INTERFAKULTÄRE
BIOMEDIZINISCHE
FORSCHUNGSEINRICHTUNG

# Suffering from aggression?
# Use cage climbers in your mouse cage!

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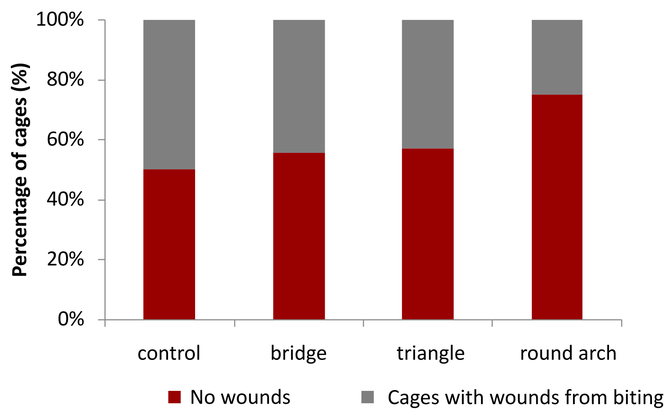
## Introduction

While many neurobiological publications describe clear effects of enrichment on the animals' physiology and behavior in an experimental context, large-dimensional implementation in rodent facilities often lacks a systematic analysis of respective refinement measures. Here, we aimed at implementing a new and innovative tool to improve wellbeing without side effects. Thus, we focused on enrichment-induced changes in behavior and stress physiology especially emphasizing effects on data variability in male and female mice. For that purpose, recycled cage lids were formed and three types of shapes examined for different effects of different structures ('cage climber'):
1.) 'Triangle' Climber,
2.) 'Bridge' Climber and
3.) 'Round Arch' Climber.

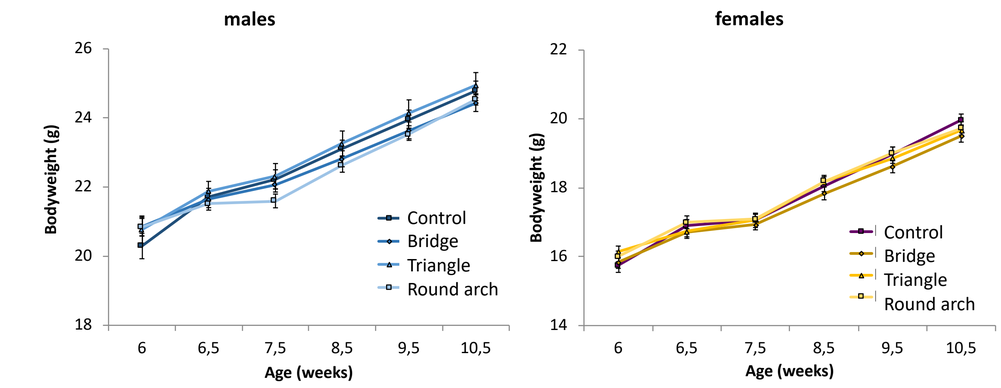

## Aggression in male mice

Aggression was measured indirectly: Every week, animals were scores for their fur status at 8 different zones of the body (head, neck, front paws, hind paws, ventral and dorsal body, tail, genital region) with a score of 0 (no change), 1 (fur change) or 2 (wounds from biting). Wounds occurred only in male mice.




Control: N=32 in 8 cages, bridge N=36 in 9 cages, triangle N=28 in 7 cages, round arch N=32 in 8 cages
Cages with animals with wounds from biting were removed from further analyses to avoid aggression as confounding factor: resulting in: females: N=24 /enrichment type, male: control: N= 16, bridge: N=20, triangle N=16, round arch N=24.

