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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

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ABSTRACT

This report provides practical guidance on refinement of the use of food and fluid control as motivational tools for macaques used in behavioural neuroscience research. The guidance is based on consideration of the scientific literature and, where data are lacking, expert opinion and professional experience, including that of the members of a Working Group convened by the United Kingdom National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs). The report should be useful to researchers, veterinarians and animal care staff responsible for the welfare of macaques used in food and fluid control protocols, as well as those involved with designing, performing and analysing studies that use these protocols. It should also assist regulatory authorities and members of local ethical review processes or institutional animal care and use committees concerned with evaluating such protocols. The report provides a framework for refinement that can be tailored to meet local requirements. It also identifies data gaps and areas for future research and sets out the Working Group's recommendations on contemporary best practice.

1. Introduction

1.1. Background

Control of food or fluid intake is commonly used to maintain extended sequences of responses on behavioural or cognitive tasks in neuroscience research using macaques (e.g. Baxter and Voytko, 1996; Gaffan and Parker, 1996; Newsome and Stein-Aviles, 1999; Foeller and Tychsen, 2002; Tootell et al., 2004). Rarely, both food and fluid are controlled (Hollerman et al., 1998). Typically the monkeys are trained to perform tasks of varying complexity for which small amounts of a food or fluid (referred to as *rewards* or *positive reinforcers*) are given each time the monkey successfully performs the task (Newsome and Stein-Aviles, 1999; Weed et al., 1999; National Institute of Mental Health, 2002; National Research Council, 2003a; Taffe, 2004). The control may involve strict scheduling of the time for which food or fluid is available, or may involve a reduction in the total amount of food or fluid provided per day; either way, hunger or thirst becomes a key motivator for reliable performance.

Depending on the manner in which they are implemented, controls of food and fluid can elicit physiological and behavioural responses in animals, potentially compromising their health and well-being (Kutscher, 1969; Hamilton and Flaherty, 1973; Albee et al., 1987; Levin et al., 1993; Heiderstadt et al., 2000; Toth and Gardiner, 2000; National Research Council, 2003a; Tucci et al., 2006; Rowland, 2007). Food or fluid control may also have an indirect impact on animal welfare if it affects husbandry (e.g. if food or fluid control for the study animals were to lead to restrictions on the food and/or fluid intake of co-housed animals that are not

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part of the study). Coupled with the relatively lengthy periods of time over which behavioural neuroscience experiments are conducted, these issues make use of food and fluid control the subject of much concern and debate. Accordingly, guidelines and policy statements have been issued by national authorities, such as the United Kingdom (UK) Home Office (2003) and United States (US) National Institutes of Health (NIH) (2005), and many institutional animal care and use committees (IACUCs). However, the rationales and methodologies for the use of food and fluid control protocols often are not well documented or understood (indeed, research continues on these topics: e.g. Taffe, 2004), and this can be problematic for those responsible for evaluating them. There is, then, a need for clear guidance on the use and refinement of such protocols to avoid or minimize animal suffering, which is an important objective for scientific, legal and ethical reasons.

1.2. Working Group aims and scope

The National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs) is the UK Government's primary mechanism for advancing the 3Rs. Its mission is to use the 3Rs to support science, innovation and animal welfare. In consultation with the Home Office and Medical Research Council, the NC3Rs convened an expert Working Group with the following terms of reference:

1. To review and summarise current practice in the use of food and fluid control as motivational tools for macaques used in behavioural neuroscience research.
2. To identify the animal welfare issues.
3. To identify refinements.
4. To make recommendations on contemporary best practice.
5. To highlight data gaps and areas for future research to support the development of best practice.

The Working Group included senior neuroscientists, Named Veterinary Surgeons and a Named Animal Care and Welfare Officer working under the Animals (Scientific Procedures) Act 1986 (ASPA) (UK Government, 1986), representatives from the Animals (Scientific Procedures) Inspectorate (ASPI), and a zoologist specialising in the welfare of non-human primates. Most of the practical experience of this UK group involves the scheduled restriction of access to food or fluid rather than programmed limits on the total amount of food or fluid available to the animal.

It was necessary for the Working Group to grapple with a particular issue. Use of food and fluid control protocols has generated a good deal of debate. The fact that this debate has been conducted antagonistically at times (see exchange between Orlans, 1991, 1992; Desimone et al., 1992) does not mean that the potential animal welfare issues can be simply addressed by a compromise somewhere in the middle between two extreme opinions. Whilst opinions are useful in generating new hypotheses to test, they do not substitute for research investigations aimed at evaluating and improving animal welfare. Consequently, the Working Group adopted an approach in which opinion was treated with scepticism and the weakness of the evidence base in many areas of current practice was acknowledged.

1.3. Report audience and contents

The Working Group's report provides a framework for refinement that can be tailored to meet local requirements. It presents both background explanation for the non-specialist reader and technical information for researchers, veterinarians and animal care staff. The goals and practices involved in behavioural neuroscience research with macaques are set out first. Next, issues to be considered during the evaluation of food and fluid control protocols are discussed and general principles for refinement are described. This is followed by detailed information about refinement of the use of food control, use of fluid control, and some associated animal husbandry practices. Conclusions, data gaps and the Working Group's recommendations for contemporary best practice are presented last.

Table 1
Keywords used in the literature search.

abnormal behaviour, adverse effects, appetitive, awake behaving, behavioural/cognitive performance/testing, body condition, body weight, brain, central nervous system, cognition, dehydration, diet, distress, drinking, electrophysiological/neurophysiological recording, feeding, food/fluid/water restriction/control/deprivation/regulation/intake/delivery, foraging, health, hunger, husbandry, learning, *Macaca*, macaque, memory, microelectrode, monkey, motivation, motor control, neuron, neuroscience, non-human/non-human primate, nutrition, operant conditioning, pair/group/social housing, pair-/group-/socially-housed, physiology, polydipsia, positive reinforcement, psychology, psychological well-being/wellbeing, refinement, reinforcer, reward, socialisation, stress, thirst, training, vision, welfare

Table 2
Levels of evidence.

Level of evidence	Type of evidence
I+	Appropriately designed, controlled trials, with a low risk of bias (e.g. objective assessment of the data)
I	Appropriately designed, controlled trials
II+	Case-control or cohort studies, with a low risk of bias (e.g. objective assessment of the data)
II	Case-control or cohort studies
III	Case reports, case series
IV	Expert opinion, formal consensus

1.4. Methodology

The foundation for the Working Group's report was a literature search conducted using the PubMed and PrimateLit databases and combinations of the keywords in Table 1. The titles and abstracts of the retrieved citations were reviewed for relevance and those deemed not relevant to the Working Group's aims were excluded. Full text copies of 191 articles published between 1927 and 2009 were obtained for assessment; and the quality of the reported studies/information was evaluated according to the criteria in Table 2. The majority of the available evidence was level IV.

Drafts of the report were developed by the lead author in accordance with the decisions of the Working Group, incorporating contributions and advice from individual members in their expert areas. The Working Group met periodically to discuss the literature, review progress with its report, and formulate and approve the recommendations. The recommendations (Section 8.2) were graded according to the criteria in Table 3. The grade of recommendation relates to the strength (i.e. highest level) of the evidence on which the recommendation is based. Where there is specific, supporting evidence for aspects of a recommendation, the individual reference and level of evidence is indicated.

Table 3
Grades of recommendation.

Grade of recommendation	Level of evidence
A	Level I or I+
B	Level II or II+
C	Level III
D	Level IV

1.5. Limitations

In writing its report and formulating its recommendations, the Working Group took account of relevant scientific literature. It was not within the remit of the Working Group to consider the use of food and fluid control for purposes other than motivation of performance on behavioural or cognitive tasks in neuroscience research (e.g. for the study of homeostatic regulation of energy metabolism or fluid balance). Because of pressure to limit the overall length of published journal articles, descriptions of the food and fluid control protocols employed in published behavioural neuroscience studies using macaques tend to be minimal. In addition, there is a lack of published data on many aspects of the successful application of such protocols, their implications for animal welfare and the available opportunities for refinement. Thus, where data is lacking, the Working Group has, of necessity, reported the views of expert committees, and the professional experience of practitioners, including from within the Working Group; this is clearly indicated at all points.

2. The use of macaques in behavioural neuroscience research

2.1. Goals of behavioural neuroscience research with macaques

The goal of behavioural neuroscience research is to understand how the structure and function of the brain contributes to perception, thinking, emotion and motor control (e.g. how brain circuits enable us to see, to remember what we have seen and where we saw it, or to reach out and pick up an object). Aside from the advancement of basic scientific knowledge, understanding of the brain is needed for the discovery of treatments for brain disease and injuries (see Weatherall et al., 2006; Society for Neuroscience brain briefings, www.sfn.org/briefings).

Many neuroscience experiments aim to explain the performance of a well-defined behavioural task in terms of the activity of the brain's neurons (nerve cells) and systems. Typically, experiments combine: (i) behavioural measurements of sensory, cognitive, motor or other psychological functions; and (ii) some method of monitoring or manipulating the activity of individual neurons, circuits or systems (see Section 2.2). Since many of these procedures used to monitor and manipulate brain elements are invasive, only rarely can they be ethically carried out in humans.

The brains of monkeys are anatomically and functionally akin to those of humans, and many of their visual and motor abilities are on a par with humans (e.g. De Valois et al., 1974; Porter and Lemon, 1993; Connor, 2000; Roy et al., 2000; Lemon and Griffiths, 2005; Pereira and Aziz, 2006). They can also be trained to perform a variety of psychological tests similar to those used with people. Therefore behavioural testing in monkeys parallels many aspects of neurological and psychological testing in humans (Roberts, 1996; Weed et al., 1999). For these reasons, monkeys, in particular various species of macaque, are often used in behavioural neuroscience research in preference to other laboratory animals (Lapin, 1996; Weatherall et al., 2006; Anderson, 2008; Crist and Lebedev, 2008). Regulatory authorities and local ethics committees require particular justification for the use of monkeys in research.

2.2. Monitoring and manipulating neural activity

The basic computing work performed in the brain is by neurons working in coordinated circuits. Much of what is known about brain computations comes from monitoring and recording the electrical signals in individual neurons, or groups of neurons, in awake, behaving macaques using fine wires called microelectrodes. At the time of writing, a search of PubMed using the search terms 'monkey', 'electrode' and 'cortex' yielded 4036 articles published between 1965 and 2009, but the techniques go back to the pioneering studies of Jasper and Evarts (e.g. Jasper et al., 1958, 1960; Evarts, 1966, 1968). Additionally, other probes can be used to measure chemicals, brain waves or even small fluctuations in temperature as the neurons perform computational work.

All of these measurement techniques are correlational, namely they establish that various brain and behavioural events occur together in time. In order to establish that a brain event is necessary and sufficient for a behavioural response, the brain activity can be manipulated and the resulting changes in behaviour observed. These manipulations include: permanent surgical destruction of part of the brain (also called *ablation*); temporary silencing of a brain area by cooling or by electrical, magnetic or drug stimulation; or temporary activation by electrical or drug stimulation. Modern molecular techniques are being developed so that specific sets of neurons can be made sensitive either to an externally applied chemical or to an intense light source, which can then be used to switch the neurons on and off (Tan et al., 2006).

Experiments measuring the brain's electrical activity place strong demands on the reliable performance of the behavioural response. Among other requirements, a daily testing session must last long enough for the following to take place:

- Safe positioning of microelectrodes in the brain to isolate and measure the activity of individual neurons.
- Preliminary work to determine the properties of the neurons before the main experiment, either to adapt the characteristics of the behavioural task to the neurons under study or to establish the type of neuron from which electrical signals are being observed.
- Recording of activity from the neuron while the monkey performs the behavioural task in order to establish how that neuron reacts to sensory stimuli presented to the monkey and how the neuron becomes activated in relation to behavioural responses.
- It may be necessary to manipulate neural function in some way (such as a pharmacological manipulation: e.g. Thiele et al., 2006) while the experimenter records data and the monkey performs the task.

