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

Behavioral management

Dr Mark Prescott, NC3Rs

NASEM NHP workshop, August 25 2022

Pioneering Better Science

Importance of the 3Rs

- NHPs are unquestionably useful models for certain types of research
- Opinion polls demonstrate large societal concern about NHP use, so careful regulation and periodic review is appropriate
- Justification for individual research projects and protocols is assessed case-by-case, incorporating harm-benefit assessment and the 3Rs
- Full and active application of the 3Rs is part of the societal contract with the public and expected by all major scientific stakeholders
- Implementation of refinement not only minimizes animal suffering but also maximizes scientific benefits (model validity and translation)

Likely harms to the animals involved

- Scientific procedures and their effects
- Contingent suffering due to housing, transport, etc.

Likely benefits to science and society

- New scientific knowledge
- Improvements in human (or animal) health or safety

Graham ML, Prescott MJ (2015) The multifactorial role of the 3Rs in shifting the harm-benefit analysis in animal models of disease. *European Journal of Pharmacology* 759: 19-29.

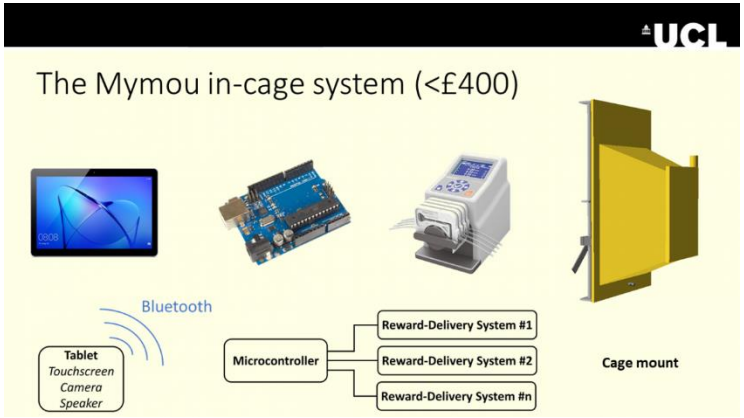
Refinement and the crucial role of the investigator

- Refinement encompasses the lifetime experience of research animals, from their breeding, acquisition and transport, through housing and management, to research procedures and their effects, and eventual fate
- Responsibility lies with investigators, veterinary and animal care staff, IACUCs, and others
- Many researchers have themselves developed new refinement methods

Buchanan-Smith HM, Rennie AE, Vitale A, et al. (2005) Harmonising the definition of refinement. *Animal Welfare* 14 379-384.
Jennings M, Prescott MJ (eds.) Joint Working Group on Refinement (2009) Refinements in husbandry, care and common procedures for non-human primates. *Laboratory Animals* 43(S1): 1-47.

New science and technologies benefiting refinement

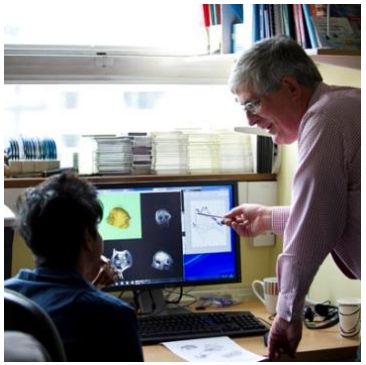
- Automated, home-cage training systems (e.g. Mymou) – avoiding the need for social separation, fluid control and delaying chair restraint
- Imaging (e.g. MRI) – enhancing animal selection and assignment, custom-fitted recording/head fixation devices, and diagnosis of health issues
- Improved cranial implant designs and protective caps – fewer health issues
- New anaesthetic regimens – faster recovery and return to the social group
- Automated behavioural recording and face recognition methods – improving welfare assessment and management
- Telemetry systems – allow social housing, earlier endpoints, increased data yield
- Improved chair restraint training and practices
- Metabolism cage designs for multiple animals
- Techniques for microsampling of blood



Butler JL, Kennerley SW (2018) *Beh Res Meth* 51: 2559–2572; Prescott MJ, Poirier C (2020) *NeuroImage* 225: 117521; McMillan J, et al. (2017) *Com Med* 67(5): 1-10; Mason S, et al. (2019) *J Neurosci Methods* 317: 82-93; Prior H, et al. (2016) *J Pharmacol Toxicological Methods* 81: 75-87; Stow R, et al. (2021) *J Pharmacol Toxicological Methods* 107: 106947; Prescott MJ, et al. (2022). *F1000Research* 11: 272; Witham CL (2018) *J Neurosci Methods* 300: 157–165; Morozov et al. (2021) *eNeuro* 8(6): ENEURO.0117-21.2021.

