

- **Group Analysis: Basic concepts**
 - Group analysis
 - Make general conclusions about some population
 - Partition/untangle data variability into various sources (effect → causes)
 - Fixed factor
 - Treated as a fixed variable in the model
 - Categorization of experiment conditions; group of subjects
 - All levels of the factor are of interest and included for all experiment replications
 - Fixed in the sense inferences
 - apply only to the specific levels of the factor
 - don't extend to other potential levels that might have been included
 - Random factor
 - Exclusively subject in fMRI
 - Treated as a random variable in the model
 - average response + effects uniquely attributable to each subject: $N(\mu, \sigma^2)$
 - Each subject is of no interest
 - Random in the sense
 - subjects serve as a random sample of a population
 - inferences can be generalized to a population
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- **Group Analysis: Types**
 - Different terminology for factorial (crossed)/nested
 - Statisticians: count subject as a random factor; Random-effect model
 - Psychologists: Within-subject (repeated measures) / between-subjects
 - Crossed and nested designs
 - Fixed effect
 - Only a few subjects as case study; can't generalize to whole population
 - Simple approach: $T = \sum t_i / \sqrt{t(n)}$
 - Sophisticated approach: v_i = variance for coefficient b_i
 - $B = \sum (b_i \sqrt{v_i}) / \sum (1/\sqrt{v_i})$, $T = B \sum (1/\sqrt{v_i}) / \sqrt{n}$
 - $B = \sum (b_i / v_i) / \sum (1/v_i)$, $T = B \sqrt{\sum (1/v_i)}$
 - Concatenate individual subject data
 - Random effect
 - "Random" refers to subject
 - Individual and group analyses: separate
 - Assumption: within-subject variation is negligible compared to between-subjects
 - Focus of this talk
 - Mixed effect
 - Ideally analyze with all subjects' data combined, but not computationally feasible
 - Bring within-subject variances to group analysis, but still not easy to do currently
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• Group Analysis: Programs in AFNI

- ¶ Parametric Tests: assumption of Gaussian distribution; 10+ subjects
 - ↳ `3dttest` (one-sample, unpaired and paired *t*)
 - ↳ `3dANOVA` (one-way between-subject)
 - ↳ `3dANOVA2` (one-way within-subject, 2-way between-subjects)
 - ↳ `3dANOVA3` (2-way between-subjects, within-subject and mixed, 3-way between-subjects)
 - ↳ `3dRegAna` (regression/correlation, hi-way or unbalanced ANOVA, ANCOVA)
 - ↳ `GroupAna` (Matlab script for up to 5-way ANOVA)
- ¶ Non-Parametric Analysis
 - ↳ No assumption of normality; Statistics based on ranking
 - ↳ Appropriate when number of subjects too few (< 10)
 - ↳ Programs
 - > `3dWilcoxon` (~ paired *t*-test)
 - > `3dMannWhitney` (~ two-sample *t*-test)
 - > `3dKruskalWallis` (~ between-subjects with `3dANOVA`)
 - > `3dFriedman` (~one-way within-subject with `3dANOVA2`)
 - > Permutation test
 - plugin on AFNI under Define Datamode / Plugins /
 - C program by Tom Holroyd
 - ↳ Can't handle complicated designs
 - ↳ Less sensitive to outliers (more robust) and less flexible than parametric tests

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• Group Analysis: Overview

- ¶ How many subjects?
 - ↳ Power/efficiency: proportional to \sqrt{n} ; $n > 10$
 - ↳ Balance: Equal number of subjects across groups if possible
- ¶ Input
 - ↳ Common brain in tirc space (resolution doesn't have to be 1x1x1 mm³)
 - ↳ % signal change (**not** statistics) or normalized variables
 - > HRF magnitude: Regression coefficients
 - > Contrasts
- ¶ Design
 - ↳ Number of factors
 - ↳ Number of levels for each factor
 - ↳ Within-subject or repeated-measures vs. between-subjects
 - > Fixed (factors of interest) vs. random (subject)
 - > Nesting: Balanced?
 - ↳ Which program?
- ¶ Contrasts and trend analysis
- ¶ Thresholding: One- or two-tail?

