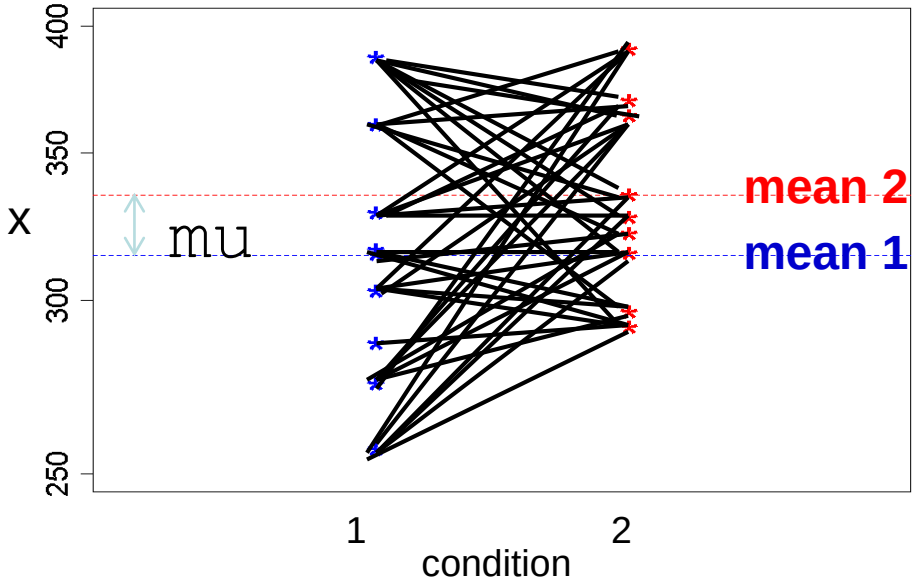
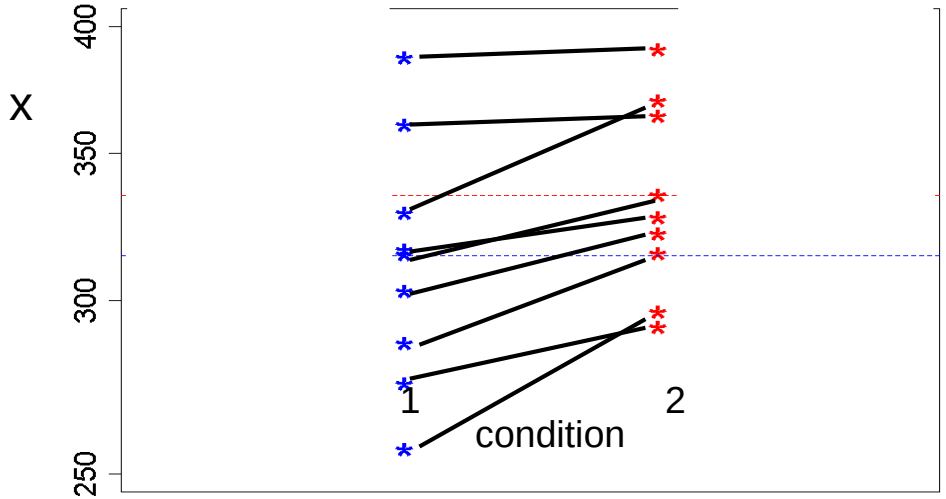
Biostatistics Week 4

- **Confidence interval, significance (≠ relevance)**
- **Testing**
  - **binomial test for proportions**
  - **t-test for means**
  - **2 group comparison: paired and unpaired tests**
- **Non-parametric tests: Wilcoxon**

Independent, unpaired

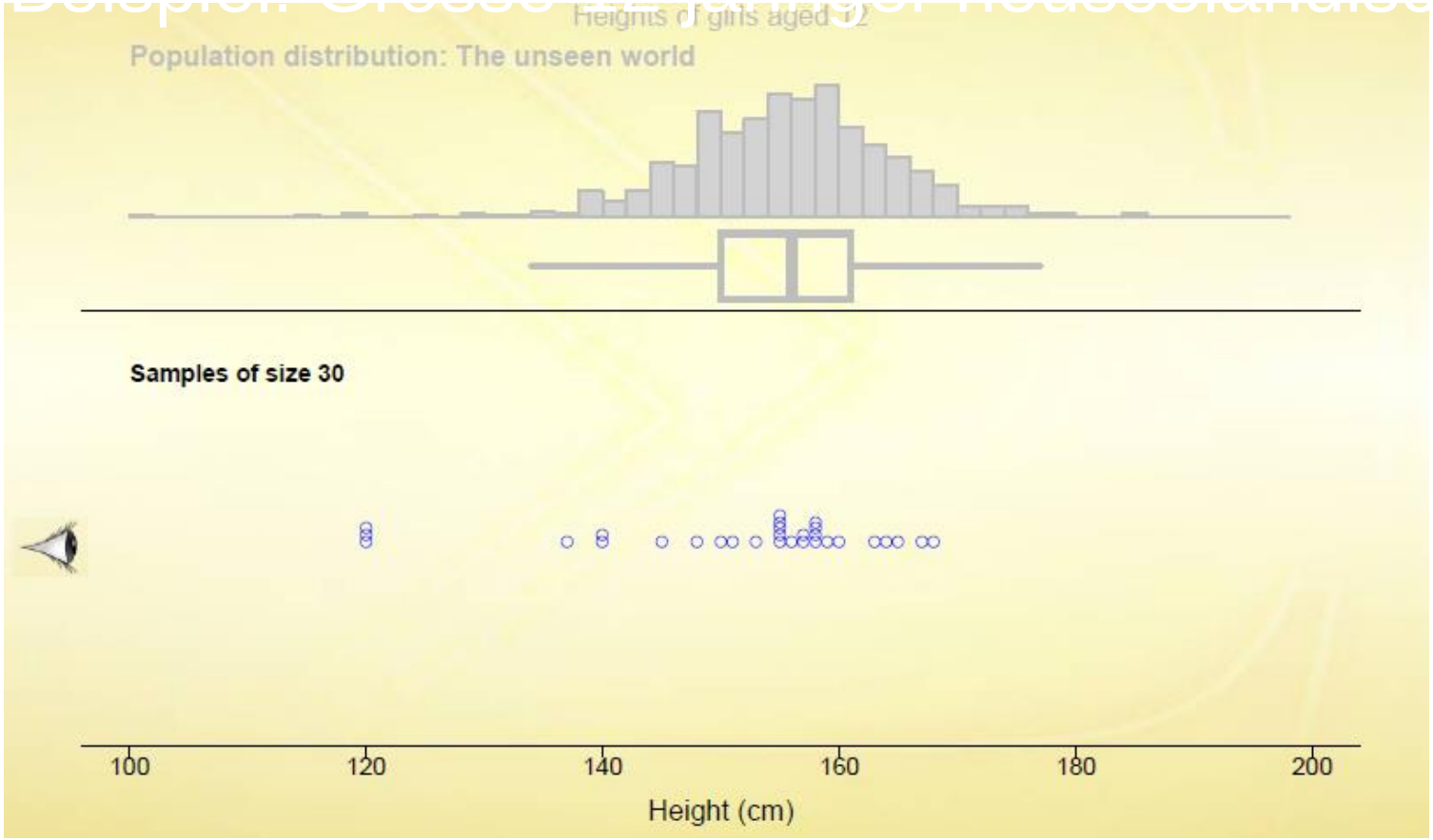


Dependent, paired



https://bsick.github.io/Biostatistics-Fall-2018/

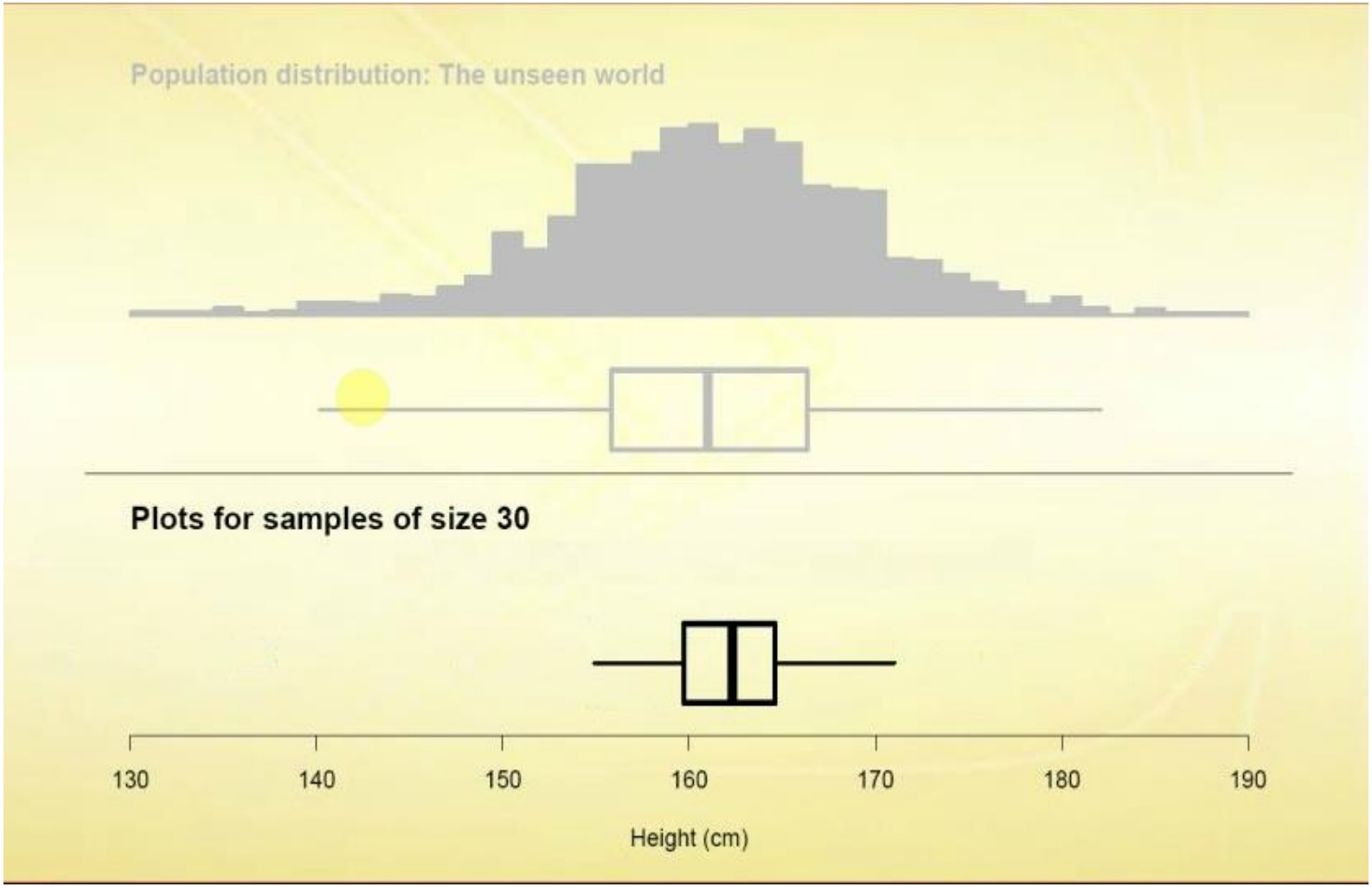
Where is the center of the population?



n=30

CtsVar_1samp_Dots30.pdf

Where is the center of the population?



