

# What about Design 3?

	Sit	Stand	Sit	# people	# measures	#EU												
3)	Jill	Bob	Amy	40	80	40												
	<table><tr><td>X</td><td>T1</td></tr><tr><td>X</td><td>T2</td></tr></table>	X	T1	X	T2	<table><tr><td>X</td><td>T1</td></tr><tr><td>X</td><td>T2</td></tr></table>	X	T1	X	T2	<table><tr><td>X</td><td>T1</td></tr><tr><td>X</td><td>T2</td></tr></table>	X	T1	X	T2			
X	T1																	
X	T2																	
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X	T2																	
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X	T2																	

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

Indirect Standard Error

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

Direct Standard Error

population = TRUE **Standing pulse**       $\mu_{Aj}$

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

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

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

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

Final Standard Error:

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

# Efficiency of Experimental Designs

## Design 2

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

## Design 1

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

## Design 3

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

Smaller population variance

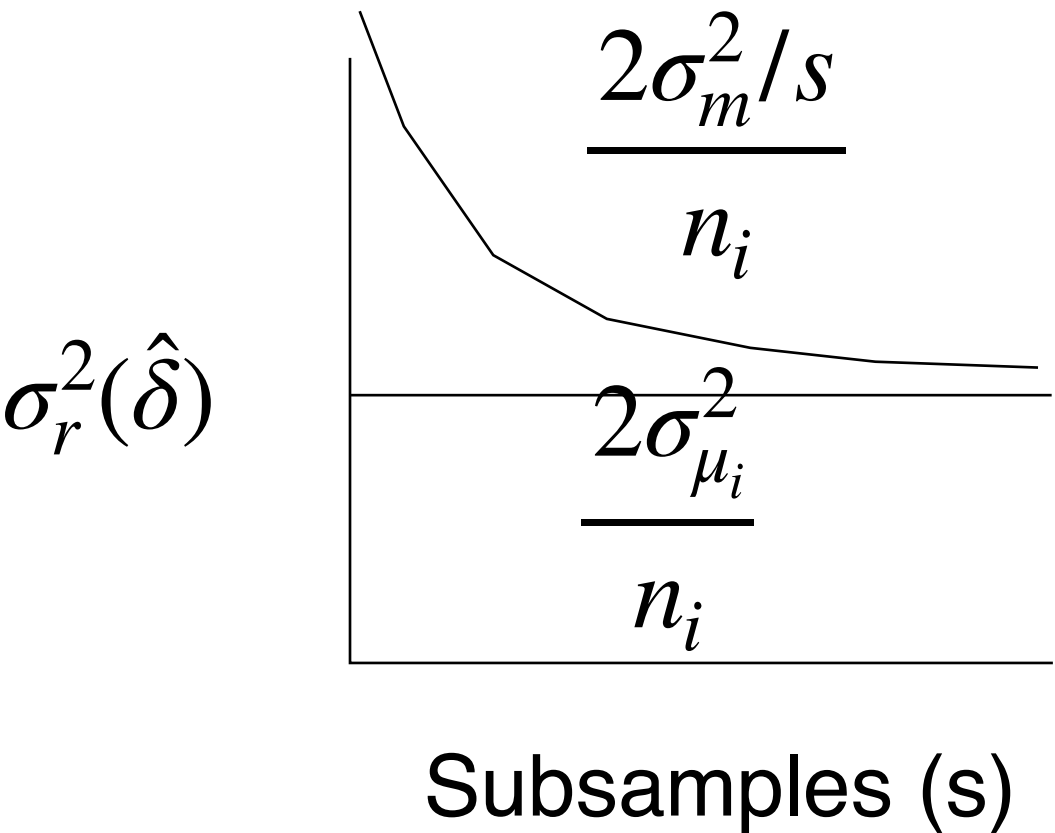
Smaller measurement error

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  $\sqrt{\frac{k}{c}}$

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

$k = \frac{\sigma_m^2}{\sigma_{\mu_i}^2} \left( \frac{\frac{\hat{\mu}_{ij1} - \hat{\mu}_{ij2}}{2}}{\frac{\mu_{i1} - \mu_{i2}}{2}} \right)^2$

half the difference among replicate measures of the same individual

half the difference among different individuals

k	c	Optimal number of subsamples	
.5	1	.7	→ 1
1	1	1	→ 1
2	1	1.4	→ 1
4	1	2	→ 2
.5	1/10	2.2	→ 2
1	1/10	3.1	→ 3
2	1/10	4.5	→ 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:

