Does sex matter ?

Or: Should we use both males and females in an experiment – and how ?

Frédéric Schütz, 2024

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UNIL | Université de Lausanne

Plate-forme de biostatistique

SIB Swiss Institute of Bioinformatics

An environmental factor that is linked to sex…

Olfactory exposure to males, including men, causes stress and related analgesia in rodents

Robert E Sorge[1,2,8], Loren J Martin[1,8], Kelsey A Isbester[1], Susana G Sotocinal[1], Sarah Rosen[1], Alexander H Tuttle[1], Jeffrey S Wieskopf[1], Erinn L Acland[1], Anastassia Dokova[1], Basil Kadoura[1], Philip Leger[1], Josiane C S Mapplebeck[1], Martina McPhail[3], Ada Delaney[4], Gustaf Wigerblad[4], Alan P Schumann[2], Tammie Quinn[2], Johannes Frasnelli[5,6], Camilla I Svensson[4], Wendy F Sternberg[3] & Jeffrey S Mogil[1,7]

We found that exposure of mice and rats to male but not female experimenters produces pain inhibition.

Nature Methods, 11 (6), June 2014. p. 629-632.

NATURE | NEWS

Sex matters in experiments on party drug — in mice

Ketamine lifts rodents' mood only if administered by male researchers.

Sara Reardon

17 November 2017

Form-A

No. .....

(to be completed by the authorities)

Application for licence to perform animal experiments

Article 18 Animal Welfare Act (TSchG),
article 141 Animal Welfare Ordinance (TSchV),
article 30 Animal Experimentation Ordinance (TVV)

§ 29: Experimental set-up and study design
§ 30: Rationale for the numbers of animals

We will use only male animals, as females often show more phenotypical variability; using both males and females would double the number of animals used, which would go against the 3R (Replacement, Reduction, Refinement).

If the results are significant, we will plan a second experiment on females.

– Adapted from one random application, a few years ago

§ 29: Experimental set-up and study design
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We will use only female animals, as males tends to fight more and require more cages; using both males and females would double the number of animals used, which would go against the 3R (Replacement, Reduction, Refinement).

If the results are significant, we will plan a second experiment on females.

– Adapted from one random application, a few years ago

You are now the Animal Experimentation (ethics) Committee.

What is your take on these arguments ?

A few points…

COMMENT

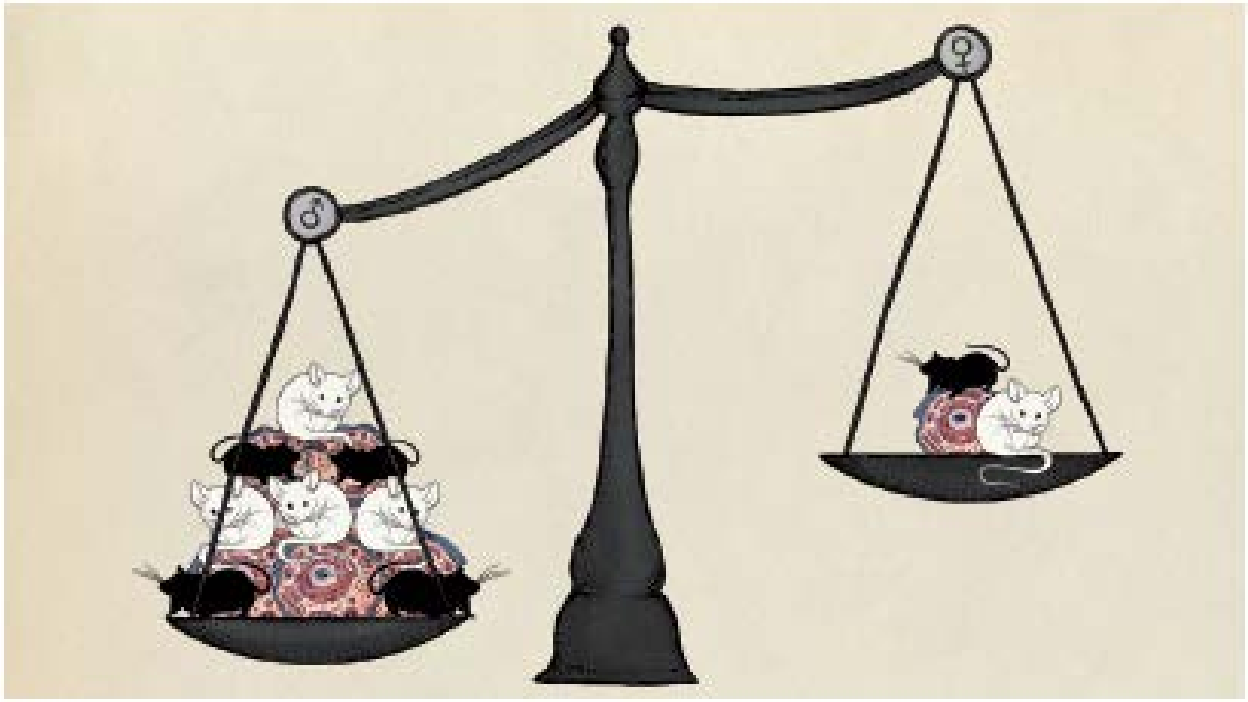

ILLUSTRATION BY ROSS SCOTT

NIH to balance sex in cell and animal studies

Janine A. Clayton and Francis S. Collins unveil policies to ensure that preclinical research funded by the US National Institutes of Health considers females and males.

JA Clayton and FS Collins. Nature 509, 15 May 2014

Partial list of rodent sex differences

anxiety
pain
aggressive behavior
stroke
food intake
depression
learning
memory
locomotor activity
response to many drugs

stress
anasthesis
neurotransmitters
brain structure
plasticity
taste
olfaction
circadian and ultradian rhythms
liver and kidney function

Inspired from Irving Zucker

Are females more variable than males in gene expression? Meta-analysis of microarray datasets

Yuichiro Itoh and Arthur P. Arnold*

Abstract

Background: The majority of preclinical biomedical research involves studies of males rather than females. It is thought that researchers have avoided females based on the idea that female traits are more variable than those of males because of cyclic variation in effects of ovarian hormones.

Methods: To test the assumption of inherently greater female variability, we analyzed 293 microarray datasets measuring gene expression in various tissues of mice and humans, comprising analysis of more than 5 million probes.

Results: Meta-analysis showed that on average, male gene expression is slightly more variable than that of females although the difference is small. We also tested if the X chromosome of humans shows greater variability in gene expression in males than in females, as might be expected because of hemizygous exposure of polymorphic X alleles but again found little sex difference.

Conclusion: Our analysis supports and extends previous studies reporting no overall greater phenotypic variability in females.

Keywords: Sexual differentiation, Sex bias, Gene expression

Biology of Sex Differences (2015), 6-18.

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If the results are significant, we will plan a second experiment on females.

– Adapted from one random application, a few years ago

Is this true ?

How can we take into account the sex variable into our design ?

Is this true ?

How can we take into account the sex variable into our design ?

(Assuming a simple F/M sex variable)

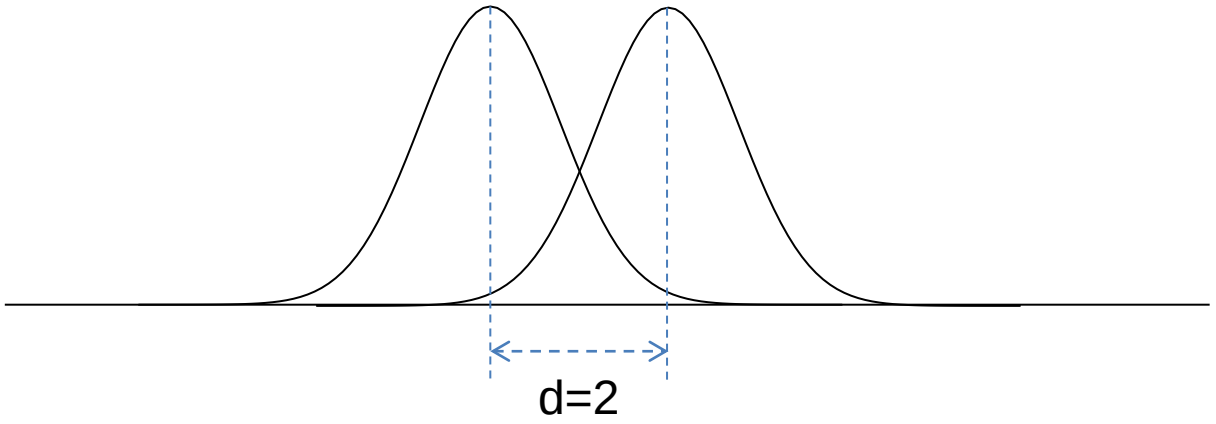
The most common statistical test used in the life sciences: Student's t-test

n=8 (per group), males or females only

sd=2.45

mean difference=2

P-value= ….



