

The implementation of tunnel handling in a mouse breeding facility revealed strain-specific behavioral responses

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Introduction

In laboratory animal facilities, mice are regularly removed from cages for cage cleaning and to perform investigations or procedures. Non-aversive methods of removal represent an improvement (refinement) in the handling of the animal compared to the classical fixation at the base of the tail. Studies show that this method sustainably reduces the stress of for the animals, even if interventions are performed in the context of the experiment¹⁻³.

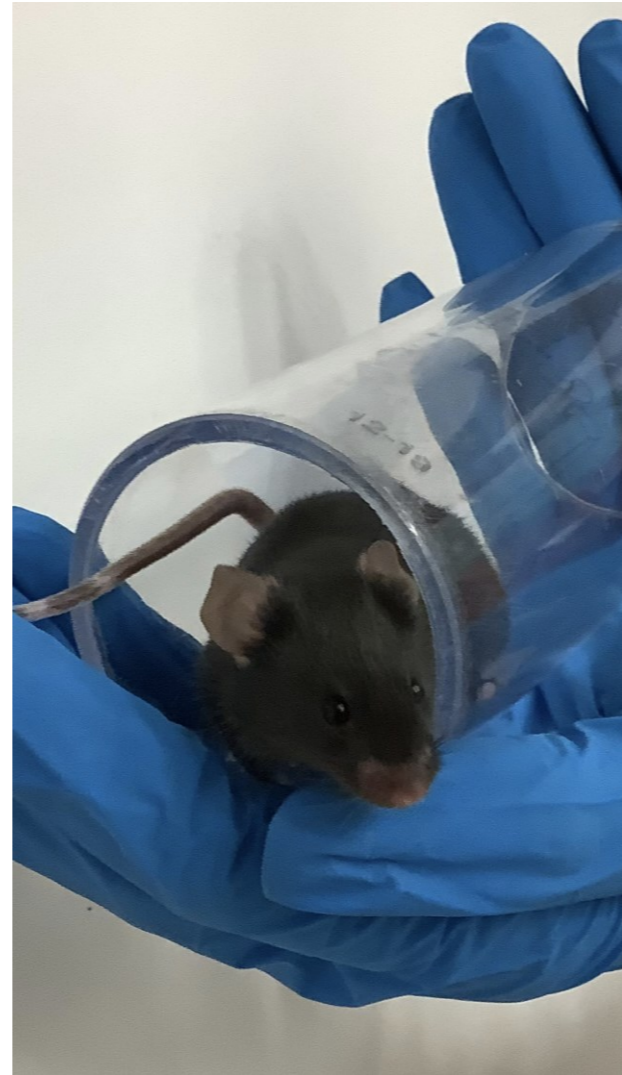


Fig.1 For the so-called tunnel handling, mice are picked up in tubes with a diameter of about 4 cm and a length of about 9 cm.

The aim of this study was to introduce this method into a large mouse breeding facility during routine business. The animals were moved by tunnel-handling or tail fixation at each weekly cage change over a period of 9 weeks, starting at weaning.

Material and Methods

During the introduction of non-aversive handling of mice, the duration of cage transfer and behavior of the inbred strains WNK 1 (Charité substrain of WNK he mice, MGI_C57BL6J_2442092)³, NZW (Charité substrain of NZWLac/J RRID: IMSR_JAX:001058, MGI:2159914)⁴, and Hello Kitty (Charité substrain of C57BL6-Cpa-Cre;Mcl-1fl/fl)⁵, which were tunnel or tail-fixed, were documented.⁶