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

Estimated  
Standard  
Error:

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

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

$$\text{SED} = \sqrt{\frac{s_{\hat{\delta}}^2}{n}}$$

This is an estimate of  $\sigma_r(\hat{\delta})$

Degrees of  
Freedom

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

# Estimated Standard Errors for each design

Design 1                      “Indirect”

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

$\hat{\mu}_A = \frac{1}{n_A} \sum \hat{\mu}_{Aj}$                       direct estimates of  $\mu_B$  and  $\mu_A$

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

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** $s_{\hat{\mu}}^2$

**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**  $s_{\hat{\mu}}^2$

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

All deviations<sup>2</sup> from the sample means

# independent deviations per treatment

Estimated  
Standard  
Error:

$$\mathbf{SED} = \sqrt{\frac{s_{\hat{\mu}}^2}{n_B} + \frac{s_{\hat{\mu}}^2}{n_A}}$$

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**  $s_{\hat{\mu}}^2$  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	<table><tr><td>X</td></tr><tr><td>X</td></tr></table> <sup>T1</sup> <sub>T2</sub>	X	X	Bob	<table><tr><td>X</td></tr><tr><td>X</td></tr></table> <sup>T1</sup> <sub>T2</sub>	X	X	Amy	<table><tr><td>X</td></tr><tr><td>X</td></tr></table> <sup>T1</sup> <sub>T2</sub>	X	X	40	80	40
X															
X															
X															
X															
X															
X															

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 \*

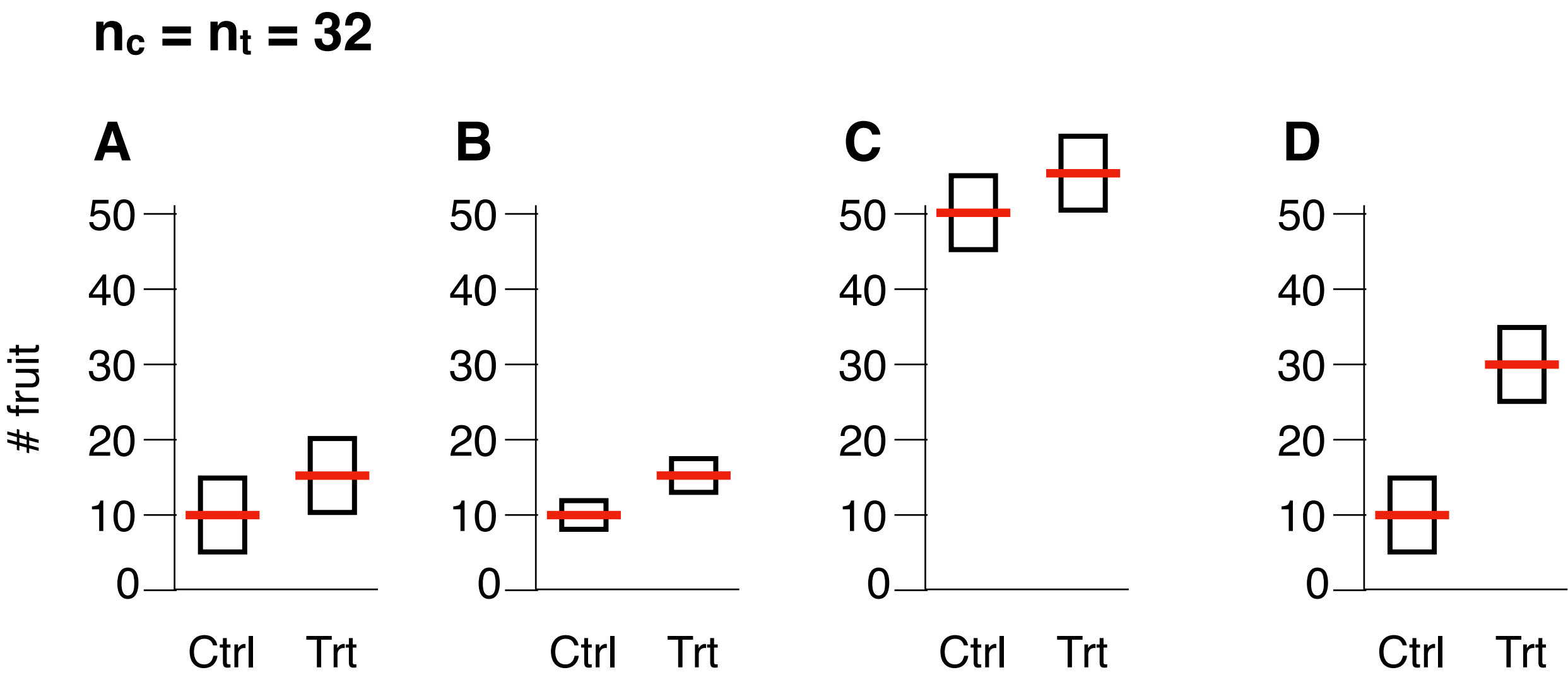
$$\hat{\delta}_{B-A} = \hat{\mu}_B - \hat{\mu}_A \quad s_{\hat{\mu}}^2 = \frac{\sum (\hat{\mu}_{Bj} - \hat{\mu}_B)^2 + \sum (\hat{\mu}_{Aj} - \hat{\mu}_A)^2}{(n_B - 1) + (n_A - 1)}$$

