Welfare of Primates in Laboratories: Opportunities for Refinement

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Abstract

The use of primates in regulated research and testing means that they are intentionally subjected to scientific procedures that have the potential to cause pain, suffering, distress, or lasting harm. These harms, combined with keeping primates in restricted laboratory conditions, are balanced against the potential (primarily human) benefits gained from their use. In this chapter, we provide a brief overview of the use of primates in laboratories, the estimated number, and purpose of use, and summarize the evidence that primates are especially vulnerable and deserve special protection compared to other animals. The 3Rs (replacement, reduction, and refinement) framework, underpinning humane science, is described, and we emphasize both the ethical and scientific needs for refinement. Refinement refers to all approaches used (by humans responsible for their care) to minimize harms and improve welfare for those primates that are still used in research after the application of the replacement and reduction principles. There is a growing body of evidence demonstrating an interplay between animals' welfare and experimental parameters, and that this interplay affects the validity and reliability of scientific output. With this perspective, we argue that it is better to collect no data than to collect poor (e.g., invalid, unreliable) data. It is, after all, unacceptable for primates to suffer in vain and violates utilitarian principles underlying animal use. Furthermore, inconsistency in experimental approach may introduce conflicting results, increasing the likelihood of using more animals, and delaying delivery of promising therapies to the clinic. We focus

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on mitigating the major welfare issues faced by primates housed in laboratories through coordinated refinements across their life spans. Drawing on examples from cynomolgus macaques (*Macaca fascicularis*), an Old World monkey commonly used during the development of medical products, we highlight the importance of understanding the critical role humans play in the laboratory, providing environments, performing husbandry, and undertaking procedures that promote welfare and decrease harms. Our theoretical premise is that if primates are to be "fit for purpose" (i.e., well suited for the designated role), we need a proactive, concerted approach for implementing refinement that spans their lifetime.

Keywords

3Rs · Fit for purpose · Regulated research · *Macaca fascicularis* · Reliable · Valid data

1 Introduction

> . . .refinement is never enough, and we should always seek further for reduction and if possible replacement. (Russell and Burch 1959, p. 66)

Using animals for research and testing in laboratories has, by its nature, the potential to cause "pain, suffering, distress or lasting harm." This is precisely how regulated scientific procedures are defined (e.g., Home Office 1986), and as such, scientific research is strictly controlled through legislation in many (but not all) countries (Bayne et al. 2010). Intentionally conducting scientific procedures that have the potential to adversely affect the welfare of animals raises its own ethical issues, and the use of nonhuman primates (hereafter primates), as opposed to other animals, is also a special case. In this chapter, we focus on regulated laboratory studies (and not unlicensed behavioral or cognitive research on primates), describing the ethical framework of the 3Rs, the importance of promoting welfare given the link with quality of scientific output, and the major welfare issues affecting primates in laboratories. Our main emphasis lies with how we can improve the welfare of laboratory-housed primates through coordinated refinements across the lifespan, recognizing the critical role humans play in devising opportunities for reducing harms, and advancing primate welfare.

Animal welfare has been the focus of scientific study for many years yet constructing a single definition and approach to measurement has been difficult (reviewed in Fraser 2009). It is accepted that welfare is broad in concept, multidimensional in nature (Dawkins 2004), and lies on a continuum from poor to good (Broom 1999). In this chapter, we adopt an integrated approach to the concept and assessment of welfare that includes both physical and psychological aspects. Defined by Broom (1986, 2010), the welfare of an animal is its state as regard its attempts to cope with its environment, such that failure to cope leads to profound deviations in biological functioning. Thus, animal welfare, as a biological state within the animal, is relevant to scientists who use primates in biomedical research and testing to benefit humans in some way. When primate welfare is poor, primates are not "fit for purpose" as models of normal functioning.

2 Differences Between Laboratories and Other Captive Settings

Factors affecting the welfare of primates housed in laboratories differ in multiple ways from those in other captive settings (see Table 2). As described in this chapter, laboratory animals have regulated procedures conducted upon them to characterize a pathophysiologic process or intentionally model clinical disease that can result in pain, suffering, or distress similar to the target patient. Factors negatively affecting welfare of primates in zoos may be high visitor numbers (Hosey 2000, 2022), a stressor not present in laboratories. There are some rare cases where zoo-housed primates are released back into the wild, with concomitant stress (such as the golden lion tamarin, *Leontopithecus rosalia*, Teixeira et al. 2007). Nonetheless, compared to laboratory-housed primates, zoo-housed primates are likely to have better welfare. They are usually housed in more natural social groupings with conspecifics of both sexes and all (or nearly all) age classes. Their enclosures are also comparatively larger, more complex, and include access to outdoor runs providing more choice and control (Coleman et al. 2022). Primates kept as pets, however, have a host of welfare-related issues related to inappropriate rearing, housing, and husbandry (Hevesi 2022), as do those who live in sanctuaries (Brent 2007) given their life experiences.

3 Primate Use in Laboratories

The number of primates used in laboratories worldwide is not known exactly, but estimates over 16 years ago were in the region of one to two hundred thousand (Carlsson et al. 2004). Primates are used as models for humans because of their genetic, physiological, and psychological similarities, primarily in the fields of microbiology, immunology, neuroscience, biochemistry, pharmacology, and toxicology (see Hau et al. 2000; Carlsson et al. 2004; Weatherall et al. 2006; Chapman et al. 2010).

The most commonly used species of primate used for research and testing are rhesus macaques (*Macaca mulatta*) and cynomolgus macaques (*M. fascicularis*). A range of other Old World monkeys are used less frequently, such as pigtail macaques (*M. nemestrina*), baboons (*Papio* spp.), and vervet monkeys (*Chlorocebus pygerythrus*). Of the New World monkeys, common marmosets (*Callithrix jacchus*) are the most frequently used, but also others such as tamarins (*Saguinus* spp.), and squirrel (*Saimiri* spp.), capuchin (*Sapajus* spp.), and night (*Aotus* spp.) monkeys. Chimpanzees (*Pan troglodytes*) were the only great ape used in biomedical research, but European legislation (European Parliament and the Council of the European Union 2010 and Turner 2022), and other bans or National Institutes of Health funding limitations mean they are now no longer or rarely used (Kaiser 2013; Graham and Prescott 2015; Grimm 2015). However, Gabon continues to conduct biomedical research on chimpanzees (Kaiser 2013).

Primates are usually purpose-bred for use in research. The use of wild-caught animals is generally no longer accepted (McCann et al. 2007; European Commission 2010). This is because of the stress of capture and transport, and the associated morbidity and mortality (McCann et al. 2007), and presence of disease (Weber et al. 1999). In addition, they may be less suitable as models when their life histories are not known (Howard 2002) as this may introduce unwanted variation and bias experimental outcomes, resulting in studies lacking in experimental rigor and that are consequently less robust in prediction (Howard 2002).

Purpose-bred primates maintain their evolved capacities (i.e., adaptations to survive and reproduce in the wild), and so an understanding of natural history is critical if we are to provide environments that may help to promote welfare. The Jennings and Prescott (2009) report chapters in the Universities Federation for Animal Welfare (UFAW) Handbook (2010; 9th ed. in prep), and Marini et al. (2019) provide important information about the natural history, veterinary, and welfare aspects of the most commonly used primates in research.

Not all animals are protected by legislation, and this varies by country (Bayne et al. 2022). For example, in Europe all vertebrates and some invertebrates are protected, and certain animals, such as primates, cats, dogs, and Equidae, get special protection (European Commission 2010). It appears that this is due to public concern for animals that humans keep as pets or close companions, and our ability to empathize with these animals. There is, however, no robust scientific evidence that the animals with special protection are capable of suffering more than those without special protection (Buchanan-Smith 2010b; Hubrecht 2014).

What evidence is there to suggest that primates require special protection? Their phylogenetic closeness to humans is exactly the reason why they are used, and this similarity is also the basis for our apprehension concerning their use (i.e., they may suffer like humans). The brains of primates are larger in relation to body size than other mammals used in laboratories (Dunbar and Shultz 2007), and brain size is associated with mental capacities and cognitive complexity. However, cognitive complexity does not necessarily mean a greater potential to suffer pain (see Mendl and Paul 2004). The sensation of pain may be the same for an individual which *experiences* it as to one which is *consciously aware* and feels pain (Bekoff 2002). An animal therefore does not need to be self-aware to experience pain. Indeed, Broom (2010) has argued that cognitive complexity may reduce suffering as it helps individuals cope with adverse conditions and allows for more possibilities of pleasure. On the other hand, the most cognitively complex primates, the great apes, may also be able to empathize with the suffering of others, and dread future events, increasing their own ability to suffer (Smith and Boyd 2002; Mendl and Paul 2004). These arguments are not fully evaluated in relation to welfare, but suggest that primates, and certainly great apes, are indeed a special case.