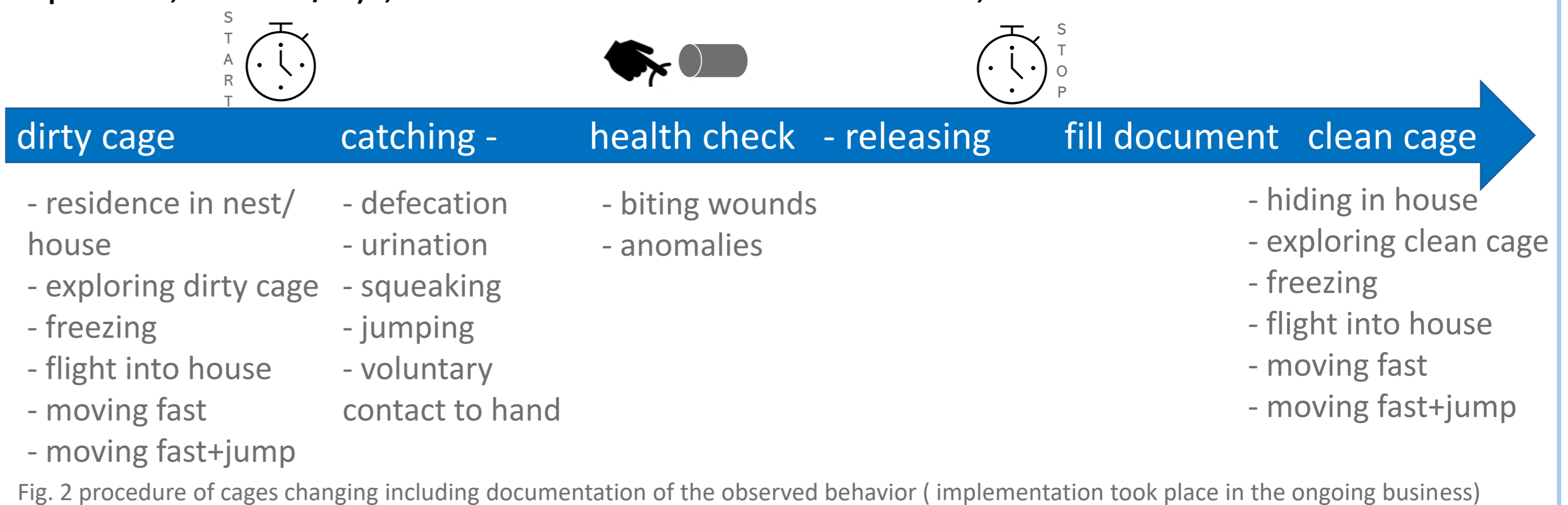


Fig. 2 procedure of cages changing including documentation of the observed behavior (implementation took place in the ongoing business)