Practical and cultural barriers to refinement

- Perceptions on the scientific value of the 3Rs
- Lack of awareness and knowledge of refinement opportunities
- Poor availability of funding for the development and application of alternative approaches
- Limitations on access to high quality infrastructure
- Weak oversight frameworks
- Inertia and resistance to change



Most can be tackled through the policies and procedures of funding bodies

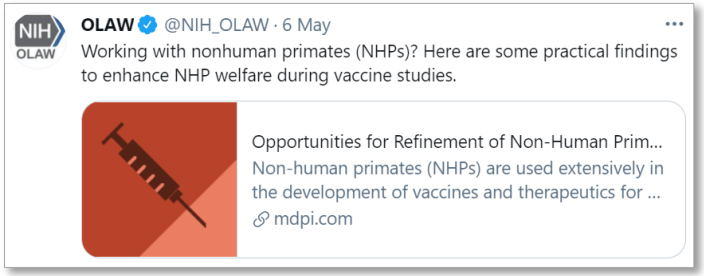
Prescott MJ, Langermans JA, Ragan I (2017) Applying the 3Rs to non-human primate research: barriers and solutions. *Drug Discovery Today: Disease Models* 23: 51-56.

Refinement of NHP vaccine and therapeutics studies

A 2019 NC3Rs-CEPI international workshop identified opportunities for refinement, even in high-level biocontainment (BSL-3/BSL-4):

- Careful animal selection
- Social housing
- Larger enclosures and more extensive enrichment
- Animal training and improved sedation
- Supportive care measures
- Early humane endpoints, including via telemetry and medical imaging

Following the workshop, CEPI contracted laboratories with high welfare standards (assessed against NC3Rs NHP guidelines) and also made investments in infrastructure, caging and training to help achieve the required standards

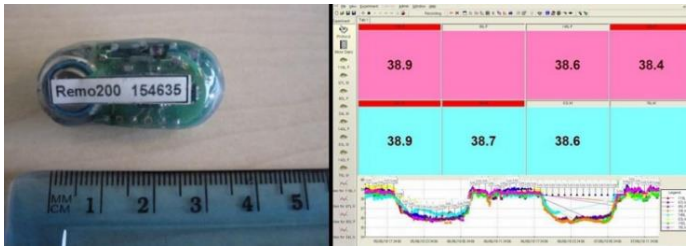


Prescott MJ, Clark C, Dowling WE, Shurtleff AC (2021) Opportunities for refinement of non-human primate vaccine studies. *Vaccines* 9: 284.

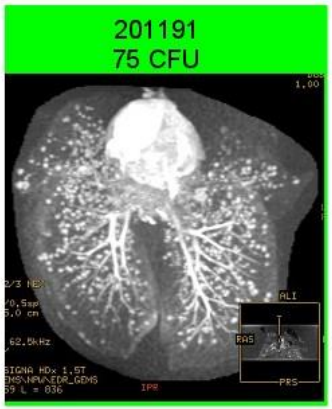
Technologies supporting refined humane endpoints

Secondary endpoint measurements (e.g. temperature change, haematology) may serve as early biomarkers for euthanasia decisions

- Telemetry is not as widely used as it could be:
  - Offers a large, accurate and objective data set to refine endpoints
  - BT, BP, EEG/ECG, HR, RR, activity, glucose
- Medical imaging (e.g. CT, PET/CT) is another useful innovation, but less widely available:
  - Portable clinical scanners can be used
  - Earlier detection of treatment effects, helps to limit suffering
  - Allows evaluation and refinement of challenge doses



Smither & Lever 2012



Mike Dennis/Macaque Website

Behavioral management

A holistic method for improving animal care and welfare that integrates species-appropriate:

- Facility design
- Husbandry and rearing practices
- Social groupings
- Environmental enrichment
- Animal training
- Behavioral monitoring and assessment

Behavioral management programs for NHPs aim to meet behavioral needs, improve psychological wellbeing and reduce stress (refinement), improving the animals' welfare and their utility as research models

Bloomsmith MA (2017) Behavioral management of laboratory primates: Principles and projections. In Schapiro SJ (ed.) Handbook of Primate Behavioral Management, 497–513. Boca Raton, FL: CRC Press.