In our view, the strongest scientific arguments that primates require special protection are (1) the intricacy of adverse effects resulting from inappropriate rearing (e.g., Parker and Maestripieri 2011) and (2) that their larger brains have evolved for dealing with the complexity of their social and physical worlds. Primates are long-lived compared with other laboratory animals (e.g., rodents), and this poses challenges for care staff who need to provide the opportunities for good welfare throughout their lives, including as their needs change. The provision of appropriate rearing, together with physical and social complexities in the laboratory environment, can be very challenging given the constraints of laboratory life and the requirements of studies. Table 2 outlines the key stages in a cynomolgus macaque life cycle, with potential negative welfare impacts and opportunities for refinement.

There is considerable debate about whether the suffering that primates experience in laboratory research is cumulative (see Honess and Wolfensohn 2010; Pickard et al. 2013; Wolfensohn et al. 2015). What is clear is that some individuals are unable to cope and are euthanized (Wolfensohn 2022). This may be due to additive stacking up when "the residual effects of repeated procedures may add up" or it may be due to additive potentiation when "suffering from earlier events may actually increase the negative impact on welfare of subsequent events" (Pickard et al. 2013, p. 6). In addition to suffering from direct scientific procedures (e.g., surgery, disease modeling, adverse effects from a test item), the intelligence of primates means they may suffer from boredom and fear. Therefore, the consequences of this, inadequate rearing histories, and environments, together with the scientific procedures conducted upon them, can lead to poor welfare (Buchanan-Smith 2010a, b) (Table 1).

4 Ethical Framework of 3Rs and Welfare

The utilitarian approach is adopted for dealing with the ethical dilemma of using animals in research and testing, is enshrined in legislation, and underpins many local ethical review processes (e.g., European Commission 2010; U.S. Department of Agriculture 2013; including China and India; see Graham and Prescott 2015). This pragmatic approach weighs the ethical importance of the individual and their capacity to suffer against the interests of the other parties concerned (Singer 1975; Sandøe et al. 1997). In practice, this approach is known as a harm-benefit assessment. It is currently applied to primate use prospectively by mandatory ethics boards (e.g., Institutional Animal Care and Use Committees, Animal Welfare and Ethical Review Body) and retrospectively where the actual costs to the animal are reviewed in light of the results of scientific study (European Commission 2010). The perceived harm to the animal in terms of its likely experience of pain, distress, or lasting harm, including intensity, duration, and frequency, is weighed against the anticipated benefits of the research for humans (or other animals or the environment) (Graham and Prescott 2015).

In addition to requiring assessment of the harms and benefits of the proposed research, the legislative framework requires the 3R principle be applied to the project

Table 1 Examples of issues that may compromise welfare of primates (and many other animals) housed and used in laboratory research and testing, and associated refinements

Housing and husbandry	Example of welfare compromise	Refinement opportunity
Individual identification.	Freeze branding, tattooing, microchip, and temporary methods may be painful and impact on behavior (reviewed in Rennie and Buchanan-Smith 2006b)	Sensitive placement of tattoos (never on face). Primates should be anesthetized for tattooing. Combined temporary and permanent methods to minimize frequency of intrusive handling, together with positive reinforcement training (PRT) to accept scanning of microchips, or accept temporary dyes (reviewed in Rennie and Buchanan-Smith 2006b)
Small enclosures lacking environmental complexity.	Space is restricted in laboratories, and few have outdoor areas, limiting the ability to perform species-typical behavioral repertoires. Primates may become bored (Buchanan-Smith 2010a, b).	Factors that should be taken into account when determining enclosure size and design include morphometric, ecological, locomotor, physiological, social, reproductive, and behavioral characteristics (see Buchanan-Smith et al. 2004). Increasing choice, complexity, and opportunities for control improve welfare and coping ability (see Buchanan-Smith and Badihi 2012).
Separation from family earlier than weaning would normally occur in the wild. Unnatural social groups and loss of social support.	Early weaning has a range of physical and psychological effects that negatively impact welfare and quality of scientific output, including behavioral disturbances (e.g., stereotypies and self-injury), growth, health and survival, and immune consequences (reviewed in Prescott et al. 2012).	Prescott et al. (2012) describe the range of factors that should determine weaning age in macaques, with a focus on behavioral, weight, and health criteria, as well as age (not normally less than 10–14 months specified). Marmosets and tamarins should remain in their natal groups until at least 8 months, and 12 months for those destined to breed, to gain experience with rearing 2 sets of younger siblings (Council of Europe Appendix A to Convention ETS 123).

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Table 1 (continued)

Housing and husbandry	Example of welfare compromise	Refinement opportunity
Regular room and cage changes, with changes in grouping. Noisy enclosures—often metal, rooms may be power-hosed.	Room and cage change (Crockett et al. 1995, 2000), and social regrouping (Shively et al. 1997) adversely impact macaques. Regular changes in rooms/social grouping may lead to instability. Cage cleaning is stressful and masks olfactory communication (Epple 1970).	Careful advance planning may increase stability of groups and rooms. For marmosets, where olfactory communication is important, ensure some continuity of familiar scents (e.g., cleaning half the cage, or keep one branch or enrichment device which has been scent marked) (Prescott 2006).
Regulated scientific procedure	Example of welfare compromise	Refinement opportunity
Use as disease models (e.g., Parkinson's disease (PD), arthritis), which may include genetic modification.	MPTP-treated monkeys used as models for PD show akinesia, rigidity, and postural abnormalities; some display a "climbing syndrome" or "obstinate progression syndrome" (reviewed in Vitale et al. 2009). The development of a transgenic model of Huntington's disease, a neurodegenerative disorder characterized in humans by motor impairment, cognitive deterioration, and psychiatric disturbances followed by death within 10–15 years of the onset of the symptoms. The transgenic rhesus macaque exhibits clinical features including dystonia and chorea (Yang et al. 2008).	Physical and social refinements for PD primates are comprehensively described by Vitale et al. (2009) at all stages, including preparation, injections, restraint, and at the various stages of disease progression, to humane end points. These include soft enclosures for individuals who climb and fall, to minimize injury. For genetically modified animals: appropriate treatment of conditions produced, restriction of gene expression to tissues of interest or to certain time periods, and clear criteria to remove primates from a study, or humanely end life to stop further suffering (Dennis 2002).
Toxicology testing	The test substance may cause sickness and health deterioration. Historically, primates used in toxicology studies have had limited social and physical environmental enrichment, given concerns about confounding or negating the study data (e.g., unstable groups, ingesting material) (Bayne 2003).	A list of refinements including social and physical enrichment, and refinements to capture, handling, restraint, and administration and sampling is provided in Rennie and Buchanan-Smith (2006a, b, Rennie and Buchanan-Smith 2006). Consideration should be given to providing comfortable, quiet areas to individuals suffering the effects of administered substances or surgery, and this impacts on types of environmental enrichment appropriate.

Regulated scientific procedure	Example of welfare compromise	Refinement opportunity
Surgery	Surgery is common, for example, in neuroscience and implanting internal telemetry devices for remote recording, but even with appropriate anesthesia and analgesics surgery may lead to complications (e.g., Rennie and Buchanan-Smith 2006b; Pickard et al. 2013).	Improvement in headposts includes biocompatible titanium, which is simpler to implant, more securely anchored, easier to maintain, and less obtrusive than devices attached with traditional acrylic (Adams et al. 2007). Morton et al. (2003) provide an account of refinements for all aspects of telemetry.
Capture, handling, and restraint to collect data (such as blood samples, electrocardiogram), administer substances, and provide medical care, which often has intrinsically aversive components for disease models.	There is extensive evidence that capture, handling, and restraint can be stressful (reviewed in Rennie and Buchanan-Smith 2006b). Drug Metabolism and Pharmacokinetics (DMPK) requires sampling at fixed intervals (e.g., waking animals at night), and sleep deprivation adversely alters biological functioning (McEwen 2006).	Human socialization and PRT are key to minimizing stress associated with restraint (see Prescott and Buchanan-Smith 2007). Careful planning of housing can minimize disruption of nonstudy animals and DMPK animals, without disruption to social groups. Methods of sampling and refinements, including PRT, long-term catheterization techniques to reduce painful catheter starts or more invasive approaches for blood collection/drug delivery (e.g., portal vascular access), and sonophoresis, are reviewed in Rennie and Buchanan-Smith (2006b). Refinement for administration of substances is reviewed in Rennie and Buchanan-Smith (2006b) and Morton et al. (2001). Caron et al. (2015) describe miniaturized blood sampling techniques to minimize volume required to be taken.
Single housing in metabolism cages, to allow collection of samples (e.g., urine).	It is widely recognized that single housing of primates is detrimental to psychological well-being (e.g., Hartner et al. 2001; McCann et al. 2007)	Primates can be trained to produce a urine sample on request (McKinley et al. 2003) or other mediums such as saliva may be collected (e.g., Lutz et al. 2000; Ash et al. 2018) obviating the need for single housing.

