Group Analysis with AFNI Programs

* Major programs
  - `3dttest, 3dANOVA, 3dANOVA2, 3dANOVA3, 3dRegAna, AlphaSim, 3dFDR`
  - Matlab package (http://afni.nimh.nih.gov/sscc/gangc)
* Help documents
  - Type the command with "-help" at the prompt: `3dANOVA2 -help`
  - Manuals in pdf format (http://afni.nimh.nih.gov/afni/doc/manual/)
  - Website for group analysis (http://afni.nimh.nih.gov/sscc/gangc)
  - Example: HowTo#5 (http://afni.nimh.nih.gov/afni/doc/howto/)
  - AFNI message board (http://afni.nimh.nih.gov/afni/community/board/)
  - Books:
    o *Applied Linear Statistical Models* by Neter, Wasserman, and Kutner (4th Ed.)
    o *Applied Regression Analysis* by Draper and Smith (3rd Ed.)
    o *Design and Analysis: A researcher's handbook* by Keppel and Wickens (4th Ed.)
* Major steps of FMRI analysis
  - Pre-processing: spatial smoothing (`3dmerge`), temporal normalization (`3dcalc`)
  - Individual subjects' analyses: Deconvolution/Regression (`3dDeconvolve`)
  - Spatial normalization (`adwarp`)
  - Group analysis (`3dttest, 3dANOVA2, 3dRegAna`, …) ⟵ Today's topic
  - Post-analysis: Multiple comparison correction (`AlphaSim`,
  `3dFDR`), cluster analysis (`3dclust, 3dmerge`), conjunction analysis (`3dcalc`), …

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* **Data Preparation I: Spatial Smoothing**
  * Purposes – reduce noise and improve sensitivity: Spatial variability of fMRI activation and the Talairach transform can result in little or no overlap of function across subjects
    ❖ Mechanism: convolution - weighted average among neighboring voxels
    ❖ How much? Kernel size usually on the order of 2 voxels
    ❖ Not sure? Try and compare several different kernel sizes
    ❖ Downside: Loss of spatial resolution, specificity, and power, but a price to be paid with inter-subject anatomical alignments
  * **Before** or **after**?
    ❖ Usually done on time series before individual subject analysis
      ➢ Comparable to group analysis results
    ❖ Alternatively smooth coefficients/contrasts after individual analysis
      ➢ Only coefficients (% signal changes) are carried over to group analysis
  * Programs
    ❖ Volume data: `3dmerge` with the `-1blur_fwhm` option
    ❖ Surface data: `SurfSmooth`

- **Data Preparation II: Parameter Normalization**
  - ★ Parameters quantifying activation must be normalized before group comparisons.
    - ➥ Signal amplitude varies for different subjects, runs, scanning sessions, regressors, image reconstruction software, modeling strategies, etc.
    - ➥ Only meaningful to compare dimensionless numbers: percent signal change
  - ★ **Before** or **after**?
    - ➥ Normalize (scale) before individual subject analysis with `3dcalc`: $100\ s_i / b_i * c$
        - s_i = signal for i-th run
        - b_i = baseline estimate i-th run (output from `3dTstat -mean`)
        - c = multiplier of 1's or 0's: mask created with `3dAutomask -dilate`
      - ❖ Regression (β) coefficients out of `3dDeconvolve`: percent signal changes
      - ❖ More convenient for comparisons between individual and group results
    - ➥ Convert to percent signal change after individual subject analysis: $100\ \beta / b * c$
        - β = coefficient for a regressor (output from `3dDeconvolve`)
        - b = **averaged** baseline estimate across runs (output from `3dDeconvolve`)???
        - c = multiplier generated from running `3dAutomask -dilate`
      - ❖ Problematic if baselines vary a lot cross runs
    - ➥ Traditionally done before individual subject analysis
      - ❖ Normalize each run separately!
      - ❖ No difference on statistics, and little difference on % signal changes (underestimate)
      - ❖ Cautionary check: baseline constants should be close to (slightly less than) 100

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- **Data Preparation III: Data Concatenation**
  - ★ Run and session: difference vague
  - ★ For sessions (not scanned consecutively), run spatial normalization if concatenation is desirable
  - ★ **Concatenate** or **not**? 3 approaches, 3 purposes
    - ➥ Real concatenation (default): concatenate all runs of data before individual subject analysis
      - ❖ Concatenate all runs, but an event is treated the same across all runs
      - ❖ Normalize before individual subject analysis to avoid cross-run variability of base line
      - ❖ Can't test cross-run variability
    - ➥ No concatenation: don't concatenate before individual analysis
      - ❖ Analyze each run with same event modeled separately with `3dDeconvolve`
      - ❖ Only possible if there are multiple repeats of an event in each run with similar design across runs
      - ❖ More desirable if variability across runs is big
      - ❖ Test cross-run difference at group level with an extra factor of run
      - ❖ Can't test cross-run difference at individual level
    - ➥ Pseudo concatenation
      - ❖ Concatenate all runs, but same event is modeled separately for each run
      - ❖ Only possible there are multiple repeats of each event in each run with similar design across runs
      - ❖ More desirable if variability across runs is big
      - ❖ Most flexible: Can test cross-run/session difference in both individual and group analyses, or
      - ❖ Test cross-run/session at individual level, and bring averaged coefficients to group level