Results

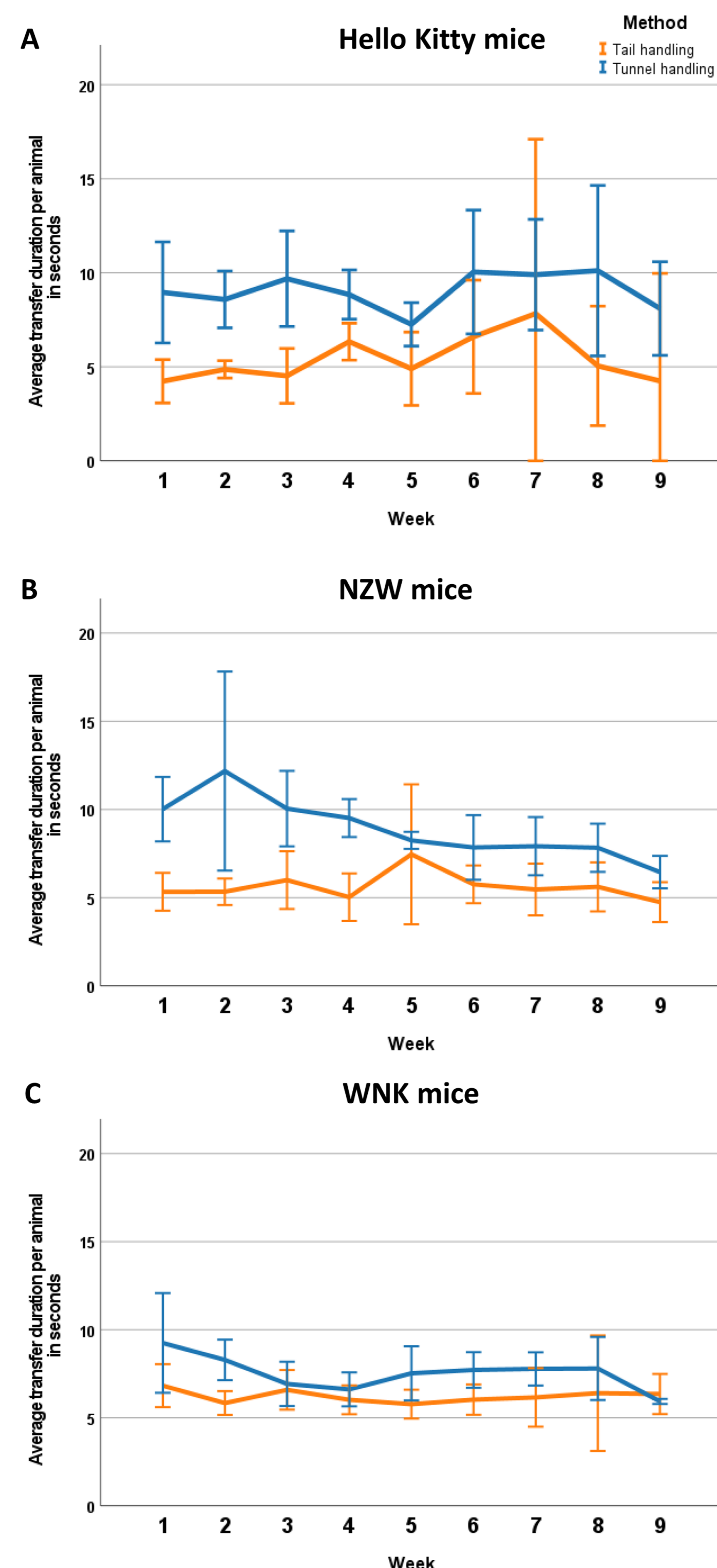


Fig. 2 Transfer duration for (A) Hello Kitty, (B) NZW, and (C) WNK mice. Data are shown as mean and 95 % confidence interval.

Mice were handled once a week during routine cage-changes with the respective handling techniques. Each transfer of one mouse from a used cage to a clean cage by tunnel or tail was analyzed as one cage-change event. A maximum of 150 mice (week 1) to a minimum of 89 mice (week 9) mice were kept in 40 (week 1) to 26 (week 9) cages.

Tunnel-handling takes longer than tail handling in all strains (Fig. 2). The time to move a mouse using a tunnel increased by about 3 seconds compared to fixation at the tail base. The transfer-time of the NZW mice for tunnel-handling is constantly decreasing over time (Fig. 2B).

The behavioral parameters examined showed positive effects using tunnels for the NZW mice. Ambiguous effects were found for Hello Kitty and WNK mice. This could be due to the fact that a weekly interval of using the tunnel by the animal caretakers was not sufficient to habituate the animals to the procedure.

Discussion

NZW: decreasing average duration of transfer shows habituation to handling technique

→ decreased defecation during capture

→ decreased hiding in the house before and after cage-change

Hello Kitty and WNK: no significant decrease of duration of transfer into the fresh cage; WNK show decreased defecation during capture

Conclusion

- The mice investigated in this study could be transferred between cages with the tunnel
- Tunnel-handling takes longer than cage-changes by tail handling and costs for the tunnel and personnel have to be considered
 - evaluation necessary if acceptable for your facility, animals, experiments
- Mouse strains show different acceptance of tunnel-handling
 - for NZW wellbeing-effects are visible
- More sophisticated methods (e.g. stress hormone levels, behavioral experiments) could give deeper insights in wellbeing effects on mice

