



Jenny Berrío & Otto Kalliokoski

FIGHTING IRREPRODUCIBILITY IN PRECLINICAL NEUROSCIENCE USING A **META-ANALYTICAL APPROACH**

The case of the Chronic Unpredictable Stress model of depression

UNIVERSITY OF
COPENHAGEN



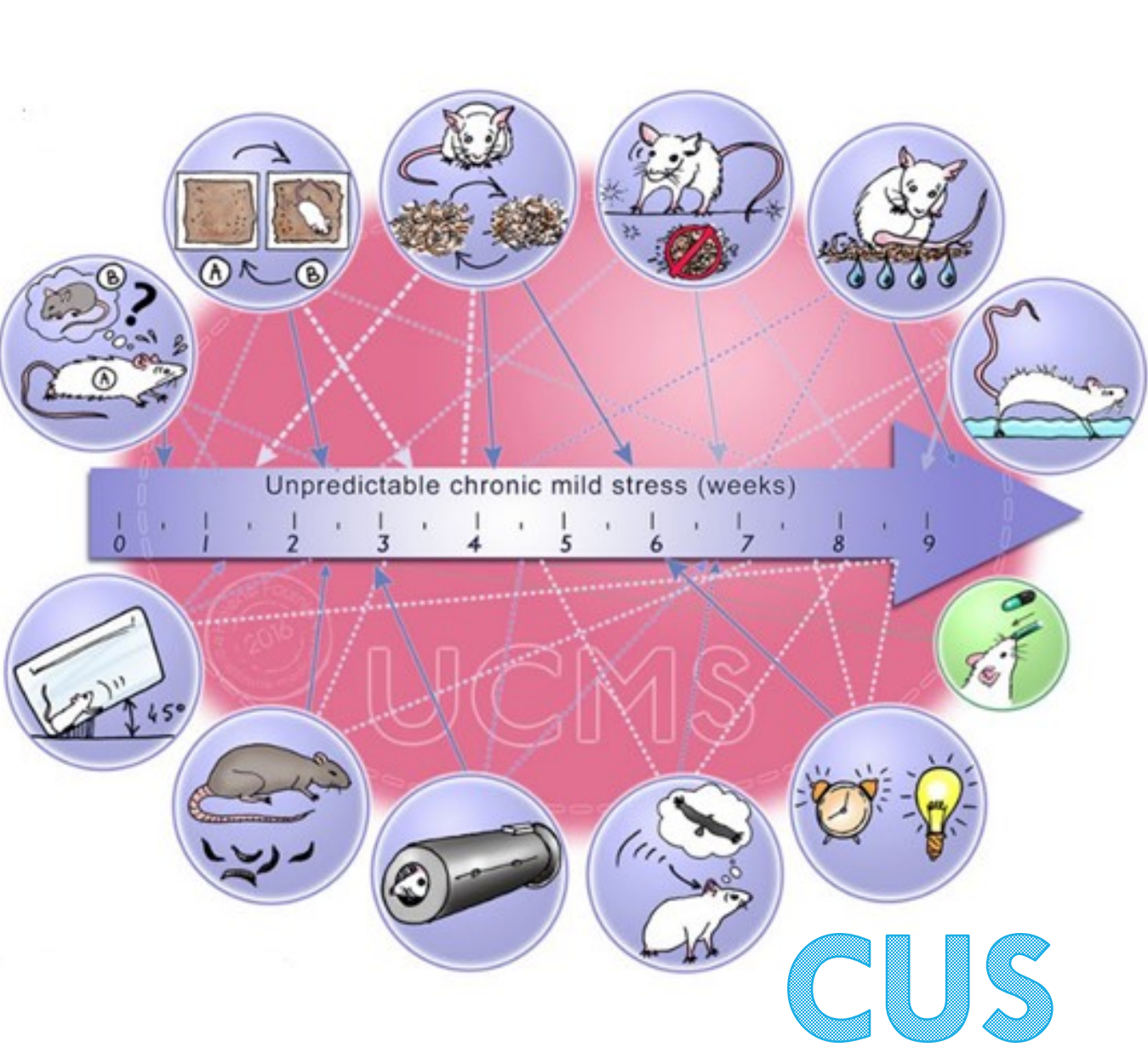
Danmarks 3R-Center

RRR

CONFLICT OF INTEREST DISCLOSURE

We have **NO potential conflict of interest to report**





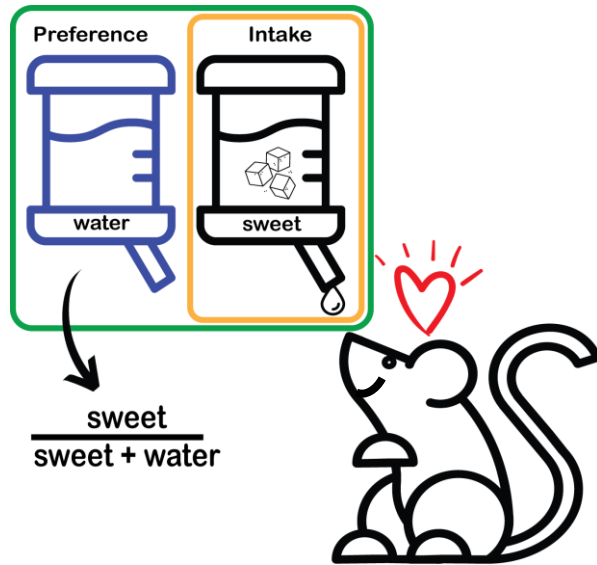
Depression

CUS

