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# Using cage ladders as a handling device reduces aversion and anxiety in laboratory mice, similar to tunnel handling

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## Summary

Handling laboratory animals for husbandry and other procedures can be an important source of anxiety and stress, compromising animal welfare as well as the reliability of research that is sensitive to background stressors. Studies have revealed that picking up laboratory mice by the tail induces aversion, anxiety, physiological stress and depression-like behaviour, but such negative responses can be reduced substantially by using a handling tunnel that mice enter readily with minimal familiarisation. It has not been tested whether anxiety and aversion can be reduced similarly by using other objects to lift up mice from their home cage. Here we compared the willingness of C57BL/6NRj mice to interact voluntarily with their handler after being picked up either on a plastic ladder present in the home cage, or inside a familiar tunnel, or lifted by the base of the tail and then returned to the home cage. We also tested anxiety in open field and elevated plus maze tests once animals were familiarised with their assigned handling method. While mice picked up briefly by the tail were unwilling to interact with the hand that picked them up, mice picked up by ladder or tunnel readily approached, climbed on or entered these devices, with no significant difference in time spent with ladder or tunnel. Anxiety in an unfamiliar open field was reduced to a similar extent in ladder and tunnel handled mice compared with those picked up by the tail. Mice handled by tunnel also showed reduced anxiety in an elevated plus maze compared to those handled by tail, while ladder handling resulted in an intermediate response. Our study shows that, like tunnels, using home cage ladders to pick up mice reduces anxiety and avoids the aversion that is induced by picking up mice by their tails. We discuss the potential practicality of using ladders and tunnels to handle mice in different contexts.

## Introduction

Mice are the most common vertebrate species used in research and are subjected to frequent manipulations and handling in captivity. It is well recognized that routine handling can cause aversion, anxiety and stress, especially when laboratory mice are picked up by the tail (Balcombe et al. 2004; Hurst and West 2010; Gouveia and Hurst 2013, 2017; Ghosal et al. 2015). The aversion and anxiety induced can influence both the behaviour and physiology of mice as well as compromising animal welfare (Brown and Winnicker 2015; Bailey 2017). Recently, two alternative methods have been developed that can be used to pick up mice instead of tail handling: the mouse can be guided into a handling tunnel and then lifted up for delivery to the hand, transferred between cages or to test apparatus; or mice can be scooped up on the open hand without restraint, in a method known as cupping, once they are familiar with being lifted (Hurst and West 2010; Gouveia and Hurst 2019). Mice picked up in a tunnel or on the open hand will interact voluntarily with their handler while those picked up by tail avoid contact, reflecting a major difference in the aversiveness of these handling methods as reported for different laboratories, handlers and mouse strains (Hurst and West, 2010; Gouveia and Hurst 2013, 2017, 2019; Clarkson et al. 2018, 2020; Nakamura and Suzuki 2018; Henderson et al. 2020a; Sensini et al. 2020). Mice picked up by the tail also show greater anxiety in standardised tests compared to those picked up by one of these non-aversive methods (Hurst and West 2010; Gouveia and Hurst 2013, 2019; Clarkson et al. 2018, 2020; Nakamura and Suzuki 2018; Henderson et al. 2020a; Sensini et al. 2020), and have elevated measures of physiological stress such as increased urination and defecation during handling (Hurst and West 2010; Nakamura and Suzuki 2018; Henderson et al. 2020a), higher plasma corticosterone in response to a stressful situation (Ghosal et al. 2015) and enlarged adrenal glands indicative of chronic stress (Clarkson et al. 2020). Furthermore, mice picked up by the tail show behaviour indicative of a depressive-like state, with reduced sucrose consumption anhedonia (Clarkson et al. 2018), greater immobility in a forced swim test and reduced burrowing behaviour (Sensini et al. 2020) compared to those picked up in a handling tunnel.

Minimising handling anxiety in laboratory animals is essential as this both negatively impacts animal welfare and can be an important source of unwanted variation and poor reliability and replicability in many areas of animal research (Brown and Winnicker 2015; Bailey 2017). For example, picking up mice by the tail can increase blood glucose stress responses and reduce glucose tolerance in diabetes research (Ghosal et al. 2015), eliminate reliable exploration of test stimuli in cognitive behavioural tests (Gouveia and Hurst 2017), increase variability in pharmacological testing (Nakamura and Suzuki 2018), and reduce responsiveness to the rewards that are used to train animals in a range of behavioural and cognitive tasks (Clarkson et al. 2018) compared to the use of non-aversive handling methods. Importantly, the positive effects of using non-aversive methods (rather than capturing and lifting mice by the tail) persist even when mice experience restraint by the tail or scruff (Hurst and West 2010; Gouveia and Hurst 2019), subcutaneous (Gouveia and Hurst 2019) or intraperitoneal (Henderson et al. 2020a) injections, oral gavage (Nakamura and Suzuki 2018), anaesthesia (Henderson et al. 2020a), or marking by tattoo or ear-tagging (Roughan and Sevenoaks 2018) once picked up. Thus, although mice are not physically restrained when picked up by tunnel or cupping, they are tolerant of restraint when this is required for health inspections or other procedures.

