The mouse in the house -

an alternative to tail handling

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Musen i huset - ett alternativ till svanshantering.

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Abstract

Refining procedures and practices for mice is an important step to ensure high standards of welfare. Previous studies have focused on refining living conditions while more recent studies are more focused on handling practices. The common practice of handling mice by their tail increases anxiety and induces aversion but non-aversive handling (e.g. tunnel handling or cupping) can mitigate the negative tail handling effects. This study investigated whether using a home cage cardboard house for brief lifts between cages would have the same positive effects on mice as tunnels, compared to body supported tail lifts.

Brief handling every other day resulted in familiarisation with the house handling technique, which resulted in only one instance of defecation for house handled mice compared to the tail handled mice that regularly defecated, urinated and vocalised during handling. House handled mice exhibited greater voluntary interactions with the house than tail handled mice did to the hand. Additionally, the house handled mice showed less anxiety and explored the open field arena more than the tail handled mice. This suggest that using the house as a handling device can be beneficial for improving and refining the welfare of laboratory mice.

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Introduction

Mice *(Mus musculus)* are the most common animal to do biomedical research on globally (Clarkson et al. 2018) and are commonly used for behaviour studies, medical research and pharmacology (Ueno et al. 2020). In the latest report from the Swedish Board of Agriculture, Sweden used 212 565 mice in experiments during 2018 (Ljung et al. 2020), the highest amount out of any mammal species.

Laboratory mice spend most of their lives in their cage and early scientific work found that cage size, environmental enrichment, room temperature and isolation have a big impact on their welfare and overall health (Clarkson et al. 2018; Ueno et al. 2020). Furthermore, more recent research has suggested that the handling technique used by laboratory staff also affects the welfare of the mice and the results of behavioural studies (Hurst & West 2010; Gouveia & Hurst 2013, 2019; Clarkson et al. 2018; Ueno et al. 2020).

1.1 Handling methods

Tail

In many research facilities, lifting mice by the base of the tail is still the traditional way to handle laboratory mice (Prescott & Lidster 2017; Hull et al. 2022). This type of handling is aversive and causes stress, which affects both the behaviour and physiology of the animal (Gouveia & Hurst 2013, 2017; Clarkson et al. 2018). In Henderson et al. (2020a), mice already interacted less with their handlers after 5 days of tail handling. In contrast to tunnel-handled mice, these mice also exhibited a greater level of anxiety after 9 days. Gouveia & Hurst (2019) concluded that picking up mice by the tail for just 2 seconds was as aversive as picking them up by the tail and supporting their body with a hand or arm for 10, 30, or 60 seconds.

Handling mice by the tail decreases exploratory behaviour and willingness to interact with handlers and can therefore impair test performance (Hurst & West 2010; Gouveia & Hurst 2017). Henderson et al. (2020a) also found that tail handled mice's response to rewards like sucrose decreased compared to tunnel handled mice.

Tunnel

Tunnelling is a non-aversive way of handling mice where the mouse voluntarily (or coaxed) moves into a tunnel and is then lifted from its cage. Gouveia & Hurst (2019) found that only brief (about 2 seconds) handling with the tunnel during cage changing was enough to familiarise the mice sufficiently. Tunnel or cupped handling reduces anxiety, increases interactions with handlers and improves performance in the behavioural test compared to tail handled mice (Gouveia & Hurst 2019; Henderson et al. 2020a; Sandgren et al. 2021). Sandgren et al. (2021) used already existing ladders in mouse cages and found that the ladders, much like the tunnel, reduced anxiety and stress-related behaviours usually seen with tail handled mice. The improved welfare from using tunnel handling over tail handling can be seen in litter loss where tunnel handled breeding pairs loose less complete litters than tail handled ones (Hull et al. 2022).

Most importantly researchers have found that the benefits of using tunnelling are not influenced by the laboratory (light or dark cycle, handler or strain of mice) and invasive procedures like tattooing, ear-tagging, single or repeated restraint, subcutaneous injection and lifting the tail for abdominal inspections do not reduce the mice willingness to interact with their handlers (Hurst & West 2010; Gouveia & Hurst 2013, 2017, 2019; Clarkson et al. 2018; Roughan & Sevenoaks 2019; Henderson et al. 2020a).

Cupping

Cupping is also a non-invasive handling method where the handler cups their hands around a mouse and scoops up the mouse which is then free to move in the hands of the handler. One small drawback to this is that mice can jump out of the hands of the handler and needs to be habituated to this form of handling (Hurst & West 2010). This is trained by having the handler close their hands loosely around the mice for up to 30s until the mouse decreases their attempt to escape (Hurst & West 2010). Still, mice handled with cupping and/or tunnelling will improve performance in behavioural tests compared to tail handled mice, but the responses for cup handled mice were more variable than tunnel-handled mice (Gouveia & Hurst 2017).

Handling techniques and the 3Rs initiative

Replace, reduce, and refine are at the heart of the 3Rs, proposed by William Russel and Rex Burch in late 1950s (Russel & Burch 1959), are today used as guidance on how to limit harm to animals in biomedical research. The goal is to avoid or replace animals used in tests, reduce the number of animals and refine the care, pain and suffering (NC3Rs 2023). The 3Rs were incorporated into EU legislation in 2010

(Díez-Solinska et al. 2022), 6 years after the National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs) was formed in the UK in 2004 (*NC3Rs* 2022). Sweden also formed a centre of excellence for 3Rs questions in 2014. However, it was not until 2017 that the centre was inaugurated.

Handling methods is an imperative step in refining the care of mice since non-aversive handling reduce background stress (Gouveia & Hurst 2019), which also reduce variation in test results (Nakamura & Suzuki 2018) and in turn can lead to a reduction in the number of animals used in experiments.

1.1.1 Measures of stress during handling

There are different ways to measure stress when handling animals. This study focused on defecation and urination as well as vocalization while similar studies have only used defecation and urination as stress measures (Henderson et al. 2004; Hurst & West 2010; Nakamura & Suzuki 2018). Although more studies are needed for audible (sonic) vocalisation in mice, Ruat et al. (2022) found that mice use sonic vocalisation as a response to anxiety. They found this by locally infusing muscimol into the midbrain periaqueductal grey (PAG) which is a gating centre for vocalisation in mice (Chen et al. 2021; Ruat et al. 2022) before a tail suspension test. This infusion removed sonic vocalization in mice during the suspension test leading the authors to conclude that there was a link between increased anxiety behaviour and high levels of sonic vocalization and the importance PAG has between the links (Ruat et al. 2022). Therefore, this study added vocalisation to the study when handling mice as an additional measure of stress.

1.2 Tests

1.2.1 Voluntarily interaction (VI)

To explore whether the handling technique influenced mice's willingness to interact with a hand or house, an interaction test was performed. There are typically three sessions evenly spaced out for the experiment length with the first session starting the first day of the experiment. During each session there are two interaction tests, the first one before the mice have been handled and the second test after the mice have been handled. This interaction test enables researchers to see if there is a difference in the amount of time the mice interact with the handling device during each session, typically three, but also immediately before and after each session. This gives an estimate of whether the handling device has a negative, neutral or positive effect on the mouse over time as well as at the moment.

1.2.2 Open Field Test (OFT)

The open field test is a well-documented, easy and readily available test to measure exploratory behaviour, anxiety and general activity in rodents (Gould et al. 2009; Seibenhener & Wooten 2015; Sturman et al. 2018). Total distance moved, type of movements, place of movements and defecation/urination are all analysed in the OFT. Although there have been discussions about the validity of emotionality from the OFT; time spent in the centre, defecation, and activity within the first 5 minutes likely do measure some aspects of emotionality (Gould et al. 2009). Test duration can differ, as well as size, light conditions, and other parameters.

1.3 Aim and hypothesis

This study aimed to assess how two different handling methods on mice (body-supported tail lifting and house lifting) affect mice willingness to interact with a hand or house, as well as exploration and anxiety in a novel environment. Based on previous research in this area the following hypotheses were tested:

- Mice picked up by their home cage house will show an increased willingness to interact with the house compared to mice picked up by their tail will towards the hand.
- Mice handled with their home cage house will be more exploratory and show less anxious behaviours in novel environments than those handled by their tail.

Methods

All procedures were approved by Uppsala regional ethics committee with ethical permit number: 5.8.18-15533/2018.

2.1 Housing

Mice were housed in Greenline IVC cages (17 x 32 x 13 cm). The substrate used in the cages was wood granulate bedding and the mice were provided with a disposable cardboard house (Biodegradable Smart Home, Datesand), and nesting paper shreds (Bed-r'Nest puck, The Andersons) as well as a wooden chewing stick (Aspen Bricks, Datesand) and nesting paper for enrichment (Fig. 1). Water and food (Ssniff, R70, Lantmännen) were given ad-lib. The mice were on a 12-hour light schedule, 06.00-18.00 light and 18.00-06.00 dark. Cage changing was performed on a Monday every two weeks and done by the same person for the duration of this experiment. During cage change old nesting material was placed in the new cage and the old house was stacked on the inside of a new house, making the house more durable.

Figure 1. Picture of the enrichment in the Greenline IVC cages for each group.