Total number of animals: 16

The most common statistical test used in the life sciences:
Student's t-test

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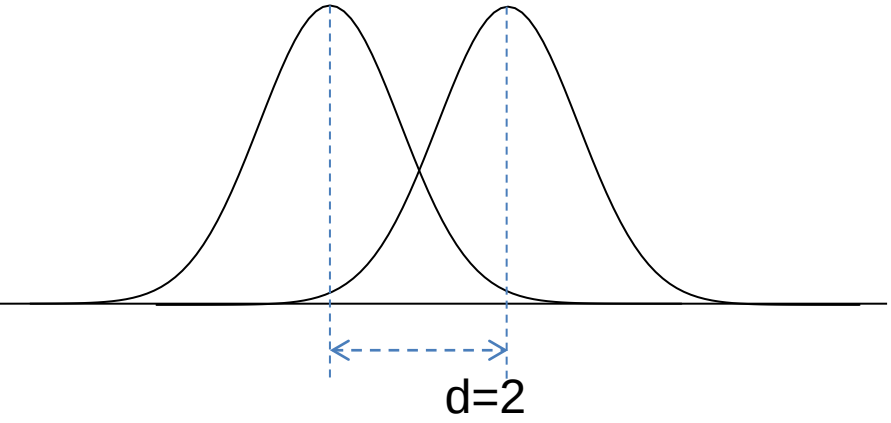
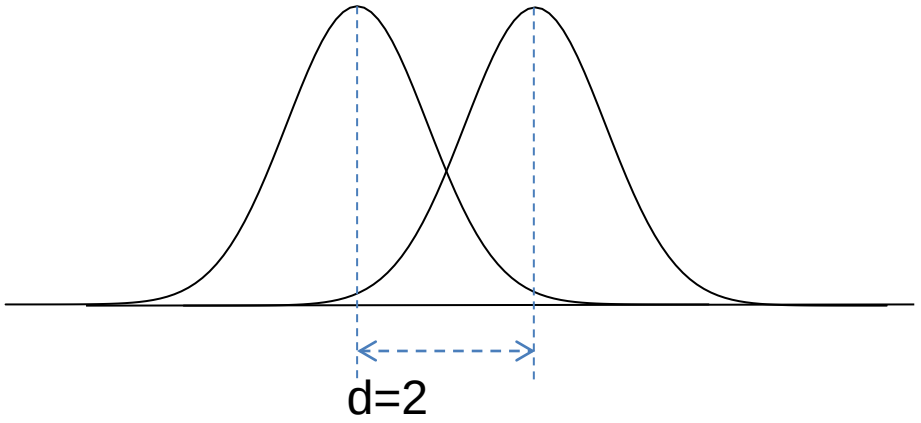


n=8 (per group), females
sd=2.45
mean difference=2
P-value= ….



Total number of animals: **32**

Can we do things in another way ?

Does sex matter ?

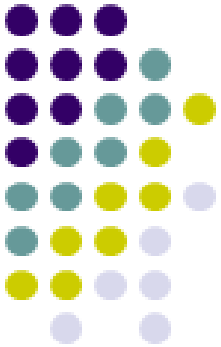
Session at the SGV
(Swiss animal laboratory association)
2015 meeting

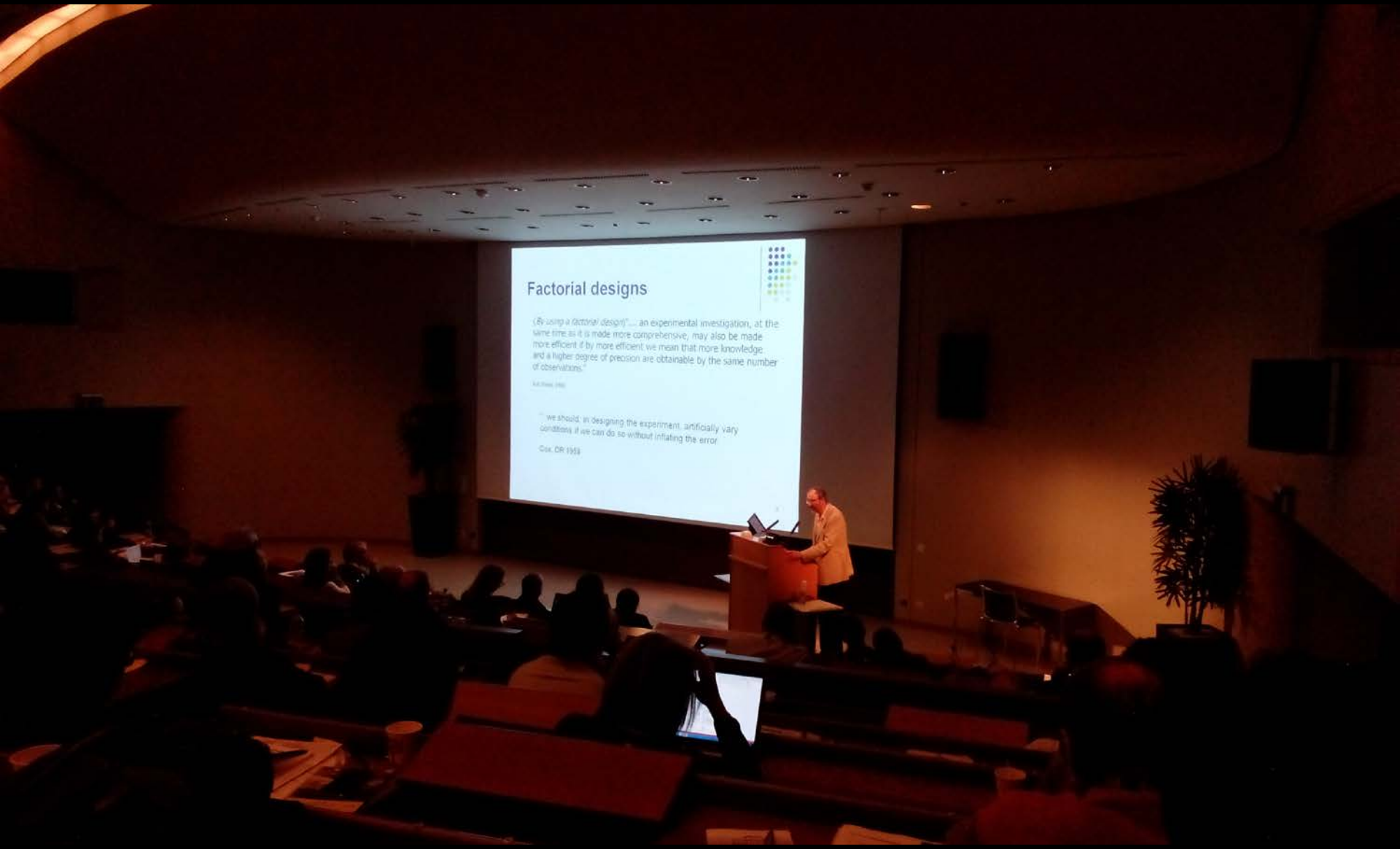
Will we be able to reduce the number of animals used if both sexes are used in research projects?

Michael FW Festing, Ph.D., D.Sc., CStat.