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Table 1 (continued)

Regulated scientific procedure	Example of welfare compromise	Refinement opportunity
Food and fluid restriction.	Prescott et al. (2010) describes the (understudied) effects of food and fluid restriction on physiological and behavioral responses in animals, which can potentially compromise their health and well-being.	Prescott et al. (2010) details refinements to food and fluid control as motivational tools for macaques used in behavioral neuroscience research, including alternatives and type of reward (e.g., appetitive rewards), level of control, and breaks in regimen.
Stress in anticipation of event.	The order in which samples are taken are known to affect blood cell counts and plasma cortisol (Capitanio et al. 1996; Flow and Jaques 1997).	Individuals should not be restrained and dosed or sampled in view of others. Reliably signaling a stressful event for individual animals may reduce stress (see Bassett and Buchanan-Smith 2007).
Intentional death—this is often required as part of the experiment, to allow postmortem analysis, or when primates are no longer required and cannot be reused.	Rennie and Buchanan-Smith (2006b) describe issues related to welfare leading up to euthanasia, and humane end points.	Extremely competent staff and use of PRT are important refinements in euthanasia—administration of the euthanizing agent must result in rapid loss of consciousness before death ensues (Rennie and Buchanan-Smith 2006b). Rehoming potential is also discussed. The OECD (2000) describes refinements, including validation, use of earlier end points, and avoidance of using death and moribundity as end points.

from its experimental design to its execution (Home Office 1986; European Commission 2010). The 3Rs are replacement, reduction, and refinement. Replacement is concerned with the absolute or relative replacement of animals for scientific use. Reduction emphasizes the need to reduce to a minimum the number of animals through good experimental design, the sharing of data and/or resources, or by using modern techniques to obtain more information from the same number of animals (thereby reducing future use of animals). Refinement has been defined as "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 well-being" (Buchanan-Smith et al. 2005, pp. 379–380). This definition highlights the need to consider all stages of an animal's life, from birth to death, including the promotion of good welfare of breeding animals not used in research, and involves not just minimization of harms, but takes a proactive stance to enhance welfare through to death with the use of humane end points as required (Wolfensohn 2022). It should also be noted that, although each R is often considered separately, they have a complex interplay (de Boo et al. 2005). For example, reuse may reduce the number of individuals used, but increase the suffering of individual animals. Table 2 highlights some of the main welfare issues that a macaque used in toxicology may experience across their lifespan and describes the opportunities for refinement.

The 3Rs provide a platform for uniting welfare together with scientific merit (e.g., refinement: Richter et al. 2010; Tasker 2012; Hall et al. 2015). They can also help increase public support for animal research by highlighting that alternatives are being sought and that animal welfare is prioritized (Leaman et al. 2014). However, despite the widespread scientific support of the 3Rs, there are barriers to uptake of refinements, including staff time, motivation, knowledge, skills, and resources. Laboratories need to have ongoing programs to critically appraise practice in light of new evidence and resources so that the most up-to-date refinements are used (Lloyd et al. 2008). Several publications provide detailed and comprehensive accounts of refinements for primates (e.g., Rennie and Buchanan-Smith 2006a, b; Rensnie and Buchanan-Smith 2006; Jennings and Prescott 2009). To implement refinement successfully requires understanding what welfare is, how it can be assessed, and having a strategy for rapid implementation of changes and their evaluation. Underlying this process should be an acceptance that refinement is a necessary and continuous process—it is a permanent challenge for care staff and scientists (Tasker 2012).

While welfare in the laboratory is formally considered in terms of refinement, one of the greatest influences on the development of animals and their resilience (i.e., their coping ability) as adults is their early rearing environment (Parker and Maestripieri 2011). Hence, the welfare of primates under study may be profoundly affected by the conditions in which they are born, reared, and kept prior to their use in a study.

In macaques, natural weaning from the mother's milk is usually seen at 10–14 months of age (Harvey et al. 1987); it is a gradual process involving withdrawal of milk and dependence on the mother for caregiving over a period of weeks or months (Lee 1996). Offspring remain with their mother beyond weaning for up to 24 months of age (Ross 1992). In captive breeding, infants are commonly removed from their mothers and natal group at about 6 months of age (Honess et al. 2010). Removal from the natal group and manipulations in the early rearing environment are stressful and result in long-term alterations in the animals' immune system and its regulation (Coe et al. 1989). More specifically, weaning and removal of the mother are known to have immunosuppressive effects (Coe et al. 1987). Toxicologists testing new pharmaceuticals that are likely to alter immunological parameters in macaques should be aware of the potential confounding effects of differential rearing histories when selecting research subjects (Tasker 2012).

Table 2 Example life cycle of a cynomolgus macaque used in toxicology in the UK, with opportunities for refinement at all stages. PRT: positive reinforcement training

Potential negative welfare impact	Opportunity for refinement
Birth and rearing environment	
Prenatal stress (i.e., during gestation) can negatively impact stress responsivity of offspring (Clarke et al. 1994) potentially making the primate less "fit for purpose."	Appropriate breeding and rearing environment (see below)
Unnatural social group and inappropriate housing and husbandry, and/or overcrowding leading to stress and poor welfare (e.g., Buchanan-Smith et al. 2004).	Natural social group in appropriate housing, providing complexity, choice, and control, with visual barriers, increasing resilience and ability to cope with challenges associated with laboratory research and testing (Buchanan-Smith 2010a, b). Human socialization and PRT—visual and auditory cues well established and required in husbandry, research, and testing.
Weaning and transport	
Capture, handling, health screening, separation, and early weaning from groups (for adverse consequences of early weaning, see Prescott et al. 2012). In captive breeding, infants are often removed early from the mother and natal group, enforcing abrupt weaning (Honess et al. 2010).	Capture, handling, and health screening are facilitated by previous PRT and good human socialization (e.g., articles in Prescott and Buchanan-Smith 2003, 2007). Decisions on timing of separation from natal group should be based on numerous factors including age, but also behavioral and health considerations (see Prescott et al. 2012). Keeping weaned macaques with familiar compatible conspecifics provides social buffering and reduces stress (Gust 1996 for rhesus macaques).
Primates imported for research may have journeys up to 58 h with evidence of heightened levels of stress for over one month after arrival at the new establishment (Prescott 2001; Honess et al. 2004).	Efforts should be made to encourage social stability, before, during, and after transport, by housing animals in socially appropriate groups, allowing environmental conditions (light, heat, etc.) to vary in a natural daily rhythm, preventing boredom with suitable environmental enrichment and sufficient space. The total duration of transport should be minimized and conditions at the destination, should as a minimum, be at least as good as those at the source (Honess et al. 2004).
Where used, a holding facility may have physically smaller enclosures, limiting range of behavior, change in routines, diet, and changes in social grouping and hierarchy	Continuation of housing and husbandry provision from breeding facility (e.g., familiarity of diet). Continuation of signals for PRT, and any prestudy training as appropriate.
Designated environment for research	
Behavioral restriction, change in routines, diet, and changes in social grouping and hierarchy.	There are a number of considerations that should determine cage size (Buchanan-Smith et al. 2004) with possibilities for enrichment, exercise areas, and providing choice, complexity, and control (Buchanan-

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Table 2 (continued)

