Week 3

Read the remainder of Hurlbert 1984

What type of **pseudoreplication** are we committing if we don't declare Person as Random in Experiment 3?

		Sit			Stand			Sit		# people	# measures	#EU
3)	Jill	X	T1	Bob	X	T1	Amy	X	T1	40	80	80
		X	T2		X	T2		X	T2			

More Design Tables and writing R models

Comparing experimental designs with Replicates and Subsamples

Standard Errors and Estimated Standard Errors

Fitting models in R

1) Write the model

2) Pass it to the function to fit the model: **lm()** or **lmer()**

3) Pass the result to a function to get estimates, SE, CI, etc

   emmeans(), **contrast()**, etc

Writing the model

1) Response ~ every variable in Treatment + Design

   with # levels < # responses

   combined with “+”

2) Any variable that is a **EU** gets declared with (1|X)

   + Variable => + (1|Variable)

   call these “random”

3) Replicate variables combined with treatments are random

   + (1|Replicate:Treatment)

   This can be omitted for narrower scope

4) If any random variable is included, use lmer(). Otherwise use lm()

   For our purposes, the only difference between these functions is the type of model statement they allow

   The underlying code is very different

   Some arguments of emmeans are different

							# people	# measures	#EU
		Sit		Stand		Sit			
1)	Jill	$\boxed{X}$	Bob	$\boxed{X}$	Amy	$\boxed{X}$	80	80	80

Structure	Variable	Type	#levels	Replicate	EU
Treatment	Posture	Cat	2	None	Person
Design	Person	Cat	80		
Response	Pulse	Num	80		

Write the model: **lm(Pulse ~ Posture)**

1) Response ~ every variable in Treatment + Design

    with # levels < # responses

    combined with "+"

2) Any variable that is a **EU** gets declared with (1|X)

    + Variable => + (1|Variable)

    call these "random"

3) Replicate variables combined with treatments are random

    + (1|Replicate:Treatment)

    This can be omitted for narrower scope

4) If any random variable is included, use lmer(). Otherwise use lm()

2)

	Jill	Bob	Amy
T1	Sit	Stand	Sit
T2	Stand	Sit	Stand

# people	# measures	#EU
40	80	80

Structure	Variable	Type	#levels	Replicate	EU
Treatment	Posture	Cat	2	Person	Person:Trial
Design	Person	Cat	40		
	Trial	Cat	2		
	Person:Trial	Cat	80		
	Person:Posture	Cat	80		
Response	Pulse	Num	80		

Write the model: **lm(Pulse ~ Posture + Person + Trial)**

1) Response ~ every variable in Treatment + Design

with # levels < # responses

combined with "+"

2) Any variable that is a **EU** gets declared with (1|X)

+ Variable => + (1|Variable)

call these "random"

3) Replicate variables combined with treatments are random

+ (1|Replicate:Treatment)

This can be omitted for narrower scope

4) If any random variable is included, use lmer(). Otherwise use lm()

	Sit		Stand		Sit		# people	# measures	#EU
3)	Jill	X T1	Bob	X T1	Amy	X T1	40	80	80
		X T2		X T2		X T2			

Structure	Variable	Type	#levels	Replicate	EU
Treatment	Posture	Cat	2	None	Person
Design	Person	Cat	40		
	Trial	Cat	2		
	Person:Trial	Cat	80		
Response	Pulse	Num	80		

Write the model: **lmer(Pulse ~ Posture + (1|Person) + Trial)**

1) Response ~ every variable in Treatment + Design

   with # levels < # responses

   combined with “+”

2) Any variable that is a **EU** gets declared with (1|X)

   + Variable => + (1|Variable)

   call these “random”

3) Replicate variables combined with treatments are random

   + (1|Replicate:Treatment)

   This can be omitted for narrower scope

4) If any random variable is included, use lmer(). Otherwise use lm()

Why don’t we use Trial as a **Replicate** in 2) and 3)?