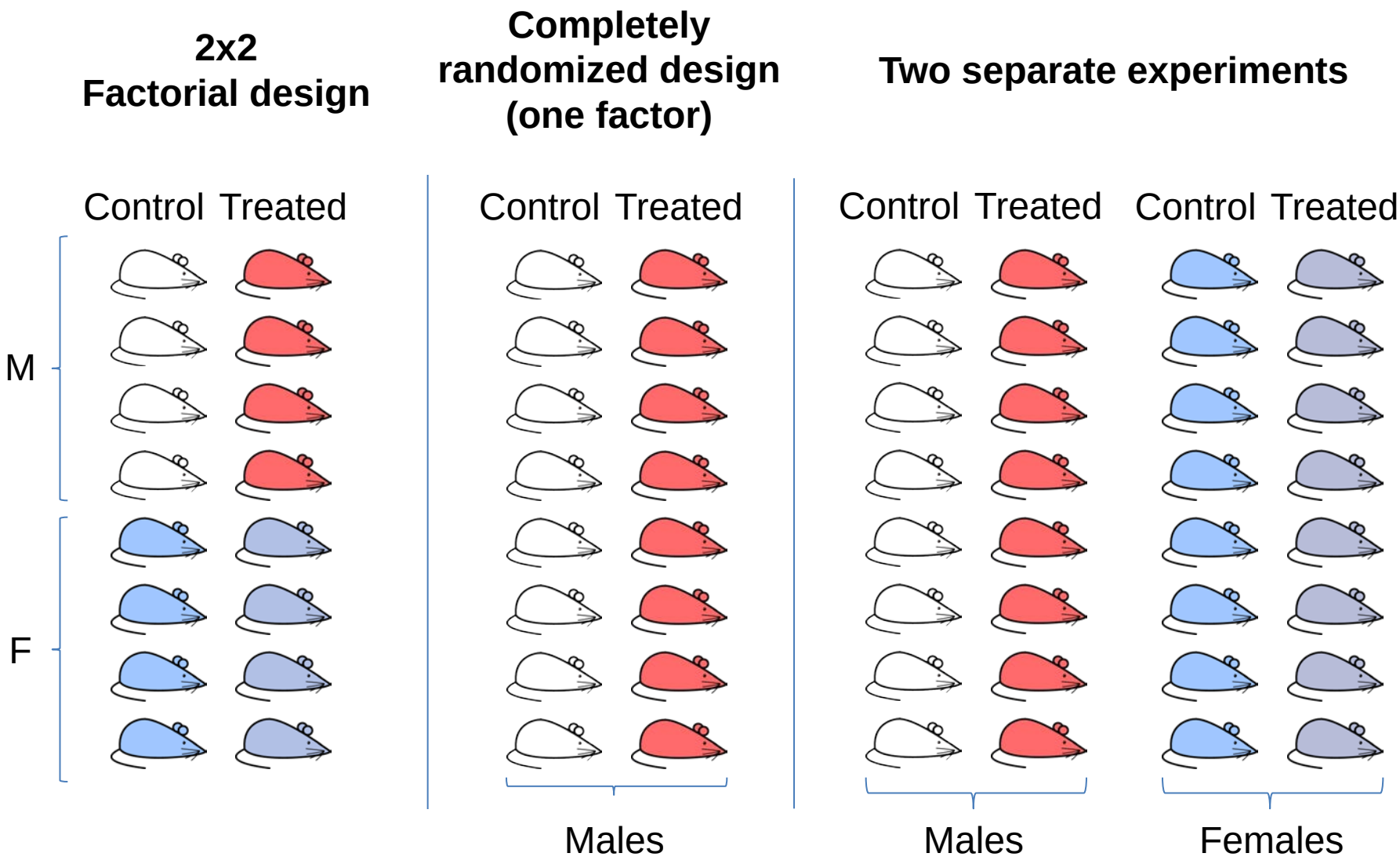
michaelfesting@aol.com

www.3Rs-reduction.co.uk




Factorial designs
(By using a factorial design)"... an experimental investigation, at the same time as it is made more comprehensive, may also be made more efficient if by more efficient we mean that more knowledge and a higher degree of precision are obtainable by the same number of observations."
we should, in designing the experiment, artificially vary conditions if we can do so without inflating the error
Cox, DR 1958

Different ways of analyzing Control/Treated with(out) sex



How can we know which design is correct ?

Simulations !!

Simulation tool: go to

t.ly/BTBl-
https://schutz.shinyapps.io/gender/

Sex power simulations

Treatment effect

1

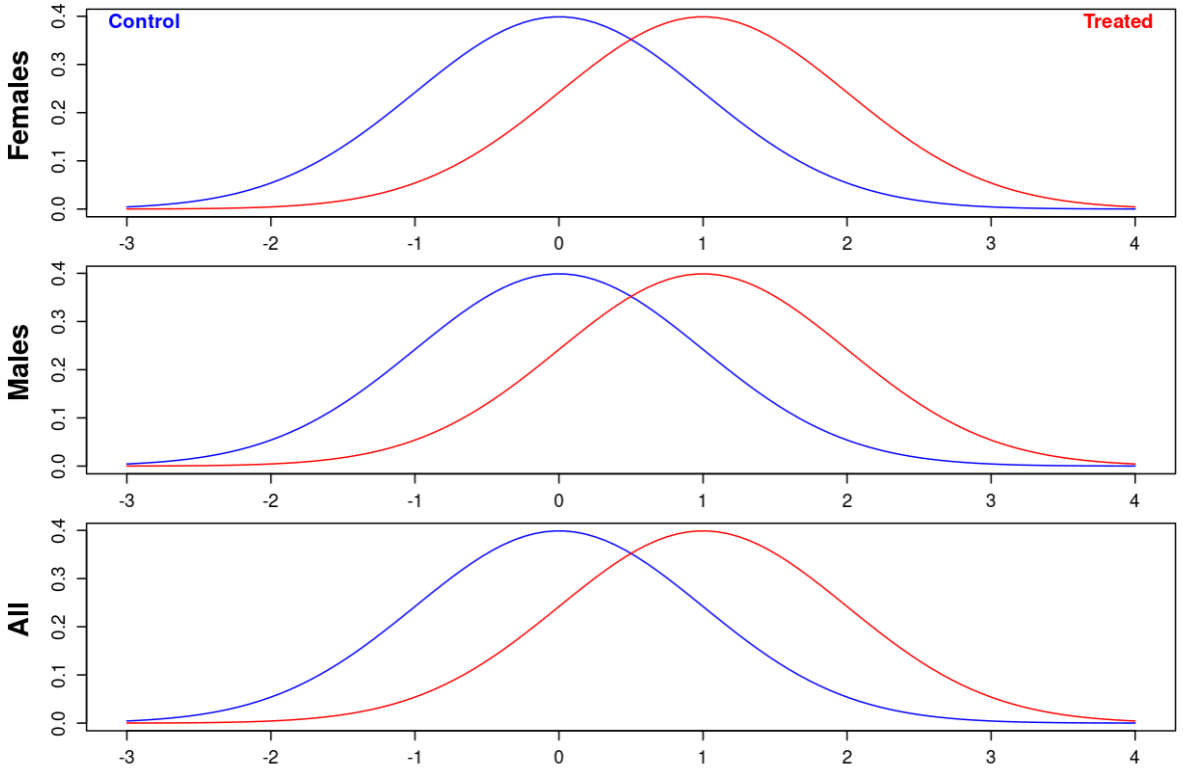
Sex effect

0

☐ Show advanced options

Simulate !

Frédéric Schütz
(frederic.schutz@sib.swiss)



Theoretical power (for n=32 units, all females): **0.78**. This is the baseline power.