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- **Data Preparation IV: Co-Registration (AKA "Spatial Normalization")**
  - ★ Improve sensitivity: Group analysis on voxel-by-voxel basis; Must be aligned/defined over same domain
  - ★ **Before** or **after**?
    - ➥ Usually done after individual subject analysis
      - ❖ Keep close to the original data
      - ❖ Save huge runtime in `3dDeconvolve`
    - ➥ Can alternatively done before `3dDeconvolve`
      - ❖ More desirable for cross-session concatenation
      - ❖ Compare group results with individual activation maps
      - ❖ Before or after, no significant difference in terms of group analysis results
      - ❖ Convert to lower resolution with roughly the same resolution as EPI (`adwarp -dxyz`)
  - ★ Steps and programs
    - ➥ Volumetric data
      - ❖ Anatomical data transformation: manual (AFNI) or automatic (@auto_tlrc)
      - ❖ Transform functional data using AFNI interactively or `adwarp` (use option `-dxyz` with about the same resolution as EPI data — do *not* have to use the default 1 mm resolution!)
    - ➥ Surface data: Standard meshes and spherical coordinate system
      - ❖ Surface models of the cortical surface are warped to match a template surface using Caret/SureFit (**http://brainmap.wustl.edu**) or FreeSurfer (**http://surfer.nmr.mgh.harvard.edu**)
      - ❖ Standard-mesh surface models are then created with `SUMA` (**http://afni.nimh.nih.gov/ssc/ziad/SUMA**) to allow for node-based group analysis
    - ➥ Once data is aligned, analysis is carried out voxel-by-voxel or node-by-node
      - ❖ Analyses and results are on the percent signal changes
      - ❖ Display resulting statistics (voxel-wise or node-wise) AFNI and/or SUMA

- **Basics about null hypothesis significance testing (NHST)**
  - ★ Controversy: Are humans cognitively good intuitive statisticians?
  - ★ Quiz: HIV prevalence = 10^{-3}, false + of HIV test = 5%, power of HIV test = 100%.
    - ➥ P(HIV+ | test+) = ?
  - ★ Cohen, J., "The Earth Is Round (p < .05)" (1994), American Psychologist, 49, 12 997-1003
    - ➥ Ritualized dichotomous decisions around a sacred number 0.05
  - ★ H_0: no task effect or no response difference between two tasks at a voxel vs H_1: there is difference
  - ★ What does it mean with a dichotomous decision and rejecting H_0 at a significant level α (e.g., 0.05)?
    - ➥ **Conditional probability** P(result | H_0) = α, not $P(H_0)$!
    - ➥ 2 types of errors: type I and type II
      - ➢ Type I error = α, Type II error = β = P(accept H_0 | H_1); Power = P(accept H_1 | H_1) = 1 – β
      - ➢ Traditional strategy: control type I error while gaining power as much as possible
      - ➢ Importance of checking **efficiency** (power) of your design with `RSFgen` before scanning
      - ➢ Norman H. Anderson: "the main function of statistics is to get more information into the data."
    - ➥ Usual misinterpretations
      - ➢ Reject H_0 ==> Prove or confirm a theory (alternative hypothesis)!   (wrong!)
      - ➢ P(result | H_0) = $P(H_0)$ (wrong!)
      - ➢ P(result | H_0) = Probability if the experiment can be reproduced   (wrong!)
  - ★ Keep in mind
    - ➥ Better plan than sorry: Spend more time on experiment design (power analysis)
    - ➥ More appropriate for detection than sanctification of a theory
    - ➥ Try to avoid unnecessary overstatement when making conclusions
    - ➥ Present graphics and report percent signal change, standard deviation, confidence interval, …
    - ➥ Replications are the best strategy on induction/generalization

A researcher tested the null hypothesis that two population means are equal (H_0: $\mu_1 = \mu_2$). A *t*-test produced *p*=0.01. Assuming that all assumptions of the test have been satisfied, which of the following statements are true and which are false? Why?

  1. There is a 1% chance of getting a result even more extreme than the observed one when H_0 is true.
  2. There is a 1% likelihood that the result happened by chance.
  3. There is a 1% chance that the null hypothesis is true.
  4. There is a 1% chance that the decision to reject H_0 is wrong.
  5. There is a 99% chance that the alternative hypothesis is true, given the observed data.
  6. A small *p* value indicates a large effect.
  7. Rejection of H_0 confirms the alternative hypothesis.
  8. Failure to reject H_0 means that the two population means are probably equal.
  9. Rejecting H_0 confirms the quality of the research design.
10. If H_0 is not rejected, the study is a failure.
11. If H_0 is rejected in Study 1 but not rejected in Study 2, there must be a moderator variable that accounts for the difference between the two studies.
12. There is a 99% chance that a replication study will produce significant results.
13. Assuming H_0 is true and the study is repeated many times, 1% of these results will be even more inconsistent with H_0 than the observed result.