Visualize boxplot with memory

Quelle: http://www.stat.auckland.ac.nz/~wild/09.USCOTSTalk.html

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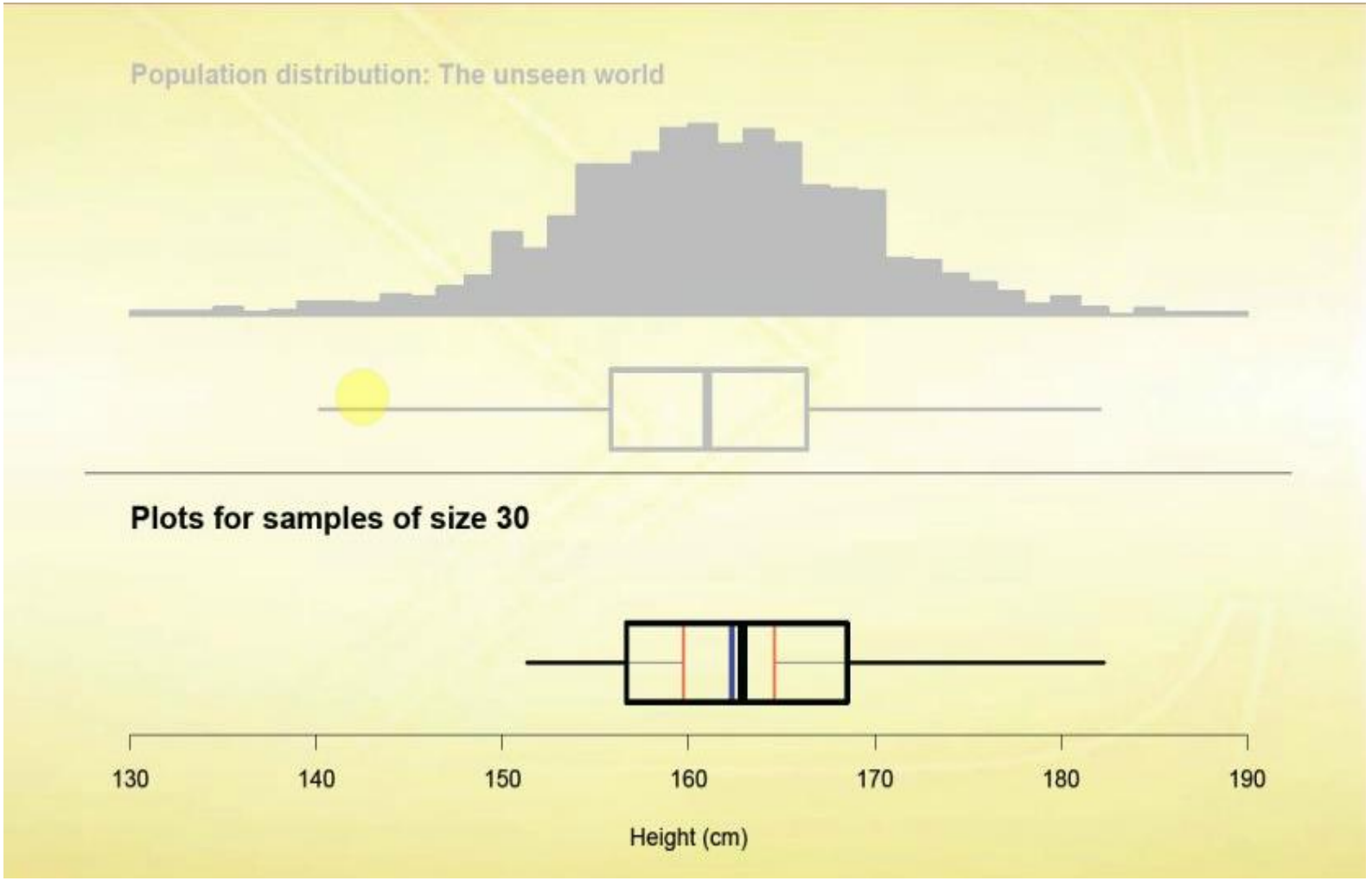

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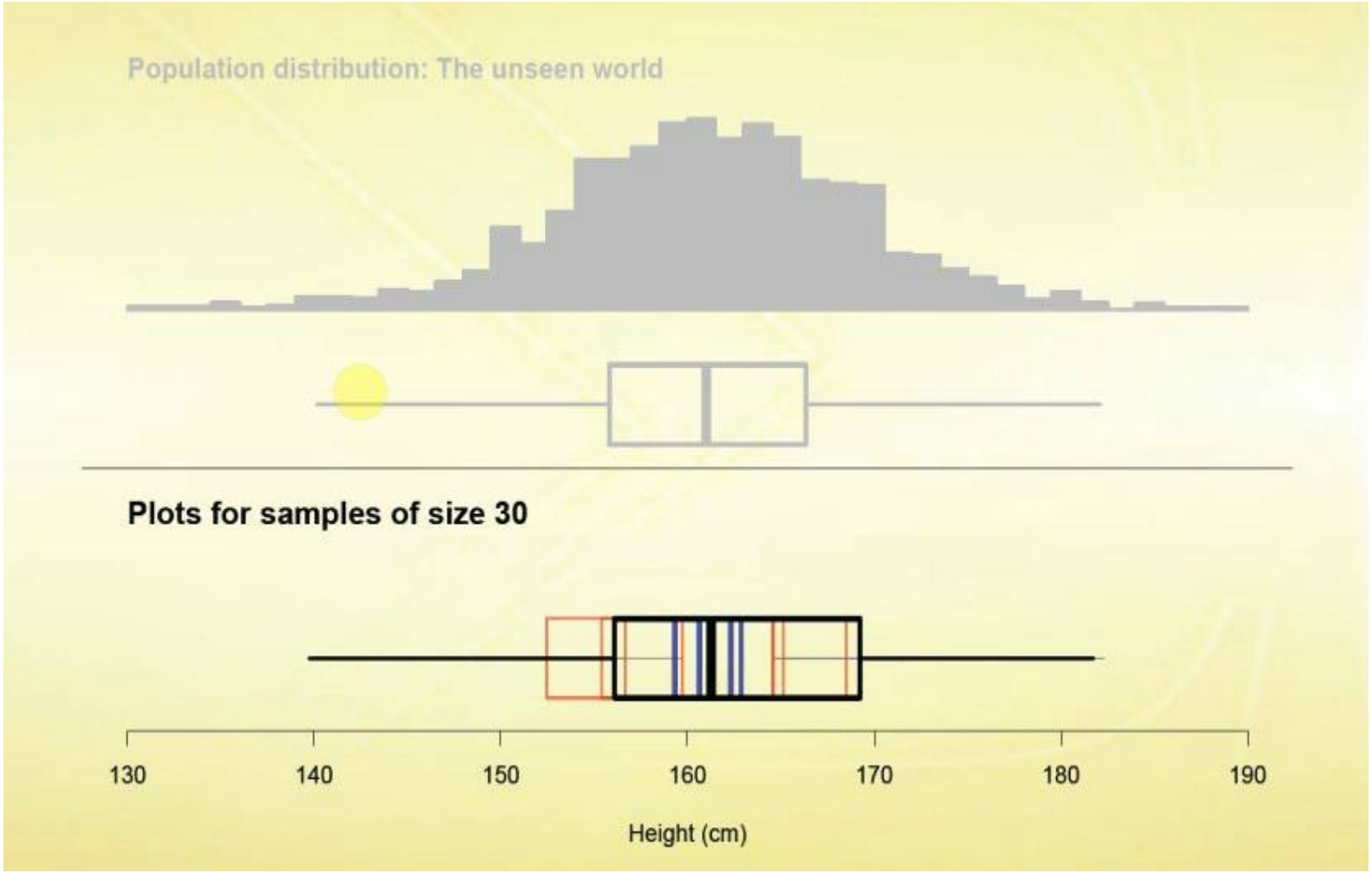

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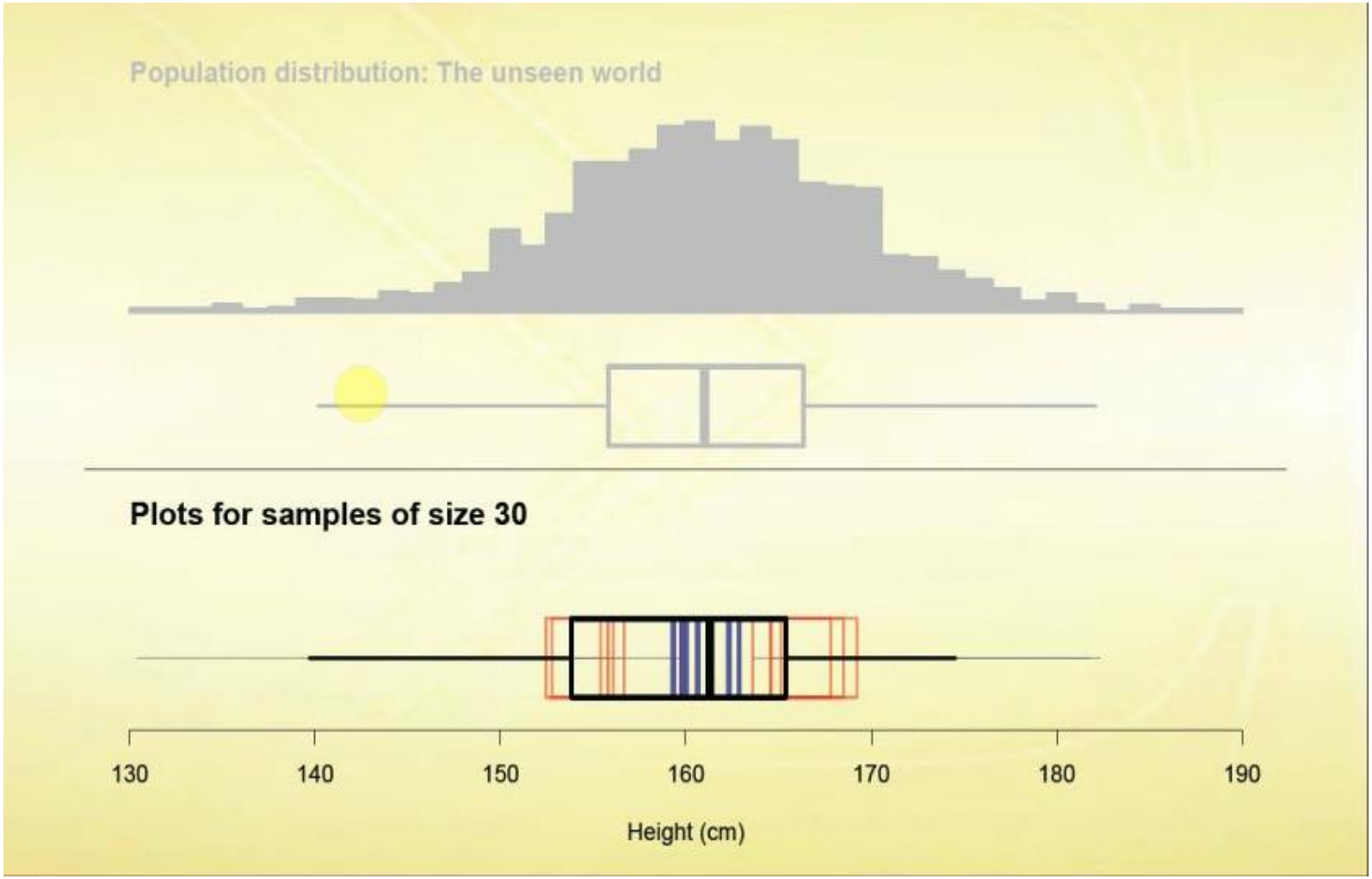

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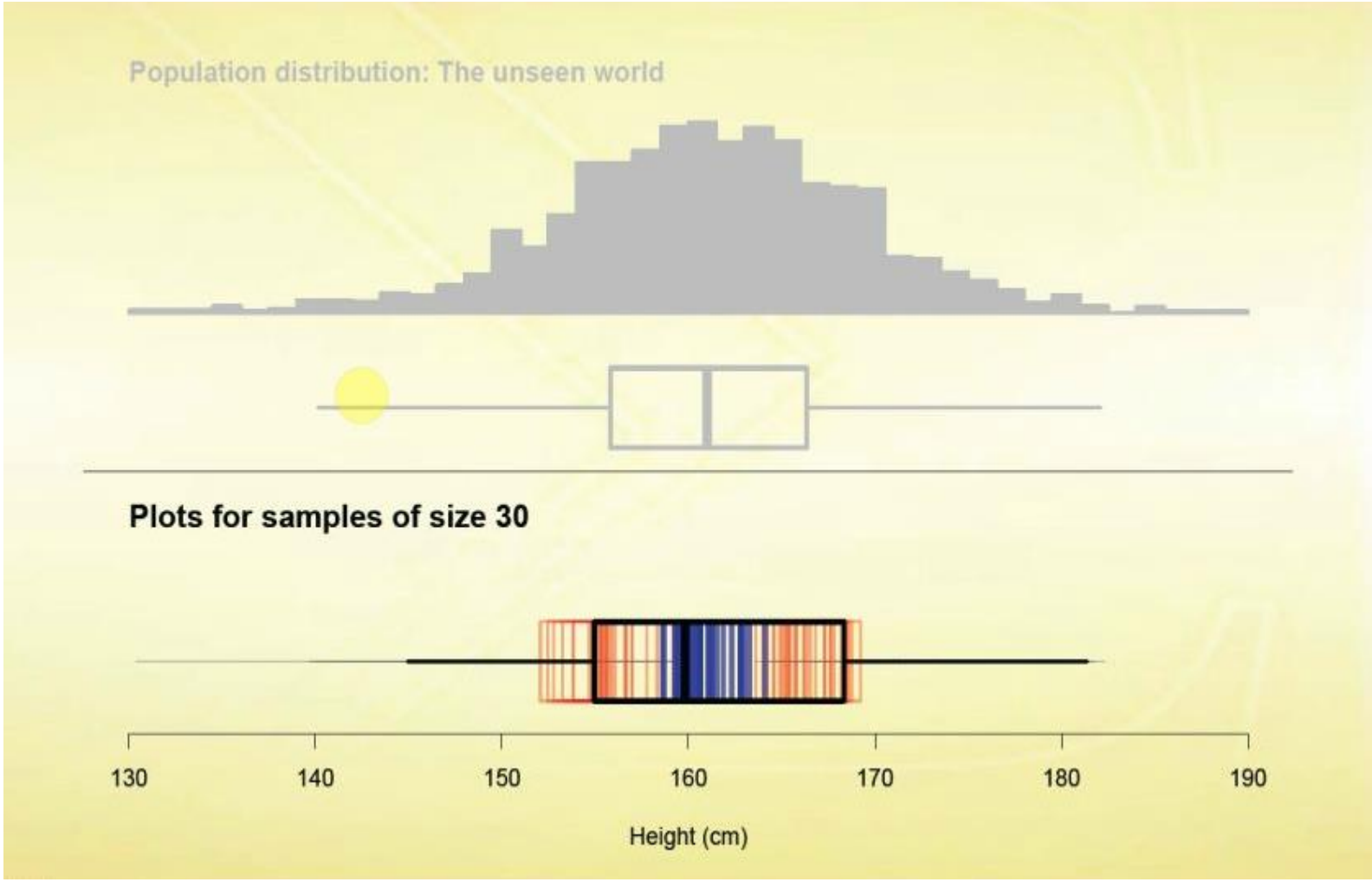

