KINE 601

Inferences concerning means

***t*-tests, different kinds of ANOVA's**

Reading: Huck pp 233 - 416

Parametric Statistics

- **Parametric Statistics:**
  - statistics performed on continuous data (interval or ratio data)
  - more powerful than non-parametric statistics when assumptions are met
    - note: previously discussed Pearson correlation is a parametric statistic

- **Assumptions:**
  - normal distributions
    - distributions for all variables involved in the statistic are normally distributed
  - independent distributions
    - in all distributions, one subjects score is not dependent any other score
  - similar (homogeneous) variances for all variables involved in the statistic
  - assumptions are usually relaxed and "statistical robustness" is cited if
    - samples are very large
    - samples have the same number of subjects (same n)
  - sometimes data are "transformed" in an attempt to "normalize" the data
    - example: log transformation (taking the natural log of each data point)

Student's *t* - test

- **for independent data (uncorrelated data or unmatched data)**

  - H_0: $y_1 = y_2$
  - tests for significant differences between 2 independent means (y_1 and y_2)
  - calculated $t = \dfrac{y_1 - y_2}{s(y_1 - y_2)}$

    difference between means

    standard error of the difference between means

  - reject H_0 if calculated t > table t at a given probability (α) level (or calculated p - value is < α)
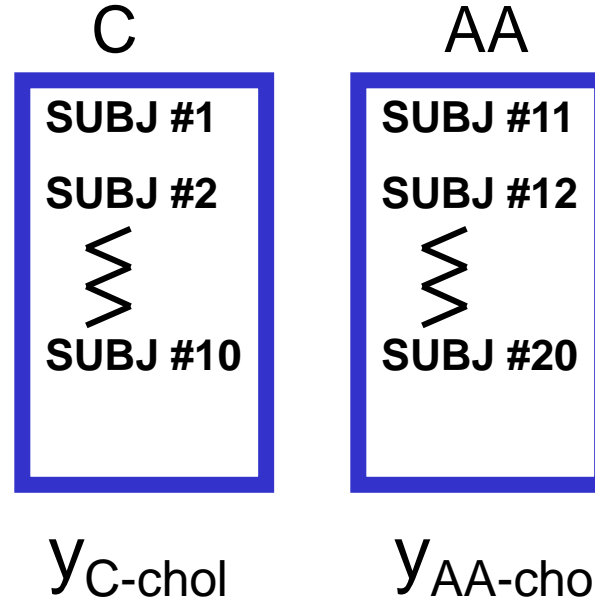
- **for dependent data (correlated data, matched pairs, pre-post testing)**

- calculated $t = \dfrac{d_y}{s(d_y)}$

  mean difference between pairs

  standard error of the mean difference between pairs

  - note: when the correlation between the correlated pairs is large, the standard error of the mean difference will be small. This makes the calculated t statistic large, making it a more powerful test.

Independent *t*-test example

- A researcher wishes to determine if a significant difference exists between a select group of hospitalized Caucasian heart disease patients and hospitalized African-American heart disease patients with respect to total cholesterol. At a local hospital, 10 Caucasian and 10 African-American patients with confirmed heart disease had their blood drawn and analyzed for total cholesterol. Data were analyzed using a student's independent t-test.

- H_0: $y_{C\text{-chol}} = y_{AA\text{-chol}}$

C	AA
SUBJ #1	**SUBJ #11**
SUBJ #2	**SUBJ #12**
⋛	⋛
SUBJ #10	**SUBJ #20**

$y_{C\text{-chol}}$ $y_{AA\text{-chol}}$

Correlated *t*-test example

- A researcher wishes to determine if a a new cholesterol lowering drug will significantly lower cholesterol in a group of hospitalized heart disease patients. A group of 20 hospitalized heart disease patients were administered the drug over a three week period. The researcher wants to first determine if the drug significantly lowered cholesterol levels during the first week of treatment. Blood from the patients was drawn and analyzed at the onset of the study and again at the end of the first week. Data were analyzed using a correlated (paired) *t*-test.
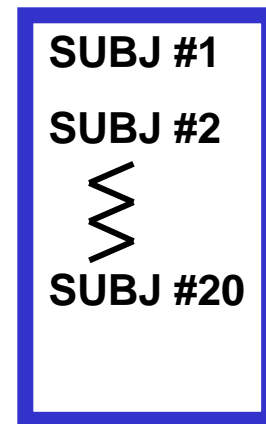
- $H_0: y_{chol-T1} = y_{chol-T2}$

Chol-T1

| SUBJ #1 |
| SUBJ #2 |
| ≶ |
| SUBJ #20 |

Chol-T2

| SUBJ #1 |
| SUBJ #2 |
| ≶ |
| SUBJ #20 |
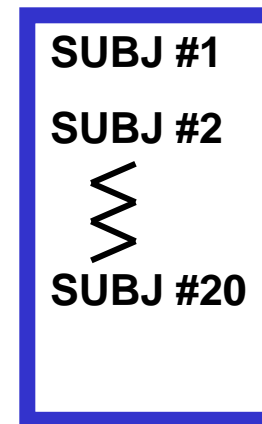
$y_{chol-T1}$ $y_{chol-T2}$

