

Methodological Refinement Options for Research Involving Rodents

Introduction

Mice and rats are the most commonly used mammalian model in biomedical research. This distinction is attributable to the diversity of well-characterized strains, ease of genetic manipulation, and abundant commercially available assays for the species. Thanks to commonly used laboratory rodents, we have unraveled mechanisms to many devastating diseases. In the pursuit of ethical and responsible research, we have a moral obligation to minimize the suffering and distress of our animal research subjects. With that in mind, the intent of the following guide is to provide resources to explore alternative methodologies for painful or distressing procedures. Deciding on the best methods for the specific research question at hand requires careful consideration on the part of the researcher as no single approach is suitable for every model system. By providing references and links for a range of potential refinements, the goal is to help researchers can find tools that improve animal welfare without compromising the scientific goals of their project.

GENERAL RESOURCES

The National Centre for the Replacement, Refinement & Reduction of Animals in Research; <https://www.nc3rs.org.uk/> (website). “The NC3Rs is a UK-based scientific organisation dedicated to replacing, refining and reducing the use of animals in research and testing ([the 3Rs](#)).”

The Animal Welfare Information Center, part of the U.S. Department of Agriculture (USDA) National Agricultural Library (NAL) in Beltsville, Maryland; <https://www.nal.usda.gov/awic> (website). “The Animal Welfare Information Center (AWIC) is mandated by the Animal Welfare Act (AWA) to provide information for improved animal care and use in research, testing, and teaching.”

AltWeb; <http://altweb.jhsph.edu/index.html> (website). “Altweb, the Alternatives to Animal Testing Web Site, was created to serve as a gateway to alternatives news, information, and resources on the Internet and beyond. Altweb now is the U.S. home of the journal ALTEX: Alternatives to Animal Experimentation, which is the official publication of the Johns Hopkins Center for Alternatives to Animal Testing (CAAT).”

Norecopa; <https://norecopa.no/> (website). “Norecopa is Norway's National Consensus Platform for the advancement of ‘the 3 Rs’ (Replacement, Reduction, Refinement) in connection with animal experiments.”

RESOURCES BY PROCEDURE

Handling

Hurst JL & West RS. Taming anxiety in laboratory mice. *Nature Methods* 7, 825–826 (2010) doi:10.1038/nmeth.1500 *Contains helpful video clips.*

Gouveia K, Hurst JL (2013) Reducing Mouse Anxiety during Handling: Effect of Experience with Handling Tunnels. *PLoS ONE* 8(6): e66401. <https://doi.org/10.1371/journal.pone.0066401>

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

Housing

Walker M, Fureix C, Palme R, Newman JA, Ahloy Dallaire J, Mason G. Mixed-strain housing for female C57BL/6, DBA/2, and BALB/c mice: validating a split-plot design that promotes refinement and reduction. *BMC Med Res Methodol.* 2016 Jan 27;16:11. doi: 10.1186/s12874-016-0113-7.

Blood collection

Techniques

Alworth LC, Berghaus RD, Kelly LM, Supakorndej P, Burkman EJ, Savadelis MD, Cooper TL, Salyards GW, Harvey SB, Moorhead AR. Assessment of Blood Collection from the Lateral Saphenous Vein for *Microfilaria* Counts in Mongolian Gerbils (*Meriones unguiculatus*) Infected with *Brugia pahangi*. *Comp Med.* 2015 Dec;65(6):492-8.

Heimann M, Käsermann HP, Pfister R, Roth DR, Bürki K. Blood collection from the sublingual vein in mice and hamsters: a suitable alternative to retrobulbar technique that provides large volumes and minimizes tissue damage. *Lab Anim.* 2009 Jul;43(3):255-60. doi: 10.1258/la.2008.007073.

Francisco CC, Howarth GS, Whittaker AL. Effects on animal wellbeing and sample quality of 2 techniques for collecting blood from the facial vein of mice. *J Am Assoc Lab Anim Sci.* 2015 Jan;54(1):76-80.

Miniaturized sampling techniques

Caron A1, Lelong C2, Pascual MH2, Benning V2. Miniaturized blood sampling techniques to benefit reduction in mice and refinement in nonhuman primates: applications to bioanalysis in toxicity studies with antibody-drug conjugates. *J Am Assoc Lab Anim Sci.* 2015 Mar;54(2):145-52.

Dainty TC, Richmond ES, Davies I, Blackwell MP. Dried blood spot bioanalysis: an evaluation of techniques and opportunities for reduction and refinement in mouse and juvenile rat toxicokinetic studies. *Int J Toxicol.* 2012 Jan-Feb;31(1):4-13. doi: 10.1177/1091581811429493.