(Katz, 1982; P. Willner et al., 1987)

Image sources : <https://www.linkedin.com/pulse/chronic-stress-lets-talk-jonny-atter/>; Planchez et al, 2019

Anhedonia : Decreased ability to feel pleasure and reduced interest in activities an individual used to enjoy



Animal's consumption of sweetened solution

SUCROSE PREFERENCE TEST (SPT)



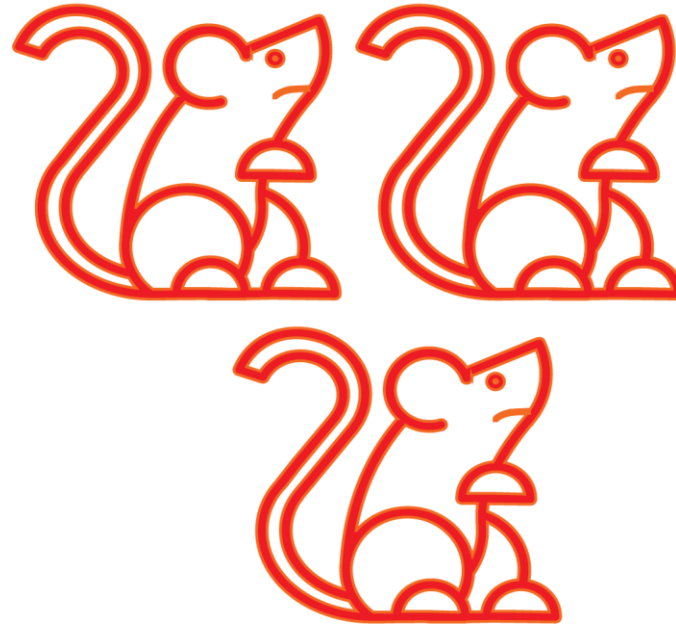
Level of the stimulation (threshold)

INTRACRANIAL SELF-STIMULATION (ICSS)