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• Group Analysis: 3dttest

- ¶ Basic usage
 - ↳ One-sample *t*
 - > One group: simple effect
 - > Example: 15 subjects under condition A with $H_0: \mu_A = 0$
 - ↳ Two-sample *t*
 - > Two groups: Compare one group with another
 - > ~ 1-way between-subject (`3dANOVA`)
 - > Unequal sample sizes allowed
 - > Assumption of equal variance across groups
 - > Example: 15 subjects under A and 13 other subjects under B - $H_0: \mu_A = \mu_B$
 - ↳ Paired *t*
 - > Two conditions of one group: Compare one condition with another
 - > ~ one-way within-subject (`3dANOVA2 -type 3`)
 - > ~ one-sample *t* on individual contrasts
 - > Example: Difference between conditions A and B for 15 subjects with $H_0: \mu_A = \mu_B$
- ¶ Output: 2 values (% and *t*) at each voxel
- ¶ Versatile program: Most tests can be done with `3dttest` -piecemeal vs. bundled

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• Group Analysis: 3dANOVA

- ¶ Generalization of two-sample *t*-test
 - ↳ One-way between-subject
 - ↳ H_0 : no difference across all levels (groups)
 - ↳ Examples of groups: gender, age, genotype, disease, etc.
 - ↳ Unequal sample sizes allowed
- ¶ Assumptions
 - ↳ Normally distributed with equal variances across groups
- ¶ Results: 2 values (% and *t*)
- ¶ 3dANOVA vs. 3dttest
 - ↳ Equivalent with 2 levels (groups)
 - ↳ More than 2 levels (groups): Can run multiple two-sample *t*-test

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• **Group Analysis: 3dANOVA2**

¶ Designs

- ↳ One-way within-subject (type 3)
 - Major usage
 - Compare conditions in one group
 - Extension and equivalence of paired t
- ↳ Two-way between-subjects (type 1)
 - 1 condition, 2 classifications of subjects
 - Similar to two-sample t
 - Unbalanced designs not allowed: Equal number of subjects across groups

¶ Output

- ↳ Main effect ($-fa$): F
- ↳ Interaction for two-way between-subjects ($-fab$): F
- ↳ Contrast testing
 - Simple effect ($-amean$)
 - 1st level ($-acontr$, $-adiff$): one-sample or paired t among factor levels
 - 2nd level (interaction) for two-way between-subjects
 - 2 values per contrast: % and t

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• **Group Analysis: 3dANOVA3**

¶ Designs

- ↳ Three-way between-subjects (type 1)
 - 3 categorizations of groups
- ↳ Two-way within-subject (type 4): Crossed design AXBXC
 - Generalization of paired t -test
 - One group of subjects
 - Two categorizations of conditions: A and B
- ↳ Two-way mixed (type 5): Nested design BXC(A)
 - Nesting factor: ≥ 2 groups of subjects (Factor A): subject classification, e.g., gender
 - One category of condition (Factor B)
 - Nesting: balanced

¶ Output

- ↳ Main effect ($-fa$ and $-fb$) and interaction ($-fab$): F
- ↳ Contrast testing
 - 1st level: $-amean$, $-adiff$, $-acontr$, $-bmean$, $-bdiff$, $-bcontr$
 - 2nd level: $-abmean$, $-abdifff$, $-abcontr$, $-Abdiff$, $-Abcontr$
 - 2 values per contrast : % and t

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• **Group Analysis: GroupAna**