Power for a Student t-test (all units included, males and females, but the sex variable is not taken into account)
Power (for n=32 units): **0.8** (2.6% from the baseline)
As there is no sex effect, this should be close to the baseline

Case study 1 (simulation)

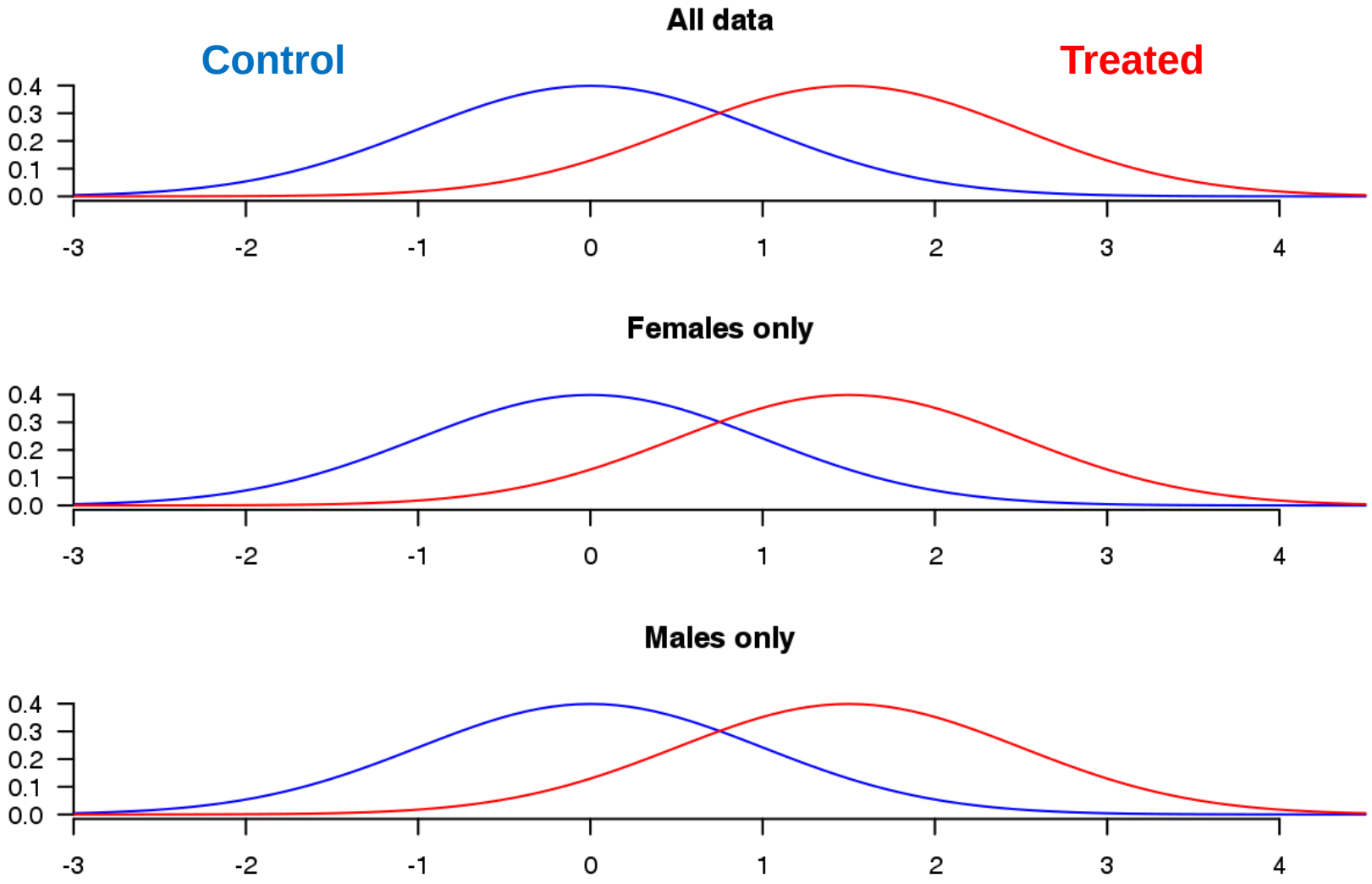
Presence of a treatment effect
No sex effect

Case study 1 (simulation)

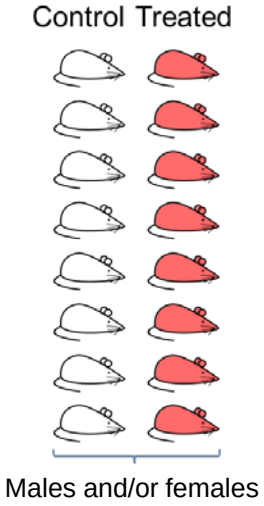
Presence of a treatment effect
No sex effect

How much does it "cost" to take account sex in your model when it would not be needed ?

What the populations look like

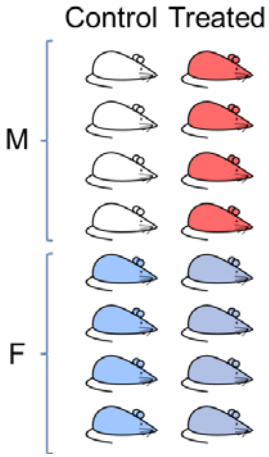


Difference between the two designs



Completely randomized design

- Analysed with a Two-sample Student t-test
- Sex is not taken into account
- Estimated power for treatment: **0.78**



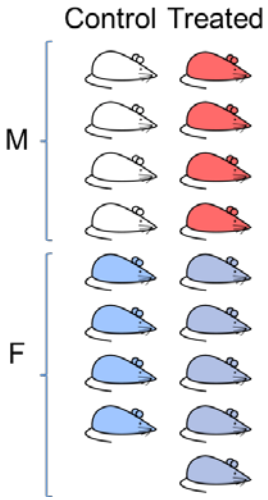
2x2 Factorial design

- Analysed with a Two-way ANOVA (treatment + sex + interaction)
- Sex is taken into account
- Estimated power for treatment : **0.765**

What happens here ?

- In the two-way ANOVA, we are estimating 2 more variables (effect of sex and interaction) than in the one-way model.
- All other parameters (variance, effect size, etc) are unchanged.
- As a result, the power decreases a little bit – but the difference is likely negligible.

Adding just **one** animal to the study (in any of the groups) would be enough to recover the original power (and even go a little bit beyond):



