

## Mouse handling research papers

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 the refined mouse handling techniques.



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

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. To alert the NC3Rs to further papers on mouse handling, please email [enquiries@nc3rs.org.uk](mailto:enquiries@nc3rs.org.uk).

## The original research

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
<p><b>Hurst JL, West RS (2010) Taming anxiety in laboratory mice. <i>Nature Methods</i> 7: 825-826. <a href="https://doi.org/10.1038/nmeth.1500">doi:10.1038/nmeth.1500</a> (full text: <a href="https://bit.ly/2JhgbJb">bit.ly/2JhgbJb</a>)</b></p> <p>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.</p> <p>The positive effects of tunnel handling and cupping generalise across strains, handlers, and the light/dark phase.</p> <p>Mice handled by their home cage tunnel or cupping are <i>much</i> 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.</p> <p>Mice picked up by the tail do not habituate to tail handling.</p>						
<p>Tail, tunnel, cupping</p> <p>(Tunnel then cupping was used for one cohort of C57BL/6 mice, producing similar results to tunnel handling: Suppl. Fig. 4, Suppl. Tables 2 &amp; 3)</p> <p>Tunnels were clear acrylic, familiar (home cage) tunnels and were present in all cages</p> <p>Measures: voluntary interaction with handling device; urination and defecation during handling; anxiety in elevated plus maze</p>	<p>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</p> <p>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</p> <p>Mice handled consistently by one of 11 handlers</p>	N/A	<p>Cages randomised into handling methods and balanced on the cage rack. Order of testing randomised but balanced across methods</p> <p>Blinding used, but not consistently</p> <p>No sample size justification</p> <p>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</p>	<p>BALB/c, ICR(CD-1), C57BL/6</p> <p>Males and females</p> <p>8-10 weeks old at start of testing; 11-15 weeks old at end</p> <p>Housed two per cage</p>	Open (MB1)	ASAB, BBSRC, NC3Rs, Wellcome
<p><b>Caveats:</b> 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?"</p>						

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
<p><b>Gouveia K, Hurst JL (2013) Reducing mouse anxiety during handling: Effect of experience with handling tunnels. <i>PLoS ONE</i> 8(6): e66401. <a href="https://doi.org/10.1371/journal.pone.0066401">doi:10.1371/journal.pone.0066401</a></b></p> <p>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.</p>						
<p>Tail v tunnel – either familiar (home cage) tunnel; external tunnel with 1-week experience in the home cage; or external tunnel without experience</p> <p>Tunnels were clear acrylic tunnels</p> <p>Measures: voluntary interaction with handling device; anxiety in elevated plus maze</p>	<p>Nine daily handling sessions of 2x30s each</p> <p>Single handler</p>	<p>Replication</p>	<p>Randomisation not mentioned (but was as per Hurst &amp; West 2010 above)</p> <p>Blinding not used</p> <p>No sample size justification</p> <p>N=8 cages per handling method x strain combination. 128 mice in total.</p>	<p>ICR(CD-1), C57BL/6</p> <p>Males and females</p> <p>7-10 weeks old when tested</p> <p>Housed two per cage</p>	<p>Open (M3)</p>	<p>NC3Rs (PhD studentship)</p>
<p><b>Caveats:</b> as above</p>						
<p><b>Gouveia K, Hurst JL (2017) Optimising reliability of mouse performance in behavioural testing: the major role of non-aversive handling. <i>Scientific Reports</i> 7: 44999. <a href="https://doi.org/10.1038/srep44999">doi:10.1038/srep44999</a></b></p> <p>Mice handled by tunnel and cupping methods showed <i>substantially</i> 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.</p> <p>The positive effects of non-aversive handling can be achieved through normal brief handling during cage cleaning.</p>						
<p>Expt. 1 (stimulus location): Tail, tunnel or cupping</p> <p>Expt. 2 (test area familiarity): Tail v tunnel</p> <p>Tunnels were clear acrylic, familiar (home cage) tunnels present in all home cages</p> <p>Measures: voluntary interaction with handling device; exploration of clean arena; habituation-dishabituation response to novel urine stimuli</p>	<p>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</p> <p>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</p> <p>Single handler</p>	<p>Replication, but handling habituation sessions were very brief (only 2s)</p>	<p>Cages randomised to handling methods</p> <p>Blinding not used</p> <p>No sample size justification</p> <p>Expt. 1: N=8 cages per handling method. 48 mice in total</p> <p>Expt. 2: N=8 cages per handling method. 32 mice in total</p>	<p>BALB/c (BALB/cOlaHsd)</p> <p>Females only</p> <p>Expt. 1: 14-15 weeks old when tested</p> <p>Expt. 2: 16 weeks old when tested</p> <p>Housed two per cage</p>	<p>Open (M3)</p>	<p>NC3Rs (PhD studentship)</p>
<p><b>Caveats:</b> as above</p>						