		Jill		Bob		Amy	# people	# measures	#EU
2)	T1	Sit	T1	Stand	T1	Sit	40	80	80
	T2	Stand	T2	Sit	T2	Stand			

		Sit			Stand			Sit		# people	# measures	#EU
3)	Jill	X	T1	Bob	X	T1	Amy	X	T1	40	80	80
		X	T2		X	T2		X	T2			

In both cases, we have 2 trials, each with observations of sitting and standing

We could get a “direct” estimate of the standing effect in each trial

Benefit

Control for changes in Pulse / treatment effect between trials

Cost

With only 2 trials, we only have (n-1) = 1 degree of freedom

$t_c(\alpha = 0.05) = 12.7$ (huge confidence intervals)

Why?

Trials are replicates if our goal is to extrapolate to **new trials**

Otherwise, we are limited to the **average** of these two trials

Are we concerned about the treatment effect **changing** between our two trials?

Are all treatment effects **the same** if we do only 1 trial?

Efficiency of Experimental Designs

	Jill		Bob		Amy	# people	# measures	#EU
2) T1	Sit	T1	Stand	T1	Sit	40	80	80
T2	Stand	T2	Sit	T2	Stand			

$$\hat{\delta}_{B-A} = \frac{1}{n} \sum \hat{\delta}_j$$

Direct estimate = average of *n* observations

$$\sigma_r(\hat{\delta}) = \sqrt{\frac{\text{Variance of population + Variance of measurements}}{\text{Sample size}}}$$

	Sit		Stand		Sit	# people	# measures	#EU
1) Jill	X	Bob	X	Amy	X	80	80	80

$$\hat{\delta}_{B-A} = \hat{\mu}_B - \hat{\mu}_A$$

Indirect estimate of δ

$$\hat{\mu}_A = \frac{1}{n_A} \sum \hat{\mu}_{Aj} \qquad \hat{\mu}_B = \frac{1}{n_B} \sum \hat{\mu}_{Bj}$$

direct estimates of μ_B and μ_A

$$\sigma_r(\hat{\mu}_i) = \sqrt{\frac{\text{Variance of population + Variance of measurements}}{\text{Sample size}}}$$

$$\sigma_r(\hat{\delta}) = \sqrt{\sigma_r^2(\hat{\mu}_B) + \sigma_r^2(\hat{\mu}_A)}$$

Add the two variances

Because errors ϵ_B and ϵ_A are independent

Use different EU

Efficiency of Experimental Designs

2)

	Jill		Bob		Amy	# people	# measures	#EU
T1	Sit	T1	Stand	T1	Sit	40	80	80
T2	Stand	T2	Sit	T2	Stand			

$$\sigma_r(\hat{\delta}) = \sqrt{\frac{\text{Variance of population + Variance of measurements}}{\text{Sample size}}}$$ **Direct** Standard Error

population = TRUE **Standing effects** $\delta_j = \mu_{Bj} - \mu_{Aj}$

measurements = Estimates of effect of each person $\hat{\delta}_j = \hat{\mu}_{Bj} - \hat{\mu}_{Aj}$

sample size = # Replicates

$$\sigma_r(\hat{\delta}) = \sqrt{\frac{\sigma_\delta^2 + \sigma_r^2(\hat{\delta}_j)}{40}}$$

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1)

	Sit		Stand		Sit	# people	# measures	#EU
Jill	X	Bob	X	Amy	X	80	80	80

$$\sigma_r(\hat{\delta}) = \sqrt{\sigma_r^2(\hat{\mu}_B) + \sigma_r^2(\hat{\mu}_A)}$$ **Indirect** Standard Error

$$\sigma_r(\hat{\mu}_i) = \sqrt{\frac{\text{Variance of population + Variance of measurements}}{\text{Sample size}}}$$ **Direct** Standard Error

population = TRUE **Standing pulse** μ_{Aj} sample size = # replicates/level

measurements = Estimates of Standing pulse $\hat{\mu}_{Aj} = y_{Aj}$

$$\sigma_r(\hat{\mu}_A) = \sqrt{\frac{\sigma_{\mu_A}^2 + \sigma_r^2(\hat{\mu}_{Aj})}{40}} \qquad \sigma_r(\hat{\delta}) = \sqrt{\frac{\sigma_{\mu_B}^2 + \sigma_r^2(\hat{\mu}_{Bj})}{40} + \frac{\sigma_{\mu_A}^2 + \sigma_r^2(\hat{\mu}_{Aj})}{40}}$$