Hotellings T² - the Multivariate t-test

- **Multivariate - having more than 1 dependent variable**
- **Hotellings T²**
  - tests for differences between two groups of means (two vectors of means)
  - H_0:
  $$\begin{pmatrix} \bar{y}_1 \\ \bar{x}_1 \\ \bar{z}_1 \end{pmatrix} = \begin{pmatrix} \bar{y}_2 \\ \bar{x}_2 \\ \bar{z}_2 \end{pmatrix}$$

  - Hotelling's T² Example:
    - A researcher wishes to determine if a significant difference exists between a select group of hospitalized Caucasian heart disease patients and hospitalized African-American heart disease patients with respect to total cholesterol, LDL cholesterol, and triglycerides. At a local hospital, 10 Caucasian and 10 African-American patients with confirmed heart disease had their blood drawn and analyzed for total cholesterol, LDL cholesterol, and triglycerides. Data were analyzed using a Hotelling's T²

analysis of variance (ANOVA)
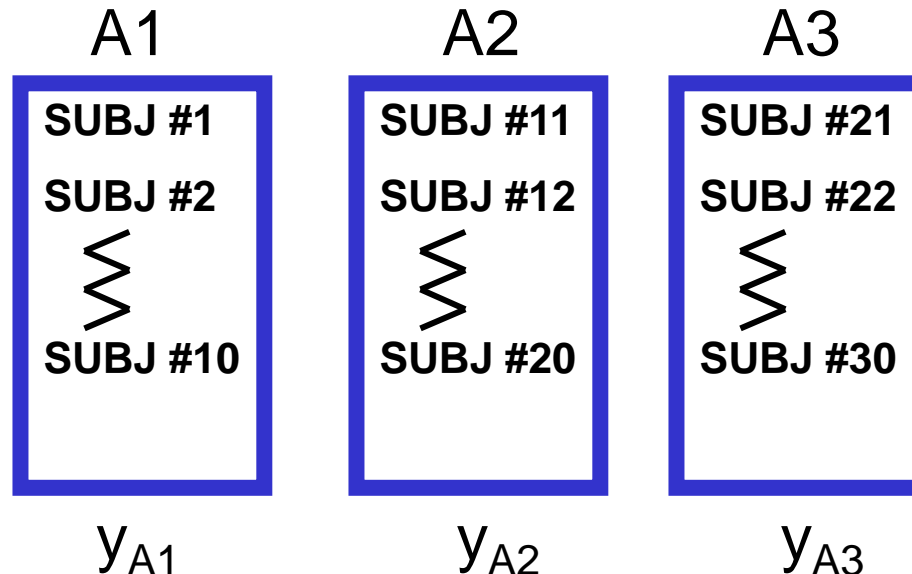
- **One-way ANOVA for independent data**

  - **H_0:** $y_1 = y_2 = y_3 \ldots\ldots\ldots y_p$

  - tests for significant differences between 2 or more means of one dependent variable with respect to one independent variable

  - calculated F = $\dfrac{\textbf{between groups variance}\ \text{(variance caused by treatment)}}{\textbf{within groups variance}\ \text{(variance caused by sampling error)}}$

    - variances are often called "mean squares" (MS) : $F = \dfrac{\overline{MSB}}{MSW}$

  - reject H_0 if calculated F > table F at a given probability (α) and **df** level (or calculated p - value is < α)

Post hoc and planned comparisons

- **"Post hoc" tests are used in ANOVA's to determine exactly which means are significantly different from one another**
  - Listed from most "liberal" to most "conservative":
    - t-test, Fischer's LSD, Duncan's test, Newman-Keuls, Tukey, Sheffe test
- Bon Ferroni adjustments for post hoc tests will d type I error chances
  - good idea but seldom done
- Apriori planned comparisons between two specific means or between a combination of means can be made using various types of post hoc tests and <u>contrasts</u>
    - Suppose you hypothesized that the average of the dependent variable means in the first two categorizations in an ANOVA is equal to the dependent variable mean of the third categorization. An apriori contrast can be constructed so as to make this comparison.
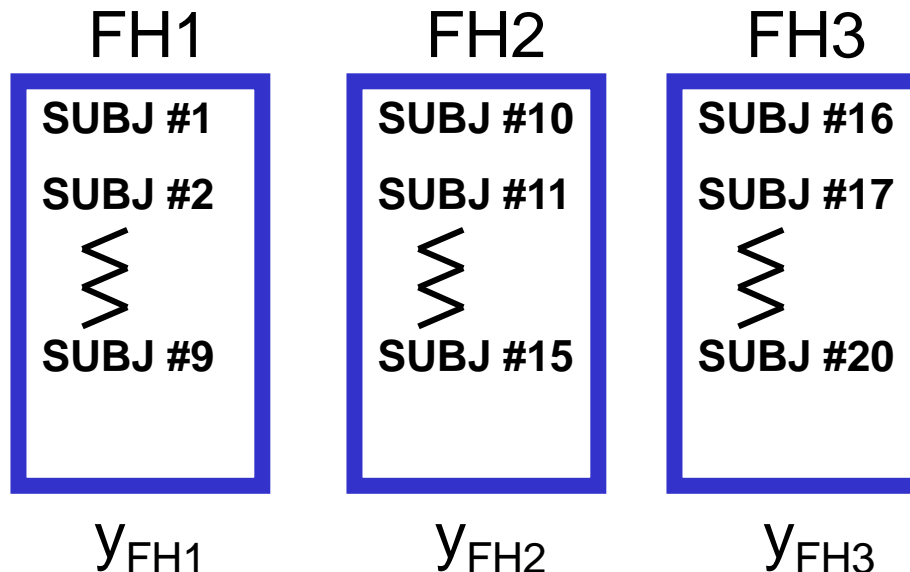
Graphical representation of one-way ANOVA