Vizualize boxplot with memory

Quelle: http://www.stat.auckland.ac.nz/~wild/09.USCOTSTalk.html

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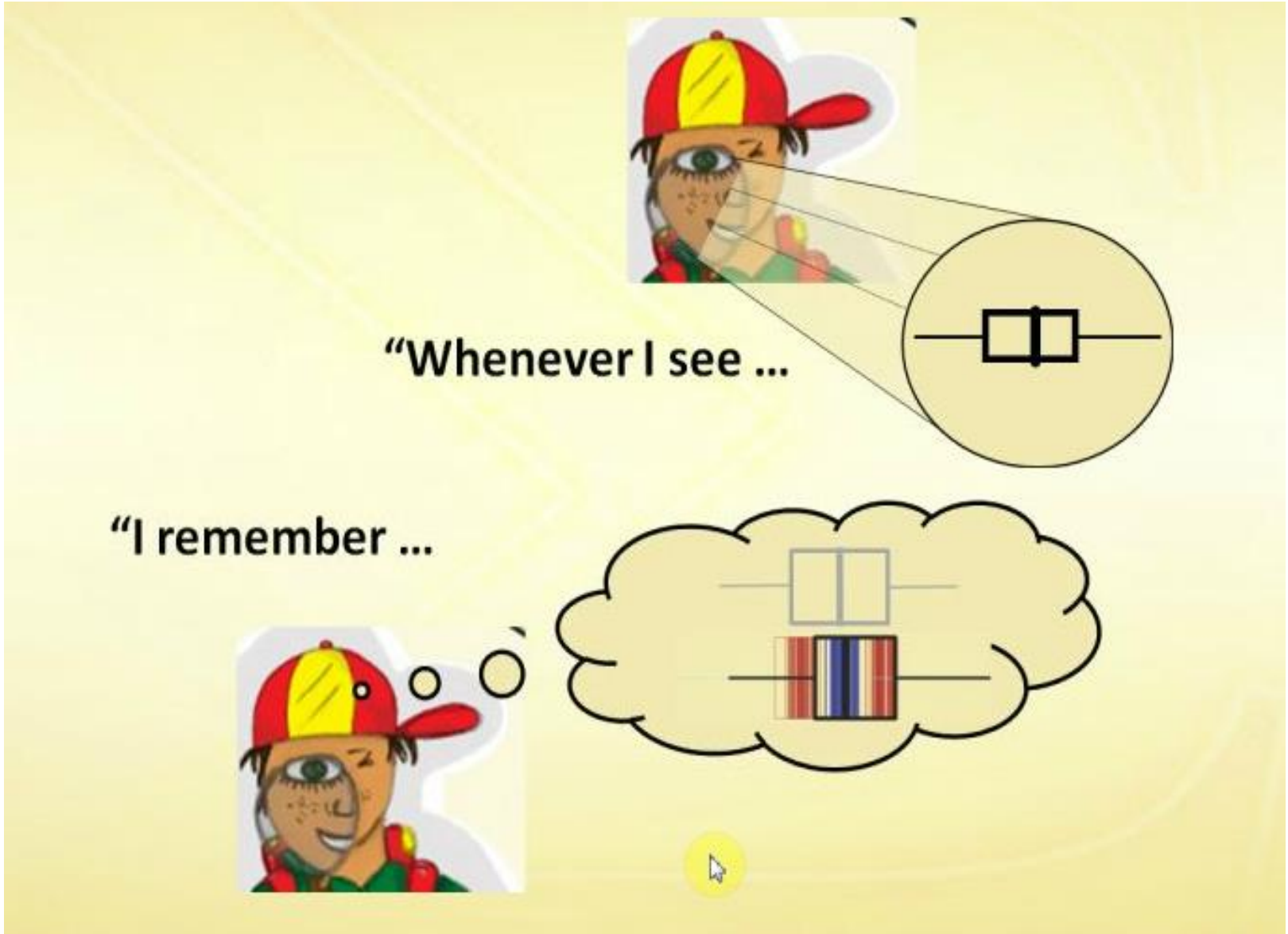
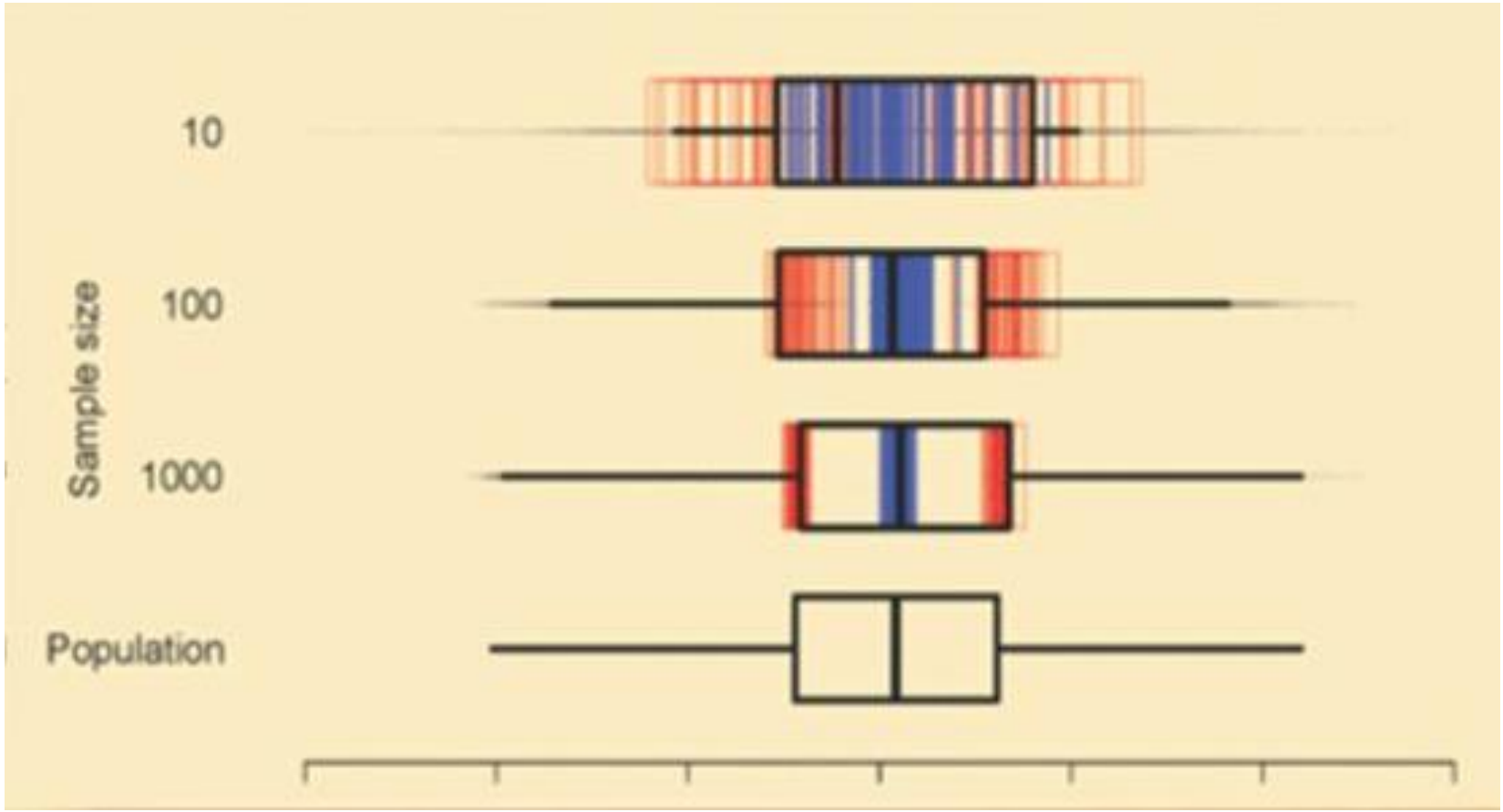


Quelle: http://www.stat.auckland.ac.nz/~wild/09.USCOTSTalk.html

Where is the center of the population?
We get more certain with increasing sample size

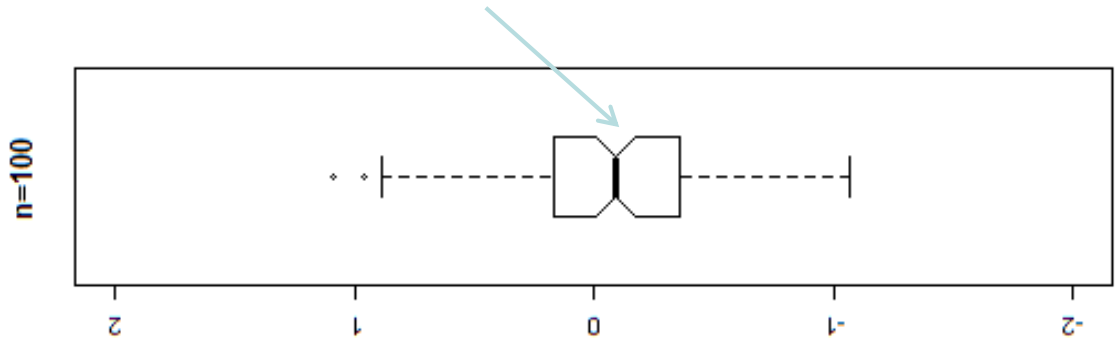


Quelle: http://www.stat.auckland.ac.nz/~wild/09.USCOTSTalk.html

How sure can I be about the true parameter value?

Goal:
We would like to determine from our sample/observations an interval, which covers the true parameter value with a probability of 95%.

```
boxplot(x,notch=TRUE)
```

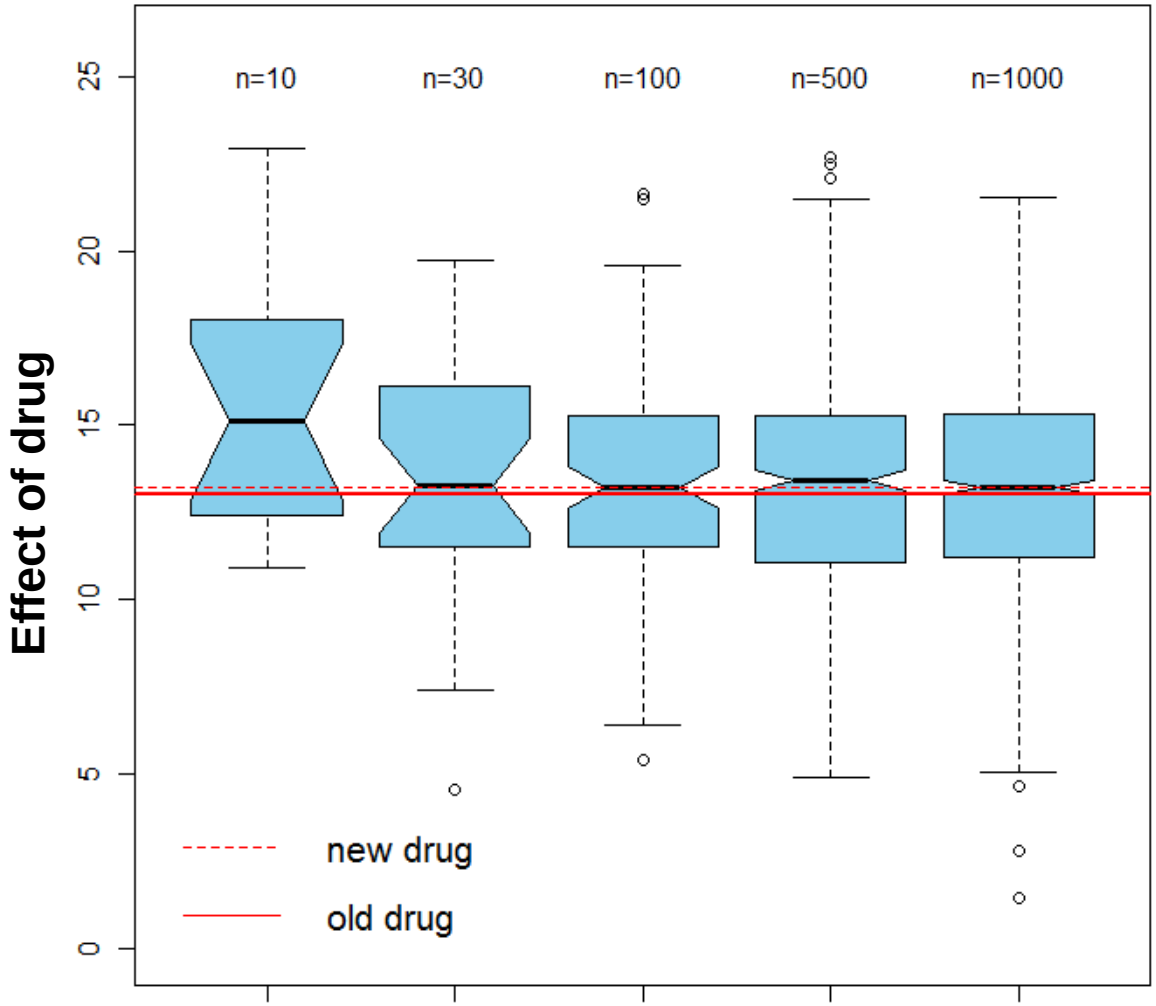


+/-1.58 IQR/sqrt(n)
The notch covers the population median «quite certain»