When working with animals, the handling method not only needs to minimise any aversion, anxiety and stress in animals but also needs to be efficient and secure from a practical perspective so that it is workable in daily routines. While both cupping and handling tunnels are non-aversive to mice once they are familiar with these methods, mice require more time to become familiarised with cupping (Hurst and West 2010; Gouveia and Hurst 2019). Further, cupping without physical restraint is not suitable for very young mice or strains that are very jumpy, or for inexperienced handlers who are less able to judge when an unrestrained mouse might jump off the open hand unless the mice concerned are very placid (Gouveia and Hurst 2019). However, mice are easily and securely picked up inside a handling tunnel (tube) once handlers are trained in this technique, while the very brief handling necessary to transfer mice between cages at cage cleaning (approximately two seconds) is sufficient to familiarise mice with this method and achieve positive effects (Gouveia and Hurst 2017, 2019). By contrast, while picking up mice by tail is secure, even brief and infrequent tail handling during cage cleaning induces strong aversion (Gouveia and Hurst 2019), tail handled mice are more difficult to handle and restrain, and handlers are at an increased risk of being bitten (Nakamura and Suzuki 2018; Gouveia and Hurst 2019). Handling tunnels overcome these issues but, nonetheless, perceived incompatibility of tunnels with a caging system, experimental apparatus, or implants carried by animals might be deterrents for using handling tunnels routinely in some facilities (Henderson et al. 2020b). This led us to explore other handling devices that might be as effective as tunnels for minimising aversion, anxiety and stress in laboratory mice while also being efficient and secure.

A major difference between picking up mice by tunnel or cupping versus the tail, besides direct restraint, is that animals face the handling device head-on and step into the tunnel or onto the hand themselves (even though guided to do so by the handler) rather than being grasped from behind and pulled up backwards. This may lead to a perception of greater choice and cooperation that might also be achieved by using other objects that animals walk on or into. Gouveia and Hurst (2013) recommended using home cage tunnels where possible, allowing mice to become highly familiar with tunnels that also provide enrichment in the home cage. However, to our knowledge, no studies have looked at whether other enrichment objects could also be used for non-aversive handling, such as different types of shelter or items added to encourage activity and increase the use of space in a cage that would otherwise be unavailable. In this study, we compare the responses of mice picked up with a home cage ladder (Figure 1A) with those of mice picked up in a familiar tunnel or by the base of the tail, to establish whether handling using an enrichment device such as a ladder is also non-aversive and as effective as tunnel handling for reducing anxiety in laboratory mice. Our animal facility at Lund University designed and developed the ladder used in this study to provide mice with the opportunity for climbing (Roemers et al. 2019) in cages that do not have bars. The ladders provide a familiar grid that mice readily climb on and grip, potentially providing a suitable device to pick up mice from the cage. The ladder is angled upwards at one end (Figure 1A,C) which makes it easier to scoop up a mouse. We predicted that mice picked up on their home cage ladder would be less anxious than those picked up by tail and readily interact with the ladder, with a positive response equal to that of mice picked up in a home cage tunnel.

## Materials and Methods

Animal use and care was in accordance with EU directive 2010/63/EU. All procedures were approved by Malmö-Lund regional ethics committee, ethical permit number: 5.8.18-02982/2020.

### Animals and housing conditions

48 C57BL/6NRj mice (24 females, 24 males) were obtained from Janvier Labs (Le Genest-Saint-Isle, France) at four weeks of age. On arrival, they were individually identified by ear punch and divided into 24 cages with two same-sex mice per cage. Microbiological health monitoring was carried out according to FELASA guidelines (Mähler et al. 2014) with negative results.

All mice where housed in disposable IVC cages (Innocage®outside dimensions 37.3 x 23.4 x 14.0 cm; Innovive, San Diego, USA) at Lund University, Sweden. Each cage contained corncob bedding, nesting material (InnorichmentTM, Innovive, San Diego, USA) and had been irradiated. Irradiated pelleted food (A40-SP25, SAFE, France) and acidified water (2.5-3.0 pH) in pre-filled water bottles (300 ml, Aquavive®/Innovive, SanDiego, USA) were provided *ad libitum*. Animals were maintained on a 12:12 h light:dark cycle (lights on at 07:00), at 22 ± 2 °C with approximately 15 air changes per hour and 52-55 % relative humidity. An aspen gnaw stick (Tapvei Estonia OÜ, Harjumaa, Estonia), a plastic ladder (Mouse climber, 200 x 60 mm polyethylene terephthalate, Innovive, San Diego, USA) and an opaque plastic tunnel (100 mm x 50 mm diameter polypropylene, local retail, Sweden) were provided as additional cage enrichment (Figure 1).

