Mouse handling research papers

National Centre for the Replacement Refinement & Reduction of Animals in Research

Below are links to the original research papers that provide the evidence-base for improved welfare and scientific outcomes with the tunnel handling and cupping methods of picking up mice. We also provide access to papers which validate or use these refined (non-aversive) mouse handling techniques. In each case, a short summary of the key findings is provided, along with notes. We recommend reading the papers in full.

We update this document as new research is published – it was last updated on **25 March 2022**. Please email enquiries@nc3rs.org.uk to alert us to relevant papers.

The original research

Hurst JL, West RS (2010) Taming anxiety in laboratory mice. *Nature Methods* 7: 825-826. doi: 10.1038/nmeth.1500 (full text: bit.ly/2JhgbJb)

Picking up mice by the tail induces aversion and high anxiety levels (i.e. avoidance of the human gloved hand, greater urination and defecation during handling, a higher frequency of protected stretch attend postures, fewer open arm entries and less time spent on the open arms of the elevated plus maze). These responses can be minimised by instead using a tunnel or cupped hands.

The positive effects of tunnel handling and cupping generalise across strains, handlers, and the light/dark phase.

Mice handled by their home cage tunnel or cupping are *much* more willing to approach the handler than those picked up by the tail, even after restraint by the scruff of the neck or lifting by the tail for abdominal inspection. Scruff restraint does not reverse the taming effects of tunnel handling or cupping.

Mice picked up by the tail do not habituate to tail handling.

What was compared?	Schedule of acclimation to handling method	Replication or modification of Hurst & West 2010 handling methods?	Study reliability	Animal characteristics	Cage type	Funders
Tail, tunnel, cupping (Tunnel then cupping was used for one cohort of C57BL/6 mice, producing similar results to tunnel handling: Suppl. Fig. 4, Suppl. Tables 2 & 3) Tunnels were clear acrylic, familiar (home cage) tunnels and were present in all cages Measures: voluntary interaction with handling device; urination and defecation during handling; anxiety in elevated plus maze	Minimum nine daily handling sessions of 2x30s. Acclimation extended variably up to 16 sessions to address specific responses. EPM anxiety tested after seven or nine handling sessions For tail handling, the base of the tail was grasped between thumb and forefinger and the mouse gently lifted onto the opposite gloved hand or laboratory coat sleeve and held there by the tail for 30s before release back into the cage; after 90s handling was repeated Mice handled consistently by one of 11 handlers	N/A	Cages randomised into handling methods and balanced on the cage rack. Order of testing randomised but balanced across methods Blinding used, but not consistently No sample size justification N=47 cages per handling method (BALB/c N=23 cages x 3 methods; ICR N=8 cages x 3 methods; C57BL/6 N=16 cages x 3 methods; tunnel to cup method, N=8 cages of C57BL/6). 298 mice in total	BALB/c, ICR(CD-1), C57BL/6 Males and females 8-10 weeks old at start of testing; 11-15 weeks old at end Housed two per cage (single sex)	Open (MB1)	ASAB, BBSRC, NC3Rs, Wellcome
Caveats: The voluntary interaction test assessed willingness to interact with the handling method, so mice in the tunnel group were tested with a hand holding a tunnel; tail and cupping groups with a hand only. These are considered the appropriate controls for the question: "Does handling method influence willingness to approach the 'device' that animals are handled with?"						

Gouveia K, Hurst JL (2013) Reducing mouse anxiety during handling: Effect of experience with handling tunnels. *PLoS ONE* 8(6): e66401. doi: 10.1371/journal.pone.0066401

Using a tunnel for routine handling reduces anxiety compared to tail handling (as assessed by willingness to approach the handler and behaviour in the elevated plus maze).

This is the case regardless of prior familiarity with the tunnels (i.e. home cage tunnel, or an external tunnel shared between cages with or without prior experience of a tunnel in the cage).

C57BL/6 mice showed a slower habituation to handling by a shared tunnel in comparison to handling by their home tunnel (voluntary interaction with the handling device), suggesting home cage tunnels can further improve response to handling in anxious strains.

What was compared?	Schedule of acclimation to handling method	Replication or modification of Hurst & West 2010 handling methods?	Study reliability	Animal characteristics	Cage type	Funders
Tail v tunnel – either familiar (home cage) tunnel; external tunnel with 1-week experience in the home cage; or external tunnel without experience Tunnels were clear acrylic tunnels Measures: voluntary interaction with handling device; anxiety in elevated plus maze	Nine daily handling sessions of 2x30s each Single handler	Replication	Randomisation not mentioned (but was as per Hurst & West 2010 above) Blinding not used No sample size justification N=8 cages per handling method x strain combination. 128 mice in total.	ICR(CD-1), C57BL/6 Males and females 7-10 weeks old when tested Housed two per cage (single sex)	Open (M3)	NC3Rs (PhD studentship)
Caveats: as above						

Gouveia K, Hurst JL (2017) Optimising reliability of mouse performance in behavioural testing: the major role of non-aversive handling. *Scientific Reports* 7: 44999. doi: 10.1038/srep44999

Mice handled by tunnel and cupping methods showed *substantially* improved performance in a simple behavioural test (habituation-dishabituation paradigm) compared to picking up by the tail. Tail-handled mice showed little willingness to explore and investigate test stimuli, leading to poor test performance that was only slightly improved by prior familiarisation to the test arena.

By contrast, mice handled by tunnel explored readily and showed robust responses to test stimuli, regardless of prior familiarisation or stimulus location (though responses were more variable for cup handling). Handling method therefore has implications for the reliability of performance in behavioural tests.

The positive effects of non-aversive handling can be achieved through normal brief handling during cage cleaning.

What was compared?	Schedule of acclimation to handling method	Replication or modification of Hurst & West 2010 handling methods?	Study reliability	Animal characteristics	Cage type	Funders
Expt. 1 (stimulus location): Tail, tunnel or cupping Expt. 2 (test area familiarity): Tail v tunnel Tunnels were clear acrylic, familiar (home cage) tunnels present in all home cages Measures: voluntary interaction with handling device; exploration of clean arena; habituation-dishabituation response to novel urine stimuli	Expt. 1: 2s handling by assigned method to transfer mice between cages during bimonthly routine cage cleaning from 5 weeks of age until testing at 14 weeks of age. Transfer of mice to and from test arena for four trials Expt. 2: 2s daily handling by assigned method over 10 days from 14 weeks of age. Transfer of mice to and from test arena for habituation and four trials Single handler	Replication, but handling habituation sessions were very brief (only 2s)	Cages randomised to handling methods Blinding not used No sample size justification Expt. 1: N=8 cages per handling method. 48 mice in total Expt. 2: N=8 cages per handling method. 32 mice in total	BALB/c (BALB/cOlaHsd) Females only Expt. 1: 14-15 weeks old when tested Expt. 2: 16 weeks old when tested Housed two per cage	Open (M3)	NC3Rs (PhD studentship)
Caveats: as above						

Papers from other groups that validate or use the improved handling techniques

Ghosal S, Nunley A, Mahbod P *et al.* (2015) Mouse handling limits the impact of stress on metabolic endpoints. *Physiology & Behaviour* 150: 31-37. doi: 10.1016/j.physbeh.2015.06.021

Mice handled by the cupping method show reduced anxiety-like behaviours in the elevated plus maze, coupled with a reduction in blood glucose levels, compared to mice handled by the tail (Expt. 1).

Cupped mice maintained on a high fat diet for 3 months exhibited improved glucose tolerance compared to tail-handled controls (Expt. 2).

A C57BL/6 cup-massage group showed lower glucose levels following an overnight fast, and decreased anxiety-like behaviours associated with lower stress-induced plasma corticosterone concentration compared to controls picked up by tail but only at cage change (Expt. 3).

The physiological evidence supports better welfare when using the cupping method. The authors also conclude use of handling methods that reduce anxiety will mitigate the confounding effect of stress on the interpretation of metabolic endpoints.