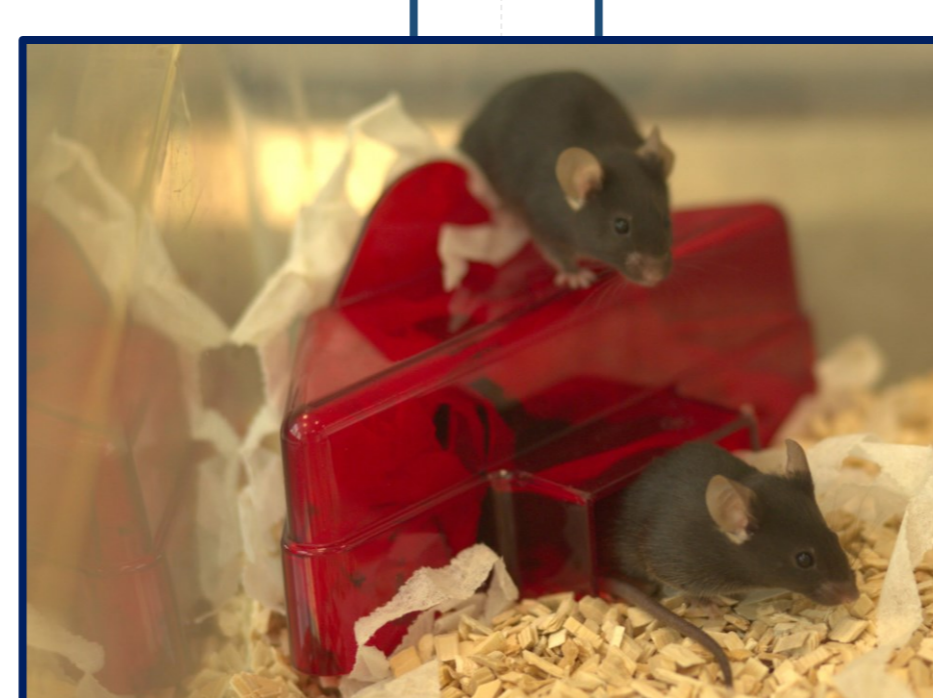


Fig. 5 Mice exploring their house

It was found that method and strain, had significant influence on the probability of defecation during capture and in the clean cage (Fig. 3). NZW an WNK mice transferred by tail handling had a 2.0 times higher risk of defecation during capture than those with tunnel handling. Significantly less Hello Kitty animals defecated during capture than NZW animals.

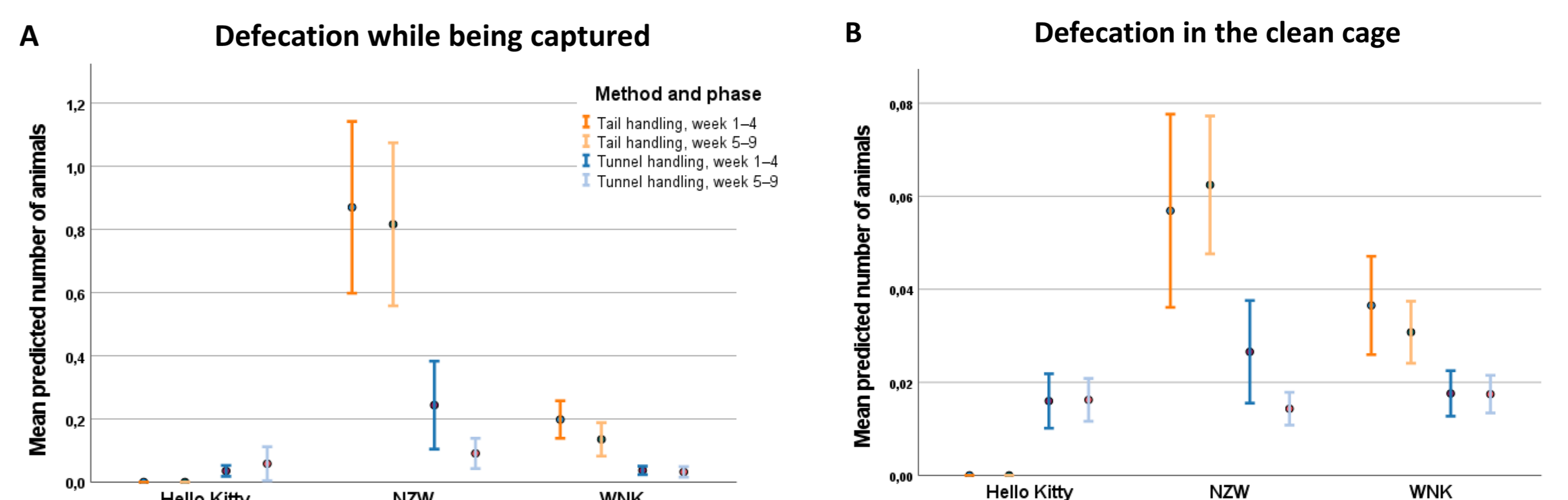


Fig. 3 Defecation observed (A) while being captured in the used cage and (B) after release in the clean cage. Data are shown as mean and 95 % confidence interval.