Potential negative welfare impact	Opportunity for refinement
	Smith 2010b). Clear temporal and signaled predictability to learn new routines (Bassett and Buchanan-Smith 2007), with possibilities for accelerated acclimatization. Positive staff interactions and socialization, using PRT (Tasker 2012; Ash and Buchanan-Smith 2016). Playback of affiliative vocalizations at a natural frequency improves welfare (Watson et al. 2014 for marmosets).
Study protocol	
Uncertainty, capture, restraint, sham dosing, dosing, effects of toxicology (see Table 1).	Use of reliable signals to inform primates of events, refined methods of capture and restraint, facilitated by socialization and PRT, removal of sham dosing that appears to sensitize primates (Tasker 2012). Quiet secluded area given to primates suffering from adverse effects of test substance.
Re-use raises particular welfare issues arising from inappropriate housing and husbandry, and their use in scientific procedures may prolong negative welfare states and impact on model suitability (see Morton et al. 2003).	Morton et al. (2003) include the following recommendations for re-use of primates in telemetry studies, over and above legal compliance: • All the ethical and welfare issues are fully addressed when making decisions about re-use, in addition to the scientific issues. • Ensure recovery and wash-out periods are adequate. • A system is set up where authority for re-use depends on veritable certification of health status that includes an assessment of behavioral, physical, psychological, and social well-being. • Consider all the potential welfare costs to each individual, including those associated with housing and husbandry, when making a decision about re-use or continued use.
Death or moribundity is used as the end points.	See Table 1

There are also potential problems with the rearing environments of common marmosets, the most frequently used New World primate. This species is characterized by twin births and the cooperation of all members of the family in rearing the young until independence and natural weaning, which occurs at approximately 8 weeks of age (Yamamoto 1993; Buchanan-Smith 2010a). In captivity, dams have higher weights than in the wild, which is associated with larger litters, birth complications, and increased infant mortality (Ash and Buchanan-Smith 2014). Supplemental feeding of litters of three or more, involving removal of the infant or infants from the natal group for hand-feeding, is often practiced during the first 2 months after birth to reduce infant mortality. Depending on how this is done, it has the potential to affect development and confound scientific output (Ash and Buchanan-Smith 2016).

Ash and Buchanan-Smith (2016) used a battery of tests to determine the impact of rearing environment in common marmoset infants in 3 conditions: family-reared marmoset twin pairs, family-reared marmosets from triplet litters where only 2 remain (2 stays), and supplementary-fed triplets. The supplementary-fed triplet infants were never isolated except for very short periods for weighing and had positive experiences with humans from an early age. Furthermore, they are naturally adapted to being passed between carriers (Ingram 1977). The infants were also returned to their family group as soon as possible after feeding, and so spent most of their time with their family group. This supplementary feeding rearing practice had no adverse effects on behavior/cognition, neophobia, nor affective state (Ash and Buchanan-Smith 2016). However, primate infants that are hand-raised entirely by humans have reduced reproductive success and often experience adverse welfare such as increased self-directed behaviors, abnormal behaviors, and inappropriate aggressive behavior (e.g., Porton and Niebruegge 2006). Dettling et al. (2002, 2007) found that early parental deprivation in common marmosets impacts endocrine responses (lower basal cortisol) and several behavioral responses (e.g., they are less mobile and make fewer contact calls than controls in response to social separation/exposure to novelty), as well as blood pressure, which is higher than in controls. These changes make them unfit models of normal healthy humans.

Ideally, the purpose for which primates are bred and subsequently used as research subjects should be known, so practices can be put in place to ensure the animals are “fit for purpose.” This might, for example, involve human socialization and positive reinforcement training for certain husbandry practices and procedures, or exposure to a range of stimuli likely to be encountered, paired with rewards to desensitize the primates. However, many primates are bred in special centers that are often overseas and require transport to the laboratory (Prescott 2001) where they will be used in research (Ha and Sussman 2022). The laboratory of end use may not have direct control over, or the ability to monitor, social groups, weaning age, and conditions, although some countries have legislation to cover designated breeding centers. Ideally, research laboratories should have a coordinated approach with the breeding centers that supply their animals to promote welfare, as well as ensure the primates are “fit for purpose” and to minimize confounding factors that may introduce unwanted variability in scientific output.

5 Importance of Welfare for Quality of Scientific Output

To achieve high-quality and reliable science, several essential conditions must be satisfied. The experiment must yield unambiguous results by minimizing unwanted variation, there must be an absence of confounding factors (Poole 1997), and the study must be undertaken to a required standard (e.g., Organisation for Economic Cooperation and Development (OECD) 1997). In addition to aspects of quality pertaining to the study, quality in its fullest sense should also include the impact of research using animals (see Bateson et al. 2011)—that is, the application of knowledge resulting from research findings.

Poole's (1997) seminal paper "Happy animals make good science" argues that good laboratory animal science is based upon normal, healthy, and happy animals, unless illness or alleviation of stress is the subject of study. Although the effects of stress and disease are easy to identify, and their confounding effects are well known (e.g., Reinhardt et al. 1995; Festing and Altman 2002; Hall 2007), Poole (1997) argues that unhappiness is also a confounding variable because its effects on biological variables produce increased variation in the data output. He goes further, asserting that most scientists working with animals assume that they have normal physiology and behavior (e.g., heart rates, blood pressure, blood values, metabolism, hormones, and immunological competence). However, these parameters can be dramatically altered by the conditions in which the animals were bred, reared, kept, transported, and the way in which experimental procedures were conducted. Experimenters may assume these parameters to be normal because they commonly encounter them and have no reference for comparison (Tasker 2012). If animals are not well acclimatized, properly characterized, and stable, there is a major risk of confounding and under- or overestimating the treatment effect with no predictive validity to the clinic (Graham and Schuurman 2017). For example, restraint may lead to maximum heart rates, preventing the detection of arrhythmias (Tasker 2012) or significant changes in glycemic control, blunting the response to treatment (Graham and Schuurman 2017). The link between welfare and scientific output is covered in Schapiro and Hau (2022).

There are strong ethical, scientific, and economic arguments to suggest that "no data are better than poor data." If the data are not of good scientific quality and results are therefore potentially unreliable, inaccurate, or inconclusive, then the primates used in the research will have suffered in vain, violating utilitarian principles underlying animal use. Poor animal welfare and quality of science may also cause delays or lead the research down the wrong path, with more animals being used (going against the reduction principle) and more unnecessary suffering. This not only has ethical implications for the animals, but also wastes time and money. Indeed, Bains (2004) estimated that it takes an average of 12.5 years and $1 billion to take a new drug to market. Recognizing the dependence of reliability of scientific outcomes on animal welfare, it is logical to conclude that these costs are likely to be reduced with improved animal welfare.

6 Welfare Assessment

Given that scientific procedures directly impact negatively on welfare, it is critical for there to be ways of accurately assessing welfare. The list of factors in Table 1 illustrates that primates in laboratories often have reduced welfare, especially in the absence of refinement. However, assessment should include measuring positive welfare states, such as comfort and contentment, as well as negatives ones such as boredom, fear, pain, and/or suffering (Buchanan-Smith et al. 2005). We should focus not only on welfare as a snapshot of the animal in time, but view the animal, as much as is possible, from birth to death, 24/7, across the lifespan (Brando and Buchanan-Smith 2018). This includes day and nighttime assessments, and assessments over weekdays and weekends. For example, Lambeth et al. (1997) found higher wounding rates for chimpanzee during weekdays when care staff are present than at weekends, suggesting something about the weekday routines, such as elevated activities of caregiving, veterinary, research, and other personnel, were causing tension. As well as this welfare may vary across seasons, particularly if there is a mating season when male aggression rises. There are also individual requirements across the different life stages. Younger individuals require special provisioning to allow them to engage more in play, to explore, and to learn contingencies between behaviors and their outcomes. It is known that having control over aspects of the environment improves the welfare of younger individuals more than it does for older individuals (Badihi 2006). Waitt et al. (2010) provide a list of considerations for designing environments for aged primates that include accessibility issues, positioning, size, and type of furnishings, to avoid poor welfare related to age-related arthritis, deteriorating vision, difficulties in thermoregulation, etc. Furthermore, given individual differences in the propensity for welfare states due to personality differences (e.g., King and Landau 2003), we must consider individuals as being unique (Robinson and Weiss 2022).