Adapted from Kline, R. B. (2004). Beyond significance testing. Washington, DC: American Psychological Association (pp. 63-69). Dale Berger, CGU 9/04

• **Basics of Group Analysis**
- ★ General Linear Model (GLM) and ANOVA
- ★ Factor and level
  - ➥ Dependant and independent variable
  - ➥ Factors: categorizing variables, e.g., subject category and stimulus class
  - ➥ Levels: nominal (qualitative) values of a factor, e.g., conditions 1, 2, and 3
- ★ Fixed/random factor
  - ➥ Fixed: effects/contrasts at specific levels are of interest
  - ➥ Random (usually subject in fMRI): each level of the factor is not of interest, but should model variance for generalizing the conclusion to whole population
- ★ Factorial (crossed)/nested (more specifically within-subject/between-subjects) design
- ★ Different terminology
  - ➥ count subject as a random factor (statisticians)
  - ➥ within-subject (repeated measures)/between-subjects   (psychologists)
- ★ Group analysis: partition/untangle data variability into various sources
  - ➥ Cell-mean (or structural) model of one-way within-subject ANOVA: $Y_{ij} = \mu + \alpha_i + \beta_j + \varepsilon_{ij}$
    - ○ Y_{ij} independent variable, percent signal change of subject *j* with task *i*;
    - ○ μ constant – grand/common mean;
    - ○ α_i constants subject to $\Sigma\alpha_i = 0$ – **simple effect** of factor A at level *i*, *i* = 1, 2, ..., *a*;
    - ○ β_j independent $N(0, \sigma_p^2)$ – **random effect** of subject *j*, *j* = 1, 2, ..., *b* (σ_p^2 - population variance);
    - ○ ε_{ij} independent $N(0, \sigma^2)$ – **interaction**: within-subject variability (σ^2 - variance of sampling error)
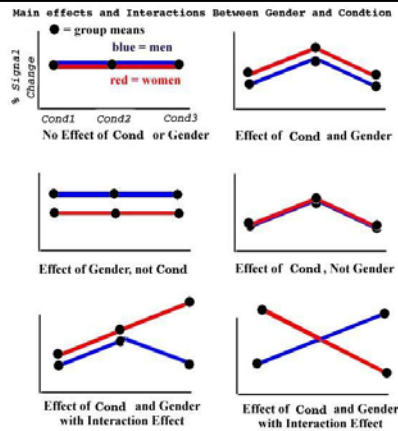
- **Basics of Group Analysis**
  - ★ Main/simple effect, interaction, and contrast
    - › Main effect: general info regarding a factor
    - › Simple effect: specific info regarding a factor level
    - › Disordinal interaction: differences reverse sign
    - › Ordinal interaction: one above another
    - › Contrast: comparison of 2 or more simple effects;
      coefficients add up to 0
  - ★ **Trend analysis**: Linear, quadratic or cubic effect
    - › Among stimuli (individual subject analysis)
    - › Among factor levels (group analysis)
    - › If **equally-spaced**, use the orthogonal polynomial coef

Main effects and Interactions Between Gender and Condtion

No Effect of Cond or Gender — Effect of Cond and Gender

Effect of Gender, not Cond — Effect of Cond , Not Gender

Effect of Cond and Gender with Interaction Effect — Effect of Cond and Gender with Interaction Effect

Main effects and interactions in 2-way mixed ANOVA

No. of Conditions/Levels	1	2	3	4	5	6
3 Linear		-1	0	1		
Quadratic		1	-2	1		
Linear		-3	-1	1	3	
4 Quadratic		1	-1	-1	1	
Cubic		-1	3	-3	1	
Linear		-2	-1	0	1	2
5 Quadratic		2	-1	-2	-1	2
Cubic		-1	2	0	-2	1
Linear		-5	-3	-1	1	3	5
6 Quadratic		5	-1	-4	-4	-1	5
Cubic		-5	7	4	-4	-7	5

  - › **If unequally-spaced**, contrast coefficients have to be specially constructed

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- **Overview of Statistical Testing of Group Datasets with AFNI programs**

  - ★ Non-parametric analysis
    - ➥ No assumption of normality
    - ➥ More appropriate when number of subjects too few
    - ➥ Tend to be less sensitive to outliers (more robust)
    - ➥ Programs: ranking
      - ❖ **3dWilcoxon** (~ paired *t*-test)
      - ❖ **3dMannWhitney** (~ two-sample *t*-test)
      - ❖ **3dKruskalWallis** (~3dANOVA)
      - ❖ **3dFriedman** (~3dANOVA2)
      - ❖ Permutation test
        plugin in AFNI under Define Datamode / Plugins / Permutation Test
    - ➥ Can't run complicated design types
    - ➥ Less sensitive and less flexible than parametric tests

- **Overview of Statistical Testing of Group Datasets with AFNI programs**
  - ★ Parametric Tests
    - ➥ Data normally and independently distributed (Gaussian) and sphericity assumption met
      - ❖ **3dttest** (one-sample, unpaired and paired) and **3dANOVA2** (one-way within-subject)
      - ❖ **3dANOVA, 3dANOVA3, 3dRegAna** (regression, unbalanced ANOVA, ANCOVA)
      - ❖ Matlab package = script for up to 5-way ANOVA
    - ➥ Sphericity: The roundness of an n-D object
      - ❖ Between-subject factors: homogeneity of variance
        - Heteroscedasticity? usually not too serious
      - ❖ Within-subject factors: homogeneity of level difference variances
        - A little more stringent but easier-to-verify assumption: compound symmetry

Tasks	A1	A2	A3	A4
A1	s_1^2	s_{12}	s_{13}	s_{14}
A2	s_{21}	s_2^2	s_{23}	s_{24}
A3	s_{31}	s_{32}	s_3^2	s_{34}
A4	s_{41}	s_{42}	s_{43}	s_4^2

