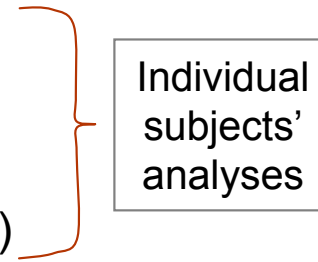# Group Analysis with AFNI Programs

- **Introduction**
  - Most of the material and notations are from Doug Ward's manuals for the programs `3dttest`, `3dANOVA`, `3dANOVA2`, `3dANOVA3`, and `3dRegAna`
  - Documentation available with the AFNI distribution
    - Lots of stuff (theory, examples) therein
  - Doug Ward's software and documentation files are based on these books:
    - *Applied Linear Statistical Models* by Neter, Wasserman, and Kutner (4th edition)
    - *Applied Regression Analysis* by Draper and Smith (3rd edition)
  - General steps
    - Smoothing (`3dmerge -1blur_fwhm`)
    - Normalization (`3dcalc`)
    - Deconvolution/Regression (`3dDeconvolve`)

      > Individual subjects' analyses
    - Co-registration of individual analyses to common "space" (`adwarp -dxyz`)
    - Group analysis (`3dttest`, `3dANOVA`, …)
    - Post-analysis (`AlphaSim`, conjunction analyses, …)
    - Interpretation

- **Data Preparation: Spatial Smoothing**
  - Spatial variability of both FMRI and the Talairach transform (the common space) can result in little or no overlap of function between subjects.
    - Data smoothing is used to reduce this problem.
      - Leads to loss of spatial resolution, but that is a price to be paid with the Talairach transform (or any current technique that does inter-subject anatomical alignments)
    - In principle, smoothing should be done on time series data, before data fitting (*i.e.*, before `3dDeconvolve` or `3dNLfim`, etc.)
      - Otherwise one has to decide on how to smooth statistical parameters.
        - In statistical data sets, each voxel has a multitude of different parameters associated with it like a regression coefficient, t-statistic, F-statistic, etc.
        - Combining some statistical parameters across voxels would result in parameters with unknown distributions
      - It is OK to blur percent signal change values that come out of the regression analysis, since these numbers depend linearly on the input data (unlike the F- and t-statistics)
      - Blurring in 3D is done using `3dmerge` with the `-1blur_fwhm` option
      - Blurring on the surface is done with program `SurfSmooth`

- **Data Preparation: Parameter Normalization**
  - Parameters quantifying activation must be normalized before group comparisons.
    - FMRI signal amplitude varies for different subjects, runs, scanning sessions, regressors, image reconstruction software, modeling strategies, etc.
  - Amplitude measures (regression coefficients) can be turned to percent signal change from baseline (do it before the individual analysis in `3dDeconvolve`).
    - Equations to use with `3dcalc` to calculate percent signal change
      - $100\ b_i / b_0$ (basic formula)
      - $100\ b_i / b_0 * c$ (mask out the outside of the brain)
        - b_i = coefficient for regressor i (output from `3dDeconvolve`)
        - b_0 = baseline estimate (output from `3dTstat -mean`)
        - c = threshold value generated from running `3dAutomask -dilate`
    - This will be included into `3dDeconvolve` in a future release
  - Other normalization methods, such as z-score transformations of statistics, can also be used.

- **Data Preparation: Co-Registration (AKA "Spatial Normalization")**
    - Group analyses are performed on a voxel-by-voxel basis
    - All data sets used in the analysis must be aligned and defined over the same spatial domain.
        - Talairach domain for volumetric data
            - Landmarks for the transform are set on high-res. anatomical data using AFNI
            - Functional data volumes are then transformed using AFNI interactively or `adwarp` from command line (use option `-dxyz` with about the same resolution as EPI data — do *not* use the default 1 mm resolution!)
        - Standard meshes and spherical coordinate system for surface data
            - 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 using AFNI's programs
        - Once data is aligned, analysis is carried out voxel-by-voxel or node-by-node
            - The percent signal change from each subject in each task/stimulus state are usually the numbers that will be compared and contrasted

- **Overview of Statistical Testing of Group Datasets with AFNI programs**
  - Parametric Tests:
    - Assume data are normally distributed (Gaussian)
      - `3dttest` (paired, unpaired)
      - `3dANOVA` (or `3dANOVA2` or `3dANOVA3`)
      - `3dRegAna` (regression, unbalanced ANOVA, ANCOVA)
      - Matlab script for one-, two-, three- and four-way ANOVA (still under development)
  - Non-parametric analyses:
    - No assumption of normality
    - Tends to be less sensitive to outliers (more robust)
      - `3dWilcoxon` (~*t*-test paired)
      - `3dMannWhitney` (~*t*-test unpaired)
      - `3dKruskalWallis` (~`3dANOVA`)
      - `3dFriedman` (~`3dANOVA2`)
      - Permutation test
    - Less sensitive and less flexible than parametric tests
    - In practice, seems to make little difference
      - Probably because number of datasets and subjects is usually small