Hawkins et al. (2011) provide an excellent review of assessment of welfare in laboratory-housed animals. Several practical issues were raised in this review, including how to set up and operate effective protocols for the welfare assessment. The need to tailor welfare assessment protocols to individual animals, as well as individual projects, is emphasized, too, in this review, together with the need to quantify objectively measures relating to the welfare state, and to intervene early to alleviate negative states and minimize them worsening. The problem is that even in our closest living relatives, the primates, it can be challenging to recognize internal states such as pain and suffering (e.g., Flecknell et al. 2011; Sneddon et al. 2014), and although the use of analgesics following potentially painful procedures is improving in primates, it is still not optimal (Coulter et al. 2009). Section 2 of this book provides a comprehensive review of the methods used to assess primate welfare.

7 The Role of Human Behavior Change in Refinement

From birth to death, the lives of primates in designated breeding and supplying establishments are under absolute human control. When seeking welfare improvements, a fully integrated approach is required to ensure refinements are implemented at every stage of the life cycle (e.g., Table 1). The stakeholders include the scientists, study directors, advising statisticians, ethical review panel staff, the veterinarians, the animal technicians, and care staff who are all responsible for the primates' day-to-day needs—they all have a stake in implementing positive welfare change.

It is often the case that primates spend a rather small amount of time directly engaged in scientific research. An exception is neuroscience, where the primates (usually macaques) may be food and fluid-controlled, restrained in chairs, and tested from 2 to 8 h at a time, 5–7 days a week depending on the requirements of the experiment, and use continues for a number of years (Prescott et al. 2005). But for most primates, the majority of time is spent living in enclosures. Given this, the social and physical complexity of the enclosures, and the control and choices that the primates can make directly impact their welfare. In all cases, the behavior of humans is critical for promoting the welfare of primates housed in laboratories (Rennie and Buchanan-Smith 2006a).

Human behavior change is a growing discipline. It refers to a process that translates knowledge into actions, so that targeted change is implemented. This process is underpinned by multidisciplinary scientific approaches and theoretical frameworks (Michie et al. 2014); it has considerable merit in improving human health (e.g., Ory 2002) and is gaining momentum as a practical way for advancing animal welfare (e.g., Van Dyne and Pierce 2004; van Dijk et al. 2013; Whay and Main 2015). However, traditional approaches to implementing refinements have not focused on stimulating changes in human behavior.

Broadly speaking, two types of intervention are employed to improve animal welfare. These are (1) enforcement of legislation, codes of practice, and supplementary, voluntary accreditation schemes or standards, and/or (2) encouragement, which includes promoting innovation that exceeds minimum standards, and regularly accessing and implementing new knowledge and scientific findings.

In the UK, the appointment of Named Animal Care and Welfare Officers and, in Europe, the appointment of the Institutional Care and Animal Welfare Responsible Person provide oversight and, together with mandatory staff training, ensure minimum standards are met. Pharmaceutical companies and Contract Research Organizations (CROs) are committed to improving animal welfare. They often undergo voluntary Assessment and Accreditation of Laboratory Animal Care accreditation to demonstrate that they meet the minimum standards required by law and are also going the extra step to improve animal care and use. Most large pharmaceutical companies and CROs are signatories of the Concordat on Openness in Animal Research, an agreement across the biomedical sciences sector to improve communication and increase transparency to the public about animal research. There is therefore considerable enthusiasm from the industry to improve animal care and use now and in future.

Good primate welfare is dependent upon creating a strong culture of care. To achieve this, we must overcome barriers, including a lack of knowledge/resources/skills, provide a robust scientific evidence base for recommendations, give ownership of improvements, and the recognition, support, and reward system for those who effectively engage with the refinements we are proposing. To tackle the lack of knowledge and skills, we see communication, training, and dissemination of findings as fundamental to moving the refinement agenda forward.

Given the range of standards worldwide, it is also critical that training is pitched at the right level for dissemination, and the effectiveness of training resources may be enhanced if created with an understanding of models of human behavior change. Considering that primate use is likely to continue until alternative technologies are developed (e.g., see Burm et al. 2014), we need to disseminate evidence-based practice and empower individuals to lead on refinement. The launches of websites, such as marmosetcare.com and the macaque website (nc3rs.org.uk/macaques/), together with other online resources (see Prescott 2016), are a good step toward this goal.

The second approach, encouragement, is where we see considerable opportunities for innovation and improvement, to promote sustainable human behavior changes that result in positive impacts on primate welfare. The cornerstone of encouraging behavior change is to transfer ownership of both the problem and solution to a person responsible for implementing change (Whay and Main 2015). There are two important components necessary for change to happen. The first is appreciating the relevance of the desired behavior change, which must be coupled with taking ownership of the process of change, rather than being told what to do, or even through demonstration. Therefore, creating opportunities for colleagues to explore issues and come up with their own solutions will be more effective than simply being instructed without individual motivation and responsibility (Cunningham et al. 2002). Indeed, there is no one-size-fits-all approach to refinement.

All key stakeholders need to be empowered toward improving welfare, while at the same time fully appreciating experimental aims and impact. The ultimate goal is to synergize better welfare and better science to elevate the quality of the research. Understanding and seeking out people who have powers of influence are important, as change requires targeting several levels. Behavior change may be encouraged using three broad approaches, namely social marketing (an extension of principles used in marketing and advertising to promote change among groups), participatory methods (such as those used in the community development sector), and the creation of action groups (self-help or discussion groups). Useful examples of how these approaches have been implemented and tested in the agricultural sector are discussed by Whay and Main (2015). In practice, both combining and coordinating approaches (e.g., Van Dyne and Pierce 2004; Pritchard et al. 2012; van Dijk et al. 2013) are required to improve welfare. We are keen to advance the evidence base showing that human behavior change techniques improve sustainable uptake of refinements for primates; hence, making primates better models for research.

8 Conclusions

There are specific welfare issues for primates used in laboratory research and testing, including painful procedures and a restricted environment. It is imperative that we apply refinement throughout laboratory primates' lives. Specifically, efforts must be made to integrate all stages of a primate's life to improve their welfare by providing opportunities for positive experiences and conditions that enable them cope with challenges. If primates are to be used as models of normal functioning humans, the promotion of welfare will also help ensure that they are "fit for purpose" and will avoid situations where a negative welfare state confounds biological data and leads to research with unreliable or faulty conclusions. By giving ownership of the resources to target audiences, and providing the evidence base underpinning benefits (welfare, scientific output, and financial), knowledge exchange within and across facilities should continue to improve, and with it animal welfare and quality of scientific output.

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References

Adams DL, Economides JR, Jocson CM, Horton JC (2007) A biocompatible titanium headpost for stabilizing behaving monkeys. J Neurophysiol 98(2):993–1001. https://doi.org/10.1152/jn.00102.2007

Ash H, Buchanan-Smith HM (2014) Long-term data on reproductive output and longevity in captive female common marmosets (*Callithrix jacchus*). Am J Primatol 76(11):1062–1073. https://doi.org/10.1002/ajp.22293

Ash H, Buchanan-Smith HM (2016) The long-term impact of infant rearing background on the affective state of adult common marmosets (*Callithrix jacchus*). Appl Anim Behav Sci 174: 128–136. https://doi.org/10.1016/j.applanim.2015.10.009

Ash H, Smith TE, Knight S, Buchanan-Smith HM (2018) Measuring physiological stress in the common marmoset (*Callithrix jacchus*): validation of a salivary cortisol collection and assay technique. Physiol Behav 185:14–22. https://doi.org/10.1016/j.physbeh.2017.12.018

Badihi I (2006) The effects of complexity, choice and control on the behaviour and the welfare of captive common marmosets (*Callithrix jacchus*). University of Stirling

Bains W (2004) Failure rates in drug discovery and development: will we ever get any better? Drug Discov World 9. https://www.ddw-online.com/failure-rates-in-drug-discovery-and-development-will-we-ever-get-any-better-1027-200410/

Bassett L, Buchanan-Smith HM (2007) Effects of predictability on the welfare of captive animals. Appl Anim Behav Sci 102(3–4):223–245. https://doi.org/10.1016/j.applanim.2006.05.029

Bateson P, Johansen-Berg H, Jones DK, et al (2011) Review of research using non-human primates: Report of a panel chaired by Professor Sir Patrick Bateson FRS. London

Bayne KAL (2003) Environmental enrichment of nonhuman primates, dogs and rabbits used in toxicology studies. Toxicol Pathol 31(Supplement):132–137. https://doi.org/10.1080/01926230390175020