      - ❖ Compound symmetry: homogeneity of variances; homogeneity of covariances/correlations
      - ❖ Compound symmetry → sphericity; not true the other direction, but rare
      - ❖ How serious is sphericity violation?
      - ❖ Sphericity test (under consideration)

- **Overview of Statistical Testing of Group Datasets with AFNI programs**
 - ★ Parametric Tests
    - ➥ Basic rule to avoid potential sphericity violation: **Simple effects and contrasts involving a portion of the data is tested exclusively based on that specific portion**
    - ➤ Variance: pooled vs. focused
    - ➥ The following 3 scenarios are immune to sphericity violation:
      - ➤ Simple effect: one-sample t test (**3dttest**) or one-way within-subject (**3dANOVA2**)
      - ➤ Contrast: one-sample, paired t test (**3dttest**) or one-way within-subject (**3dANOVA2**)
      - ➤ With 2 levels for all factors, no issue of sphericity violation in multi-way ANOVA
    - ➥ Multiple coefficients for each condition (IRF with basis functions including deconvolution)
      - ➤ "Area" Under the Curve (AUC) with t test
        - Simplifying: reduce multiple numbers to one for group H_0: $\Sigma c_i = 0$
      - ➤ Take all coefficients to group analysis: A possible new option of F test in **3dANOVA2**
        - H_0: $c_1 = 0$, $c_2 = 0$, ..., $c_k = 0$
      - ➤ Comparisons between the 2 approaches
        - AUC is better:
          - Simple and easy for group analysis, especially important in multi-ANOVA
          - Effect directionality with t test (F doesn't indicate any direction)
          - Available now in both individual and group analysis
        - Downside of AUC:
          - How to interpret the "area" in cases of other basis functions: tent, gamma, Fourier, etc.?
          - Usually ignore head and tail (undershoot) of IRF
            - Assuming similar shape of IRF across brain and across subjects
            - Bias on some specific interval of IRF: not all coefficients treated equally
        - Both vulnerable to sphericity departure?
          - Homogeneity of correlation: Similarly sequentially-correlated
          - Homogeneity of variance

Overview of Statistical Testing of Group Datasets with AFNI programs

- ★ Parametric Tests
  - ➢ What are the differences between the two approaches?
    - ❖ Rejection areas slightly different: 2 non-overlapping areas
      - I and III quadrants: smaller p value with AUC (Var(sum)>average Var)
      - II and IV quadrants): bigger p value with AUC (Var(sum)>average Var)
      - Only along the two axes, the 2 approaches match
  - ➢ AUC is good for direction while F-test is better for subtle difference. Ideally run both.
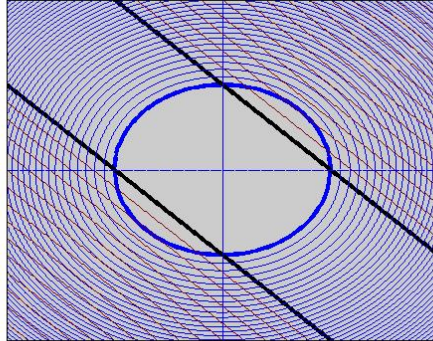
_t_-Test: `3dttest`

- ★ Student's _t_ distribution, developed by Gossett, tests if the mean of a set of data is different from a constant (usually 0) or the mean of another set of data.
- ★ 3 usages: one-sample, two-sample, and paired _t_ test.
- ★ Assumptions
  - ➥ Values in each set are normally distributed with equal variance
  - ➥ Sphericity issue? No issue of sphericity violation
  - ➥ Values in each set are independent? Compare a task between 2 groups: _unpaired_ _t_-test
  - ➥ Values in each set are dependent? Compare two tasks for one group: _paired_ _t_-test
- ★ Example: effects of 2 conditions _A_ and _B_ at group level
  - ➥ Case 1: 15 subjects were given condition _A_
    - ❖ **One-sample** _t_ test: effect of A is significant at group level $\mu_A = 0$?
  - ➥ Case 2: 15 subjects under condition _A_ and 13 other subjects under _B_
    - ❖ **Two-sample** _t_ test: $\mu_A = \mu_B$ (average response is the same?)
    - ❖ OK with unequal sample sizes
    - ❖ Equivalent to 1-way between-subject ANOVA
  - ➥ Case 3: 15 subjects under both conditions
    - ❖ **Paired** _t_ test is used to test: $\mu_A = \mu_B$?
    - ❖ Equivalent to one-way within-subject (`3dANOVA2 -type 3`)
    - ❖ Equivalent to run one-sample _t_ test on individual differences/contrasts

- **t-Test**: `3dttest`
  - ★ Example: 2-way (2X2) within-subject design (16 subjects) - AXBXC
    - ➢ 4 tasks (word, picture, spoken, sound) categorized as the following:
      - Modality: visual, auditory
      - Format: verbal, nonverbal
      - visual verbal = word, visual nonverbal = picture,
      - auditory verbal = spoken, auditory nonverbal = sound
    - ➢ What is the word or word-picture effect at group level?
      - H_0: word = 0, or H_0: word-picture = 0 (one-sample t test!)
    - ➢ `3dANOVA3 -type 4` would not work because of lack of 2nd contrast testing option
    - ➢ Can also run GroupAna
    - ➢ Script of paired t test for H_0: word-picture = 0

    ```
    3dttest -prefix Wd-Pic                        \       Name of output dataset
    -paired                                       \       We want a paired t test
    -set1 Wd_S1+tlrc ... Wd_S16+tlrc              \       Input of first datasets
    -set2 Pic_S1+tlrc ... Pic_S16+tlrc \                  Input of second datasets
    ```

    - ➢ Can also run a one-sample t test for H_0: word-picture = 0
    - ➢ Picture/spoken/sound/ or spoken-sound/word-spoken/picture-sound effect? (quiz!)