Efficiency of Experimental Designs

Design 2

$$\sigma_r(\hat{\delta}) = \sqrt{\frac{\sigma_\delta^2 + \sigma_r^2(\hat{\delta}_j)}{40}}$$

people

Design 1

$$\sigma_r(\hat{\delta}) = \sqrt{\frac{\sigma_{\mu_B}^2 + \sigma_r^2(\hat{\mu}_{Bj})}{40} + \frac{\sigma_{\mu_A}^2 + \sigma_r^2(\hat{\mu}_{Aj})}{40}}$$

people Standing

people Sitting

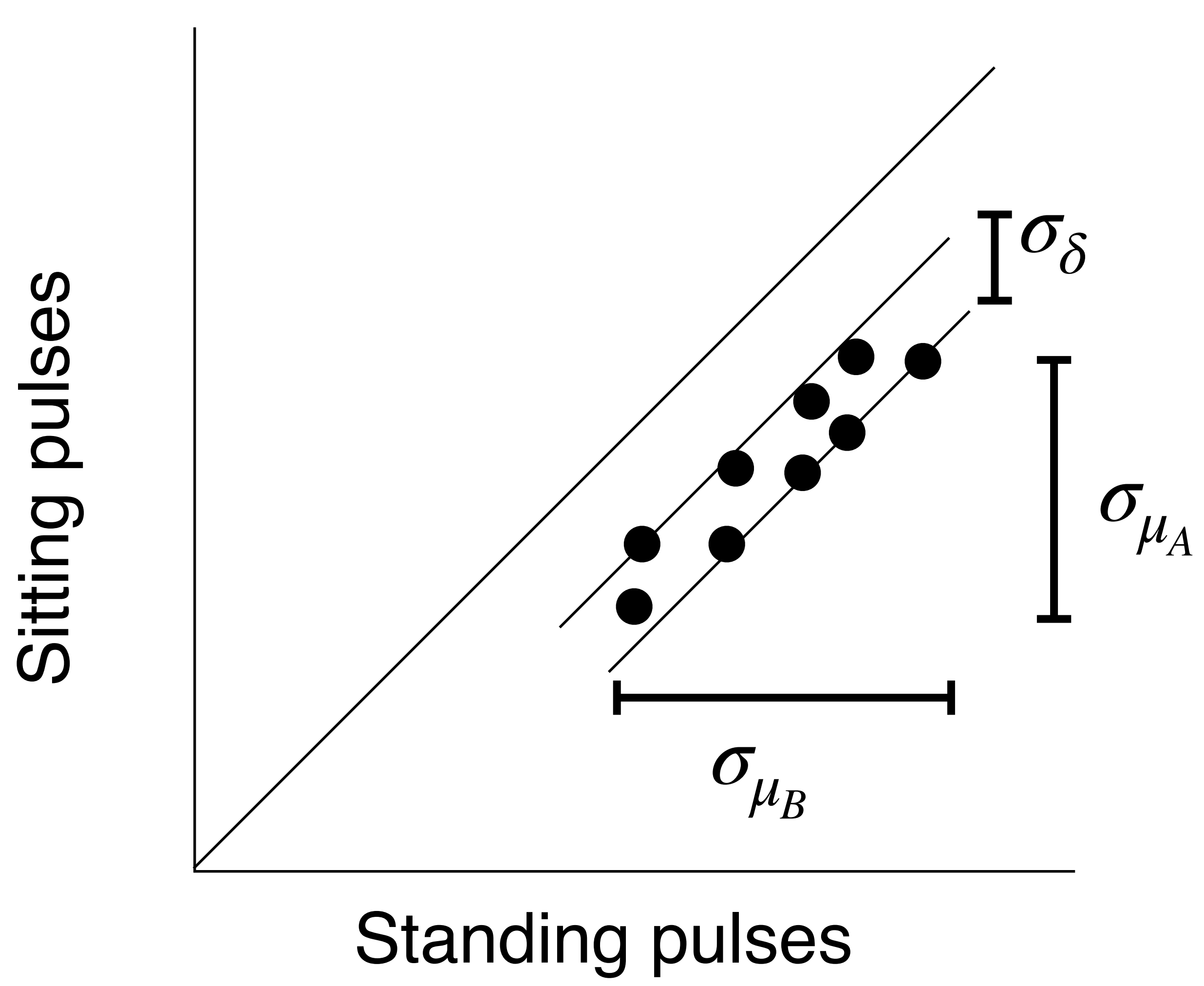
Which is bigger: measurement error for $\hat{\delta}_j$ or measurement error for $\hat{\mu}_{Bj}$ or $\hat{\mu}_{Aj}$?