What was compared?	Schedule of acclimation to handling method	Replication or modification of Hurst & West 2010 handling methods?	Study reliability	Animal characteristics	Cage type	Funders
Expt. 1: Tail v cupping Expt. 2: Tail v cupping Expt. 3: Tail v cup-massage Measures: anxiety in elevated plus maze; plasma glucose and corticosterone responses; glucose tolerance test or fasted blood glucose	Tail and cupping: ten sessions of 2x30s over 2 weeks Cup-massage: at least daily for 5 days, then approx. twice in the following week. Control group not handled except for pick up by tail during weekly cage changes	Replication for tail and cupping methods Cup-massage is a new method	Randomisation not mentioned Blinding not mentioned No sample size justification N=10 mice per handling method (but N=5 for tail-handled in Expt. 3); number of cages not specified. 20 mice in total for Expts. 1 & 2, 15 mice in total for Expt. 3	Expts. 1 & 2: CD1.C57BL/6 Expt. 3: C57BL/6 Males only Mice aged 10-19 weeks (Expt. 1), 6-7 months (Expt. 2), 10-21 weeks (Expt. 3) Housed two per cage for tail and cup; single housing during cup-massage training	Not stated	NIH (First author also holds American Heart Association fellowship and Albert J Ryan Foundation award)
Caveats: Potential pseudoreplication (the experimental unit is arguably the cage, not the animal). In Expt. 3, the control tail group were only handled briefly during cage changes, so differences could be due to cup-massage method or frequency of handling. No comparison made between cupping and cup-massage.						

Miller AL, Leach MC (2015) The effect of handling method on the mouse grimace scale in two strains of laboratory mice. *Laboratory Animals* 50(4): 305-307. doi: 10.1177/0023677215622144

The mouse grimace scale (MGS) uses changes in facial expression to assess pain. No significant difference in MGS scores were found between mice handled using a tunnel compared with the tail. (No interventions were applied other than routine husbandry and handling).

These methods of handling are therefore not confounding factors when establishing baseline MGS scores.

The authors recommend that tunnel handling should be used when handling mice to minimize anxiety and doing so will have no impact on the implementation of the MGS.

What was compared?	Schedule of acclimation to handling method	Replication or modification of Hurst & West 2010 handling methods?	Study reliability	Animal characteristics	Cage type	Funders
Tail v tunnel Tunnels used were cardboard, familiar (home cage) tunnels Measures: mouse grimace scores in a 3 min session after handling	All routine husbandry over a 1-week period used either tail or tunnel handling using the Hurst & West 2010 methods	Replication, but duration of handling not indicated	Cages randomised to handling methods Blinding used where possible No sample size justification N=8 mice per handling method. 16 mice in total	CBA, DBA/2 Males only Age not stated Housed four per cage	IVC (Type II)	NC3Rs
Caveats: Potential pseudoreplication (the experimental unit was arguably the cage; however individual animal data were assessed with one cage per method)						

Novak J, Bailoo JD, Melotti L, *et al.* (2015) An exploration based cognitive bias test for mice: effects of handling method and stereotypic behaviour. *PLoS ONE* 10(7): e0130718. doi: 10.1371/journal.pone.0130718

This study aimed to validate an exploration based cognitive bias test, using two different handling methods, tail and cupping.

Mice from both handling groups displayed a similar pattern of exploration in the radial arm maze, suggesting no difference in affect (but see Caveats).

The authors speculate the test may not be appropriate or sensitive enough to detect changes in affective state, the effects of handling may have been too subtle to induce changes in maze performance, or the mice may have habituated to the handling methods.

What was compared?	Schedule of acclimation to handling method	Replication or modification of Hurst & West 2010 handling methods?	Study reliability	Animal characteristics	Cage type	Funders
Tail v cupping Measures: discrimination between positively and negatively cued arms of radial maze; response to ambiguous arms; home cage activity and stereotypy	Daily handling for 15 weeks from 3 weeks old (30s of tail handling or cupping); 6 weeks with only weekly cage change and health check; new experimenter then handled the mice daily for 9 days during testing	Replication, but mice were handled daily for many weeks	Animals randomised to different handling methods Blinding used where possible No sample size justification N=14 mice per handling method. 28 mice in total	CD-1; Females only Habituated from 3 weeks old, with spatial discrimination training and tested at 26-27 weeks old Housed two per cage	Type II	DFG, ERC

Caveats: The mice were housed with one tail and one tunnel handled mouse per cage. If stress is communicated amongst cage-mates, this might confound the results.

This is a novel cognitive bias test that has not been validated, for example using antidepressants as a positive control. The authors suggest that future work should do this.

Cupping may have increased the number of arms entered in the maze during training ($F_{1,21}$ Handling = 4.50, which is larger than the critical F-value of 4.325; p value would be 0.046).

Ono M, Sasaki H, Nagasaki K, *et al.* (2016) Does the routine handling affect the phenotype of disease model mice? *Japanese Journal of Veterinary Research* 64(4): 265-271. doi: 10.14943/jjvr.64.4.265

This study compared the impact of handling methods on the severity of symptoms in the ICGN glomerulonephritis mouse – a model for the human idiopathic nephrotic syndrome. Female tail-handled mice showed higher glomerulus lesion scores than controls (approximately 2.4-fold higher).

In a second experiment, plasma corticosterone levels were higher in tail-handled C57BL/6 male mice compared to controls, and higher in tunnel-handled BALB/c male mice compared to tail-handled and controls.

What was compared?	Schedule of acclimation to handling method	Replication or modification of Hurst & West 2010 handling methods?	Study reliability	Animal characteristics	Cage type	Funders
Expt. 1: Tail v tunnel (voluntary entry) v cupping v undisturbed controls Expt 2: Tail v tunnel (voluntary entry) v undisturbed control Tunnels were transparent red, polycarbonate, familiar (home cage) tunnels Measures: Expt. 1: kidney histopathology; blood haematocrit; creatinine and urea nitrogen Expt. 2: plasma corticosterone 20 min after handling	Handled 5 days per week for 20s over 4 weeks Mice were picked up by their tails and lifted up (tail method); lifted up after voluntarily entering into the plastic tunnel (tunnel method); or hand-scooped and lifted up by both hands moving freely over the palm (hand method). In each case, mice were lifted up for 20s. Control mice were undisturbed except for tail handling for a short period during weekly cage changing	Modification Tunnel handling varies from Hurst & West 2010, where the mouse is guided into the tunnel from behind with the hand In this study, BALB/c mice took more than 5 min to enter tunnels voluntarily during handling, C57BL/6 took 10-15s to enter voluntarily. Tail handled mice were not supported during the 20s lifting	Randomisation not mentioned Blinding not mentioned No sample size justification Expt. 1: N=5 mice per sex and handling method. 40 mice in total Expt. 2: N=5 males per handling method x strain combination. 30 mice in total	Expt. 1: ICGN glomerulonephritis mouse, males and females Expt. 2: C57BL/6 and BALB/c, males only 8 weeks old when tested Two or three mice per cage (single sex?)	IVC	JSPS KAKENHI (Grants-in-Aid for Scientific Research No. 25925011), the Ministry of Education, Culture, Sports, Science and Technology, Japan.
Caveats: Potential pseudoreplication (the experimental unit is arguably the cage, not the animal). Requirement for voluntary entry to tunnels (in contrast to Hurst & West 2010 and recommended practice) led to substantially longer disturbance of mice during handling, particularly among BALB/c mice. During cage changing, the undisturbed control mice were actually handled by the tail. Number of animals of each sex in each group/cage not mentioned (but sex differences are reported).						

Wilde E, Aubdool AA, Thakore P, *et al.* (2017) Tail-cuff technique and its influence on central blood pressure in the mouse. *Journal of the American Heart Association* 6(6): e005204. doi: 10.1161/JAHA.116.005204

This study investigated the effects of tail-cuff plethysmography on central blood pressure (BP), heart rate (HR) and core body temperature (BT) in C57Bl/6J mice, as measured by telemetry. The mice were handled by one of three methods in turn for delivery to the tail-cuff restraint tube.