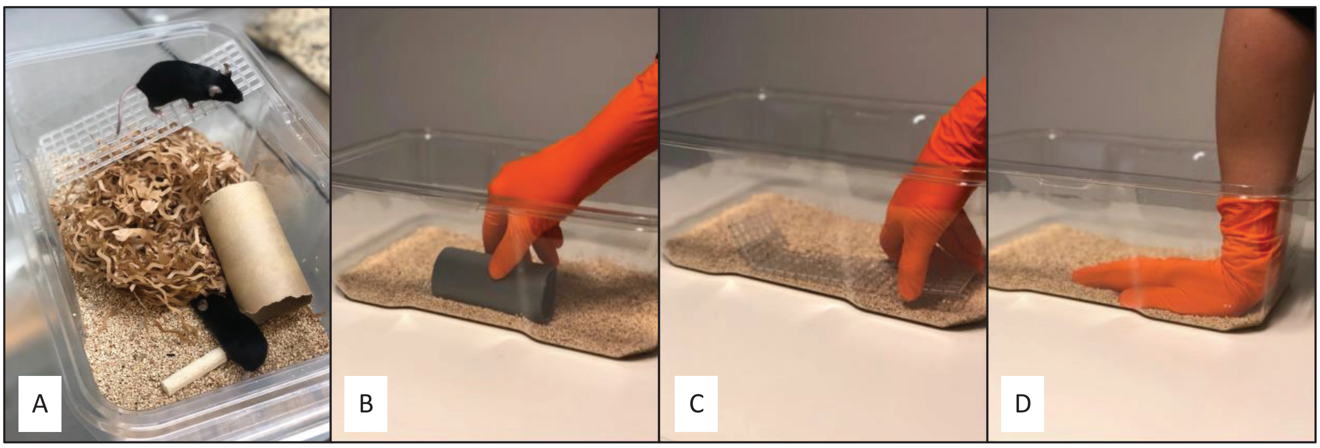


**Figure 1.** Standard enrichment in mouse cages at Lund University **(A)**. Placement of handling device during the test of voluntary interaction with the handler for tunnel **(B)**, ladder **(C)** and tail **(D)** methods.

Cages were assigned randomly to one of three handling methods (tail, tunnel or ladder) using random numbers, with four female and four male cages per method (16 mice per handling group). Mice were handled only by their assigned method from arrival. Cages were arranged in the cage rack such that handling method and sex were distributed in a balanced design. For the first three weeks, mice were acclimatized to the laboratory and cages were opened only if food required replenishment. Cages were changed once during the study, with mice placed into a clean cage after the first voluntary interaction test. All handling and testing were carried out during the light phase of the circadian cycle.

## Handling methods

To simulate routine handling of mice at cage change, all lifts were for approximately two seconds then mice were lowered back into their cage. Mice assigned to tail handling were grasped at the base of the tail between thumb and forefinger by a gloved hand and gently lifted into the air. Mice handled by tunnel were gently guided by a gloved hand into their home cage tunnel and then lifted into the air without covering the ends of the tunnel. For ladder handling, mice were guided gently onto the ladder by a gloved hand and lifted into the air. The mice were handled by two female handlers alternating between days. For anxiety tests, all mice were handled by one of the two handlers. Gloves (Powder-free orange nitrile glovesTM, SHIELDskinTM, SHIELD Scientific, ND Bennekom, Netherlands) were disinfected (Contec® ProChlor, Vita Verita AB, Kungsängen, Sweden) between cages and allowed to air dry, and all enrichments were removed from the cage prior to handling. In each handling session, the order of cages was randomised but the males were always handled first as female scents can promote increased androgen levels and competitive aggression within male groups (Koyama 2004) and then gloves changed before handling females to avoid any impact of male scents on females. The first mouse picked up in each cage was alternated between daily handling sessions. When mice were transported to test arenas by tail, their body weight was supported, and then lifted by the tail unsupported for delivery to the arena.

## Handling and test schedule

The study started when mice were seven weeks old. Mice were briefly handled once per day on week days (Monday-Friday) for nine handling sessions. Voluntary interaction with the handler was tested immediately after handling sessions 1, 5 and 9 (days 1, 5 and 11). An open field test was conducted on day 15 or 16 from the start of the study, and an elevated plus maze on day 17 or 18. Gloves were worn during all tests and disinfected between mice.

## Voluntary interaction with handler

We carried out tests of voluntary interaction with the handler immediately after both mice in a cage had been handled in sessions 1, 5 and 9. The device used to handle the animals (gloved hand holding a home cage tunnel, gloved hand holding a home cage ladder, or gloved hand only for tail handled animals) was introduced into the home cage and held on the substrate without movement for 60s (Figure 1B-D). The session was video recorded and transcribed using BORIS event logging software (Friard and Gamba 2016). The total time when none, one or both mice in the cage interacted with the hand, tunnel or ladder (close sniffing or physical contact, including the gloved hand holding a tunnel or ladder) was recorded. These data were then used to calculate the proportion of test time spent in voluntary interaction per mouse averaged over both mice in the same cage.

## Open field test

To assess anxiety in an open field test, mice were picked up by their assigned method and delivered to an unfamiliar open field arena (40 x 40 x 40 cm opaque black walls, Stoelting Co., Illinois, USA) for a 5 min test. As anxious animals are reluctant to spend time in open areas (Gould et al. 2009), we measured time spent in the central 7.5 x 7.5 cm zone of the arena using a video tracking system (ANY-maze, Stoelting Co., Illinois, USA). Cages, and mice within cages, were tested in random order, with no difference in test order between treatments (Kruskal-Wallis $H2 = 1.14$, $p = 0.57$). The test arena was disinfected using ethanol and wiped off with water between mice.

## Elevated plus maze test

Anxiety was also assessed in an elevated plus maze consisting of two closed arms (35 x 5 cm) with 15 cm high side walls and two open arms (35 x 5 cm) with no side walls, connected by a central hub (5 x 5 cm), elevated 60 cm above ground and constructed of grey painted steel. Mice were picked up and delivered to the maze by their assigned method for a 5 min test. Cages, and mice within cages, were tested in random order, with no difference in test order between treatments (Kruskal-Wallis $H2 = 0.37$, $p = 0.83$). A video tracking system (ANY-maze, Stoelting Co., Illinois, USA) recorded the number of entries and total time spent on the open arms of the maze as measures of anxiety (Pellow et al. 1985; Walf and Frye 2007), and the number of entries to the closed arms and total distance moved on the maze as measures of general activity.