$\sigma_r^2(\hat{\delta}_j)$ is 2x as big because it's from 2 separate pulse measurements (σ_m^2)

$$\sigma_r^2(\hat{\delta}_j) = 2\sigma_m^2,\ \sigma_r^2(\hat{\mu}_{ij}) = \sigma_m^2$$

Which is bigger: TRUE variance of **effects** or TRUE variance of **sitting(standing) pulses?**

$\sigma_{\mu_i}^2$ is generally bigger because μ_{Bj} and μ_{Aj} are **correlated**



$$\delta_j = \mu_{Bj} - \mu_{Aj}$$

When you add two **correlated** variables, the total variance is less than the sum of each variance

Efficiency of Experimental Designs

Design 2

$$\sigma_r(\hat{\delta}) = \sqrt{\frac{\sigma_\delta^2 + 2\sigma_m^2}{40}}$$

40 ← # people

Design 1

$$\sigma_r(\hat{\delta}) = \sqrt{\frac{\sigma_{\mu_B}^2 + \sigma_m^2}{40} + \frac{\sigma_{\mu_A}^2 + \sigma_m^2}{40}}$$

first 40 ← # people Standing; second 40 ← # people Sitting

If the variances of Sitting and Standing are the same

And the sample sizes are the same, we can simplify

$$\sigma_r(\hat{\delta}) = \sqrt{\frac{2\sigma_{\mu_i}^2 + 2\sigma_m^2}{40}}$$

Since $\sigma_\delta^2 < 2\sigma_{\mu_i}^2$, Design 2 has a smaller standard error

With same # measurements and #EU, but fewer people

What about Design 3?

		Sit		Stand		Sit	# people	# measures	#EU
3)	Jill	X T1 X T2	Bob	X T1 X T2	Amy	X T1 X T2	40	80	40

Direct or Indirect?

What is the formula for $\sigma_r(\hat{\delta})$?

Is the total population variance less than designs 1 or 2?

Is the measurement error of each estimate less than designs 1 or 2?

What about Design 3?

		Sit			Stand			Sit		# people	# measures	#EU
3)	Jill	X	T1	Bob	X	T1	Amy	X	T1	40	80	40
		X	T2		X	T2		X	T2			

$$\sigma_r(\hat{\delta}) = \sqrt{\sigma_r^2(\hat{\mu}_B) + \sigma_r^2(\hat{\mu}_A)}$$ **Indirect** Standard Error

$$\sigma_r(\hat{\mu}_i) = \sqrt{\frac{\text{Variance of population + Variance of measurements}}{\text{Sample size}}}$$ **Direct** Standard Error

population = TRUE **Standing pulse** μ_{Aj}

population variance = $\sigma^2_{\mu_i}$ (same as Design 1)

measurements = Estimates of Standing pulse $\hat{\mu}_{Aj} = \dfrac{y_{Aj_1} + y_{Aj_2}}{2}$

measurement variance is 1/2 that of Design 1: $\dfrac{\sigma_m^2}{2}$

samples / treatment level is 1/2 that of design 1

Final Standard Error:

$$\sigma_r(\hat{\delta}) = \sqrt{\frac{2\sigma^2_{\mu_i} + 2/2\sigma_m^2}{20}}$$

Efficiency of Experimental Designs

Design 2

$$\sigma_r(\hat{\delta}) = \sqrt{\frac{\sigma_\delta^2 + 2\sigma_m^2}{40}}$$

Smaller population variance

Design 1

$$\sigma_r(\hat{\delta}) = \sqrt{\frac{2\sigma_{\mu_i}^2 + 2\sigma_m^2}{40}}$$

Smaller measurement error

Design 3

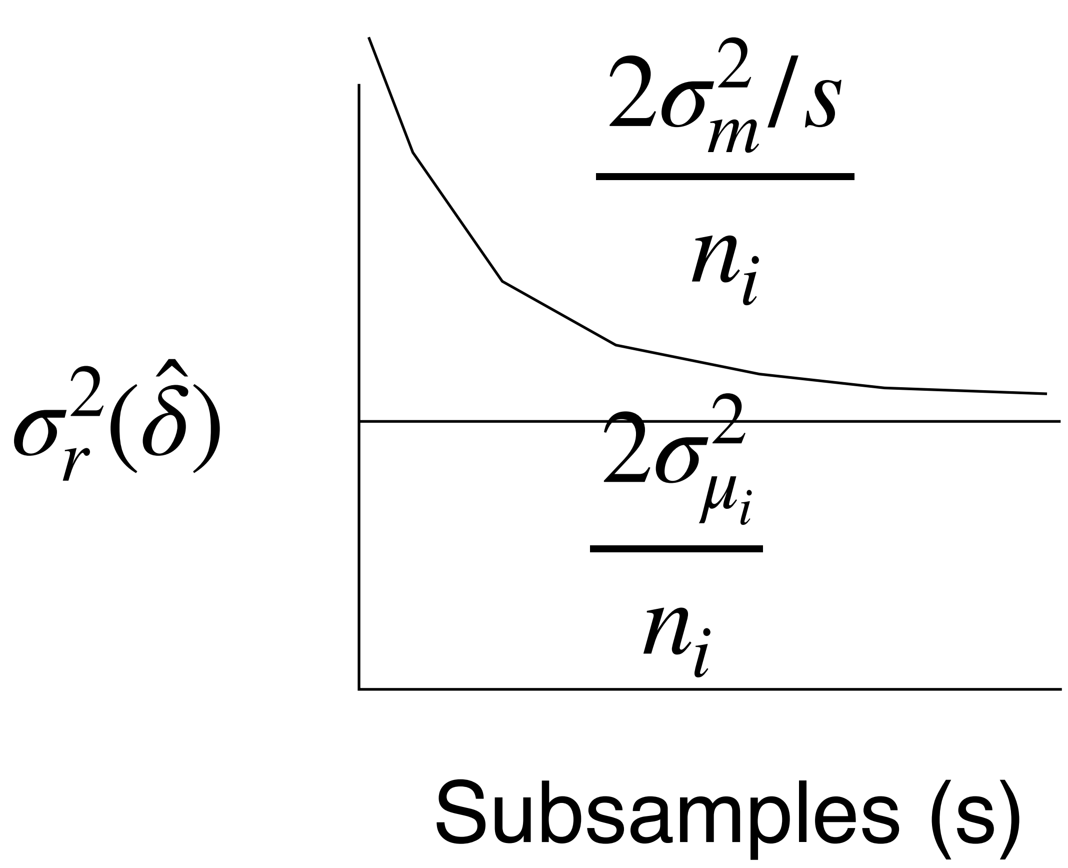
$$\sigma_r(\hat{\delta}) = \sqrt{\frac{2\sigma_{\mu_i}^2 + 2/2\sigma_m^2}{20}}$$

Smaller sample size (#EU / level)

	Sit			Stand			Sit	
Jill	X	T1	Bob	X	T1	Amy	X	T1
	X	T2		X	T2		X	T2

Repeated measures of the same sample are **subsamples**

Not declaring EU causes **pseudoreplication**



More subsamples = less measurement error / EU

More subsamples = fewer EU

Optimal number of subsamples

Tradeoff between EU and subsamples based on cost

Optimal number of subsamples $\sqrt{\frac{k}{c}}$

$$c = \frac{\text{cost/(subsample + measurement)}}{\text{cost/(EU)}}$$

$$k = \frac{\sigma_m^2}{\sigma_{\mu_i}^2} \qquad \left(\frac{\dfrac{\hat{\mu}_{ij1} - \hat{\mu}_{ij2}}{2} \ \text{half the difference among replicate measures of the same individual}}{\dfrac{\mu_{i1} - \mu_{i2}}{2} \ \text{half the difference among different individuals}} \right)^2$$

k	c	Optimal number of subsamples
.5	1	.7 $\longrightarrow$ 1
1	1	1 $\longrightarrow$ 1
2	1	1.4 $\longrightarrow$ 1
4	1	2 $\longrightarrow$ 2
.5	1/10	2.2 $\longrightarrow$ 2
1	1/10	3.1 $\longrightarrow$ 3
2	1/10	4.5 $\longrightarrow$ 4