2.2 Animals and experimental design

Animals

The mice used in this study were 24 male and 12 female excess CBB (C57BL/6 and BALB/c) mice bred at SLU (Sveriges Lantbruksuniversitet). They were crossed for their friendly and docile behaviour, making them good teaching animals for inexperienced students. These mice had not been handled by the tail but had been handled with tunnel or cupping every two weeks during cage changing. Therefore, both tail and house lifting were novel methods for the mice.

Male and female mice were housed separate in different cages, and males were handled before females since their scent could increase competitive aggression in male groups (Koyama 2004). The cages were then randomly selected into two sub-groups: tail (body-supported tail lifting) and house (house lifting) with equal number of individuals in each group (Table 1). In each handling session, the order of cages was randomised (Sandgren et al. 2021). The mice were between 21 and 11 weeks old at the start of this experiment (Table 1).

Table 1. Table describing the experimental groups per cage; Tail (body-supported tail lift) and House (house lift), the number of individuals in each cage, the age rounded down in weeks at the start of the experiment, the strain of mice used and if they were male or female.

Experiment	**Individuals**	**Age**	**Strain**	**Sex**
Tail	2	21	CBB[1]	M
Tail	3	18	CBB	M
Tail	4	12	CBB	M
Tail	3	11	CBB	M
House	2	18	CBB	M
House	4	17	CBB	M
House	3	14	CBB	M
House	3	13	CBB	M
Tail	2	15	CBB	F
Tail	4	12	CBB	F
House	4	17	CBB	F
House	2	14	CBB	F

[1] CBB refers to the mix-strain between C57BL/6 and BALB/c bred at SLU

Experimental design

The mice were handled twice on Mondays, Wednesdays, and Fridays from 09.00 to 12.00 (Fig. 2). In total there were 11 experiment days: a baseline test on day 0 before a cage change, a voluntary interaction (VI) test on days 1, 5 and 9 and an open field test (OFT) on experiment day 10 (Fig. 2). After the last voluntary interaction test on test day 9, mice were marked with Farber-Castell multimark permanent marker on the base of their tail during the last lift which resulted in a longer handling time.

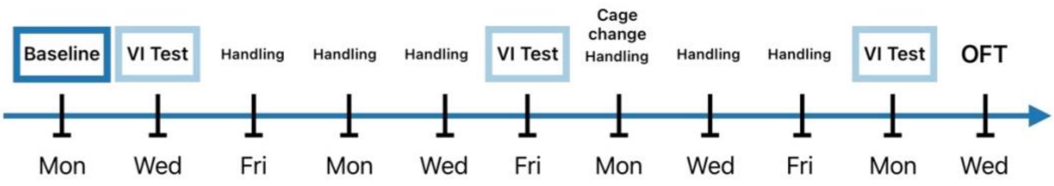

Figure 2. Schematic diagram of the experimental design for this study.
Baseline refers to the first baseline test which also contained a cage change. The VI Test is the voluntary interaction test, Handling refers to days where the mice were only handled and OFT is the Open Field Test. The test started on a Monday and ended on a Wednesday, totalling 3.5 weeks. Illustration made by Julia Wallin.

2.2.1 Handling method and handling sessions

Mice were handled twice during each handling session and a total of 18 handling sessions (including one handling session during the baseline and cage changing session and excluding the cage to arena transfer during the OFT test) was counted for. Those handled by the tail were picked up at the base of the tail with the thumb and forefinger. In contrast to previous studies (Hurst & West 2010; Gouveia & Hurst 2013, 2017, 2019), the body was immediately supported with the middle, little and ring fingers or the palm area below during the lift (Fig. 3). Mice handled with the house had the house turned upside down, creating an L-like shape, and were motivated to climb on and into the house with another free hand (Fig. 4).

Figure 3. Picture showing how the tail-handled mice were picked and their back body immediately supported before being completely lifted off the ground.

Figure 4. Picture showing how the cardboard house has been turned upside down to an L-shape and how the mice can move onto it.

Before the mice were lifted, all environmental enrichment was removed from their cage. The mice were then transferred from their home cage using the assigned handling method to an empty cage and transferred back to their home cage with the same handling method. These transfers took approximately 2 seconds per mouse and only one mouse was lifted at a time. The use of a corresponding empty cage was to prevent picking up the same mice more than once since the mice were not individually marked before the experiment. This was done for each experiment day except during cage changing on day 6 when the previous empty cage became the new home cage. This resulted in only one lift session that day.

Any occurrence of urination, defecation, and vocalisation during, and up to two seconds after transferring the mice to their new cage was recorded and was counted per individual since there were an equal number of individuals in the two handling groups. Gloves (Powder-free, soft white nitrile, Magic Touch, GranberG, Norway) were disinfected with 70% ethanol between each cage.

2.2.2 Voluntary interactions

An interaction test was performed on day 0 to measure the baseline interest to interact with the hand in both groups before any handling methods were applied (Fig. 5). Thus, willingness to interact with the hand or house was therefore assessed on experiment days 1, 5 and 9, immediately before and after each handling session (Fig. 5). The test was conducted after Hurst & West (2010) with some modifications.

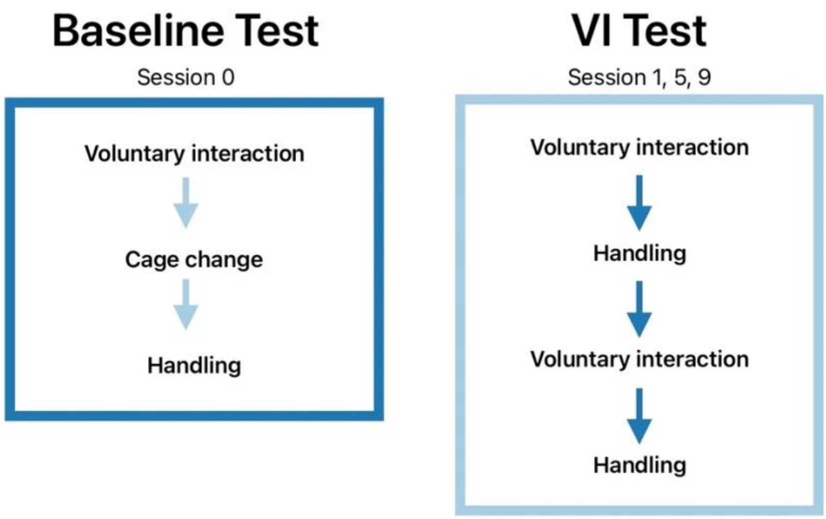

Figure 5. Schematic diagram of the Baseline test and Voluntary Interaction Test days.
The baseline test was conducted on experiment day 1, and due to cage change, only one voluntary interaction test was performed before any handling was conducted that day. The mice were only handled once, from their old cage to a new.
VI Test days contained a voluntary interaction test before any handing. After the mice were handled once, a second voluntary interaction test was performed. The mice were then handled again, and the test day was complete. Illustration made by Julia Wallin.

Gloves were disinfected between each cage; males were tested first, and the order of the tests was randomised for each experiment day. The handler removed the cage lid and all environmental enrichment in the cage and stood still in front of the cage for 60 seconds. One gloved hand (Fig. 6) or one gloved hand with the house (Fig. 7) was then placed on the substrate in the front half of the cage for 60 seconds while recording from the top with a mobile phone (iPhone 8 mini). The mice were then transferred, using the handling method, to the other empty cage and the interaction test was repeated. The first interaction test was therefore done in their home cage and the second test in the other empty cage so that the same mouse was not picked up multiple times since they were not individually marked. The baseline test was done due to a cage change which meant that the mice could only be handled once that day. Since the mice were also unfamiliar with tail handling, this test could indicate if only one instance of tail handling (in the tail group) could influence their willingness to interact with the hand two days later.

Due to the house and hand placement (Fig. 7) in the house group there was an area underneath the hand that the mice had access to, either through the entry hole in the house (Fig. 4) or by the side of the house, where they could not be seen by the video recordings. No interaction was registered for mice in that area.

Figure 6. Placement of the gloved hand inside the cage during the interaction test.

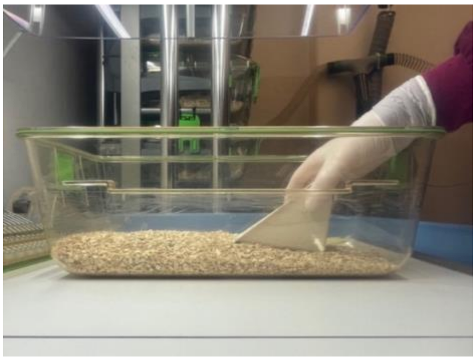

Figure 7. Placement of the recycled cardboard house and gloved hand during the interaction test.

The video material was transcribed using BORIS event logging software (Friard & Gamba 2016). Close interaction with the house or gloved hand was summarised as "Interaction" and consisted of time spent sniffing with nose less than 0.5cm from the glove or glove/house and physical contact (one or more paws on or inside the glove or glove/house as well as chewing on the glove/house) (Hurst & West 2010; Gouveia & Hurst 2019). To not overestimate the amount of time spent interacting with the house or hand, mice that engaged in both sniffing and physical contact at the same time were only registered as Interaction once. The Interaction would start when the first behaviour started and finish when the last interaction behaviour stopped. The duration of Interaction for each individual was then summarised and averaged across the number of mice in each cage, making the cage the experimental unit.