The effect of handling on BP and HR did not differ between the three handling methods (nor between handler's sex and habituation [to repeated tail-cuff measurements]).

The authors concluded this was because the various handling methods preceded restraint in the tail-cuff restraint tube, which is associated with a high level of stress.

What was compared?	Schedule of acclimation to handling method	Replication or modification of Hurst & West 2010 handling methods?	Study reliability	Animal characteristics	Cage type	Funders
Expt. 1: Tail v tunnel v tail-cup Expt. 2: female vs male handler Expt. 3: restraint, heating and handling interventions Expt. 4: angiotensin infusion (hypertension model) v saline Tunnels used were cardboard, familiar (home cage) tunnels Measures: blood pressure and heart rate measured by telemetry	No information is given about how mice were handled until the experiment began (nor about their origin) Expt. 1: Tail-cuff plethysmography was carried out on 6 mice for 5 consecutive days using each handling technique, with 6 days of rest given between each handling method. Duration of handing was typically 10-30s followed by 30-60s of handling to place the animals in the restraint tubes and 5 min acclimatisation to the tube before each recording session Expt. 2: Male or female researchers handled mice on consecutive days for tail-cuff plethysmography by their own preferred technique (method not reported) Expts. 3 & 4: No information on handling method given	Modification Tail-cup is a new method, involving grasping by the tail and scooping into the palm Few details are given of tunnel handling technique used	Mice randomised to handling methods Blinding not mentioned No sample size justification Handling method order semi-randomised for Expt. 1; method not indicated for other experiments Expt. 1: N=6 male mice in total; data averaged over 5 days of recording per mouse and method Expt. 2: N=3 male and N=4 female researchers; alternation of individual researchers unclear Expt. 3: N=4 mice, mixed sexes, for a series of interventions Expt. 4: N=12 mice	C57Bl/6J Males and females 13-22 weeks old when tested Singly housed Telemetry implanted	Assumed IVC ("filtered positive pressure ventilation")	NC3Rs (PhD studentship), BHF, King's College London

Caveats: Not comparable to other studies comparing handing methods, for several reasons:

Did not use cupping method used by other authors.

Different methods were not implemented until mice were 13-15 weeks old, and then mice were not handled by a consistent method but swapped between methods on a weekly basis for 3 weeks. Crossover design assumes the mice do not habituate to the handling method.

Those mice picked up in a tunnel are presumed to have been tail handled for 12 weeks prior to the experiment, plus daily tail handling for a further 5 or 10 days (4/6 mice) during the experiment, before mice were picked up in a tunnel for assessment. Responses were then averaged over 5 days of testing, going from completely naïve to 5 days tunnel handling experience.

Only 6 mice were used to examine effect of handling method on BP and HR. This compromises interpretation of any non-significant differences (as reported) given the very low power to detect any differences and absence of power tests.

Occlusion cuff for recording was placed at the base of the tail.

Mice were singly housed after telemetry implantation.

Clarkson JM, Dwyer DM, Flecknell PA, *et al.* (2018) Handling method alters the hedonic value of reward in laboratory mice. *Scientific Reports* 8: 2448. doi: 10.1038/s41598-018-20716-3

Tail-handled mice showed more anhedonic responses (consumed less sucrose, in smaller licking bouts) compared to tunnel-handled mice, indicating a more depressive-like state.

This finding that tail handling reduces responsiveness to reward has scientific as well as animal welfare implications.

The study again replicated Hurst's findings at a different research institution: tail-handled mice interacted substantially less with the handler and showed greater levels of anxiety in behavioural tests (elevated plus maze – EPM; open field test – OFT) compared to tunnel-handled mice.

What was compared?	Schedule of acclimation to handling method	Replication or modification of Hurst & West 2010 handling methods?	Study reliability	Animal characteristics	Cage type	Funders
Tail v tunnel Tunnels used were clear Perspex, familiar (home cage) tunnel Measures: consumption of sucrose at 4% and 16% concentration; lick cluster size; voluntary interaction with handling device; anxiety in EPM and OFT	Nine daily 2x30s handling sessions, then handling to transfer mice during testing and training (day 10 for EPM, days 15-26 and 29-33 for sucrose tests, day 36 for EPM) and at weekly cage cleaning	Replication	Cages randomised to handling methods Blinding used where possible No sample size justification N=16 mice per handling method. 32 mice in total	C57BL/6J Males only 7 weeks old at start, 12 weeks old at end Two mice per cage	Open (M3)	BBSRC
Caveats: Potential pseudoreplication (the experimental unit was arguably the cage, however for most of the tasks individual animal data were assessed).						

Nakamura Y, Suzuki K (2018) Tunnel use facilitates handling of ICR mice and decreases experimental variation. *Journal of Veterinary Medical Science* 80(6): 886-892.
doi: 10.1292/jvms.18-0044

This study investigated whether tunnel handing can improve welfare during persistent stress from repeated oral drug administration. During 1 week of handling acclimation and 1 week of gavage, voluntary interaction with experimenter (handling device) was much greater in tunnel-handled mice compared to mice picked up by the tail.

Tunnel-handled mice were also easier to handle (as assessed by an independent rating scale), which the authors suggest could reduce workload for experimenters, and defecation and urination during handling were reduced compared to mice picked up by the tail.

Mice handled by the tunnel showed greater exploration in the open field test (OFT) and elevated plus maze (EPM), and reduced anxiety in the OFT (but not EPM) compared to tail-handled mice.

Variation in the behavioural test data was reduced in tunnel-handled mice compared to tail-handled, after intraperitoneal administration of saline (placebo) or diazepam, suggesting tunnel-handling might decrease variation in pharmacological tests.

No differences were found between sexes.

What was compared?	Schedule of acclimation to handling method	Replication or modification of Hurst & West 2010 handling methods?	Study reliability	Animal characteristics	Cage type	Funders
Tail v tunnel Tunnels used were clear, acrylic tunnels not present in home cage Measures: urination and defecation during handling; ease of handling rating; voluntary interaction with handling device; open field test; elevated plus maze test	Seven sessions of daily handling for 2x30s, followed by capture by assigned method and daily oral gavage of saline when restrained by scruff for seven sessions, then handled for OFT and EPM tests after intraperitoneal administration of diazepam or saline	Replication	Cages were randomised into two handling methods Order of handling of cages balanced across sessions Blinding not mentioned No sample size justification N=40 mice per handling method. 80 mice in total	Jcl:ICR Males and females 3 weeks old at start of handling, daily handling sessions started at 4 weeks old, 6 weeks old at end of study Housed four per cage (single sex?)	Open	Funder not specified
Caveats: Potential pseudoreplication (the experimental unit was arguably the cage, particularly during voluntary interaction tests; however individual animal data were assessed). Animals are juvenile to subadult (4-6 weeks old). Tail-handled mice showed significantly higher coefficient of variation on the EPM compared to tunnel-handled mice, and the different measures of anxiety conflicted with each other – suggests a potential problem in the EPM test (e.g. low entries but high time on open arms and high coefficient of variation can result from 'freezing' behaviour in some animals).						

Roughan J, Sevenoaks T (2018) 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. doi: 10.30802/AALAS-JAALAS-18-000057 (Full text: bit.ly/2uauUeF)

This study investigated if handling method differentially affected anxiety before assessing responses to restraint and tattooing using the Labstamp device, or ear-tagging.

Tunnel-handled mice showed significantly greater voluntary interaction with the handler's hand (less fearful) compared to tail-handled mice, increasing substantially over the study period in tunnel-handled mice despite experience of restraint, tattooing or ear tagging.

The apparent anti-neophobic effect of tunnel handling was long lasting and robust.

Tunnel-handled mice were more active across all assessment times. Change in body weight from before to after handling acclimation was no different between the tunnel- and tail-handled groups. (Handling method also had no significant impact on response to tattooing/restraint).

Grimace scale scores were higher in tail-handled compared to tunnel-handled mice after handling acclimation and throughout subsequent testing.