- graphical example of One-way ANOVA :
  - 30 subjects…..10 in each independent variable classification
  - tests for differences in means for the dependent variable **y** among the three different levels of the independent variable (treatments) **A**:
    - **treatment A1**, **treatment A2**, and **treatment A3**
  - **H_0**: y_{A1} = y_{A2} = y_{A3} (follow up post hoc comparisons)

A1	A2	A3
SUBJ #1	**SUBJ #11**	**SUBJ #21**
SUBJ #2	**SUBJ #12**	**SUBJ #22**
$\lessgtr$	$\lessgtr$	$\lessgtr$
SUBJ #10	**SUBJ #20**	**SUBJ #30**
y_{A1}	y_{A2}	y_{A3}

one-way ANOVA example

- A researcher wishes to determine if there is any difference in cholesterol among a select group of hospitalized heart patients with respect to 3 different genetic levels of heart disease risk. The risk stratification is based on how many first degree relatives have documented heart disease before the age of 50. A group of 20 patients so stratified yielded a sample of 9 patients with 1 relative with heart disease, 6 patients with 2 relatives, and 5 patients with 3 relatives. Blood was drawn from all patients and analyzed for cholesterol. Data were analyzed using a one-way analysis of variance.

- H_0: $y_{FH1} = y_{FH2} = y_{FH3}$ (follow up post hoc comparisons)

FH1	FH2	FH3
SUBJ #1	SUBJ #10	SUBJ #16
SUBJ #2	SUBJ #11	SUBJ #17
⋛	⋛	⋛
SUBJ #9	SUBJ #15	SUBJ #20

y_{FH1} y_{FH2} y_{FH3}

Source Table for One-Way ANOVA

- One-way ANOVA source Table (Microsoft Excel):

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	11013.64444	2	5506.822222	5.088500888	0.01854	3.591538
Within Groups	18397.55556	17	1082.20915			
Total	29411.2	19				

- One-way ANOVA source Table (SAS):

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model (between)	2	11013.64444444	5506.82222222	5.09	0.0185
Error (within)	17	18397.55555556	1082.20915033		
Corrected Total	19	29411.20000000			

R-Square	C.V.	Root MSE	WEIGHT1 Mean
0.374471	19.37394	32.8969474	169.80000000

Source	DF	Anova SS	Mean Square	F Value	Pr > F
FAMHIST	2	11013.64444444	5506.82222222	5.09	0.0185

Post Hoc analysis for One-Way ANOVA (Duncan's test)

Means with the same letter are not significantly different.

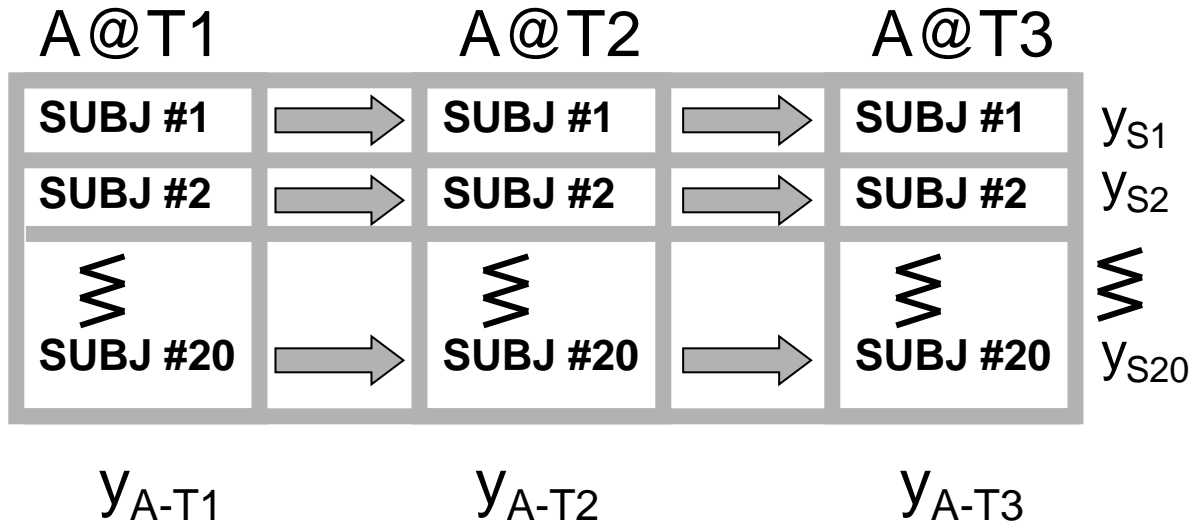| Duncan Grouping	Mean (CHOL)	N	# of REALATIVES
A	187.56	9	3
A			
A	176.33	6	2
B	130.00	5	1