Consequently, the number of repeated responses (or "trials") required from the monkey each day for the successful characterisation of a single neuron's properties may be of the order of 700–800, since a neuron cannot necessarily be re-identified in a subsequent recording session. In general, the scientific requirement is that these trials need to be completed according to the temporal schedule driven primarily by the experimental protocol. Delays in the performance of the behavioural task by the monkey accumulate the risk that there will be some slight relative movement between the electrode and the neuron under study, with the possible consequence that the entire day's findings may be unusable. Some experiments require over 1000 trials per daily session to be performed by the animal, given that there is an overhead associated with searching for suitable neurons to record and, in some cases, studying the responses of these neurons in relation to performance of a task. Experimental sessions can thus last anything from 2 to 8 h, depending on the requirements of the experiment. The monkey's co-operation and motivation to perform the task are central elements to the enterprise. Many experiments require months of experimental sessions (5–7 days per week), with several months of training before data collection can begin. Consequently, each animal will normally be used in experiments for a number of years after being trained.

2.3. The need for preparation and training of the animals

The critical dependence on the active co-operation of the monkey means that researchers take care to bring about a stable situation in which the monkey can achieve its goal (to acquire a steady stream of rewards, thereby satisfying its physiological needs without frustration) and the researchers can achieve their goals (to acquire a steady stream of informative data). This requires careful preparation and training of the animals, to form positive relationships with staff, to accept the necessary degrees of restraint required by the experiment, to work for reward, to learn the experimental task and to perform it repeatedly (Laties, 1987; Newsome and Stein-Aviles, 1999; Sauceda and Schmidt, 2000; Fairhall et al., 2006; Scott et al., 2003; Rennie and Buchanan-Smith, 2006; Crist and Lebedev, 2008).

Training involves the *shaping* of the monkey's behaviour by successive approximation until the animal performs the required task well (see Section 4.6). Initially the monkey is required to do something simple to obtain its reward (e.g. to get into a restraint chair where a visible water bottle is located). Progressively, more conditions are added such that at the end of the training the animal is making, say, a specific eye or hand movement in response to the arrival of particular stimulus sequence.

2.4. The need for reliable and stable behavioural performance

Reliable and stable performance from the animal is crucial for the success of behavioural neuroscience experiments with monkeys. Early in training, there will be many factors that may lead to errors in the experimental task, such as lack of attention, misdirected attention, and too much or too little motivation. After training, when the monkey performs the experimental task reliably and consistently, errors in performance can reveal valuable information about exactly how the animal performs the task and which brain structures are functionally related to the requirements of the task.

Training and testing regimens must reach three goals, which require the monkey to be motivated to:

- Learn the task to a stable level of performance–this may require as little as 2–3 weeks for simple tasks (e.g. retrieval of food) but can take 1 year or more for sophisticated tasks involving perceptual threshold measurements or intricate manipulations of attention and memory.
- Perform a sufficient number of trials each day to provide precise and reliable answers to the questions under investigation–for very simple measures this may require a few tens of trials per day; for more detailed characterisations it can require more than 1000 trials per day (see Section 2.2).
- Complete the trials promptly, where the experimental design requires it.

3. Evaluating food and fluid control protocols

3.1. Cost/benefit assessment and ethical review

In most countries, regulatory authorities, whether national or institutional, conduct some form of *cost/benefit assessment* prior to using animals in experiments, to ensure that the animal use is justified and that any suffering that may arise is evaluated and minimized. For example, in the UK, in determining whether to licence a scientific project using animals, the government minister acts on the advice of independent inspectors who conduct a cost/benefit assessment in which the likely adverse effects on the animals concerned are weighed against the benefit likely to accrue as a result of the programme of work to be specified in the licence (Home Office, 2000). This decision takes account of the conclusions of a local Ethical Review Process (ERP) within the host institution (Home Office, 1998).

Cost/benefit assessments are normally carried out implementing the 3Rs principles developed by Russell and Burch (1959), which are reflected in many legislative texts on the protection of animals used for experimental and other scientific purposes (European Community, 1986, currently undergoing revision—see European Commission, 2008; UK Government, 1986; United States Department of Agriculture, 1990; Shoji, 2007).

- Replacement of animals with non-animal methods (or methods that avoid protected species).
- Reduction in the number of animals used to gain information of a particular amount and precision.
- Refinement of scientific procedures and husbandry to minimize pain, suffering, distress or lasting harm.

Consideration of refinement of food and fluid control protocols typically involves addressing the following questions:

- Might the means of motivating the animals cause pain, suffering, distress or lasting harm? If so, are there alternative ways to motivate the animals that are less likely to cause adverse effects yet allow successful completion of the research goals?
- Can the protocols be refined to minimize the potential animal welfare costs?
- Are there rigorous procedures in place for monitoring of animal welfare?

In order to address these questions, all those involved in conducting, supporting, regulating or evaluating the research activities should inform themselves fully about current practice. This will involve searches of subject-specific literature databases and expert advice from multiple perspectives. The scope of the enquiries should include the scientific protocols that are in use or under review but should also be wider. Thus consideration needs to be given to whether food or fluid control is necessary, the level of any food or fluid control to be used, the implications of such control for animal health and psychological well-being, and the opportunities for refinement (National Institute of Mental Health, 2002; Willems, 2009). Seeking and comparing policy statements on food and fluid control produced by individual IACUCs (e.g. University of California Davis, 2001) can also be useful, although detailed practice may vary from one institution to another and all involved should be open to further refinements, even of established protocols.

Macaques in the wild spend a large portion of their daily activity budget engaged in foraging behaviour (Seth and Seth, 1986; Malik and Southwick, 1988; Lindburg, 1991) and in the judgement of many researchers, performing a complex task for food reward in the laboratory is behaviourally enriching for these animals as it causes them to use their highly developed cognitive abilities in a similar way to natural foraging behaviour. Laboratory-housed rhesus and stump-tailed macaques will voluntarily "work" for food, even when food is freely available, which indicates that the act of foraging in itself may have intrinsic appeal for these animals (Anderson and Chamove, 1984; O'Connor and Reinhardt, 1994; Reinhardt, 1994).

Naturally-occurring periods of restricted access to food or water are challenges faced by some wild macaque populations, to which the animals must adapt by changing their ranging and feeding behaviour (e.g. Lindburg, 1971, 1977; Bernstein, 1986; Malik and Southwick, 1988). For example, macaques in forest habitats usually obtain water from succulent foods and by licking morning dew or rain from leaves; by contrast, in the hot season in Uttar Pradesh, India, rhesus macaques were observed by Lindburg to confine their activity to the vicinity of the two remaining permanent springs in their range.

Many individual macaques will become obese under free-feeding conditions (Kemnitz and Francken, 1986; Schwartz et al., 1993), where obesity is defined as values >2 standard deviations above the mean in at least two of the following parameters: body weight, body mass index, abdominal fat fold, and abdominal circumference. Calorie restriction has been shown to extend lifespan and reduce the incidence of age-related disease in short-lived laboratory animal species, and the same may be true for macaques (Lane et al., 1992; Kemnitz et al., 1993; Roberts et al., 2001; Colman et al., 2009).

These facts may be used to argue that restricted access *per se* is not unduly stressful (Desimone et al., 1992) or undersirable (Taffe, 2004). However, the fact that animals suffer potentially life-threatening hardships in the wild does not by itself provide justification to impose such hardships on captive research animals. Hunger and thirst are powerful motivating agents and therefore must be used carefully. The Society for Neuroscience has adopted the US Public Health Service Policy on Humane Care and Use of Laboratory Animals (2002), which recommends that unless the contrary is established researchers should consider that procedures that cause pain or distress in humans may cause pain or distress in other animals.

In the experience of the Working Group, robust cost/benefit assessment and refinement of protocols require the regulatory authority and research institution's ethical review to investigate the following information points:

- The nature of the task, the experience of the research group in training monkeys on the proposed task, the monkey's past experience and the future level of performance necessary for the scientific goals of the study.
- The protocol to be adopted, including descriptions of any proposed control of the quantity of the food or fluid to be provided (compared against baseline *ad libitum* intake), any restrictions on the time or place in which food or fluid will be made available (even if there is no limit on the quantity available), and any proposals for periods of time when control will be relaxed, including a description of the protocol for moving safely from a physiological state in which food or fluid is controlled to a state in which it is not and vice versa (see Section 6.3.5).
- An estimate of the level of control (which should clearly follow from the previous description).
- Justification for any particular level of control, which should include the reasons why a lesser degree of control is likely to be ineffective, unless already clear from information provided. The justification should consider the feasibility of adopting a lesser degree of control to start with, with a description of the staged increases in the level of control, if required, to achieve the target performance level. Such a system allows a demonstration that the level of control is no more than is required for each individual animal. As training proceeds, it may be possible to relax the control whilst maintaining adequate performance. The description of the protocol should address this point.
- Criteria for the determination of intervention endpoints, such as relaxation of the level of control, or temporary/permanent removal of the animal from the protocol (e.g. temporary removal from the protocol if the animal unexpectedly develops illness that results in, for example, fever, diarrhoea or vomiting).

It may be that strong evidence in favour of or against a particular level of control is not available, in which case a view should be taken on balance of probability that the protocol selected will be the most refined. In such cases, there should be careful ongoing review.

Aversive stimuli: In general, controlled access to food or fluid confers motivating value to these rewards. Thus, when using food or fluid control protocols, it is normal practice to signal incorrect responses to the animal by withholding the food or fluid reward and to provide a sensory cue (e.g. a beep) and a brief *time out* (Weed et al., 1999; Foeller and Tychsen, 2002). The sensory cue signals the failure clearly to the animal and the withholding of the reward adds to its motivating value on subsequent trials. Any circumstances where it is proposed to use aversive stimuli ("punishers") to eliminate errors or unwanted behaviour, and the special reasons why they may be needed, should be clearly described and justified to the regulatory authority and research institution's ethical review. The Working Group considers that, when fluid control is being used to motivate animals to work for fluid, saline or quinine should not be given in response to performance errors merely to improve the rate of learning and expedite data collection. However, for studies of the neural mechanisms underlying the behavioural response to aversive stimuli, the use of these or similar aversive stimuli is clearly mandated.

3.2. Alternatives to food or fluid control

Given the potential animal welfare issues associated with the use of food or fluid control (see Sections 5.1 and 6.2 for details), it is important to consider alternative options for motivating the animals to work; some examples are given below. However, many different types of study currently use food and fluid control; it is unlikely that all of them would be amenable to an alternative approach, especially those that utilize challenging tasks that are difficult to learn or perform, or require animals to work steadily for extended periods. It must also be recognized that the adoption of alternative methods of reward may raise new, unforeseen animal welfare issues.

3.2.1. Appetitive reinforcement without food or fluid control or restriction

Monkeys can be trained to voluntarily co-operate with a variety of scientific, veterinary or husbandry procedures for "treats" (e.g. peanuts, raisins, sweets) (see Prescott and Buchanan-Smith, 2003, 2007; Prescott et al., 2005, for examples). In some circumstances (e.g. if the reward is sufficiently attractive and the task is undemanding) performance can be maintained on behavioural and cognitive tasks without the need for food or fluid control or restriction. The skill of the trainer and his/her level of rapport with the individual animal are considered to be important factors for successful use of reward only methods of animal training (Prescott et al., 2005; Association for the Study of Animal Behaviour/Animal Behavior Society, 2006).