The data suggest tunnel handling overcame anxiety-like behaviour following restraint, tattooing or ear-tagging.

What was compared?	Schedule of acclimation to handling method	Replication or modification of Hurst & West 2010 handling methods?	Study reliability	Animal characteristics	Cage type	Funders
Tail v tunnel Restraint v tattooing v ear tagging Tunnels were clear, Plastiglas tunnels. Each cage had its own tunnel, but these were not left in situ. Home cages had cardboard tubes Measures: light/dark conditioned place preference; voluntary interaction with gloved hand; novel arena; mouse grimace score; tail inflammation; body weight change; agitation during tattooing or restraint	For first 2 weeks, all mice tail handled during weekly cage clean, weighing every 2 days and pre-acclimation testing Daily handling by assigned method for seven sessions (tunnel handling was 60s, tail handling 10s), then for pick up during post-acclimation testing, procedure (tattooing, restraint or ear tagging), post-procedure testing and cage cleaning	Modification Tunnel handling for 2x30s but not with home cage tunnel Tail handling for 1x10s	Animals were randomly allocated for restraint or tattooing, and tail or tunnel handling Rack placement of each cage and order of treatments/procedures was counterbalanced Blinding used where possible No sample size justification (based on previous work) N=16 mice per handling method (half experiencing tattooing and half restraint and ear tags). 32 mice in total	BALB/cAnCrl. Males and females 12-19 weeks old when tested Housed four per cage (single sex)	IVC Type II	Newcastle University
Caveats: Potential pseudoreplication (the experimental unit is arguably the cage, not the animal). The tails of tunnel handled mice were more inflamed following tattooing, possibly due to improved tail circulation through lack of tail handling.						

Mertens S, Vogt MA, Gass P, *et al.* (2019) Effect of three different forms of handling on the variation of aggression-associated parameters in individually and group-housed male C57BL/6NCrl mice. *PLOS ONE* 14(4):e0215367. doi: 10.1371/journal.pone.0215367

This study investigated the effect of three different handling methods (tail, forceps and tunnel) on aggression-associated parameters in single- and group-housed male C57BL/6NCrl mice over 8 weeks.

The authors report that picking up mice by the tail with forceps appears to stimulate aggressive behaviour within groups of familiar mice more than picking them up by tail between fingers or using a handling tunnel.

Overall, tunnel handled mice displayed reduced anxiety (light/dark box test and social novel-object test, but not open field test). The authors conclude that tunnel handling should be used when minimization of anxiety in experimental mice is desired.

What was compared?	Schedule of acclimation to handling method	Replication or modification of Hurst & West 2010 handling methods?	Study reliability	Animal characteristics	Cage type	Funders
Tail v forceps v tunnel Tunnels used were transparent, polycarbonate tunnels Measures: Behavioural measures included aggression (spontaneous aggression and resident intruder test), anxiety (open field test, light/dark box, number of fecal boli), sociality (social novel-object test), reaction to thermal pain (hotplate test) and well-being (nest building assay) Clinical parameters included: barbering and bite wounds, body weight, blood glucose levels, body temperature, stress-induced hyperthermia, fecal corticosterone metabolites (FCM) and final organ weight	Handled by one female experimenter four times per week over 8 weeks, and in week 9 for behavioural tests All restrained by tail each week for tail venepuncture and 2 x rectal temperature assessment	Modification of tail and tunnel methods Mice were picked up and placed on cage wire lid then returned to home cage (duration not indicated) No tunnels present in home cage; not stated whether a clean or shared handling tunnel was used Handling by forceps was not used by Hurst & West 2010	Allocation of animals to treatment groups was haphazard (not random) Blinding not mentioned No sample size justification N=6 cages per treatment (handling x housing method); 72 mice in total. For behavioural tests, N=6 mice per treatment	C57BL/6NCrl; males only Habituated from 3 weeks old, with measurements taken at 10, 11 and 12 weeks old Housed three per cage (18 cages) or individually (18 cages)	Macrolon II (370cm^2)	Deutsche Forschungsgemeinschaft (DFG)
Caveats: Conclusions are drawn from a small sample size (N=6 cage groups or mice per treatment) which compromises interpretation of the data given the low power to detect differences, particularly in behavioural studies (e.g. aggression after cage cleaning observed in 0/6 tail-handled, 1/6 tunnel-handled [once in 7th week], and 2/6 forceps-handled groups [from 5th and 6th week until the end of recording]). For many measures, animals were assessed weekly over 8 weeks but separate data analyses often found differences only in a single week, from which a broad conclusion is drawn (p values do not appear to be corrected for multiple comparisons). Repeated measures in non-parametric tests appear to be treated as independent data points. Degrees of freedom for ANOVAs appear incorrect.						

Gouveia K, Hurst JL (2019) Improving the practicality of using non-aversive handling methods to reduce background stress and anxiety in laboratory mice. *Scientific Reports* 9: e20305. doi: 10.1038/s41598-019-56860-7

Very brief handling (2s) is sufficient to familiarise mice with tunnel handling, even when only experienced during fortnightly cage cleaning. Brief but more frequent handling is needed for familiarisation with cupping. Picking up mice by the tail induces strong aversion even when handling is brief and infrequent.

Experience of repeated immobilisation (scruff restraint) and subcutaneous injection does not reverse the positive impact of tunnel handling for avoiding handling stress.

Replacing tail handling with tunnel handling during routine cage cleaning and procedures provides a major refinement. The time cost for familiarising mice to the non-aversive methods is negligible.

What was compared?	Schedule of acclimation to handling method	Replication or modification of Hurst & West 2010 handling methods?	Study reliability	Animal characteristics	Cage type	Funders
Expt. 1, stage 1 (hold duration): Tail v tunnel v cupping Measures: Voluntary interaction immediately before and after handling on 1st and 5th daily handling sessions. Open field test two days after 5th handling session Expt. 1, stage 2 (response to scruff restraint): Tail v tunnel v cupping Measures: Voluntary interaction immediately before and 60s after first and third scruff, and again 24 hrs after third scruff. Elevated plus maze test two days after third scruff Expt. 2 (handling frequency): Tail v tunnel v cupping Measures: Voluntary interaction immediately before and after 1st, 4th and 5th cage clean. Reluctance to be handled after 1st, 4th and 5th cage clean tested immediately after voluntary interaction, in the old home cage. Elevated plus maze test two days after 5th cage clean Expt. 3 (subcutaneous injection): Tail v tunnel Measures: Voluntary interaction immediately before and after 10th handling session, 1st injection or control lift, 5th injection or control lift. Modified open field test after 1st and 5th injection or control lift	Expt. 1, stage 1: Mice were picked up by their assigned method (tail, tunnel or tail) and held above their home cage for 2, 10, 30 or 60s for five daily handling sessions. Mice assigned to 60s daily handling were picked up twice and held for 30s each time Expt. 1, stage 2: Mice were scruffed for 10s for three consecutive days. Before scruffing, animals were picked by the method assigned during Expt. 1, stage 1 Expt. 2: All mice were handled for 2s at fortnightly cage clean by their assigned method for five cages cleans. Half of the mice received an additional nine daily 2s handling by the assigned method between 4th and 5th cage cleans Expt. 3: Mice were handled for 2s daily by their assigned method (tail or tunnel). 1st injection or control pick up at 15-16 weeks of age. 2nd – 5th injection (daily injections) or control pick up at 21-22 weeks Single handler for all experiments	Modification (shortened hold duration)	Cages randomly allocated to handling method/ treatment. Order of testing randomly determined Blinding not achievable Sample size justification for Expt. 1 and Expt. 3 cites previous papers. Details of calculations not reported in this manuscript Expt. 1: n=8 cages per hold duration for tunnel and cup method, and n=4 cages per hold duration for tail method (two mice per cage, single sex pairs, equal number of each sex tested for each handling method and hold duration). 160 mice in total Expt. 2: n=8 cages per frequency and handling method (equal number of each sex). 96 mice in total Expt. 3: n=10 cages per group (females only). 80 mice in total	Expt. 1 stage 1: C57BL/6JOla/ Hsd, males and females, housed in single sex pairs, 7-8 weeks of age when tested Expt. 1, stage 2: As for stage 1, 8-9 weeks of age when tested Expt. 2: BALB/ cOlaHsd, males and females, housed in single sex pairs, from 5-6 weeks of age until 15-16 weeks of age Expt. 3: BALB/ cOlaHsd mice, females, housed in pairs, from 13-14 weeks of age until 21-22 weeks of age	Open (M3)	NC3Rs (PhD studentship)
Caveats: As for Hurst & West 2010 above.						