Significance does not imply relevance

Everything gets significant if the sample size is large enough

Sample with different sample sizes drawn from a Normal distribution with expected value of 13.1



H_0: $\mu_0 = \mu_{old\text{-}drug} = 13$

H_A: $\mu > 13$

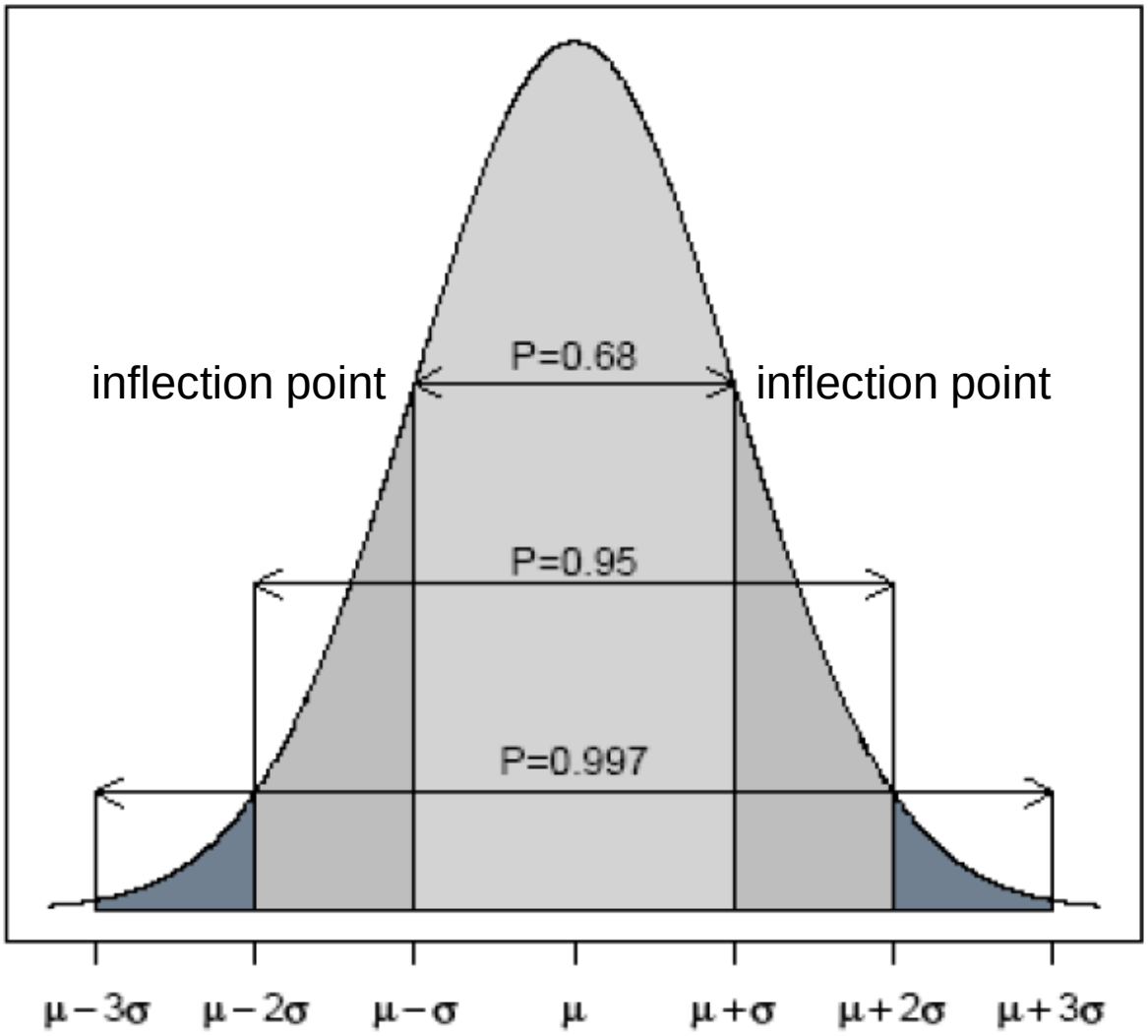
Assume true median of the new drug is13.1 which would be no relevant improvement compared to old drug value 13

- To ensure relevance of an significant test result one should form a confidence interval.
- Non-significance could be caused by a too small sample size.

With increasing sample size the 95%

Density of the Normal distribution

$$f(x) = \frac{1}{\sqrt{2\pi}\cdot\sigma}\cdot e^{-\frac{1}{2}\cdot\frac{(x-\mu)^2}{\sigma^2}}$$



Rule of thumb:

A random value $X \sim N(\mu,\sigma^2)$ has 95% of its probability mass within the following interval:

$$[\mu - 2\sigma, \mu + 2\sigma]$$

Or equivalently:

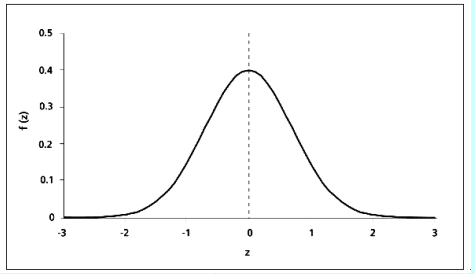
$$P(\mu - 2\sigma \le X \le \mu + 2\sigma) = 95\%$$

Distribution of the standardized mean in case of normal distributed observations

$X_1, X_2,...,X_n \sim N(\mu_x,\sigma_x^2)$ i.i.d.

Variance σ_x^2 is known.

$$T = \frac{\bar{X} - \mu_x}{\sigma_x / \sqrt{n}} \sim N(0,1)$$



$$n \to big(> 25)\ \ t_{df=n-1} \to N(0,1)$$

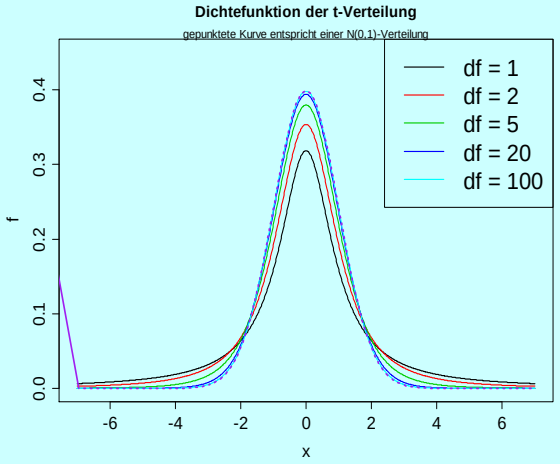
$X_1, X_2,...,X_n \sim N(\mu_x,\sigma_x^2)$ i.i.d.

Variance σ_x^2 is unknown and is estimated from the data

$$T = \frac{\bar{X} - \mu_x}{s_x / \sqrt{n}} \sim t_{n-1}$$

$$s_x^2 = \hat{\sigma}_x^2 = \frac{1}{n-1}\sum_{i=1}^{n}(X_i - \bar{X})^2$$

$$se(\bar{x}) = sd(x) / \sqrt{n}$$



se: standard error of the mean
variation of the estimator

Remark: Since beside the mean also the variance is derived from the random sample we have some additional variation when determining T and the distribution of T gets broader and is given by the $t_{df=n-1}$

Construct an exact confidence interval for the expected value if values are normally distributed

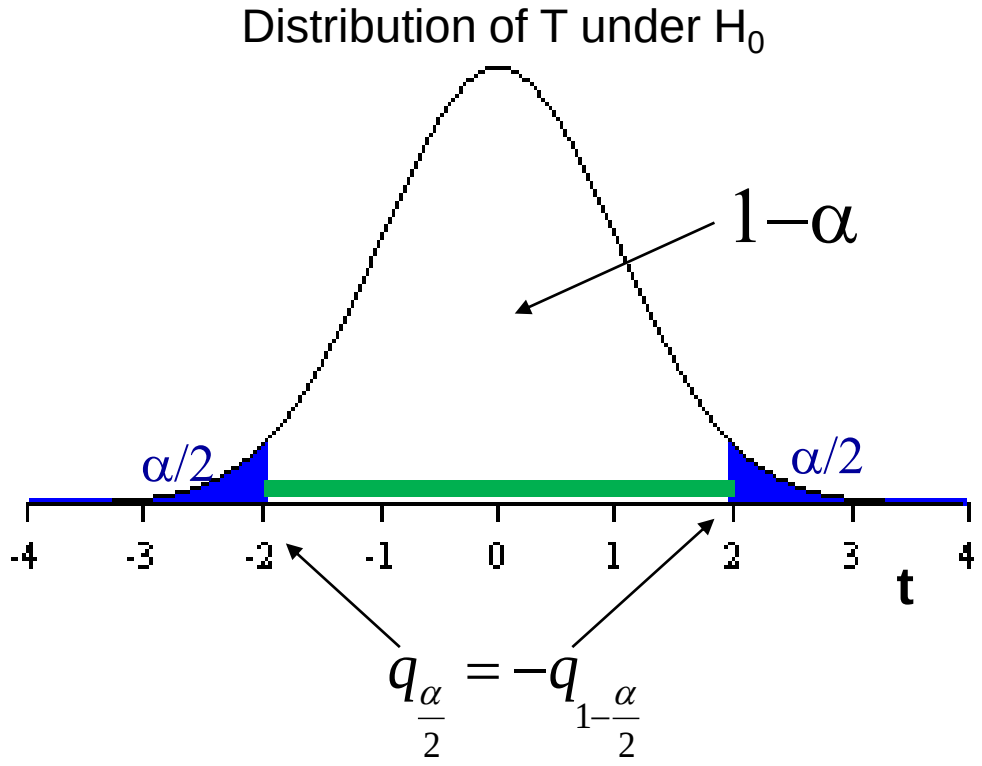
$$X_i \; i.d.d. \;\; \sim N(\mu, \sigma^2) \;, E(X) = \mu_x \;, \; Var(X) = \sigma_x^2$$

$$\Rightarrow \; T = \frac{\bar{X} - \mu_x}{s_x / \sqrt{n}} \sim t_{df=n-1}$$

$$P(q^t_{\frac{\alpha}{2}} \le \frac{\bar{X} - \mu_x}{\sigma_x / \sqrt{n}} \le q^t_{1-\frac{\alpha}{2}}) = 1 - \alpha$$

$$P(-\frac{\sigma_x}{\sqrt{n}} \cdot q^t_{1-\frac{\alpha}{2}} \le \bar{X} - \mu_x \le \frac{\sigma_x}{\sqrt{n}} \cdot q^t_{1-\frac{\alpha}{2}}) = 1 - \alpha$$

$$P(\bar{X} - \frac{\sigma_x}{\sqrt{n}} \cdot q^t_{1-\frac{\alpha}{2}} \le \mu_x \le \bar{X} + \frac{\sigma_x}{\sqrt{n}} \cdot q^t_{1-\frac{\alpha}{2}}) = 1 - \alpha$$



95% CI for μ_x

$$\left[\bar{X} - \frac{\sigma_x}{\sqrt{n}} \cdot q^{t_{n-1}}_{0.975} \; ; \bar{X} + \frac{\sigma_x}{\sqrt{n}} \cdot q^{t_{n-1}}_{0.975} \right]$$

The exact 95% CI for the expected value

if values are normally distributed

$$\bar{x} \pm {}^{t_{n-1}}q_{97.5\%} \cdot \frac{sd(x)}{\sqrt{n}} \overset{n>25}{\approx} \bar{x} \pm {}^{z}q_{97.5\%} \cdot \frac{sd(x)}{\sqrt{n}}$$