- **1-Way ANOVA**: `3dANOVA`
  - ★ Generalization of two-sample t-test
    - ➥ One-way between-subject
    - ➥ Null hypothesis H_0: no difference across all the factor levels (groups)
    - ➥ Examples of *factor*: subject group such as gender, age group, genotype, disease, *etc.*
    - ➥ OK with unequal sample sizes
  - ★ Assumptions
    - ➥ Values are normally distributed with equal variances across levels (groups)
    - ➥ Unlike regression analysis, no assumptions about relationship between dependent and independent variables (e.g., not necessarily linear)
    - ➥ Independent variable (e.g., gender, disease, genotype, etc.) is qualitative
  - ★ `3dANOVA` versus `3dttest`
    - ➥ Equivalent when there are only two levels
    - ➥ More than 2 levels
      - ❖ Can still run two-sample t-test on paired levels with `3dttest` to obtain contrasts
      - ❖ Results should be similar unless there is significant heteroscedasticity across levels
      - ❖ Pooled variance vs. between-levels variance
    - ➥ `3dttest` is better if heteroscedasticity is significant across groups
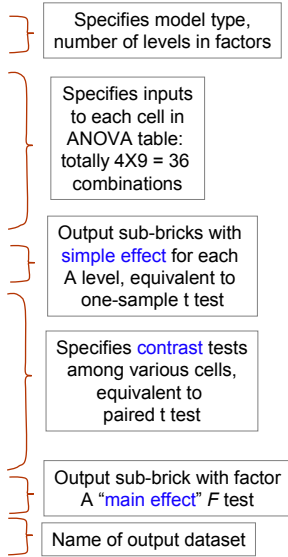
- **2-Way ANOVA**: **3dANOVA2**
  - ★ 3 design types, most frequently used design: **3dANOVA2** –type 3 (one-way within-subject)
    - ➥ Extension of paired *t* test
    - ➥ No concern of sphericity violation for simple effect and contrast testing
  - ★ Example: **3dANOVA2 script**

```
3dANOVA2 -type 3 -alevels 4 -blevels 9              \

-dset 1 1 ED+tlrc'[0]' -dset 2 1 ED+tlrc'[1]'       \
-dset 3 1 ED+tlrc'[2]' -dset 4 1 ED+tlrc'[3]'       \
-dset 1 2 EE+tlrc'[0]' -dset 2 2 EE+tlrc'[1]'       \
-dset 3 2 EE+tlrc'[2]' -dset 4 2 EE+tlrc'[3]'       \
…
-dset 1 9 FN+tlrc'[0]' -dset 2 9 FN+tlrc'[1]'       \
-dset 3 9 FN+tlrc'[2]' -dset 4 9 FN+tlrc'[3]'       \

-amean 1 TM -amean 2 HM -amean 3 TP  -amean 4 HP    \

-acontr 1 1 1 1 AllAct                              \
-acontr -1 1 -1 1 HvsT                              \
-acontr 1 1 -1 -1 MvsP                              \
-acontr 0 1 0 -1 HMvsHP                             \
-acontr 1 0 -1 0 TMvsTP                             \
-acontr 0 0 -1 1 HPvsTP                             \
-acontr -1 1 0 0  HMvsTM                            \
-acontr  1 -1 -1 1 Inter                            \

-fa StimEffect                                      \

-bucket AvgANOVA
```

	Specifies model type, number of levels in factors
	Specifies inputs to each cell in ANOVA table: totally 4X9 = 36 combinations
	Output sub-bricks with simple effect for each A level, equivalent to one-sample t test
	Specifies contrast tests among various cells, equivalent to paired t test
	Output sub-brick with factor A "main effect" *F* test
	Name of output dataset

- **3-Way ANOVA**： 3dANOVA3
  - ★ Most frequently used designs
    - ➥ Type 4 (two-way within-subject) - crossed design (AXBXC)：generalization of paired *t*-test
    - ➥ Type 5 (two-way mixed) - classification factor A can't be varied within a subject: BXC(A)
  - ★ Has new concept: *nested design* (vs. fully crossed design) or between-subjects factor
    - ➥ Nested design: each level of one factor contains a unique set of levels of the other
  - ★ Example
    - ➥ Design type 5 BXC(A)
      - ❖ factor A = subject type; level #1=wild type P, #2=genotype Q, #3=genotype R
      - ❖ factor B = stimulus type; levels #1–4=different types of tasks
      - ❖ factor C = subject; 30 different subjects, 10 for each genotype; C is "nested" inside A
    - ➥ Mixture of unpaired and paired tests with one between-subject and one within-subject
      - ❖ Paired across stimulus type (factor B) – within-subject (repeated-measures) factor
      - ❖ Unpaired across subject types (factor A levels) - between-subjects
  - ★ Limitation: no 2nd-order contrasts; balanced design for type 5 (mixed design)
    - ➔ Matlab package GroupAna