- **_t_-Tests** [starting easy, but contains most of the ideas]
  - Program `3dttest`
    - Used to test if the mean of a set of values is significantly different from a constant (usually 0) or the mean of another set of values.
  - Assumptions
    - Values in each set are normally distributed
    - Equal variance in both sets
    - Values in each set are independent $\Rightarrow$ _unpaired_ _t_-test
    - Values in each set are dependent $\Rightarrow$ _paired_ _t_-test
  - Example: 20 subjects are tested for the effects of 2 drugs _A_ and _B_
    - Case 1: 10 subjects were given drug _A_ and the other 10 subjects given drug _B_.
      - Unpaired _t_-test is used to test: $m_A = m_B$? (mean response is different?)
      - Equivalent to one-way ANOVA with between-subjects design of equal sample size $\Rightarrow$ can also run `3dANOVA` (treating subjects as repeated measures)
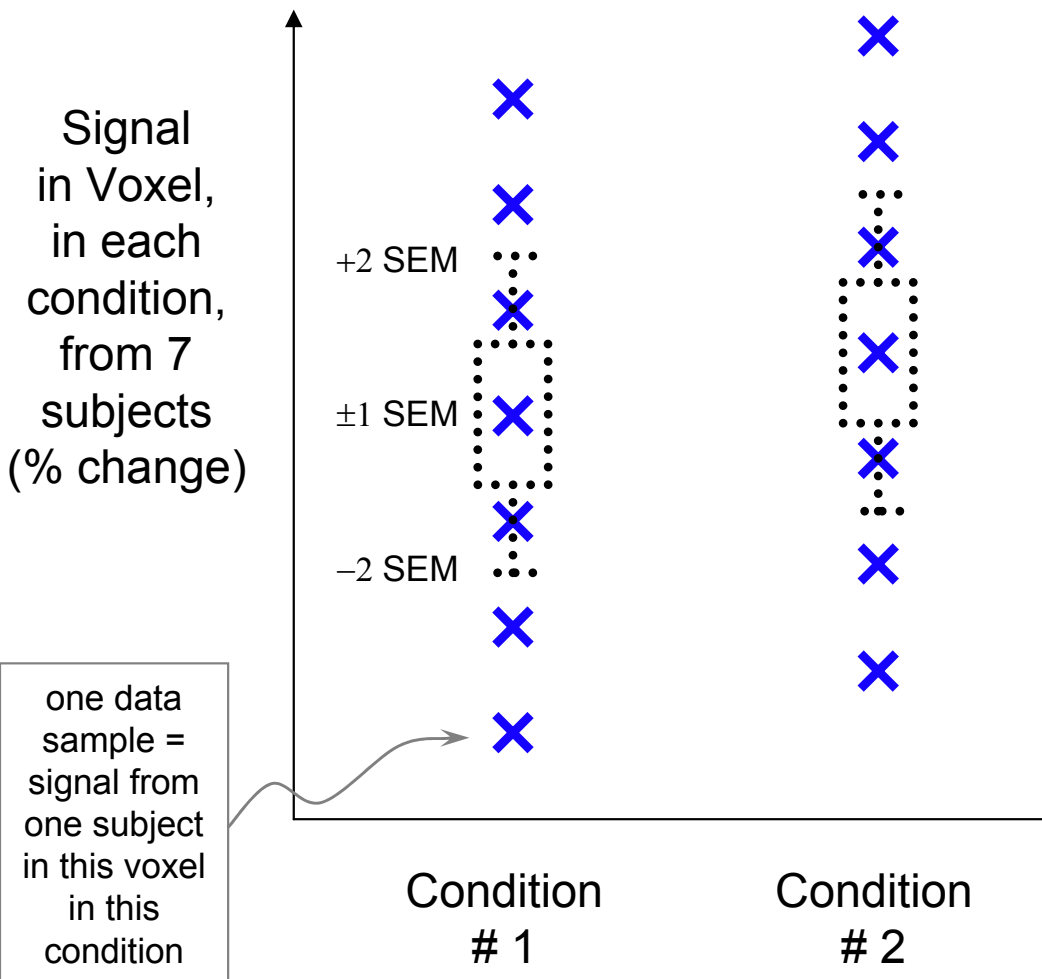   - Case 2: 20 subjects were given both drugs at different times.
      - Paired _t_-test is used to test: $m_A = m_B$?
    - Case 3: 20 subjects were given drug _A_.
      - _t_-test is used to test if drug effect is significant at group level: $m_A = 0$?
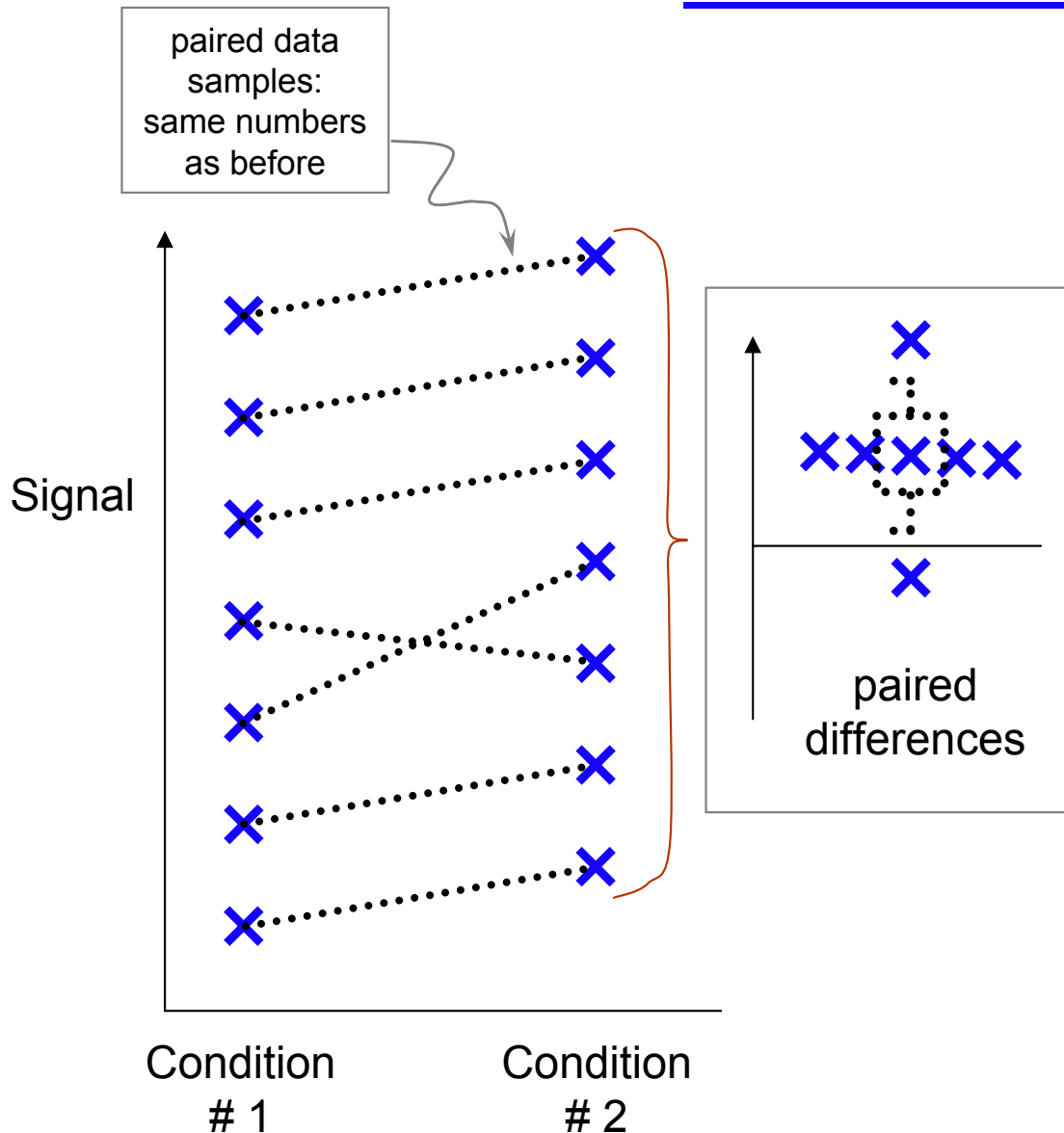
Unpaired 2 Sample *t*-Test: Cartoon