$se(\bar{x})$: standard error of the mean

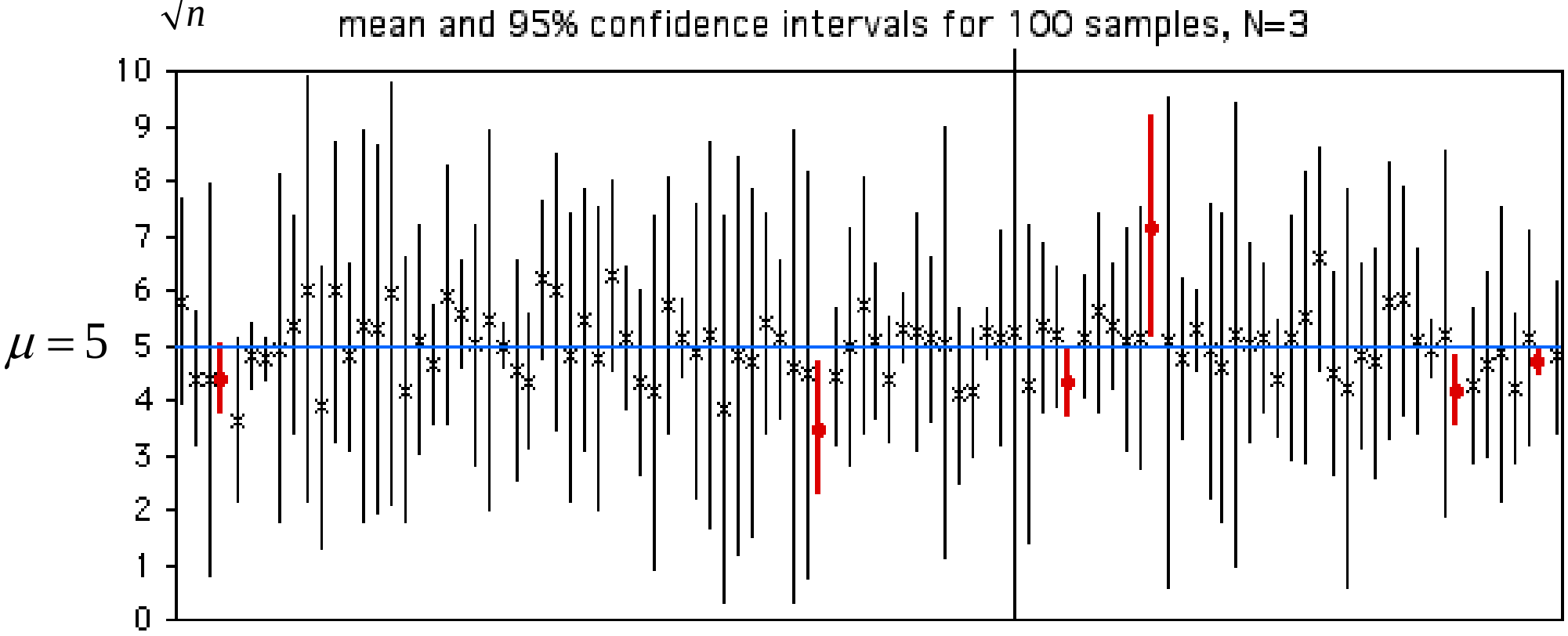
Please note that for this CI the quantiles of the t-distribution are used. The t-distribution has a parameter df (degree of freedom), which must be set on n-1, where n is the number of observations in the sample.

If n gets large (>25) the quantiles of the t-distribution can be approximated by the quantiles of the N(0,1) distribution. In the large sample case (n>25) the assumption $x \sim N(\mu,\sigma^2)$ is not essential!

The reason is the Central Limit Theorem that ensures that the mean is approximately normally distributed and therefor also the standardized mean.

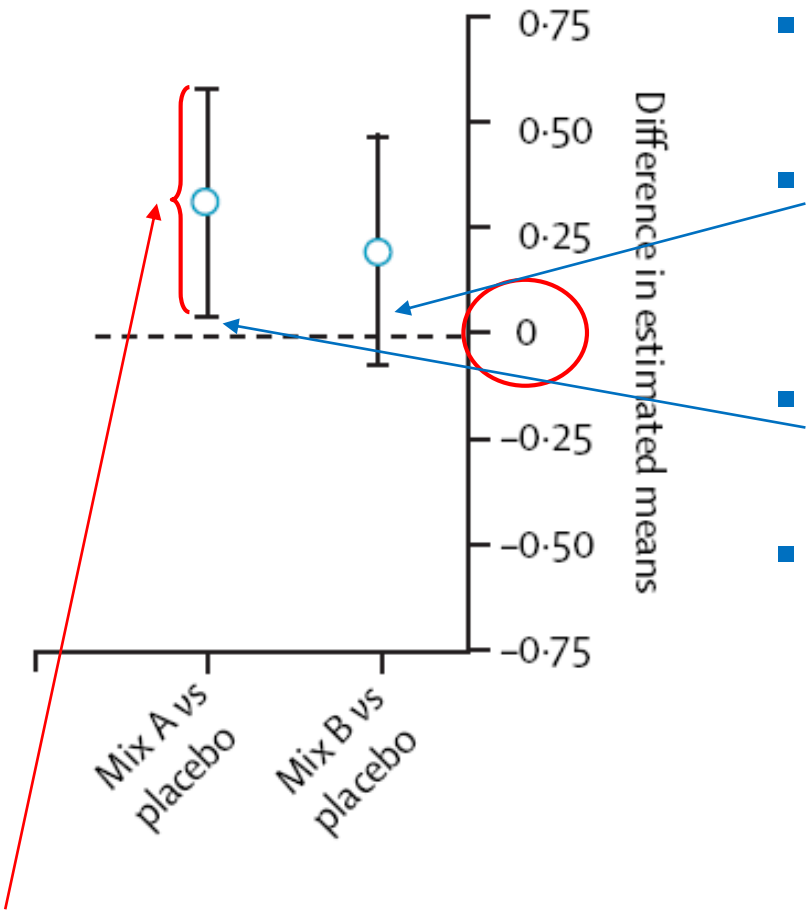
The CI is as random as the sample

$$\bar{x} \pm 2 \cdot \frac{sd(x)}{\sqrt{n}}$$



95 out of 100 95%-CI for μ do cover the true population parameter μ=5 when simulating 100 random samples from a population following N(μ=5,σ^2).
With a 95%-CI we have a risk of 5% that our random sample was not typical for the population and the true population parameter is not contained by the CI.

Interpretation of a confidence interval
Example from paper on hyperactivity form McCann et al.

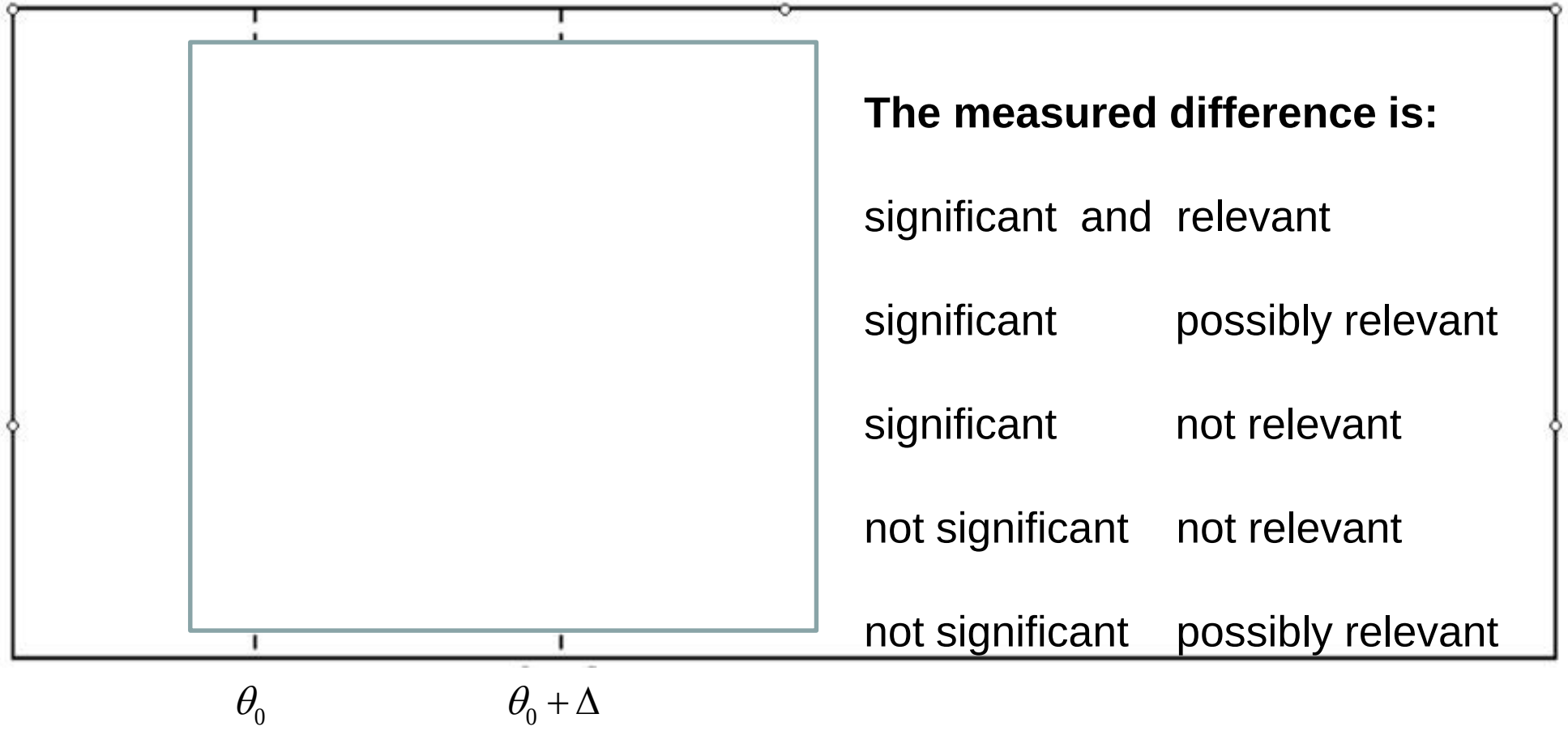


- The CI covers all plausible values for the true mean-difference – here the true treatment effect
- If 0 is covered by the CI it is plausible that the treatment effect is 0 – we have no evidence against H_0, that the treatment has no effect.
- If 0 is **not** covered by the CI, we say that the treatment effect is **significantly** different from 0.
- To have a reasonable chance (80%) to claim a relevant treatment effect to be significant we must plan the sample size to be large enough to be able to find a the effect to be significant if existing.

Here we see a 95% CI of the difference of the mean hyperactivity under placebo and under treatment with Mix A indicating a significant effect of Mix A.

With a confidence interval we can decide:
Is there a significant difference to a postulated value θ_0 ?
Is the difference relevant (>Δ)?

Draw CIs that correspond to the description on the right

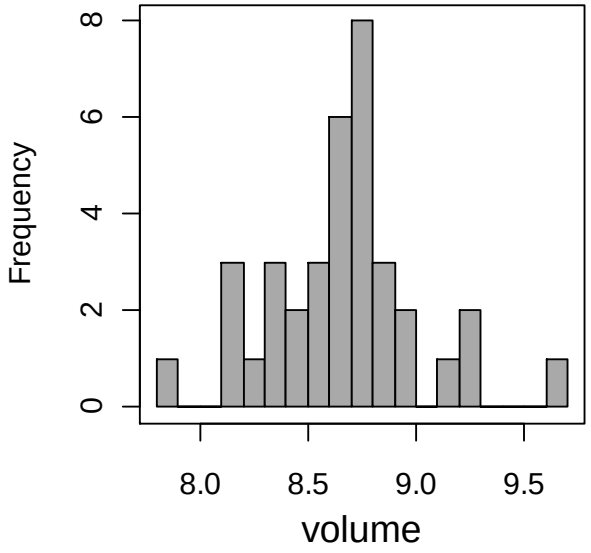


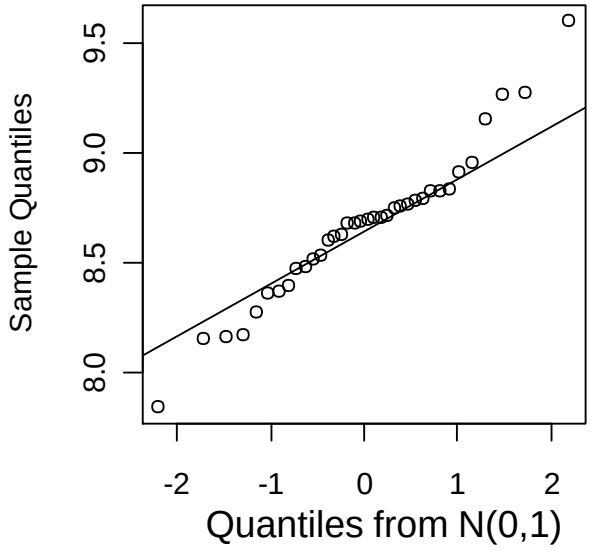
The measured difference is:

significant and	relevant
significant	possibly relevant
significant	not relevant
not significant	not relevant
not significant	possibly relevant

Example for a test problem

A new stem of bacteria was designed to produce a certain enzyme. A tube of bacteria can produce within 1 day in average a certain volume X. The vendor of these bacteria kit claims a volume of 8.2 ml per day. A purchaser wants to check this claim and measures for n=36 tubes the produced volume within a day. He gets to the following results:





From data viszalization roughly extimated:

$$\hat{\mu} = \bar{x} = 8.7$$

$$\hat{\sigma} = sd = 0.2$$

$X_i \sim ?$, $H_0 : ?$, $H_A : ?$, $T = ?$, $T \sim ?$, $^{95\%}VI = ?$

Example for a test problem

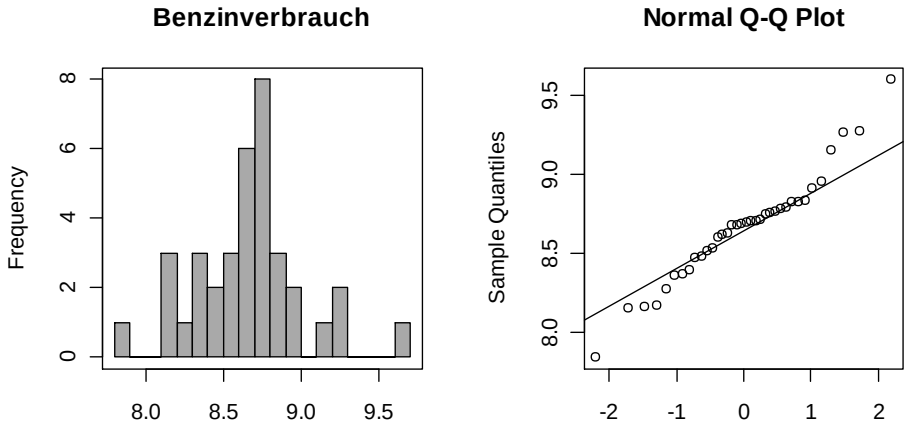
Postulated expected value μ of 8.2 should be tested.

sample: dayly-production of n=36 tubes of bacteria

model for individual values:

$$X_i \; i.i.d. \; X_i \sim N(\mu, \sigma^2)$$

model verification:



Null-hypothesis H_0: $\mu = \mu_0 = 8.2$

Alternative-hypothesis H_A: $\mu \neq \mu_0$

$$T = \frac{\bar{x} - \mu_0}{s/\sqrt{n}} \sim t_{n-1}$$

Teststatistik	$t = \frac{\bar{x} - \mu_0}{s/\sqrt{n}}$	Einstichproben–t–Test

$$^{T}VI^{1-a} = [-\,{}^{t_{35}}q_{1-\frac{a}{2}},\; {}^{t_{35}}q_{1-\frac{a}{2}}] \Leftrightarrow$$

$$^{m}VI^{1-a} = [\bar{x} - s/\sqrt{n} \cdot {}^{t_{35}}q_{1-\frac{a}{2}},\; \bar{x} + s/\sqrt{n} \cdot {}^{t_{35}}q_{1-\frac{a}{2}}]$$

$$= [8.6\;,\; 8.8]$$

$$8.2 \notin [8.6\;,\; 8.8]$$

$$m_0 \notin {}^{m}VI^{1-a} \Rightarrow H_0 \text{ rejected}$$

The one-sample t-test in R

The name t-test comes from the use of t-distribution for test statistic T. The most important results are the CI for the parameter and the p-value.

```
>t.test(voulume, alternative="two.sided", mu=8.2, conf.level=0.95 )
```

```
        One Sample t-test

data:  benzin$benzin
t = 8.1649, df = 35, p-value = 1.283e-09
alternative hypothesis: true mean is not equal to 8.2
95 percent confidence interval:
 8.550504 8.782479
sample estimates:
mean of x
 8.666492
```

p-value of the test:
if $p<\alpha$ ja H_0 will be rejected.

θ_0 (check if $\notin$ $^{\theta}$VI?)

H_A in words (no decision!)

$^{\theta}$CI of the parameter θ

The estimate of the parameter θ

Historical Excursion: Who has invented the t-test?

... the Guinness brewery in Dublin, Ireland.

The t-test was a by-product of Student / Gosset's efforts to select the best yielding varieties of barley.

S-PLUS - Commands

File Edit View Insert Data Statistics Graph Options Window Help

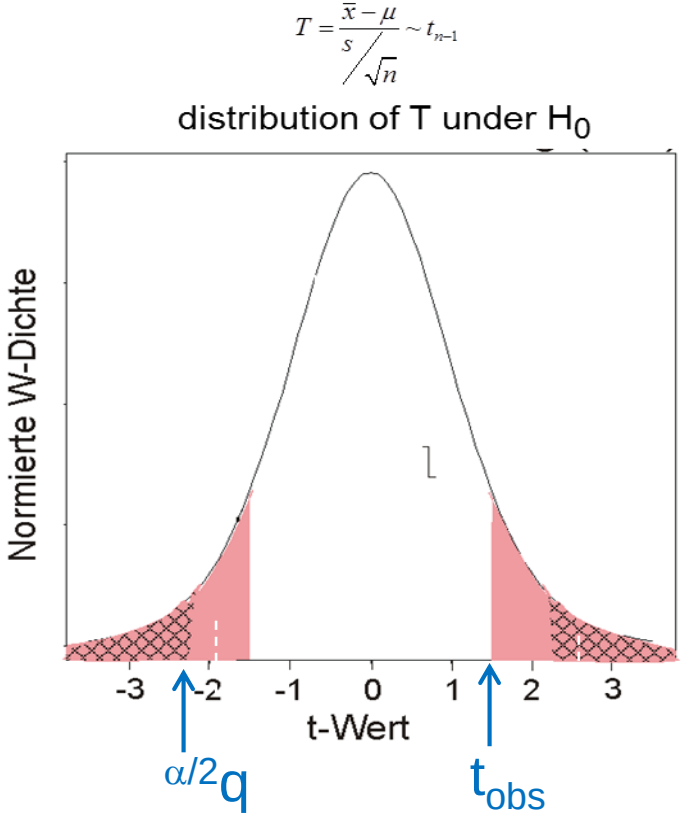
Commands

```
> barley
      yield          variety year            site
 1 27.00000        Manchuria 1931 University Farm
 2 48.86667        Manchuria 1931          Waseca
 3 27.43334        Manchuria 1931          Morris
 4 39.93333        Manchuria 1931       Crookston
 5 32.96667        Manchuria 1931    Grand Rapids
 6 28.96667        Manchuria 1931          Duluth
 7 43.06666          Glabron 1931 University Farm
 8 55.20000          Glabron 1931          Waseca
 9 28.76667          Glabron 1931          Morris
10 38.13333          Glabron 1931       Crookston
11 29.13333          Glabron 1931    Grand Rapids
12 29.66667          Glabron 1931          Duluth
13 35.13333         Svansota 1931 University Farm
14 47.33333         Svansota 1931          Waseca
```

Interpretation of the p-value

The p-value corresponds to the probability to get an at least such extreme result as the seen one if we assume that the Null-Hypothesis is valid. (Therefore we reject H_0 if this probability is small)

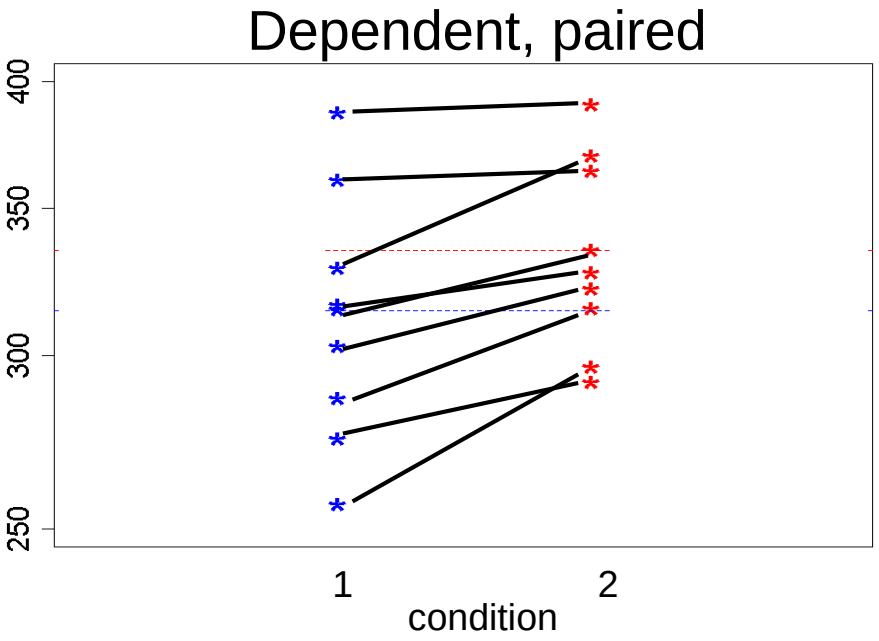
Graphically: the p-value corresponds to the area in the extreme tails (from the observed t-value outwards) under the density of the test-statistic distribution which is taken for a true H0.

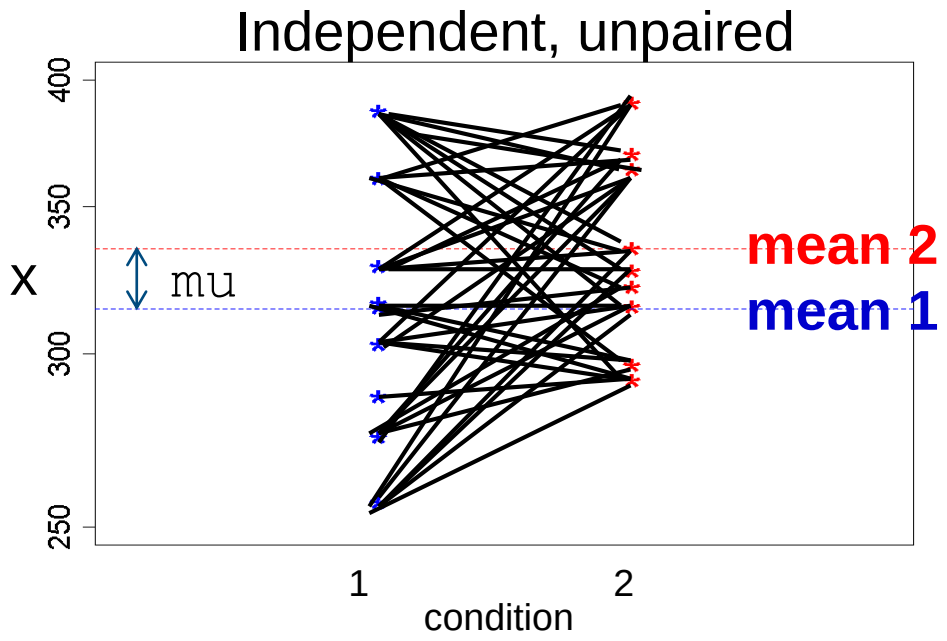


$$p = P\left(|t| \geq |t_c| \;\middle|\; H_0 \text{ is true}\right)$$
$$= P\left(p_{new} \leq p \;\middle|\; H_0 \text{ is true}\right)$$

p-value > 0.1 : no evidence for H_A
p-value < 0.1 : weak evidence for H_A
p-value < 0.05 : evidence for H_A
p-value < 0.01 : clear evidence for H_A
p-value < 0.001 : strong evidence for H_A

Is there a significant difference between 2 groups? What are paired/dependent samples?

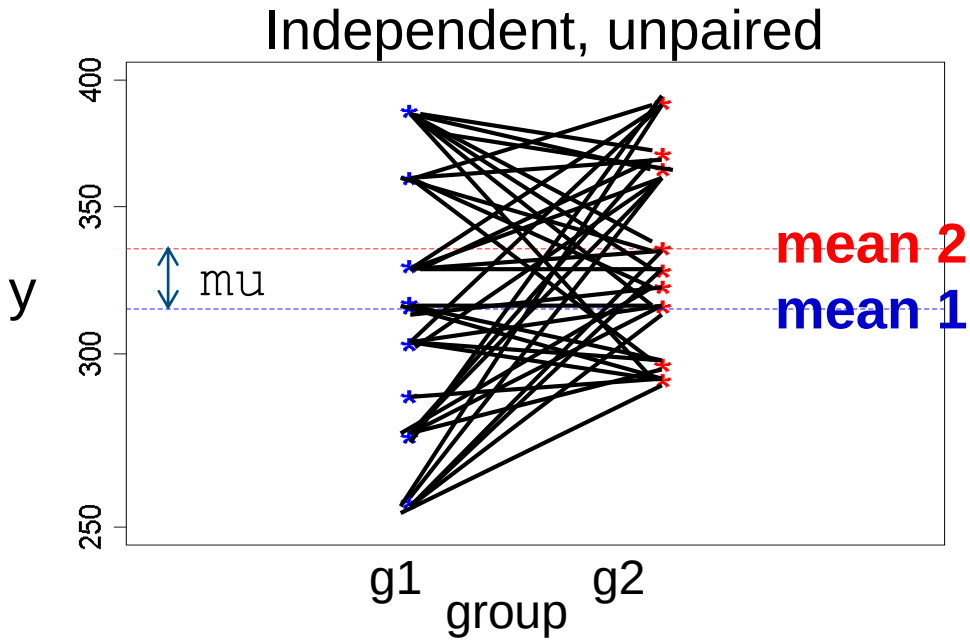




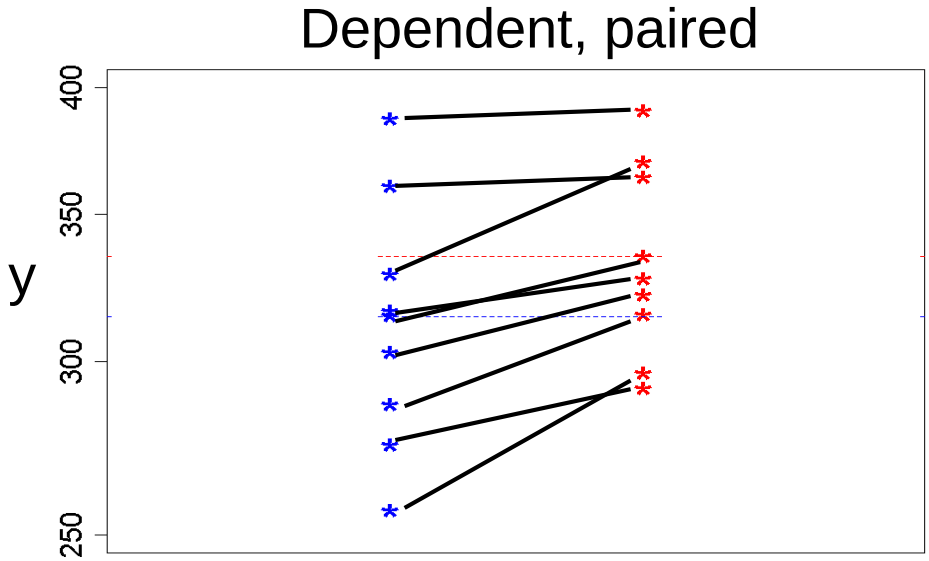
In a **paired design always a pair of values from group1 and group2 correspond to each other** (often 2 treatments were applied to each unit or person) ~> $n_1=n_2$
In a paired design we test if the population **differences within pairs** are zero (mu=0).