### Data analysis

Statistical analyses were carried out using SPSS version 25 (IBM software). Shapiro-Wilks tests and qqnorm plots checked for any deviation of residuals from normality for each parametric model, using data transformation where appropriate. The raw data analysed in this study are provided in Supplementary Table 1. One male mouse from the tunnel group died before the Open Field and Elevated Plus Maze tests were performed. Also, one male mouse from the tail group was not recorded in the Open field test due to technical problems.

The total time spent in voluntary interaction with the handler at each time point was calculated as a proportion of the 60s test and averaged across the two mice in the same cage to reflect the lack of independence of animals tested together. A repeated measures ANOVA analysed the effects of handling method and sex on time spent interacting with the handling device in tests repeated after handling sessions 1, 5 and 9. As the duration of voluntary interaction across sessions lacked sphericity, a Greenhouse-Geisser correction was applied when assessing the effect of session.

To assess anxiety in the open field test, a repeated measures ANOVA assessed the effects of handling method and sex on the proportion of test time spent in the inner zone of the open arena, using cage as a within-subjects factor to account for lack of independence between mice in the same cage. Data were log transformed to meet assumptions of parametric analysis. To assess anxiety in the elevated plus maze, repeated measures ANOVAs assessed the effects of handling method and sex on the number of entries and total time on the open arms of the maze, using cage as a within-subjects factor. A similar analysis assessed the number of entries to closed arms as a measure of general activity, while non-parametric Kruskal-Wallis and Mann-Whitney tests assessed the effects of handling method and sex respectively on the total distance moved during the test. As problems with recording meant that some tests were slightly shorter than 5 min (elevated plus maze test duration 289 ± 3s), all data were adjusted to an equivalent rate for a 5 min test.

Where a significant effect of handling method was found, Bonferroni post hoc comparisons checked which methods differed significantly from each other. Throughout, $p < 0.05$ was regarded as statistically significant.

## Results

### Voluntary interaction with handler

We compared the willingness of mice to interact voluntarily with their assigned handling device immediately after they had been picked up briefly by the device (a gloved hand holding a tunnel or ladder, or a gloved hand only for mice picked up by the tail). This was assessed as the amount of time spent in close interaction (close sniffing or physical contact) during a 60 s test, averaged over both mice in a cage. Voluntary interaction differed substantially according to the handling device in both sexes ($F_{2,18} = 129.6$, $p < 0.0001$; Figure 2). This difference was evident from the first handling session and did not change significantly over handling sessions 1, 5 and 9 (effect of session: $F_{1.4, 36} = 1.56$, $p = 0.23$; interaction between method and session: $F_{2.7, 36} = 0.38$, $p = 0.75$; Figure 2). While mice spent very little time interacting with the hand after being picked up by the tail (2.9 ± 0.3 s per 60 s test), those picked up by tunnel or ladder spent much longer interacting with their handling device (20.3 ± 0.7 s per test). There was no significant difference in the duration of interaction between ladder and tunnel (Bonferroni *post hoc* comparison, $p = 1.0$; Figure 2). There was also a qualitative difference in the type of interaction shown: while tail handled mice only sniffed the hand and rarely made paw contact, those handled by ladder or tunnel readily climbed on the handling devices, went inside the tunnel and made paw contact with the gloved hand that held the ladder or tunnel.

### Anxiety

Handling method influenced anxiety in an open field test, assessed as the amount of time spent in the inner zone of the unfamiliar arena ($F_{2,16} = 25.4$, $p < 0.0001$). While males spent more time in the inner zone overall compared to females ($F_{1,16} = 6.7$, $p = 0.02$), handling method had a similar effect on both sexes (interaction between sex and method: $F_{2,16} = 1.20$, $p = 0.33$). Mice handled by either tunnel or ladder spent much more time in the inner zone than those handled by tail, with no significant difference between tunnel or ladder (Bonferroni *post hoc* comparison, $p = 0.69$; Figure 3A).

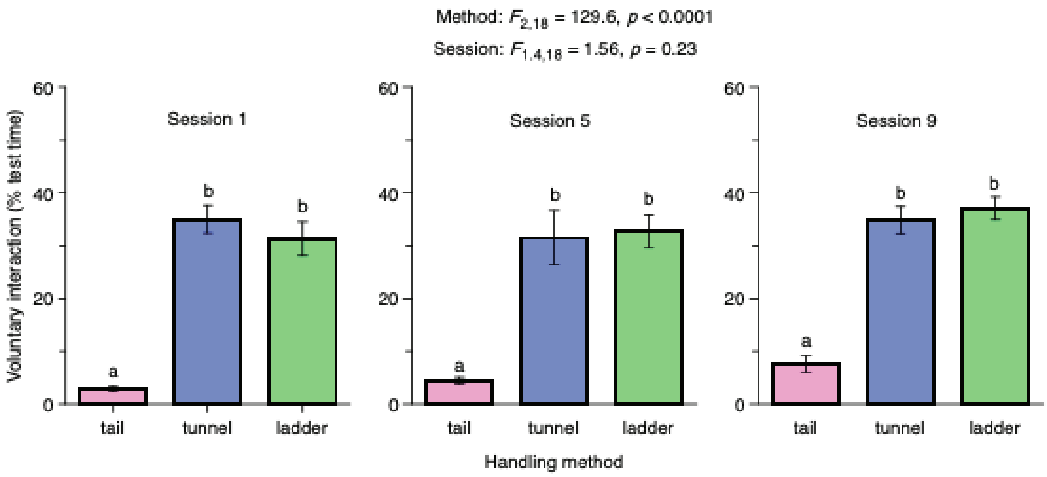


**Figure 2.** The effect of brief handling by different methods on voluntary interaction with the handling device (mean ± sem per cage). Mice were picked up for approximately 2 s (similar to a cage transfer) by the tail (pink), home cage tunnel (blue) or ladder (green) in nine daily handling sessions. Voluntary interaction was assessed after handling on first, fifth and ninth session (% of 60 s test sniffing or contacting the device, averaged over both mice in the same cage). N = 8 cages per method (four male, four female). *P* values from a repeated measures ANOVA across the three repeated tests. Different letters (a,b) indicate significant differences between methods from Bonferroni post hoc comparisons ($p < 0.0001$).