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

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
<p><b>Ghosal S, Nunley A, Mahbod P et al. (2015) Mouse handling limits the impact of stress on metabolic endpoints. <i>Physiology &amp; Behaviour</i> 150: 31-37. <a href="https://doi.org/10.1016/j.physbeh.2015.06.021">doi:10.1016/j.physbeh.2015.06.021</a></b></p> <p>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).</p> <p>Cupped mice maintained on a high fat diet for 3 months exhibited improved glucose tolerance compared to tail-handled controls (Expt. 2).</p> <p>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).</p> <p>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.</p>						
<p>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</p>	<p>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</p>	<p>Replication for tail and cupping methods  Cup-massage is a new method</p>	<p>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 &amp; 2, 15 mice in total for Expt. 3</p>	<p>Expts. 1 &amp; 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</p>	<p>Not stated</p>	<p>NIH (First author also holds American Heart Association fellowship and Albert J Ryan Foundation award)</p>
<p><b>Caveats:</b> 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.</p>						
<p><b>Miller AL, Leach MC (2015) The effect of handling method on the mouse grimace scale in two strains of laboratory mice. <i>Laboratory Animals</i> 50(4): 305-307. <a href="https://doi.org/10.1177/0023677215622144">doi:10.1177/0023677215622144</a></b></p> <p>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).</p> <p>These methods of handling are therefore not confounding factors when establishing baseline MGS scores.</p> <p>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.</p>						
<p>Tail v tunnel  Tunnels used were cardboard, familiar (home cage) tunnels  Measures: mouse grimace scores in a 3 min session after handling</p>	<p>All routine husbandry over a 1-week period used either tail or tunnel handling using the Hurst &amp; West 2010 methods</p>	<p>Replication, but duration of handling not indicated</p>	<p>Cages randomised to handling methods  Blinding used where possible  No sample size justification  N=8 mice per handling method. 16 mice in total</p>	<p>CBA, DBA/2  Males only  Age not stated  Housed four per cage</p>	<p>IVC (Type II)</p>	<p>NC3Rs</p>
<p><b>Caveats:</b> Potential pseudoreplication (the experimental unit was arguably the cage; however individual animal data were assessed with one cage per method)</p>						