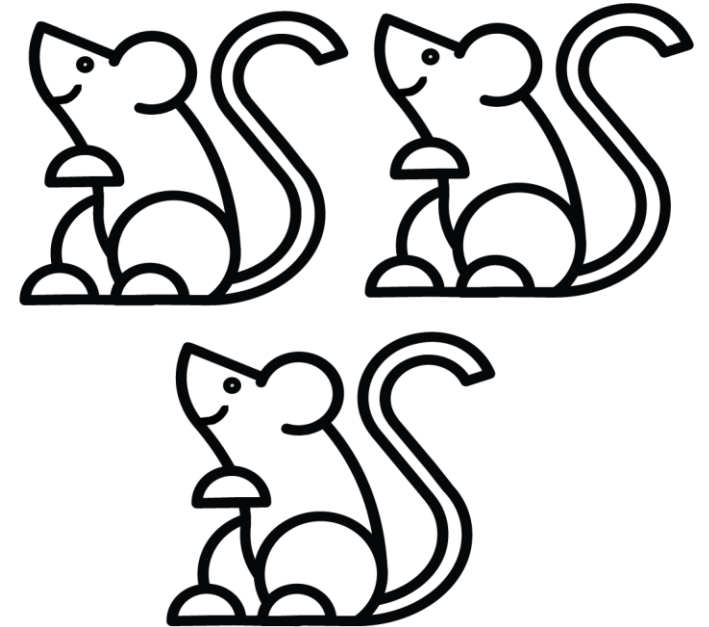
Expected
outcomes

STRESSED

CONTROL



VS



SPT

ICSS

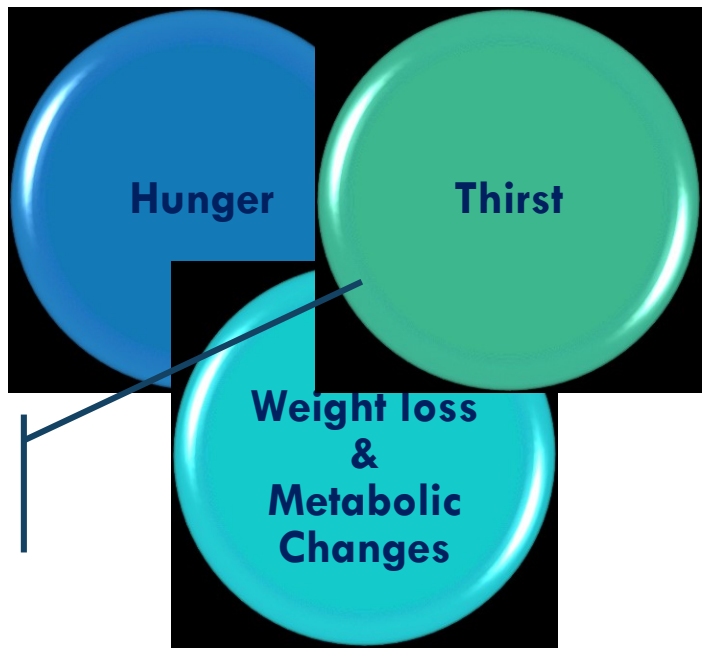
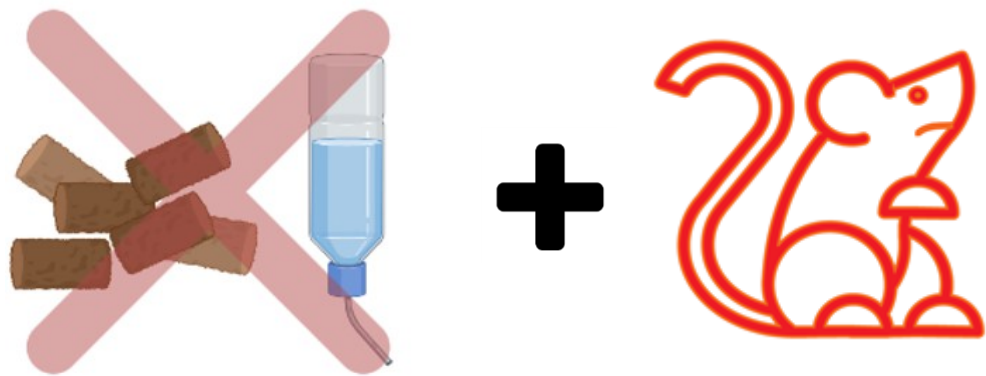
DECREASED SWEET
CONSUMPTION

INCREASED STIMULATION
THRESHOLD

Anhedonia =

**Lack of reproducibility
and inconsistency in
results**





Liking of caloric solution



What Are We Really Measuring?

