Session 2.4
Non-human primates – housing, enrichment, positive reinforcement training

Primates in Laboratories: Standardisation, Harmonisation, Variation and Science

Hannah M. Buchanan-Smith
Department of Psychology, University of Stirling, Scotland, UK

Summary
Many animals are bred specifically for use in laboratories; the genetic variation between individuals is minimised, and housing and husbandry is often standardised. The rationale is to decrease the variation of the scientific findings, and allow a reduction in number of animals used, although these relationships are questioned. Non-human primates used in laboratories present a different case; there are genetic differences, and considerable variation in rearing practices, housing, enrichment and training both among, and often within, facilities. The impact of this variation on both welfare and science warrants further investigation.

Keywords: three Rs, welfare, enrichment

Introduction

Animals are housed in laboratories for scientific research and testing. Russell and Burch's three Rs (replacement, reduction and refinement) are now widely accepted, and provide a systematic framework to progress humane animal use. Setting standards, standardisation and harmonisation have the potential to impact on science and on the three Rs. The aims of this paper are to:

- clarify the terms "setting standards", "standardisation" and "harmonisation", and to summarise their potential impact on the three Rs and science;
- describe how housing and husbandry practices vary for non-human primates (hereinafter primates);
- argue that the impact of such variation should be quantified with the goal of striving towards well-adjusted animals for sound scientific results.

Agreeing upon definitions of terms

There is a need to agree upon definitions before one can proceed! In the 45 years since Russell and Burch (1959) first proposed the three Rs, there have been considerable advances in animal ethics and animal welfare science. Despite the progressive nature of the three Rs as described by Russell and Burch, a recent analysis found over 15 different definitions of refinement, some of which are regressive from the intended meaning. A suggested definition has been proposed: "*any approach which avoids or minimises the actual or potential pain, distress and other adverse effects experienced at any time during the life of the animals involved, and which enhances their wellbeing*" (Buchanan-Smith et al., 2005). This not only makes it explicit that refinement applies to all stages of the life of an animal destined for use in the laboratory (i.e. the birth to death experience), but also takes a proactive stance of enhancing wellbeing. There has been less confusion about the meaning of replacement and reduction and Russell and Burch's original definitions of "*any scientific method employing non-sentient material which may, in the history of experimentation, replace methods which use conscious living vertebrates*" and "*reduction in the number of animals used to obtain information of a given amount and precision*" (Russell and Burch, 1992) are used here.

A further confusion in the literature is that standardisation has been interpreted in three ways. The first is the setting of standards – referred to here as "setting standards". The second is defining the properties of experimental animals and their environments to increase the reproducibility of results (Beynen et al., 2001), referred to here as "listing" (see Würbel, 2002). The third refers to keeping experimental conditions the same for all animals (within-experimental standardisation) or all experiments (between-experiment standardisation – see Würbel, 2002). In this paper, the latter will be referred to as standardisation. The difference between setting standards and harmonisation can also be confused (see below).

Setting standards and harmonisation

There is debate about the need for setting standards and harmonisation in laboratory animal science (e.g. ILAR, 2003). One of the problems is they can refer to a multitude of levels (e.g. opinions, animal welfare, environments, practices, processes, scientific protocols, tests), and whether they are a desirable goal surely lies with the proposed level, taking into account the broader context, including the ability to implement, and the means of ensuring the desired outcome has been fulfilled.

One interpretation of the difference between setting standards and harmonisation is that harmonisation refers to performance outcomes whilst setting standards is prescriptive and refers specifically to how to get to the outcome. So for example, a legal requirement to adhere to minimum cages sizes based on body weight of the animal is a standard, and standards of this sort are set in an attempt to ensure a minimum level of welfare. They can be easily quantified, and monitored, which constitutes an advantage for legal oversight/regulation. Such prescriptive standards should be based, wherever possible, on scientific findings or at least well-established practice. It is also important to note that they should be treated as the minimum required and providing more than these minimum standards is encouraged to enhance welfare (i.e. minimum standards do not necessarily imply standardisation).