Animal welfare and quality of science are linked

Benefits of positive reinforcement training to animals and staff:

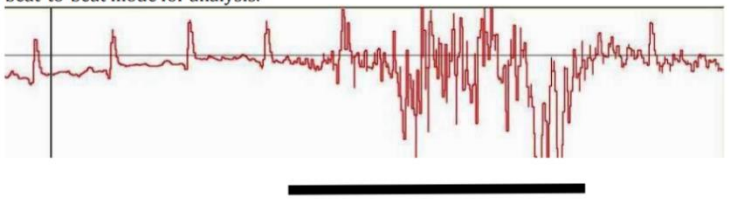
- Reduced animal stress and data variability, enhancing model validity
- Improved animal care and wellbeing (e.g. reduced abnormal behaviour)
- Greater ease, speed and safety for scientific procedures, treatments, etc.

Refinement of restraint and training of macaques leads to better outcomes in safety assessment studies:

- reduced fear responses to care staff
- lower baseline HR and BP values
- reduced between-animal variation
- fewer artefacts in ECG traces

Prescott MJ, Buchanan-Smith HM (2007) Training laboratory-housed non-human primates, Part 1: A UK survey. *Animal Welfare* 16: 21-26.
Prescott MJ, Bowell VA, Buchanan-Smith HM (2005) Training laboratory-housed non-human primates, Part 2: Resources for developing and implementing training programmes. *Animal Technology and Welfare* 4: 133-148.
Graham ML, Rieke EF, Mutch LA, et al. (2012) Successful implementation of cooperative handling eliminates the need for restraint in a complex non-human primate disease model. *Journal of Medical Primatology* 41: 89-106.
Palmer S, Oppler SH, Graham ML (2022). Behavioral management as a coping strategy for managing stressors in primates: the influence of temperament and species. *Biology* 11(3): 423.

Figure 1.3.7b Artefacts in ECG trace from a macaque as a consequence of muscle tension (manual restraint); baseline is not clean and difficult to identify all waveforms. Trace seen in beat-to-beat mode for analysis.



Muscle tension affects waveform morphology on the trace.

Figure 1.3.7f A 'clean' trace from a macaque with reduced muscle tension and minimal animal movement (tube restraint). Trace seen in beat-to-beat mode for analysis.

Normal waveform morphology. All wave forms are distinguishable for analysis.

Tasker L (2012) Linking welfare and quality of scientific output in cynomolgus macaques (*Macaca fascicularis*) used for regulatory toxicology. *PhD thesis, University of Stirling*

Features of a high-quality pen enclosure system

Floor-to-ceiling enclosure height, with high resting areas, where monkeys may feel more secure

Vertical dividers function as visual barriers for privacy and refuge from other monkeys

Horizontal, wide bars enable climbing and increase useable space, visibility and interaction

Greater space for enrichment, natural behaviour, exercise and group housing/socialization

Sufficient shelving/perches for all occupants. Hardwood is warm, non-slip and chewable

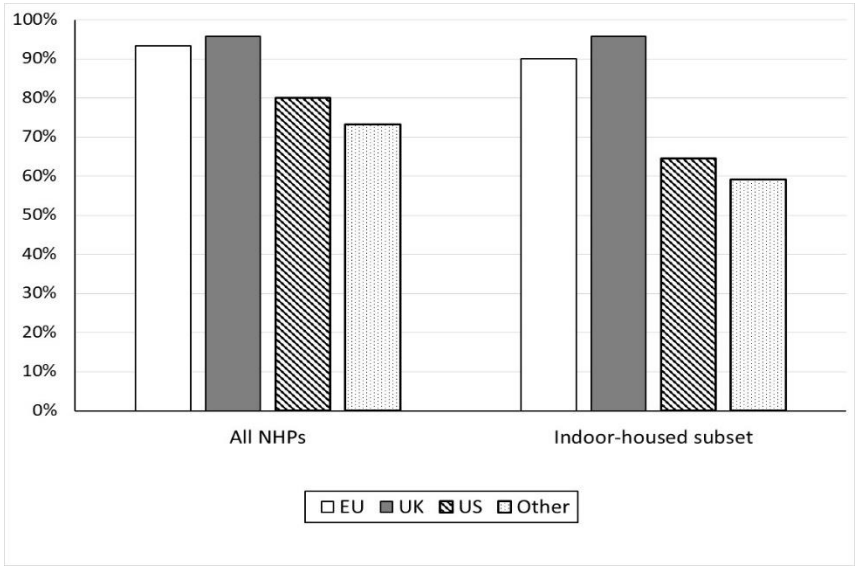
Verandas/balconies and/or bay fronts give good visibility into the room, reducing anxiety