HEDONIC DRIVE

Metabolic
drives



1 Do chronically stressed rats show a decreased sweet consumption when none or short periods of fasting are used instead?

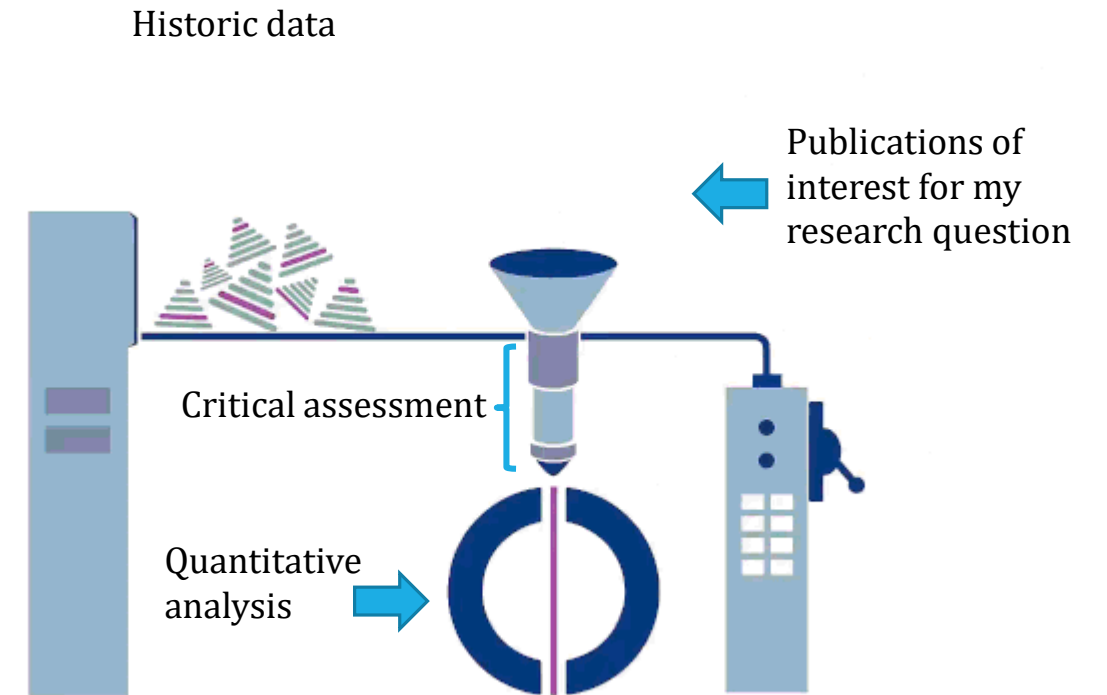
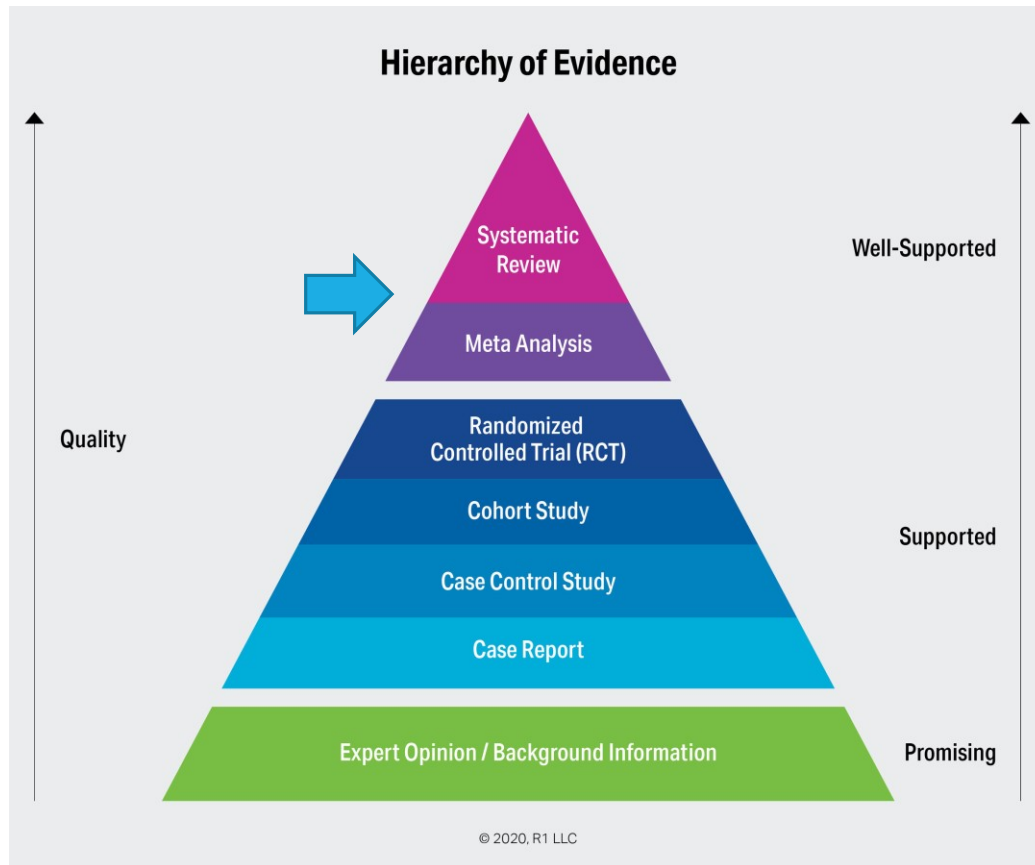
ICSS