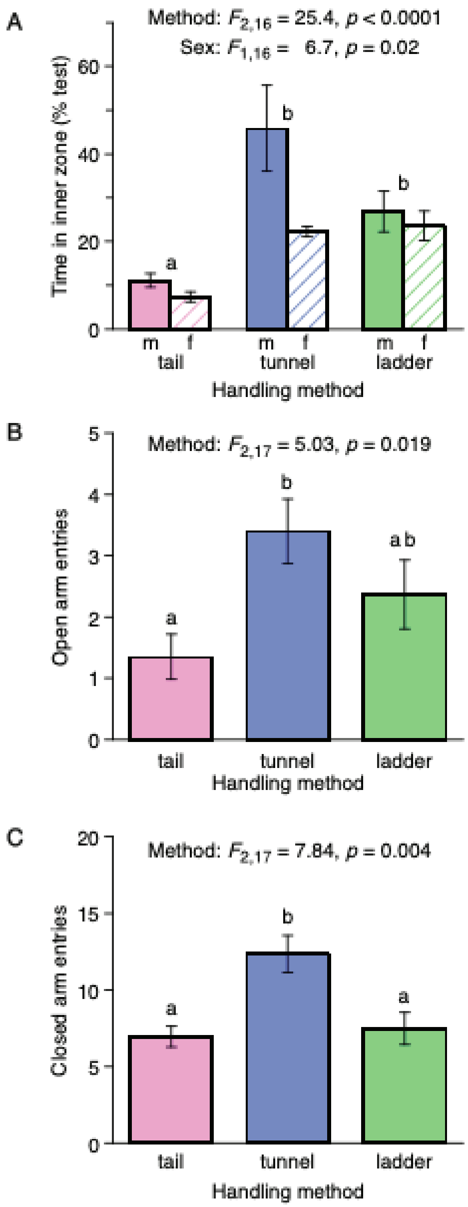


**Figure 3.** Responses of mice handled by different methods in open field and elevated plus maze tests of anxiety (mean ± sem). After being picked up for approximately 2 s over nine daily sessions to familiarise mice with their designated handling method (pink: tail, blue: tunnel, green: ladder), mice were tested in an open field test **(A)** and an elevated plus maze test **(B,C)**. Mice picked up using a tunnel or ladder spent more time in the inner zone of an open field, indicating reduced anxiety compared to those picked up by tail **(A)**. Entries to the open arms of an elevated plus maze were more frequent in tunnel compared to tail handled mice but intermediate in ladder handled mice **(B)**. Tunnel handled mice also entered closed arms more frequently than tail or ladder handled mice **(C)**. P values from repeated measures ANOVAs with mice from the same cage as a within-subjects factor (time in inner zone of an open field log transformed to meet assumptions of parametric analysis). Different letters (a,b) indicate significant differences between methods from Bonferroni post hoc comparisons.

Handling method also influenced the number of entries to the open arms of an elevated plus maze ($F_{2,17}$ = 5.03, $p$ = 0.019), with a similar response in both sexes ($F_{1,17}$ = 0.76, $p$ = 0.40; interaction between sex and method: $F_{2,17}$ = 4.35, $p$ = 0.24). *Post hoc* comparisons indicated that mice picked up by the tail made significantly fewer entries to the open arms than those picked up in a tunnel (Bonferroni test, p = 0.011, Figure 3B), consistent with greater anxiety among tail handled mice. Mice picked up on a ladder showed an intermediate response that did not differ significantly from either tunnel or tail handled mice (Figure 3B). The mean time that mice spent on the open arms followed a similar pattern (tail: 10.4 ± 2.8 s; tunnel: 19.6 ± 5.8 s; ladder: 15.3 ± 2.7s per cage) but this did not differ significantly between handling methods ($F_{2,17}$ = 1.90, $p$ = 0. 18) as only female mice picked up in a tunnel tended to spend more time on the open arms than those handled by other methods (interaction between method and sex, $F_{2,17}$ = 2.90, $p$ = 0. 08).

As measures of general activity in the elevated plus maze test, we also examined the number of entries into closed arms and the total distance moved on the maze during the test. Handling method significantly influenced the number of closed arm entries ($F_{2,17}$ = 7.84, $p$ = 0. 004; Fig. 3C) but not the total distance moved during the test (Kruskal-Wallis $H_2$ = 3.30, $p$ = 0.19). Mice handled by tunnel made more entries to the closed arms of the maze than those handled by tail (Bonferroni $p$ = 0.004) or ladder (Bonferroni $p$ = 0.009), although they did not travel a greater total distance during the test. This suggests that tunnel handled mice were more active in exploring both the open and closed arms of the maze but were not generally more active overall.