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
<p><b>Novak J, Bailoo JD, Melotti L, et al. (2015) An exploration based cognitive bias test for mice: effects of handling method and stereotypic behaviour. <i>PLoS ONE</i> 10(7): e0130718. doi:10.1371/journal.pone.0130718</b></p> <p>This study aimed to validate an exploration based cognitive bias test, using two different handling methods, tail and cupping.</p> <p>Mice from both handling groups displayed a similar pattern of exploration in the radial arm maze, suggesting no difference in affect (but see Caveats).</p> <p>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.</p>						
<p>Tail v cupping</p> <p>Measures: discrimination between positively and negatively cued arms of radial maze; response to ambiguous arms; home cage activity and stereotypy</p>	<p>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</p>	<p>Replication, but mice were handled daily for many weeks</p>	<p>Animals randomised to different handling methods</p> <p>Blinding used where possible</p> <p>No sample size justification</p> <p>N=14 mice per handling method. 28 mice in total</p>	<p>CD-1; Females only</p> <p>Habituated from 3 weeks old, with spatial discrimination training and tested at 26-27 weeks old</p> <p>Housed two per cage</p>	<p>Type II</p>	<p>DFG, ERC</p>
<p><b>Caveats:</b> 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.</p> <p>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.</p> <p>Cupping may have increased the number of arms entered in the maze during training (F1,21 Handling = 4.50, which is larger than the critical F-value of 4.325; p value would be 0.046).</p>						
<p><b>Ono M, Sasaki H, Nagasaki K, et al. (2016) Does the routine handling affect the phenotype of disease model mice? <i>Japanese Journal of Veterinary Research</i> 64(4): 265-271. doi:10.14943/jjvr.64.4.265</b></p> <p>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).</p> <p>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.</p>						
<p>Expt. 1: Tail v tunnel (voluntary entry) v cupping v undisturbed controls</p> <p>Expt 2: Tail v tunnel (voluntary entry) v undisturbed control</p> <p>Tunnels were transparent red, polycarbonate, familiar (home cage) tunnels</p> <p>Measures:</p> <p>Expt. 1: kidney histopathology; blood haematocrit; creatinine and urea nitrogen</p> <p>Expt. 2: plasma corticosterone 20 min after handling</p>	<p>Handled 5 days per week for 20s over 4 weeks</p> <p>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</p>	<p>Modification</p> <p>Tunnel handling varies from Hurst &amp; 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</p>	<p>Randomisation not mentioned</p> <p>Blinding not mentioned</p> <p>No sample size justification</p> <p>Expt. 1: N=5 mice per sex and handling method. 40 mice in total</p> <p>Expt. 2: N=5 males per handling method x strain combination. 30 mice in total</p>	<p>Expt. 1: ICGN glomerulonephritis mouse, males and females</p> <p>Expt. 2: C57BL/6 and BALB/c, males only</p> <p>Males and females</p> <p>8 weeks old when tested</p> <p>Two or three mice per cage</p>	<p>IVC</p>	<p>JSPS KAKENHI (Grants-in-Aid for Scientific Research No. 25925011), the Ministry of Education, Culture, Sports, Science and Technology, Japan.</p>
<p><b>Caveats:</b> Potential pseudoreplication (the experimental unit is arguably the cage, not the animal). Requirement for voluntary entry to tunnels (in contrast to Hurst &amp; 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).</p>						

What was compared?	Schedule of acclimation to handling method	Replication or modification of Hurst & West 2010 handling methods?	Study reliability	Animal characteristics	Cage type	Fundors
<p><b>Wilde E, Aubdool AA, Thakore P, et al. (2017) Tail-cuff technique and its influence on central blood pressure in the mouse. <i>Journal of the American Heart Association</i> 6(6): e005204. doi:10.1161/JAHA.116.005204</b></p> <p>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.</p> <p>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]).</p> <p>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.</p>						
<p>Expt. 1: Tail v tunnel v tail-cup</p> <p>Expt. 2: female vs male handler</p> <p>Expt. 3: restraint, heating and handling interventions</p> <p>Expt. 4: angiotensin infusion (hypertension model) v saline</p> <p>Tunnels used were cardboard, familiar (home cage) tunnels</p> <p>Measures: blood pressure and heart rate measured by telemetry</p>	<p>No information is given about how mice were handled until the experiment began (nor about their origin)</p> <p>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 handling 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</p> <p>Expt. 2: Male or female researchers handled mice on consecutive days for tail-cuff plethysmography by their own preferred technique (method not reported)</p> <p>Expts. 3 &amp; 4: No information on handling method given</p>	<p>Modification</p> <p>Tail-cup is a new method, involving grasping by the tail and scooping into the palm</p> <p>Few details are given of tunnel handling technique used</p>	<p>Mice randomised to handling methods</p> <p>Blinding not mentioned</p> <p>No sample size justification</p> <p>Handling method order semi-randomised for Expt. 1; method not indicated for other experiments</p> <p>Expt. 1: N=6 male mice in total; data averaged over 5 days of recording per mouse and method</p> <p>Expt. 2: N=3 male and N=4 female researchers; alternation of individual researchers unclear</p> <p>Expt. 3: N=4 mice, mixed sexes, for a series of interventions</p> <p>Expt. 4: N=12 mice</p>	<p>C57Bl/6J</p> <p>Males and females</p> <p>13-22 weeks old when tested</p> <p>Singly housed</p> <p>Telemetry implanted</p>	<p>Assumed IVC ("filtered positive pressure ventilation")</p>	<p>NC3Rs (PhD studentship), BHF, King's College London</p>
<p><b>Caveats:</b> Not comparable to other studies comparing handling methods, for several reasons:</p> <p>Did not use cupping method used by other authors.</p> <p>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.</p> <p>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.</p> <p>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.</p> <p>Occlusion cuff for recording was placed at the base of the tail.</p> <p>Mice were singly housed after telemetry implantation.</p>						