Solid floors with substrate encourage extended bouts of calm foraging behaviour

Variation in behavioral management practices

- Largest survey of NHP behavioral management practices (unpublished)
- Conducted 2019-2020 in collaboration with Dr Kate Baker, Tulane NPRC
- Information on management of >53,000 NHPs at 55 facilities internationally
- Large variation in practices between world regions – UK and EU at forefront of best practice in many aspects:
  - proportion of NHP populations housed socially
  - provision of pen enclosures
  - caging exceeding regulatory/ accreditation minimum floor space
  - provision of destructible enrichment and floor substrate
  - later ages of removal of infants from the dam

Use of social housing, by region (percent of facilities)



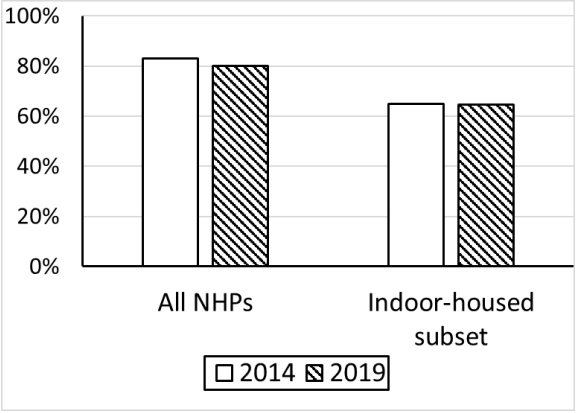
Baker KC, Prescott MJ. An international survey of behavioral management programs for laboratory nonhuman primates (in prep)

Progress in the US over time

- Positive changes and progress are being made in the US since previous surveys – e.g. greater proportion of facilities providing access to exercise enclosures, and more often
- However, no increase in use of social housing since 2014 (though clinical, instrumentation, and staff/time constraints less often reported in 2019) and no change in provision of destructible enrichment and flooring substrates

Overall these survey data demonstrate with knowledge transfer and sufficient resources, perceived constraints on key aspects of behavioral management can be overcome to improve NHP welfare and facilitate good science

Use of social housing in the US, over time (percent of facilities)



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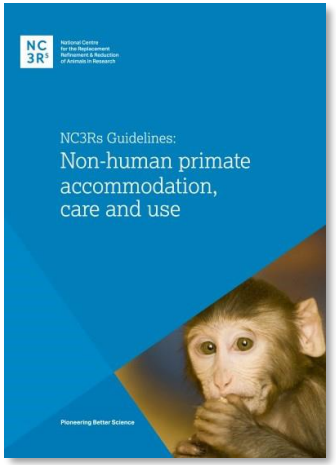
Steps funders can make to advance refinement and behavioral management

NC3Rs and the major UK biomedical funders of NHP research have:

- Established genuine high standards for NHP research and made implementation a condition of grant funding
- Incorporated a parallel, detailed 3Rs review into the scientific peer review process for grant proposals
- Provided funding for the development and application of 3Rs approaches – >$2.5 million committed to-date
- Established expert committees to publish recommendations on best practice in specific fields
- Hosted annual symposia, forums and dedicated websites for sharing of knowledge and practical information



Prescott MJ (2016) Online resources for improving the care and use of non-human primates in research. *Primate Biology* 3: 33-40.
Prescott MJ, Brown VJ, Flecknell PA, et al. (2010) Refinement of the use of food and fluid control as motivational tools for macaques used in behavioural neuroscience research. *Journal of Neuroscience Methods* 193: 167-188.

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Thank you

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Thanks to collaborators and NC3Rs grant holders.