Mice have been significantly more often in the house in the first 4 weeks of the investigation (Fig 4A, 4B) The number decreased over time. Tail-handled mice were also more likely to sit in the house as tunnel-handled individuals in the used cage (Fig. 4A).

This was also observed after transfer to the clean cage in tail-handled NZW. The occurrence of this behavior decreased over time (Fig. 4B).

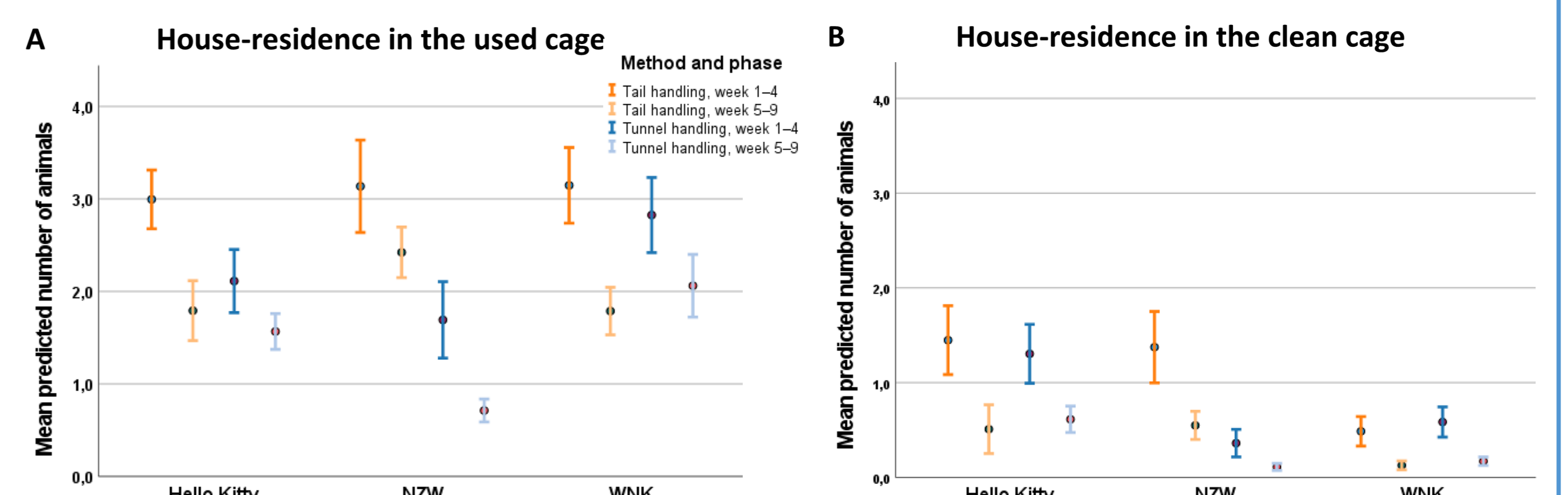


Fig. 4 Number of mice located in the house in the (A) used cage and (B) clean cage. Data are shown as mean and 95 % confidence interval.

References

- Hurst JL, West RS. Taming anxiety in laboratory mice. *Nat Methods* 2010; 7: 825–826.
- Henderson LJ, Dani B, Serrano EMN, et al. Benefits of tunnel handling persist after repeated restraint, injection and anaesthesia. *Sci Rep* 2020; 10: 14562. DOI: 10.1038/s41598-020-71476-y.
- Gouveia K, Hurst JL. Improving the practicality of using non-aversive handling methods to reduce background stress and anxiety in laboratory mice. *Sci Rep* 2019; 9: 20305. DOI: 10.1038/s41598-019-56860-7.
- Susa K, Kita S, Iwamoto T, Yang SS, Lin SH, Ohta A, Sohara E, Rai T, Sasaki S, Alessi DR, Uchida S. Effect of heterozygous deletion of WNK1 on the WNK-OSR1/ SPAK-NCC/NKCC1/NKCC2 signal cascade in the kidney and blood vessels. *Clin Exp Nephrol.* 2012 Aug;16(4):530-8.
- Lilla JN, Chen CG, Mukai K, et al. Reduced mast cell and basophil numbers and function in Cpa3-Cre; Mcl-1fl/fl mice. *Blood* 2011; 118: 6930–6938.
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