Signal in Voxel, in each condition, from 7 subjects (% change)

+2 SEM

±1 SEM

−2 SEM

one data sample = signal from one subject in this voxel in this condition

Condition # 1

Condition # 2

• Condition = some way to categorize data (*e.g.*, stimulus type, drug treatment, day of scanning, subject type, …)

• SEM = Standard Error of the Mean = standard deviation of sample divided by square root of number of samples = estimate of uncertainty in sample mean

• Unpaired *t*-test determines if sample means are "far apart" compared to size of SEM

• Not <u>significantly</u> different!

Paired *t*-Test: Cartoon

paired data samples: same numbers as before

Signal

Condition # 1   Condition # 2

paired differences

• <u>Paired</u> means that samples in different conditions should be linked together (*e.g.*, from same subjects)

• Test determines if differences between conditions in each pair are "large" compared to SEM of the differences

• Paired test can detect systematic *intra*-subject differences that can be hidden in *inter*-subject variations

• <u>Lesson</u>: properly separating ***inter***-subject and ***intra***-subject signal variations can be very important!

• Significantly different!
• Condition #2 > #1, per subject

- **1-Way ANOVA**
  - Program `3dANOVA`
    - Determine whether treatments (levels) of a single factor (independent parameter) has an effect on the measured response (dependent parameter, like FMRI percent signal change due to some stimulus).
    - Examples of *factor*: subject type, task type, task difficulty, drug type, drug dosage, *etc.*
    - Within a factor are *levels*: different sub-categorizations
      - Example: factor=subject type; level 1=normals, level 2=patients with mild symptoms, level 3=patients with severe symptoms
    - The various AFNI ANOVA programs differ in the number of factors they allow: `3dANOVA` allows 1 factor, comprising up to 100 levels
  - Assumptions
    - Values are normally distributed
    - No assumptions about relationship between dependent and independent variables (e.g., not necessarily linear)
    - Independent variables are qualitative
  - Can also use `3dttest` if there are only two levels
    - The 1-way `3dANOVA` analysis is a generalization to multiple levels of an unpaired `3dttest` (for generalization of *paired*, wait for `3dANOVA2`)
  - Example: *r* different types of subjects performed the same task in the scanner

Data from Voxel V	Factor levels (*e.g.*, subject types)			
	$\underline{1}$	$\underline{2}$	…	$\underline{r}$
Measurements (*e.g.*, percent signal change)	$Y_{1,1}$	$Y_{2,1}$	…	$Y_{r,1}$
	$Y_{1,2}$	$Y_{2,2}$	…	$Y_{r,2}$
	…	…	…	…
	$Y_{1,n1}$	…	…	…
		…	…	$Y_{r,nr}$
		$Y_{2,n2}$	…	

e.g., Subjects are repeated measurements within each level

Null Hypothesis: $H_0 : m_1 = m_2 = … = m_r$

i.e., subject type has no effect on mean signal in this voxel

Alternative Hypothesis: $H_a : $ *not all m_i are equal*

i.e., at least one subject type had a different mean FMRI signal

- `3dANOVA` is effectively a generalization of the unpaired *t*-test to multiple columns of data (a further refinement will be introduced with `3dANOVA3`)
  - As such, `3dANOVA` is probably not appropriate when comparing results of different tasks on the same subjects (need a generalization of the paired *t*-test: `3dANOVA2`)

- **<u>ANOVA: Which levels had an effect or were different from one another?</u>**

  - Usually, just knowing that there is a <u>*main effect*</u> (some of the means are different, but no information about *which* ones) isn't enough, so there is a number of options to let you look for more detail

  - Which treatment means (m_i) are $\neq 0$?