2.2.3 Open Field Test

To test anxiety and exploratory behaviour, an Open Field Test (OFT) was conducted on experiment day 10. The arena was made of grey plastic with the measurement W58.5 x L58.8 x H45cm and had previously been used to test rats, but since activity patterns are relatively stable between different arena sizes (Gould et al. 2009) it was used without any modifications. The test was performed under white light in a quiet room and a mobile phone (iPhone 8 mini) recorded from above. The video material was transcribed in BORIS.

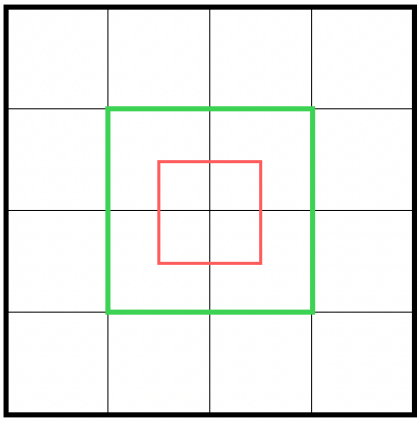

Figure 8. Arena of the Open Field. The arena was divided into 16 smaller equal size areas. The red square represents the inner area, the green represents the middle area and the black represent the outer area. Not to scale. Illustration made by Julia Wallin.

Only one mouse per cage was tested in the OFT. Both the cage order and the mice within each cage were randomly selected, however, males were tested before females. The mice were handled using their assigned handling method and placed in the middle of the arena and the test lasted for 5 minutes. Once the mouse finished the 5-minute test they were placed in an empty cage with the assigned handling method until everything was checked to make sure the video recorded and was saved. They were then placed back into their home cage.

The OFT arena was divided into 16 equal-sized areas where time spent and number of entries into the inner area, middle area and outer area was summarised (Fig. 8). Crossing lines (all four feet moved from one area to another) and rearing (only hind feet on the ground) were counted. Faecal boli and urination were counted after each mouse before the box was cleaned with 70% ethanol between each mouse.

Due to limited time and area, this study conducted the OFT in an adjacent area to where the mice were kept so pre-transportation for acclimatisation was not necessary.

2.2.4 Cage changing

To check if one handling technique was faster than the other, cage changing was timed on experiment day 6 using the assigned handling method. Several mice were allowed onto the house during the timed cage changing. The whole duration of each cage change was recorded. However, since each cage had different requirements (some needed new houses while others needed a new biting stick) only the time from when the hand had reached the top of the open cage before lifting the first mouse until the last mouse had all four feet on the substrate in the new cage was measured. During this day, the mice were only handled once from their old home cage to the new clean cage.

2.3 Statistics

All data were tested for normality and homogeneity of variance and where non-significant Shapiro-Wilks test results were found (normality) a subsequent F-test was done. Descriptive statistics in the text are represented as mean ± SD unless otherwise stated.

Voluntary interactions were summed and averaged within each cage due to the different number of individuals in each cage and calculated as a proportion of the 60 second test length. This resulted in the use of cage units instead of individuals. A mixed model analysis of variance (ANOVA) using handling methods × experiment day × pre/post handling was tested in the voluntary interaction test. Handling method was treated as a between-subject factor while handling session (experiment day) and pre/post handling were treated as a within-subject factor. Data sets were grouped and tested for normality and only one: tail x session 9 x pre handling had a non-normal distribution according to the Shapiro-Wilks test ($W = 0.755$, $p = 0.022$). Since ANOVA can handle some disturbance to normality, the decision was made to continue with the planned mixed model ANOVA test. Two extreme outliers were found: house x session 9 x post handling x cage 273 and tail x session 9 x pre handling x cage 272. However, due to the small sample size, these were kept in the dataset. Mauchly's Test for Sphericity found no significant differences in variance. Subsequent pairwise Bonferroni post hoc tests with adjusted p-value were used to investigate differences between significant groups from the mixed model ANOVA results. Pairwise test with between factors from the mixed anova was treated as independent t-tests while within factors used paired t-tests, both with Bonferroni adjustments.

Factors tested for in the open field test were the total time spent in the different areas; centre area, middle area, and outer area, as well as the number of entries into those areas between the two handling types. These were tested between groups using a two-sample t-test due to the normality scores. Latency to exit the inner area and rearing showed non-normality scores and a Mann-Whitney test was used. Descriptive statistics for this was median and interquartile range (IQR) between first and third quartiles. Line crossings and faecal boli count was normally distributed and a two-sample t-test was used. Urination rarely occurred; therefore, no comparison test was made. One male mouse from the tail group was removed due to excessive manipulation of bedding material that fell into the arena.

As for the timed cage changing, the average time taken for each cage was used and then tested with a two-sample t-test between the two handling groups.

All analyses were conducted in RStudio (RStudio Team 2022) and the rststix package (Kassambara 2022) was mainly used. Alpha level was set to 0.05 for all statistical tests.

Results

The mice used in this study were divided into two groups based on how they were handled: tail or house, and the study compared the two handling groups with each other. This study used mice of different ages and group sizes in the voluntary interaction test and open field test. Due to the different cage group sizes and the uneven number of sexes, this study did not investigate the effect of sex or group sizes in the various tests.

3.1 Handling

Tail handled mice were more likely to defecate (Mann-Whitney: $U = 3.5$, $p = 0.016$) and vocalise (Mann-Whitney: $U = 3$, $p = 0.009$) during the very brief 2 second handling compared to house handled mice. Mice lifted using the house only defecated once during this study while handled (excluding the open field test day – experiment day 10) compared to the 27 times tail handled mice defecated. Similarly, tail handled mice vocalised in total 18 times during this experiment, compared to house handled mice that never did. Due to no urinations in the house handled group and only three occasions in the tail handled group, no comparison was made between the two handling groups.

3.2 Voluntary interaction

The baseline test (session 0 before any handling) was the first test that the mice experienced in this study and it compared the mice’s voluntary interaction with the gloved hand for both handling groups (tail and house). There was no difference in the amount of time spent interacting with the gloved hand between both handling groups (t-test: $t_{10} = 0.099$, $p = 0.923$). The tail and house handled groups spent about an equal amount of time interacting with the gloved hand (tail: 6.8% ± 5.51 & house: 7.1% ± 4.09). There was also no difference between session 0 and session 1 before the mice were handled (Mann-Whitney: $U = 28$, $p = 0.128$) although there were some differences in the median time spent interacting with the gloved hand (session 0: median = 4.1%, IQR 3-11 & session 1 pre handling: median = 2.6%, IQR 1-4), indicating a small decrease in the time spent interacting with the hand after being handled by the tail only two days before the voluntary interaction test at session 1. Since the baseline test was performed with the hand only, this study did not investigate any differences between session 0 and session 1 pre handling for the house handled group.

Voluntary interactions differed according to handling device ($F_{1, 10} = 853.873$, $p < 0.001$), session ($F_{2, 20} = 7.327$, $p = 0.004$) and pre/post handling ($F_{2, 20} = 56.603$, $p < 0.001$) according to the mixed ANOVA. The results also found that handling method x handling session x pre and post handling had no effect on time spent interacting with the handling device ($F_{2, 20} = 0.008$, $p = 0.992$).

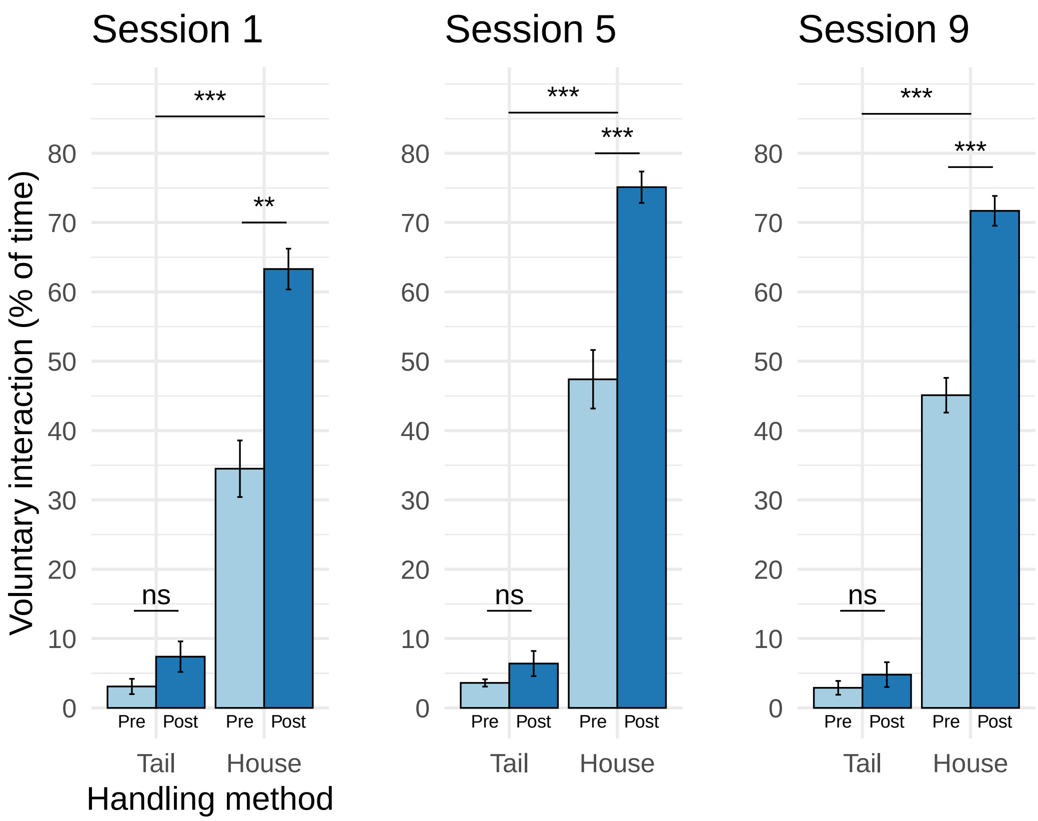

Figure 9. Effects of handling method on voluntary interaction duration as a percentage of 60 seconds.