Analysis of variance with repeated measures

- **One-Way ANOVA with repeated measures**
  - also called: within subjects ANOVA, randomized block design
  - tests for differences between means of the dependent variable for the <u>same subjects</u> over a period of time and / or under different treatment conditions
  - like the dependent *t*-test, RM ANOVA is modified to account for the correlation of successive measurement on the same subject
  - repeated measure ANOVA partitions out the within subjects variation (error) in a one-way ANOVA into variation due to treatment and variation due to subject treatment interaction (error variation). This makes the total error variation in RM designs smaller r larger F-ratio r more powerful test.
  - RM ANOVA assumes that, since extraneous variation due to different subjects should not exist to a significant extent, differences found between treatments or time points should primarily be due to the action of the independent variable
  - must be cautious of "practice effect", "fatigue effects", "confounding effects"
    - extraneous variation due to participating in the treatment or the passage of time

  - calculated F = $\dfrac{\textbf{between timepoints variance} \text{ (variance caused by treatment)}}{\textbf{error variance} \text{ (treatment - subject interaction variance)}}$

Graphical representation of RM ANOVA
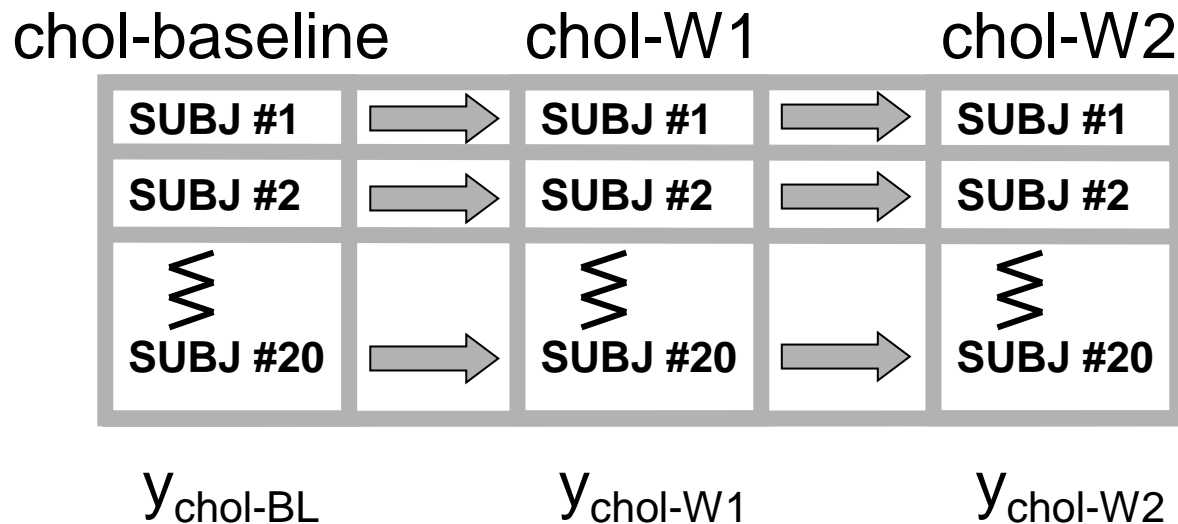
- **One-Way ANOVA with Repeated Measures**
    - 2 or more repeated assessments of a dependent variable are taken from the same subjects (n = 20 in this graphical illustration) at selected time points along the course of the administration of some independent (treatment) variable A.

    - H_0: $y_{A-T1} = y_{A-T2} = y_{A-T3}$ (follow up post hoc comparisons)

A@T1		A@T2		A@T3	
SUBJ #1	→	**SUBJ #1**	→	**SUBJ #1**	y_{S1}
SUBJ #2	→	**SUBJ #2**	→	**SUBJ #2**	y_{S2}
⧩		⧩		⧩	⧩
SUBJ #20	→	**SUBJ #20**	→	**SUBJ #20**	y_{S20}

y_{A-T1} y_{A-T2} y_{A-T3}

variation between subjects is expected and is not of experimental importance. Although it is given in most stat program printouts it has nothing to do with data interpretation

Repeated Measures ANOVA example

- A researcher wishes to determine if a a new cholesterol lowering drug will significantly lower cholesterol in a group of hospitalized heart disease patients. A group of 20 hospitalized heart disease patients were administered the drug over a 2 week period. The researcher wants to determine if the drug significantly lowered cholesterol levels from week to week during the 2 week period. Blood from the patients was drawn and analyzed at the onset of the study, again at the end of the first week, and finally, at the end of the 2nd week. Data were analyzed using a repeated measures ANOVA.