$$\text{SED} = \sqrt{\frac{s_{\hat{\mu}}^2}{n_B} + \frac{s_{\hat{\mu}}^2}{n_A}}$$

$s_{\hat{\mu}}^2$  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



Which effect is largest?

Which effect is most important?

Which effect is most significant?

$\hat{\delta}$	5	5	5	20
$s_{pooled}$	8	2	8	8
SED	2	0.5	2	2
$\frac{\hat{\delta}}{\hat{\mu}_c}$	5/10=0.5	0.5	5/50=0.1	2
$\frac{\hat{\delta}}{SED}$	5/2=2.5	5/0.5=10	2.5	10

Hypothesis tests deal with significance, not importance



# Hypothesis testing

Unlike confidence intervals, do not report effect sizes

Instead, report **evidence** or a **decision** about whether an effect could be 0

T-test:

1) Calculate t-statistic:  $\frac{\hat{\delta}}{SED}$

2) Calculate **p-value** from T-distribution with  $df$   
 $2 * pt(t, df, lower.tail=F)$

Outcomes: Decision about plausibility of  $H_0$

1) Weigh evidence

Is **p** small?

The smaller **p**, the stronger the evidence that  $\delta \neq 0$

Report: more/less significant

**p-value** is one piece of evidence

weigh this with effect size, plausibility, other data

2) Decide Yes/No

Determine consequences of being wrong

Choose a threshold  $\alpha$

If  $p < \alpha$ , declare **significant**, state  $\delta \neq 0$

If  $p > \alpha$ , declare **not significant**, state  $\delta$  **could be 0**

Don't report the p-value itself!

# Hypothesis testing

Determine consequences of being wrong

Table of outcomes

		<b>Fail to Reject</b>	<b>Reject</b>
		Declare $\delta$ may be 0	Declare $\delta \neq 0$
<b>TRUE</b>	$\delta = 0$	+	X - False Positive
<b>FALSE</b>	$\delta \neq 0$	X - False Negative	+

$\alpha$  = Probability of **False Positive** (Reject when  $\delta = 0$ )

If  $\delta = 0$ , and our  $p < \alpha$ , we'll make a False Positive mistake

Probability of this is  $\alpha$

$\beta$  = Probability of **False Negative** (Accept when  $\delta \neq 0$ )

Lost opportunities

If  $\delta \neq 0$ , and our  $p > \alpha$ , we'll make a False Negative mistake

$1 - \beta$  = **Power** (Reject when  $\delta \neq 0$ )

**Power:** Change of declaring significant when  $\delta \neq 0$

**Goal: Power > 80%**

# What determines the Power of an experiment?

Declare significant if  $p < \alpha$

## What goes into p?

$2 * pt(t, df, lower.tail=F)$

$$t = \frac{\hat{\delta}}{SED} \quad \text{TRUE effect size } \delta$$
$$\sqrt{\frac{s_{pooled}^2}{n_B} + \frac{s_{pooled}^2}{n_A}} \quad \sigma_y^2 = \sigma_\mu^2 + \sigma_m^2$$

Sample size

$df$  Denominator of  $s_{pooled}^2$   $(n_A - 1) + (n_B - 1)$

## What controls $\alpha$ ?

You choose  $\alpha$ !