## Discussion

In agreement with our predictions, mice picked up on a home cage ladder voluntarily approached and climbed on the ladder immediately after handling, showing the same lack of aversion towards the ladder and hand holding the ladder that mice show towards the tunnel. By contrast, those picked up by tail avoided contact with the handler's gloved hand. Mice picked up on a ladder also showed reduced anxiety in an unfamiliar open field compared to tail handled mice, spending a very similar length of time in the open inner zone as those handled by tunnel. However, measures of anxiety in ladder handled mice in an elevated plus maze test were intermediate between tunnel and tail handled animals.

When testing voluntary interaction immediately after handling, the level of aversion shown by tail handled mice was very similar to that shown in previous studies (Hurst and West 2010; Gouveia and Hurst 2013, 2017, 2019; Clarkson et al. 2018, 2020; Nakamura and Suzuki 2018; Henderson et al. 2020a; Sensini et al. 2020). When tail handled mice did approach, they only sniffed the hand and rarely made paw contact, as described previously (Hurst and West 2010; Sensini et al. 2020), with an observed tendency to also stay close to the cage walls. By contrast, both tunnel and ladder handled mice moved around freely and were more explorative in the cage, climbing on or in these devices and making contact with the gloved hand. These findings are in accordance with the behaviour patterns that differentiate anxious from non-anxious behaviour in mice (Simon et al.1994; Prut and Belzung 2003). In our study, the voluntary interaction test was designed to assess whether mice found the handling device itself aversive after handling, so we did not test how mice picked up by ladder responded when only the handler's gloved hand was presented as for tail handling. However, other studies have shown that mice picked up by tunnel interact readily with just a gloved hand as well as with the tunnel (Hurst and West 2010; Roughan and Sevenoaks 2018; Henderson et al. 2020a). Given the similarity of response between tunnel and ladder handled mice, and their willingness to make paw contact with the hand holding the ladder, it is likely that mice picked up with a ladder will respond in the same positive way that tunnel handled mice do when they need to be manipulated and restrained in the hand (Hurst and West 2010; Gouveia and Hurst 2019).

Mice interacted freely with the tunnel or ladder right from their first brief handling session. By contrast, mice scooped up by cupping on the open hand require familiarisation through multiple handling sessions before they interact freely with the gloved hand that has just picked them up (Hurst and West 2010; Gouveia and Hurst 2019). Several factors may influence this immediate positive response to ladder handling. First, mice were highly familiar with climbing on the ladder in their home cage. However, prior familiarity may only play a minor role given that familiarity with handling tunnels in the home cage slightly improves interaction in a first handling session, but mice also respond positively to handling tunnels even when these are completely unfamiliar (Gouveia and Hurst 2013). Secondly, the rigid surface of ladders and tunnels may be preferred by animals as these will provide much less variable surface movement when mice are lifted up compared to a hand. Thirdly, mice will be more familiar with rigid plastic surfaces compared to the material, warmth and odour of gloves (López-Salesansky et al. 2015). Lastly, the ladder grid is designed for easy gripping, which may provide a greater perception of safety when mice balance on a moving ladder compared to the smooth moving surface of a gloved hand, while mice in tunnels are protected by the encompassing tunnel walls. All of these factors combined may help to attract mice more readily to tunnels and ladders than to the hand, and to reduce anxiety as mice are lifted such that initial familiarisation is less necessary than for cupping on the open hand.

Time spent in the inner zone of an open field suggests that mice handled by ladder experienced similar low levels of anxiety as tunnel handled animals, with both moving freely around the arena, while mice picked up by tail showed high anxiety and were reluctant to enter the inner zone as reported previously (Gouveia and Hurst 2017, 2019; Clarkson et al. 2018, 2020; Nakamura and Suzuki 2018; Henderson et al. 2020a). In our tests, males generally spent more time in the inner zone of the open field compared to females, suggesting that males were less anxious or bolder during exploration. However, both sexes showed a similar reduction in time spent in the inner zone after tail handling compared to those handled by either ladder or tunnel. Sex differences have been reported previously in some measures of anxiety, although whether males or females show greater anxiety varies between strains and between studies (Augustsson et al. 2005; An et al. 2011). In C57BL/6J mice, An et al. (2011) found no sex difference in time spent in the inner zone of an open field but did not report the handling method used. By contrast, when examining the effects of handling by tail or tunnel on anxiety in an open field among C57BL/6J mice, Gouveia and Hurst (2019) report very similar results to our study, with males spending more time overall in the central zone while both sexes spent less time in the inner zone when picked up by tail compared to those picked up in a tunnel. In the elevated plus maze test, mice of both sexes handled by tail also showed significantly increased anxiety as evidenced by fewer entries onto open arms compared to tunnel handled mice, which was consistent with previous reports (Hurst and West 2010; Gouveia and Hurst 2013, 2019; Ghosal et al. 2015; Clarkson et al. 2018, 2020; Nakamura and Suzuki 2018; Henderson et al. 2020a). However, those handled by ladder showed an intermediate response and did not move as freely around the maze as tunnel handled mice. In this test, males and females did not differ overall in the number of entries to the open arms, in agreement with Gouveia and Hurst (2013), where tail handled mice of both sexes made fewer entries onto open arms compared to tunnel handled mice. Other studies have found more entries to open arms among C57BL/6J females compared to males overall (Hurst and West 2010; Gouveia and Hurst 2019), suggesting that males of this strain may sometimes demonstrate greater anxiety in this test than females, which is opposite to the sex difference found in open field tests discussed above. This may explain why only females handled by tunnel spent more total time on the open arms as well as entering these arms more frequently, as these were the least anxious animals in this test. However, regardless of the specific test and measures used, all of these studies (including the study reported here) have consistently found greater anxiety among mice of both sexes picked up by the tail compared to those picked up in a tunnel. Mice of both sexes picked up by a ladder were clearly less anxious in the open field test than those picked up by the tail and not significantly more anxious than tunnel handled mice in this test. They also readily approached and interacted with the ladder, which was similar to their response to handling tunnels and without the aversion shown by tail handled mice, but effects on anxiety in the elevated plus maze were less clear cut.