Common marmosets have been trained to respond to stimuli in order to gain access to a preferred fluid (banana milkshake) that is not encountered elsewhere in the diet (Crofts et al., 1999; Scott et al., 2003). This reward can sustain daily performance on touch-screen-mediated discrimination tasks presented from the Cambridge Neuropsychological Test Automated Battery (CANTAB) for up to 15 months (Pearce et al., 1999). Similarly, rhesus macaques have been trained to respond to CANTAB tasks presented twice daily using 45 mg banana-flavoured pellets, achieving around 60 trials per session (Crofts et al., 1999). Wilson et al. (2005) report that certain rhesus macaques will work for 300 trials per day for varied food rewards without food restriction, but typically less if the task is demanding. Similar results were reported by Brannon et al. (2004) with socially-housed bonnet macaques. These studies made no comparisons with other reward schedules, however the number of trials achieved would be too low and the rate of responding too haphazard for the majority of electrophysiological studies.

3.2.2. Social and other visual stimuli

Social stimuli can be potent rewards for operant behaviour in non-human primates (Anderson, 1998). Macaques will perform a variety of behaviours to gain visual access to conspecifics, including pressing a lever (Sackett, 1966; Swartz and Rosenblum, 1980), moving the head into a viewing channel (Haude et al., 1976), and using a joystick to direct the movements of a cursor on a computer monitor (Andrews and Rosenblum, 1993, 1994; Washburn and Hopkins, 1994; Brannon et al., 2004). Video clips of conspecifics appear to constitute particularly effective rewards for individually-housed monkeys, producing 205–1210 behavioural responses per 12-h period (Andrews and Rosenblum, 1993, 2002), but are less effective for socially-housed monkeys (Brannon et al., 2004). Where social and appetitive reward methods have been compared directly (e.g. video versus pellet rewards), reliable inter-monkey preferences have been found (Andrews and Rosenblum, 1993, 1994; Andrews et al., 1995; Washburn et al., 1997; Brannon et al., 2004).

Movies have been used also as rewards for performance of operant tasks (Blatter and Schultz, 2006). Blatter and Schultz report that response rates with movies are similar to those for sequences of still images; these response rates are similar or inferior to those obtained with rewards of food pellets to monkeys that have *ad libitum* access to food in the home enclosure. As expected, higher response rates are obtained with rewards provided when monkeys have controlled food or fluid intake. They write that (p. 546) “The current maximal rates of 50 responses per 15 min with movies would be at the lower end of the rates necessary for electrophysiological experiments.”

One possibility for exploiting the rewarding effect of certain visual stimuli is to present them in conjunction with appetitive rewards (Washburn et al., 1997; Deaner et al., 2005; Blatter and Schultz, 2006). Although this may not be scientifically appropriate in cases where the visual system is being studied, this technique might be expected to increase the incentive value of the food or fluid rewards, thus potentially reducing the level of food or fluid control necessary outside of the experimental task. Evidence on this point is limited but it is worthy of further investigation.

Washburn and Hopkins (1994) found that when non-restricted rhesus macaques were rewarded with fruit-flavoured pellet alone or pellets plus 30 s of video of conspecifics, no reliable performance differences were found between these conditions. Furthermore, when given the choice between these two reward conditions presented conjointly (Washburn et al., 1997), 70% of the animals preferred pellets in conjunction with 10 s of blank video over pellets plus 10 s of live video. Thus, video reinforcement appears to add little to the motivating value of food incentives for promoting task performance in non-restricted macaques.

3.3. Choice of food or fluid control

Where control is necessary, the choice of whether to use food or fluid control should be made carefully and is usually governed by a complex interaction involving: (i) animal welfare considerations, such as the potential impact on food and fluid intake, body weight and behaviour; and (ii) experimental considerations, such as the research question, requirements of the experimental apparatus, typical length of the experimental session and total period of time for which the individual animal will be under experimental study.

3.3.1. Animal welfare considerations

There is a perception that the margin of error (i.e. risk to animal health and psychological well-being) is greater with the use of fluid control than with the use of food control, based on the observation that animals can withstand food deprivation for longer than water deprivation (Forbes, 1995). Consequently, on the diurnal timescale of many research or husbandry activities, the consequences of the animal not drinking for 24 h are generally taken to be more severe that not eating for 24 h. However, the Working Group is not aware of evidence suggesting that a properly-managed fluid control protocol is, for this particular reason, necessarily more severe than a properly-managed food control protocol (in this context, this means scheduled access to food/fluid with the opportunity to obtain many small food/fluid rewards, such that the animal can achieve all, or a substantial fraction, of its daily food/fluid requirement).

One important issue in evaluating the animal welfare effects of such protocols is that they are generally used for prolonged periods of time, thus allowing physiological and behavioural adaptation to the protocol. Studies are less relevant in this context if they have examined the immediate short-term effects of fluid or food restriction on animals previously maintained on *ad libitum* food and fluid. It is important to stress that there are no studies directly comparing the effects on macaque welfare of equivalent regimens of food control and fluid control, but there are studies of this type with rats and mice.

In a study with cage-adapted rats, Gillette-Bellingham et al. (1986) found that the suppression of feeding induced by fluid control (i.e. scheduled access to water for either 40, 20, 10, or 5 min per day) was temporary and relatively mild overall in comparison to that with food control (i.e. scheduled access to food for either 2, 1.5, 1 or 0.5 h per day), and that body weight depletion was more severe under food control than fluid control.

Hamilton and Flaherty (1973) compared the food intake, water intake and body weight loss of rats that were permitted access to either food (with *ad libitum* water) or water (with *ad libitum* food) for up to 1 hour each day provided that they did not drink while the food was available or, in the case of *ad libitum* food, feed while the water was available (i.e. access to food/water was allowed until the onset of drinking/eating, respectively). They found that food-controlled rats lost more weight and ate less food than water-controlled rats. However, since these authors were not specifically interested in induced restriction they, like many others, did not include *ad libitum* controls against which the restriction effects might have been observed.

Similar results were obtained by Tucci et al. (2006) for females of one inbred mouse strain (C57BL6/J). Fluid-controlled mice appeared to cope well with only 2 or 4 h *ad libitum* access to water per day and their recovery during the *ad libitum* period was more efficient as the days progressed, while food-controlled mice tended to lose weight progressively over the course of the study, although their weight rarely fell below 85% free fed weight.

Heiderstadt et al. (2000) compared the effects of chronic food and water restriction on behaviour in the open field test (a measure of anxiety) and serum corticosterone levels in rats. Activity in the open field was significantly greater in food-restricted animals (maintained at 80% of their baseline weight) than in water-restricted animals (15 min free access to water every 24 h) and in controls (*ad libitum* food and water). Plus, food-restricted animals had a higher mean serum corticosterone level than water-restricted and control animals 37 days after the start of the experimental period. These data suggest that chronic food restriction led to behavioural changes and physiological stress in contrast to water restriction. Adjustment of the feeding regimen for food-restricted animals (to maintenance at 80% of an *ad libitum* fed control rat's weight) eliminated physiological evidence of chronic stress.

Rats, mice and macaques are adapted to different ecological niches, and none of these studies exactly matches the circumstances presented to the animal in most behavioural neuroscience studies with macaques. In particular, for all the studies, the restrictions on access to food or fluid were not placed in the context of a behavioural task. Nonetheless, these data for rats and mice suggest that the welfare differences between the withholding of food as opposed to fluid may be less significant than initial intuitions might suggest. It is clear that a direct comparison of the animal welfare implications and induced restriction effects of use of food control versus fluid control with macaques would be of considerable value. One would need to record a number of indices of animal welfare, such as body weight fluctuations and changes in appearance, activity, responsiveness to human caregivers and behavioural performance, in three groups of monkeys (food-controlled, fluid-controlled and controls) over a number of weeks.

One advantage of food reward is the variety that it allows in the reward scheme. Behavioural responses rewarded by different foods can be combined (Wilson et al., 2005) and, in the experience of the Working Group, by keeping back favourite foods until late in the session, macaques can be persuaded to perform extra trials after becoming satiated on less-highly-favoured rewards, which may permit a less severe food control regimen.

3.3.2. Experimental considerations

For some research, food rewards are preferred; for instance, solid food rewards may become an integral part of research on motor performance because they can be used to explore spontaneous motor actions other than the trained motor task (e.g. retrieval of the reward, manipulation of the reward, release of the reward at the mouth, use of either hand) (Lemon et al., 1976). Food rewards may also be used to encourage animals to relax and allow sensory stimuli to be applied to various body parts (Lemon and Porter, 1976). However, food rewards are not suitable for all experimental designs, including those that require long sessions with a large number of small reward deliveries (National Institutes of Mental Health, 2002). Other reasons for choosing fluid rewards are:

- With food, chewing or crunching movements of the teeth and jaws can introduce electrical noise in electrophysiological recording, and the recorded neuron(s) may be lost by the motion artefacts.
- Some experiments require an event time resolution of 0.5–3 s which is better achieved with fluid delivery.
- Fluid rewards are more easily quantified than food rewards.
- Different fluid rewards can be delivered in the same controlled way using the same standard apparatus and equipment.
- Food is difficult to deliver without the animal seeing it, which can represent a considerable problem for data interpretation in some studies.

These problems can be overcome in some cases. For example, liquid gruel/slurry (e.g. a mixture of standard non-human primate dried pellets or biscuits, fresh fruit and fruit juice: Economides et al., 2007) delivered via a peristaltic pump can be used to overcome problems of destabilization of electrode recording due to chewing movements. Applesauce supplementedwith protein powder and STAT^{TM} (PRN Pharmacal) has also been used (Kaneko, 1997). With an experienced animal, reward size can be as small as 0.3 ml, comfortably yielding more than 2000 trials per daily session. Whether this approach is better than controlled delivery of water or fruit juice from the perspective of animal welfare can only be judged in the context of a specific protocol and future research.

4. General principles for refinement of food and fluid control protocols

Determining a single standard by which all food or fluid control protocols can be evaluated or performed is difficult. Therefore, the most important general principles for their refinement are summarised below. More specific information for refinement of the use of food and fluid control protocols, respectively, is given in Sections 5 and 6.

4.1. Type of reward

The desirability ("incentive value") of the reward offered will influence the animal's performance (Wu et al., 1986; Watanabe et al., 2001; Brannon et al., 2004) and hence the requirement for, or level of, food or fluid control (Franowicz and Arnsten, 1998; Wilson et al., 2005), so rewards should be chosen with care. There may be differences between individual macaques in their preference for certain rewards (e.g. Kaneko, 1997; Schultz et al., 2000). For example, some animals offered fruit juice rather than water as a reward may perform well with a lesser level of fluid restriction, at least initially (see Section 6.3.1). Before choosing rewards for performance of specific operant behaviours, researchers should consult the recent literature on the particular species and seek advice from the attending veterinarian, animal care staff and experienced colleagues about rewards tried and discarded and knowledge of the preferences of individual animals. Stale rewards should not be used and the reward delivery system should be cleaned regularly.

4.2. Rate of reward

Measures that increase the rate at which the animals gain reward should be carefully explored, given that this will mitigate somewhat the effect of restricted access to food/fluid. These include, increasing the volume of food/fluid reward, minimizing the delay between the reward and the response required to obtain it, decreasing the interval between opportunities to work for reward within a session (i.e. reducing inter-trial intervals), pre-exposing the animal to the reward freely under conditions in which it causes satiety, and increasing the palatability of the reward.

4.3. Nature and level of control

The nature and level of food or fluid control can have important consequences for animal welfare (Heiderstadt et al., 2000; Tucci et al., 2006). The level of control should be kept to the minimum required for achieving the scientific objective whilst maintaining an acceptable standard of animal welfare, as recommended by the National Research Council of the US National Academy of Sciences (1996) and the UK Home Office (2003). The veterinarian and senior animal care staff should provide advice on the animal welfare issues, and the level of control should be approved by the regulatory authority and research institution's ethical review. The onus is on the researcher to show that the proposed level of food or fluid control for each individual animal, rather than a lower (less restricted) level, is scientifically necessary, and that any ill effects that may result will be minimized (see Section 3.1). Faster accumulation of data *per se* (i.e. without appeal to valid justifications, such as the need for many repetitions while recording from one neuron in order to obtain the required level of statistical power, or the need to acquire data during a steady state level of a drug effecting neurotransmitter function) should not generally be considered good justification for use of a higher (more restricted) level of control, unless there are other animal welfare issues to be considered.