- H_0: $y_{chol-BL} = y_{chol-W1} = y_{chol-W2}$ (follow up post hoc comparisons)

chol-baseline	chol-W1	chol-W2
SUBJ #1 →	SUBJ #1 →	SUBJ #1
SUBJ #2 →	SUBJ #2 →	SUBJ #2
⧩	⧩	⧩
SUBJ #20 →	SUBJ #20 →	SUBJ #20

$y_{chol-BL}$ $\qquad$ $y_{chol-W1}$ $\qquad$ $y_{chol-W2}$

- **RM ANOVA source Table (SAS)**:

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	21	86750.08333333	4130.95634921	1069.56	0.0001
Error	38	146.76666667	3.86228070		
Corrected Total	59	86896.85000000			

R-Square	C.V.	Root MSE	CHOL Mean
0.998311	1.187832	1.9652686	165.45000000

Source	DF	Anova SS	Mean Square	F Value	Pr > F
SUBJ	19	85518.18333333	4500.95701754	1165.36	0.0001
TIME	2	1231.90000000	615.95000000	159.48	0.0001

- **One-way ANOVA source Table (SAS) for same data:**

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	1231.90000000	615.95000000	0.41	0.6657
Error	57	85664.95000000	1502.89385965		
Corrected Total	59	86896.85000000			

R-Square	C.V.	Root MSE	CHOL Mean
0.014177	23.43135	38.7671750	165.45000000

Source	DF	Anova SS	Mean Square	F Value	Pr > F
TIME	2	1231.90000000	615.95000000	0.41	0.6657

Analysis of Variance (ANOVA)

- **two-way factorial ANOVA for independent data**
  - tests for significant differences between 2 or more means of one dependent variable with respect to 2 or more independent variable **(factors)**
  - results in 3 **F**-tests….1 for each **main effect** and 1 for possible **interaction** of the treatments

Factor (treatment) A - 3 levels

	A1	A2	A3	
B1	SUBJ #1 SUBJ #2 y_{A1B1} SUBJ #10	SUBJ #21 SUBJ #22 y_{A2B1} SUBJ #30	SUBJ #41 SUBJ #42 y_{A3B1} SUBJ #50	y_{B1}
B2	SUBJ #11 SUBJ #12 y_{A1B2} SUBJ #20	SUBJ #31 SUBJ #32 y_{A2B2} SUBJ #40	SUBJ #51 SUBJ #52 y_{A3B2} SUBJ #60	y_{B2}
	y_{A1}	y_{A2}	y_{A3}	

Factor (treatment) B
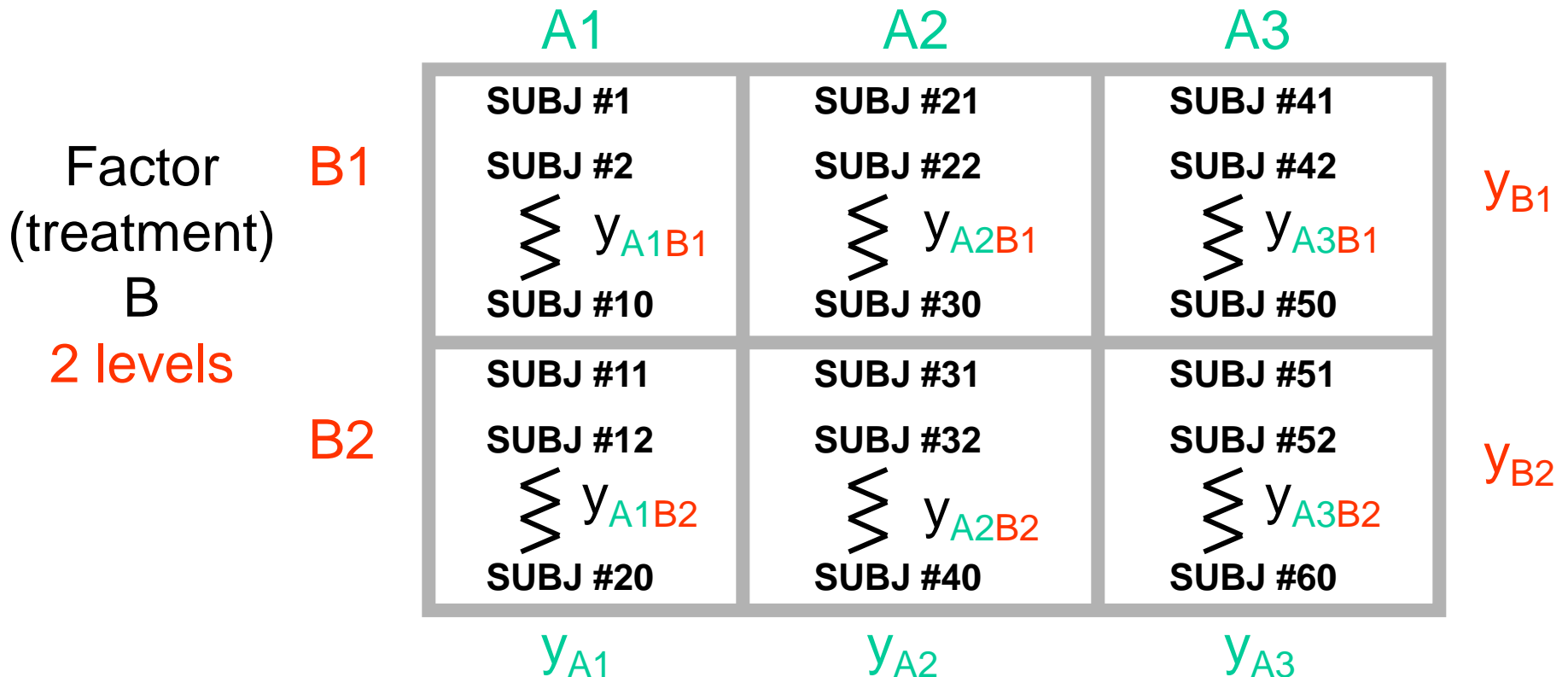
2 levels

2 X 3 Factorial ANOVA Example

- a researcher wishes to determine if post-participation plasma norepinephrine levels (pg/ml) differ among ropes course participants that A: participate in different "elements" of the course and B: are shown a video of participation prior to actually participating