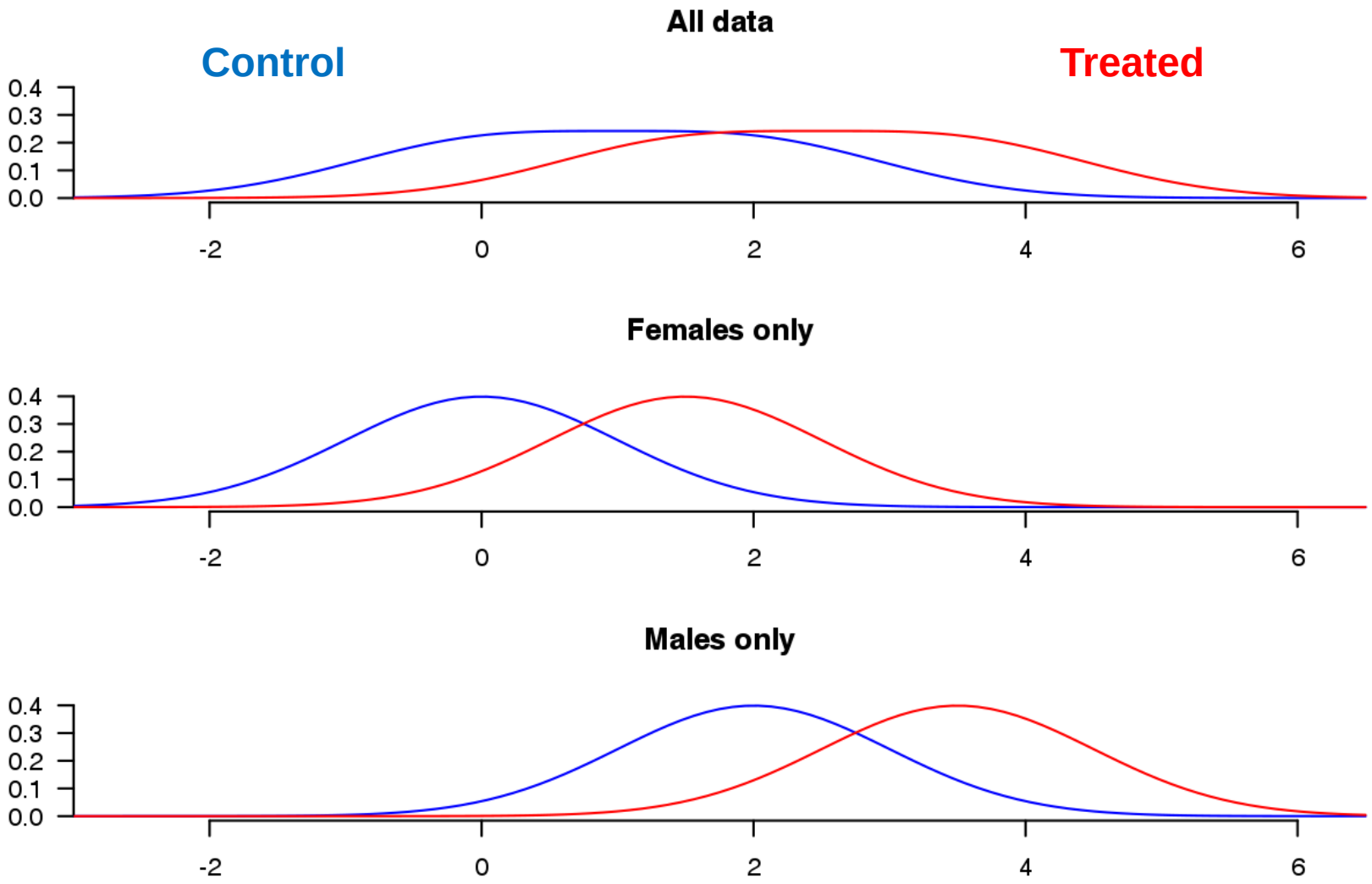
2x2 Factorial design

- Analysed with a Two-way ANOVA (treatment + sex + interaction)
- Sex is taken into account
- Estimated power for treatment : **0.80**

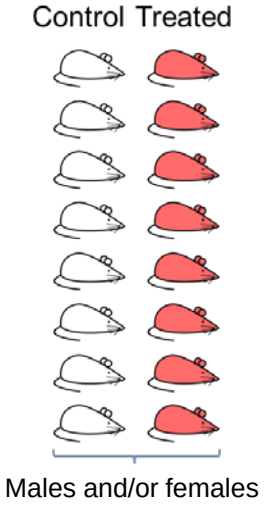
Case study 2:
16 mice (8 males, 8 females)
(simulation)

Presence of a treatment effect
Presence of a sex effect

What the populations look like

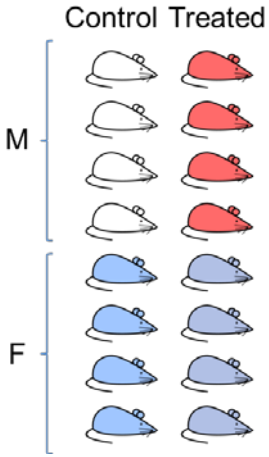


Difference between the two designs



Completely randomized design

- Analysed with a Two-sample Student t-test
- Sex is not taken into account
- Estimated power for treatment : **0.47**



2x2 Factorial design

- Analysed with a Two-way ANOVA (treatment + sex + interaction)
- Sex is taken into account
- Estimated power for treatment : **0.79**

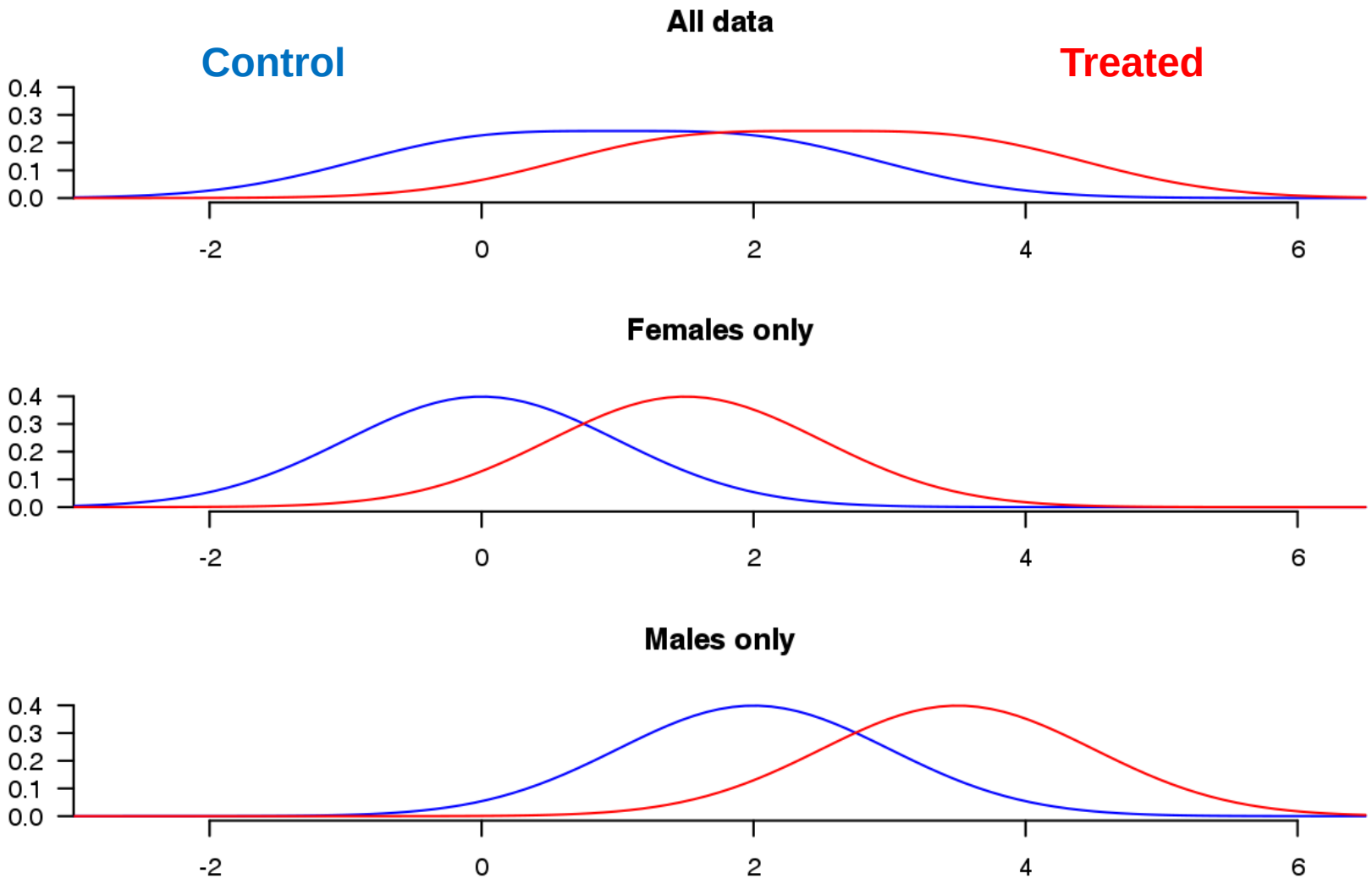
What happens here ?

- In the two-way ANOVA, we are still estimating 2 more variables (effect of sex and interaction) than in the one-way ANOVA.
- Again, this **decreases** the power very slightly.
- However, the presence of the "sex" parameter explains some of the variability of the data, and removes much of the within-group variance.
- In other words: with some "noise" removed, it becomes easier for the model to identify the effect of the treatment.
- As such, the **power increases drastically**.