    - ↙ *e.g.*, is the response of subjects in level #3 different from 0 ?
    - ↙ *t*-statistic with option **-mean** in **3dANOVA**
    - ↙ Similar to using **3dttest -base1 0** (single sample test) to test only the data from those subjects

  - Which treatment means are different from each other ?

    - ↙ *e.g.*, is the response of subjects in level #3 different from those in level #2 ?
    - ↙ *t*-statistic with option **-diff** in **3dANOVA**
    - ↙ Similar to using **3dttest** (unpaired) between the data from these sets of subjects

  - Which linear combination of means (<u>*contrasts*</u>) are $\neq 0$?

    - ↙ *e.g.*, is the average response of subjects in level #1 different from the combined average of subjects in levels #2 and #3 ?
    - ↙ *t*-statistic with option **-contr** in **3dANOVA**

- **2-Way ANOVA**: **test for effects of two independent factors on measurements**
  - This is a _fully crossed_ analysis: all combinations of factor levels are measured
    - In particular, if one factor is "subject", then all subjects are tested in all levels of the other factor
    - Program is limited to _balanced designs_: Must have same number of measurements in each "cell" (combinations of factor levels)
  - Example: Stimulus type for factor A and subject for factor B
    - Each subject is a level within factor B (1 measurement per cell)
    - This is a _fixed effect_ $\times$ _random effect_ model = "mixed effect" model
  - Example: Stimulus type for factor A and drug treatment for factor B
    - Each subject is a repeated measurement for both factors, all levels
    - This is a _fixed effect_ $\times$ _fixed effect_ model
    - If you also want to treat subject as a separate factor, need `3dANOVA3`

    see next pages for description of _fixed_ and _random effects_

  - Example: Stimulus type for factor A, stimulus day for factor B
    - With one fixed subject, for a longitudinal study (_e.g._, training between scan days)
    - This also is a _fixed effect_ $\times$ _fixed effect_ model
    - Again, multiple subjects could be treated as repeated measurements in `3dANOVA2` or as a third factor in `3dANOVA3`

- Choose between two types of analysis for each factor: _fixed_ and _random effects_
- _Fixed effects_ factor = differences between levels in this factor are modeled as deterministic differences in the mean measurements (as in `3dANOVA` and `3dttest`)
  - Useful for most categories under the experimenter's control or observation
  - Allows same type of statistics as `3dANOVA`:
    - factor main effect (are all the mean activations of each level in this factor the same?)
    - differences between level pairs (_e.g._, level #2 same as #3?)
    - more complex contrasts (_e.g._, average of levels #1 and #2 same as level #3?)
  - If both factors are modeled as fixed effects with repeated measurements (_e.g._, subjects):
    - Can also test for _interaction_ between the factors
      - Are there any combinations of factor levels whose means "stick out" [_e.g._, mean of cell #(A_1,B_2) differs from (#A_1 mean)+(#B_2 mean)]?
      - Example: A=stimulus type, B=drug type; then cell #(A_1,B_2) is FMRI response (in each voxel) to stimulus #1 and drug #2
      - Interaction test would determine if any individual combination of drug type and stimulus type was abnormal
        - e.g., if stimulus #1 averages a high response, and drug #2 averages no effect on response, but when together, value in cell #(A_1,B_2) averages small
        - no interaction means the effects of the factors are always just additive
    - Inter-factor contrasts can then be used to test individual combinations of cells to determine which cell(s) the interaction comes from

- *Random effects* factor = differences between levels in this factor are modeled as random fluctuations
  - Useful for categories not under experimenter's control or observation
  - In FMRI, is especially useful for subjects; a good rule is

    treat subjects as a separate random effects factor rather than

    as repeated measurements inside fixed-effect factors

  - In such a case, usually have 1 measurement per cell (each cell is the combination of a level from the other factor with 1 subject)
  - Treating subjects as a random factor in a fully crossed analysis is a generalization of the paired *t*-test
    - intra-subject and inter-subject data variations are modeled separately
    - which can let you detect small intra-subject changes due to the fixed-effect factors that might otherwise be overwhelmed by larger inter-subject fluctuations
  - Main effect for a random effects factor tests if fluctuations among levels in this factor have additional variance above that from the other random fluctuations in the data
    - *e.g.*, Are inter-subject fluctuations bigger than intra-subject fluctuations?
    - Not usually very interesting when random factor = subject
  - It is hard to think of a good FMRI example where both factors would be random
  - `3dANOVA2`: Usually have 1 fixed factor and 1 random factor = *mixed effects* analysis

|	Data from Voxel V	factor B levels (e.g., subject)			
		1	**2**	**...**	**b**
Factor A levels (e.g., stimulus type, drug dose, ...)	**1**	Y_{111} Y_{112} ... Y_{11n}	Y_{121} Y_{122} ... Y_{12n}	... ...	Y_{1b1} Y_{1b1} ... Y_{1bn}
	2	Y_{211} Y_{212} ... Y_{21n}	Y_{221} Y_{222} ... Y_{22n}	... ...	Y_{2b1} Y_{2b1} ... Y_{2bn}
	...	.	.	.	.
	a	Y_{a11} Y_{a12} ... Y_{a1n}	Y_{a21} Y_{a22} ... Y_{a2n}	... ...	Y_{ab1} Y_{ab1} ... Y_{abn}