In contrast, harmonisation of acceptable welfare might refer to providing animals with cage dimensions that allow them to meet species-specific and individual needs. For example, Buchanan-Smith et al. (2004) have argued that setting minimum cage sizes for primates based on body weight alone is inappropriate to ensure acceptable welfare, but that a range of characteristics such as morphometric, physiological, ecological, locomotor, social, reproductive and behavioural characteristics, together with the primate's age, sex and individual history should be taken into account. Harmonisation is therefore less prescriptive than minimum standards and allows for greater flexibility in how to achieve the goal of enhancing welfare.

This flexibility can be beneficial to allow innovation and to challenge and improve conditions. Further it encourages one to take the individual needs of animals into account. This flexibility may contribute positively to refinement but flexibility may be inappropriate due to discrepancy in the interpretation of acceptable welfare as a consequence of differences among cultures, religions, legislation, and regulations across countries. Therefore specific guidance, and specific standards, may need to be imposed and strictly monitored to increase the likelihood that there is a minimum standard of animal care that is adhered to on an international basis.

Protocols and legally required safety tests may also be harmonised internationally. This not only represents an important strategy to reduce the number of animals used in experimentation in individual countries, but it also helps to ensure that obsolete invasive animal studies are replaced by the most advanced techniques using insentient material (de Boo et al., 2005). Therefore there is the potential to act positively on both reduction and replacement. Further, setting standards and harmonising animal care and use internationally has been viewed as a socio-political and economic imperative, essential to the elimination of trade barriers and to promote multinational interactions (Miller, 1998).

As already mentioned, there are two further interpretations of "standardisation". The benefits of "listing" and "standardisation" are also debated (see Würbel, 2002). For example, rigorous attempts to equate test apparatus, testing protocols, and all possible features of animal husbandry have not succeeded. Crabbe et al. (1999) found that there were significant differences between the behaviour of mice of identical strains, across three test laboratories, and their findings demonstrate the difficulty, or indeed impossibility, of standardisation to guarantee reproducibility of results across experiments. Würbel (2002) argues that standardisation is done at the expense of external validity. By limiting the experiment to the effects of the variable under question on a sample of, for example, a specific genetic strain, under specific standardised conditions, the generalisability of the results to other conditions, populations or species is limited.

Variation

From the discussion above, we get a flavour of the debate and the potential impact of setting standards, standardisation and harmonisation on the three Rs, and on science. However, there is a complex interplay between the three Rs (de Boo et al., 2005), and there is also the potential for standardisation to create a conflict between reduction and refinement. For example, providing housing to enhance individual wellbeing will have a positive impact on refinement, but the potential for a negative impact on reduction. One reason is because enriched housing may confound experimental results (enrichment is known to impact on a wide range of parameters), increasing variance and the number of animals required (e.g. Bayne, 2005; Benefiel et al., 2005; Mering et al., 2001). A negative consequence of such reasoning is that some scientists are reluctant to provide enrichment for their research animals. However, although there are some studies that have concluded that enrichment increases variation in results (e.g. see review in Van de Weerd et al., 2002) a most systematic and comprehensive study has shown that enrichment does not disrupt standardisation and reproducibility of results (Wolfer et al., 2004). Wolfer et al. (2004) compared female mice housed in barren cages with those in large enriched cages on a range of behavioural tests. They replicated their tests three times, in each of three laboratories. They conclude that housing in enriched cages, which should improve the wellbeing, does not increase variation in the findings, and should therefore be used as the norm. This conclusion is supported by Garner (2005) who argues that abnormal repetitive behaviours, which are seen more often in barren conditions, is likely to compromise validity and reproducibility of results.

Primates in laboratories

Whilst the debate about whether enrichment is a desirable goal for non-primates (e.g. rodents, rabbits etc.) used in laboratories continues, due to the potential effects on science (e.g. Benefiel et al., 2005), primates used in laboratories often present a very different case. The most commonly used primates are the rhesus macaque (*Macaca mulatta*), the long-tailed or cynomolgus macaque (*M. fascicularis*) and the common marmoset (*Callithrix jacchus*) (see Rennie and Buchanan-Smith (2005) for an analysis of primate use in Europe). Primate use makes up a very small percentage of total animal use in scientific research and testing. However, due to the genetic similarity with humans, their intelligence, sentience and sociality, the complexity of their physical as well as social needs, and the clear temperament differences both among (e.g. Clarke et al., 1988) and within species (e.g. Capitanio, 2004) they have been treated differently to other animals used in laboratories.