Higher  $\alpha \rightarrow$  higher power

But also greater chance of a False Positive

# Calculating Power

```
power.t.test(n = NULL, delta = NULL, sd = 1, sig.level = 0.05,  
             power = NULL,  
             type = c("two.sample", "one.sample", "paired"),  
             alternative = c("two.sided", "one.sided"),  
             strict = FALSE, tol = .Machine$double.eps^0.25)
```

$n$  = # samples **per treatment**

$\delta$  = **TRUE** effect size

$sd$  = **TRUE** standard deviation of observations

$\text{sig.level} = \alpha$

$\text{power} = 1 - \beta$

Choose 1 of these to set to NULL

R will calculate its value

Need to guess at  **$\delta$**  and  **$sd$**

Questions:

What happens to **Power** when you **increase** each of the other parameters?

List 4 ways in **increase Power** in an experiment

# Calculating Power

```
power.t.test(n = NULL, delta = NULL, sd = 1, sig.level = 0.05,  
             power = NULL,  
             type = c("two.sample", "one.sample", "paired"),  
             alternative = c("two.sided", "one.sided"),  
             strict = FALSE, tol = .Machine$double.eps^0.25)
```

$n$  = # samples **per treatment**

$\delta$  = **TRUE** effect size

$sd$  = **TRUE** standard deviation of observations

$\text{sig.level} = \alpha$

$\text{power} = 1 - \beta$

Other options:

**type:** two.sample = Replicate Level

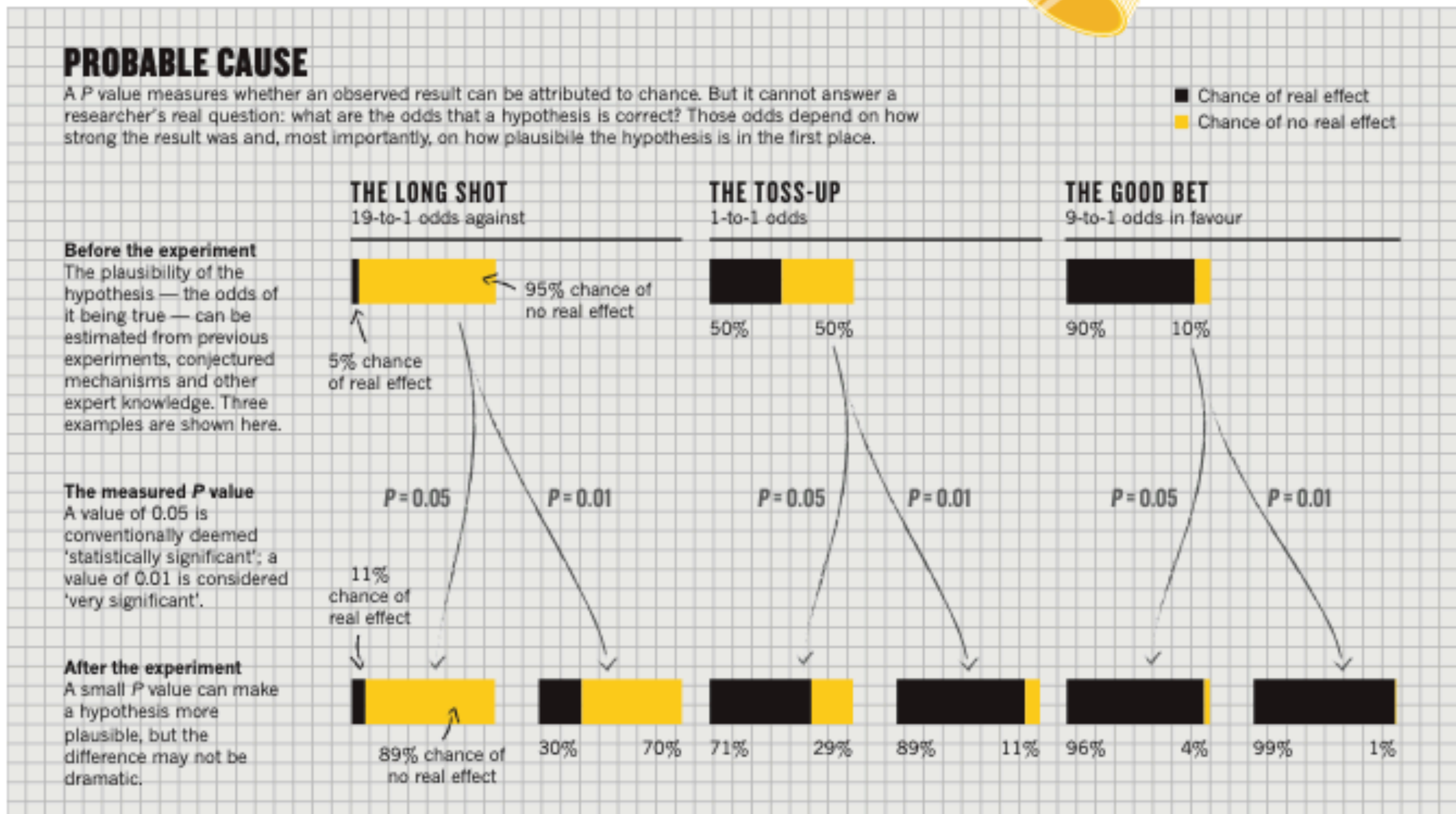
paired = Replicate Effect

one.sample = Test if  $\mu_A = 0$

**alternative:** two.sided: test if  $\delta \neq 0$

one.sided: test if  $\delta > 0$

# Statistical Errors paper



## Key points

small p-value from a implausible treatment is not strong evidence

small p-value from an experiment with low power won't replicate

you can get a small p-value with a meaningless effect if your experiment is large

If your experiment is small and your p-value is small, your effect size is probably over-estimated

# 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

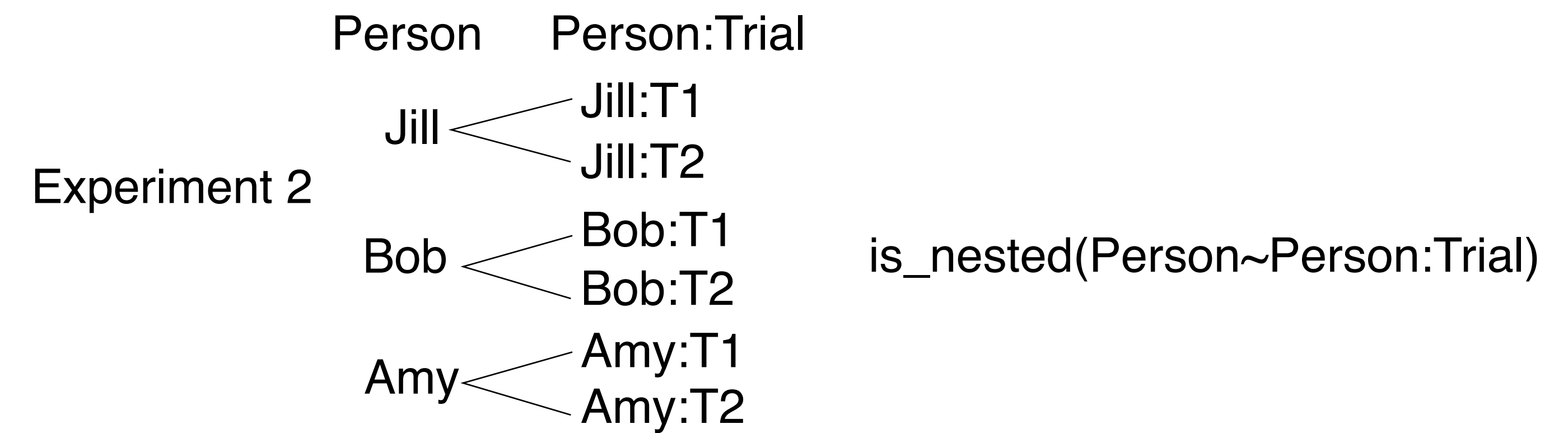
EU Variable must be **nested** in the Treatment variable