Doerning CM, Thurston SE, Villano JS, *et al.* (2019) Assessment of mouse handling techniques during cage changing. *JAALAS* 58(6): 767-773. doi: 10.30802/AALAS-JAALAS-19-000015

This study looked at the effect of handling methods (tail - gloves, tail - forceps, home cage tunnel, novel tunnel, plastic cup) on efficiency of cage change by one handler, biosecurity, breeding and animal welfare.

Time taken to change cages was significantly faster for the gloved hands and forceps groups as compared with the other methods, for every timepoint (but see caveats below). The speed of cage changing did not increase consistently for any handling method. Contamination levels were the same for all handling methods; however, when the different handling tools (forceps, novel tunnel and gloves) were disinfected, gloves had the highest levels of microbial and organic contamination.

Home tunnels did not inhibit the caretaker's ability to perform cageside health checks. The overall health status and breeding success (pups per litter) where similar between the refined handling and tail handling groups.

What was compared?	Schedule of acclimation to handling method	Replication or modification of Hurst & West 2010 handling methods?	Study reliability	Animal characteristics	Cage type	Funders
Expt. 1: Tail - gloves v tail - forceps v home cage tunnel v novel tunnel v plastic cup Measures: Time taken to change each cage. There were 14 cages per group and the experiment was repeated four times with a minimum of four weeks between timing assessments Expt. 2: Tail - gloves v tail - forceps v home cage tunnel v novel tunnel v plastic cup Measures: Microbial and organic contamination levels on the surface of handling tools throughout cage change process and after sanitization, using an ATP-based assay Expt. 3: Tail - gloves v home cage tunnel v novel tunnel v plastic cup Measures: Retrospective analysis of health status and breeding success (number of pups per litter)	No details are given regarding acclimation, although the mice were handled using their allocated handling method throughout the study, i.e. including the four-week intervals (minimum) between the four experimental sessions. During these interval periods, handling techniques were used once every two weeks, which is when cage-change sessions occurred. A single handler was used for all experiments.	Modification Few details are given of the tunnel handling technique used compared to Hurst & West 2010 Handling by forceps was not used by Hurst & West 2010 Cup handling here refers to the use of a plastic cup, unlike Hurst & West 2010 where cupping refers to cupped hands	Order of testing randomly determined but no randomisation of mice into handling groups Blinding not mentioned No sample size justification No justification for choice of statistical analyses. Primary outcome measure not identified Groups of animals compared were of different strain, sex, age and cage density. Additional variation introduced to the data due to mouse turnover in breeding cages.	70 cages of mice of varied strains, ranging from neonatal pups to animals six months old (N = 242 adults) Sex and cage densities varied between cages Housed either singly or in groups of as many as five adult mice per IVC cage Density of breeding cages varied during study	IVC (P/NV IVC, Allentown)	University of Michigan Animal Care and Use Office Quality Compliance Assurance and Validation Fund. Mouse colony funded by the JDRF and National Institute of Diabetes and Digestive and Kidney Diseases

Caveats: The caretaker was naïve to refined (tunnel) handling but experienced in tail-forceps and tail-glove handling, which will have affected how quickly cage change was performed for the different handling groups and the rate with which handling performance changed over the duration of the experiment.

There was no replication of handler, so findings may not generalise to other individuals.

Handling techniques were used infrequently (~ every two weeks, during cage changing and experimental sessions), which may have delayed or negated the acclimation effect on the animals.

The total number of animals (i.e. presence of pups) in the breeding pair cages is not specified, which adds a potential confounder when comparing the speed of cage changing.

Not clear if all cages for all handling methods were changed on the same day. If cages with different handling methods were changed on different days, this adds a potential confounder.

Not clear which strains were used in this study, and if different strains were evenly distributed across the different handling method groups. If not evenly distributed, this becomes a potential confounder. Same for animal sex and age.

Health and breeding assessment data from animals in the forceps group was not included.

Sanitisation duration for gloves ("dipped" in solution) was different to the other objects (2 min) and much shorter than the recommended contact time (5 min), making the biosecurity results unreliable.

Ueno H, Takashi Y, Suemitsu S, *et al.* (2020) Effects of repetitive gentle handling of male C57BL/6NCrl mice on comparative behavioural test results. *Scientific Reports* 10: 3509. doi: 10.1038/s41598-020-60530-4

Repeated daily capture of mice by the tail followed by placing on an experimenter's hand unrestrained for 30s for 21 days led to a gain in body weight compared to mice that were not handled repeatedly but only picked up by the tail during cage changes; and allowed mice to become more likely to interact with a water-filled glove in a habituation test.

In behavioural testing, mice acclimatised to daily tail capture followed by 30s on an experimenter's hand demonstrated reduced anxiety to high altitudes (elevated plus maze) and improved spatial cognitive functions (Y-maze test), compared to mice that were not acclimatised to this daily handling procedure. These differences were not observed in any other behaviour test for anxiety, depression-like behaviour or social hierarchy.

What was compared?	Schedule of acclimation to handling method	Replication or modification of Hurst & West 2010 handling methods?	Study reliability	Animal characteristics	Cage type	Funders
Repeated handling acclimation v no handling acclimation for 3 weeks. Behavioural tests performed within at least a day of each other, from day 22 onwards, with two behavioural tests per day Measures: Habituation test: number of entries into habituation area (water-filled unfamiliar glove placed inside unfamiliar cage), distanced travelled and time spent in habituation area General characterisation: body weight; grip strength and latency to fall from rotarod Behavioural tests: elevated plus maze test; light/dark transition test; open-field test; Y-maze test; sociability test; tail suspension test; forced swim test; passive avoidance test	Daily for 21 days the handled mice were removed from the cage by grasping the base of the tail and placing the mouse on the experimenter's hand, where it was allowed to move freely for 30s. The experimenter's hand was held 50cm above cage height. After 30s the hand was moved into the cage and the mouse allowed to jump off the hand. Non-handled mice were not handled by the experimenter, except for cage changes, during this same period. A single (male) handler was used for all experiments.	Modification. Mice picked up by the tail were placed unrestrained on the hand and allowed to explore the hand for 30s. This represents a combination of the tail and cupping methods used by Hurst & West The authors refer to Hurst & West's cupping method incorrectly – this involves picking mice up on cupped hands without tail restraint, not use of cups. The authors also fail to understand that the tail can be touched during the conduct of subsequent dosing and sampling procedures when using refined handling methods, and that mice picked up in a tunnel are tipped out onto the hand or other surface to conduct procedures	Experimental unit not identified explicitly. Implicitly the paper identifies it as the animal (based on both the manuscript text and the way the F-statistic is reported) Order of behavioural testing of mice was randomised; however, not clear if this refers to animals or handling groups No explanation if randomisation of animals into handled or non-handled groups is blocked by cage – how this was done will affect the reliability of results Blinding not mentioned No sample size justification No justification for choice of statistical analyses N=10 mice per handling experience group. 20 mice in total	C57BL/6N Males only 13-14 weeks of age when tested Housed in groups of five	Open plastic cage 20 x 30 x 20 cm with stainless steel lid	Grant-in-Aid from the Sanyo Broadcasting Foundation and from the Okayama Medical Foundation

Caveats: The handled and non-handled groups differed in the frequency and duration of handling experience, and in the experience of being held on the hand. It is not possible to interpret which of these factors, or a combination, were responsible for differences between groups.