Bayne K, Morris TH, France MP (2010) Legislation and oversight of the conduct of research using animals: a global overview. In: Hubrecht R, Kirkwood J (eds) The UFAW handbook on the care and management of laboratory and other research animals. Wiley-Blackwell, Oxford, pp 107–123

Bayne K, Hau J, Morris T (2022) The welfare impact of regulations, policies, guidelines and directives and nonhuman primate welfare. In: Robinson LM, Weiss A (eds) Nonhuman primate welfare: from history, science, and ethics to practice. Springer, Cham, pp 629–646

Bekoff M (2002) Awareness: Animal reflections. Nature 419(6904):255–255. https://doi.org/10.1038/419255a

Brando S, Buchanan-Smith HM (2018) The 24/7 approach to promoting optimal welfare for captive wild animals. Behav Process 156:83–95. https://doi.org/10.1016/j.beproc.2017.09.010

Brent L (2007) Life-long well being: applying animal welfare science to nonhuman primates in sanctuaries. J Appl Anim Welf Sci 10(1):55–61. https://doi.org/10.1080/10888700701277626
Broom DM (1986) Indicators of poor welfare. Br Vet J 142(6):524–526. https://doi.org/10.1016/0007-1935(86)90109-0
Broom DM (1999) Animal welfare: the concept and the issues. In: Dolins FL (ed) Attitudes to animals: views in animal welfare. Cambridge University Press, Cambridge, pp 129–143
Broom DM (2010) Cognitive ability and awareness in domestic animals and decisions about obligations to animals. Appl Anim Behav Sci 126(1–2):1–11. https://doi.org/10.1016/j.applanim.2010.05.001
Buchanan-Smith HM (2010a) Marmosets and tamarins. In: Hubrecht R, Kirkwood J (eds) The UFAW handbook on the care and management of laboratory and other research animals. Wiley-Blackwell, Oxford, pp 543–563
Buchanan-Smith HM (2010b) Environmental enrichment for primates in laboratories. Adv Sci Res 5(1):41–56. https://doi.org/10.5194/asr-5-41-2010
Buchanan-Smith HM, Badihi I (2012) The psychology of control: effects of control over supplementary light on welfare of marmosets. Appl Anim Behav Sci 137(3–4):166–174. https://doi.org/10.1016/j.applanim.2011.07.002
Buchanan-Smith HM, Prescott MJ, Cross NJ (2004) What factors should determine cage sizes for primates in the laboratory? Anim Welf 13(Supplement 1):S197–S201
Buchanan-Smith HM, Rennie AE, Vitale A et al (2005) Harmonising the definition of refinement. Anim Welf 14(4):379–384
Burm SM, Prins J-B, Langermans J, Bajramovic JJ (2014) Alternative methods for the use of non-human primates in biomedical research. ALTEX-Altern Anim Ex 31(4):520–529. https://doi.org/10.14573/altex.1406231
Capitanio JP, Mendoza SP, McChesney M (1996) Influences of blood sampling procedures on basal hypothalamic-pituitary-adrenal hormone levels and leukocyte values in rhesus macaques (*Macaca mulatta*). J Med Primatol 25(1):26–33. https://doi.org/10.1111/j.1600-0684.1996.tb00189.x
Carlsson H, Schapiro SJ, Farah I, Hau J (2004) Use of primates in research: a global overview. Am J Primatol 63(4):225–237. https://doi.org/10.1002/ajp.20054
Caron A, Lelong C, Pascual M-H, Benning V (2015) 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 54(2):145–152
Chapman KL, Pullen N, Andrews L, Ragan I (2010) The future of non-human primate use in mAb development. Drug Discov Today 15(5–6):235–242. https://doi.org/10.1016/j.drudis.2010.01.002
Clarke AS, Wittwer DJ, Abbott DH, Schneider ML (1994) Long-term effects of prenatal stress on HPA axis activity in juvenile rhesus monkeys. Dev Psychobiol 27(5):257–269. https://doi.org/10.1002/dev.420270502
Coe CL, Rosenberg LT, Fischer M, Levine S (1987) Psychological factors capable of preventing the inhibition of antibody responses in separated infant monkeys. Child Dev 58(6):1420–1430. https://doi.org/10.1111/j.1467-8624.1987.tb03855.x
Coe CL, Lubach GR, Ershler WB, Klopp RG (1989) Influence of early rearing on lymphocyte proliferation responses in juvenile rhesus monkeys. Brain Behav Immun 3(1):47–60. https://doi.org/10.1016/0889-1591(89)90005-6
Coleman K, Timmel G, Prongay K, Baker KC (2022) Common husbandry, housing, and animal care practices. In: Robinson LM, Weiss A (eds) Nonhuman primate welfare: from history, science, and ethics to practice. Springer, Cham, pp 317–348
Coulter CA, Flecknell PA, Richardson CA (2009) Reported analgesic administration to rabbits, pigs, sheep, dogs and non-human primates undergoing experimental surgical procedures. Lab Anim 43(3):232–238. https://doi.org/10.1258/la.2008.008021

Crockett CM, Bowers CL, Shimoji M et al (1995) Behavioral responses of longtailed macaques to different cage sizes and common laboratory experiences. J Comp Psychol 109(4):368–383. https://doi.org/10.1037/0735-7036.109.4.368

Crockett CM, Shimoji M, Bowden DM (2000) Behavior, appetite, and urinary cortisol responses by adult female pigtailed macaques to cage size, cage level, room change, and ketamine sedation. Am J Primatol 52(2):63–80. https://doi.org/10.1002/1098-2345(200010)52:2<63::AID-AJP1>3.0.CO;2-K

Cunningham CE, Woodward CA, Shannon HS et al (2002) Readiness for organizational change: a longitudinal study of workplace, psychological and behavioural correlates. J Occup Organ Psychol 75(4):377–392. https://doi.org/10.1348/096317902321119637

Dawkins MS (2004) Using behaviour to assess animal welfare. Anim Welf 13(Supplement 1):S3–S7

de Boo MJ, Rennie AE, Buchanan-Smith HM, Hendriksen CFM (2005) The interplay between replacement, reduction and refinement: considerations where the Three Rs interact. Anim Welf 14(4):327–332

Dennis MB (2002) Welfare issues of genetically modified animals. ILAR J 43(2):100–109. https://doi.org/10.1093/ilar.43.2.100

Dettling AC, Feldon J, Pryce CR (2002) Early deprivation and behavioral and physiological responses to social separation/novelty in the marmoset. Pharmacol Biochem Behav 73(1): 259–269. https://doi.org/10.1016/S0091-3057(02)00785-2

Dettling AC, Schnell CR, Maier C et al (2007) Behavioral and physiological effects of an infant-neglect manipulation in a bi-parental, twinning primate: Impact is dependent on familial factors. Psychoneuroendocrinology 32(4):331–349. https://doi.org/10.1016/j.psyneuen.2007.01.005

Dunbar RI, Shultz S (2007) Understanding primate brain evolution. Philos T Roy Soc B 362(1480): 649–658. https://doi.org/10.1098/rstb.2006.2001

Epple G (1970) Quantitative studies on scent marking in the marmoset. (*Callithrix jacchus*). Folia Primatol 13(1):48–62. https://doi.org/10.1159/000155308

European Commission (2010) Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes OJL276/33

Festing MFW, Altman DG (2002) Guidelines for the design and statistical analysis of experiments using laboratory animals. ILAR J 43(4):244–258. https://doi.org/10.1093/ilar.43.4.244

Flecknell P, Leach M, Bateson M (2011) Affective state and quality of life in mice. Pain 152(5): 963–964. https://doi.org/10.1016/j.pain.2011.01.030

Flow BL, Jaques JT (1997) Effect of room arrangement and blood sample collection sequence on serum thyroid hormone and cortisol concentrations in cynomolgus macaques (*Macaca fascicularis*). Contemp Top Lab Anim Sci 36(1):65–68

Fraser D (2009) Assessing animal welfare: different philosophies, different scientific approaches. Zoo Biol 28(6):507–518. https://doi.org/10.1002/zoo.20253

Graham ML, Prescott MJ (2015) The multifactorial role of the 3Rs in shifting the harm-benefit analysis in animal models of disease. Eur J Pharmacol 759:19–29. https://doi.org/10.1016/j.ejphar.2015.03.040

Graham ML, Schuurman H-J (2017) Pancreatic islet xenotransplantation. Drug Discov Today Dis Model 23:43–50. https://doi.org/10.1016/j.ddmod.2017.11.004