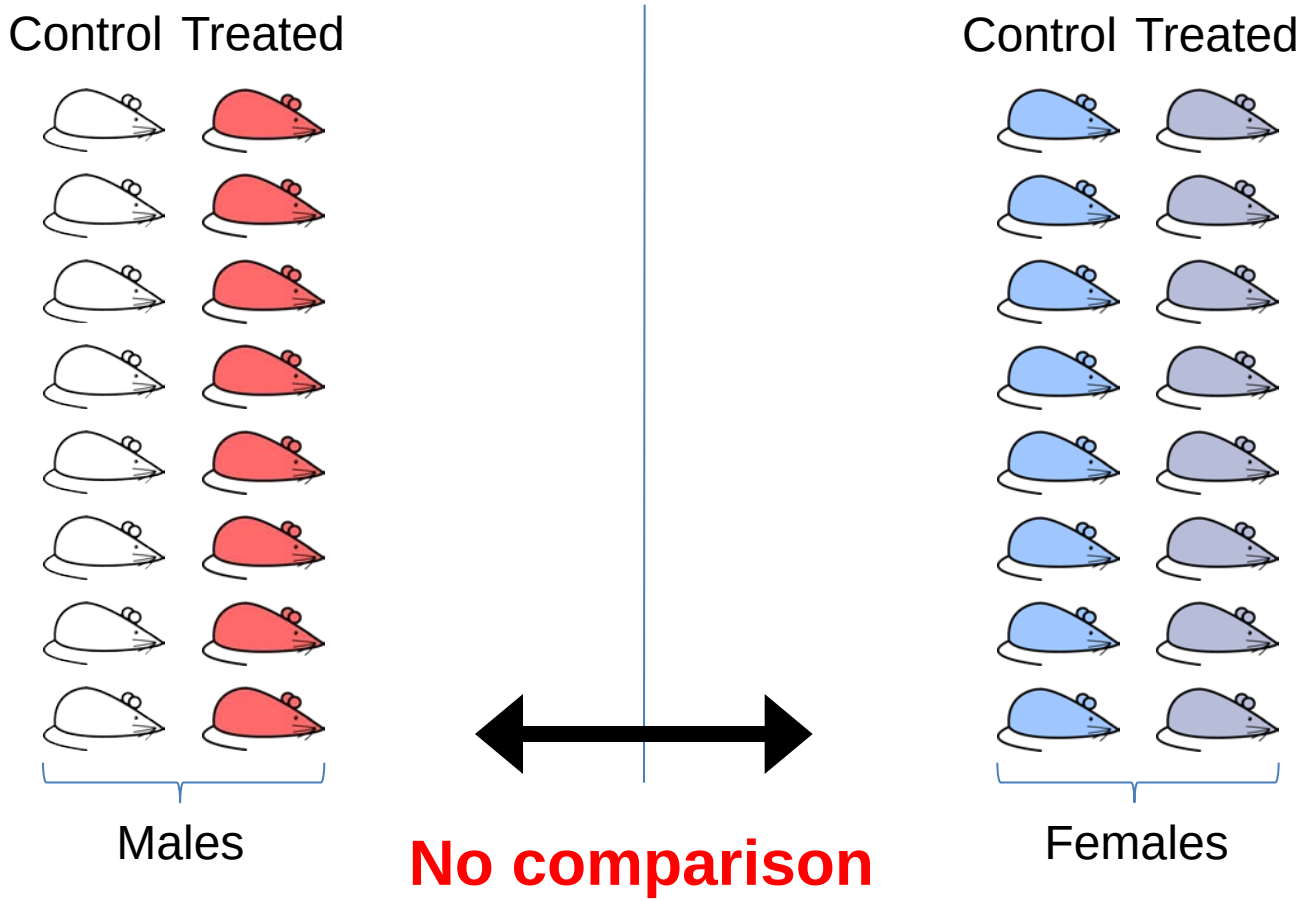
Case study 3:
32 mice (16 males, 16 females)
(simulation)