While conducting these tests, we noted a difference in the ease of placing mice onto the elevated plus maze between the handling methods. Use of a tunnel provided the smoothest delivery to the maze as mice easily could be tipped out backwards onto the central hub. By contrast, mice delivered to the maze on a ladder clung to the handling device and sometimes had to be pushed off firmly, resulting in less voluntary cooperation and a less precise placement. This problem did not occur when handling animals in cages, where animals are very familiar with the environment and readily leave the ladder. Nor did it occur when delivering animals by ladder to the open field test, suggesting that mice found the elevated plus maze a more unfamiliar and potentially threatening environment to enter. We also found that delivering mice to the elevated plus maze by tail was sometimes difficult, given the very small central hub and the tendency of mice held by the tail to twist. Some tail handled mice ran straight out onto one of the open arms of the maze when released. This did not appear to be a voluntary action to enter the open arms, but instead an attempt to run away when the tail grip was released. Thus, delivery of mice to this particular test by either tail or ladder may result in potential confounding effects which can be avoided by using a handling tunnel.

As the ladder is provided as standard enrichment in cages at our Lund facility and frequently is used for handling, we have seen that it has many practical advantages. As it is already in each cage, it is easily accessible and provides a biosecure way to transfer mice into new cages as shown for other handling devices (Doerning et al. 2018). We have observed that mice are easy to inspect when on the ladder, making it possible to see the mouse from all angles including the ventral view without restraining the animal as it grips onto the ladder. When opaque handling tunnels are used, such inspection is not possible without tipping the animal out onto the hand, although animals can usually be observed well through clear handling tunnels. Having the mice already gripping on to the ladder also allows for scruffing directly from the ladder. The construction of the ladder, as an "open" device, may also make it particularly suitable to pick up animals that have implants, surgical staples or similar devices on their body. However, as the ladder provides an open surface, there is a potential risk that jumpy young animals or strains might jump off the ladder, which we have not investigated in this study. However, we did not find that mice attempted to jump from the ladder during our handling and tests and this has not been reported as an issue in our animal facility where ladders are used routinely to pick up mice from the cage, and it is possible to cover the mice with a hand where this might be a risk. Although we have not collected data systematically on the time it takes to transfer mice between cages using ladders and other enrichment objects in the cage, we have observed that using these non-aversive handling methods to transfer mice instead of picking them up by the tail does not prolong the time spent for cage change.

In this study we have shown that using a home cage ladder to handle mice is non-aversive, similar to using a tunnel. Like tunnel handling, this can also reduce anxiety and provides a refinement compared to tail handling. However, tunnels might be better for transferring mice in some contexts. Our findings suggest that mice can be picked up on or in a variety of objects present in the cage, making handling easier and avoiding aversion and high anxiety in the animals. This will benefit animal welfare and the practicality of animal management, and may also improve scientific reliability in animal models that are susceptible to background stress and anxiety.

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## Conflict of interest

The authors declared no potential conflicts of interest.

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**Supplementary Dataset 1.** Raw data used in Sandgren, Grims, Water and Hurst (excel spreadsheet).