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
<p><b>Clarkson JM, Dwyer DM, Flecknell PA, et al. (2018) Handling method alters the hedonic value of reward in laboratory mice. <i>Scientific Reports</i> 8: 2448. <a href="https://doi.org/10.1038/s41598-018-20716-3">doi:10.1038/s41598-018-20716-3</a></b></p> <p>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.</p> <p>This finding that tail handling reduces responsiveness to reward has scientific as well as animal welfare implications.</p> <p>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.</p>						
<p>Tail v tunnel</p> <p>Tunnels used were clear Perspex, familiar (home cage) tunnel</p> <p>Measures: consumption of sucrose at 4% and 16% concentration; lick cluster size; voluntary interaction with handling device; anxiety in EPM and OFT</p>	<p>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</p>	<p>Replication</p>	<p>Cages randomised to handling methods</p> <p>Blinding used where possible</p> <p>No sample size justification</p> <p>N=16 mice per handling method. 32 mice in total</p>	<p>C57BL/6J</p> <p>Males only</p> <p>7 weeks old at start, 12 weeks old at end</p> <p>Two mice per cage</p>	<p>Open (M3)</p>	<p>BBSRC</p>
<p><b>Caveats:</b> Potential pseudoreplication (the experimental unit was arguably the cage, however for most of the tasks individual animal data were assessed).</p>						

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
<p><b>Nakamura Y, Suzuki K (2018) Tunnel use facilitates handling of ICR mice and decreases experimental variation. <i>Journal of Veterinary Medical Science</i> 80(6): 886-892. doi:10.1292/jvms.18-0044</b></p> <p>This study investigated whether tunnel handling 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.</p> <p>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.</p> <p>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.</p> <p>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.</p> <p>No differences found between sexes</p>						
<p>Tail v tunnel</p> <p>Tunnels used were clear, acrylic tunnels not present in home cage</p> <p>Measures: urination and defecation during handling; ease of handling rating; voluntary interaction with handling device; open field test; elevated plus maze test</p>	<p>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</p>	<p>Replication</p>	<p>Cages were randomised into two handling methods</p> <p>Order of handling of cages balanced across sessions</p> <p>Blinding not mentioned</p> <p>No sample size justification</p> <p>N=40 mice per handling method. 80 mice in total</p>	<p>Jcl:ICR</p> <p>Males and females</p> <p>3 weeks old at start of handling, daily handling sessions started at 4 weeks old, 6 weeks old at end of study</p> <p>Housed four per cage</p>	<p>Open</p>	<p>Funder not specified</p>
<p><b>Caveats:</b> Potential pseudoreplication (the experimental unit was arguably the cage, particularly during voluntary interaction tests; however individual animal data were assessed).</p> <p>Animals are juvenile to subadult (4-6 weeks old).</p> <p>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).</p>						