## Bodyweight development




## Methods

- C57BL/6N mice
- Female and male in groups (4/cage)
- 6 weeks old at beginning

### Preference for the enrichment

- Housing in Macrolon®standard cages type III with nesting material
- Test in novel object test for preference of a cage climber vs. Standard object
- N=6/object/sex

### Evaluation with the animal-caretakers

- Scoring of Handling in daily routine
  - Daily use
  - Visibility of animals
  - Cleaning/hygienic aspects
  - Changes in animal handling
  - Storage
  - Standardization

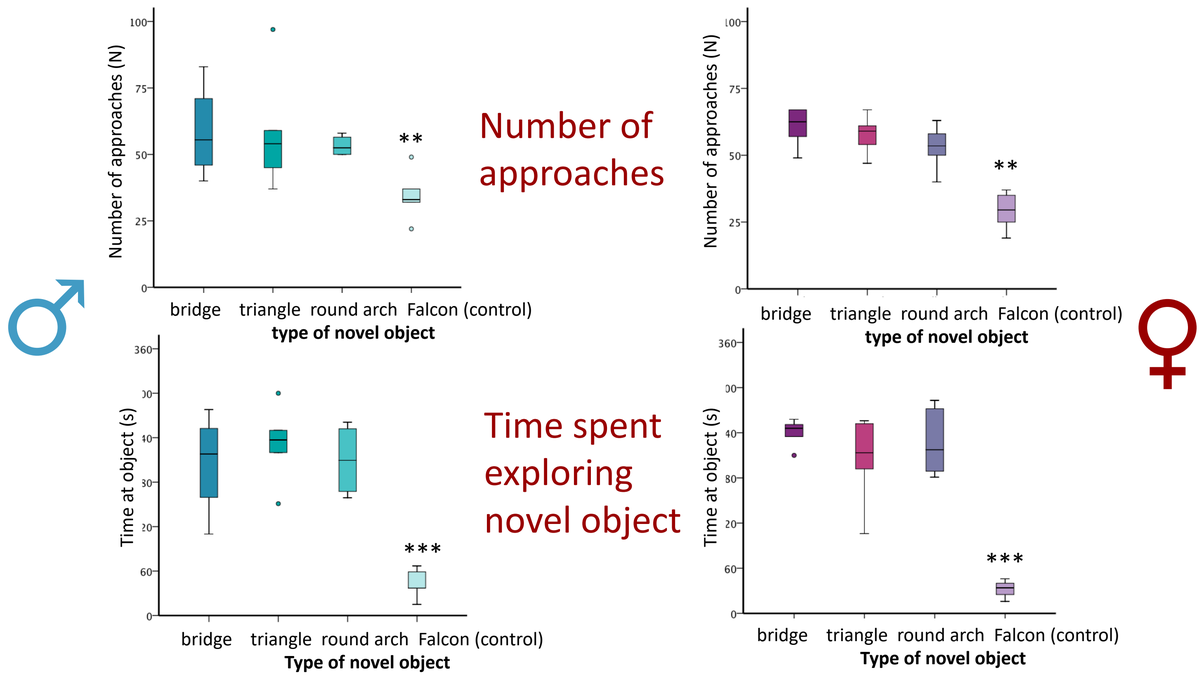
### Housing with cage climber

- Housing in cages with cage climbers and nesting material (control with nesting material only)
- 5 weeks housing with cage climber
- Weekly test for bodyweight and fur status (aggression, changes of well-being)

### Behavioral test battery

- after 5 weeks of housing with cage climber
- Locomotion /exploration
- Anxiety
- Social interest to unfamiliar conspecific
- Social memory
- Balance
- Stress hormone level in Feces (R. Palme)

## Preference for cage climbers as novel object

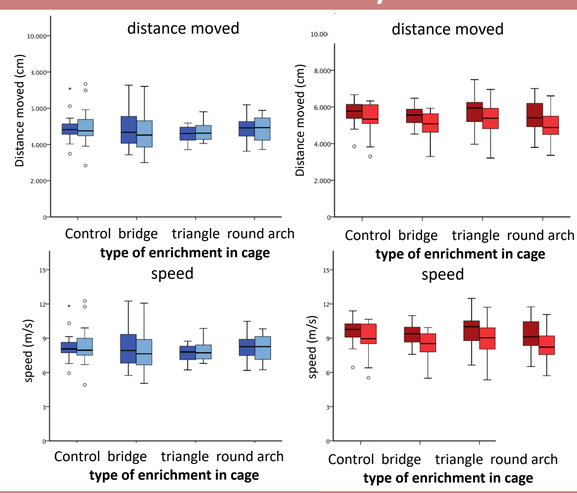


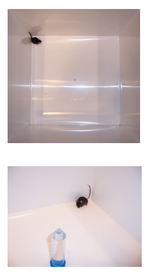

Openfield (50 x 50 cm²) Ethovision X8 tracking parameters:
Locomotion (distance moved, speed, time spent in center area)
Object-related (Time at object, latency to explore, no. of approaches)