Presence of a treatment effect
Both sex considered separately

What the populations look like

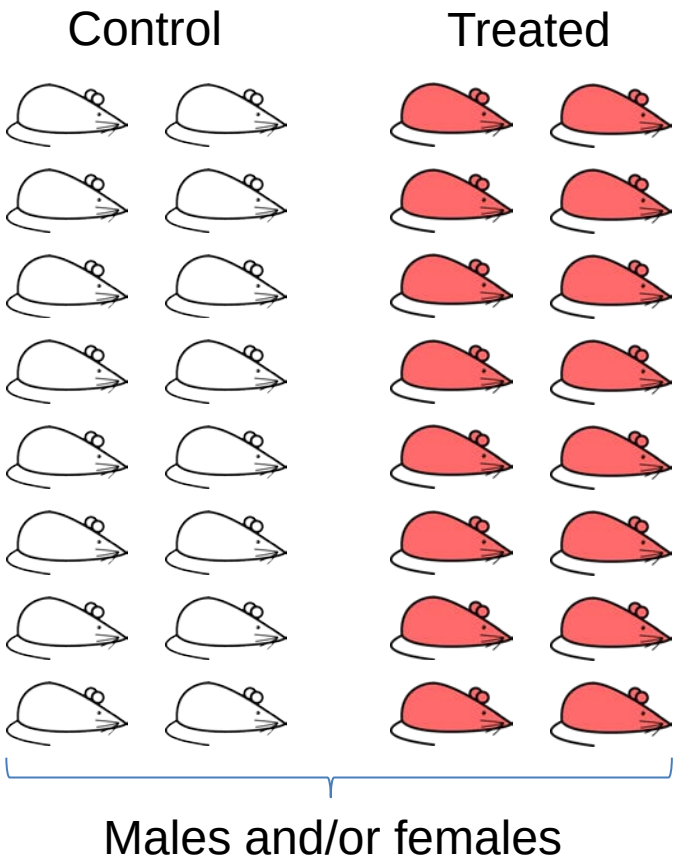


Two separate experiments

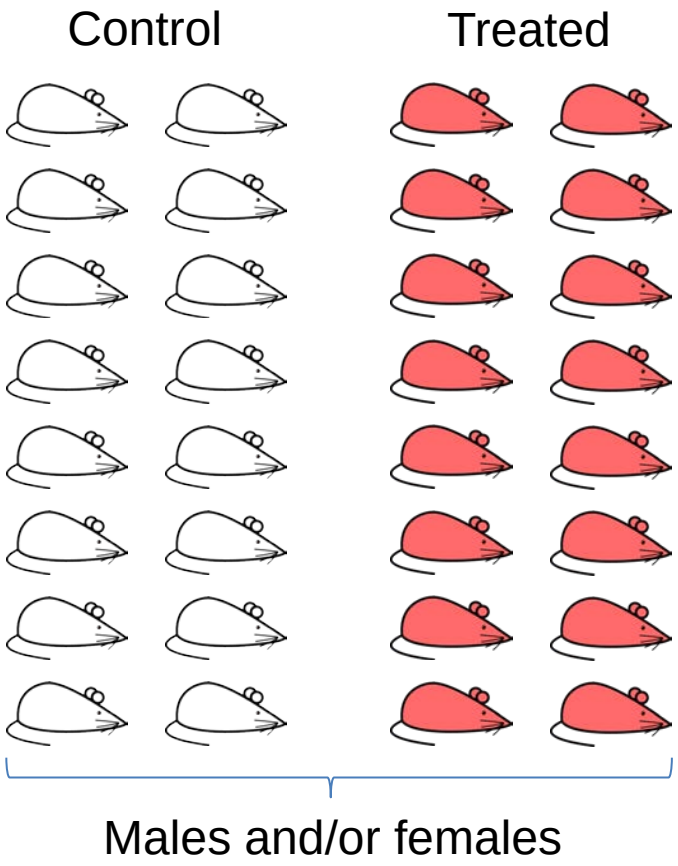


Power for treatment:
0.796

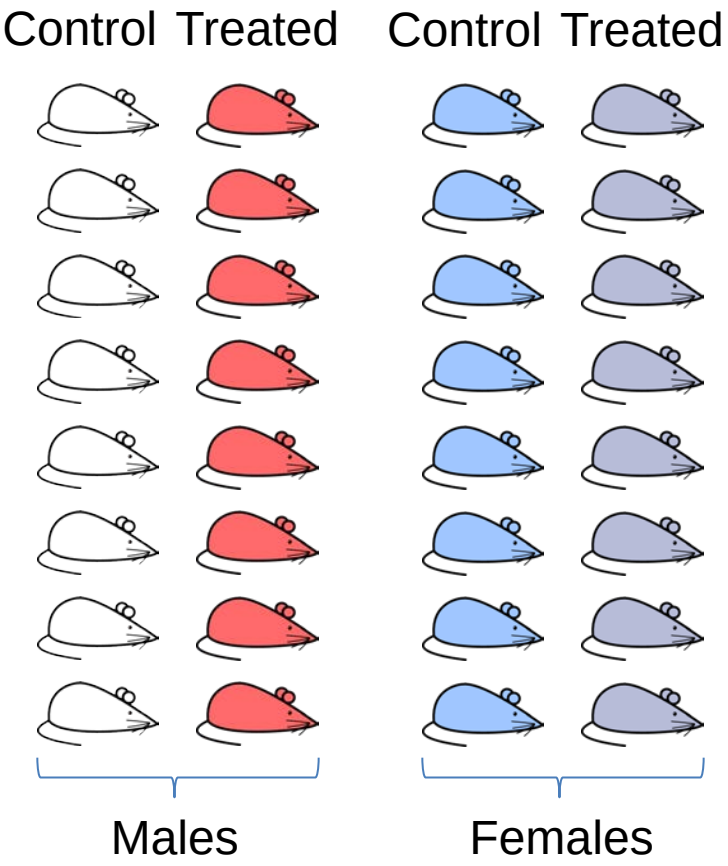
Power for treatment:
0.796



Two-sample T-test:
Treatment only

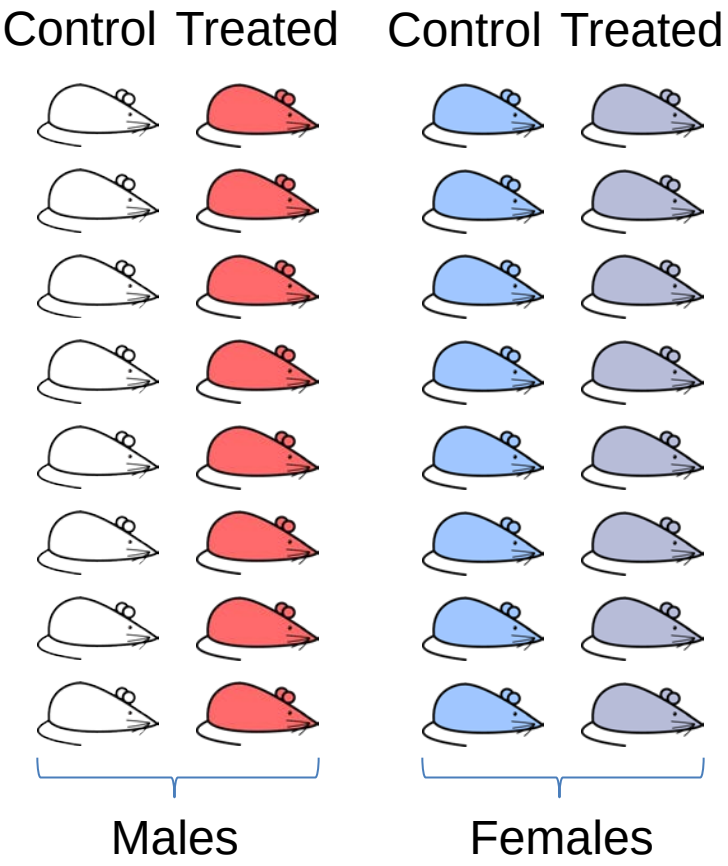


Two-sample T-test:
Treatment only
Power: **0.886**



Two-sample T-test:
Treatment only
Power: **0.886**

Two-way ANOVA:
Treatment + Sex



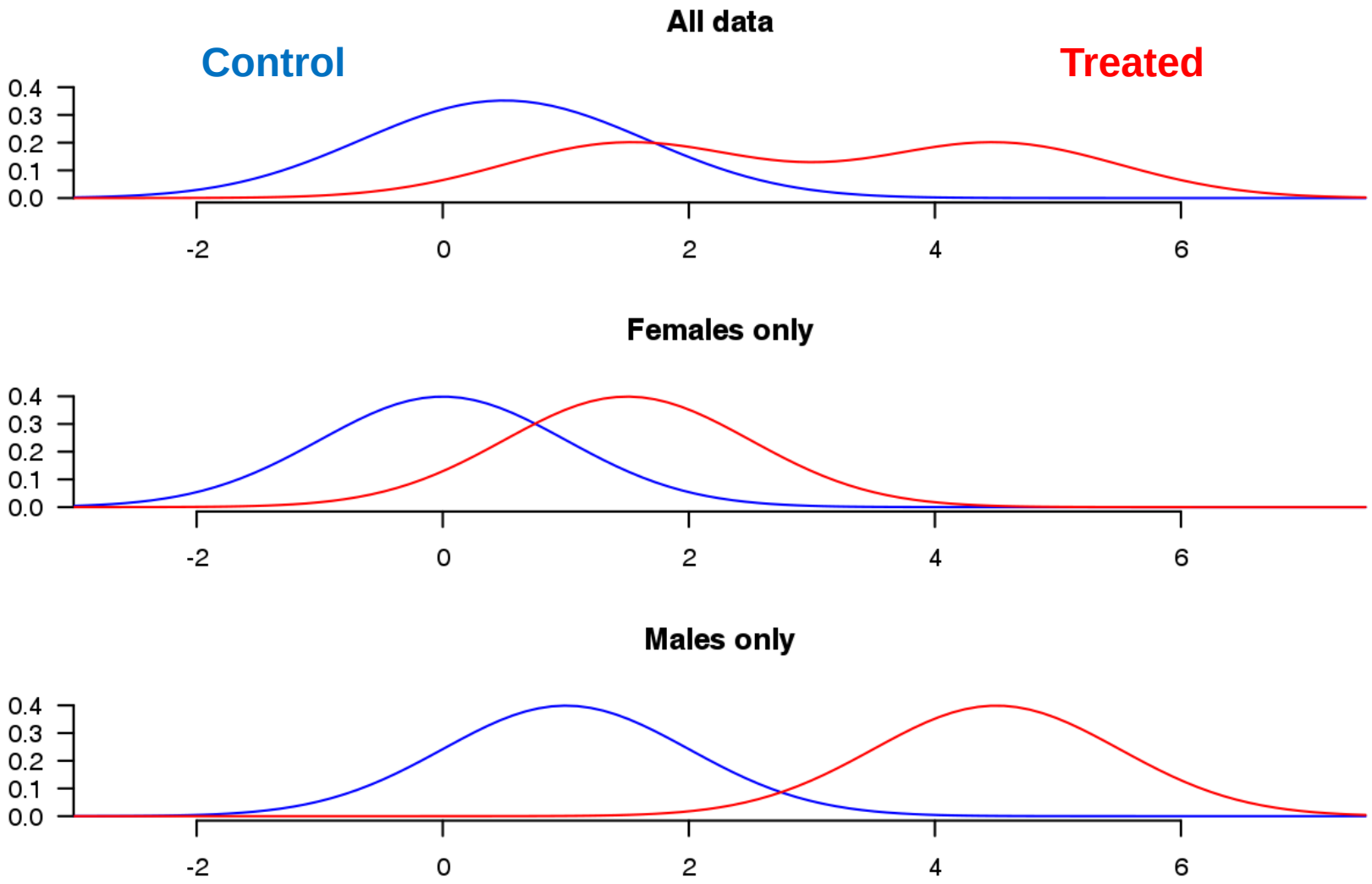
Two-sample T-test:
Treatment only
Power: **0.886**

Two-way ANOVA:
Treatment + Sex
Power: **0.983**

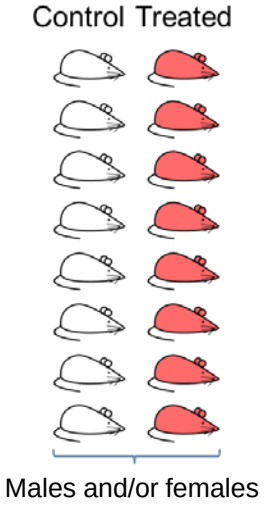
Case study 4:
16 mice (8 males, 8 females)
(simulation)

Presence of a treatment effect
Presence of a sex effect
Presence of an interaction