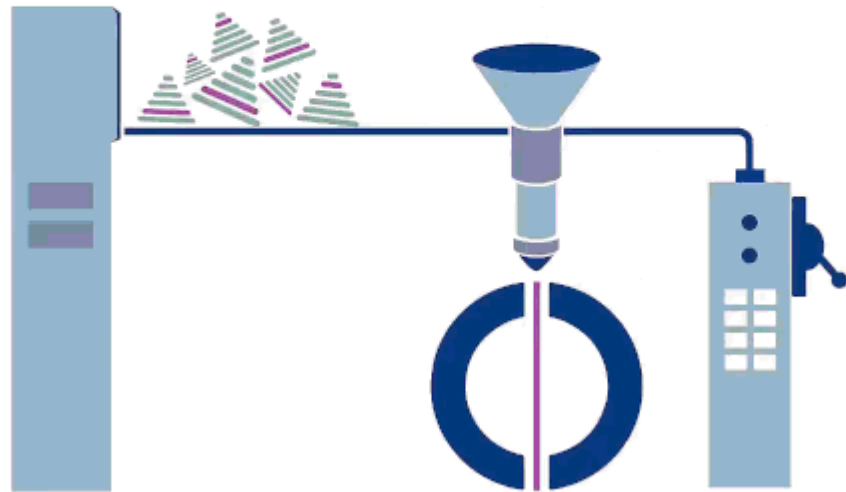
HEDONIC DRIVE

2 Do chronically stressed rats show an increased stimulation threshold?

SYSTEMATIC REVIEWS AND META-ANALYSIS



SYSTEMATIC REVIEWS AND META-ANALYSIS



Available
data

Replacement

Reduction

Reconcile conflicting
data

Assess quality of the
evidence

Refinement

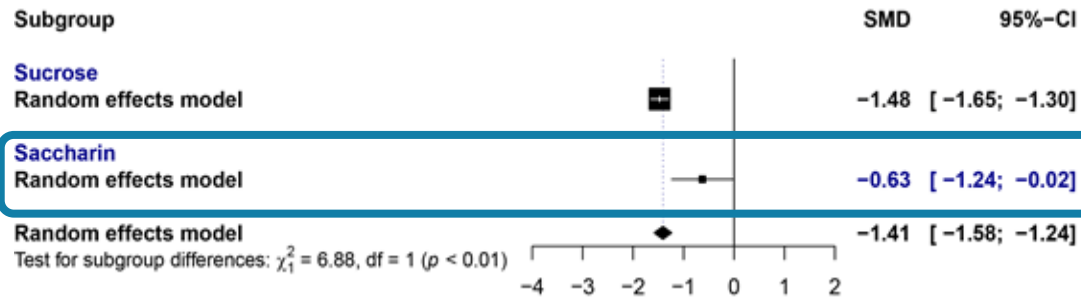
1 Do chronically stressed rats show a decreased sweet consumption when none or short periods of fasting are used instead?