Grimm D (2015) New rules may end U.S. chimpanzee research. Science 349(6250):777. https://doi.org/10.1126/science.349.6250.777

Gust D (1996) Effect of companions in modulating stress associated with new group formation in juvenile rhesus macaques. Physiol Behav 59(4–5):941–945. https://doi.org/10.1016/0031-9384(95)02164-7

Ha JC, Sussman AF (2022) Primate breeding colonies: colony management and welfare. In: Robinson LM, Weiss A (eds) Nonhuman primate welfare: from history, science, and ethics to practice. Springer, Cham, pp 303–316

Hall RL (2007) Clinical pathology of laboratory animals. In: Gad S (ed) Animal models in toxicology, 2nd edn. CRC Press, Boca Raton, FL, pp 826–828

Hall LE, Robinson S, Buchanan-Smith HM (2015) Refining dosing by oral gavage in the dog: a protocol to harmonise welfare. J Pharmacol Toxicol Methods 72:35–46. https://doi.org/10.1016/j.vascn.2014.12.007

Hartner M, Hall J, Penderghest J, Clark LP (2001) Group-housing subadult male cynomolgus macaques in a pharmaceutical environment. Lab Anim (NY) 30(8):53–57. https://doi.org/10.1038/5000167

Harvey PH, Martin RD, Clutton-Brock TH (1987) Life histories in comparative perspective. In: Smuts BB, Cheyney DL, Seyfarth RM et al (eds) Primate societies. University of Chicago Press, Chicago, pp 181–196

Hau J, Farah IO, Carlsson H-E, Hagelin J (2000) Opponents' statement: non-human primates must remain accessible for vital biomedical research. In: Balls M, van Zeller AM, Halder M (eds) Progress in the reduction, refinement and replacement of animal experimentation: Developments in animal and veterinary sciences, book 31b. Elsevier, Oxford, pp 1593–1601

Hawkins P, Morton DB, Burman O et al (2011) A guide to defining and implementing protocols for the welfare assessment of laboratory animals: eleventh report of the BVAAWF/FRAME/RSPCA/UFAW Joint Working Group on Refinement. Lab Anim 45(1):1–13. https://doi.org/10.1258/la.2010.010031

Hevesi R (2022) The welfare of primates kept as pets and entertainers. In: Robinson LM, Weiss A (eds) Nonhuman primate welfare: from history, science, and ethics to practice. Springer, Cham, pp 121–144

Home Office (1986) Animals (Scientific Procedures) Act 1986. Her Majesty's Stationary Office, London

Honess P, Wolfensohn S (2010) The extended welfare assessment grid: a matrix for the assessment of welfare and cumulative suffering in experimental animals. ATLA-Altern to Lab Anim 38(3):205–212. https://doi.org/10.1177/026119291003800304

Honess PE, Johnson PJ, Wolfensohn SE (2004) A study of behavioural responses of non-human primates to air transport and re-housing. Lab Anim 38(3):119–132. https://doi.org/10.1258/002367704322968795

Honess P, Stanley-Griffiths MA, Narainapoulle S et al (2010) Selective breeding of primates for use in research: consequences and challenges. Anim Welf 19(Supplement 1):57–65

Hosey G (2000) Zoo animals and their human audiences: what is the visitor effect? Anim Welf 9(4):343–357

Hosey G (2022) The history of primates in Zoos. In: Robinson LM, Weiss A (eds) Nonhuman primate welfare: from history, science, and ethics to practice. Springer, Cham, pp 3–30

Howard BR (2002) Control of variability. ILAR J 43(4):194–201. https://doi.org/10.1093/ilar.43.4.194

Hubrecht RC (2014) The welfare of animals used in research: practice and ethics. Wiley, Blackwell, Chichester, West Sussex

Ingram JC (1977) Interactions between parents and infants, and the development of independence in the common marmoset (*Callithrix jacchus*). Anim Behav 25(4):811–827. https://doi.org/10.1016/0003-3472(77)90035-5

Jennings M, Prescott MJ (eds) (2009) Refinements in husbandry, care and common procedures for non-human primates. Lab Anim 43(Supplement 1):1–47. https://doi.org/10.1258/la.2008.007143

Kaiser J (2013) NIH to phase out most chimp research. Science 341(6141):17–18. https://doi.org/10.1126/science.341.6141.17

King JE, Landau VI (2003) Can chimpanzee (*Pan troglodytes*) happiness be estimated by human raters? J Res Pers 37(1):1–15. https://doi.org/10.1016/S0092-6566(02)00527-5

Lambeth SP, Bloomsmith MA, Alford PL (1997) Effects of human activity on chimpanzee wounding. Zoo Biol 16(4):327–333. https://doi.org/10.1002/(SICI)1098-2361(1997)16:4<327::AID-ZOO4>3.0.CO;2-C

Leaman J, Latter J, Clemence M (2014) Attitudes to animal research in 2014. Ipsos Mori, 1–54

Lee PC (1996) The meanings of weaning: Growth, lactation, and life history. Evol Anthropol Issues News Rev 5(3):87–98. https://doi.org/10.1002/(SICI)1520-6505(1996)5:3<87::AID-EVAN4>3.0.CO;2-T

Lloyd MH, Foden BW, Wolfensohn SE (2008) Refinement: promoting the three Rs in practice. Lab Anim. 42(3):284–293.https://doi.org/10.1258/la.2007.007045

Lutz CK, Tiefenbacher S, Jorgensen MJ et al (2000) Techniques for collecting saliva from awake, unrestrained, adult monkeys for cortisol assay. Am J Primatol 52(2):93–99. https://doi.org/10.1002/1098-2345(200010)52:2<93::AID-AJP3>3.3.CO;2-2

Marini RP, Wachtman LM, Tardif SD et al (eds) (2019) The common marmoset in captivity and biomedical research. Academic, Cambridge, MA

McCann C, Buchanan-Smith HM, Jones-Engel L et al (2007) IPS International guidelines for the acquisition, care and breeding of nonhuman primates, 2nd edn. Primate Care Committee, International Primatological Society

McEwen BS (2006) Sleep deprivation as a neurobiologic and physiologic stressor: allostasis and allostatic load. Metabolism 55(Supplement 2):S20–S23. https://doi.org/10.1016/j.metabol.2006.07.008

McKinley J, Buchanan-Smith HM, Bassett L, Morris K (2003) Training common marmosets (*Callithrix jacchus*) to cooperate during routine laboratory procedures: ease of training and time investment. J Appl Anim Welf Sci 6(3):209–220. https://doi.org/10.1207/S15327604JAWS0603_06

Mendl M, Paul ES (2004) Consciousness, emotion and animal welfare: insights from cognitive science. Anim Welf 13(Supplement 1):17–25

Michie S, West R, Campbell R et al (2014) ABC of behaviour change theories: an essential resource for researchers, policy makers and practitioners. Silverback Publishing, London

Morton DB, Jennings M, Buckwell A et al (2001) Refining procedures for the administration of substances. Lab Anim 35(1):1–41. https://doi.org/10.1258/0023677011911345

Morton DB, Hawkins P, Bevan RM et al (2003) Refinements in telemetry procedures. Lab Anim 37:261–299

Organisation for Economic Cooperation and Development (1997) OECD series on principles of good laboratory practice and compliance monitoring. Number 1. OECD principles on good laboratory practice. Guideline

Organisation for Economic Cooperation and Development (2000) Guidance document on the recognition, assessment, and use of clinical signs as humane endpoints for experimental animals used in safety evaluation (ENV/JM/MONO(2000)7). ENV/JM/MONO(2000)7, Organisation for Economic Cooperation and Development, Paris

Ory MG (2002) The behavior change consortium: setting the stage for a new century of health behavior-change research. Health Educ Res 17(5):500–511. https://doi.org/10.1093/her/17.5.500

Parker KJ, Maestripieri D (2011) Identifying key features of early stressful experiences that produce stress vulnerability and resilience in primates. Neurosci Biobehav Rev 35(7):1466–1483. https://doi.org/10.1016/j.neubiorev.2010.09.003

Pickard J, Buchanan-Smith HM, Dennis M, et al (2013) Review of the assessment of cumulative severity and lifetime experience in non-human primates used in neuroscience research. Research report of the animal procedures committee's Primate Subcommittee Working Group. London

Poole T (1997) Happy animals make good science. Lab Anim 31(2):116–124. https://doi.org/10.1258/002367797780600198