As no alternative handling method (e.g. tunnel handling or cupping) was used for comparison, the study provides no insight into the impact of different handling methods.

'Repeated handling' acclimatisation procedure involved daily capture by tail before placement on experimenter's hand for 30s at 50cm above cage height. This sequence of events may have negatively impacted welfare of the 'repeated handled' mice due to the stress of daily tail capture, whilst also habituating them to height, introducing a potential confounder for the elevated plus maze test.

Potential pseudoreplication (the experimental unit is arguably the cage, not the animal).

Only one sex used, limiting the generalisability of the results.

No enrichment structures in the cage, which could negatively impact animal welfare and affect behavioural observations.

The belief that immobility in the forced swim test measures depressive-like behaviour is contentious and not supported by evidence (Stanford 2020).

Clarkson JM, Leach MC, Flecknell PA, *et al.* (2020) Negative mood affects the expression of negative but not positive emotions in mice. *Proceedings of the Royal Society B* 287: 20201636. doi: 10.1098/rspb.2020.1636

Tail-handled mice demonstrated increased anxiety in the elevated plus maze (EPM) and open field test (OFT) compared with tunnel-handled mice. They also showed an anhedonic response (reduced sucrose consumption) compared with tunnel-handled mice, indicating a depressive-like state. Tail-handled mice also showed increased expression of a discrete negative emotion (disappointment), meaning that they were less resilient to negative events. However, their capacity to express a discrete positive emotion (elation) was unaffected relative to control mice. Finally, tail-handled mice had much less voluntary interaction with the handler compared with tunnel-handled mice and had larger adrenal glands, indicating a chronic stress response in tail-handled mice.

These results again replicate both this group's and Hurst's previous findings that tail-handled mice interact less with the handler and display signs of anxiety and depression in behavioural tests compared with tunnel-handled mice.

What was compared?	Schedule of acclimation to handling method	Replication or modification of Hurst & West 2010 handling methods?	Study reliability	Animal characteristics	Cage type	Funders
Tail v tunnel Tunnels were clear Perspex Measures: anxiety in EPM and OFT; voluntary interaction with handler; sucrose consumption (anhedonia and depression); lick cluster size and response to changes in sucrose reward value ("disappointment" or "elation"); size of adrenal glands as an indicator of chronic stress	Mice were handled twice daily (for 30s with a 60s interval) by their allocated method (tail or tunnel) for 9 days Mice were also handled by their designated method for routine husbandry, transferring for behavioural testing (day 10 EPM, day 33 OFT), and prior to the voluntary interaction tests on days 1, 5, 9, 19 and 27 Not clear whether a single handler was used	Replication	Cages randomised to handling method Randomisation not mentioned in allocation to contrast experiment treatment groups. Not clear if cages or individual mice were allocated to contrast experiment groups Order of testing of tail-handled and tunnel-handled mice counterbalanced throughout the day. Testing cohorts balanced by handling method and test group. Blinding not mentioned No sample size justification N=32 mice per handling method (missing datapoints for EPM). N=8 mice per treatment and handling method combination. 64 mice in total	C57BL/6 Males only Approximately 7 weeks on arrival (estimated ~ 9 weeks at the beginning of experiments) Housed two per cage	Open (M2)	BBSRC

Caveats: Potential pseudoreplication (arguably the experimental unit is the cage; cage was used as the experiment unit for voluntary interaction, but individual animal data was analysed for all other measures).

Only one sex used, limiting the generalisability of the results.

Missing data points mentioned, with no explanation of why the data were missing / the criteria used to exclude data (and if the exclusion criteria were decided *a priori*).

Henderson LJ, Dani B, Serrano EMN, *et al.* (2020) Benefits of tunnel handling persist after repeated restraint, injection and anaesthesia. *Scientific Reports* 10: 14562.
doi: 10.1038/s41598-020-71476-y

Experience of stressful procedures does not reverse the positive impact of tunnel handling on animal welfare. After repeated scruff restraint, intraperitoneal injections or short isoflurane anaesthesia, tunnel-handled mice showed increased willingness to interact with a handler, and reduced anxiety in standard behavioural tests compared with tail-handled mice.

What was compared?	Schedule of acclimation to handling method	Replication or modification of Hurst & West 2010 handling methods?	Study reliability	Animal characteristics	Cage type	Funders
Expt. 1 (response to scruff restraint): Tail v tunnel Measures: voluntary interaction (VI) on day 1 and day 5 of handling, followed by VI on day 9 (after 4 days of scruff restraint); elevated plus maze (EPM) and open field test (OFT) on days 10 and 11 Expt. 2 (response to intraperitoneal injection): Tail v tunnel Measures: voluntary interaction (VI) on day 1 and day 5 of handling; VI immediately after injection (days 7, 11 and 15) and on day after each injection (days 8, 12 and 16); OFT on day 6 and EPM on day 19 Expt. 3 (response to isoflurane anaesthesia): Tail v tunnel Measures: as for Expt. 2 but after anaesthesia rather than injection Tunnels were clear Perspex	Mice were not handled regularly before experimentation, but if handling was necessary, then the assigned method was used Expt. 1: twice daily handling (for 30s with a 60s interval) by the assigned method (tail or tunnel) for 5 days before restraint was introduced Expts. 2 & 3: mice had been handled in the previous experiment; however, several weeks passed between experiments when mice were not handled daily. Mice were handled daily by the assigned method for 5 days prior to undergoing any procedures Each experiment used one female handler. Mice were handled by one male handler for anaesthesia.	Replication Voluntary interaction assessed towards gloved hand for both tunnel and tail handled mice	Cages randomly assigned to handling method Handling method and sex counterbalanced across the experimental time Videos blinded for analysis No sample size justification Expt. 1: for VI test, experimental unit was the cage (n=4 cages per restraint and handling method combination). For OFT and EPM, experimental unit was the mouse (n=8 mice per restraint and handing method combination, 48 mice in total) Expt. 2: for VI test, experimental unit was the cage (n=5 cages per handling method). For OFT and EPM, experimental unit was the mouse (n=10 mice per handing method, 20 mice in total) Expt. 3: for VI test, experimental unit was the cage (n=6 cages per handling method). For OFT and EPM experimental unit was the mouse (n=12 mice per handing method, 24 mice in total)	BALB/c Males and females (equal numbers of each at the start of the study) Aged 4-7 weeks on arrival (assumed to be ~17-20 weeks in Expt. 1 and ~30-33 weeks in Expts. 2 & 3) Weight reported in supplementary information (raw data) Housed two per cage in single sex pairs	IVC 420	NC3Rs
Caveats: Potential pseudoreplication for OFT and EPM tests (the experimental unit is arguably the cage, not the animal).						

Sensini F, Inta D, Palme R, *et al.* (2020) The impact of handling technique and handling frequency on laboratory mouse welfare. *Scientific Reports* 10: 17281. doi: 10.1038/s41598-020-74279-3

This study looked at the effects of daily or weekly handling by tail or tunnel (not present in the home cage) on voluntary interaction with the handler, tests of positive and negative affective behaviour, and faecal corticosterone metabolites.

Mice picked up in a tunnel showed much higher frequencies of voluntary touching or climbing on the hand or tunnel compared to tail-handled mice responding to the hand, and less defensive digging, indicating a more positive response to the tunnel.

Handling method influenced measures of positive and negative affect in males but not females. Tail-handled males showed greater immobility in a forced swim test compared to those handled by tunnel, while frequent tail handling also led to reduced burrowing behaviour in males. Females handled weekly by either method had higher faecal corticosterone metabolites than those handled daily.