If two variables are **aliased**, keep only 1 of them

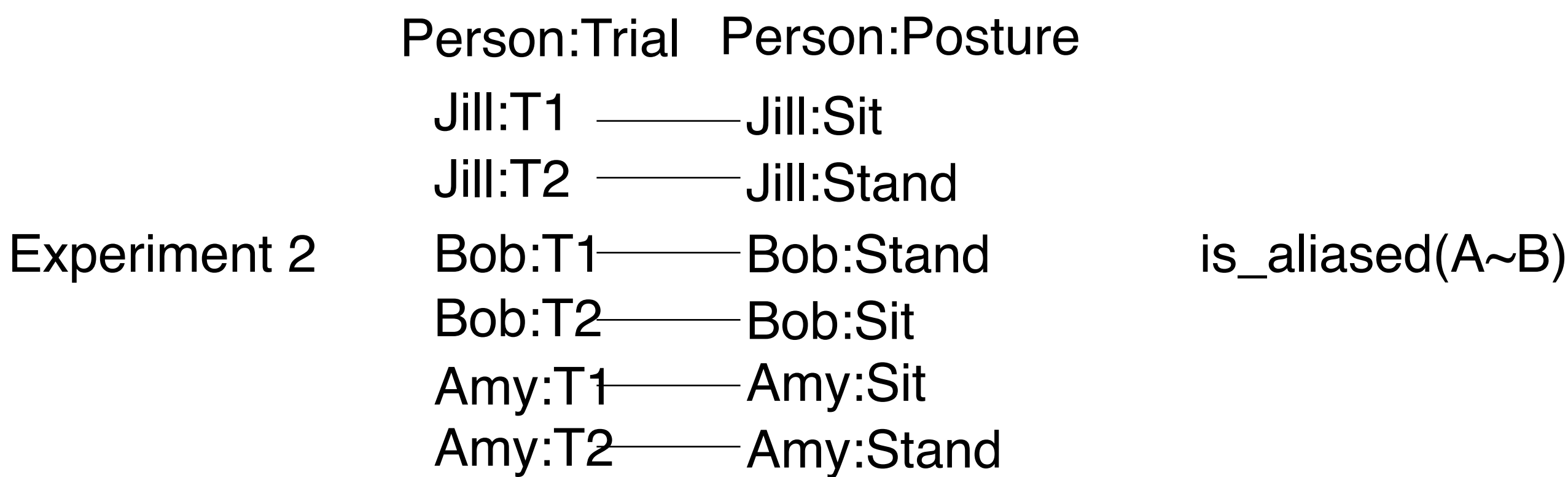
If two variables are **crossed**, keep only both

# Relationships among variables

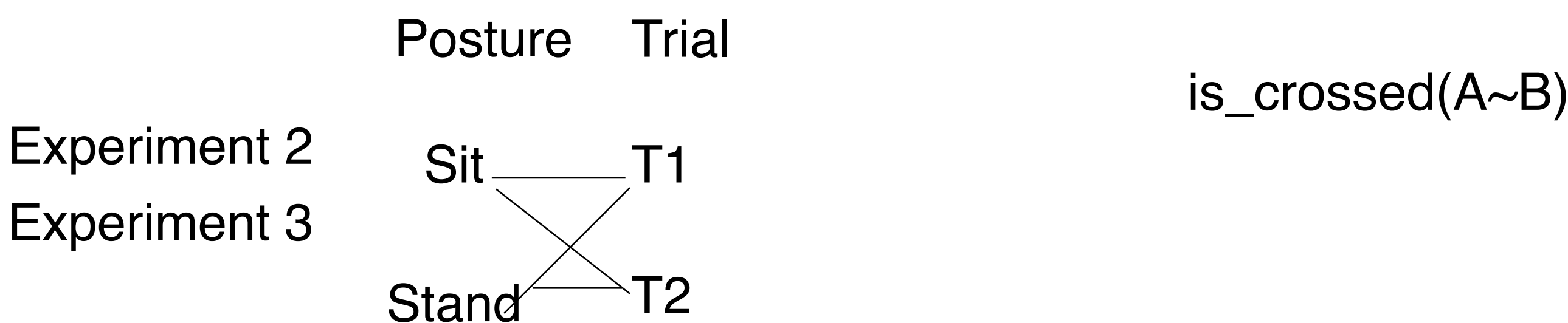
**nested**      many:one      Keep both, if 1 first is random, so is second



**aliased**      one:one      Keep one, particularly EU



**crossed**      many:many      Keep both





# 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

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

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		