NOTE WELL: Must have same number of observations ("n") in each cell

Can use `3dRegAna` if you don't have the same number of values in each cell (program usage is much more complicated)

- **3dANOVA2**: A test case
  - Michael S. Beauchamp, Kathryn E. Lee, James V. Haxby, and Alex Martin, *fMRI Responses to Video and Point-Light Displays of Moving Humans and Manipulable Objects*, Journal of Cognitive Neuroscience, **15**: 991-1001 (2003).
  - Purpose is to study the organization of brain responses to different types of complex visual motion (the 4 levels within factor A) from 9 subjects (the levels within factor B)
  - Data from 3 of the subjects, and scripts to process it with AFNI programs, are available in AFNI HowTo #5 (hands-on)
    - Available for download at the AFNI web site:
      http://afni.nimh.nih.gov/afni/doc/howto/
    - If you want *all* the data, it is at the FMRI Data Center at Dartmouth:
      http://www.fmridc.org

- **Stimuli: Video clips of the following**

<u>Human whole-body motion (HM)</u>
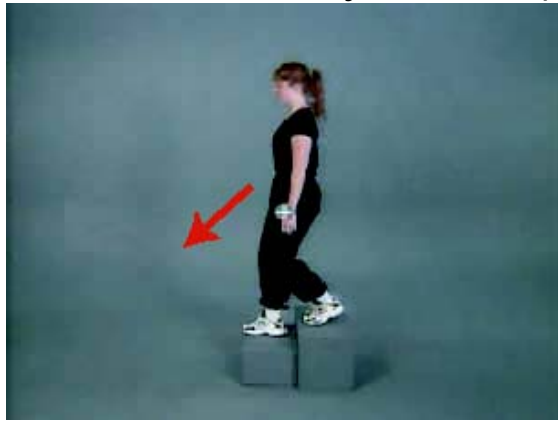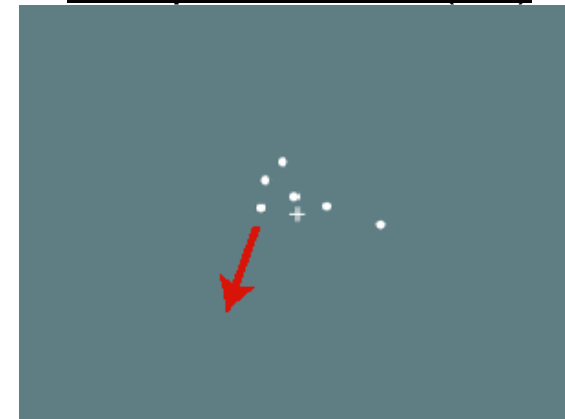

<u>Tool motion (TM)</u>
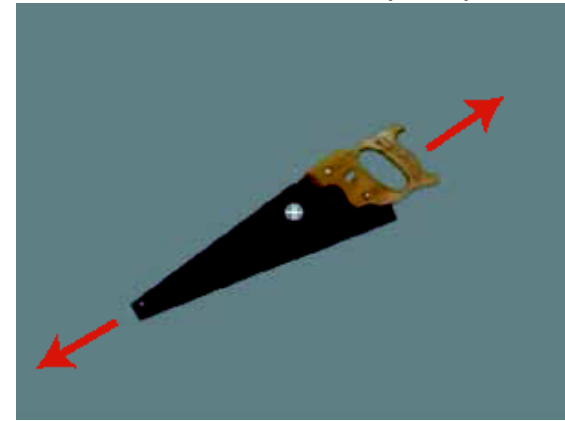
<u>Human point motion (HP)</u>

From Figure 1
Beauchamp et al. 03

<u>Tool point motion (TP)</u>

<u>Hypotheses to test</u>:
- Which areas are differentially activated by any of these stimuli (main effect)?
- Which areas are differentially activated for point motion versus natural motion?
  (type of image)
- Which areas are differentially activated for human-like versus tool-like motion?
  (type of motion)

- **Data Processing Outline**
  - Image registration with `3dvolreg`
  - Images smoothed (4 mm FWHM) with `3dmerge`
  - IRF for each of the 4 stimuli were obtained using `3dDeconvolve`
  - Regressor coefficients (IRFs) were normalized to percent signal change (using `3dcalc`)
  - An average activation measure was obtained by averaging IRF amplitude from the 4th through the 10th second of the response (using `3dTstat`)
    - Capturing the positive blood-oxygenation level dependent response but not any post-stimulus undershoot
    - These activation measures will be the measurements in the ANOVA table
      - After each subject's results are warped to Talairach coordinates, using `adwarp` program
  - `3dANOVA2` was carried out with:
    - Factor *A*, fixed effects: levels = HM, TM, HP, TP (4 types of stimuli)
    - Factor *B*, random effects: levels = 9 subjects
    - 1 measurement per cell

- **3dANOVA2 script**

```
3dANOVA2 -type 3 -alevels 4 -blevels 9                      \
```
Specifies mixed effects, number of levels in factors

```
-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]'    \
```
Specifies inputs to each cell in ANOVA table

```
-amean 1 TM -amean 2 HM -amean 3 TP  -amean 4 HP \
```
Output sub-bricks with mean activation for each A level (*i.e.,* each task)

```
-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                             \
```
Specifies contrast tests amongst various cell combinations

```
-fa StimEffect                                       \
```
Output sub-brick with factor A "main effect" F test

```
-bucket AvgANOVA
```
Name of output dataset