What the populations look like

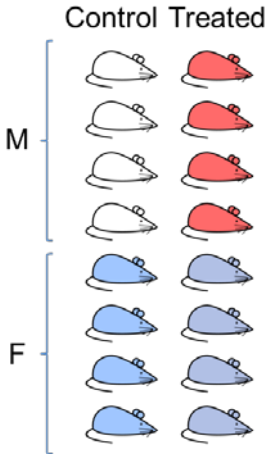


Difference between the two designs



Completely randomized design

- Analysed with a Two-sample Student t-test
- Sex is not taken into account
- Estimated power for treatment : **0.70**



2x2 Factorial design

- Analysed with a Two-way ANOVA (treatment + sex + interaction)
- Sex is taken into account
- Estimated power for treatment : **0.78**

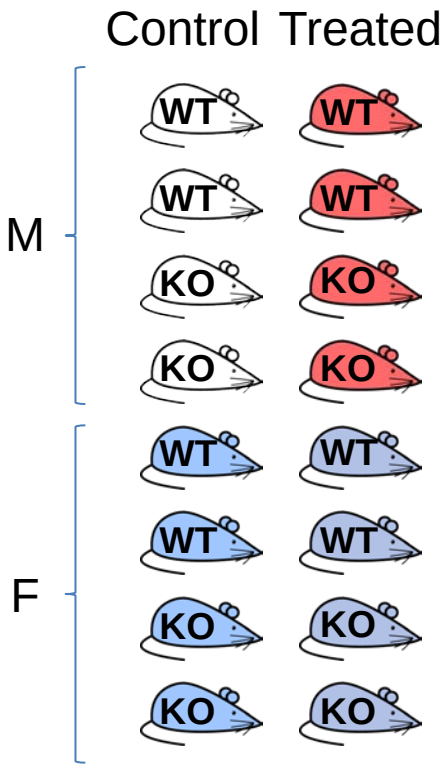
What if we have more than two variables ?

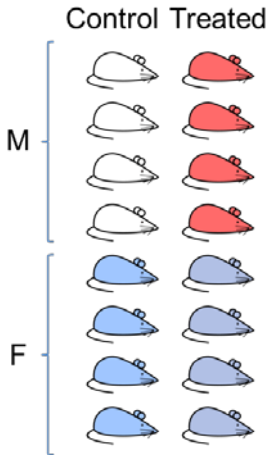
Case study 5
16 mice
(8 males, 8 females)
(8 WT, 8 KO)

Presence of a treatment effect
Presence of a sex effect
No interaction
No effect of genotype

Adding one more variable

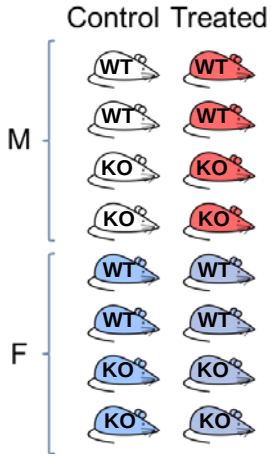
2x2x2
Factorial design





2x2 Factorial design

- Analysed with a Two-way ANOVA (treatment + sex + interaction)
- Sex is taken into account
- Estimated power for treatment : **0.795**

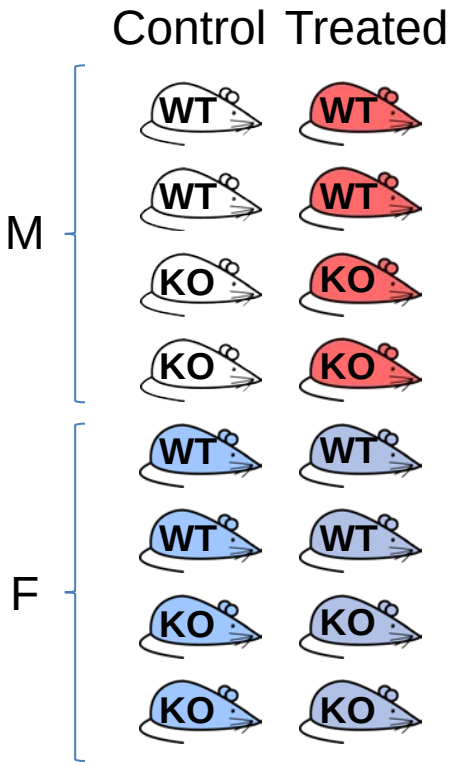


2x2x2 Factorial design

- Analysed with a Three-way ANOVA (treatment + sex + genotype + 4 interactions)
- Sex and genotype are taken into account
- Estimated power for treatment : **0.77**

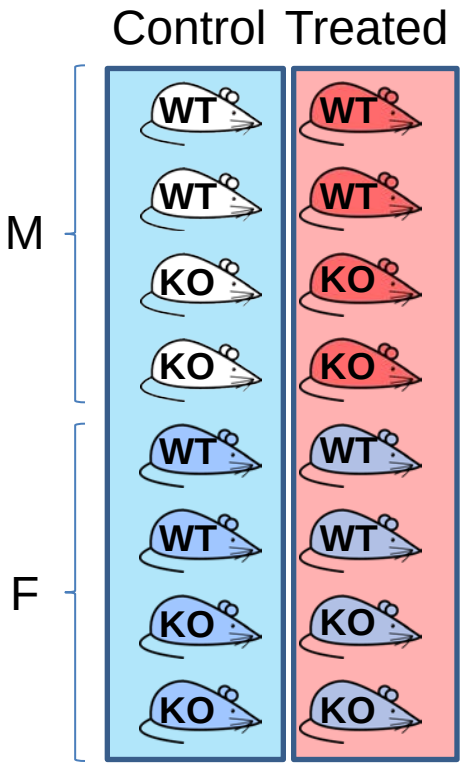
Why does the factorial design work ?

2x2x2
Factorial design



We still have …

2x2x2
Factorial design



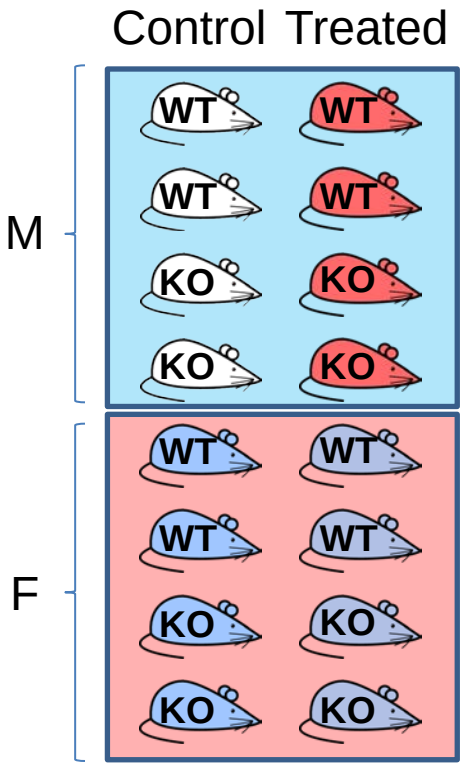
8 controls

VS

8 treated

We still have …

2x2x2
Factorial design



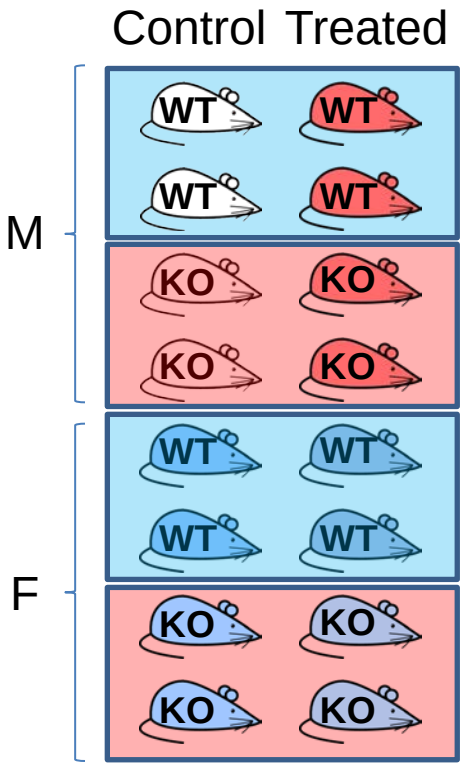
8 males

VS

8 females

We still have …

2x2x2
Factorial design



8 WT

VS

8 KO

Thank you !