¶ Multi-way ANOVA

- ↳ Matlab script package for up to 5-way ANOVA
- ↳ Requires Matlab plus Statistics Toolbox
- ↳ GLM approach (slow): regression through dummy variables
- ↳ Powerful: Test for interactions
- ↳ Downside
 - Difficult to test and interpret simple effects/contrasts
 - Complicated design, and compromised power
- ↳ Heavy duty computation: minutes to hours
 - Input with lower resolution recommended
 - Resample with `adwarp -dxyz #` or `3dresample`
- ↳ Can handle both volume and surface data
- ↳ Can handle following unbalanced designs (two-sample t type):
 - 3-way ANOVA type 3: BXC(A)
 - 4-way ANOVA type 3: BXCXD(A)
 - 4-way ANOVA type 4: CXD(AXB)

- ¶ See <http://afni.nimh.nih.gov/sscc/gangc> for more info
- ¶ Alternative: `3dRegAna`

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• **Group Analysis: Example**

¶ Design

- ↳ 4 conditions (TM, TP, HM, HP) and 8 subjects
- ↳ 2-way within-subject: 2x2x8
 - A (Object), 2 levels: Tool vs Human
 - B (Animation), 2 levels: Motion vs Point
 - C (subject), 8 levels
 - AXBXC: Program? `3dANOVA3 -type 4`

¶ Main effects (A and B): 2 F values

¶ Interaction AXB: 1 F

¶ Contrasts

- ↳ 1st order: TvsH, MvsP
- ↳ 2nd order: TMvsTP, HMvsHP, TMvsHM, TPvsHP
- ↳ 6 contrasts x 2 values/contrast = 12 values

¶ Logistic

- ↳ Input: 2x2x8 = 32 files (4 from each subject)
- ↳ Output: 18 sub-bricks

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• **Group Analysis:** Example

```

Script
3dANOVA3 -type 4 -alevels 2 -blevels 2 -clevels 8 \
-dset 1 1 1 ED_TM_irf_mean+tlrc \
-dset 1 2 1 ED_TP_irf_mean+tlrc \
-dset 2 1 1 ED_HM_irf_mean+tlrc \
-dset 2 2 1 ED_HP_irf_mean+tlrc \
-
-adiff 1 2 TvsH1 \ (indices for difference)
-acontr -1 -1 TvsH2 \ (coefficients for contrast)
-bdiff 1 2 MvsP1 \
-abdiff 1 2 : 1 TMvsHM \ (indices for difference)
-aBcontr 1 -1 : 1 TMvsHM \ (coefficients for contrast)
-abdiff -1 1 : 2 HPvsTP \
-Abdiff 1 : 1 2 TMvsTP \
-Abcontr 2 : 1 -1 HMvsHP \
-
-fa ObjEffect \
-fb AnimEffect \
-fab ObjXAnim \
-bucket Group

```

Model type, number of levels for each factor

Input for each cell in ANOVA table: totally 2X2X8 = 32

1st order Contrasts, paired t test

2nd order Contrasts, paired t test

Main effects & interaction F test; Equivalent to contrasts

Output: bundled

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• **Group Analysis:** Example

```

Alternative approaches
- GroupAna
- 3dRegAna
- Paired t: 6 tests
  > Program: 3dttest -paired
  > For TM vs HM: 16 (2x8) input files (beta coefficients: %) from each subject
    3dttest -paired -prefix TMvsHM \
    -set1 ED_TM_irf_mean+tlrc ... ZS_TM_irf_mean+tlrc \
    -set2 ED_HM_irf_mean+tlrc ... ZS_HM_irf_mean+tlrc
- One-sample t: 6 tests
  > Program: 3dttest
  > For TM vs HM: 8 input files (contrasts: %) from each subject
    3dttest -prefix TMvsHM \
    -base1 0 \
    -set2 ED_TMvsHM_irf_mean+tlrc ... ZS_TMvsHM_irf_mean+tlrc

```

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• **Group Analysis:** ANCOVA (ANalysis of COVAriances)