- <u>3dANOVA2</u>: specifying input datasets

  ☞ 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]'      \
```

	Data from Voxel V	**Factor A levels** (stimulus type, fixed effects)			
		TM	**HM**	**TP**	**HP**
Factor B levels (9 Subjects, random effects)	ED	ED[0]+tlrc	ED[1]+tlrc	ED[2]+tlrc	ED[3]+tlrc
	EE	EE[0]+tlrc	EE[1]+tlrc	EE[2]+tlrc	EE[3]+tlrc
	.	.	.	.	.
	FN	FN[0]+tlrc	FN[1]+tlrc	FN[2]+tlrc	FN[3]+tlrc

- **3dANOVA2**: specifying which statistics to output

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

- **-amean 1 TM**: estimate mean of factor *A*, level 1 and label it TM in the output dataset
- **-acontr** : specifies contrast matrix and label in output dataset

  1 1 1 1: all of factor *A*'s levels summed = 0?

  -1 1 -1 1: contrast between human and tools (HM + HP) − (TM + TP)

  1 1 -1 -1: contrast between motion and points (HM + TM) − (HP + TP)

  0 1 0 -1: contrast between human motion and points (HM − HP)

  … …

- **-fa StimEffect**: *F*-statistic for main effect of factor *A* (any differences among stimuli?)
- **-bucket AvgANOVA**: prefix of output dataset containing statistical results

- **3dANOVA2**: viewing results
  - <u>Main effect</u>: Regions showing presence of differences in activation due to changes in stimulus type (which differences must be determined via later contrasts)
    - ↙ view **StimEffect** sub-bricks for function and threshold (F-stat = 15, p = 10^{-5})
  - <u>Factor Means</u>: Activation in response to each category
    - ↙ view **TM**, **HM**, etc. sub-bricks (t-stat = 10.6, p = 10^{-10})
    - ↙ all categories appear to activate same areas
  - Choose **AllAct** sub-bricks for finding regions activated by at least one of the stimuli
    - ↙ this region of activation is often used to select an ROI which is examined for subtler effects
  - Choose **HvsT** (human versus tools) sub-bricks
    - ↙ note small range of t-values (subtler effects, if any)
    - ↙ lower t-stat threshold to 4, p ~ 5×10^{-4}
    - ↙ might want to restrict hypothesis testing to region activated by stimuli
  - Look for interactions that might complicate your fairy tale (AKA hypothesis)
    - ↙ view the **Inter** sub-bricks to determine if some areas for which the contrast `(TM+HP)-(HM+TP)` is significant
    - ↙ Hopefully you'll find few/none, or be prepared to explain such activations

- **3-Way ANOVA: `3dANOVA3`** (again, balanced designs only)
  - ☞ Read the manual first and understand what options are available
    - ↙ It is important to understand 2-way ANOVA before moving up to the big time show!
  - ☞ Has several fixed effects and random effects combinations
  - ☞ Has new concept: _nested design_ (vs. fully crossed design)
    - ↙ Nested design is for use when you have 2 fixed effects factors and 1 random effects factor where the subjects for the random effects factor depend on one of the fixed effect factors; <u>example</u>:
      - ↙ factor A = subject type; level #1=normal, #2=genotype Q, #3=genotype R
      - ↙ factor B = stimulus type; levels #1–4=different types of videos
      - ↙ factor C = subject; levels #1–10 = 30 different subjects, 10 in each of the factor A levels; C is "nested" inside A
    - ↙ Nested design is a mixture of unpaired and paired tests
      - ↙ Will be like "paired" for tests across stimulus type (factor B levels)
      - ↙ Will be like "unpaired" across subject types (factor A levels)
    - ↙ Fully crossed design is when the subjects are common across the other factors
      - ↙ As was said before, un-nested design is a generalization of paired _t_-test
    - ↙ Treating the subjects correctly is a crucially important decision
  - ☞ Unlike `3dANOVA2`, `3dANOVA3` does not currently allow for arbitrary contrasts between random cells in different factors/different levels

- **4-Way ANOVA: ready to rock-n-roll** (for the daring and intrepid)
  - Interactive Matlab script
  - Can run both crossed and nested (*i.e.,* subject nested into gender) design
  - Heavy duty computation + Matlab: expect to take 10s of minutes to hours
  - Same script can also do `ANOVA`, `ANOVA2`, and `ANOVA3` analyses
  - Includes contrast tests across all factors
  - At present, must have a balanced design with no missing data
    - equal number of entries in each cell
    - can be a problem when studying patients (*e.g.,* hard to find some genotypes)
  - Working now to implement more options, such as
    - ANCOVA (ANOVA plus regression with continuous covariates; *e.g.,* age)
    - unbalanced designs (uneven numbers of entries in cells, or levels in factors)
    - missing data (some subjects couldn't perform certain tasks)
  - Goal: be a user-friendly alternative to running `3dRegAna` for most complicated analyses of group datasets