The required task should be compatible with the monkeys' behavioural capabilities and be designed so that they accrue the maximum rate/amount of reward that is possible given the scientific aims of the experiment. There is variation between individual monkeys as to whether or not they will perform a particular task under a particular control and reward regimen. Consequently, researchers should individualize food or fluid control protocols such that monkeys willing to work for larger amounts of food or fluid are provided with access to these (e.g. Baxter and Voytko,

1996) (see Sections 5.2.2 and 6.3.2 for details). Where restriction is significant, once the monkey is trained and experienced, attempts should be made to manipulate the reward amount upwards to boost total food or fluid intake, consistent with achieving the required number of behavioural trials (Toth and Gardiner, 2000).

The National Institute of Mental Health (2002) notes that inexperienced personnel may presume that a source of problems in training or maintaining a food-motivated or fluid-motivated behaviour is that the restriction is not strict enough or, in some cases, that it is too strict. It recommends that other types of variables should be considered first, such as equipment malfunctions, programming errors, illness, non-programmed water restriction (in the case of food-motivated behaviour), non-programmed food restriction (in the case of fluid-motivated behaviour), poor animal training skills on the part of the researcher, or task criteria that are raised rapidly or set too high for the animal's level of training (see Section 4.4). In the experience of the Working Group, excessive deprivation can be detrimental to task performance, often well before it becomes a health problem due to dehydration or weight loss, to the extent that an animal can refuse to work. Indeed, refusal to work in a well-trained animal may be an early indicator of other health problems (Smith et al., 2006).

4.4. Introduction of the protocol

After arrival in the laboratory each macaque should first be acclimatised to the new living conditions. Monkeys should not normally begin preliminary training until they are eating well in the home enclosure and familiar and well-socialised with staff. During these preliminary phases, temperaments and other preliminary individual observations can be recorded. During the restraint chair training phase, body weights and individual water intake (see Section 6.3.2) can be acquired.

In order that the monkey can use the experimental situation as a means of satisfying its physiological needs, the food/fluid control needs to be introduced in such a way that the animal has time to recognize and anticipate the limited availability of free food/fluid and adapt its patterns of intake (e.g. over a period of several days or weeks) so as to maintain good health (Toth and Gardiner, 2000) (see Section 6.3.5). Task complexity can be gradually increased as the animal becomes accustomed to the regimen and as it learns each component of the task (Weed et al., 1999). This approach based on the performance of the individual animal is superior to one based on numerical standards (e.g. increasing task complexity after a given number of sessions or days). If task performance is not adequately supporting minimal intakes, it will be necessary to re-evaluate and perhaps simplify the training strategy to facilitate the animal's ability to learn and master the task.

4.4.1. Dealing with poor performance during training

During training, the criteria for successful performance need to be set so as to allow the monkey to be within reach of a level of performance that sustains regular rewards and helps to establish a rhythm of working. The criteria will need to be raised as the monkey trains towards the level of performance that the experiment demands. If the monkey fails to progress, repeated returns to a lower criterion of performance may undermine the training programme, and an alternative strategy needs to be adopted to prevent *failure set*: the situation that arises when the monkey, despite being highly motivated by control of food or fluid intake, fails to attempt to perform the task.

The intervention point might occur when the number of successful trials within a session is less than a third of the minimum needed at the final stage. Many laboratories adopt a strategy of finishing the session soon after the monkey "fails" and returning the monkey to the home enclosure. No further food/fluid is given at this point. Another training session can then be given later in the day. When food/fluid is given as a supplement to maintain the daily intake, it is important to dissociate this from the termination of an unsuccessful session (e.g. by allowing a reasonable interval, say 1–2 h, before the supplement is provided). It has been suggested that finishing the training session on a positive note, for example, after a short run of good trials, even if the rest of the session has been poor, can enhance training progress (Laule, 1999).

Learning situations with behavioural errors and erroneous reward expectations are usually accompanied by increased arousal, which can affect performance (Hollerman et al., 1998). For example, the performance of monkeys can decline if they are required to work without receiving rewards (for instance, in fixed-ratio schedules of reinforcement when a reward is delivered only after a given number of responses, or when an external cue indicates that multiple response will be required to earn the reward: see Bowman et al., 1996). Under such circumstances, the reward is delayed with respect to the behavioural response and more effort is required to earn the reward. The effect of any arousal or frustration should be considered when designing training protocols. Situations where rewards are unpredictably available need to be distinguished clearly from situations in which rewards are predictably available at the end of a long and possibly complex sequence of choices, as might occur in a working memory paradigm.

4.5. Monitoring of animal health and psychological well-being

Staff should undertake such monitoring of food- or fluid-controlled animals as is necessary to evaluate their health and psychological well-being (see Sections 5.2.3 and 6.3.3). The measures and monitoring regimen to be used and the intervention points should be specified in the protocol and agreed with the veterinarian, animal welfare officer and ERP/IACUC. Each animal should be evaluated at the start of the experiment and regularly thereafter. The Working Group recommends use of the following measures to evaluate animal health and well-being:

- Stability in the rate of body weight gain (growing animals) or body weight (fully-grown animals) after the initiation/training phase or following a shift from *ad libitum* access to programmed control (see Section 5.2.3).
- Absence of signs of dehydration (see Section 6.3.3).
- Relative stability, or incremental improvement, of performance on the experimental protocol (see Section 6.3.3).
- Behavioural indicators of psychological well-being (see Section 6.3.3).

Attention to psychological well-being is always important for animal welfare reasons; in addition, there may be implications for research programmes where the monkey under study is assumed to represent a valid model of normal behavioural function. To take a specific example, adult female long-tailed macaques may show behaviours similar to those observed in humans with depression (Shively et al., 2005). Furthermore, there are common neurobiological substrates (Shively et al., 2006). Macaques exhibiting depressive behaviours are thus unlikely to be good subjects and may not generate normal data.

Smith et al. (2006) have developed and validated an objective and quantitative system for assessing and monitoring the health and well-being of laboratory rhesus macaques; specifically those used in long-term neurophysiology studies with fluid control. Observations are made twice a day by an experienced observer and checklists used to record: (1) potentially life-threatening clinical concerns; (2) developing clinical issues; (3) atypical behaviours; and (4) laboratory performance. The authors demonstrate with two case studies the utility of their multidimensional system for identifying incipient clinical and behaviour problems before they become serious. Although observations were made twice daily, the authors comment that a single daily measure would be sufficient.

There should be a simple and readily visible system for making clear to all staff whether or not a particular monkey is currently maintained on a food or fluid control protocol. As a minimum, records should be kept of food intake (for food and fluid control regimens), fluid intake (for fluid control regimens) and body weight in a form that is easily interpreted and is acceptable to the regulatory authority, veterinarian and animal care staff (Smith et al., 2006; Willems, 2009). These records should be readily accessible to all those involved with the welfare of the animals. In practice, it can be difficult to obtain a quantitative estimate of food consumption if food is provided *ad libitum* in the home enclosure and animals are housed in social groups, but see Section 5.2.2 for solutions to this problem.

4.6. Preliminary training

The preliminary training phase presents a number of challenges to the experimenter working with macaques in behavioural neuroscience. Many of these challenges are identical, regardless of whether food or fluid is used as a reward. For example, most experimental procedures require the monkey to enter an enclosed space, such as a testing box or restraint chair. The monkey needs to be trained gradually to accept this restraint. It is no use presenting, say, a complex visual discrimination task to an animal that is distressed on account of simply being within the box or chair. Thus, it is important that researchers take widespread advice before embarking on the training of a naïve monkey.

As mentioned in Section 2.3, the basic principle is that of "shaping" the monkey's behaviour by successive approximation. This involves breaking down the ultimately desired goal into a series of small, individual steps that build upon each other, eventually leading to the completed goal. The trainer rewards performance of each step that leads towards the final goal and reliable performance of each step needs to be stably established before the next, more complex step is attempted. The key to successful shaping is the ability to identify steps that are appropriate to the goal and the animal learning it; steps which are too large can confuse and frustrate the animal (Laule, 1999).

The training of a naïve monkey begins by socialisation of the monkey to the presence and behaviour of the researcher, usually involving hand feeding. The familiarity of the monkey with the researcher, and the positive association of the researcher with food reward developed during this period, can reduce the amount of anxiety the monkey experiences during subsequent training and facilitate the training process (Prescott and Buchanan-Smith, 2007; Crist and Lebedev, 2008).

Normal practice for chair training in the UK is to allow the monkey to adjust gradually to confinement within the chair by initially encouraging the animal to approach the chair in order to take liquid from a bottle attached to it, whilst the chair is attached to the home cage. Fluid control is sometimes used but motivation of this kind should not be employed before alternatives have been explored (see Section 3.2). Once the monkey is regularly entering the chair, it is confined within the chair for short periods and rewarded simply for staying calm within the chair, until this stage is no longer a challenge.

Handling and restraint procedures should be refined to avoid welfare problems and confounding variables. Experience in the UK has shown that monkeys can be trained through shaping and treat rewards to voluntarily enter a chair or transport box on verbal command, such that food or fluid control, or manipulation with the pole-and-collar system, is unnecessary; the training protocols have been published (Prescott et al., 2005; Smith et al., 2005). Placing the chair or box in the animal's home enclosure for a few days prior to training, so that it has the opportunity to explore the apparatus and become familiar with it, can facilitate the training process (Prescott and Buchanan-Smith, 2007).

After learning that the chair signifies imminent treats such as juice, fruit or nuts, most monkeys become quite cooperative with the entire process (Newsome and Stein-Aviles, 1999). Occasionally there are animals that are unwilling to enter the chair and/or accept restraint even after several weeks of training, in which case sedatives have been used successfully (e.g. Skoumbourdis and Potratz, 2005), including by members of the Working Group. Animals that do not adapt without continued distress (e.g. persistent vocalisation or agitated behaviour on entering the chair, increasing over the period of training) are unlikely to be suitable for behavioural neuroscience experiments whilst ensuring acceptable welfare standards and so are generally transferred to a non-recovery procedure with terminal anaesthesia.

After becoming comfortable with the chairing process, the chaired animal is moved to the laboratory each day for extended training on specific behavioural tasks. Again, the principle of successive approximation is used. When a lever is to be used for the animal to make a response, the early phase of training simply rewards the animal for touching the lever. Only later are the constraints tightened so that, for example, pressing a lever brings a reward only when certain stimuli are presented on a computer screen in front of the animal. If an eye movement is the chosen form of response, then animals should be rewarded initially simply for looking in the right direction. Only later do other considerations come in, such as a requirement to hold gaze steadily on one spot for several seconds. These stages are introduced gradually as the animal learns to reliably perform each behaviour.

5. Use of food control

Food rewards are often used to motivate macaques to perform behavioural tasks. In some cases, the food reward is a "treat" for which the monkey will work without control of the daily food ration, because the treat is sufficiently desirable and motivating in itself (see Section 3.2.1). In other cases, control of the monkey's access to food outside of the experimental session is necessary to maintain its motivation for food rewards in the session. In the UK, the requirement for legislative regulation depends on the level of food control.[1]

One approach is to schedule access to food (e.g. making high-calorie food available only during the daily training/testing session), rather than to reduce the daily amount of food that the monkey receives each day. This approach is usually used to motivate macaques to work for food rewards in the UK and generally results in stable behavioural performance without weight loss. Researchers working with rats and mice have commented that these animals find learning about limitations on access to food easier than learning about limitations on the amount of food provided, and hence are more likely to familiarise with the schedule of the experiment (Gillette-Bellingham et al., 1986; Tucci et al., 2006).