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
<p><b>Roughan J, Sevenoaks T (2018) Welfare and scientific considerations of tattooing and ear-tagging for mouse identification. <i>Journal of the American Association for Laboratory Animal Science</i> 58(2):142-153. doi:10.30802/AALAS-JAALAS-18-000057 (Full text: <a href="http://bit.ly/2uauUeF">bit.ly/2uauUeF</a>)</b></p> <p>This study investigated if handling method differentially affected anxiety before assessing responses to restraint and tattooing using the Labstamp device, or ear-tagging.</p> <p>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.</p> <p>The apparent anti-neophobic effect of tunnel handling was long lasting and robust.</p> <p>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).</p> <p>Grimace scale scores were higher in tail-handled compared to tunnel-handled mice after handling acclimation and throughout subsequent testing.</p> <p>The data suggest tunnel handling overcame anxiety-like behaviour following restraint, tattooing or ear-tagging.</p>						
<p>Tail v tunnel</p> <p>Restraint v tattooing v ear tagging</p> <p>Tunnels were clear, Plastiglas tunnels. Each cage had its own tunnel, but these were not left in situ. Home cages had cardboard tubes</p> <p>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</p>	<p>For first 2 weeks, all mice tail handled during weekly cage clean, weighing every 2 days and pre-acclimation testing</p> <p>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</p>	<p>Modification</p> <p>Tunnel handling for 2x30s but not with home cage tunnel</p> <p>Tail handling for 1x10s</p>	<p>Animals were randomly allocated for restraint or tattooing, and tail or tunnel handling</p> <p>Rack placement of each cage and order of treatments/procedures was counterbalanced</p> <p>Blinding used where possible</p> <p>No sample size justification (based on previous work)</p> <p>N=16 mice per handling method (half experiencing tattooing and half restraint and ear tags). 32 mice in total</p>	<p>BALB/cAnCrI.</p> <p>Males and females</p> <p>12-19 weeks old when tested</p> <p>Housed four per cage (single sex)</p>	<p>IVC Type II</p>	<p>Newcastle University</p>
<p><b>Caveats:</b> 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.</p>						



## Mouse handling research papers

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.



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

To connect with these laboratories, please email [enquiries@nc3rs.org.uk](mailto: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?	<a href="#">Hurst &amp; West 2010</a> ; <a href="#">Gouveia &amp; Hurst 2013</a> ; <a href="#">Ghosal et al. 2015</a> ; <a href="#">Gouveia &amp; Hurst 2017</a> ; <a href="#">Clarkson et al. 2018</a> ; <a href="#">Nakamura &amp; Suzuki 2018</a>
For welfare benefits of the refined handling methods from laboratories other than the Hurst laboratory?	<a href="#">Ghosal et al. 2015</a> ; <a href="#">Ono et al. 2016</a> ; <a href="#">Clarkson et al. 2018</a> ; <a href="#">Nakamura &amp; Suzuki 2018</a> ; <a href="#">Roughan &amp; Sevenoaks 2018</a>
For improved welfare from tunnel handling/cupping, using physiological (as opposed to behavioural) measures?	<a href="#">Ono et al. 2016</a> (tunnel) <a href="#">Ghosal et al. 2015</a> (cupping)
That duration of tail restraint is what causes the highly stressful response to tail handling?	There is no such evidence in the literature, or indeed any data to our knowledge, to support this assumption. The available data show that duration of restraint (up to 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?	<a href="#">Gouveia &amp; Hurst 2017</a>
That tunnel handling/cupping takes no longer than tail handling, once staff members are competent?	Gouveia, Waters & Hurst 2016 <a href="#">mouse handling tutorial</a> ; 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 <a href="#">mouse handling tutorial</a> )
That tunnel handling can be performed in IVCs?	<a href="#">Miller &amp; Leach 2015</a> ; <a href="#">Ono et al. 2016</a> ; <a href="#">Roughan &amp; Sevenoaks 2018</a>
That scruff restraint does not reverse the taming effects of tunnel handling/cupping?	<a href="#">Hurst &amp; West 2010</a> ; <a href="#">Roughan &amp; Sevenoaks 2018</a>
That tunnel handling/cupping improves performance on behavioural tests compared to tail handling?	<a href="#">Gouveia &amp; Hurst 2017</a>
That cupping improves glucose tolerance compared to tail-handled controls?	<a href="#">Ghosal et al. 2015</a>
That tail handling reduces responsiveness to reward compared to tunnel handling/cupping?	<a href="#">Clarkson et al. 2018</a>
That handling method (tail, tunnel, tail-cup) does not differentially affect blood pressure and heart rate in mice undergoing tail-cuff plethysmography?	<a href="#">Wilde et al. 2017</a>