Sources of variation for primates

Despite the potential benefits of setting standards, standardisation and harmonisation described above, for primates there is great variation in, for example, sourcing, rearing practices, weaning policy, housing, enrichment and training both among, and often within, facilities. Below these differences are briefly outlined, and it is highlighted that if the harmonised goal is to produce well adjusted primates suitable for good scientific research, the impact of this variation must be investigated.

Although common marmosets are generally bred in-house, macaques are sourced from many different countries, and often bred in countries where the macaques occur naturally (for UK supply see Prescott, 2001). There are several, potentially important differences between individuals from these overseas establishments, and those that are bred in the user establishment. For example, those bred overseas are often first generation from wild- caught macaques, whilst those bred in house are from captive-bred parents. Those from overseas are likely to have had greater exposure to diseases, and there is less potential for preparation for future use (e.g., socialisation to humans, positive reinforcement training). Further, if bred in house, there may be no, or limited, transport required, whilst those from overseas often have long transport and relocation, which is known to impact negatively on welfare (Honess et al., 2004). Furthermore there are known genetic differences between macaques from different habitats (Zhang and Shi, 1993), yet, unlike the reporting of the exact strain of the rodent used, few details are given in scientific articles on the source of the primate used in the experiment.

Similarly, there is considerable variation in weaning policy, when offspring are removed from their mothers. Most guidelines refer to age as the sole criterion and the guidelines of minimum weaning age vary considerably (6-12 months, Prescott et al., in prep.) as do policies between overseas establishments (e.g. from 90 days, Welshman, 1999). It is well known that early weaning has serious consequences for behaviour, physiology and immune function. Again, like body weight as a measure for determining minimum cage sizes, age has the advantage of being unambiguously quantified, but in order to achieve the harmonised goal of socially well adjusted primates that can cope with the challenges they will face in laboratory experimentation, a range of measures should be used. These might include age, in addition to weight, behaviour and physiological measures, and any guidance should be based on sound evidence.

Given the known impact of early separation, further research is required on the use of rotational hand-rearing, a practice that is used widely in marmosets. Although twins are the norm in the wild, triplet births are increasingly common in captivity, probably due to the rich diet (Tardif and Jaquish, 1997). In some laboratories, the triplets are rotationally hand-reared. In these instances, two infants are left with the marmoset family, whilst one is removed for hand-feeding on a rotational basis. Occasionally, mothers will die or reject their infants and the offspring are entirely human hand-reared. Even brief early social deprivation (9 hours/week on postnatal days 2-28) is known to adversely affect endocrine and behavioural responses to psychosocial challenges in the long-term (Dettling et al., 2002a, 2002b). This suggests that the highly productive management practice of rotational hand-rearing which necessitates early deprivation from the family for long periods is likely to have a significant impact on experimental results and should be quantified.

Housing, husbandry and enrichment may also impact on experimental results. There is a vast literature on the diversity of "enrichment" that has been provided for primates in laboratories. These include housing (e.g. the provision of outside runs and exercise cages), structural (e.g. perches and swings, "toys" and manipulanda, visual barriers, water baths), feeding (e.g. grooming boards, puzzle feeders, gum trees for marmosets), visual and auditory (e.g. television or video, computer tasks, music, mirrors), and social enrichment (e.g. pair or group housing) (e.g. see Honess and Marin, 2006; Lutz and Novak, 2005). However, although the benefits of such enrichment in enhancing welfare are regularly reported, no comprehensive attempt has been made to determine how different enrichment techniques impact upon variation in experimental findings. In this respect, the primate literature lags behind that for other animals used in laboratories.