- **4,5-Way ANOVA**
  - ★ Multi-ANOVA
    - ➥ Test for interactions
    - ➥ Difficult to test and interpret simple effects/contrasts
  - ★ Interactive Matlab script: http://afni.nimh.nih.gov/sscc/gangc
  - ★ Requires Matlab plus its Statistics Toolbox (pricey)
  - ★ GLM approach, different from programs 3dANOVAx
  - ★ Heavy duty computation: minutes to hours
    - ➥ input with lower resolution recommended
    - ➥ use `adwarp -dxyz #` and `3dresample`
  - ★ Same script can also run 1,2,3-way ANOVA
  - ★ Includes contrast tests across all factors
  - ★ Can handle both volume and surface data
  - ★ Can handle the following unbalanced designs (two-sample *t* test type):
    - ➥ 3-way ANOVA type 3: BXC(A)
    - ➥ 4-way ANOVA type 3: BXCXD(A)
  - ★ See http://afni.nimh.nih.gov/sscc/gangc for more info

5 Design Types of 4-Way ANOVA

$A_F \times B_F \times C_F \times D_F$ All factors fixed; fully crossed	A,B,C,D=stimulus category, drug treatment, etc. All combinations of subjects and factors exist; Multiple subjects: treated as multiple measurements; One subject: longitudinal analysis
☼ $A_F \times B_F \times C_F \times D_R$ Last factor random; fully crossed	A,B,C=stimulus category, etc. D=subjects, random Fully crossed design
☼ $B_F \times C_F \times D_R(A_F)$ Last factor random, and nested within the first (fixed) factor	A=subject class: genotype, sex, or disease B,C=stimulus category, etc. D=subjects nested within A levels
$B_F \times C_R \times D_F(A_F)$ Third factor random; fourth factor fixed and nested within the first (fixed) factor	A=stimulus type (*e.g.*, repetition number) B=another stimulus category (*e.g.*, animal/tool) C=subjects (a common set among all conditions) D=stimulus subtype (*e.g.*, perceptual/conceptual)
$C_F \times D_R(A_F \times B_F)$ Doubly nested!	A, B=subject classes: genotype, sex, or disease C=stimulus category, etc. D=subjects, random with two distinct factors dividing the subjects into finer sub-groups (*e.g.*, A=sex × B=genotype)

3 Design Types of 5-Way ANOVA

$A_F \times B_F \times C_F \times D_F \times E_F$ All factors fixed; fully crossed	A,B,C,D,E=stimulus category, drug treatment, etc. All combinations of subjects and factors exist; Multiple subjects: treated as multiple measurements; One subject: longitudinal analysis
☼ $A_F \times B_F \times C_F \times D_F \times D_R$ Last factor random; fully crossed	A,B,C,D=stimulus category, etc. E=subjects, random Fully crossed design
☼ $B_F \times C_F \times D_F \times E_R(A_F)$ Last factor random, and nested within the first (fixed) factor	A=subject class: group, genotype, sex, or disease B,C,D=stimulus category, etc. E=subjects nested within A levels

•A real example with 5-way mixed design (neural mechanism for category-selective response):
  ▪Factors
      ➢Task (between-subject): semantic decision, naming
      ➢Modality: visual, auditory
      ➢Format: verbal, nonverbal
      ➢Category: animal, tool
      ➢Subject (random)
  ▪4 stimuli (2X2) for animal and tool - visual verbal = word, visual nonverbal = picture,

  auditory verbal = spoken, auditory nonverbal = sound

  ▪4-way mixed design: Only 2 levels for all 3 within-subject factors: no sphericity violation

---

>> **GroupAna**

How many factors? 5
Available design types:
   Type 1: Factorial (crossed) design AXBXCXDXE - all 5 factors are fixed.
   Type 2: Factorial (crossed) design AXBXCXDXE - only factor E is random. If E is subject it is also
        called 4-way design with all 4 factors A, B, C and D varying within subject.
   Type 3: Mixed design BXCXDXE(A)- only the nested (5th) factor E (usually subject) is random.
        Also called 4-way design with factors B, C and D varying within subject and factor A between subjects.
Choose design type (1, 2, 3, 4, 5): 3
Is the design balanced? (1 - Yes; 0 - No) 1
Label for No. 1 factor: task
  How many levels does factor A (task) have? 2
  Label for No. 1 level of factor A (task) is: sem
  Label for No. 2 level of factor A (task) is: nam
Label for No. 2 factor: mod
  How many levels does factor B (mod) have? 2
  Label for No. 1 level of factor B (mod) is: vis
  Label for No. 2 level of factor B (mod) is: aud
Label for No. 3 factor: format
  How many levels does factor C (format) have? 2
  Label for No. 1 level of factor C (format) is: dir
  Label for No. 2 level of factor C (format) is: ind
Label for No. 4 factor: cat
  How many levels does factor D (cat) have? 2
  Label for No. 1 level of factor D (cat) is: animal
  Label for No. 2 level of factor D (cat) is: tool
Label for No. 5 factor: subj
  How many levels does factor E (subj) have? 16
  Label for No. 1 level of factor E (subj) is: S1
  ......
  All input files are supposed to contain only one subbrik.
  There should be totally 256 input files. Correct? (1 - Yes; 0 - No) 1
(1) factor combination:
    factor A (task) at level 1 (sem)
    factor B (mod) at level 1 (vis)
    factor C (format) at level 1 (dir)
    factor D (cat) at level 1 (animal)
    factor E (subj) at level 1 (S1)
    at repeat 1
is: ss03.a_word+tlrc.BRIK

