - Voodoo? (more later)
- May be more realistic to perform correlations on a small set of regions-of-interest

CORRELATIONS AND OUTLIERS

Null-hypothesis data, N = 50



Same data, with one outlier

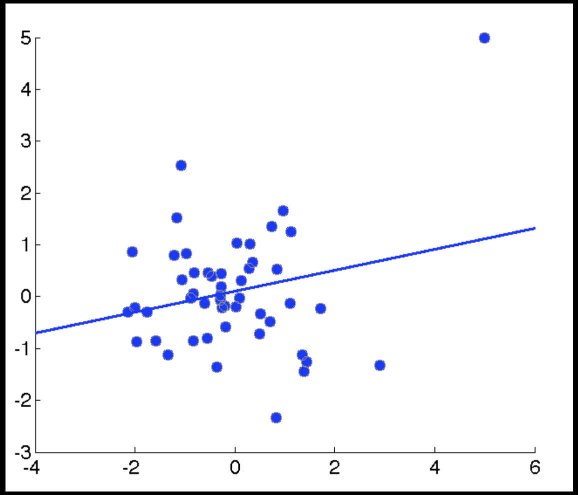


Courtesy of Tor Wager

ROBUST REGRESSION

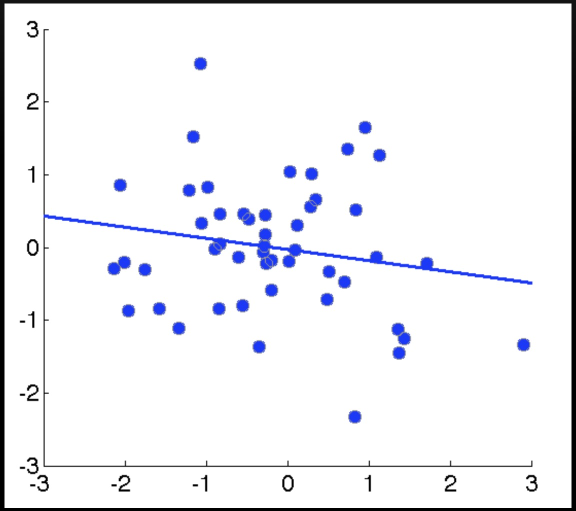
- Outliers can be problematic, especially for correlations

- Robust regression reduces the impact of outliers
  - 1) Weight data by inverse of leverage
  - 2) Fit weighted least squares model
  - 3) Scale and weight residuals
  - 4) Re-fit model
  - 5) Iterate steps 2-4 until convergence
  - 6) Adjust variances or degrees of freedom for p-values



- Can be applied to simple group results or correlations
  - http://wagerlab.colorado.edu/

Null-hypothesis data, N = 50



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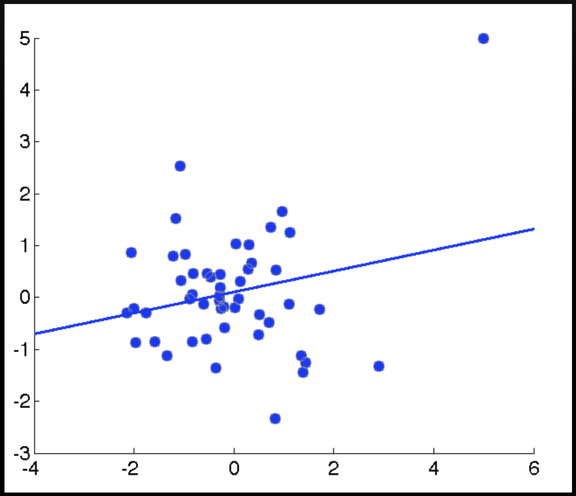
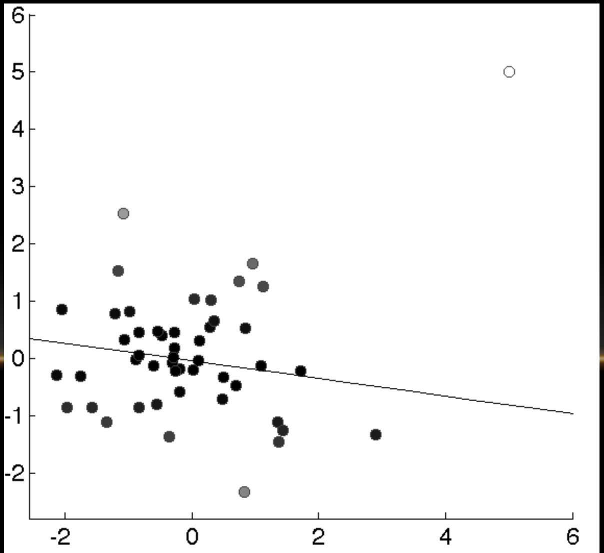


Robust IRLS solution



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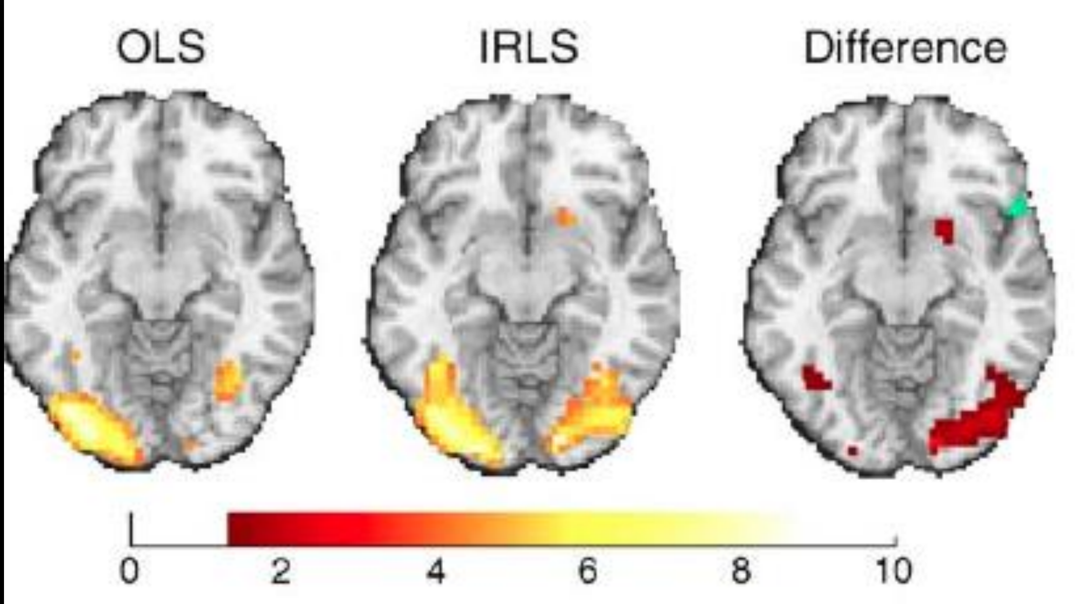
Case study: Visual Activation

Visual responses



Courtesy of Tor Wager

TAKE HOME

- Best approach is to keep it simple
  - Simpler designs will typically be estimated for effectively at 1st level
  - Simpler designs will be easier to handle at the 2nd level

- Condense where possible
  - If a factor can be collapsed through a contrast at the 1st level, do so and use the simplest possible 2nd level model
  - T-tests at 2nd level are preferred

- Correlations can be done in a mass bivariate method, but may be more appropriate on ROI by ROI basis
  - Robust regression can compute more outlier-resistant correlations
    - Can also perform outlier-correction on simple t-tests!