Factor (treatment) A

Ropes Course Participation Levels

		low elements	high elements	both low and high elements	
Factor (treatment) B Prior Video Exposure	Shown Video	y Plasma NE	y Plasma NE	y Plasma NE	y_{B1}
	Not Shown Video	y Plasma NE	y Plasma NE	y Plasma NE	y_{B2}
		y_{A1}	y_{A2}	y_{A3}	

Analysis of Variance (ANOVA)

- **Two-Way factorial ANOVA for independent data**
  - Main Effects (row effects and column effects)
    - **Main effect for factor A (columns)**
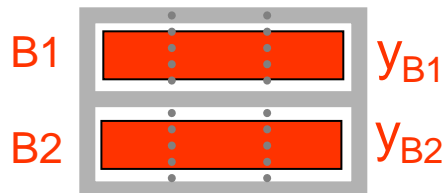   - is there significant differences among y_{A1}, y_{A2}, & y_{A3} (H_0: y_{A1} = y_{A2} = y_{A3})

      A1   A2   A3

      y_{A1} y_{A2} y_{A3}
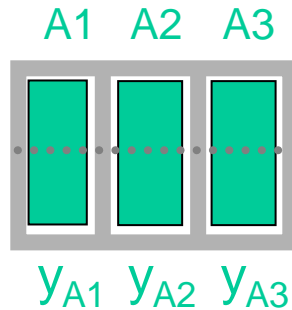
      in example: does participation in different elements of the ropes course affect plasma NE levels?

      (follow up Post hoc comparisons)

   - **Main effect for factor B (rows)**
    - is there significant differences between y_{B1}, & y_{B2} (H_0: y_{B1} = y_{B2})

      B1 y_{B1}

      B2 y_{B2}

      in example: does being shown a video prior to participation affect plasma NE levels

Analysis of Variance (ANOVA)

- **Two-Way factorial ANOVA for independent data: <u>Interaction Effects</u>**
  - occurs when there is a directionally different response of the dependent variable at different levels of the independent variable
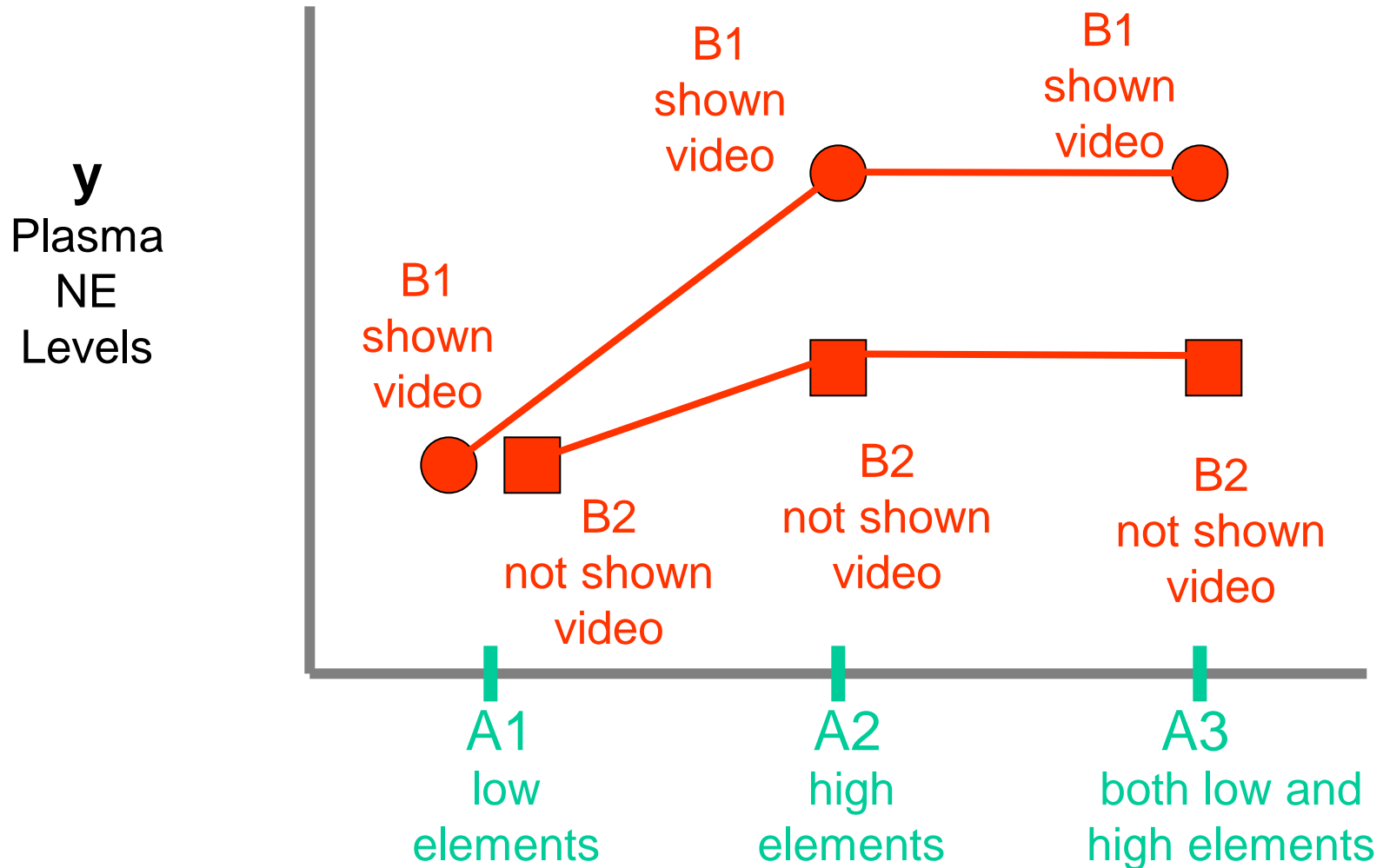
analysis of variance (ANOVA)