*Brief handling by cage transfers, by the handling method tail or house, was assessed during session 1, 5 and 9 before and after the handling during each session. N = 6 cages per handling method (n= 4 male and n = 2 female cages). Data are represented as mean ± SEM. Results from the post hoc Bonferroni pairwise test with corresponding asterisks indicating significance level (ns – not significant, * p < 0.05, ** p < 0.01, *** p < 0.001).*

There was, however, a difference in time spent interacting with the gloved hand or house & gloved hand between the two handling methods and sessions ($F_{2, 20} = 9.051\ 7$, $p = 0.002$). To investigate it further, a post hoc Bonferroni comparison test revealed that house handled mice interacted more with the house than the tail handled mice did with the hand across all handling sessions (Bonferroni: session 1: $p < 0.001$, session 5: $p < 0.001$ & session 9: $p < 0.001$; Fig. 9). House handled mice interacted on average 48.9% (± 17.18) with the house in session 1 while tail handled mice only interacted with the hand for 5.3 % (± 4.65) of the test. When comparing the two handling methods in session 5, the average time spent interacting with the house rose to 61.3% (± 16.51) for the house handling group while the tail handled groups interaction time decreased to 5.0% (± 3.42). During session 9 the house handled group interaction decreased to 58.4% (± 14.95), similar to the tail handled group that decreased their average interaction to 3.9% (± 3.53).

Comparing each session within the two different handling groups revealed differences in voluntary interaction in the house handled group between sessions 1 & 5 (Bonferroni: $p = 0.002$) and sessions 1 & 9 (Bonferroni: $p = 0.004$). There was no difference in the house handled group's voluntary interaction between sessions 5 and 9 (Bonferroni: p = 0. 543) and no difference was found within the tail handled groups voluntary interactions between sessions (Bonferroni: sessions 1 & 5: $p = 1$, sessions 1 & 9: $p = 1$ and session 5 & 9: $p = 0.642$).

Handling method and pre and post handling also affected time spent interacting with the device ($F_{2, 10} = 36.710$, $p < 0.001$) and post hoc revealed differences in pre and post handling on device interaction for both handling groups (Bonferroni: house x pre and post handling: $p < 0.001$ & tail x pre and post handling: $p = 0.009$). Mice picked up by the house were more willing to interact with the house immediately after their handling experience during each session (Bonferroni: session 1 x pre & post handling: $p = 0.004$, session 5 x pre & post handling: $p < 0.001$ & session 9 x pre & post handling: $p < 0.001$; Fig. 9). There were no changes in voluntary interactions after handling session during sessions 1, 5 and 9 for the tail handled group (Fig. 9). There were only minimal changes found in the tail handled group, but the time interacting with the hand increased only slightly immediately after handling session. No difference between handing session x pre & post handling ($F_{2, 20} = 0.617$, $p = 0.549$) was found.

There were qualitative differences in the interactions too. Tail handled mice rarely made paw contact and would also sniff from a further distance than qualifying as a physical sniff in this study. House handled mice would readily climb, make paw contact and stay on the house. They also utilised the hole in the house that was directly underneath the gloved hand to go in and out of the house. However, due to the gloved hand's placement while holding the house during this test, there is a possibility of underestimation of time spent in physical contact with the house due to the mice not being visible.

3.3 Open Field Test

There was no difference in the latency to leave the inner area between the two handling groups (Mann-Whitney: $U = 8$, $p = 0.234$), although the tail handled mice exhibited a longer latency to leave the inner area (median = 4.2 seconds, IQR 3-11) compared to the house handled mice (median 2.7 seconds, IQR 1-5). However, there were variations within each group.

The results from the open field test showed differences between the two handling groups in the time spent in the inner area, middle area and outer area (Fig. 10) as well as the number of entries into each of them (Fig. 11).

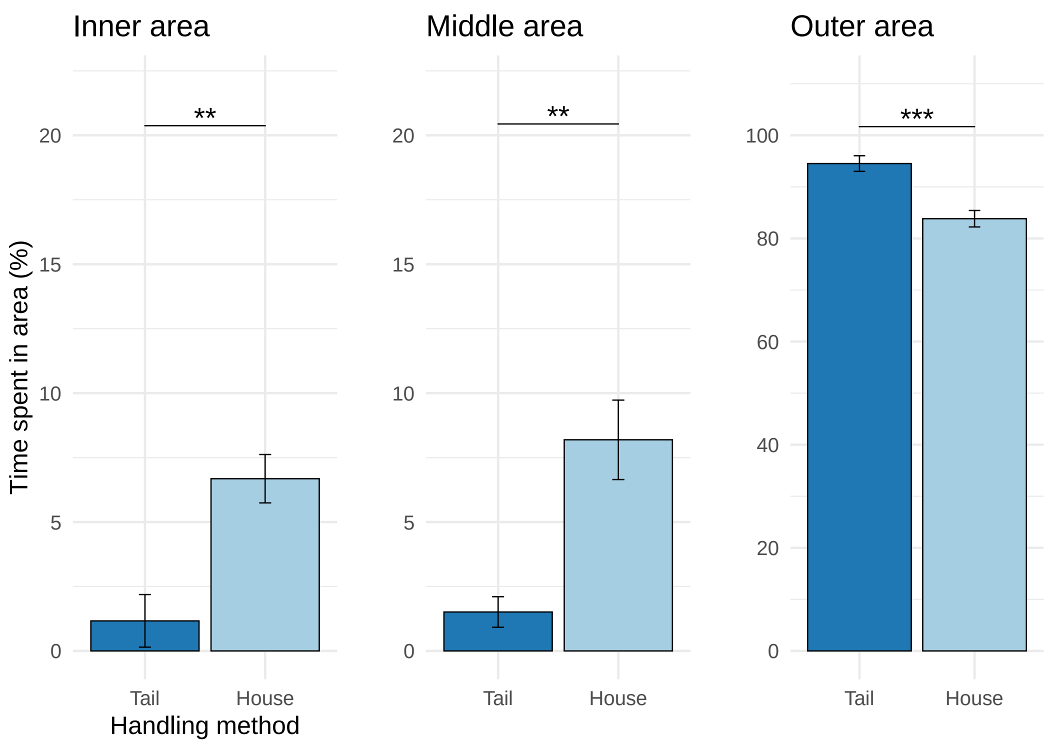

Figure 10. Effect of handling method (tail and house) on anxiety behaviour in the three different areas in the open field arena.

Results show the percentage of time spent from the 5-minute test in the inner area, middle area and outer area, as well as the number of entries into the inner area, middle area and outer area during the 5-minute test. N = 11 individual mice (n = 6 house handled and n = 5 tail handled mice) were used in this test. Data are represented as mean ± S.E.M. Results from the t-test resulted in corresponding asterisks indicating significance between tail versus hand (p < 0.05, ** p < 0.01, *** p < 0.001).*

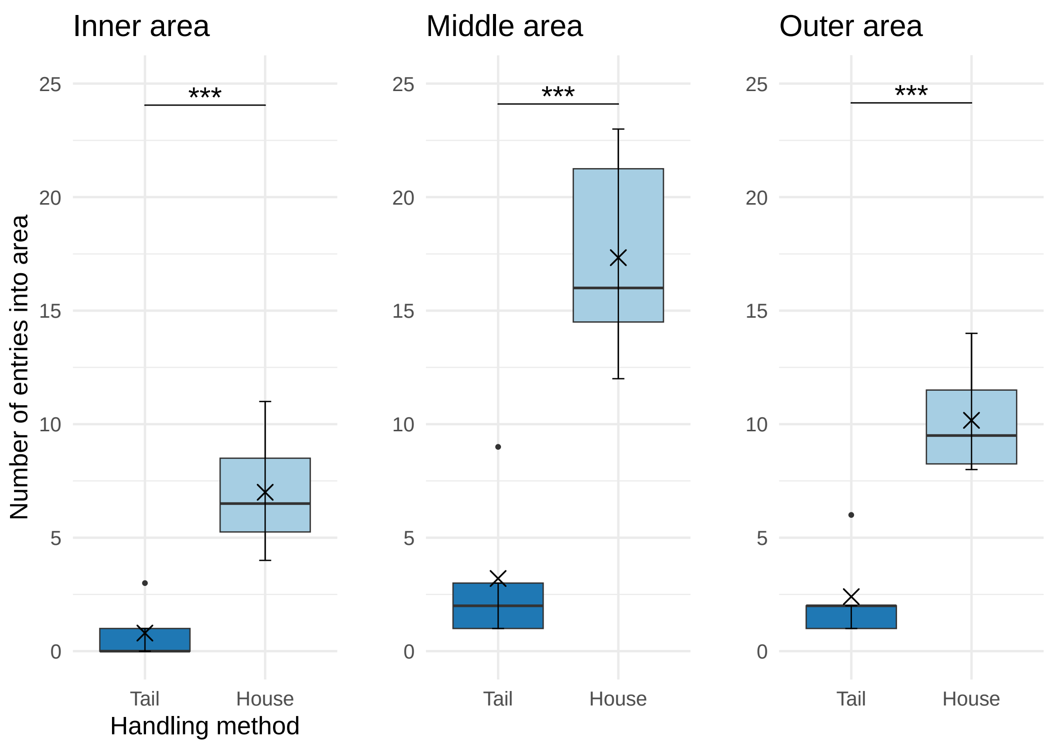

Figure 11. Effect of handling method (tail and house) on locomotor behaviour in the three different areas in the open field arena.