[1] Project licence authority, which clearly justifies the work and the benefits that should result from it, is required for work with any protected species: (i) which restricts food intake to a point where weight loss, or reduced weight gain, of more than 15% of age- and sex-matched non-deprived animals, might occur; (ii) where animals are to be maintained below 85% of body weight for age- and sex-matched controls fed *ad libitum* (Home Office, 2003).

In most experiments with macaques, the monkeys are typically allowed to work to satiety during the experimental session. In a minority of others, some fraction of the daily food requirement is earned in the experimental session. Where a monkey earns less than its daily food requirement, a measured *supplement* of food is usually given after the end of the session. The monkey is given constant access to water in the home enclosure, and also has daily access to a small amount (e.g. 10–20 g) of low-calorie forage mix (see Section 7.1). On days when there are no experimental sessions (e.g. weekends) the monkey receives its daily food requirement "free" (without the necessity of earning it), but all food apart from a limited amount of forage is withdrawn 18–24 h prior to the next experimental session, in order to re-establish hunger and a willingness to perform.

Outside the UK, macaques are sometimes motivated to work for food rewards by limiting the amount of food provided daily such that the monkeys are maintained at a "target weight" below normal free-feeding body weight (programmed restriction) (e.g. Platt et al., 2001; Cosgrove and Carroll, 2002; France et al., 2006). This approach is usually used for initial training or for the study of certain experimental questions. The particular percentage reduction in body weight necessary to motivate varies for individual monkeys, depending on body size and other factors (Taffe, 2004). For example, some monkeys will work well at the normal point of the growth curve for their age, whereas others need to be at less than 90% of the average weight for their age in order to work reliably. Therefore, the Working Group recommends that a target weight is not used, but rather individual requirements should be established for each animal. In all cases, the goal should be to permit the food reward to maintain performance during the experimental session while maintaining the individual monkey's physical health and psychological well-being.

5.1. Animal welfare issues

The primary animal welfare concerns associated with the use of food control protocols are: (i) the risk of nutritional imbalances, depending on the reward type and diet used; (ii) the potential for weight loss (or poor growth, in the case of growing animals) from programmed or non-programmed restriction; and (iii) the aversive experience of hunger. There is interdependency between food and water intake in many animal species, such that food-deprived animals may voluntarily drink less water, but this is probably in response to reduced demand for water by the digestive system and thus does not imply thirst (e.g. Kleitman, 1927; Adolph, 1947; Finger and Reid, 1952; Bolles, 1961; Cizek and Nocenti, 1965; Natelson and Bonbright, 1978). Depending on the nature and level of food control and restriction, well-trained animals on food control protocols ought to be able to satisfy their daily energy and nutrient requirements and maintain good health.

5.2. Considerations for refinement

5.2.1. Type of food reward

Whenever an animal obtains any portion of its food requirement through food reward, the researcher should ensure that the sum of the nutritional value of the food earned through reward and of the food provided "free" is sufficient to maintain the animal in a healthy state. There should be constant access to water in the home enclosure. The National Research Council (2003b) has published information on the nutrient requirements of primate species, which can be used to design a nutritionally adequate and balanced diet.

Small (20 or 45 mg) balanced-diet food pellets can provide both rewards that will be consistently worked for and a large proportion of the monkey's daily food ration. In such circumstances, a supplement of fruit and/or vegetables daily may be necessary to prevent vitamin C deficiency[2] and to provide environmental enrichment in the home enclosure after the testing session; vitamin supplements can also be used (Buffalo et al., 1994; Weed et al., 1999; Cosgrove and Carroll, 2002; National Institutes of Mental Health, 2002).

Many monkeys work more readily for more appetising foodstuffs than pellets. Identifying preferred "treat" foods for a given monkey is advantageous because the animal may work reliably for these foods on a less severe food control regimen (e.g. Franowicz and Arnsten, 1998; Wilson et al., 2005; Association of Primate Veterinarians, accessed 2009). Where treats are used, for the animal's general health, nutritionally balanced rewards, such as small pieces of fresh or dried fruit, nuts, pulses or yogurt, are better than others such as sucrose pellets, sugar-coated chocolate or marshmallows. In addition, high-sugar rewards are relatively filling and so limit the number of trials for which the monkeys will work (Wilson et al., 2005). The possibility of dental caries is another disadvantage with frequent consumption of sugared food, particularly when the animals are used for many months or years.

It can be motivationally useful to provide a single, large food reward (called a "bonus") composed of a significant portion of the animal's daily food ration, or more appetitive rewards (such as a combination of fruit, nuts and other foods according to the individual animal's preference), in the training equipment when the animal works to the end of the training session (e.g. Gaffan and Parker, 1996; Browning et al., 2007). Switching of food reward type within the experiment can also help to maintain motivation (Franowicz and Arnsten, 1998) and, in the experience of the Working Group, allow high performance levels to be sustained with less severe control regimens. Wilson et al. (2005) have designed an automated food delivery system that permits researchers to reward monkeys with a variety of foods within a single experimental session, which can be difficult with commercially available feeders.

5.2.2. Level of control

Determining parameters of food control that do not produce weight loss or poor growth requires careful consideration of energy requirements (for maintenance, growth and exercise) and of growth patterns. Animals should not be at risk if the total amount of food obtained is appropriate for the species and individual, and the animal receives a balanced diet. Whilst information on the energy requirements of macaques is available from the scientific literature (e.g. National Research Council, 2003b) and from diet manufacturers, caution should be exercised when using published figures because energy requirements will vary between species and individuals and with local environmental conditions. Rations should therefore be adjusted for individual animals based on consumption, body condition, rate of weight gain, and life stage (Association of Primate Veterinarians, accessed 2009). As a guide, Wolfensohn and Lloyd (2003) report that an adult macaque requires approximately 420 kJ/kg/day for maintenance. Nicolosi and Hunt (1979) recommend 315–500 kJ/kg body weight daily for adults and 800–1134 kJ/kg/day for infants (age ranges not specified).

Special considerations for young/growing animals: Many laboratories find it difficult to obtain macaques older than 2–3 years of age and therefore use young animals (Taffe, 2004). Young macaques (e.g. 2–5 years of age) should still be increasing their body weight and will have additional food consumption requirements for normal growth as well as for maintenance. Researchers working with young animals should therefore ensure that sufficient food is pro-

[2] The ability to synthesise vitamin C is lacking in macaques and other higher primates. Early signs of vitamin C deficiency in macaques include weakness, lethargy, anorexia, weight loss and muscle and joint pain (National Research Council, 2003b).

vided to maintain normal growth whilst continuing to perform the behavioural task/s effectively.

The growth and development patterns of the macaque species used should be evaluated prior to beginning food control. For example, rhesus macaques experience a 'growth spurt' between 3 and 4 years of age during which they gain weight faster than when they are 1 or 2 years of age (Turnquist and Kessler, 1989). Macaques of this species on food control and experiencing this growth spurt may be at increased risk of poor condition and should be monitored with increased vigilance at this time (Association of Primate Veterinarians, accessed 2009).

Measuring individual baseline food intake: Macaques show much inter-animal variability in food intake (e.g. Hamilton, 1972). Consequently, some researchers measure individual food consumption over a number of days or weeks when the monkey has *ad libitum* access to food prior to any food control and its weight is stable or its growth is normal in order to establish a baseline for total daily necessary food intake for that animal (e.g. Smith et al., 2006). In some circumstances quantitative measurement of food consumption is not necessary because monitoring of body weight against normal growth curves (see Section 5.2.3) and observation of eating and drinking habits can suffice.

Where quantitative measurement of food consumption is necessary, it is important to balance this requirement against the needs of macaques for social contact (see Section 7.2) by use of prudent husbandry. Housing animals in pairs and measuring food consumption in the home enclosure at times when one animal of the pair is working in the laboratory, being weighed or examined by the veterinarian is one such husbandry option that has been used in the UK. Modular caging systems have been used also to separate animals for short periods during the day to allow individual food to be given yet still allow visual, olfactory and tactile contact with other monkeys.

Wilson et al. (2008) have validated a method for reliably quantifying individual food intake in macaques permanently housed in complex social groups. Radio-frequency microchips are implanted subcutaneously into both forearms of each monkey. A custom-made, automated feeding device dispenses a pellet of food when activated by one of the chips. Each chip contains a unique ID number that is read by a reader positioned around the pellet dispenser and recorded by computer. In this way, the system records intake of diet by individual members of the social group continuously 24 h per day, 7 days per week. The monkeys also receive a limited amount of food enrichment daily (i.e. a piece of fruit or vegetable and forage mix).

Special considerations with young/growing animals: For young/growing animals, baseline food intake should be re-established periodically as the animal ages. The Working Group recommends that this should be no less frequently than every 6 months, but account should be taken of the age of the animal, expected rate of growth and other issues judged relevant by the veterinarian and animal care staff. Where possible, breaks in the regimen to re-establish food intake should be scheduled such that scientific objectives are disrupted as little as possible.

5.2.3. Monitoring animals and record keeping

The animals' body weight should be monitored throughout all stages of the procedure and during breaks in the regimen. The frequency of weighing should be sufficient to pick up significant fluctuations in body weight (e.g. at least weekly and usually at the same time each day, as the animal enters the restraint chair prior to training/testing).

In the experience of the Working Group, comparison of body weight records against normal growth curves (e.g. Figs. 1 and 2) is an effective way of ensuring that macaques are receiving adequate nutrition, are not overweight or underweight, and that young

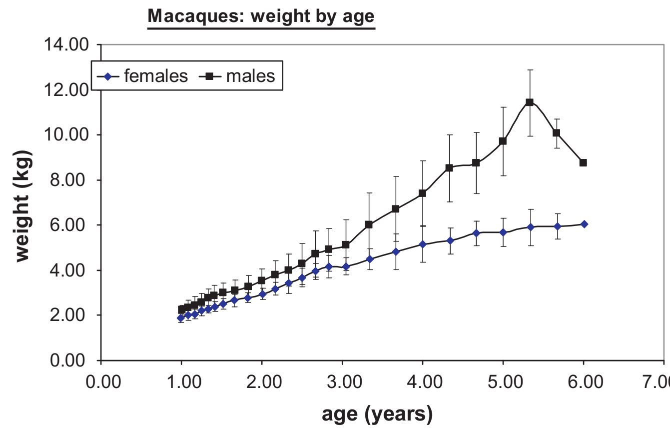

Fig. 1. Growth curve for the rhesus macaque (captive-bred males and females) based on groups of between 1 and 23 cases per data point (mean ± 1SD) (University of Rochester).

monkeys are growing normally. Colony-specific growth curves determined from historical data are preferable for this purpose but, if these are not available, growth curves and morphometric data published in the scientific literature can be used (e.g. van Wagenen and Catchpole, 1956; Turnquist and Kessler, 1989; Schapiro and Kessell, 1993; Baskerville, 1999; Ribeiro Andrade et al., 2004), bearing in mind differences in local conditions such as food availability, type of housing, opportunity for exercise, provision of environmental enrichment and ambient temperature. Condition scoring can also be used for this purpose (see Figures 5.4 and 5.5 in Wolfensohn and Honess, 2005).

Fig. 2 shows a growth curve with centiles for male rhesus macaques working for food reward in one UK laboratory. The curve is used in this laboratory in two ways for monitoring growth. Any young monkey that ceases to gain weight at a normal rate, assessed by the monkey's past weight records in conjunction with the figure (e.g. crossing centiles), is discussed by the researcher and veterinarian and an appropriate cause of action is taken. The growth curve can also be used to determine if an animal is small but growing normally, as shown by the weight records which should follow the relative centile, in which case no intervention is needed. However, if there is evidence that the food control is harming the monkey, then the control is relaxed.

Fig. 2. Growth curve for the rhesus macaque (captive-bred males) based on historical records for animals working for food reward in a UK laboratory ($n = 40$).