Habituation: Minute 0-10 (no object)
→ No behavioral differences
Novel object test: min 10-20
Introduction of cage climber or falcon tube (control object)

## Behavioral test battery - locomotion

No changes in locomotion or exploration in the openfield - novel object exploration!

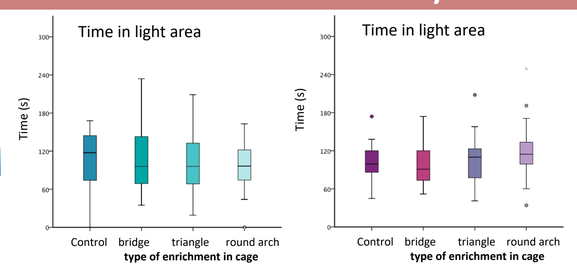


Dark bar: min 0-10 (openfield test)
Light bar: min 10-20 (novel object test with falcon tube as novel object)

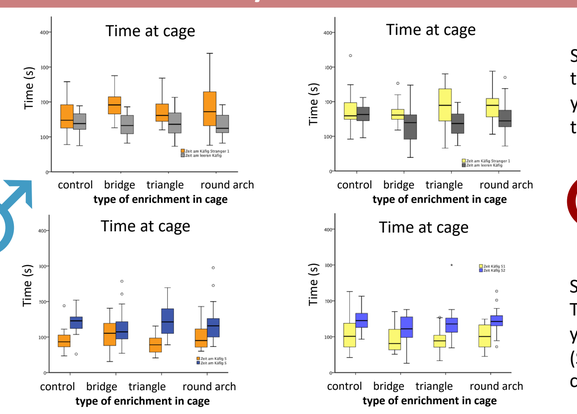
## Behavioral test battery - anxiety

No changes in anxiety in the Dark-Light-Box Test!




## Behavioral test battery – social interest / memory

No changes in social interest and social memory performance!

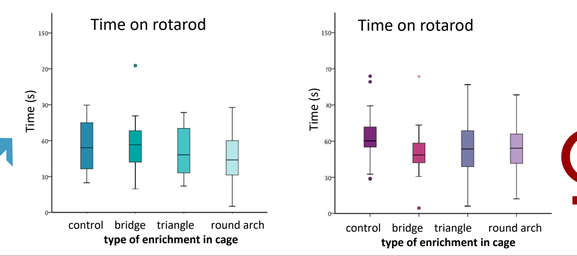


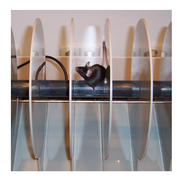

Sociability test (social interest): time at encaged unfamiliar (S1, yellow/orange) conspecific vs. time at empty cage (grey).

Social memory test:
Time at encaged **familiar** (S1, yellow/orange) vs. **unfamiliar** (S2, blue) encaged conspecific.

## Behavioral test battery – balance performance

No changes in balance on an accelerating rotarod!



## Conclusion

The results demonstrate significant preferences of C57BL/6N mice for any of the three structures in comparison with a neutral object. Despite observable intense use of enrichment, there were no behavioral alterations detectable in a test battery assessing anhedonia (sucrose consumption) locomotion (openfield, rotarod), exploration (novel object exploration), anxiety (dark-light box) and sociability as well as social memory. The structural supplement neither affected levels of fecal corticosterone metabolites nor general variability of data in both male and female mice. The only detectable effect was a 50% reduction in male aggression in cages equipped with 'round arch' type of enrichment in comparison to control cages with only nesting material. To promote well-being of mice in a 3R-matched context, our study recommends the use of properly assessed structural enrichment, such as 'cage climbers' combined with nesting material to satisfy physical and thermal needs in the cage environment.

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