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- **ANCOVA: Analysis of Covariance**
  - ★ Subjects might not be an ideally randomized representation of a group
    - ➥ Such uncontrollable variables called covariates
    - ➥ Typical covariates in fMRI: age, behavioral data (response time), cortex thickness, …
    - ➥ If no controlled, analysis would be confounded by those covariates
  - ★ Indirect (statistical) control by untangling covariate effect: ANCOVA = Regression + ANOVA
  - ★ Assumption: Linear relation between percent signal change and the covariate
  - ★ Try to avoid multi-way ANCOVA and analyze partial data with a simple one-way ANCOVA
    - ➥ Counterpart of a one-sample or two-sample t test
    - ➥ **Centralize** your covariate first so that it would not confound with other effects
  - ★ Example: Running ANCOVA
    - ➥ Two groups: 15 normal vs. 13 patients
    - ➥ Model $Y_i = \beta_0 + \beta_1 X_{1i} + \beta_2 X_{2i} + \beta_3 X_{3i} + \varepsilon_i$, i = 1, 2, ..., n
    - ➥ Remove the mean from the covariate X_1 first!
    - ➥ Code the factor (group) with a dummy variable

      $X_{2i} = \{$

            0, when the subject is a patient;

            1, when the subject is normal.
    - ➥ If covariate X_1 is centralized, β_0 reflects the effect of patient group
    - ➥ $X_{3i} = X_{1i} X_{2i}$ models interaction (optional) between covariate and factor (group)

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- **ANCOVA : Analysis of Covariance**

  *3dRegAna -rows 28 -cols 3 * ⊐— Specifies parameters

  *-workmem 1000 * ⊐— Specifies RAM = 1GB

  *-xydata 0.1 0 0 patient/Pat1+tlrc.BRIK *
  *-xydata 7.1 0 0 patient/Pat2+tlrc.BRIK *
  …
  *-xydata 7.1 0 0 patient/Pat13+tlrc.BRIK *   Specifies covariates, factor levels,
  *-xydata 2.1 1 2.1 normal/Norm1+tlrc.BRIK *   interaction, and input files
  *-xydata 2.1 1 2.1 normal/Norm2+tlrc.BRIK *
  …
  *-xydata -8.9 1 -8.9 normal/Norm14+tlrc.BRIK *
  *-xydata 0.1 1 0.1 normal/Norm15+tlrc.BRIK *

  *-model 1 2 3 : 0 * ⊐— Specifies reduced model for F and R^2

  -bucket 0 Pat_vs_Norm * ⊐— Specifies output format: Total subbriks = 2#coef + F + R^2

  *-brick 0 coef 0 'Pat' *
  *-brick 1 tstat 0 'Pat t' *
  *-brick 2 coef 1 'Age Effect' *
  *-brick 3 tstat 1 'Age Effect t' *   Labels output subbriks
  *-brick 4 coef 2 'Norm-Pat' *
  *-brick 5 tstat 2 'Norm-Pat t' *
  *-brick 6 coef 3 'Interaction' *
  -brick 7 tstat 3 'Interaction t'

  See http://afni.nimh.nih.gov/sscc/gangc/ANCOVA.html for more information

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- **Multiple comparison correction: `AlphaSim`**
  - ⋆ 2 types of errors in statistical tests
    - ↪ What is H_0 in FMRI studies?
    - ↪ Type I = P (reject H_0|when H_0 is true) = false positive = so-called *p* value
      Type II = P (accept H_0|when H_1 is true) = false negative = β
      (power = 1- β = probability to detect true activation)
    - ↪ Significance level = α: $p < \alpha$
    - ↪ Dilemma: Usual strategy – controlling type I error (similar to the legal system)
  - ⋆ Sex ratio at birth as an example for multiple comparison issue
  - ⋆ Almost all statistical analyses in AFNI are done voxel-wise ➜ multiple comparison problem: the increase of the chance that at least one detection is wrong in cluster analysis
    - ↪ 3 occurrences of multiple comparisons: individual, group, and conjunction
      - ➢ Group analysis is the most concerned
    - ↪ For an ROI of *n* **independent** voxels of no activation, the chance to mistakenly label at least one active voxel: $\alpha_{FW} = 1-(1-\alpha)^n \sim n\alpha$ ➜ α_{FW} becomes big as *n* increases
    - ↪ **multiple comparison correction** - controlling the severity of family-wise error: making α_{FW} reasonably small without losing too much power!
      - ➢ Bonferroni correction: to achieve an overall significance level of α, take α/n as individual significance level ➜ $\alpha_{FW} = \alpha$
      - ➢ Bonferroni correction is too stringent and overly conservative for FMRI analysis ➜ not many voxels can survive ➜ lose statistical power, failing to detect true activations!
      - ➢ Assumption of independence vs. brain structure