In a unpaired design we test if the population means of two independent samples, e.g. corresponding to 2 treatment groups, are different (mu=0). The 2 groups might have different sizes.

Unpaired and paired data with continuous outcome




```
t.test(g1,g2, mu=0,
       var.equal=T, paired=FALSE)
```

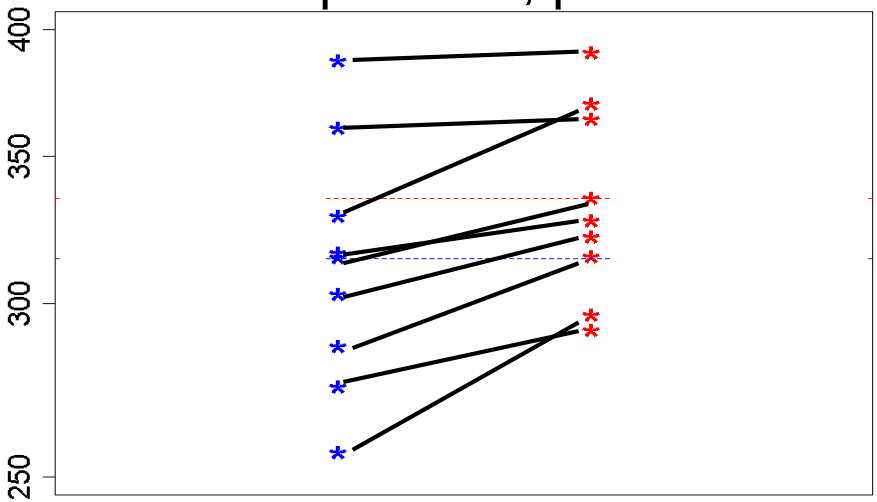



```
t.test(g1,g2, mu=0,
       var.equal=T, paired=TRUE)
```

Breaking the match results in a valid group/treat effect but invalid p-values.

Pros and Cons of a paired compared to unpaired design

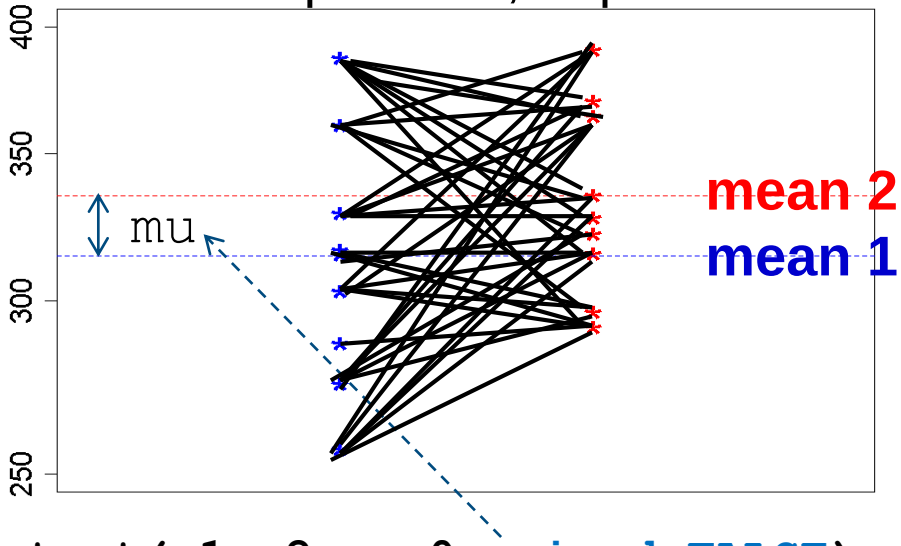
Dependent, paired



`t.test(g1,g2,mu=0,paired=TRUE)`

$$T = \frac{\bar{\Delta}_{pair}}{se(\bar{\Delta}_{pair})} \sim t_{(n_i-1)}$$

Independent, unpaired



`t.test(g1,g2,mu=0,paired=FALSE)`

$$T = \frac{\bar{X}_1 - \bar{X}_2}{se_{pooled}} \sim t_{(n_1+n_2-2)}$$

+ In a paired design we can exclude the individual differences of the investigated persons or units and therefore **a paired design is preferable in cases where the individual differences are bigger than the treatment effect.**

+ We need less persons (observations units) to enroll for the same total size $n=n_1+n_2$

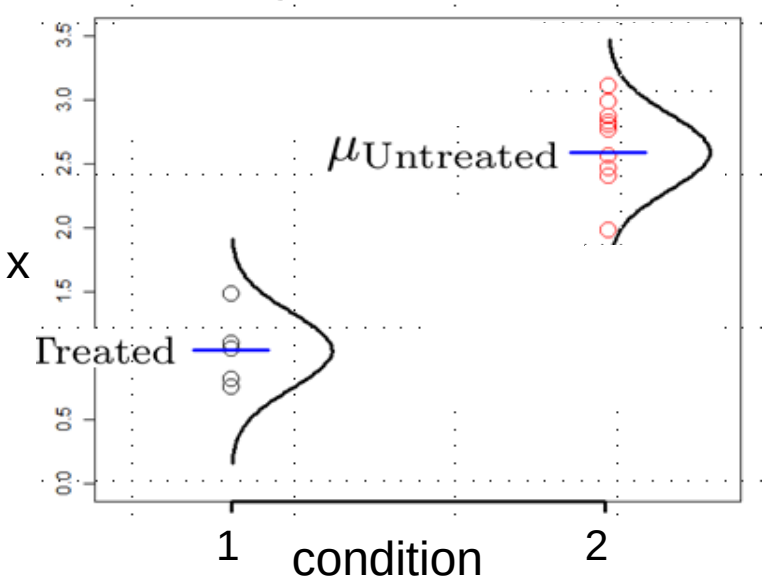
- If the total number of observations $n=n_1+n_2$ is the same and the effect size is much larger than the individual differences, then the standard error of the estimated group difference is larger in the paired design (compared to the unpaired design) since it relies on less comparisons.

Test for differences between treatments, unpaired design

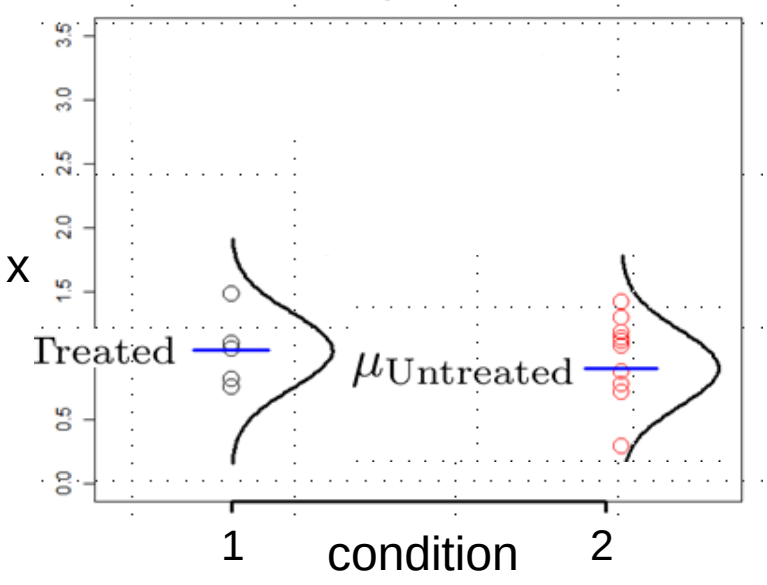
Zurich University of Applied Sciences

zh aw

Drug is effective



drug fails



p=0.0001

X: outcome of interest should be normally distributed ➔

- t-Test (comparison of 2-conditions)

$$T = \frac{\bar{X}_1 - \bar{X}_2}{se_{pooled}} \sim t_{(n_1+n_2-2)}$$

equal variance (t-test): $se_{pooled} = \sqrt{\frac{s^2}{n_1} + \frac{s^2}{n_2}}$

different variance (Welch test): $se_{pooled} = \sqrt{\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2}}$

CI interpretation in case of a unpaired group comparison

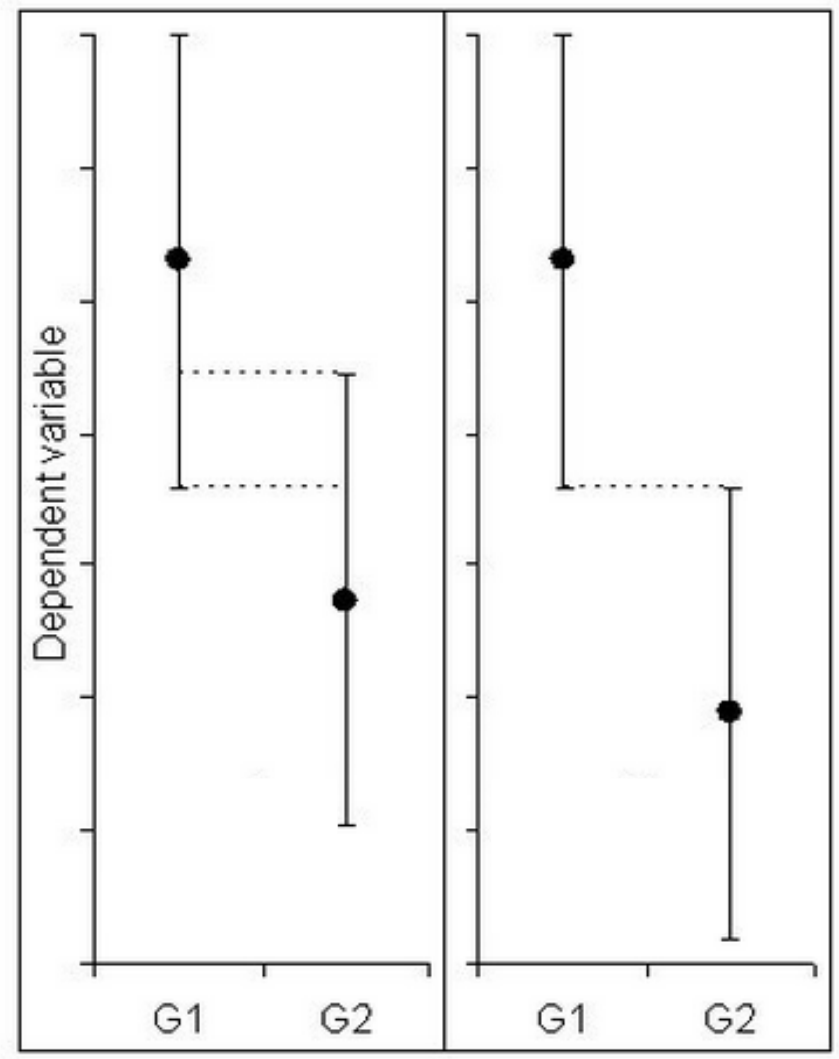


Figure 2: The CIs on the left overlap by about 1/4, half the average margin of error, which corresponds to a p value of ≈ .05. The CIs on the right are just touching. This corresponds toa p value of ≈ .01 (Cumming and Finch, 2005).

If the 95%-CI of two populations means (derived from independent samples) are overlapping less than 25% than the difference is significant, i.e. there is a high data based evidence for a real difference which is not due to sample variation.

Has caffeine intake influence on the reaction time?

- 10 “patients”
- We measure reaction times after treatment with coffee.
- Once coffee contains coffeine once not.