- Why ANCOVA?
 - Subjects might not be an ideally randomized representation of a population
 - If not controlled, cross-subject variability will lead to loss of power and accuracy
 - Direct control through experiment design: balanced selection of subjects
 - Indirect (statistical) control: untangling covariate effect
 - Factor of no interest - covariate: uncontrollable/confounding variable, usually continuous
 - Age, IQ, Cortex thickness
 - Behavioral data, e.g., response time, correct rate, symptomatology score, ...
 - Gender
- ANCOVA = Regression + ANOVA
 - Assumption: linear relation between % signal change and the covariate
 - GLM approach: accommodate both categorical and quantitative variables
 - Can model interaction between covariate and other factors
 - Centralize covariate so that it would not confound with other effects
- 3dRegAna
 - Flexible program that can run all sorts of group analysis
 - Miserable to write script, but hopeful: python scripting in future

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• **Group Analysis:** ANCOVA Example

- Example: Running ANCOVA
 - Two groups: 15 normal vs. 13 patients
 - Analysis: comparing the two groups
 - Running what test?
 - Two-sample t with 3dttest
 - Controlling age effect
 - GLM model
 - $Y_i = \beta_0 + \beta_1 X_{1i} + \beta_2 X_{2i} + \beta_3 X_{3i} + \epsilon_i, i = 1, 2, \dots, n (n = 28)$
 - Demean covariate (age) X_1
 - Code the factor (group) with a dummy variable
 - 0, when the subject is a patient;
 - 1, when the subject is normal.
 - With covariate X_1 centralized:
 - β_0 = effect of patient; β_1 = age effect (correlation coef); β_2 = effect of normal
 - $X_{3i} = X_{1i} X_{2i}$ models interaction (optional) between covariate and factor (group)
 - β_3 = interaction

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Cluster Analysis: Multiple testing correction

- Multiple testing problem in fMRI: voxel-wise statistical analysis
 - Increase of chance at least one detection is wrong in cluster analysis
 - 3 occurrences of multiple testings: individual, group, and conjunction
 - Group analysis is the most concerned
- Two approaches
 - Control **FWE**: $\alpha_{FW} = P(\geq \text{one false positive voxel in the whole brain})$
 - Making α_{FW} small but without losing too much power
 - Bonferroni correction too conservative: $p=10^{-8} \sim 10^{-6}$
 - Too stringent and overly conservative: Lose statistical power
 - Something to rescue? Correlation and structure!
 - Voxels in the brain are not independent
 - Structures in the brain
 - Control false discovery rate (FDR)
 - $FDR = \text{expected proportion of false + voxels among all detected voxels}$
 - Concrete example: individual voxel $p = 0.001$ for a brain of 25,000 EPI voxels
 - Uncorrected \rightarrow 25 false + voxels in the brain
 - FWE: corrected $p = 0.05 \rightarrow$ 5% false + hypothetical brains for a fixed voxel location
 - FDR: corrected $p = 0.05 \rightarrow$ 5% voxels in those positively labeled ones are false +

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Cluster Analysis: AlphaSim

- FWE: Monte Carlo simulations
 - Named for Monte Carlo, Monaco, where the primary attractions are casinos
 - Program: **AlphaSim**
 - Randomly generate some number (e.g., 1000) of brains with white noise
 - Count the proportion of voxels are false + in ALL brains
 - Parameters:
 - ROI - mask
 - Spatial correlation - FWHM
 - Connectivity - radius
 - Individual voxel significant level - uncorrected p
 - Output
 - Simulated (estimated) overall significance level (corrected p -value)
 - Corresponding minimum cluster size
 - Decision: Counterbalance among
 - Uncorrected p
 - Minimum cluster size
 - Corrected p

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Cluster Analysis: AlphaSim

- See detailed steps at <http://afni.nimh.nih.gov/sscc/gangc/mcc.html>
- Example


```
AlphaSim \
-mask MyMask+orig \
-fwmx 4.5 -fwmy 4.5 -fwmz 6.5 \
-rmm 6.3 \
-pthr 0.0001 \
-iter 1000
```