  - Goal: once program is stabilized, re-write in C for speed and independence from the commercial product Matlab

5 Types of 4-Way ANOVA Now Available!

$A_F \times B_F \times C_F \times D_F$ 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 repeated measures; 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, typically treated as random (more powerful than treating them as repeats) Good for an experiment where each fixed factor applies to **all** subjects;
$B_F \times C_F \times D_R(A_F)$ 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 $\times$ B=genotype)

Further Directions for Group Analysis Developments

- In a mixed effects model, ANOVA cannot deal with unequal variances in the random factor between different levels of a fixed factor
  - ☞ Example: 2-way layout, factor A=stimulus type (fixed effect), factor B=subject (random effect)
    - ↙ As seen earlier, ANOVA can detect differences in means between levels in A (different stimuli)
    - ↙ But if the measurements from different stimuli **also** have significantly different variances (*e.g.,* more attentional wandering in one task vs. another), then the ANOVA model for the signal is wrong
    - ↙ In general, this "heteroscedasticity" problem is a difficult one, even in a 2-sample *t*-test; there is no exact *F*- or *t*-statistic to test when the means and the variances might differ simultaneously
- Although ANOVA does allow somewhat for intra-subject correlations in measurements, it is not fully general
  - ☞ Example: 2-way layout as above, 3 stimulus types in factor A; general correlation matrix between the 3 different types of responses is $\begin{bmatrix} 1 & \rho_{12} & \rho_{13} \\ \rho_{12} & 1 & \rho_{23} \\ \rho_{13} & \rho_{23} & 1 \end{bmatrix}$ but ANOVA only properly deals with the case $\rho_{12}=\rho_{13}=\rho_{23}$ (recall we are assuming subject effects are random; this is the correlation matrix for the intra-subject random responses).
- Possible solution: general linear-quadratic minimum variance mixed effects modeling
  - ☞ A statistical theory not yet much applied to FMRI data (but it will be, someday)
  - ☞ Questions of sample size (number of subjects needed) will surely arise

And Now for Something Completely Different

- ## Regression Analysis: `3dRegAna`

    - Simple linear regression:

        - $Y = \beta_0 + \beta_1 X_{1,} + \varepsilon$
        - where Y represents the FMRI measurement (*i.e.*, percent signal change) and X is the independent variable (*i.e.*, drug dose)

    - Multiple linear regression:

        - $Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \ldots + \varepsilon$

    - Regression with qualitative and quantitative variables (ANCOVA)

        - *i.e.*, drug dose (5mg, 12mg, 23mg, etc.) is quantitative while drug type (Nicotine, THC, Cocaine) or age group (young vs. old) or genotype is qualitative, and usually called dummy (or indicator) variable

    - ANOVA with unequal sample sizes (with indicator variables)

    - Polynomial regression:

        - $Y = \beta_0 + \beta_1 X_1 + \beta_2 X_1^2 + \ldots + \varepsilon$

    - Linear regression: model is a linear function of its unknowns β_i , *NOT* its independent variables X_i

    - Not for fitting time series, use `3dDeconvolve` (or `3dNLfim`) instead

- *F*-test for Lack of Fit (*lof*)
  - If repeated measurements are available (and they should be), a **Lack Of Fit** *(lof)* test is first carried out.
    - Hypothesis:

      H_0: $E(Y) = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \ldots, + \beta_{p-1} X_{p-1}$

      H_a: $E(Y) \neq \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \ldots, + \beta_{p-1} X_{p-1}$

    - Hypothesis is tested by comparing the variance of the model's lack of fit to the measurement variance at each point (pure error).
    - If F_{lof} is significant then model is inadequate. STOP HERE.
      - Reconsider independent variables, try again.
    - If F_{lof} is insignificant then model appears adequate, so far.
    - It is important to test for the lack of fit:
      - The remainder of the analysis assumes an adequate model is used
      - You will not be visually inspecting the goodness of the fit for thousands of voxels!

- Test for Significance of Linear Regression
  - This is done by testing whether additional parameters significantly improve the fit
    - For simple case

      $Y = \beta_0 + \beta_1 X_1 + \varepsilon$

      $H_0: \beta_1 = 0$

      $H_1: \beta_1 \neq 0$

    - For general case

      $Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \ldots + \beta_{q-1} X_{q-1} + \beta_q X_q + \ldots + \beta_{p-1} X_{p-1} + \varepsilon$

      $H_0: \beta_q = \beta_{q+1} = \ldots = \beta_{p-1} = 0$

      $H_a: \beta_k \neq 0$, for some k, $q \leq k \leq p-1$