Teilmann AC, Kalliokoski O, Sørensen DB, Hau J, Abelson KS. Manual versus automated blood sampling: impact of repeated blood sampling on stress parameters and behavior in male NMRI mice. *Lab Anim.* 2014 Oct;48(4):278-91. doi: 10.1177/0023677214541438.

Gavage

Voluntary oral ingestion of test article or pharmaceutical:

Gonzales C, Zaleska MM, Riddell DR, Atchison KP, Robshaw A, Zhou H, Sukoff Rizzo SJ. Alternative method of oral administration by peanut butter pellet formulation results in target engagement of BACE1 and attenuation of gavage-induced stress responses in mice. *Pharmacol Biochem Behav.* 2014 Nov;126:28-35. doi: 10.1016/j.pbb.2014.08.010.

Walker MK1, Boberg JR, Walsh MT, Wolf V, Trujillo A, Duke MS, Palme R, Felton LA. A less stressful alternative to oral gavage for pharmacological and toxicological studies in mice. *Toxicol Appl Pharmacol.* 2012 Apr 1;260(1):65-9. doi: 10.1016/j.taap.2012.01.025.

Küster T, Zumkehr B, Hermann C, Theurillat R, Thormann W, Gottstein B, Hemphill A. Voluntary ingestion of antiparasitic drugs emulsified in honey represents an alternative to gavage in mice. *J Am Assoc Lab Anim Sci.* 2012 Mar;51(2):219-23.

Refinements to habituation procedures:

Kärrberg L, Andersson L, Kastenmayer RJ, Ploj K. Refinement of habituation procedures in diet-induced obese mice. *Lab Anim.* 2016 Oct;50(5):397-9. doi: 10.1177/0023677216631459.

Breeding management

Heyne GW, Plisch EH, Melberg CG, Sandgren EP, Peter JA, Lipinski RJ. A Simple and Reliable Method for Early Pregnancy Detection in Inbred Mice. *J Am Assoc Lab Anim Sci.* 2015 Jul; 54(4): 368–371.

Seaborn T, Moulin JA, Côté M, Tremblay Y. Promoting the "3Rs" principle in developmental biology with early and convenient diagnosis of pregnancy in mice. *J Reprod Dev.* 2011 Oct;57(5):655-9.

Telemetry

Device size/type refinement:

Braga VA, Prabhakar NR. Refinement of telemetry for measuring blood pressure in conscious rats. *J Am Assoc Lab Anim Sci.* 2009 May;48(3):268-71.

Humane endpoints

Paster EV, Villines KA, Hickman DL. Endpoints for mouse abdominal tumor models: refinement of current criteria. *Comp Med.* 2009 Jun;59(3):234-41.

Nunamaker EA, Artwohl JE, Anderson RJ, Fortman JD. Endpoint refinement for total body irradiation of C57BL/6 mice. *Comp Med*. 2013 Feb;63(1):22-8.

Koch A, Gulani J, King G, Hieber K, Chappell M, Ossetrova N. Establishment of Early Endpoints in Mouse Total-Body Irradiation Model. *PLoS One*. 2016 Aug 31;11(8):e0161079. doi: 10.1371/journal.pone.0161079.

Hankenson FC1, Ruskoski N, van Saun M, Ying GS, Oh J, Fraser NW. Weight loss and reduced body temperature determine humane endpoints in a mouse model of ocular herpesvirus infection. *J Am Assoc Lab Anim Sci*. 2013;52(3):277-85.

Adamson TW1, Diaz-Arevalo D, Gonzalez TM, Liu X, Kalkum M. Hypothermic endpoint for an intranasal invasive pulmonary aspergillosis mouse model. *Comp Med*. 2013;63(6):477-81.

Franco NH1, Correia-Neves M, Olsson IA. How "humane" is your endpoint? Refining the science-driven approach for termination of animal studies of chronic infection. *PLoS Pathog*. 2012 Jan;8(1):e1002399. doi: 10.1371/journal.ppat.1002399.

Cates CC1, McCabe JG2, Lawson GW2, Couto MA2. Core body temperature as adjunct to endpoint determination in murine median lethal dose testing of rattlesnake venom. *Comp Med*. 2014 Dec;64(6):440-7.

Study design

Smith AJ, Clutton RE, Lilley E, Hansen KEA, Brattelid T. PREPARE: guidelines for planning animal research and testing. *Lab Anim*. 2018 Apr;52(2):135-141. doi: 10.1177/0023677217724823.

Festing MF. On determining sample size in experiments involving laboratory animals. *Lab Anim*. 2018 Aug;52(4):341-350. doi: 10.1177/0023677217738268.