 - Program
 - Restrict correcting region: ROI
 - Spatial correlation
 - Connectivity: how clusters are defined
 - Uncorrected p
 - Number of simulations
- Output: 5 columns
 - Focus on the 1st and last columns, and ignore others
 - 1st column: minimum cluster size in voxels
 - Last column: alpha (α), overall significance level (corrected p value)

Cl Size	Frequency	Cum Prop	p/Voxel	Max Freq	Alpha
2	1226	0.999152	0.00509459	831	0.859
5	25	0.998382	0.00015946	25	0.137
10	3	1.0	0.00002432	3	0.03

 - May have to run several times with different uncorrected p : uncorrected $p \rightarrow$ cluster size

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Cluster Analysis: 3dFDR

- Definition:

$$FDR = \frac{N_{fa}}{D_a} = \frac{N_{fa}}{N_{fa} + N_{ta}}$$
 - proportion of false + voxels among all detected voxels
- Doesn't consider
 - spatial correlation
 - cluster size
 - connectivity
- Again, only controls the expected % false positives among declared active voxels
- Algorithm: statistic (t) \rightarrow p value \rightarrow FDR (q value) \rightarrow z score
- Example:


```
3dFDR -input 'Group+tlrc[6]' \
-mask_file mask+tlrc \
-cdep -list \
-output test
```

 - One statistic
 - ROI
 - Arbitrary distribution of p
 - Output

	Declared Inactive	Declared Active	
Truly Inactive	N_{di}	$N_{da}(I)$	T_i
Truly Active	$N_{di}(II)$	N_{da}	T_a
	D_i	D_a	

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• **Cluster Analysis:** FWE or FDR?

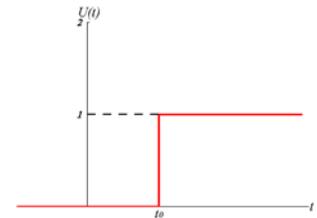
- Correct type I error in different sense
 - ↳ FWE: $\alpha_{FW} = P(\geq \text{one false positive voxel in the whole brain})$
 - Frequentist's perspective: Probability among **many** hypothetical activation brains
 - Used usually for parametric testing
 - ↳ FDR = **expected % false + voxels among all detected voxels**
 - Focus: controlling false + among detected voxels in **one** brain
 - More frequently used in non-parametric testing
- Fail to survive correction?
 - ↳ At the mercy of reviewers
 - ↳ Analysis on surface
 - ↳ Tricks
 - One-tail?
 - ROI – cheating?
 - ↳ Many factors along the pipeline
 - Experiment design: power?
 - Sensitivity (power) vs specificity (small regions)
 - Poor spatial alignment among subjects

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• **Cluster Analysis:** Conjunction analysis

- Conjunction analysis: HM vs TM
 - ↳ Common activation area
 - ↳ Exclusive activations
- Double/dual thresholding with AFNI GUI
 - ↳ Tricky
 - ↳ Only works for two contrasts
 - ↳ Common but not exclusive areas
- Conjunction analysis with **3dcalc**
 - ↳ Flexible and versatile
 - ↳ **Heaviside unit (step function)** defines a *On/Off* event

$$U(x - t_0) = \begin{cases} 1 & x \geq t_0 \\ 0 & x < t_0 \end{cases}$$



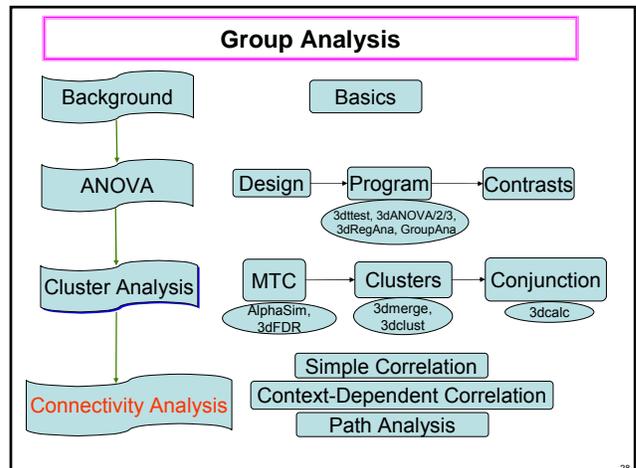
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• **Cluster Analysis:** Conjunction analysis