A final example of variation is from the increasing use of positive reinforcement to train primates to co-operate during laboratory procedures. Although there is little doubt that this practice is beneficial (e.g. see Prescott and Buchanan-Smith, 2003) in terms of reduction of stress, improvement in welfare and facilitation of experimental protocols, there is considerable variation in its uptake (Prescott et al., 2005). Further, standardisation of overall length of time that animals are trained for may be inappropriate as primates learn at different rates, and temperament differences will impact on training success (e.g. Coleman et al., 2005). As Videan et al. (2005) demonstrate, chimpanzees that have been trained over 21 months vary considerably in what they can be trained to do, and in the time taken to be successfully trained. Not all are able to be trained for certain tasks, and those successfully trained to present for injection do not necessarily have lower stress levels than those that could not be trained and requiring darting. The level of co-operation was reported to be a more important factor in determining the amount of stress experienced during anaesthetisation (Videan et al., 2005). Again the impact on science is not well understood, but a desirable goal would be harmonisation of training procedures to ensure a similar level of co-operation is achieved. Similar principles apply to habituation to novel environments or tests procedures (such as restraint chairs).

Conclusions

Primates have, to some extent, escaped the same genetic, rearing and environmental standardisation that is seen with the majority of laboratory animals. This has been a mixed blessing. On the one hand, there has been far greater innovation in enrichment (e.g. outside runs, manipulanda, visual and auditory enrichment) designed to enhance wellbeing, and these have been implemented liberally. On the other hand, this variation in housing, together with the numerous different rearing conditions, weaning policies, husbandry and other practices have not been systematically quantified and their impact on the scientific outcome is not known. They may increase variation in results, and thus more primates may be used in experiments than are needed (conflicting with reduction), although the external validity (generalisability) of the findings might be increased. The impact of such variation certainly warrants systematic investigation. It is concluded that greater harmonisation is needed in primate use and care, with the goal of ensuring socially well adjusted primates that are able to cope with the challenges they will face in experimentation and to ensure sound scientific results.

References

Bayne, K. (2005). Potential for Unintended Consequences of Environmental Enrichment for Laboratory Animals and Research Results. *ILAR Journal 46*, 129-139.

Benefiel, A. C., Dong, W. K. and Greenough, W. T. (2005). Mandatory "Enriched" Housing of Laboratory Animals: The Need for Evidence-based Evaluation. *ILAR Journal 46*, 95-105.

Beynen, A. C., Gartner, K. and Van Zutphen, L. F. M. (2001). Standardization of animal experimentation. In L. F. M. Van Zutphen, V. Baumans and A. C. Beynen (eds.), *Principles of Laboratory Animal Science, revised edition* (103-110). Amsterdam: Elsevier.

Buchanan-Smith, H. M., Rennie, A. E., Vitale, A. et al. (2005). Harmonising the Definition of Refinement. *Animal Welfare 14*, 379-384.

Buchanan-Smith, H. M., Prescott, M. J. and Cross, N. J . (2004). What factors should determine cage sizes for primates in the laboratory? *Animal Welfare 13 (Suppl.)*, 197-201.

Capitanio, J. P. (2004). Personality factors between and within species. In B. Thierry, M. Singh and W. Kaumanns (eds.), *Macaque Societies: A Model for the Study of Social Organization* (13-33). New York: Cambridge University Press.

Clarke, A. S., Mason, W. A. and Moberg, G. P. (1988). Differential behavioral and adrenocortical responses to stress among three macaque species. *American Journal of Primatology 14*, 37-52.

Coleman, K., Tully, L. A. and McMillan, J. L. (2005). Temperament correlates with training success in adult rhesus macaques. *American Journal of Primatology 65*, 63-71.

Crabbe, J. C., Wahlsten, D. and Dudek, B. C. (1999). Genetics of mouse behavior: Interactions with laboratory environment. *Science 284*, 1670-1672.

de Boo, M. J., Rennie, A. E., Buchanan-Smith, H. M. and Hendriksen, C. F. M. (2005). The Interplay between Replacement, Reduction and Refinement. *Animal Welfare 14*, 327-332.

Dettling, A. C., Feldon, J. and Pryce, C. R. (2002a). Early deprivation and behavioral and physiological responses to social separation/novelty in the marmoset. *Pharmacology, Biochemistry and Behavior, 73*, 259-269.

Dettling, A. C., Feldon, J. and Pryce, C. R. (2002b). Repeated parental deprivation in the infant common marmoset (*Callithrix jacchus*, Primates) and analysis of its effects on early development. *Biological Psychiatry 52*, 1037-1046.

Garner, J. P. (2005). Stereotypies and Other Abnormal Repetitive Behaviors: Potential Impact on Validity, Reliability, and Replicability of Scientific Outcomes. *ILAR Journal 46*, 106-117.