Special considerations with young/growing animals: Macaques that are not fully-grown should be expected to gain weight throughout the training and recording period. If an animal undergoing food control deviates from its growth trajectory, consideration should be given to relaxing the food control. Persistent divergence (particularly when significant weight loss is seen) is a cause for concern. Other causes may underlie this, such as infection. Review by the veterinarian should be undertaken promptly. It may be that the weight loss does not endanger the health of the animal (e.g. in the case of an animal that was initially overweight) but the case should be kept under regular review.

5.2.4. Breaks in the regimen

Macaques trained for behavioural tasks are sometimes used for a number of years and a factor to consider is whether there should be periodic breaks in the regimen, commonly termed "holidays/vacations" and lasting several days or weeks, during which the monkeys are not required to perform the learned task and have free access to food. The Working Group recommends that the need for such breaks should be assessed with the veterinarian and animal care staff, weighing the advantages and disadvantages for animal welfare. This recommendation is based on expert opinion in the absence of relevant studies addressing the issues. Practices vary and there are several considerations, including:

- Level of control—many food control regimens do not need breaks as the restriction is not significant (i.e. the monkey can achieve all or most of its daily food requirement and therefore does not need to be provided with the opportunity to make up any deficit in order to maintain normal weight or growth).
- Whether there are problems created by abrupt shifts between restricted and non-restricted feeding (e.g. rapid return to *ad libitum* food may be a contributory factor to bloat in some monkeys)—in general, sudden changes in the amount and pattern of feeding (e.g. the number of feeds per day, timing and location of feeds) should be avoided.
- Duration of expected break—macaques can become obese with prolonged *ad libitum* feeding only to lose weight on restricted feeding, causing dramatic shifts in body weight for obese animals; experimental and stock animals should be managed in such a way as to prevent obesity.
- Whether the opportunity to work on the task and to interact with staff outside of the home enclosure is stimulating for the monkey; environmental enrichment in the home enclosure should be provided at all times to promote psychological well-being.
- For weight control regimens, the extent to which weight was controlled below an *ad libitum* weight during the study and the probability that a new *ad libitum* weight is desirable because of the age of the monkey at the time of original determination.

6. Use of fluid control

Fluid control is most commonly used to provide a large number of trials with a behavioural response within a single day of experimentation (but see Section 3.3). This requirement may arise either from the complexity of the experimental design or the need to acquire enough data to reach statistical significance (see Section 2.2). Depending on the country in which the experiment is conducted, control of water intake may be a regulated procedure under animal protection legislation. For example, in the UK, regulation under the ASPA is necessary when "the programme of work to be applied requires water withdrawal that may result in suffering, distress or lasting harm and is applied for a scientific purpose" (Home Office, 2003).

The macaque may not be deprived of its daily fluid requirement; access to fluid in the home enclosure is controlled and the monkey earns a substantial fraction of its daily requirement as a series of small rewards for performing a behavioural task. Food and treats (such as raisins, peanuts or, in some cases, high water content fruit) may be offered at suitable points in the experimental session or immediately afterwards. Body weight is normally recorded each day that the monkey undertakes the behavioural task and is used in conjunction with other data to assess the welfare of the animal (see Sections 6.2.2 and 6.3.3).

6.1. Protocols in use

It is clear that the bodies of animals engaged in fluid control protocols undergo physiological changes to the control of fluid intake (Toth and Gardiner, 2000; Rowland, 2007). Many of these changes are adaptive, which is to say that they improve the animal's capacity to meet the challenge of controlled fluid intake. It is also clear that the behavioural responses of the animal (which are not just limited to behavioural performance on the required task) strongly indicate that there is a psychological aspect to adaptation to fluid restriction protocols, as there is to other elements of the experimental procedure. In other words, the animal comes to expect and anticipate the availability of fluid. It can be difficult to identify these changes accurately and to gauge their precise impact on animal welfare, particularly over the extended period for which these protocols take place. Consequently, a good deal of the welfare advice in this area attempts to derive an evaluation of particular protocols with reference to basic underlying principles, rather than detailed evidence about the effects of particular protocols.

There is a wide range of protocols in use with macaques, which vary in their approach to the management of fluid control. Some of these protocols focus on the changes in environmental circumstances for the animal, so that they provide for periods when the animal has *ad libitum* access to fluid to compensate for the restrictions on access that are in place at other times. Others focus on how the animal adapts to the changed circumstances and aim to bring about a gradual and stable adjustment to a new state of programmed availability of fluid, whilst monitoring indices of health and well-being. Some protocols mix the two approaches. Examples that have been used recently in the UK are:

1. *Ad libitum* access to fluid in the home enclosure for at least 1 hour within any 24 hour period, normally following the experimental session; *ad libitum* access to fluid on days when the monkey is not required to work (e.g. at weekends).
2. A limited volume of fluid in the home enclosure, normally following the experimental session; *ad libitum* access to fluid on non-working days.
3. Requiring the monkey to obtain the substantial fraction of its daily fluid requirement by earning it as a reward for performing a behavioural task; *ad libitum* access to fluid on non-working days.
4. As 3, but on non-working days providing a limited volume of fluid consistent with the volume the monkey is normally prepared to work for in the experimental apparatus; the fluid provision may be via a mixture of liquid and dietary sources.

Whilst there may be reason to distinguish between the desire to approximate *ad libitum* and the desire to stabilize around a controlled restricted regimen, currently there is no published evidence which suggests either system is better for animal welfare, or more likely to achieve scientific goals. Physiological variables (e.g. osmotic balance, volume regulation, clearance rates, urine concentrating functions) are likely to differ under each circumstance, and temporally.

In all the different protocols, the common ground is that: (i) the monkey has to work effectively for the required behavioural task (otherwise the protocol is pointless); and (ii) the monkey has to take in at least a minimum volume of fluid over a certain time period in order to maintain physiological functioning. One problem arises in assessing the welfare costs to the monkey in approaching any minimum level (if that should happen). Another problem is understanding what is the relevant time period over which intake must be assessed: there is clearly a short-term need over 24–48 h, but it is not known whether there are longer-term effects, leading to an argument for the beneficial effect of "holidays" (i.e. periods of time during which the fluid control protocol is removed: see Section 6.3.4).

The Working Group is not able to recommend use of any particular fluid control protocol over another due to the lack of published data on the relative merits and welfare costs of those in use. Also, researchers report that individual monkeys may work better on certain protocols than others. A direct comparison of the effectiveness and animal welfare implications of commonly used protocols would be of considerable value to researchers and others having to decide which of the many protocols to use. One would need to record daily body weights and fluid consumption (total and fraction during testing) as well as quantitative measures of work habits (e.g. trials completed, psychometric functions, accuracy). Of particular value would be a comparison of protocols currently approved in the UK in which monkeys may perform the experimental task to obtain fluid 5 or 6 days per week with a day or two of non-working each week with free access to fluid, compared with protocols which involve working every day continuously or for periods longer than 6 days (e.g. 13 days per fortnight), which is claimed to allow faster and better data collection and to avoid repeated fluctuations in physiology. It might be that a continuous or prolonged regimen is appropriate for some monkeys (e.g. fully-grown animals that are maintaining steady body weight) or is necessary for certain stages of experiments (e.g. the initiation/training phase).

In the following appraisal of current practice and considerations for refinement, the Working Group begins by commenting on the case of the well-trained monkey, which is stably performing the required task and is gaining a substantial fraction or its entire regular fluid intake in this way. We then comment on the monkey in the training phase. Issues raised by changes in the monkey's routine, such as moving to fluid control away from no control (and back again) are discussed in Section 6.3.5. Special considerations for working with young/growing animals are highlighted in the text.

6.1.1. Assessing satiation

In most protocols, well-trained monkeys will establish a stable pattern of work, providing a stable level of fluid intake. When a well-trained monkey ceases to perform a task that requires no physical effort beyond eye movements, the rational conclusion is that the animal's motivation for water is exceeded by the motivation to terminate the experiment. If the experimenter has minimized or eliminated physical discomfort, then the relevant desires and costs would seem to be limited: a desire to be in the home enclosure; or boredom (or something comparable) with the testing protocol and the extended time in the restraint chair (including head restraint). What is commonly referred to as thirst can be defined more precisely this way. When a monkey terminates an experiment, it does not imply that there is no desire for water, only that this desire is less than the other factors.

This insight is in agreement with principles based on a behavioural economics approach, which is increasingly being used in farm and laboratory animal science to investigate the strength of motivation of animals for specific resources (e.g. Mason et al., 2001; Cosgrove and Carroll, 2002; Wakita, 2004; Hansen and Jensen, 2006; Seaman et al., 2008). In the experience of members of the Working Group and most researchers, all monkeys will drink more water if it is given to them freely in the home enclosure immediately after they return from an experimental session. The importance of this observation is that termination of an experiment task by the monkey indicates that the monkey is now satisfied up to a criterion level, at which the costs of acquiring more fluid by performing more trials is balanced against the benefit from taking more of the fluid that is available. Thus, a useful practice by some laboratories, supported by the Working Group, is to routinely supplement the amount of fluid obtained in the experiment by a further amount (whether by water bottle or wet fruit in the home enclosure). The extra amount is titrated to the point that it does not reduce the incentive for behaviour in the experiment the following day. This approach respects the principle of refinement; that the impact of the scientific procedure on the welfare of the animal should be adjusted to the minimum compatible with the effective conduct of the science. Although called a *supplement* (being supplemental to the "earned" fluid), this fluid forms part of the total daily intake of the animal.

6.1.2. Use of supplements

The Working Group considers that, for the reasons given above, a well-trained monkey that is stably adapted to the fluid control protocol should routinely receive a supplement after the experimental session, normally via free access to a measured volume of water in the home enclosure. If the monkey fails to achieve its usual fluid intake in the experimental session it should receive a supplement, but the temporal separation of the supplement and work should normally occur in this circumstance (see Section 4.4.1). Where the monkey achieves less than 50% of its daily intake (see Section 6.3.2), it should normally receive a supplement at least up to this amount, and its health and psychological well-being should be monitored particularly closely.

During the training phase, the provision of additional fluid, above that for which the monkey is prepared to work, may be detrimental to performance, in some cases thereby thwarting the scientific goals of the experiment (e.g. the animals fails to learn the task). If this is considered to be the case, the animal is sometimes encouraged to work for more fluid by receiving only the fluid it has earned in the session; the supplement is delayed or even withheld. Only one UK establishment has found it necessary to delay the supplement. In such cases, the regulatory authority has approved a limit on the number of times that postponement of the supplement can be used (e.g. a maximum of two 24 hour delays per week for a maximum of 4 weeks in series before reverting to daily supplementation for 1 week). If the supplement is withheld, the monkey does not receive its full daily intake and may not be able to regain the deficit until free water is supplied. In the view of the Working Group, delay or withholding of the daily intake of fluid should be used rarely and only with the agreement of the veterinarian. Particular attention should be paid to the individual monkey's hydration status and consideration should be given to its long-term suitability for further training and use in the experimental protocol.

An alternative strategy for dealing with poor performance during training is to provide a supplement but to reduce the amount provided (as detailed above for well-trained animals). The amount of reduction should be agreed with the veterinarian and should be such that the monkey's welfare is not compromised. Effective monitoring of the monkey's health and psychological well-being will be necessary to ensure this (see Section 6.3.3).

Various factors may result in a monkey failing to learn a task or to perform as expected (e.g. it may cease to work if it is uncomfortable in the restraint chair or if it does not know what is expected of it). Alternative explanations for poor performance should always be considered before assuming that greater food or fluid control will increase motivation and improve performance.