Results show the number of times the mice entered the inner area, middle area and outer area during the 5-minute test. N = 11 individual mice (n = 6 house handled mice and n = 5 tail handled mice) were used in this test. X represents the mean. Results from the t-test resulted in corresponding asterisks indicating significance between tail versus house handling ($p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).*

The house handled mice were more active, entering the inner area more times than the tail handled (t-test: $t_9 = 4.81$, $p < 0.001$; Fig.11), yet the house handled group also spent more time in the inner area compared to the tail handled mice group (t-test: $t_9 = 3.91$, $p = 0.005$; Fig.10). This pattern was also found for the middle area where the house handled mice entered the middle more than the tail group (t-test: $t_9 = 5.68$, $p < 0.001$; Fig.11) yet also spent more time in the middle area compared to the tail handled mice (t-test: $t_9 = 3.74$, $p = 0.005$; Fig.10).

There was a difference between house and tail handled mice when comparing entries and time spent in the outer area. House handled mice were more active and entered into the outer area more than tail handled mice (t-test: $t_9 = 5.76$, $p < 0.001$; Fig.11), however, house handled mice spent less time in the outer area compared to the tail handled mice (t-test: $t_9 = -4.79$, $p < 0.001$; Fig.10).

House handled mice were more active in exploring the OF arena than tail handled mice. This could be seen by the overall number of lines crossed (t-test: $t_9 = 4.52$, $p = 0.001$) where the house handled mice crossed on average 117.2 lines (± 25.75) compared to tail handled mice which only crossed 49.2 (± 23.63).

Rearing did not differ between tail and house handled mice (Mann-Whitney: $U = 23$, $p = 0.169$), even though tailed handled mice reared at a median of 11 times (IQR 9-17), less than house handled mice at 19.5 (IQR 14-23). There was also no difference in faecal boli count between the mice in the two handling groups (t-test: $t_9 = -1.66$, $p = 0.1308$), still, the tail handled mice boli count was 27 in total compared to the 15 in the house group even though one male mouse from the tail group was excluded. Urination only occurred once in each handling group and therefore no statistical analysis was made.

3.4 Cage changing time

Timed cage changing sessions found that handling mice by the house took longer (t-test: $t_{10} = 4.77$, $p < 0.001$) than handling the tail. The average time taken to lift and place the mice in the house handled group lasted almost twice as long as the tail handled group (10.4s ± 2.33 vs 5.6s ± 1.24). Despite this, no difference was observed between females in the house and tail groups (t-test: $t_2 = 0.99$, $p = 0.428$), but was observed in males (t-test: $t_6 = 7.6285$, $p < 0.001$), however, this could be due to the small sample size in the female handling group. Interestingly, in the house group, handling female mice were on average quicker than handling males (house females: 9.3s ± 4.15 & house males: 11.1s ± 1.15) compared to the tail group where handling males was quicker than females (tail males: 5.3s ± 0.48 & tail females: 6.3s ± 0.42).

Discussion

Previous research has found that mice handled with a tunnel, cup or cage ladder are more willing to interact with their handlers, show a reduction in anxiety and an increase in exploration (Hurst & West 2010; Clarkson et al. 2018; Nakamura & Suzuki 2018; Gouveia & Hurst 2019; Henderson et al. 2020a; Sandgren et al. 2021). This study investigated a new way of handling mice by using a home cage house and compared it to handling mice by the tail with the body supported. As predicted, the house handled group interacted more with the house compared to the tail group and the hand during the interaction tests. The house handled mice were also more exploratory and showed less anxious behaviours in the open field arena.

This study started by measuring a baseline (session 0) response to a gloved hand for each cage, indicating that there was no difference in voluntary interaction between the two different handling groups from the start or between session 0 and session 1 pre-handling for the tail group. Mice lifted with a home cage house voluntarily approached and interacted with the house before and immediately after handling, indicating a lack of aversion towards the house. Comparatively, those picked up by the tail showed similarly low voluntary contact with the gloved hand both between sessions and before and immediately after being handled. Mice lifted by the house also showed reduced anxiety in an unfamiliar open field arena compared to tail handled mice and showed more behaviours indicative of exploration. Transferring mice by the house during cage changing took nearly twice as long compared to transferring the mice by the tail.

4.1 Handling method

Nakamura & Suzuki (2018) and Hurst & West (2010) found that tunnel handling decreased urination and defecation and this study also found that mice lifted by the house were less likely to defecate and vocalise during the handling sessions than those lifted by the tail. This indicates a lower level of anxiety in house handled mice compared to the tail handled mice. However, using defecation alone as an indicator of anxiety in tests has not been entirely consistent and has also been found to be strain and sex-specific (O’Leary et al. 2013). Defecation alone should therefore be used with caution when measuring anxiety. Still, in combination with the amount of sonic vocalisation (Ruat et al. 2022), defecation, urination and escape attempts that occurred during the brief handling of the tail handled mice suggest that they did not find handling by the tail a positive experience. It is noteworthy however, that mice can vocalise on an ultrasonic level in which humans cannot hear (Ruat et al. 2022) and that potential valuable data from ultrasonic vocalisation during and after the lifts were not tested for in this study.

Similarly to the tunnel handled mice (based on Hurst & West (2010) methodology), the house handled mice in this study were guided to climb onto the house by blocking an alternative route, forcing the mice to make contact with the house. However, guiding the mice onto the house was rarely needed and would only occur in their home cage after the nesting material was removed (before being placed in the other already empty cage). A slow initial contact with the house during the first handling could be because the mice were asleep when the experiments started in the morning. However, once they were placed in the other empty cage, it was more difficult to only lift one mouse at a time instead of 2-4 mice all at once due to the immense interest they had in climbing the house. At that time, the house had to be picked up and placed back down in the cage until only one mouse was on the house.

4.2 Voluntary interaction

Results from the voluntary interaction test found that mice handled briefly (2s) with a house for three days in three weeks interacted more with the house than mice handled by the tail interacted with a hand. Gouveia & Hurst (2017) found that brief (2s) tunnel handling of mice during bi-monthly routine cage cleaning was enough to achieve similar results to this study, indicating that short handling during cage changes would be enough to familiarise mice with tunnel handling and the benefits it has over tail handling. Using the house mice are already familiar with in the cage as a handling method would therefore not add any additional workload for animal technicians wanting to implement tunnel or house handling since they would not have to interact more with the mice than what the facility protocol requires.

From the baseline test, the results indicated that there were no differences between the two handling groups in their willingness to interact with the hand. Since these mice were handled with different non-aversive handling techniques the expected interaction time would therefore decrease after the aversive handling session. This study tested this by comparing the baseline test to the first pre-handling session in the tail group. The tail handling group was handled only once after the baseline test and the first pre-handling session during session 1 occurred two days after. Comparing the results, no difference was found between the tail handled group's willingness to interact with the hand after just one handling session.

However, there were differences in the median time spent interacting with the hand. This could indicate that just one handling session with aversive techniques could elicit a negative response to the hand.

In each interaction test, mice handled by the house increased their time spent with the device directly after the first test. Mice handled by the tail did not exhibit the same behaviour. This indicates that they did not find being lifted by the tail a positive experience and did not want to interact more with the hand.

Time spent interacting with the device increased in the house handled mice between sessions 1 & 5 and sessions 1 & 9, while for tail handled mice it did not. This was a similar pattern seen by Clarkson et al. (2018) and indicates that there was positive habituation to the house as a handling device. During each interaction test, mice handled by the house also increased their time spent with the device directly after the first test. Mice handled by the tail did not exhibit the same behaviour. This indicates that they did not find being lifted by the tail a positive experience and did not want to interact more with the hand.

Gouveia & Hurst (2013) investigated whether a familiar or unfamiliar tunnel would affect the mice willingness to interact with the tunnel. They initially found that mice spent a longer time interacting with a familiar home cage tunnel than with an unfamiliar shared tunnel that was not present in their home cage. However, the willingness to interact with an unfamiliar tunnel increased substantially over the handling sessions, indicating that the experience of being handled with the tunnel no matter if it was familiar or unfamiliar in the cage was a positive experience (Gouveia & Hurst 2013). This is also something that should be tested for when using the house since it is still unclear if the reduction in stress and anxiety responses to house handling depends on their familiarisation with their home cage house.