- Example with 3 contrasts: A, B, and C
 - ↳ Map 3 contrasts based on binary system: A: 001(1); B: 010(2); C: 100(4)
 - ↳ Create a mask with 3 subbricks of t (threshold = 4.2)


```
3dcalc -a func+tlrc'[5]' -b func+tlrc'[10]' -c func+tlrc'[15]' \
                    -expr 'step(a-4.2)+2*step(b-4.2)+4*step(c-4.2)' \
                    -prefix ConjAna
```
- ↳ Interpret output - 8 ($=2^3$) scenarios:
 - 000(0): none;
 - 001(1): A but no others;
 - 010(2): B but no others;
 - 011(3): A and B but not C;
 - 100(4): C but no others;
 - 101(5): A and C but not B;
 - 110(6): B and C but not A;
 - 111(7): A, B and C
- Downsides: no p associated with conjunctions and no MTC

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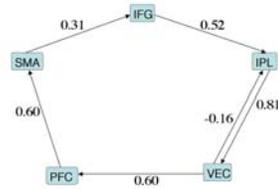
• **Connectivity: Correlation Analysis**

- Similarity between a seed and the rest of the brain
 - ↳ Says nothing about causality/directionality
 - ↳ Voxel-wise analysis
 - ↳ Both individual subject and group levels
- Steps at individual subject level
 - ↳ Extract seed time series: 3dmaskdump
 - ↳ Remove trend: 3dDetrend
 - ↳ Correlation analysis: 3dfim+ or 3dDeconvolve
- Steps at group level
 - ↳ Convert correlation coefficients to Z (Fisher transformation): 3dcalc
 - ↳ One-sample t test on Z scores: 3dttest
- More details: <http://afni.nimh.nih.gov/sscc/gangc/SimCorrAna.html>

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• **Connectivity: Path Analysis**

- Causal model approach on a network of ROI's
- Minimizing discrepancies
 - ↳ btw correlation based on data and one estimated from model



- Input: Model specification, correlation matrix, residual error variances, DF
- Output: Path coefficients, various fit indices

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• **Connectivity: Path Analysis – 1dSEM**

- AFNI program **1dSEM**
 - ↳ Written in C
 - ↳ Not dependent on FMRI analysis platform
- Two modes
 - ↳ Validate a theoretical model
 - Accept, reject, or modify the model?
 - ↳ Search for 'best' model
 - Start with a minimum model (can be empty): 1
 - Some paths can be excluded: 0
 - Model grows by adding one extra path a time: 2
 - 'Best' in terms of various fit criteria
- Script: `1dSEM -theta testthetasfull.1D -C testcorr.1D -psi testpsi.1D -DF 30`
- Caveats:
 - ↳ Causal relationship modeled through correlation (covariance) analysis
 - ↳ Valid only with the data and model specified
 - ↳ If one critical ROI is left out, things may go awry
- More details
 - ↳ <http://afni.nimh.nih.gov/sscc/gangc/PathAna.html>
 - ↳ 1dSEM -help

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• **Need Help?**

- ⊗ Command with "-help"
 - `3dANOVA3 -help`
- ⊗ Manuals
 - <http://afni.nimh.nih.gov/afni/doc/manual/>
- ⊗ Web
 - <http://afni.nimh.nih.gov/sscc/gangc>
- ⊗ Examples: HowTo#5
 - <http://afni.nimh.nih.gov/afni/doc/howto/>
- ⊗ Message board
 - <http://afni.nimh.nih.gov/afni/community/board/>
- ⊗ Appointment

➤ **Contact us @1-800-NIH-AFNI**

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