YES, BUT!

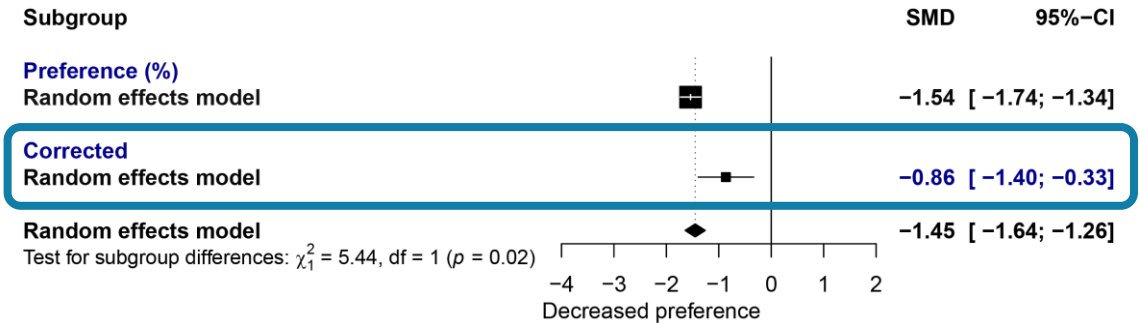


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Non caloric solution



Correction for body weight



EFFECT SIGNIFICANTLY REDUCED

1

Do chronically stressed rats show a decreased sweet consumption when none or short periods of fasting are used instead?

YES, BUT!

EFFECT SIGNIFICANTLY REDUCED

Non caloric solution

Correction for body weight

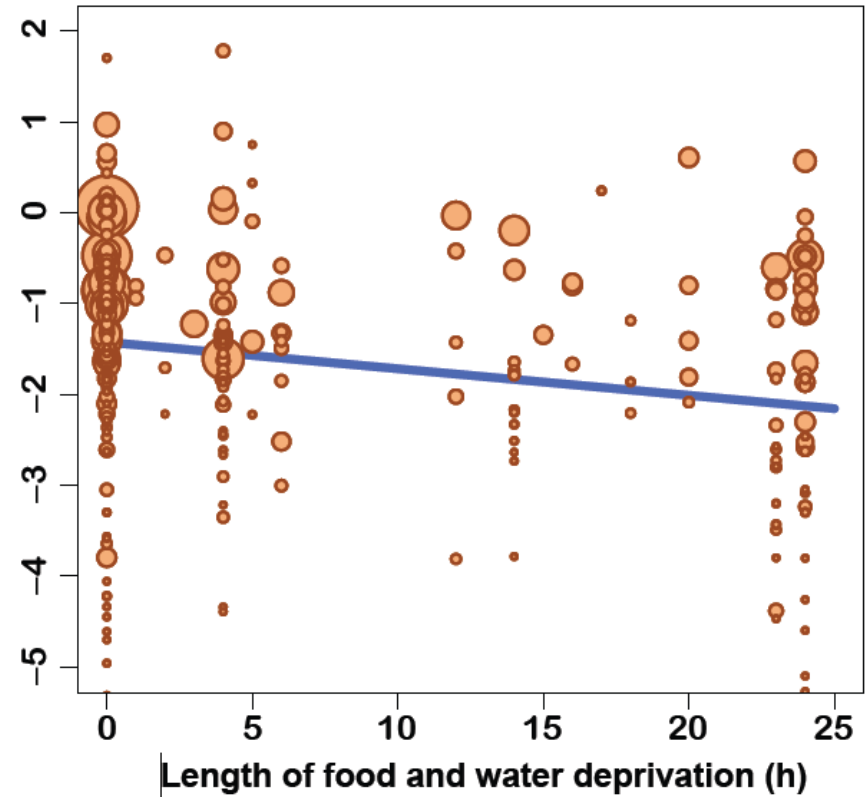
&

LONGER PERIODS OF FASTING CONFOUNDS EFFECT



Not reliable

Effect on SPT outcome (SMD)



Cut-off for susceptibility and resilience?