Porton I, Niebruegge K (2006) The changing role of hand rearing in zoo-based primate breeding programs. In: Sackett GP, Ruppentahal GC, Elias K (eds) Nursery rearing of nonhuman primates in the 21st century. Developments in primatology: progress and prospects. Springer, Boston, pp 21–31

Prescott MJ (2001) Counting the cost: Welfare implications of the acquisition and transport of non-human primates for use in research and testing. Royal Society for the Prevention of Cruelty to Animals (RSPCA), Horsham, West Sussex

Prescott M (2006) Primate sensory capabilities and communication signals: implications for care and use in the laboratory. The National Centre for the Replacement, Refinement, and Reduction of Animals in Research, London
Prescott MJ (2016) Online resources for improving the care and use of non-human primates in research. Primate Biol 3(2):33–40. https://doi.org/10.5194/pb-3-33-2016
Prescott MJ, Buchanan-Smith HM (2003) Training nonhuman primates using positive reinforcement techniques: guest editors' introduction. J Appl Anim Welf Sci 6(3):157–161. https://doi.org/10.1207/S15327604JAWS0603_01
Prescott MJ, Buchanan-Smith HM (2007) Training laboratory-housed non-human primates, part I: A UK survey. Anim Welf 16(1):21–36
Prescott MJ, Buchanan-Smith HM, Smith AC (2005) Social interaction with non-averse group-mates modifies a learned food aversion in single- and mixed-species groups of tamarins (*Saguinus fuscicollis* and *S. labiatus*). Am J Primatol 65(4):313–326. https://doi.org/10.1002/ajp.20118
Prescott MJ, Brown VJ, Flecknell PA, Gaffan D, Garrod K, Lemon RN, Parker AJ, Ryder K, Schultz W, Scott L, Watson J, Whitfield L (2010) Refinement of the use of food and fluid control as motivational tools for macaques used in behavioural neuroscience research: report of a Working Group of the NC3Rs. J Neurosci Methods 193(2):167–188. https://doi.org/10.1016/j.jneumeth.2010.09.003
Prescott MJ, Nixon ME, Farningham DAH et al (2012) Laboratory macaques: when to wean? Appl Anim Behav Sci 137(3–4):194–207. https://doi.org/10.1016/j.applanim.2011.11.001
Pritchard JC, Van Dijk L, Ali M, Pradhan SK (2012) Non-economic incentives to improve animal welfare: positive competition as a driver for change among owners of draught and pack animals in India. Anim Welf. 21(Supplement 1):25–32 https://doi.org/10.7120/096272812X13345905673566
Reinhardt V, Liss C, Stevens C (1995) Restraint methods of laboratory non-human primates: a critical review. Anim Welf 4(3):221–238
Rennie AE, Buchanan-Smith HM (2006a) Refinement of the use of non-human primates in scientific research. Part I: the influence of humans. Anim Welf 15(3):203–213
Rensnie AE, Buchanan-Smith HM (2006b) Refinement of the use of non-human primates in scientific research. Part II: housing, husbandry and acquisition. Anim Welf 15(3):215–238
Rennie AE, Buchanan-Smith HM (2006c) Refinement of the use of non-human primates in scientific research. Part III: refinement of procedures. Anim Welf 15(3):239–261
Richter SH, Garner JP, Auer C et al (2010) Systematic variation improves reproducibility of animal experiments. Nat Methods 7(3):167–168. https://doi.org/10.1038/nmeth0310-167
Robinson LM, Weiss A (2022) Primate personality and welfare. In: Robinson LM, Weiss A (eds) Nonhuman primate welfare: from history, science, and ethics to practice. Springer, Cham, pp 387–402
Ross C (1992) Life history patterns and ecology of macaque species. Primates 33:207–215. https://doi.org/10.1007/BF02382750
Russell W, Burch R (1959) The principles of humane experimental technique. Methuen & Co, London
Sandøe P, Crisp R, Holtug N (1997) Ethics. In: Appleby MC, Hughes BO (eds) Animal welfare. CAB International, Wallingford, Oxford, pp 3–18
Schapiro SJ, Hau J (2022) Benefits of improving welfare in captive primates. In: Robinson LM, Weiss A (eds) Nonhuman primate welfare: from history, science, and ethics to practice. Springer, Cham, pp 433–450
Shively CA, Laber-Laird K, Anton RF (1997) Behavior and physiology of social stress and depression in female cynomolgus monkeys. Biol Psychiatry 41(8):871–882. https://doi.org/10.1016/S0006-3223(96)00185-0
Singer P (1975) Animal liberation. Random House, New York

Smith JA, Boyd KM (2002) The Boyd Group papers on: the use of non-human primates in research and testing. British Psychological Society Scientific Affairs Board Standing Advisory Committee on the Welfare of Animals in Psychology
Sneddon LU, Elwood RW, Adamo SA, Leach MC (2014) Defining and assessing animal pain. Anim Behav 97:201–212. https://doi.org/10.1016/j.anbehav.2014.09.007
Tasker L (2012) Linking welfare and quality of scientific output in cynomolgus macaques (*Macaca fascicularis*) used for regulatory toxicology. University of Stirling
Teixeira CP, de Azevedo CS, Mendl M et al (2007) Revisiting translocation and reintroduction programmes: the importance of considering stress. Anim Behav 73(1):1–13. https://doi.org/10.1016/j.anbehav.2006.06.002
Turner PV (2022) The history of chimpanzees in biomedical research. In: Robinson LM, Weiss A (eds) Nonhuman primate welfare: from history, science, and ethics to practice. Springer, Cham, pp 31–56
U.S. Department of Agriculture (2013) Animal Welfare Act and Animal Welfare Regulations, Section 3.81–Environmental enhancement to promote psychological well-being. Animal Welfare Act and Animal Welfare Regulations ("Blue Book"), USA
Universities Federation for Animal Welfare (2010) The UFAW handbook on the care and management of laboratory and other research animals, 8th edn. Wiley-Blackwell, Chichester, West Sussex
van Dijk L, Pradhan SK, Ali M (2013) Sustainable animal welfare: community-led action for improving care and livelihoods. Particip Learn Action 66:37–50
Van Dyne L, Pierce JL (2004) Psychological ownership and feelings of possession: three field studies predicting employee attitudes and organizational citizenship behavior. J Organ Behav 25 (4):439–459. https://doi.org/10.1002/job.249
Vitale A, Manciocco A, Alleva E (2009) The 3R principle and the use of non-human primates in the study of neurodegenerative diseases: the case of Parkinson's disease. Neurosci Biobehav Rev 33 (1):33–47. https://doi.org/10.1016/j.neubiorev.2008.08.006
Waitt CD, Bushmitz M, Honess PE (2010) Designing environments for aged primates. Lab Prim Newsl 49(3):5–9
Watson CFI, Buchanan-Smith HM, Caldwell CA (2014) Call playback artificially generates a temporary cultural style of high affiliation in marmosets. Anim Behav 93:163–171. https://doi.org/10.1016/j.anbehav.2014.04.027
Weatherall DJ, Goodfellow P, Harris J, et al (2006) The use of non-human primates in research: a working group report. London
Weber H, Berge E, Finch J et al (1999) Health monitoring of non-human primate colonies. Lab Anim 33(Supplement 1):S3–S18. https://doi.org/10.1258/002367799780640002
Whay HR, Main DCJ (2015) Improving animal welfare: practical approaches for achieving change. In: Grandin T (ed) Improving animal welfare: a practical approach, 2nd edn. CABI International, Wallingford, Oxford, pp 291–312
Wolfensohn S (2022) Humane end points and end of life in primates used in laboratories. In: Robinson LM, Weiss A (eds) Nonhuman primate welfare: from history, science, and ethics to practice. Springer, Cham, pp 369–386
Wolfensohn S, Sharpe S, Hall I et al (2015) Refinement of welfare through development of a quantitative system for assessment of lifetime experience. Anim Welf 24(2):139–149. https://doi.org/10.7120/09627286.24.2.139
Yamamoto ME (1993) From dependence to sexual maturity: the behavioural ontogeny of Callitrichidae. In: Rylands AB (ed) Marmosets and tamarins: systematics behaviour and ecology. Oxford University Press, Oxford, pp 235–254
Yang SH, Cheng PH, Banta H et al (2008) Towards a transgenic model of Huntington's disease in a non-human primate. Nature 453(7197):921–924. https://doi.org/10.1038/nature06975