The interaction test during session 9 started on a Monday. This meant that the mice were not handled for two days before the test. In contrast, session 5 which was on a Friday, occurred with only one day of no handling before the test. It could therefore be argued that the decrease in time spent interacting with the house during session 9 was due to how the test was scheduled. The time spent interacting with the house might have been the same as session 5 or increased if the test was done only one day after not being handled. However, a similar pattern emerges in other studies (Gouveia & Hurst 2013; Clarkson et al. 2018), while others have not found similar results (Nakamura & Suzuki 2018; Sensini et al. 2020; Sandgren et al. 2021) and some only did the two first interaction tests (Gouveia & Hurst 2019; Henderson et al. 2020a). There appears to be no definitive answer. An investigation into the relationship between time spent interacting with the device between the time from the last handling session and the interaction test should be further explored in the future.

4.3 Exploration and anxiety

The open field test is the most widely used test for anxiety and exploration (Sturman et al. 2018) and it is tested by observing where the animal is and what the animal is doing in an open arena. This study found that mice handle with the house were more active in exploring the open field arena compared to tail handled mice. This was reflected in the number of line crossings where house handled mice were more active and crossed more lines compared to tail handled mice. Similar results were found in other studies that also investigated line crossings but between tunnel and tail handled mice (Nakamura & Suzuki 2018; Gouveia & Hurst 2019). Exploratory activity is typically measured with automatic programs and can track the total distance travelled (Lipkind et al. 2004). Clarkson et al. (2018) used an automatic program which could analyse the time spent travelling, the velocity of travel and the pattern of travel in the arena between the tunnel and tail handled mice. They found no difference in the time spent moving, however, they did find that tunnel handled mice travelled significantly further and at a higher velocity than tail handled mice. Since this study did not investigate the activity pattern inside the arena, due to the test being measured manually, it is impossible to say whether house or tail handled mice were equally active, if one group travelled further or faster than the other. What this test does indicate for this study is that house handled mice moved around more between the lines and areas than the tail handled mice.

Animals that spend the most time near or in the centre of the arena are considered less fearful and anxious, compared to those that stay close to the walls (Sturman et al. 2018; Himanshu et al. 2020) and this has been tested by administering mice with anti-anxiety medication where the mice respond by increasing the time spent in the centre area (Lipkind et al. 2004). This study found that the house handled mice entered the three different areas (inner, middle and outer) more often than the tail handled mice. House handled mice also spent more time in the inner and middle areas compared to the tail handled mice which spent more time in the outer area indicating a greater level of anxiety by staying closer to the walls. These results are in line with previous studies on non-aversive vs tail handled mice (Nakamura & Suzuki 2018; Gouveia & Hurst 2019; Sandgren et al. 2021).

Rearing, faecal boli and urination count did not differ between the two handling groups. However, rearing in this study could have been divided into two categories: wall-leaning and unsupported rearing. It is argued that wall-leaning rearing is indicative of locomotion/activity while unsupported rearing measures emotionality and that separating the two can improve the assessment of emotional state in mice (Sturman et al. 2018).

It is important to note that this study only used one mouse per cage and only 11 mice to test the open field test while other studies had mice in pairs and tested both (Hurst & West 2010; Sandgren et al. 2021). While using two mice per cage would have given an average score in each cage for this test, this study had some cages with three to four individuals and only selecting two mice to represent the cage unit might not be representative either way. Therefore, randomly selecting one mouse for each cage to represent the cage unit was the most suitable approach for this test for this study.

It was difficult to conclude how the open field test should be designed and for how long the test should run. There is little to no standard for how many squares per arena there should be or how many areas the study should include since many studies use different test lengths and areas in their open field tests. The design of the arena might also be influenced by how data is recorded, whether done so manually or with an automatic program. Nakamura & Suzuki (2018) used a 40x40cm base, divided into 25 squares with 9 squares in the middle comprising the inner area and tested the mice for 10 minutes. Sandgren et al. (2021) also used a 40x40cm base, but they used a 7.5x7.5cm area as the inner area and only examined the mice for 5 minutes. Gouveia & Hurst (2019) used three areas with a 70x60cm base; a 10x10cm inner area, a 54x44cm middle area, and an outer area which only entailed a 3cm distance from the wall while testing mice for 5 minutes. This study, therefore, opted to do a 5-minute test based on it being a novel assessment (Himanshu et al. 2020) and a centre area consisting of about 33% of the test area since in general, the centre area covers about 25-50% (Gould et al. 2009).

4.4 Cage changing

The most common concern that researchers and animal care workers have regarding implementing non-aversive handling is that it will increase the time spent handling the animals (Henderson et al. 2020b). This study found that moving house handled mice from one cage to another during cage changing, took roughly twice as long as tail handled mice after 5 handling sessions and that there were sex differences between the two groups. Doerning et al. (2019) compared the time taken to change cages depending on handling technique and found that across all four sessions, tail handling with a gloved hand was on average faster than forceps, plastic cup, home tunnel and foreign tunnel. The speed at which cages were changed also did not differ between the first and last sessions (Doerning et al. 2019). Therefore, one cage changing session during this study might roughly reflect the average time it takes in general. However, to my knowledge this is the only study that included timed cage changing sessions. This is an area that should be studied more and would not require much more additional work.

Since the time spent handling the animals is such a common concern among animal care workers and researchers, timing or recording the changing of the cages would provide more insight into the time spent handling the different groups and better comparisons could be made. Due to the different numbers of mice in this study, as well as the small sample size, different results might be found in the future.

Sensini et al. (2020) found female C57BL/6NCrl mice to be less sensitive to tail handling since they did not find despair-like behaviours as they did in male mice. Comparing the average time taken to handle the female and male mice in this study, the females were quicker to handle using the house than males, yet it was the females that took longer to handle when handled by the tail. Although this study did not investigate sex differences when comparing time spent interacting with the hand/house or sex differences regarding movements in the OFT arena, the female mice were quicker to climb onto the house and would collectively move on the house compared to the male mice. As for the tail group, female mice made more escape attempts from the hand approaching, making it more difficult to catch them than male mice. To better understand how sex can affect the results of this study, future research should observe the mice behaviour in response to anticipated tail handling to find any sex or strain differences.

4.5 Limitations

There were a few limitations to this study. For example, this study had a small sample size and also used an unequal amount of male and female mice and different group sizes of 2 to 4 individuals (12 cages total, 8 male cages and 4 female cages). This is in comparison to other studies like Hurst & West (2010) which used 128 mice (8 cages, 2 mice per cage, method and strain combination with equal division between sexes) or Nakamura & Suzuki (2018) which used 80 mice in total (40 mice per handling method, 4 mice per cage with equal division between sexes). This study might have produced different results if there had been only two mice per cage with a bigger sample size and an equal number of male and female mice, which would have made this a larger more standardised test. It is not uncommon, however, to have more than two individuals in one cage in animal research facilities. Since the excess mice used in this study were already allocated in stable groups of 2 to 4 individuals the decision to not disturb them before starting the experiments was made, as it might have affected the results. Therefore, this study opted to not compare sex differences between or within the two handling groups during the tests because of the uneven numbers due to the limited number of excess mice available, as well as the principle of the 3Rs Reduce by not breeding more female mice.

This study was also limited in funding, and line crossings and time spent were manually logged in BORIS. Programs like ANY-maze remove this bias by having the program log everything which allows for a more thorough investigation into locomotor behaviour such as distance travelled and speed (*ANY-maze* 2023). However, this comes at a huge cost (538.00 to 2,495.00 euros depending on licence type) (*ANY-maze* 2023) and was not feasible. Even though there are a few free alternatives to ANY-maze, these alternatives require multiple programs and coding to operate and with sub-par instructions, it was not an alternative for this study.

The mice used in this study were a cross between BALB/c and C57BL/6, two inbred mouse strains that are commonly used in behavioural studies relating to anxiety, stress, fear and differ in coping with stressful stimuli (Julio-Pieper et al. 2012). BALB/c strains have reduced levels of serotonin and have been proposed to model pathological anxiety (Julio-Pieper et al. 2012), leaving them less active in response to anxiogenic stimuli, while the C57BL/6 strain does not show a reduction in activity as a response to anxiogenic stimuli (An et al. 2011). This means that genetics have a role in how active the mice are in the open field test and how they respond to aversive situations. However, even though there were individual differences within the handling groups that might be explained by genetic differences, there was a larger difference between the two handling groups in this study.

Future studies

Since this study used a strain mixed of C57BL/6 and BALB/c mice, continuing future studies by using more common non-mixed strains C57BL/6, BALB/c or ICR that have been used in other studies (Hurst & West 2010) should be done to explore the relationship between strain and stress/anxiety responses to house handling.

Henderson et al. (2020a) used a gloved hand for both groups (tail and tunnel) when testing voluntary interactions with the handler, compared to this study that used a gloved hand for tail handled mice and house & gloved hand for the other group. They stated that “mice are more likely to interact with a tunnel than with a hand regardless of handling techniques” due to their innate preference for hiding in narrow spaces when disturbed (Henderson et al. 2020a). Due to this, this study decided to refrain from using “interaction with the handler” since it was the interaction with the house that was tested for and not the handler's hand in the house group. However, the house used in this study does not offer narrow crevices and is more alike to that of the hand. Still, the removal of the device (tunnel, ladder, or house) during voluntary interaction may also remove potential bias from these kinds of studies, allowing investigation into how different devices can produce different results with the gloved hand only approach.