What was compared?	Schedule of acclimation to handling method	Replication or modification of Hurst & West 2010 handling methods?	Study reliability	Animal characteristics	Cage type	Funders
Tail v unfamiliar tunnel; weekly v daily handling Measures: voluntary interaction (VI) on 1st, 3rd and 6th session of daily handling; immobility in a forced swim test and burrowing (food pellets removed from a plastic bottle) after 10 daily or 2 weekly handling sessions; faecal corticosterone metabolites 12 days from start of handling schedule Tunnels were opaque (material and colour not specified)	All handled by tail initially for weekly cage changes. Handled for 2 x 30s either daily (Monday to Friday) or once per week for two weeks Number and sex of handlers not specified	Modification. Tunnels were not present in the home cages. An opaque tunnel was used of similar diameter to Hurst & West 2010 (not indicated whether tunnel or gloves were cleaned between cages)	Cages randomly assigned to handling method; random assignment to handling frequency not indicated. Blinding not mentioned. No sample size justification n=8 mice of each sex for each handling method and frequency (four groups, 32 males and 32 females in total)	C57BL/6NCrl Males and females (equal numbers of each at the start of the study) Aged 8-9 weeks on arrival, 13-14 weeks at the start of burrow training, 14-17 weeks during handling treatments Body weight reported (no effects of handling treatment) Housed singly (no justification provided)	Macrolon type II	DFG, Italian Ministry of University and Research, Ingeborg Ständer Foundation, German Federal Ministry of Education and Research, Swiss National Science Foundation.

Caveats: Responses of weekly handled mice not reported for interaction tests.

Details of data analyses are unclear. The statistical analysis section states that repeated measures ANOVAs were used where appropriate for repeated tests, but the degrees of freedom provided for tests are not consistent with this. However, where reported differences between tail and tunnel responses were large, this is unlikely to affect the main conclusions drawn.

The belief that immobility in the forced swim test measures depressive-like behaviour is contentious and not supported by evidence (Stanford 2020).

Sandgren R, Grimms C, Waters J & Hurst JL (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. doi: 10.23675/sjlas.v47i0.1083

Using either a tunnel or a plastic ladder to pick up mice, present in the home cage as enrichment devices, reduces their aversion to handling compared to brief pick up by tail.

Anxiety in an open field test was reduced to a similar extent in ladder- and tunnel-handled mice compared with those picked up by tail.

In an elevated plus maze test, mice handled by tunnel showed reduced anxiety compared to those handled by tail, while those handled by ladder showed an intermediate response. The authors note that mice readily left ladders when handled in cages, but delivery to an unfamiliar plus maze was more difficult because mice clung to the ladder. While pick up on a ladder reduces aversion and anxiety during routine handling, tunnels may be better for transferring mice in some situations.

What was compared?	Schedule of acclimation to handling method	Replication or modification of Hurst & West 2010 handling methods?	Study reliability	Animal characteristics	Cage type	Funders
Tail v tunnel v cage ladder Tunnels were opaque plastic (polypropylene) and ladders were clear plastic (polyethylene terephthalate), both present in the home cage and familiar to the mice Measures: voluntary interaction with handling device; anxiety in open field and elevated plus maze	Mice picked up by assigned handling method from arrival. Mice then picked up for 2s each weekday for nine handling sessions; also picked up by assigned method for delivery to anxiety tests the following week. Two female handlers alternating between days; only one of the two performed the anxiety tests	Modification (shortened hold duration) Cage ladders were not investigated by Hurst & West 2010	Cages analysed as experimental unit Cages randomly assigned to handling method Cages arranged on cage rack in balanced design for method and sex Cages and mice handled and tested in randomised order within sexes Automatic video tracking used to measure behaviour in open field and elevated plus maze tests Blinding not mentioned (not achievable in voluntary interaction tests) No sample size justification provided n=8 cages per handling method. 48 mice in total	C57BL/6NRj Males and females Habituated to handling method from 4 weeks old, 7-9 weeks of age when tested Housed two per cage (single sex)	Innocage® disposable IVC (Innovive)	Lund University, University of Liverpool

Redaelli V, Bosi A, Luzi F, *et al.* (2021) Neuroinflammation, body temperature and behavioural changes in CD1 male mice undergoing acute restraint stress: An exploratory study. *PLoS ONE* 16(11): e0259938. doi: 10.1371/journal.pone.0259938

This study explored the effects of restraint in a brightly lit enclosed chamber, similar to those commonly used for substance injection, and whether these might be mitigated via non-aversive handling

Brown adipose tissue temperature was significantly decreased in all handling groups (tail, tunnel and mechanoreceptive) following exposure to restraint and bright light

After the stressful procedure, mice undergoing non-aversive handling (tunnel, mechanoreceptive) showed improved bodyweight maintenance compared to those exposed to tail handling

Mice exposed to mechanosensitive handling spent a significantly longer time on the open arms of the elevated plus maze

Regardless of handling method, exposure to the stressful procedure resulted in a significant reduction in walking and rearing, but not in total distance travelled. All mice also groomed more

No difference among the handling groups was found in nest score, in bone marrow CCL2 and CXCL12 BM content or in brain activated microglia

What was compared?	Schedule of acclimation to handling method	Replication or modification of Hurst & West 2010 handling methods?	Study reliability	Animal characteristics	Cage type	Funders
Tail v tunnel v mechanoceptive handling (low force and low velocity stroking of the fur with brush) No details on the type of tunnel used and whether it was familiar to the mice (e.g. home cage tunnel) Measures: body weight changes; temperature of brown adipose tissue; concentration of cytokines CXCL12 and CCL2 in bone marrow; activated microglia in the brain; nest complexity scoring; automated homecage behaviour analysis; elevated plus maze test	No details on acclimation	Tail and tunnel handling replication of Hurst & West 2010	Experimental unit not explicitly defined in the paper. Implicitly it was the individual animal n=10 per group, plus 12 sentinels as control mice. 42 mice in total Mice randomly assigned to the four groups No information on blinding No information on the order in which different groups were tested Justification of sample size via a power calculation with most parameters provided for intervention groups. No justification of sample size for the sentinel animal group Justification provided for choice of statistical analysis methods. Non-parametric statistical analysis method chosen; however, power calculation was based on parametric tests. The study is potentially underpowered for this analysis method Statistical analysis method not mentioned for one analysis. p-values given without explanation of analysis used and error bars on graph undefined	CD1 8 weeks of age on arrival (9 weeks of age at the beginning of experiments) Males only Housed singly (no justification provided)	Open poly-carbonate cages	Italian Ministry of Health and NC3Rs

Caveats: During cage changing, the undisturbed control mice (sentinels) were actually handled by the tail. The mice in the mechanoceptive handling group were also tail handled for the duration of the experiment

No acclimation to the handling method and only short and infrequent handling sessions. Not clear how the mice were handled before the start of the experiments, during the 6-day acclimation period after arrival

Stressful procedure (restraint and light exposure) took place in a Plexiglas tube, similar to the tunnel used for non-aversive handling, which could have negatively predisposed the mice against tunnel handling

Generalisability limited by only using one sex

Study potentially underpowered as parametric based power calculation used but data analysed with non-parametric methods

Authors state they will not use significance testing as study is exploratory and sample size is small increasing the risk of false positives, but they go on to produce p-values and interpret them with regards to significance

Miller AL, Roughan JV (2022) Welfare assessment, end-point refinement and the effects of non-aversive handling in C57BL/6 mice with Lewis lung cancer. *Animals* 12(1): 23. doi: 10.3390/ani12010023

This study used a variety of welfare monitoring methods to determine when mice developing lung cancer experienced poor welfare, so that similar future studies can be ended before this occurs

Using non-aversive handling (combination of tunnel and cupping) on tumour-bearing and control mice did not have any beneficial effect either on data quality/variability or welfare

Regardless of cancer status, walking activity over time was modestly more reduced in females that had been handled using non-aversive handling