SWEET PREFERENCE TEST SIMULATOR

SPT simulator

Consumptions
Input your average consumption values and the average leak of your bottles with their respective standard deviations:

Sweet consumption (mL)

Standard deviation

Water consumption (mL)

Standard deviation

Bottle leak (mL)

Standard deviation


Experiment
Input the sample sizes of each group (minimum 6 per group), desired sweet preference cut-off, and number of experiments to simulate:

Control (n)

Stressed (n)

Threshold (%)

Number of experiments



Background Normal experiments Data exclusions Data splitting

The Sweet Preference Test (SPT) is often used to validate stress-induced animal models of depression.

Setting a sweet preference cut-off as the criterion for anhedonia is becoming a common practice in the field.

Based on this threshold, researchers either opt to **exclude values** in the group exposed to stress from analysis or to **split** this exposed group into two subgroups: **sensitive** or **resilient** to stress. In the simplest of studies, these modified groups are later compared to a single unstressed control, using parametric tests such as the t-test (former case) or a One-way ANOVA (later case), in order to measure the effect of stress.

Practices like these can have **hidden effects** that may confound the results of your experiment by **overestimating the effect of stress**. Taking these effects into consideration when planning the data analysis of your experiment is crucial to limit the biases of your study.

This Shiny app allows you to simulate a series experiments in which two cohorts of animals are compared in their simulated sweet preference under the assumption that any resulting significant difference arise by no other means (i.e. stress) than chance.

Three type of experiments will be simulated:

Normal experiments: both cohorts are unmodified and compared with a t-test

Data exclusions: one of the cohorts is modified by removing preferences above the selected threshold, then the unmodified cohort is compared to the modified cohort with a t-test

Data splitting: one of the cohorts is split into two subcohorts, preferences above the selected threshold are grouped in a "resilient" cohort, whereas those below into a "susceptible" cohort. Then, the unmodified cohort and the two modified subcohorts are compared using the One-way ANOVA.

You will be able to input your own baseline consumption data and calculate the false positive rate (FPR- proportion of experiments with p values <0.05) of the desired number of experiments.