6.2. Animal welfare issues

The primary animal welfare concerns associated with fluid control are: (i) the potential for dehydration; (ii) the potential for significant weight loss (or poor growth, in the case of growing animals) as a result of 'voluntary' food restriction following programmed or non-programmed fluid restriction; and (iii) the aversive experience of thirst. In addition, there is a concern that animals on fluid control protocols may be less able to cope with stresses caused by disease. Another concern is that some animals may be temperamentally unsuited to these protocols; such that they become withdrawn and do not perform well, in which case their continued use in such protocols must be questioned. However, well-trained and healthy animals on fluid control protocols performing their regular tasks are not limited in the fluid intake available to them, only in the schedule of its availability and, in the view of the Working Group, ought to be able to cover all their fluid needs during the experimental session.

6.2.1. Dehydration

Dehydration can be acute or chronic, resulting from less than adequate rehydration of daily water losses over time. The primary physiological stimuli that elicit thirst and mediate fluid intake in mammals are cellular dehydration and hypovolaemia (Fitzsimons, 1998). Dehydration results initially in an increase of the osmolality of extracellular fluids and a decrease in the volume of fluid in the circulatory system. The increased osmotic load draws water out of the body's cells, resulting in cellular dehydration. The hypovolaemic status of an animal can be ascertained by measuring hematocrit or plasma protein, both of which increase when fluid is lost from the plasma. For example, Wood et al. (1982) found that a period of 24 hours without water in rhesus macaques caused increased plasma osmolality and plasma sodium concentrations.

Comparable data for macaques undergoing controlled access to water, as opposed to the acute and total deprivation used by Wood et al. (1982), are not available from the published literature. There is a clear need for research and data sharing on this topic because these data could be used, in conjunction with clinical signs and daily body weights, to monitor animal health. Potentially they could be used to define limits in chronically restricted animals, which may assist in identifying a potentially detrimental situation in this context. In the experience of one member of the Working Group who has carried out such tests under relevant conditions, the reported values for plasma can be within the published normal ranges for healthy animals.[3] Testing of urine samples reveals the expected greater solute concentration of urine. Although such testing confirms that the kidneys are functioning as expected, it does not provide a reliable index for assessing animal welfare on fluid control protocols. Significant changes in plasma values would be a cause for concern, but these are not expected with properly managed fluid control protocols. Nonetheless, it would be valuable for refinement purposes to establish acceptable ranges of haematology and standard serum chemistry (Na, Cr, Blood Urea Nitrogen, etc.) for healthy macaques on a high level of fluid control (i.e. animals with stable weights, stable behaviour, whose clinical records indicate that their health is not deteriorating and who are required and able to perform >1000 trials per day).

6.2.2. Weight loss

Some weight loss may be expected in moving macaques from no control to fluid control. The animals drink less overall and it is normal in mammals for water deprivation to lead to a 'voluntary' reduction in food intake (Adolph, 1947; Bolles, 1961; Finger and Reid, 1952; Cizek and Nocenti, 1965; Collier and Levitsky, 1967; Kutscher, 1969; Engell, 1988). A certain amount of water is required to absorb and digest food, since absorption of food tends to elevate plasma osmolality. If the animal's water intake is limited, its physiological systems tend to control osmolality by limiting food intake, which can lead secondarily to hunger and weight loss. Reduced food consumption and consequent weight loss, in the context of a previously stable situation, are therefore signs that an animal may be receiving an inadequate amount of water, and hence both parameters should be monitored and recorded in addition to water consumption, and acted upon if a deficit is apparent (see Sections 4.5 and 6.3.3).

6.2.3. Thirst

Thirst is a physiologically adaptive sensation, which is exploited during the time frame of the experimental session to motivate the animal's behaviour. For a macaque on a fluid control protocol thirst is aversive in the sense that the monkey is working to eliminate it. Prolonged, unrelieved thirst is an animal welfare issue; the continuing failure of an animal to meet its water requirements, despite directing its behaviour towards finding water, can be seen as a chronic stressor (Kyriazakis and Savory, 1997). In the experience of the Working Group, excessive thirst in macaques may be indicated by urine drinking and/or very hasty licking at the fluid dispenser in the laboratory combined with hasty/unsteady task performance. If either of these behaviours is observed, consideration should be given to relaxing the fluid control.

6.3. Considerations for refinement

6.3.1. Type of fluid reward

Identifying a preferred fluid for a given monkey is advantageous because the animal may work reliably on a less severe water control regimen (Newsome and Stein-Aviles, 1999). Thus, the use of positive incentives in the form of flavoured fluid should be explored carefully on an individual basis for each monkey. The relative efficacy of water versus other fluids as rewards varies from monkey to monkey; some monkeys are more motivated to work for fruit juices or syrup solutions of a particular flavour. In cases where the monkey has no preference, many researchers choose water as a reward for reasons of hygiene and because water is readily distinguishable from calorific rewards (National Research Council, 2003a). The appropriate dilutions for fruit juices and syrups can be assessed in preference tests with thirsty animals (preferably the experimental animals themselves). Where high sugar fluid rewards are used, the animals should be monitored for dental caries.

6.3.2. Level of control

Whenever an animal obtains any portion of its fluid requirement through fluid reward, the researcher must ensure that the sum of the fluid earned through the reward and any supplemental fluid and fruit and/or vegetables provided is sufficient to maintain the animal in a healthy state. Animals need not be at risk if the total amount of fluid obtained and intervals of fluid access are appropriate to the species and individual (Institute of Laboratory Animal Research, 1995; Toth and Gardiner, 2000). The water content and osmotic load of food is also an important factor. The amount of

[3] Note that the finding of normal values within an individual monkey is not a guarantee of the animal's health, merely its physiological status. For normal ranges of physiological variables in macaque species see: Stanley and Cramer, 1968; Matsumoto et al., 1980; Kessler and Rawlins, 1983; Yoshida et al., 1989; Buchl and Howard, 1997; Wolfensohn and Lloyd, 2003; Ribeiro Andrade et al., 2004; Smucny et al., 2004; Association of Primate Veterinarians Nonhuman Primate Formulary, www.primatevets.org/pub_downloads.aspx; internet Primate Aging Database (iPAD), http://ipad.primate.wisc.edu.

fluid earned in the task plus any supplements and fruit or vegetables given, and some indices of hydration status (see Section 6.3.3), should be recorded daily for each animal. Where supplements are provided, these should be the maximum consistent with maintaining reliable performance (see Section 6.1.1).

As noted by Newsome and Stein-Aviles (1999), baseline fluid intake varies depending on body size, age and physiological factors that are idiosyncratic to each animal (like humans, monkeys appear to regulate hydration more or less efficiently, leading to substantial variation in amount of fluid intake required each day), but there are also non-physiological factors such as behavioural preferences. In standard, indoor laboratory conditions, *ad libitum* water consumption may be in the order of 30–60 ml/kg/day but there is great inter-animal variability (e.g. Hamilton, 1972; Suzuki et al., 1989). The amount taken may be greater than the amount needed to stay healthy. The researcher needs to know the level at which the animal's thirst is abolished and water is no longer effective as a reward. Published figures are available (e.g. Maddison et al., 1980; Wood et al., 1980) but the Working Group recommends determination of precise figures for each animal over a period of several days (e.g. 10 days), when the animal has a stable profile of behaviour and physiology.

Once the protocol has begun, the question of whether the animal's fluid requirements are being met can be addressed by careful inspection of daily weight records and a daily health check (see Section 6.3.3). The total daily requirement is assessed with reference to the continued bodily health of the animal using indicators (such as changes from normal weight and skin and faecal appearance) as advised by the veterinarian.

Special considerations with young/growing animals: For young/growing animals, baseline fluid intake should be re-established periodically as the animal grows. The Working Group recommends that this be no less frequently than every 6 months. Where possible, breaks in the regimen to re-establish fluid intake should be scheduled such that scientific objectives are disrupted as little as possible. With the use of prudent husbandry, it is possible to acquire information on baseline fluid intake for individual animals with minimal social isolation, as for baseline food intake (see Section 5.2.2).

Feeding: To encourage feeding and prevent weight loss, food should be given in close temporal proximity to the access to fluid (e.g. in between blocks of trials or immediately after the training/testing session). Monkey "treats" (e.g. chow mixed with banana ± yogurt ± peanut butter) are sometimes provided but nuts, dried fruits, fresh fruits and vegetables are better to mitigate any impact of fluid control on proper nutrition (see Section 5.2.1). Providing food with high water content can often be given without adverse effects on fluid control and can also help to ameliorate a decrease in appetite for solid food (Newsome and Stein-Aviles, 1999). In some cases, the water content of such foods is subtracted from the allotted amount of fluid for each animal. Tables showing the water content of fruit and vegetables can be found in Wolfensohn and Honess (2005) and Bastin and Henken (1997).

6.3.3. Monitoring animals and record keeping

Regular monitoring of the monkey's body weight, clinical evaluation of its health and careful observation of its behaviour are critical for ensuring successful application of fluid control protocols.

Body weight: Body weight should be monitored throughout all stages of the procedure and during breaks in the regimen (see Section 6.3.4). The frequency of weighing should be sufficient to pick up significant fluctuations in body weight (e.g. normally daily, at the same time each day, usually whilst the animal is in the restraint chair before the experimental schedule begins). Smith et al. (2006) recorded weight daily when validating their welfare assessment scheme for macaques on fluid control. Under this scheme, weight loss <10% compared with the previous days measure is recorded on a checklist and monitored. Weight loss >10% compared with the previous day's measure or the baseline weight for the monkey is recorded and reported to the veterinary staff for immediate assessment. Baseline weight is defined as the monkey's mean weight for the 31 days prior to the implementation of the assessment scheme.

Body weight records should be monitored regularly both for evidence of sudden short-term weight loss and for long-term evidence of weight decline or failure to thrive (see Section 5.2.3 for details). It may be that the weight loss does not endanger the health of the animal (e.g. in the case of an animal that was initially overweight) but the case should be kept under regular review. A plan of action should be in place in advance and implemented in case weights decline to unhealthy levels under a fluid control regimen (e.g. food and water made available *ad libitum* for at least 1 week until the animal has regained its normal weight). The Working Group recommends that fluid provision should be increased if an animal:

- Is exhibiting progressive weight loss during the testing/maintenance phase.
- Is exhibiting excessive weight loss during the training/initiation phase. The UK experience is that some weight loss may be seen during this phase as the animal adapts. Useful trigger points for intervention are weight loss of >10% between routine (weekly) measurements or >15% over longer time periods (National Institutes of Health, 2005; Smith et al., 2006). Researchers should be especially vigilant for signs and symptoms of physiological and psychological distress at this time because body weight itself is an unreliable indicator of well-being in this phase. Typically animals approach an asymptotic stable weight within a few weeks or months of fluid control but there is variability. Similarly there is variability in the amount of weight loss that the monkeys experience.
- Is young and is failing to gain a reasonable weight during the time when it should be growing–macaques that are not fully-grown should be expected to gain weight throughout the training and testing phases. If an animal undergoing fluid control deviates from its growth trajectory, consideration should be given to relaxing the fluid control. Persistent divergence (particularly when significant weight loss in a growing animal is seen) is a cause for concern. Other causes may underlie this, such as infection. Review by the veterinarian should be undertaken promptly.
- Is judged, by the veterinarian, to be compromised due to some other circumstance (e.g. a minor cold carrying the risk of insensible fluid loss).

Clinical evaluation of health: The physical health of macaques on fluid control protocols should be checked daily by an experienced member of the animal care staff using a scoring system agreed with the veterinarian and animal welfare officer (e.g. Smith et al., 2006). This should include an assessment of hydration status. Variables that can be used include food intake, urine output/presence, sequential analysis of blood, moisture content of fresh faeces and assessment of skin turgor (elasticity), as well as the general appearance, demeanour, activity level, behaviour and willingness of the animal to work (Newsome and Stein-Aviles, 1999; Toth and Gardiner, 2000; Wolfensohn and Honess, 2005). Multiple indicators of health should be used (Smith et al., 2006).