    - *Freg* is the *F*-statistic for determining if the Full model significantly improved on the reduced model
      - *NOTE:* This *F*-statistic is assumed to have a central *F*-distribution. This is **not** the case when there is a lack of fit

- **3dRegAna**: Other statistics

  - How well does model fit data?

    - R^2 (coefficient of multiple determination) is the proportion of the variance in the data accounted for by the model $0 \leq R^2 \leq 1$.

    - *i.e.*, if $R^2 = 0.26$ then 26% of the data's variation about their mean is accounted for by the model. So this might indicate the model, even if significant, might not be that useful (depends on what use you have in mind)

      - Having said that, you should consider R^2 relative to the maximum it can achieve given the pure error which cannot be modeled. [*cf.* Draper & Smith, chapter 2].
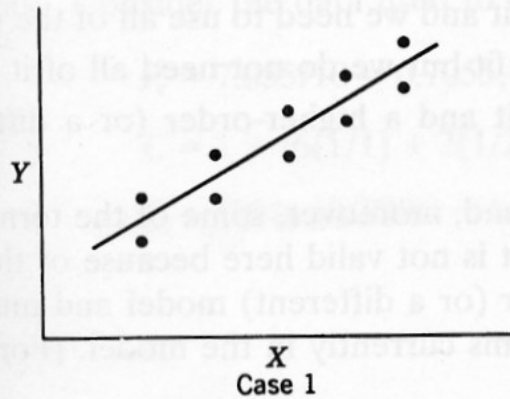
  - Are individual parameters β_k significant?

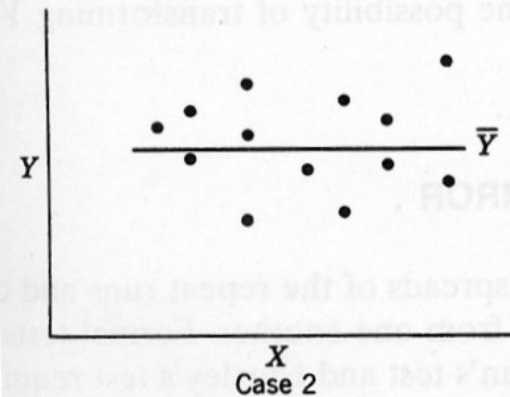   - t-statistic is calculated for each parameter

    - helps identify parameters that can be discarded to simplify the model

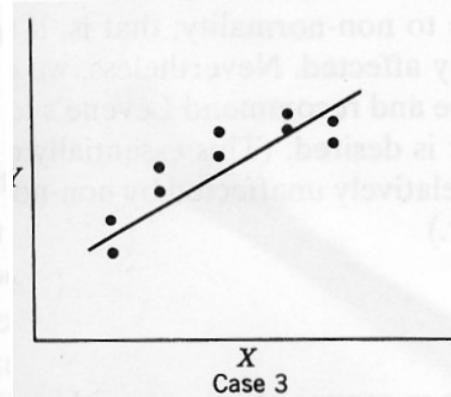 - R^2 and t-statistic are computed for full (not reduced) model

Examples from Applied Regression Analysis by Draper and Smith (third edition)

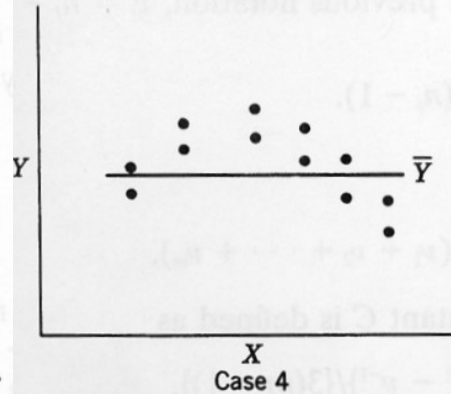*Case 1:*
(1) Try $Y = \beta_0 + \beta_1 X + \varepsilon$.
(2) No lack of fit.
(3) Significant linear regression.
(4) Use model $\hat{Y} = b_0 + b_1 X$.

Case 2:
(1) Try $Y = \beta_0 + \beta_1 X + \varepsilon$.
(2) No lack of fit.
(3) Linear regression not significant.
(4) Use model $\hat{Y} = \overline{Y}$.

Case 3:
(1) Try $Y = \beta_0 + \beta_1 X + \varepsilon$.
(2) Significant lack of fit.
(3) Try model $Y = \beta_0 + \beta_1 X + \beta_{11} X^2 + \varepsilon$.

Case 4:
(1) Try $Y = \beta_0 + \beta_1 X + \varepsilon$.
(2) Significant lack of fit.
(3) Try model $Y = \beta_0 + \beta_1 X + \beta_{11} X^2 + \varepsilon$.
(*Note:* β_1 may be significantly different from zero when residual error term is reduced by taking out $\beta_{11} X^2$. See Chapter 6.)

Figure 2.3. Typical straight line regression situations.

- **`3dRegAna`**: Qualitative Variables (ANCOVA)
  - Qualitative variables can also be used
    - *i.e.,* We're modeling the response amplitude to a stimulus of varying contrast when subjects are either young, middle-aged or old.
    - X_1 represents the stimulus contrast (quantitative): continuous covariate
    - Create indicator variables X_2 and X_3 to represent age:
      - X_2 = 1 if subject is middle-aged
               = 0 otherwise
      - X_3 = 1 if subject is old (*i.e.*, at least 1 year older than Bob Cox)
               = 0 otherwise
    - Full Model (no interactions between age and contrast)
      - $Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \varepsilon$

        $E(Y) = \beta_0 + \beta_1 X_1$ *for young subjects*

        $E(Y) = (\beta_0 + \beta_2) + \beta_1 X_1$ *for middle-aged subjects*

        $E(Y) = (\beta_0 + \beta_3) + \beta_1 X_1$ *for old subjects*
    - Full Model (with interactions between age and contrast)
      - $Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_4 X_2 X_1 + \beta_5 X_3 X_1 + \varepsilon$

        $E(Y) = \beta_0 + \beta_1 X_1$ *for young subjects*

        $E(Y) = (\beta_0 + \beta_2) + (\beta_1 + \beta_4) X_1$ *for middle-aged subjects*

        $E(Y) = (\beta_0 + \beta_3) + (\beta_1 + \beta_5) X_1$ *for old subjects*
  - Will be easier to run analysis in Matlab script for 3dANOVA4, when ready!

- **`3dRegAna`**: ANOVA with unequal samples
  - `3dANOVA2` and `3dANOVA3` do not allow for unequal samples in each combination of factor levels
    - Can use `3dRegAna` to look for main effects and interactions
    - The analysis method involves the use of indicator variables so it is practical for small for small number (~3) of factor levels
  - Details are in the 3dRegAna manual
    - method is significantly more complicated than running ANOVA; you *must* understand the math
    - avoid this, if you can, especially if you have more than 4 factor levels or more than 2 factors
    - Interactions hard to interpret, and contrast tests unavailable
  - Will be easier to run analysis in Matlab script for 3dANOVA4, when ready!

- ## **Conjunction Junction: What's Your Function?**
  - The program `3dcalc` is a general purpose program for performing logic and arithmetic calculations
    - command line is of the format

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

    - 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 = **1 – bool(v – n)**
    - create masks to apply to functional datasets
      - two values both above threshold (*e.g.,* active in both tasks)

        expression = **step(v-A)*step(w-B)**