What was compared?	Schedule of acclimation to handling method	Replication or modification of Hurst & West 2010 handling methods?	Study reliability	Animal characteristics	Cage type	Funders
Tail vs a combination of tunnel and cupping (if mouse was already tunnel, then picked up by tunnel; if not, then picked up using cupping) Cardboard tunnels were present in all cages Measures: The coefficient of variation for each daily welfare measure (i.e. body weight, food and water consumption and nest quality) was used to determine whether variability was lowered by non-aversive handling and an average CoV was then calculated (pooled across the four parameters). The CoV of the average tumour growth rate was calculated to determine if handling method had any effect on tumour developmental consistency. All CoV values were expressed as percentages (±1 SEM)	No formal pre-study acclimation to the different handling methods	Modification Mice were only handled as necessary (e.g. during routine husbandry procedures) and not as part of a pre-determined experimental handling protocol Tunnel handling and cupping were used interchangeably	Experimental unit not explicitly defined in the paper. Implicitly it was the individual animal (n=8 animals per cancer/handling method combination) Mice randomly allocated to handling groups and cancer/no cancer groups (blocked by handling method) Tumour-free control animals were not injected with vehicle for welfare reasons Recordings or observations were made by treatment blinded scorers/observers Sample size justified with a power calculation with all parameters provided. Calculation determined n=10 per group to reach 80% power. Attrition during the experiment resulted in lower power n=8 animals per group at the end of the experiment (cancer and no cancer groups sex matched within each handling method). 40 mice in total at the start of the study, 32 mice in total at the end The reason why some mice were excluded from the study/analysis is not clear for all cases	C57BL/6NCrl Age not stated Equal numbers of males and females to start with; numbers slightly unbalanced due to losses as study progressed, but proportion of each sex the same within each handling method Singly housed to allow collection of data on food and water consumption and nest	IVCs (19 × 40 × 18 cm; Arrowmight, UK)	NC3Rs

Caveats: No acclimation to handling method

Used a combination of tunnel handling and cupping

Mice singly housed which could impact welfare

Mice placed in unfamiliar cages for scoring of behaviour

Attrition during study led to potentially underpowered experiments

Data expressed as +/- SEM rather than +/-SD. SD is a measure of data variability, SEM is a measure of the precision of the mean

Hull MA, Reynolds PS, Nunamaker EA (2022) Effects of non-aversive versus tail-lift handling on breeding productivity in a C57BL/6J mouse colony. *PLoS ONE* 17(1): e0263192. doi: 10.1371/journal.pone.0263192

Compared to tail-handled mice, tunnel-handled mice averaged one extra pup per pair born and weaned during their reproductive lifespan

Loss of one or more complete litters by a given pair was significantly associated with handling method: more tunnel-handled pairs successfully weaned all litters produced, averaged fewer litter losses prior to weaning, and had a 20% lower risk of recurrent litter loss

What was compared?	Schedule of acclimation to handling method	Replication or modification of Hurst & West 2010 handling methods?	Study reliability	Animal characteristics	Cage type	Funders
Tail (using rubber-tipped forceps) v tunnel Tunnels were 8.89 x 6.35 x 5.08 cm, clear, medical-grade polycarbonate, square, familiar (home cage) tubes Measures: number of pups born and weaned per breeding pair during their reproductive lifespan; number of entire litters lost prior to weaning; inter-litter interval; pair non-productivity	No details on acclimation	Modification Tunnel handling performed as described by Hurst & West 2010 (i.e. mice guided with hand in the home cage tunnel) but the transfer tunnel was of different shape and size Tail handling performed using forceps Mice were only handled as necessary (e.g. during routine husbandry procedures) and not as part of a pre-determined experimental handling protocol	Randomised controlled trial (breeding pairs randomised to handling method and cages randomised to position on the cage rack) Blinding incorporated in study Sample size justification was based on practical constraints (i.e., the space available for the breeding pairs and pups over the course of the experiment) Study underpowered to detect effect size of interest for the primary outcome measure (one extra pup per breeding pair over reproductive lifetime) as sample size would have been unfeasibly large Experimental unit was the breeding pair (n=30 and n=29 breeding pairs for tail-handled and tunnel-handled respectively) Total of 59 breeding pairs (170 mice), giving birth to 1,950 pups during the course of the study	59 Bl6C57/6J (monogamous) breeding pairs Mice selected from existing colony Males 6-8 weeks when paired with females and females where nulliparous	IVC (JAG75; Allentown, NJ)	No specific funding received for this work

Caveats: Constraints on space and production led to the study being underpowered for the primary outcome measure. Authors explained this in the paper

Determination of neonate numbers might have been imprecise due to welfare concerns regarding disturbing dams immediately post birth, possibly underestimating pup mortality

Group differences in home cage configuration; tunnel-handled animals had one extra item in their cage (tunnel) compared to tail-handled

Before enrollment in the study all mice had been handled using forceps

Mouse handling research papers

National Centre for the Replacement Refinement & Reduction of Animals in Research

The table below provides quick links to published evidence addressing common questions about the refined mouse handling techniques (please also see our FAQs page). We are also aware of many UK laboratories that have practical, unpublished experience of using the refined techniques.

To connect with these laboratories, please email enquiries@nc3rs.org.uk. For caveats relating to each of the studies referenced below, please see the main table.

Where is the evidence?	Reference
For increased voluntary interaction with the handler, and lower anxiety, from tunnel handling/cupping compared to tail handling?	Hurst & West 2010; Gouveia & Hurst 2013; Ghosal *et al.* 2015; Goveia & Hurst 2017; Clarkson *et al.* 2018; Nakamura & Suzuki 2018; Gouveia & Hurst 2019; Clarkson *et al.* 2020; Henderson *et al.* 2020; Sensini *et al.* 2020
For the welfare benefits of the non-aversive handling methods from laboratories other than the Hurst laboratory?	Ghosal *et al.* 2015; Ono *et al.* 2016; Clarkson *et al.* 2018; Nakamura & Suzuki 2018; Roughan & Sevenoaks 2018; Clarkson *et al.* 2020; Henderson *et al.* 2020; Sensini *et al.* 2020; Redaelli *et al.* 2021
For improved welfare from tunnel handling/cupping, using physiological measures (as opposed to behavioural measures)?	Ghosal *et al.* 2015 (cupping); Ono *et al.* 2016 (tunnel); Clarkson *et al.* 2020 (tunnel)
That duration of tail restraint is what causes the highly stressful response to tail handling?	Gouveia & Hurst 2019 (The available data show that duration of restraint [2-60s] is not an important factor in response. It is picking up mice by the tail that causes aversion and anxiety.)
That only brief experience of tunnel handling (e.g. 2 secs. during cage cleaning for 10 days) is sufficient to ensure lack of aversion to handling and low anxiety?	Goveia & Hurst 2017; Gouveia & Hurst 2019
That tunnel handling/cupping takes no longer than tail handling, once staff members are competent?	Gouveia, Waters & Hurst 2016 mouse handling tutorial; many UK labs have similar data.
That tunnel handling/cupping can be performed with jumpy strains?	Cupping may be unsuitable for jumpy strains or young mice but tunnel handling can be used (Gouveia, Waters & Hurst 2016 mouse handling tutorial)
That tunnel handling can be performed in IVCs?	Miller & Leach 2015; Ono *et al.* 2016; Roughan & Sevenoaks 2018; Doerning *et al.* 2016; Henderson *et al.* 2020; Miller & Roughan 2022
That scruff restraint does not reverse the taming effects of tunnel handling/cupping?	Hurst & West 2010; Roughan & Sevenoaks 2018; Gouveia & Hurst 2019; Henderson *et al.* 2020
That tunnel handling/cupping improves performance on behavioural tests compared to tail handling?	Goveia & Hurst 2017; Clarkson *et al.* 2020
That cupping improves glucose tolerance compared to tail-handled controls?	Ghosal *et al.* 2015
That tail handling reduces responsiveness to reward and increases depressive-like behaviour compared to tunnel handling/cupping?	Clarkson *et al.* 2018; Clarkson *et al.* 2020; Sensini *et al.* 2020
That handling method (tail, tunnel, tail-cup) does not differentially affect blood pressure and heart rate in mice undergoing tail-cuff plethysmography?	Wilde *et al.* 2017
That handling with a plastic ladder can be used as an alternative to tunnel handling?	Sandgren *et al.* 2021
That non-aversive handling helps maintain bodyweight after a stressful procedure?	Redaelli *et al.* 2021
That tunnel handling can improve breeding performance?	Hull *et al.* 2022