Estimated Standard Errors for each design

Design 2 "Direct"

$$\hat{\delta}_{B-A} = \frac{1}{n}\sum \hat{\delta}_j$$ Direct estimate = average of *n* observations

TRUE Standard Error:

$$\sigma_r(\hat{\delta}) = \sqrt{\frac{\text{Variance of population + Variance of measurements}}{\text{Sample size}}}$$

Estimated Standard Error:

Sample Variance of $\hat{\delta}_i \approx$ Variance of TRUE values + Variance of error

$$s^2_{\hat{\delta}} = \frac{\sum (\hat{\delta}_j - \hat{\delta})^2}{n-1}$$

SED = $\sqrt{\frac{s^2_{\hat{\delta}}}{n}}$ This is an estimate of $\sigma_r(\hat{\delta})$

Degrees of Freedom

(n-1) from denominator of $s^2_{\hat{\delta}}$

Estimated Standard Errors for each design

Design 1 "Indirect"

$$\hat{\delta}_{B-A} = \hat{\mu}_B - \hat{\mu}_A$$

Indirect estimate of δ

$$\hat{\mu}_A = \frac{1}{n_A} \sum \hat{\mu}_{Aj}$$

direct estimates of μ_B and μ_A

TRUE Standard Error:

$$\sigma_r(\hat{\mu}_i) = \sqrt{\frac{\text{Variance of population + Variance of measurements}}{\text{Sample size}}}$$

Estimated Standard Error:

Sample Variance of $\hat{\mu}_{ij} \approx$ Variance of TRUE values + Variance of error

$$s^2_{\hat{\mu}_i} = \frac{\sum (\hat{\mu}_{ij} - \hat{\mu}_i)^2}{n_i - 1}$$

Observed variance of estimates around their mean

SEM = $\sqrt{\frac{s^2_{\hat{\mu}_i}}{n_i}}$

TRUE Standard Error:

$$\sigma_r(\hat{\delta}) = \sqrt{\sigma_r^2(\hat{\mu}_B) + \sigma_r^2(\hat{\mu}_A)}$$

Estimated Standard Error:

SED = $\sqrt{\frac{s^2_{\hat{\mu}_B}}{n_B} + \frac{s^2_{\hat{\mu}_A}}{n_A}}$

Problem:

Degrees of Freedom

$(n_B - 1)$ from $s^2_{\hat{\mu}_B}$ or $(n_A - 1)$ from $s^2_{\hat{\mu}_A}$?

Can't use 2 degrees of freedom for the t-distribution

Best Df is closer to the **smaller** of $(n_B - 1)$ and $(n_A - 1)$

Solution: **Pooled** $s^2_{\hat{\mu}}$

Sample Variance of $\hat{\mu}_{ij} \approx$ Variance of TRUE values + Variance of errors

if Variances of pulses are similar for both treatments

And measurement errors are similar

We can **pool** all deviations together into a **pooled** $s^2_{\hat{\mu}}$

$$s^2_{\hat{\mu}} = \frac{\sum(\hat{\mu}_{Bj} - \hat{\mu}_B)^2 + \sum(\hat{\mu}_{Aj} - \hat{\mu}_A)^2}{(n_B - 1) + (n_A - 1)}$$

All deviations² from the sample means

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independent deviations per treatment

Estimated Standard Error:

SED = $\sqrt{\frac{s^2_{\hat{\mu}}}{n_B} + \frac{s^2_{\hat{\mu}}}{n_A}}$

If variances are equal, this is a **better** (more accurate) estimate of $\sigma_r(\hat{\delta})$

Here, we have a single df to use for confidence intervals:

Degrees of Freedom $(n_B - 1) + (n_A - 1)$

Key points:

Follow the sample sizes for **each treatment level**

Each is used 2x

We will always use the **pooled** $s^2_{\hat{\mu}}$ in this class because of limitations of the lm() and lmer() functions

Estimated Standard Errors for each design

		Sit			Stand			Sit		# people	# measures	#EU
3)	Jill	X	T1	Bob	X	T1	Amy	X	T1	40	80	40
		X	T2		X	T2		X	T2			

Which equation for s^2 and SED?