| method | sex | cage | VI_testduration | VI_%interact_1 | VI_%interact_5 | VI_%interact_9 | OF_testduration_mousea | OF_%inners_mousea | OF_testduration_mouseb | OF_%inners_mouseb | EPM_testduration_mousea | EPM_openentries_mousea | EPM_openduration_mousea | EPM_closedentries_mousea | EPM_totaldistance_mousea | EPM_testduration_mouseb | EPM_openentries_mouseb | EPM_openduration_mouseb | EPM_closedentries_mouseb | EPM_totaldistance_mouseb |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| tail | female | 1 | 60,00 | 3,19 | 7,50 | 6,90 | 299,9 | 2,03 | 300,0 | 1,53 | 298,6 | 0 | 0,0 | 6 | 7,01 | 297,4 | 4 | 46,4 | 9 | 7,64 |
| tail | female | 13 | 60,00 | 1,33 | 3,77 | 7,34 | 299,9 | 3,27 | 298,6 | 1,47 | 299,0 | 2 | 12,9 | 5 | 7,41 | 274,0 | 0 | 0,0 | 3 | 7,56 |
| tail | female | 22 | 60,00 | 1,81 | 3,45 | 3,31 | 299,5 | 4,74 | 299,8 | 2,64 | 184,3 | 0 | 0,0 | 3 | 8,34 | 264,8 | 0 | 0,0 | 9 | 10,50 |
| tail | female | 23 | 60,00 | 5,67 | 4,05 | 1,94 | 299,4 | 2,07 | 299,8 | 1,97 | 268,0 | 2 | 10,9 | 8 | 6,92 | 286,7 | 0 | 0,0 | 6 | 6,67 |
| tail | male | 5 | 60,00 | 2,01 | 5,88 | 12,10 | 298,6 | 3,68 | 298,0 | 3,62 | 299,6 | 0 | 0,0 | 9 | 4,79 | 284,7 | 2 | 8,1 | 6 | 7,73 |
| tail | male | 12 | 60,00 | 2,21 | 4,78 | 15,47 | 297,8 | 2,42 | | | 298,1 | 1 | 4,1 | 11 | 10,22 | 282,1 | 1 | 15,5 | 7 | 10,59 |
| tail | male | 20 | 60,00 | 1,97 | 3,33 | 8,21 | 299,2 | 5,35 | 299,5 | 5,75 | 293,2 | 4 | 8,6 | 1 | 4,46 | 294,6 | 3 | 25,9 | 9 | 7,92 |
| tail | male | 24 | 60,00 | 4,30 | 2,56 | 4,97 | 298,9 | 1,87 | 299,7 | 3,35 | 271,5 | 0 | 0,0 | 5 | 3,62 | 296,2 | 2 | 30,5 | 8 | 7,17 |
| tunnel | female | 2 | 60,00 | 37,90 | 51,33 | 44,40 | 299,1 | 6,89 | 299,1 | 7,05 | 298,3 | 2 | 9,7 | 9 | 7,38 | 293,0 | 6 | 63,9 | 12 | 8,76 |
| tunnel | female | 6 | 60,00 | 27,86 | 18,98 | 29,02 | 299,9 | 10,24 | 300,0 | 5,74 | 298,2 | 4 | 19,4 | 22 | 11,34 | 289,1 | 4 | 21,2 | 17 | 13,10 |
| tunnel | female | 9 | 60,00 | 40,28 | 29,88 | 27,26 | 298,9 | 6,99 | 299,9 | 7,69 | 297,0 | 5 | 47,0 | 14 | 9,29 | 299,3 | 7 | 53,7 | 12 | 8,37 |
| tunnel | female | 10 | 60,00 | 27,75 | 54,21 | 41,15 | 299,6 | 6,17 | 298,1 | 8,54 | 293,8 | 0 | 0,0 | 9 | 7,34 | 296,4 | 4 | 22,0 | 15 | 9,56 |
| tunnel | male | 3 | 60,00 | 48,91 | 15,99 | 29,17 | 295,5 | 12,39 | 299,6 | 4,91 | 296,3 | 4 | 13,1 | 10 | 6,23 | 297,7 | 2 | 6,3 | 8 | 8,43 |
| tunnel | male | 8 | 60,00 | 27,16 | 35,51 | 39,91 | 296,5 | 29,78 | 298,0 | 13,25 | 299,3 | 2 | 12,3 | 17 | 9,78 | 297,3 | 2 | 6,6 | 12 | 6,23 |
| tunnel | male | 15 | 60,00 | 35,83 | 25,48 | 26,79 | 298,5 | 16,55 | | | 297,6 | 1 | 3,0 | 8 | 8,64 | | | | | |
| tunnel | male | 16 | 60,00 | 34,22 | 20,84 | 40,75 | 299,6 | 7,58 | 298,7 | 23,40 | 299,5 | 1 | 3,0 | 8 | 7,62 | 298,3 | 4 | 25,9 | 6 | 7,92 |
| ladder | female | 11 | 60,00 | 41,26 | 20,59 | 39,55 | 299,5 | 5,38 | 298,5 | 9,82 | 283,2 | 0 | 0,0 | 10 | 8,61 | 295,9 | 3 | 11,2 | 2 | 7,60 |
| ladder | female | 14 | 60,00 | 27,24 | 42,74 | 44,60 | 298,8 | 8,70 | 299,7 | 12,22 | 297,5 | 1 | 5,0 | 4 | 7,34 | 291,6 | 7 | 37,0 | 14 | 12,89 |
| ladder | female | 17 | 60,00 | 40,96 | 33,53 | 35,21 | 299,9 | 4,00 | 299,9 | 10,60 | 286,2 | 3 | 17,2 | 5 | 8,77 | 276,0 | 1 | 2,8 | 5 | 7,74 |
| ladder | female | 19 | 60,00 | 23,62 | 45,72 | 38,31 | 299,6 | 3,70 | 299,7 | 8,61 | 290,3 | 0 | 0,0 | 3 | 10,64 | 296,6 | 5 | 31,2 | 11 | 9,93 |
| ladder | male | 4 | 60,00 | 24,95 | 32,96 | 39,73 | 299,5 | 9,92 | 299,5 | 8,98 | 287,7 | 1 | 3,6 | 5 | 7,08 | 299,1 | 5 | 40,2 | 7 | 11,64 |
| ladder | male | 7 | 60,00 | 42,75 | 26,27 | 40,18 | 300,0 | 13,97 | 299,9 | 3,40 | 284,3 | 0 | 0,0 | 4 | 6,93 | 294,6 | 4 | 38,7 | 16 | 9,90 |
| ladder | male | 18 | 60,00 | 32,36 | 24,29 | 24,22 | 299,4 | 9,45 | 299,8 | 15,10 | 298,2 | 0 | 0,0 | 5 | 7,82 | 289,1 | 1 | 8,1 | 6 | 11,20 |
| ladder | male | 21 | 60,00 | 19,80 | 35,19 | 34,95 | 298,5 | 8,41 | 298,3 | 2,55 | 272,4 | 2 | 11,1 | 7 | 6,05 | 294,4 | 4 | 32,6 | 12 | 7,33 |