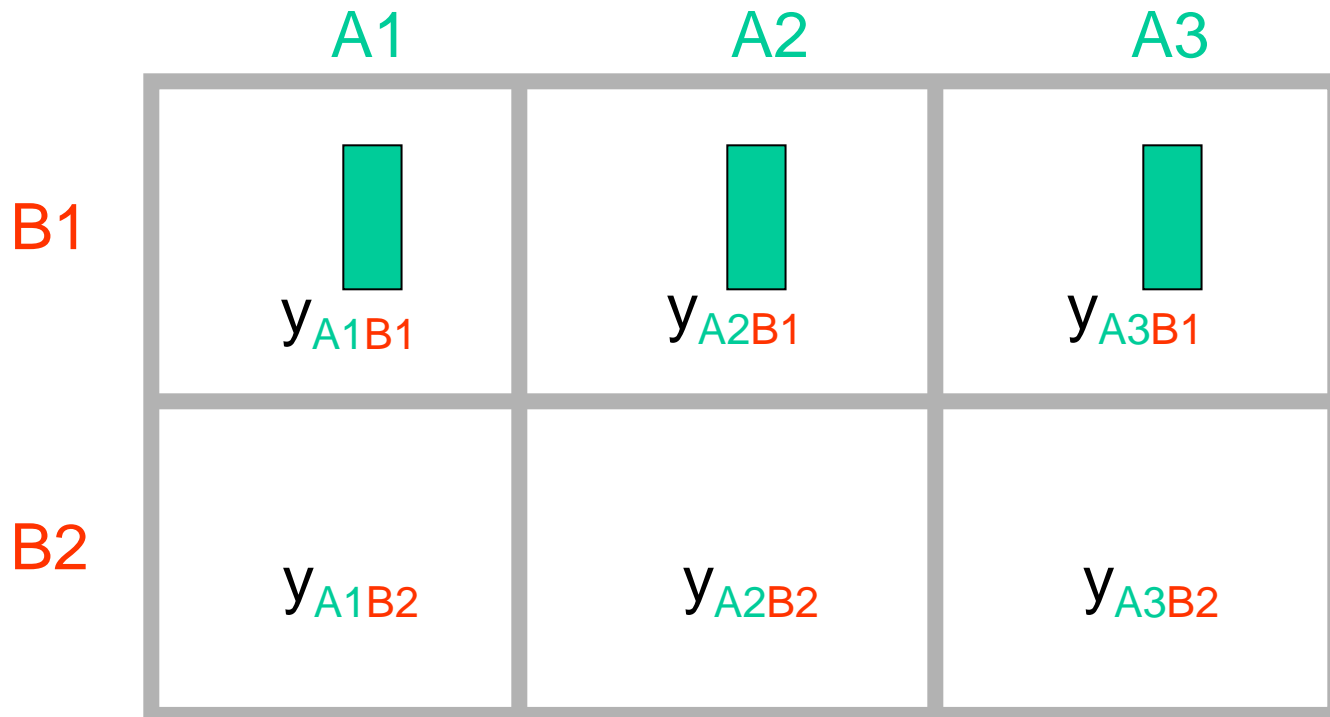- **Two-way Factorial ANOVA for independent data**
  - Testing Interaction Effects:
    - When an interaction is present, you must test **simple main effects (SME)**
      - comparison of dependent variable means at individual levels of each independent variable (SME of **A** at **B1** and SME of **A** at **B2**)
      - example below illustrates testing the simple main effects of **A** at **B1**
        - is there any significant difference in y_{A1B1}, y_{A2B1}, & y_{A3B1}

	A1	A2	A3
B1	y_{A1B1}	y_{A2B1}	y_{A3B1}
B2	y_{A1B2}	y_{A2B2}	y_{A3B2}

Analysis of Variance (ANOVA)