Which components will **tend to be** different from Design 1 / 2 ?

* our data will be different, so all estimates will be different *

$$\hat{\delta}_{B-A} = \hat{\mu}_B - \hat{\mu}_A \qquad s^2_{\hat{\mu}} = \frac{\sum(\hat{\mu}_{Bj} - \hat{\mu}_B)^2 + \sum(\hat{\mu}_{Aj} - \hat{\mu}_A)^2}{(n_B - 1) + (n_A - 1)}$$

$$\textbf{SED} = \sqrt{\frac{s^2_{\hat{\mu}}}{n_B} + \frac{s^2_{\hat{\mu}}}{n_A}}$$

$s^2_{\hat{\mu}}$ will **tend to be** smaller because of less measurement error

n_B and n_A will be smaller (or equal) because of costs

If so, SED *might be* larger and DF would be smaller

Rules for making Design Table

Include all variable necessary to describe the experiment

- Treatments: Variables we want to study
- Response: One Variable, always numeric
- Design
  - EU of the Treatment variable(s)
  - any Replicate and Replicate:Treatment
  - Variable with a unique level for each observation (Response)
  - Any other variable to describe the experiment

Check variable relationships: nested, aliased and crossed

- EU Variable must be **nested** in the Treatment variable
- If two variables are **aliased**, keep only 1 of them
- If two variables are **crossed**, keep only both

Relationships among variables

nested many:one Keep both, if 1 first is random, so is second

Experiment 2

Person	Person:Trial
Jill	Jill:T1
	Jill:T2
Bob	Bob:T1
	Bob:T2
Amy	Amy:T1
	Amy:T2

is_nested(Person~Person:Trial)

aliased one:one Keep one, particularly EU

Experiment 2

Person:Trial	Person:Posture
Jill:T1	Jill:Sit
Jill:T2	Jill:Stand
Bob:T1	Bob:Stand
Bob:T2	Bob:Sit
Amy:T1	Amy:Sit
Amy:T2	Amy:Stand

is_aliased(A~B)

crossed many:many Keep both

Experiment 2

Experiment 3

Posture	Trial
Sit	T1
Stand	T2

is_crossed(A~B)

Rules for making Design Table

Include all variable necessary to describe the experiment

- Treatments: Variables we want to study
- Response: One Variable, always numeric
- Design
  - EU of the Treatment variable(s)
  - any Replicate and Replicate:Treatment
  - Variable with a unique level for each observation (Response)
  - Any other variable to describe the experiment

Check variable relationships: nested, aliased and crossed

- EU Variable must be **nested** in the Treatment variable
  - Label as EU:Treatment
- If two variables are **aliased**, keep only 1 of them
  - If one is an EU, keep that one!
- If two variables are **crossed**, keep both
  - Treatments are crossed with their Replication variable

3)

	Sit		Stand		Sit	# people	# measures	#EU
Jill	X T1	Bob	X T1	Amy	X T1	40	80	40
	X T2		X T2		X T2			

Person	Posture	Pulse	Trial	Person:Trial
Jill	Sit	60	T1	Jill:T1
Jill	Sit	64	T2	Jill:T2
Bob	Stand	72	T1	Bob:T1
Bob	Stand	68	T2	Bob:T2
Amy	Sit	106	T1	Amy:T1
Amy	Sit	112	T2	Amy:T2
⋮				

Structure	Variable	Type	#levels	Replicate	EU
Treatment	Posture	Cat	2	None	Person
Design	Person	Cat	40		
	Trial	Cat	2		
	Person:Trial	Cat	80		
Response	Pulse	Num	80		