Honess, P. E., Johnson, P. J. and Wolfensohn, S. E. (2004). A study of behavioural responses of non-human primates to air transport and re-housing. *Laboratory Animals 38*, 119-132.

Honess, P. E. and Marin, C. M. (2006). Enrichment and aggression in primates. *Neuroscience and Biobehavioral Reviews 30,* 413-436.

Institute for Laboratory Animal Research (2004). *The Development of Science-Based Guidelines for Laboratory Animal Care*. Proceedings of the November 2003 International Workshop, The National Academies Press, Washington, D.C.

Lutz, C. K. and Novak, M. A. (2005). Environmental Enrichment for Nonhuman Primates: Theory and Application. *ILAR Journal 46*, 178-191.

Mering, S., Kaliste-Korhonen, E. and Nevalainen, T. (2001). Estimates of appropriate number of rats: Interaction with housing environment. *Lab Animal 35*, 80-90.

Miller, J. (1998). International harmonization of animal care and use: The proof is in the practice. *Lab. Animal 27*, 28-31.

Prescott, M. J. (2001). Counting the cost: *Welfare implications of the acquisition and transport of non-humans primates for use in research and testing*. Horsham, U.K.: RSPCA.

Prescott, M. J. and Buchanan-Smith, H. M. (eds.) (2003). Training nonhuman primates using positive reinforcement techniques. *Journal of Applied Animal Welfare Science 6*, 157-261.

Prescott, M. J., Rennie, A. E. and Buchanan-Smith, H. M. (2005). Training of laboratory-housed non-human primates in the UK. *Anthrozoos 18,* 288-303.

Prescott, M. J., Bard, K., Brend, S. et al. (in prep.). An investigation of the scientific basis for weaning policy for macaques bred for use in research and testing.

Rennie, A. E. and Buchanan-Smith, H. M. (2005). Report on the extent and character of primate use in scientific procedures across Europe in 2001. *Laboratory Primate Newsletter 44*, 6-12.

Russell, W. M. S. and Burch, R. L. (1959). *The principles of Humane Experimental Technique*. London: Methuen.

Russell, W. M. S and Burch, R. L. (1992). *The Principles of Humane Experimental Technique*. Universities Federation for Animal Welfare (2nd printing). Herts, England.

Tardif, S. D. and Jaquish, C. E. (1997). Number of ovulations in the marmoset monkey (*Callithrix jacchus*): Relation to body weight, age and repeatability. *American Journal of Primatology 42*, 323-329.

Van de Weerd, H. A., Aarsen, E. L., Mulder, A. et al. (2002). Effects of environmental enrichment for mice: Variation in experimental results. *Journal of Applied Animal Welfare Science 5*, 87-109.

Videan, E. N., Fritz, J., Murphy, J. et al. (2005). Does training chimpanzees to present for injection lead to reduced stress? *Laboratory Primate Newsletter 44*, 1-2.

Welshman, M. (1999). Breeding macaques in source countries. In T. Poole and P. English (eds.), *The UFAW Handbook on the Care and Management of Laboratory Animals*, 7th Edition, Vol 1: Terrestrial Vertebrates (636-642), Oxford: Blackwell Science.

Wolfer, D. P., Litvin, O., Morf, S. et al. (2004). Cage enrichment and mouse behaviour: test responses by laboratory mice are unperturbed by more entertaining housing. *Nature 432*, 821-822.

Würbel, H. (2002). Behavioral phenotyping enhanced – beyond (environmental) standardization. *Genes, Brain and Behavior 1*, 3-8.

Zhang, Y. P. and Shi, L. M. (1993). Phylogeny of rhesus monkeys (*Macaca mulatta*) as revealed by mitochondrial DNA restriction enzyme analysis. *International Journal of Primatology 14*, 587-605.

Acknowledgements

I thank Drs Mark Prescott and Hanno Würbel for helpful comments on the manuscript.

Correspondence to

Dr. Hannah M. Buchanan-Smith
Scottish Primate Research Group
Department of Psychology
University of Stirling
Stirling FK9 4LA
Scotland UK
e-mail: h.m.buchanan-smith@stir.ac.uk