- **Two-Way factorial ANOVA for independent data**
  - <u>Testing Interaction Effects:</u>
    - When an interaction is present, you must test simple main effects (SME)
      - comparison of dependent variable means at individual levels of each independent variable (SME of B at A1, SME of B at A2, SME of B at A3)
      - example below illustrates testing the simple main effects of B at A2
        - is there any significant difference in yA2B1 & yA2B2

	A1	A2	A3
B1	y_{A1B1}	y_{A2B1}	y_{A3B1}
B2	y_{A1B2}	y_{A2B2}	y_{A3B2}

- **Factorial ANOVA source Table (SAS)**:

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	17961132.56666660	3592226.51333334	589.80	0.0001
Error	24	146174.39999998	6090.60000000		
Corrected Total	29	18107306.96666660			

R-Square	C.V.	Root MSE	NE Mean
0.991927	2.661712	78.0422962	2932.03333333

| Source	DF	Anova SS	Mean Square	F Value	Pr > F
VIDEO	1	7178520.83333331	7178520.83333331	1178.62	0.0001
ELEMENT	2	7079881.66666666	3539940.83333333	581.21	0.0001
VIDEO*ELEMENT	2	3702730.06666672	1851365.03333336	303.97	0.0001

Other types of ANOVA's

- **Multi-Factor factorial ANOVA**
  - **example 2 X 2 X3**
  - **more than two independent variables**
  - **F-ratio is obtained for each factor & interaction**

|	A1		A2		A3	
	B1	B2	B1	B2	B1	B2
C1						
C2						

y_{A1B1C1}
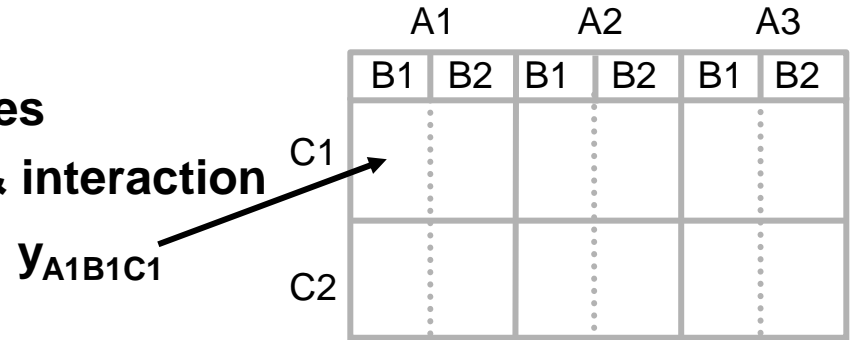
- **Factorial AVOVA's with repeated measures**
  - **Split plot (the illustrated design) is popular**
  - **F-ratio obtained for each timepoint (T),**
    for each classification (B) of subjects,
    and for interaction

		T1	T2	T3
B1	s1			
	s2			
B2	s3			
	s4			
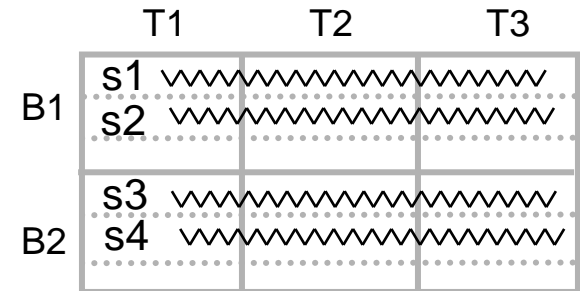
- **Multivariate ANOVA's**
  - **One-Way or Factorial ANOVA's with more**
    than one dependent variable
    - obtain overall test statistic (Wilks λ)
    - follow up with univariate ANOVA's for each
      dependent variable then do all post hocs

	A1	A2	A3
B1	y_1 y_2		
B2			
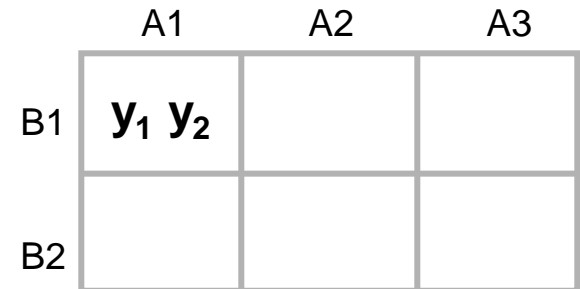
Other types of ANOVA's

- **Analysis of Covariance (ANCOVA) - mixture of ANOVA and regression**
  - Adjust the means of the dependent variable for the influence of another variable
    - Least Square Means - means adjusted for influence of covariate allowing for comparisons
  - Used when
    - checking for differences between means taking into account information on another variable
  - Can be one-way or factorial
    - Covariate variable must be continuous
    - Relationship of covariate variable to dependent variable must be linear and be the same in all classifications of the independent variable

- In our ropes course example: Suppose we knew that plasma NE levels were linearly related to the time spent on the course. We would enter minutes on the course as a covariate.

- Suppose we wish to test a control group and an experimental group on measures of strength after putting the experimental group through a weight training regimen. Pre-training scores for strength would be entered as a covariate to adjust the post-training scores for scores on the pre-training assessment.

post training control group	post training experim. group
$y1_{\text{strength measure}}$	$y2_{\text{strength measure}}$
$x_{\text{pre-test score}}$	$x_{\text{pre-test score}}$