Houses can come in different materials, shapes and sizes and future studies should investigate if mice prefer one over another. For example, plastic houses might make it easier to handle mice and remove them from the house. However, it might also make mice less keen to climb and explore the house compared to a cardboard house that provides more grip for their paws. To find potential preferences for different house types, future studies should test different sizes, shapes and materials as this could lead to more willingness to use the house as an alternative handling method to tail handling.

Education and welfare

3R education

To be able to work with research animals, a formal licence is typically obtained by both researchers and employees called LAS (Laboratory Animal Science) (Franco et al. 2018). These courses promote raised animal welfare standards and increase competence surrounding laboratory animals while also including the 3R in the curricula (Franco et al. 2018).

Franco et al. (2018) sent a survey to European participants, and only about half of the respondents were able to correctly name the 3R before the LAS course started, while about 40% did not know of the 3R principles. In contrast to the follow-up survey 6 months after the LAS course with the same participants, about 90% could correctly name the 3R principles (Franco et al. 2018). The participants were also asked about which topics impacted them the most in their work, and of the 3Rs, Refinement had the strongest impact followed by Reduction and lastly Replacement (Franco et al. 2018). Within the refinement topic, handling technique had the highest self-assessed knowledge gains from the LAS course (Franco et al. 2018). While Russel and Burch placed Replacement above Reduction and lastly Refinement in a hierarchy of importance (Russel & Burch 1959), the European participant reversed it by placing Refinement at the top, Reduction in the middle and Replacement last (Franco et al. 2018). This reverse hierarchy could be due to a caveat in the LAS course itself, where the focus is on improving the knowledge and ethical use of research animals and not on also improving the knowledge and use of non-animal methods (Franco et al. 2018). Participating in LAS courses seemingly had a big impact on the knowledge of the 3R with Refinement and handling techniques leaving the biggest impact on participants and is an important step in implementing more non-aversive handling techniques to a larger number of facilities.

Human and animal welfare

About 80% of respondents in a European LAS course survey had ethical concerns about their work and have discussed this with peers at least once (Franco et al. 2018). In a survey of personnel working with laboratory animals in the United States and Canada only about half of the participants stated that they never or sometimes talk to someone about their work (LaFollette et al. 2020). However, due to how the question is asked, it does not differentiate talk between co-workers or people outside of work (LaFollette et al. 2020). Due to the social stigma around using animals in research, it may be difficult for many people to gain social support outside of work (LaFollette et al. 2020).

The respondents that reported that they had less social support also had a higher desire to provide more enrichment to their animals (LaFollette et al. 2020). It is also important for animals to have sufficient enrichment. Since 2010, the EU require specie-specific provision of nesting materials or sleeping structures for all animals used for scientific purposes (European Parliament and of the Council 2010/63). However, this only meets one biological need and might not be stimulating enough. Hobbiesiefken et al. (2021) used tunnel handled mice to test their behaviour to different types of enrichment (categorised as structural, housing and foraging enrichment). They added these enrichments to conventional housing and compared it to conventional housing only, (the conventional housing consisted of bedding, nesting and paper material and a red plastic house). The mice living in conventional housing were more inactive and showed more stereotypical behaviours compared to the mice in enriched housing. Inactivity could be indicative of decreased well-being in mice, but it is not yet known if it is a direct indicator of impaired welfare and well-being (Hobbiesiefken et al. 2021). Stereotypical behaviours have long been an established parameter for animal welfare and are associated with understimulating living conditions and can be prevented by increasing enrichment (Hobbiesiefken et al. 2021). Even though this study provides an alternative to tunnel handling, the use of tunnels or other environmental enrichments in the cages should still be a priority. Both for the increased welfare it brings to the mice, but also for the well-being of personnel working in the facility since enriching the lives of the animals they work with can increase the well-being of the workers. This is because the respondents that reported that they have less social support and a higher desire to provide more enrichments for their animals also reported higher compassion fatigue; a psychological syndrome where the person experiences a secondary trauma (exposure to trauma of others) and burnout (exhaustion, depression, anger and frustration towards a work environment) (LaFollette et al. 2020). In another survey, almost 70% of the United States and Canadian participants had experienced compassion fatigue at some point in their careers where exhaustion was the most common feeling (Randall et al. 2021). Negative feelings or attitudes towards the job that can arise from compassion fatigue can also negatively affect an individual’s ability to perform their job (Randall et al. 2021). The animal-human relationships might then be affected negatively and therefore decreasing the care and animal welfare (Randall et al. 2021). It is therefore important to not only focus on the welfare of laboratory animals, but also on the people that work with the animals. If they do not get the support that they need, their work might be affected which in turn would affect the animals negatively and that is not sustainable, for either people or animals.

Conclusion

This study used home cage cardboard houses and tail lifts to handle mice every other day. Handling mice with the house proved to be advantageous in decreasing defecation, urination and sonic vocalisation when handled and increased their willingness to interact with the handling device. Using the house also increased exploration and decreased anxiety-related behaviours in the open field test. If there is no space for a tunnel or concerns about having to clean a tunnel between cages, then the house might be an option to use as a handling device to increase the welfare of laboratory mice even though this study indicated an increase in handling time for each cage.

References

An, X.-L., Zou, J.-X., Wu, R.-Y., Yang, Y., Tai, F.-D., Zeng, S.-Y., Jia, R., Zhang, X., Liu, E.-Q. & Broders, H. (2011). Strain and Sex Differences in Anxiety-Like and Social Behaviors in C57BL/6J and BALB/cJ Mice. *Experimental Animals*, 60 (2), 111–123. https://doi.org/10.1538/expanim.60.111

ANY-maze (2023). *ANY-maze*. https://www.any-maze.com/ [2023-01-27]

Chen, J., Markowitz, J.E., Lilascharoen, V., Taylor, S., Sheurpukdi, P., Keller, J.A., Jensen, J.R., Lim, B.K., Datta, S.R. & Stowers, L. (2021). Flexible scaling and persistence of social vocal communication. *Nature*, 593 (7857), 108–113. https://doi.org/10.1038/s41586-021-03403-8

Clarkson, J.M., Dwyer, D.M., Flecknell, P.A., Leach, M.C. & Rowe, C. (2018). Handling method alters the hedonic value of reward in laboratory mice. *Scientific Reports*, 8 (1), 2448. https://doi.org/10.1038/s41598-018-20716-3

Díez-Solinska, A., Vegas, O. & Azkona, G. (2022). Refinement in the European Union: A Systematic Review. *Animals*, 12 (23), 3263. https://doi.org/10.3390/ani12233263

Doerning, C.M., Thurston, S.E., Villano, J.S., Kaska, C.L., Vozheiko, T.D., Soleimanpour, S.A. & Lofgren, J.L. (2019). Assessment of Mouse Handling Techniques During Cage Changing. *Journal of the American Association for Laboratory Animal Science*, 58 (6), 767–773. https://doi.org/10.30802/AALAS-JAALAS-19-000015

European Parliament and of the Council (EU) 2010/63 of 22 September 2010 on the protection of animals used for scientific purposes (“Directives”) (20.10.2010, L 276/33, 1-47). http://data.europa.eu/eli/dir/2010/63/oj

Franco, N.H., Sandøe, P. & Olsson, I.A.S. (2018). Researchers’ attitudes to the 3Rs—An upturned hierarchy? *PLOS ONE*, 13 (8), e0200895. https://doi.org/10.1371/journal.pone.0200895

Friard, O. & Gamba, M. (2016). BORIS: a free, versatile open-source event-logging software for video/audio coding and live observations. *Methods in Ecology and Evolution*, 7 (11), 1325–1330. https://doi.org/10.1111/2041-210X.12584

Gould, T.D., Dao, D.T. & Kovacsics, C.E. (2009). The Open Field Test. In: Gould, T.D. (ed.) *Mood and Anxiety Related Phenotypes in Mice: Characterization Using Behavioral Tests*. Totowa, NJ: Humana Press. 1–20. https://doi.org/10.1007/978-1-60761-303-9_1

Gouveia, K. & Hurst, J.L. (2013). Reducing Mouse Anxiety during Handling: Effect of Experience with Handling Tunnels. Mintz, E.M. (ed.) (Mintz, E. M., ed.) *PLoS ONE*, 8 (6), e66401. https://doi.org/10.1371/journal.pone.0066401