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- **Multiple comparison correction**: `AlphaSim`
  - ★ Monte Carlo simulation: **`AlphaSim`**
    - ➥ Named for Monte Carlo, Monaco, where the primary attractions are casinos
    - ➥ Randomly generates values for uncertain variables over and over to simulate a model for data in EPI resolution
    - ➥ Obtain simulated (estimated) overall significance level (corrected *p*-value) for a minimum cluster size and individual voxel significance level: counterbalance among all 3!
    - ➥ Should be done in original space
  - ★ General steps
    - ➥ Create mask or ROI ➔ reducing voxel number avoids unnecessary sacrifice and leads to relaxation of cluster threshold; masking always recommended, ROI for small regions such as amygdala
    - ➥ Obtain spatial correlation: number in option **`-1blur_fwhm`** of **`3dmerge`**
    - ➥ Calculate connectivity radius: Consider EPI (not tlrc) resolution; e.g., use a number slightly larger than the diagonal length of a voxel
    - ➥ Example:
      ```
      AlphaSim \
      -mask brain_mask+tlrc \
      -fwhmx 10 -fwhmy 10 -fwhmz 8 \
      -rmm 8.1 -pthr 0.005 -iter 1000 \
      -out AlphaSim_out
      ```

- **Multiple (simultaneous) comparison correction**: `AlphaSim`
  - ★ Output – 5 columns
    - ➥ Cluster size: in voxels
    - ➥ Frequency: number of clusters for a specific cluster size
    - ➥ Cumulative probability
    - ➥ p/Voxel: ignore it!
    - ➥ Maximum frequency: occurrences of maximum cluster with the specified size
    - ➥ Alpha (α): overall significance level (corrected *p* value)
  - ★ Example

Cl Size	Frequency	Cum Prop	p/Voxel	Max Freq	Alpha
1	1826	0.984897	0.00509459	831	0.859
2	25	0.998382	0.00015946	25	0.028
3	3	1.0	0.00002432	3	0.003

- **False Discovery Rate:** `3dFDR`
  - ★ FDR = $N_{ia}/D_a = N_{ia}/(N_{aa}+N_{ia})$ – proportion of voxels declared as active but truly inactive
  - ★ Does not consider cluster size and connectivity
  - ★ Does not control the chance of *any* false positives
  - ★ Only controls the **expected** percent of false positives among declared active voxels
  - ★ More lenient and sensitive than other correction methods
  - ★ Algorithm: statistic ➔ *p* value ➔ FDR (*q* value) ➔ z score
  - ★ Example:
    ```
    3dFDR -input 'Group+tlrc[6]'          \
        -mask_file mask+tlrc -mask_thr 1   \
        -cdep -list                        \
        -output test
    ```
  - ★ Index   p-value   q-value   z-score

    2821453   0.000001   0.004033   2.875584

    2852202   0.000003   0.005840   2.756648

    2355533   0.001513   0.564017   0.576886

    ......

	Declared Inactive	Declared Active	
Truly Inactive	N_{ii}	N_{ia} (I)	T_i
Truly Active	N_{ai} (II)	N_{aa}	T_a
	D_i	D_a	

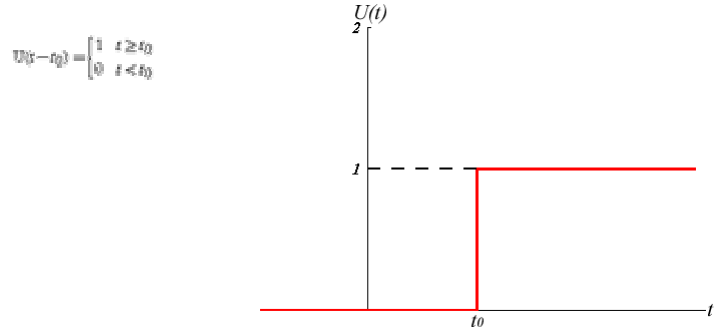- **Conjunction Analysis: What's Your Function?**

  - ★ `3dcalc`: a general purpose program for performing logic and arithmetic calculations
    - ↳ Command line is of the format

      ```
      3dcalc -a Dset1 -b Dset2 ... -expr "(a * b ...)"
      ```

      | values from `Dset1` are to be called '`a`' in `-expr` |

      | mathematical expression combining input dataset values |

    - ↳ The **Heaviside Unit Step Function** defines functions encountering ideal *On/Off*:

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

- **Conjunction Analysis: What's Your Function?**
  - ➥ Some expressions can be used to select voxels with values *v* meeting certain criteria:
    - ❖ Find voxels where $v \geq th$ and mark them with value=1 expression = *step (v – th)*
    - ❖ In a range of values: $th_{min} \leq v \leq th_{max}$ expression = *step (v – th_{min}) * step (th_{max} - v)*
    - ❖ Exact value: $v = n$ expression = **equals***(v – n)*
  - ➥ Create masks to apply to statistical (*t* or *F*) subbriks
    - ❖ Two values both above threshold (*e.g.* "conjunction") expression = *step(v-A)*step(w-B)*
  - ★ Example of conjunction analysis with 3 contrasts: A vs D, B vs D, and C vs D
    - ➥ Map 3 contrasts to 3 numbers: A > D: 1; B > D: 2; C > D: 4
    - ➥ Create a mask with 3 *t* statistical subbriks (with *t* threshold of 4.2):

      ```
      3dcalc -a func+tlrc'[5]' -b func+tlrc'[10]' -c func+tlrc'[15]' -expr
      'step(a-4)+2*step(b-4)+4*step(c-4)' -prefix cond_mask
      ```
    - ➥ 2^3 = 8 possible combinations:

      0 – none;

      1 - A > D but no others;

      2 - B > D but no others;

      3 - A > D and B > D but not C > D;

      4 - C > D but no others;

      5 - A > D and C > D but not B > D;

      6 - B > D and C > D but not A > D;

      7 – all 3 contrasts