We hope you enjoy and find our app useful



Berrio et al. 2023b

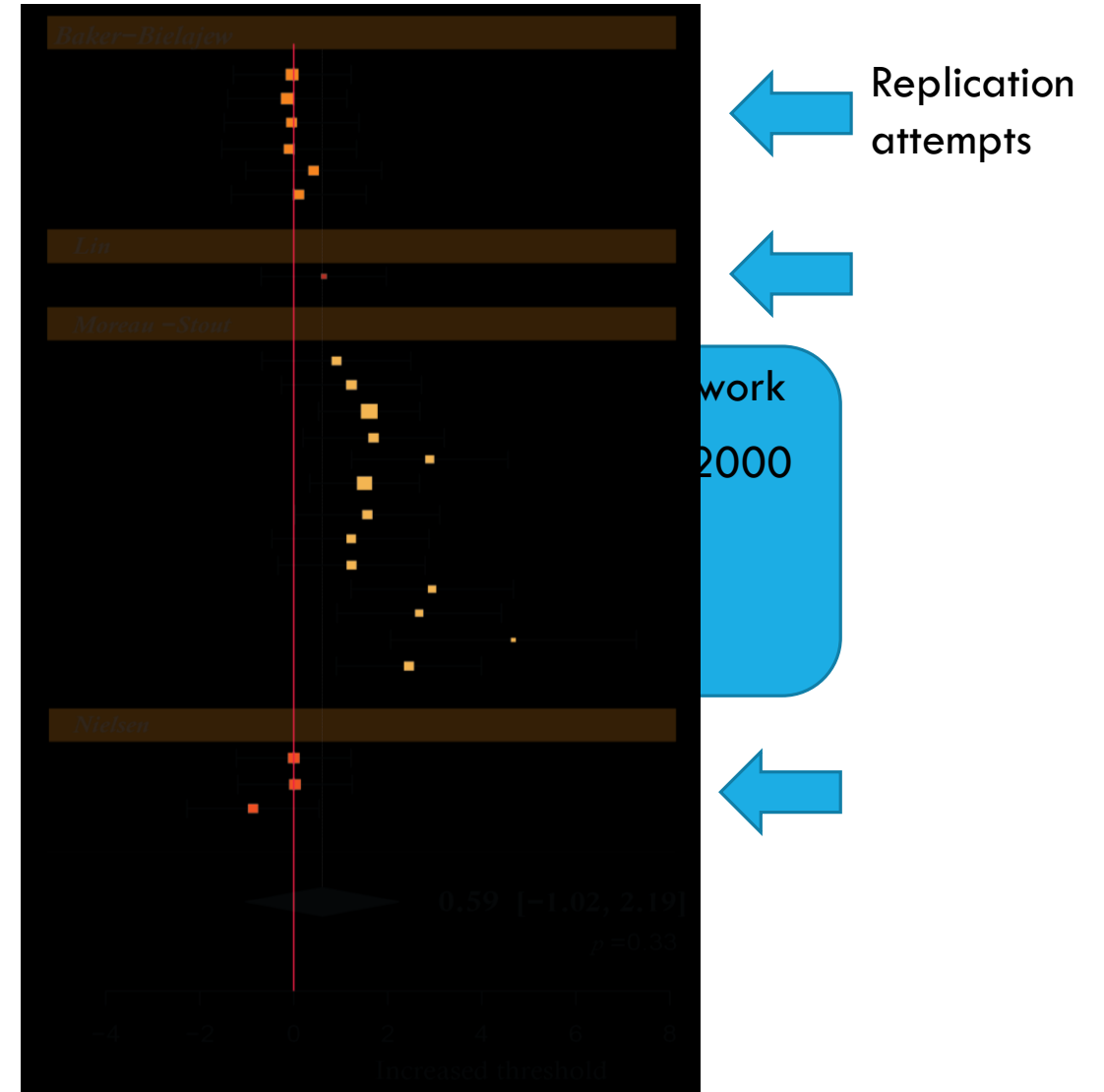
<http://jenb.shinyapps.io/App-1>

2 Do chronically stressed rats show an increased stimulation threshold and is it correlated with sweet consumption?

NO

LACK OF REPLICATION OF INITIAL RESULTS

Specific factors not identified



Conclusions

- ✓ Depriving the animals of food and water is not necessary nor advised (**Test Refinement**)
- ✓ Even without long deprivations, a **metabolic component** seem to underlie the effect. Needs to be controlled for (**Test & Model Refinement**)
- ✓ Reliability of the **SPT** is still questioned
- ✓ No evidence in support that the model is associated with a change in self-stimulating behavior
- ✓ Reliability of the **model** for stress-induced anhedonia?

Anhedonia =

SPT
DECREASED SWEET
CONSUMPTION



ICSS
INCREASED STIMULATION
THRESHOLD



MARTY, WE'VE GOT TO GO BACK...

BACK THROUGH OUR OLD LEADS!



Thank you



Otto Kalliokoski, PhD

Associate Professor

Department of experimental Medicine
Section of Research and Education



Sara Hestehave, PhD & DVM

Postdoctoral Associate in Dr. Rajesh
Khanna's group, NYU Pain Research
Center.



Jenny Wilzopolski, DVM

Researcher

German Federal Institute for Risk Assessment, German
Centre for the Protection of Laboratory Animals



Katharina Hohlbaum, PhD

Postdoctoral Researcher

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