Gouveia, K. & Hurst, J.L. (2017). Optimising reliability of mouse performance in behavioural testing: the major role of non-aversive handling. *Scientific Reports*, 7 (1), 44999. https://doi.org/10.1038/srep44999
Gouveia, K. & Hurst, J.L. (2019). Improving the practicality of using non-aversive handling methods to reduce background stress and anxiety in laboratory mice. *Scientific Reports*, 9 (1), 20305. https://doi.org/10.1038/s41598-019-56860-7
Henderson, L.J., Dani, B., Serrano, E.M.N., Smulders, T.V. & Roughan, J.V. (2020a). Benefits of tunnel handling persist after repeated restraint, injection and anaesthesia. *Scientific Reports*, 10 (1), 14562. https://doi.org/10.1038/s41598-020-71476-y
Henderson, L.J., Smulders, T.V. & Roughan, J.V. (2020b). Identifying obstacles preventing the uptake of tunnel handling methods for laboratory mice: An international thematic survey. *PLOS ONE*, 15 (4), e0231454. https://doi.org/10.1371/journal.pone.0231454
Henderson, N.D., Turri, M.G., DeFries, J.C. & Flint, J. (2004). QTL Analysis of Multiple Behavioral Measures of Anxiety in Mice. *Behavior Genetics*, 34 (3), 267–293. https://doi.org/10.1023/B:BEGE.0000017872.25069.44
Himanshu, Dharmila, Sarkar, D. & Nutan (2020). A Review of Behavioral Tests to Evaluate Different Types of Anxiety and Anti-anxiety Effects. *Clinical Psychopharmacology and Neuroscience*, 18 (3), 341–351. https://doi.org/10.9758/cpn.2020.18.3.341
Hobbiesiefken, U., Mieske, P., Lewejohann, L. & Diederich, K. (2021). Evaluation of different types of enrichment - their usage and effect on home cage behavior in female mice. *PLOS ONE*, 16 (12), e0261876. https://doi.org/10.1371/journal.pone.0261876
Hull, M.A., Reynolds, P.S. & Nunamaker, E.A. (2022). Effects of non-aversive versus tail-lift handling on breeding productivity in a C57BL/6J mouse colony. *PLOS ONE*, 17 (1), e0263192. https://doi.org/10.1371/journal.pone.0263192
Hurst, J.L. & West, R.S. (2010). Taming anxiety in laboratory mice. *Nature Methods*, 7 (10), 825–826. https://doi.org/10.1038/nmeth.1500
Julio-Pieper, M., O'Mahony, C.M., Clarke, G., Bravo, J.A., Dinan, T.G. & Cryan, J.F. (2012). Chronic stress-induced alterations in mouse colonic 5-HT and defecation responses are strain dependent. *Stress*, 15 (2), 218–226. https://doi.org/10.3109/10253890.2011.607524
Kassambara, A. (2022). *rstatix: Pipe-Friendly Framework for Basic Statistical Tests*. https://CRAN.R-project.org/package=rstatix
Koyama, S. (2004). Primer effects by conspecific odors in house mice: a new perspective in the study of primer effects on reproductive activities. *Hormones and Behavior*, 46 (3), 303–310. https://doi.org/10.1016/j.yhbeh.2004.03.002
LaFollette, M.R., Riley, M.C., Cloutier, S., Brady, C.M., O'Haire, M.E. & Gaskill, B.N. (2020). Laboratory Animal Welfare Meets Human Welfare: A Cross-Sectional Study of Professional Quality of Life, Including Compassion Fatigue in Laboratory Animal Personnel. *Frontiers in Veterinary Science*, 7, 114. https://doi.org/10.3389/fvets.2020.00114

Lipkind, D., Sakov, A., Kafkafi, N., Elmer, G.I., Benjamini, Y. & Golani, I. (2004). New replicable anxiety-related measures of wall vs. center behavior of mice in the open field. *Journal of Applied Physiology*, 97 (1), 347–359. https://doi.org/10.1152/japplphysiol.00148.2004

Ljung, P.E., Udén, E., Van Den Weghe, J. & Bornestaf, C. (2020). *Användning av försöksdjur i Sverige*. (Dnr: 5.2.17-17593/2019). Jordbruksverket. https://jordbruksverket.se/download/18.248e13d0175bd920a1b3dbc9/1605267998567/Rapport-forsoksdjursstatistik-2018.pdf [2022-12-04]

Nakamura, Y. & Suzuki, K. (2018). Tunnel use facilitates handling of ICR mice and decreases experimental variation. *Journal of Veterinary Medical Science*, advpub. https://doi.org/10.1292/jvms.18-0044

NC3Rs (2022). *The National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs)*. https://www.nc3rs.org.uk/who-we-are

NC3Rs (2023). *The 3Rs*. *The 3Rs*. https://nc3rs.org.uk/who-we-are/3rs [2023-03-14]

O'Leary, T.P., Gunn, R.K. & Brown, R.E. (2013). What are We Measuring When We Test Strain Differences in Anxiety in Mice? *Behavior Genetics*, 43 (1), 34–50. https://doi.org/10.1007/s10519-012-9572-8

Prescott, M. & Lidster, K. (2017). Improving quality of science through better animal welfare: The NC3Rs strategy. *Lab Animal*, 46, 152–156. https://doi.org/10.1038/laban.1217

Randall, M.S., Moody, C.M. & Turner, P.V. (2021). Mental Wellbeing in Laboratory Animal Professionals: A Cross-Sectional Study of Compassion Fatigue, Contributing Factors, and Coping Mechanisms. *Journal of the American Association for Laboratory Animal Science*, 60 (1), 54–63. https://doi.org/10.30802/AALAS-JAALAS-20-000039

Roughan, J.V. & Sevenoaks, T. (2019). Welfare and Scientific Considerations of Tattooing and Ear Tagging for Mouse Identification. *Journal of the American Association for Laboratory Animal Science*, 58 (2), 142–153. https://doi.org/10.30802/AALAS-JAALAS-18-000057

RStudio Team (2022). *RStudio: Integrated Development Environment for R* (2022.07.2). Boston, MA: RStudio, PBC. http://www.rstudio.com/

Ruat, J., Genewsky, A.J., Heinz, D.E., Kaltwasser, S.F., Canteras, N.S., Czisch, M., Chen, A. & Wotjak, C.T. (2022). Why do mice squeak? Toward a better understanding of defensive vocalization. *iScience*, 25 (7), 104657. https://doi.org/10.1016/j.isci.2022.104657

Russel, W.M.S. & Burch, R.L. (1959). *The Principles of Humane Experimental Technique by W.M. Russel and R.L. Burch*. Methuen.

Sandgren, R., Grims, C., Waters, J. & Hurst, J.L. (2021). Using cage ladders as a handling device reduces aversion and anxiety in laboratory mice, similar to tunnel handling. *Scandinavian Journal of Laboratory Animal Science*. 47 (5), 11. https://doi.org/10.23675/sjlas.v47i0.1083

Seibenhener, M.L. & Wooten, M.C. (2015). Use of the Open Field Maze to Measure Locomotor and Anxiety-like Behavior in Mice. *Journal of Visualized Experiments: JoVE*, (96), 52434. https://doi.org/10.3791/52434

Sensini, F., Inta, D., Palme, R., Brandwein, C., Pfeiffer, N., Riva, M.A., Gass, P. & Mallien, A.S. (2020). The impact of handling technique and handling frequency on laboratory mouse welfare is sex-specific. *Scientific Reports*, 10 (1), 17281. https://doi.org/10.1038/s41598-020-74279-3

Sturman, O., Germain, P.-L. & Bohacek, J. (2018). Exploratory rearing: a context- and stress-sensitive behavior recorded in the open-field test. *Stress*, 21 (5), 443–452. https://doi.org/10.1080/10253890.2018.1438405

Ueno, H., Takahashi, Y., Suemitsu, S., Murakami, S., Kitamura, N., Wani, K., Matsumoto, Y., Okamoto, M. & Ishihara, T. (2020). Effects of repetitive gentle handling of male C57BL/6NCrl mice on comparative behavioural test results. *Scientific Reports*, 10 (1), 3509. https://doi.org/10.1038/s41598-020-60530-4

Popular science summary

The laboratory mouse likes its house

Mice used in scientific research spend their whole life in cages and are typically lifted out of that cage by being picked up at the base of their tail. When the cage needs to be cleaned or when the mouse participates in a research project are instances when mice need to be picked up and handled. However, mice do not like to be picked up by their tail. It makes them stressed and they then remember that the hand is something bad. This can be changed by using a tunnel, like a toilet roll or one made of plastic, or by lifting their whole body with cupped hands. The mice can then enter the tunnel or hands on their own and that way be placed somewhere else or in a new clean cage, and instead associate the tunnel and hands with something positive.

This project wanted to investigate, based on the knowledge we already have about tunnels and cupped hands, if the same results can be found for the mouse house since many cages already have houses in them. This is an important project for increasing the well-being of mice so that more research facilities can use this lifting technique.

This project did two tests. The first test was to see if the mice that were lifted by the house remembered the house as good or bad and to see if the mice lifted by their tail remembered the hand as good or bad. This was tested by putting the house or hand down in the cage, depending on what the mice were lifted with, and watching for how long the mice wanted to sniff or touch the house or hand. This was done three times to see if there were changes further in the future. The second test was to see if lifting the mice with the house or by the tail would affect how anxious mice would be in a new environment: a large grey plastic box. Depending on where the mice go and what they do in the box assumptions about how anxious they are can be made.

The mice that were lifted by their house spent longer time with the house than those lifted by the tail did with the hand, and they also increased the time they spent with the house between the first and second time the test was done. Mice that were lifted by the tail spent very little time with the hand, and this suggests that the mice that were lifted by the tail did not like it. When the house lifted mice were placed in the grey box they spent more time in the middle, indicating that they were less anxious than mice lifted by the tail which spend most of their time close to the walls, indicating that they were more anxiety. With the results from this project, it can be suggested that the laboratory mouse does like its house.

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