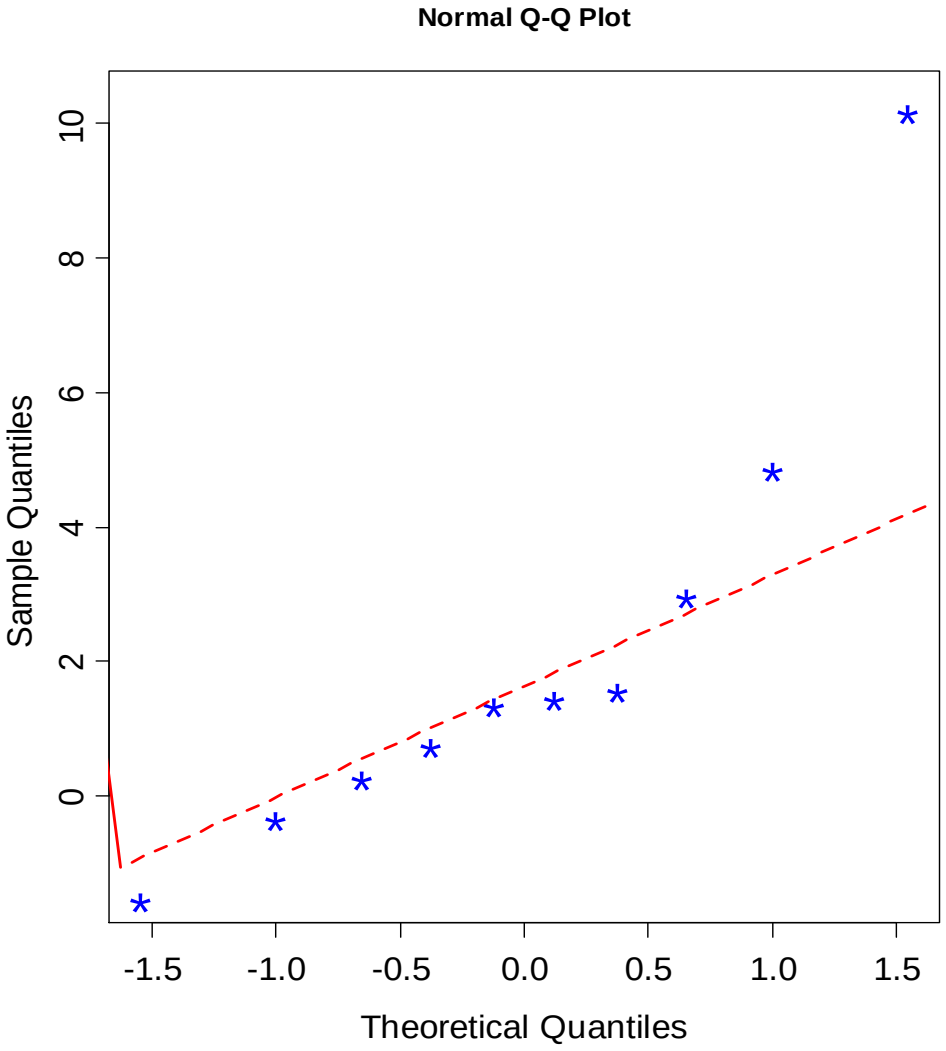
paired design

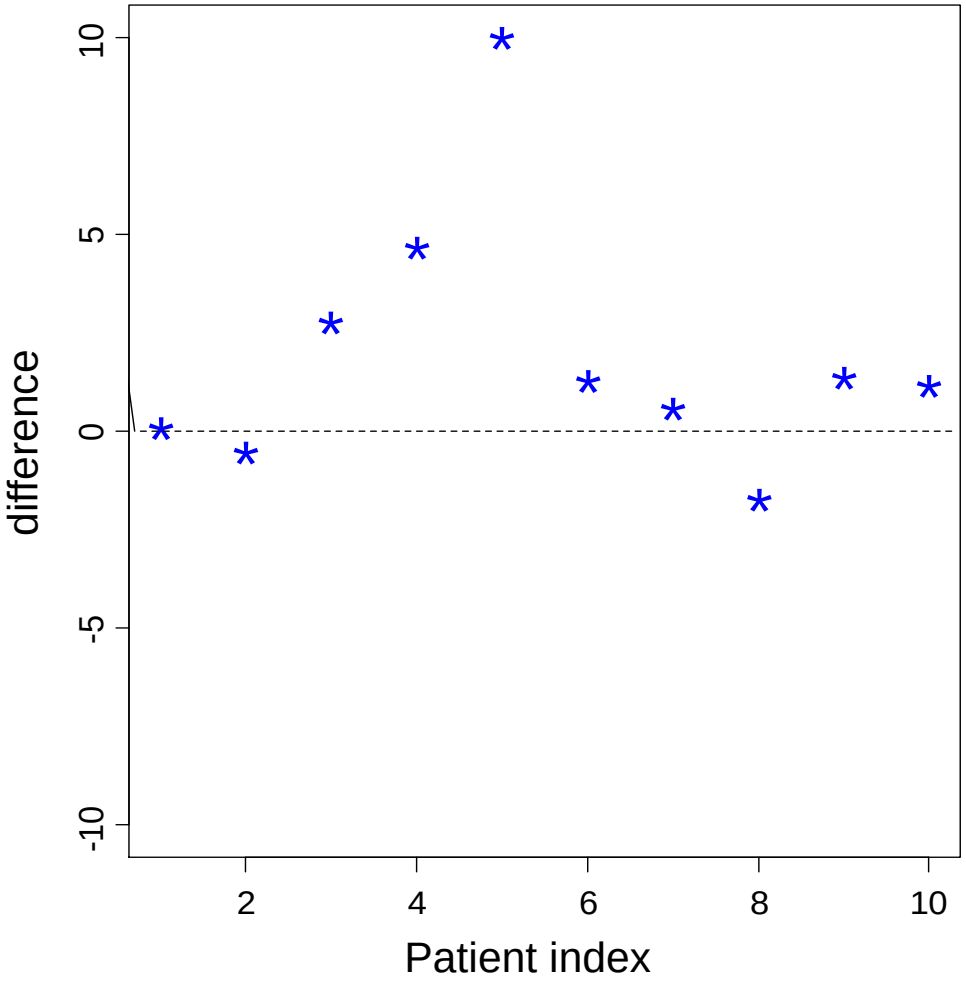
H_0: no difference with placebo or drug
population center is the same

```
> t.test(exp$Differenz, mu=0, conf.level=0.95)

        One Sample t-test

data:  exp$Differenz
t = 2.1842, df = 9, p-value = 0.05678
alternative hypothesis: true mean is not equal to 0
95 percent confidence interval:
 -0.08171953  4.66171953
sample estimates:
mean of x
     2.29
```

Patient	Reaction time with coffeine	Reaction time with decof	diff
1	44.5	44.9	0.4
2	55.0	54.8	-0.2
3	52.5	55.6	3.1
4	50.2	55.2	5.0
5	45.3	55.6	10.3
6	46.1	47.7	1.6
7	52.1	53.0	0.9
8	50.5	49.1	-1.4
9	50.6	52.3	1.7
10	49.2	50.7	1.5

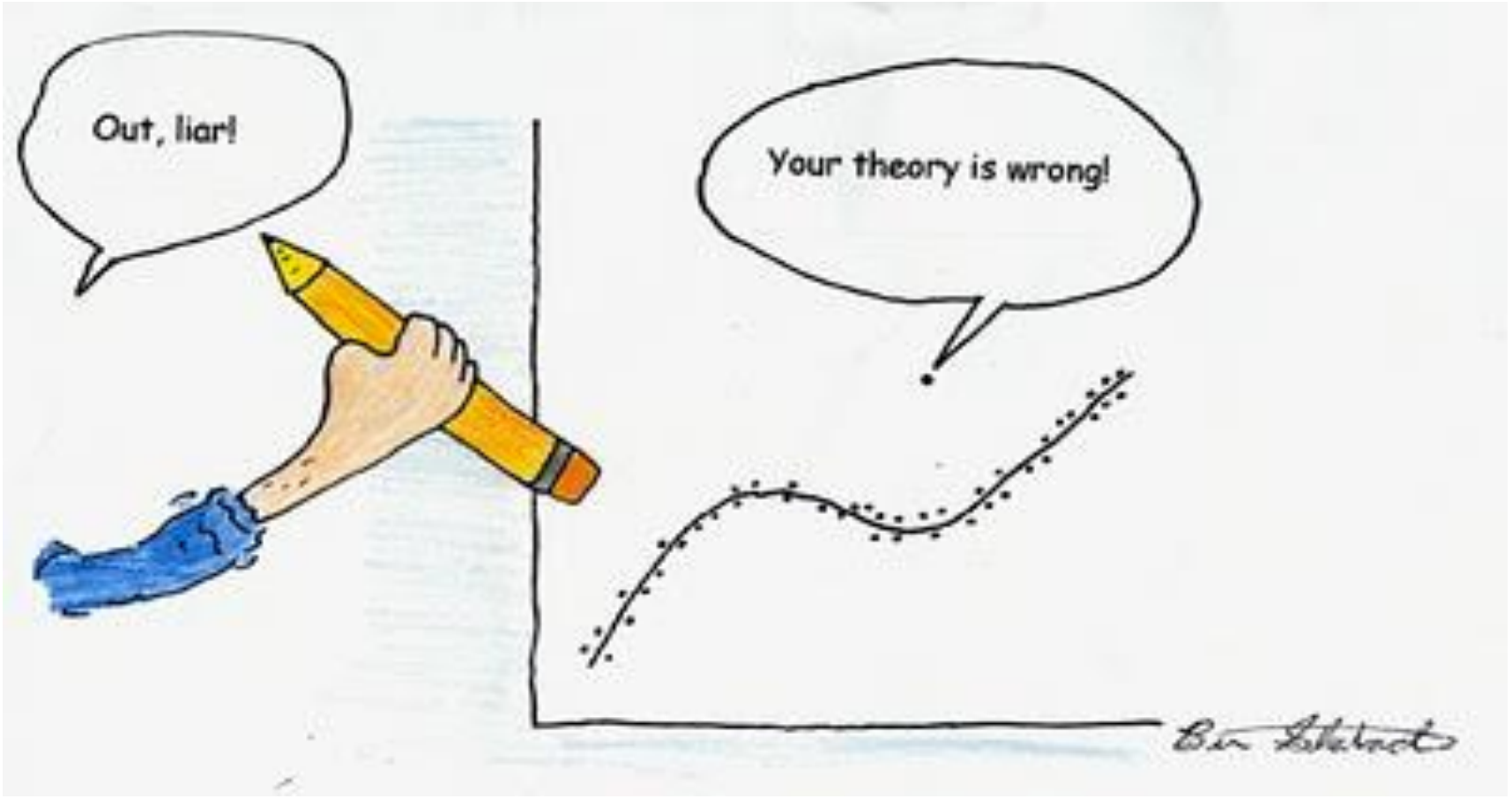
Visualization of the data





There is a outlier! We must not perform a t-test!

How to handle outliers?



Remove an outlier only, if you are sure that there was an error, e.g. the measurement went wrong.
Otherwise keep outlier an adapt your theory or use methods which can handle extreme values in an adequate way.

Look on ranks of the absolute differences

index	abs(d)= | d |	Rank(|d|)	sign(d)
1	0.2	1	-
2	0.4	2	+
3	0.9	3	+
4	1.4	4	-
5	1.5	5	+
6	1.6	6	+
7	1.7	7	+
8	3.1	8	+
9	5.0	9	+
10	10.3	10	+

Idea: Look at sum of ranks of positiv and negative difference – they should be similar if the expected value of d is zero.

$$U^+ = \sum R^+ \quad , \quad U^- = \sum R^-$$

$$\textit{Teststatistik}: \; U = \min(U^+, U^-)$$

Under H_0:

$$\sum R^+ \approx \sum R^- \approx \frac{1}{2}\sum_{k=1}^{n} k = \frac{1}{2}\cdot\frac{n}{2}\cdot(n+1)$$

$$\textit{reject } H_0, \textit{ if } \quad U << \frac{1}{2}\cdot\frac{n}{2}\cdot(n+1)$$

t-test or Wilcoxon-test?

```
> d=c(0.4,-0.2,3.1,5.0,10.3,1.6,0.9,-1.4,1.7,1.5)
> t.test(d)

 One Sample t-test

data:  d
t = 2.1842, df = 9, p-value = 0.05678
alternative hypothesis: true mean is not equal to 0
95 percent confidence interval:
 -0.08171953  4.66171953
sample estimates:
mean of x
     2.29
```

The normality assumption for the t-test is strongly violated, therefore the t-test must not be used.

If the t-test is performed anyway then the results are not reliable and can be completely wrong

(especially with small sample sizes).

```
> wilcox.test(d,my=0,conf.level=0.95)

 Wilcoxon signed rank test

data:  d
V = 50, p-value = 0.01953
alternative hypothesis: true location is not equal to 0
```

p<5% ~> H_0 is rejected and we have shown a significante effect of coffein on the reaction time.

The 1-sample wilcoxon-test requires only a symmetric distribution, which is for difference from paired values always fulfilled.

When to use non-parametric tests like the wilcoxon-tests?

- If data do **not** follow a Normal-Distribution (and sample is not large)
- If there might be outliers
- If the sample size is very small (<≈10) and don't know if data come from $N(\mu,\sigma^2)$

Remark 1: in an unpaired situation there exists also a wilcoxon test, which is known as U-test or Mann-Whitney-test and which also uses a test statistic relying on the ranks of the data.

Remark 2: if the data (in each group) follow a Normal-Distribution, than the t-test has more power than the wilcoxon-test.

Remark 3: for small samples (<10) the normality of data can hardly be checked and the wilcoxon-test should be used if normality is questionable.

Two-sample tests