In addition to daily health inspections by researchers and animal care staff, each animal should be inspected periodically (e.g. every 3 months) by a veterinarian; tests of kidney function, clinical disease and general physical fitness may be undertaken. The Working Group recommends that fluid control should *cease*:

- If an animal shows clinical signs of dehydration (e.g. dryness of mucous membranes, loss of skin elasticity [tenting in response to skin pinching], reduced urine output, lethargy).
- If an animal is being treated for disease.
- If recommended by the veterinarian for other animal health reasons (e.g. vomiting).

Assessment of behaviour: The number of daily trials performed by the monkey should be monitored. If it reliably performs the number of trials required by the experiment and thus earns the full fluid allowance, then the amount of allowance (or supplement) should be gradually increased, while monitoring performance. Behavioural indicators of psychological well-being should also be monitored; these include (Novak and Suomi, 1988; National Research Council, 1998; International Primatological Society, 2007; Joint Working Group on Refinement, 2009):

- a broad range of species-typical patterns of behaviour.
- absence of abnormal behaviour (e.g. excessive self plucking of hairs or picking of scabs, foot/thumb sucking, cage chewing, urine drinking, prolonged immobility, depression; see Bayne, 1996).
- absence of chronic signs of distress (e.g. chronic or excessive fear, grimacing, withdrawal, altered breathing, distress vocalisations, anorexia, or unusual postures; see Morton and Griffiths, 1985; National Research Council, 1992).
- ability to respond effectively to environmental challenges (e.g. novel stimuli).

Assessment of a change in any individual animal's behaviour is facilitated by use of a well-designed checklist by an experienced member of the animal care staff with a detailed knowledge of the behaviours that are 'normal' for that animal (Morton and Griffiths, 1985; Scientific Committee on Animal Health and Welfare, 2002; Smith et al., 2006).

6.3.4. *Breaks in the regimen*

In many fluid control protocols, the animals are given periodic breaks, commonly termed "holidays/vacations", of several weeks duration or longer, in which they are not required to perform the learned task and have *ad libitum* access to water (e.g. 1 week with no fluid control for every 6 weeks that the animal has experienced control of fluid intake). The Working Group recommends that the need for such breaks, if any, should be decided with the veterinarian and animal care staff, weighing the advantages and disadvantages for animal welfare. This recommendation is based on expert opinion in the absence of relevant studies addressing the issues. Practices vary and there are several considerations, including:

- Level of control—some fluid control regimens do not need breaks as the restriction is not significant (i.e. the monkey can achieve all of its daily fluid requirement and therefore does not need to be provided with the opportunity to make up any deficit in order to maintain normal weight or growth).
- Whether there are problems created by abrupt shifts between restricted and non-restricted fluid access (see Section 6.3.5)—there are concerns, based on experience and physiological considerations, that rapid return from a highly-restricted water ration to *ad libitum* access might produce behavioural depression or deleterious gastrointestinal complications (e.g. bloat) due to polydipsia[4] or water intoxication, if the kidneys continue to concentrate the urine and retain free water. Further research is needed in this area.
- Duration of expected break—macaques can become obese with prolonged *ad libitum* feeding only to lose weight on restricted fluid access, causing dramatic shifts in body weight for obese animals; experimental and stock animals should be managed in such a way as to prevent obesity.
- Whether the opportunity to work on the task and to interact with staff outside of the home enclosure is stimulating for the monkey.

[4] Haematocrit can drop overnight from 42–47% to 25% if the animals gorge on water (communication from Dr James Raber, Animal Program Director, NIH National Eye Institute).

6.3.5. *Transitioning from/to fluid control*

When transitioning an animal from *ad libitum* water to controlled intake (e.g. at the initiation of the protocol or at the end of a break in the regimen), the amount of water available in the home enclosure should be gradually decreased over a period of several days or weeks to prevent rapid changes in serum chemistry and to allow the kidneys and other system to accommodate to low levels. In such circumstances, establishing a routine will help the animal anticipate what is likely to happen each day. The animal should be monitored closely (see Section 6.3.3).

Evidence supporting the need to initiate gradual fluid control comes from research on thirst using rhesus macaques (Wood et al., 1982). Acute water deprivation for 24-hours caused a 5.8% elevation in plasma osmolality which is evidence of significant cellular dehydration and twice as great as that necessary to cause thirst (Toth and Gardiner, 2000).

When control of water intake is no longer necessary, animals should not be immediately returned to *ad libitum* water, but should have their water ration increased in increments over a period of several days or weeks and be monitored closely (Toth and Gardiner, 2000). This will aid in the prevention of gastrointestinal complications (e.g. bloat). For scheduled access protocols, increments of 20 ml/kg/day over 3–7 days have been used in the UK without problems. Providing access to water in a controlled manner (e.g. providing one water bottle at a time and waiting for it to be finished before providing another) can also help avoid complications.

Procedures under general anaesthesia: It is good practice for an animal to be returned to *ad libitum* water before a procedure that requires general anaesthesia and to remain on *ad libitum* water until the veterinarian advises that the fluid control can recommence. This will help ensure that the animal is in the optimal physiological state for anaesthesia and hence avoid the risk of complications that could compromise the experimental programme. As noted above, transitioning to *ad libitum* water should be done gradually.

No animals should undergo major surgical procedures when they are also being actively maintained on a fluid control protocol, except in emergency situations. Where necessary, minor procedures (e.g. dura debridement) can be performed without returning the animals to *ad libitum* water; any existing fluid deficit can be corrected rapidly using intravenous fluids, together with replacement of any 'insensible' fluid losses during the procedure. In such circumstances, any mild fluid deficit is unlikely to be exacerbated by excessive periods of water withdrawal prior to anaesthesia.

7. Refinement of husbandry practices

7.1. *Provision of foraging*

Foraging is an important requirement for the psychological well-being of non-human primates (e.g. Chamove and Anderson, 1989; National Research Council, 1998; Wolfensohn and Honess, 2005) and provision of opportunity to forage is required or recommended by professional guidelines on primate care and use (National Research Council, 1998; National Centre for the Replacement, Refinement and Reduction of Animals in Research, 2006; International Primatological Society, 2007). Macaques on food and fluid control protocols can be provided with foraging enrichment to meet this requirement. The use of a low calorie forage mix (e.g. a mixture of sesame seeds, poppy seeds, rice, hemp and millet) and/or chopped fruit and vegetables will help prevent this having an effect on the scientific programme by causing the monkeys to lose their motivation to work for food or fluid (Cosgrove and Carroll, 2002). Wolfensohn and Honess (2005) and the Joint Working Group on Refinement (2009) provide further advice on diet, feeding schedules and foraging enrichment strategies for non-human primates.

7.2. Social housing

The need for positive social interaction with conspecifics is recognized as a critical requirement for the psychological well-being of non-human primates (e.g. Novak and Suomi, 1988, 1991; Bellanca and Crockett, 2002; Wolfensohn and Honess, 2005; Seelig, 2007). Accordingly, social housing is the recommended standard housing configuration for macaques used in research, unless precluded by specific scientific, medical or behavioural constraints (Home Office, 1989; National Research Council, 1998; Scientific Committee on Animal Health and Welfare, 2002; Council of Europe, 2006). Macaques with cranial implants have been successfully socially-housed without damage to the device (Reinhardt, 1991; Roberts and Platt, 2005; Wolfensohn and Peters, 2005). Although social housing of implanted macaques may not be possible or practicable with all animals at all times, it should always be considered as the ideal arrangement.

Careful selection of group mates and ongoing monitoring is necessary for establishing and maintaining stable pairs and groups of macaques (e.g. Reinhardt et al., 1989, 1995; Line et al., 1990). Whilst some laboratories have experienced an increase in the incidence of aggression with social housing, this can often be successfully addressed through the provision of sufficient space and stimulation and by structuring the enclosure space to allow the monkeys to establish and maintain social hierarchies, using different levels of perches, vantage points and visual barriers (Honess and Marin, 2006). Further advice on creating and maintaining compatible social pairs/groups of non-human primates can be found in Joint Working Group on Refinement (2009).

It is possible to house experimental macaques on a food or fluid control protocol with one or more companions throughout the period of training and data collection. Some form of food or fluid control may need to be in place for the companion animals (e.g. providing access to water in the home enclosure only when the experimental animal is working in the laboratory). The companions should be monitored as closely as the experimental animals to ensure that there are no appreciable welfare costs to them from such a husbandry practice. Advice may need to be sought from the regulatory authority about whether such husbandry practices are permissible under animal welfare legislation (e.g. Home Office, 2003).

An increase in the frequency of abnormal behaviours (pacing, circling) has been observed when groups of restricted animals are able to observe the feeding and watering of groups of non-restricted animals maintained in the same colony. This can be avoided by housing the restricted animals in separate rooms from the non-restricted animals, or by providing food or water to non-restricted animals within the same room only when the restricted animals are removed for training/testing. Care must be taken to ensure adequate time is given for the non-restricted animals to eat and drink so that they are not unintentionally restricted.

8. Conclusions, data gaps and recommendations

Ongoing refinement of scientific procedures and husbandry to minimize pain, suffering, distress and lasting harm for macaques used in behavioural neuroscience research is necessary not only for good animal welfare but also to increase the likelihood of obtaining good quality scientific data. The development and refinement of research protocols that use food or fluid control require that researchers, in conjunction with the veterinarian, animal care staff and research institution's ethical review, address three fundamental issues:

- the requirement for, and level of, food or fluid control.
- the potential animal welfare consequences of the food or fluid control.
- the methods for monitoring and maintaining the health and psychological well-being of the animals.

Consideration of these issues for each *individual* animal will facilitate the establishment of appropriate intervention endpoints to maintain the animal's welfare.

8.1. Data gaps and future research

There is a paucity of published data on many aspects of the use of food and fluid control as motivational tools for macaques used in behavioural neuroscience research. The Working Group considers that refinement of such protocols would be greatly facilitated if more knowledge were available about the following:

- The potential reinforcement value of social stimuli, and whether social rewards can be used in conjunction with appetitive rewards as a means of reducing the required level of food/fluid control (Section 3.2.2).
- The potential impact on both physiology and animal welfare (e.g. as evidenced by changes in body weight, growth, behaviour, activity and responsiveness) of food control protocols compared with equivalent protocols of fluid control, including any suppression of feeding induced by fluid control (Section 3.3.1).
- Whether use of liquid slurry is preferable to water or other fluid reward from the standpoint of animal welfare and the long-term physiological implications of maintaining monkeys on a semi-solid diet (Section 3.3.2).
- The rate of acquisition of quality data and the physiological and psychological effects on the animals (during the training and testing phases) of fluid control protocols with a day or two of non-working each week and free access to water compared with fluid control protocols which involve extended periods working every day of the week or involve non-working days with limited access to water on those days (Section 6.1).
- Haematology and serum chemistry values for healthy macaques with stable weights and behaviour undergoing controlled access to fluid, stratified by level (stringency) of control (Section 6.2.1). Collection of blood parameter values which have been associated with defined clinical problems may also be useful for objective monitoring of animal welfare.
- The potential impact on both physiology and psychology of food and fluid control regimens with and without breaks to determine whether a difference can be discriminated. If a difference is seen, it would be useful to determine how frequently breaks should be given and how long such breaks should be in order to improve animal welfare. (In this context, breaks would be longer than 1 or 2 days). (Sections 5.2.4 and 6.3.4).
- Appropriate rates of transitioning to and from fluid control which retain normal physiological, behavioural and psychological states (Section 6.3.5).

- The research needed to supply the missing data on many aspects of food and fluid control will, in all likelihood, need to be undertaken by neuroscience laboratories and neuroscience researchers themselves. The NC3Rs and other bioscience research funders should provide the encouragement and resources for this research to take place and should facilitate greater data sharing aimed at refinement.

8.2. Recommendations

The majority of recommendations are Grade D (see Section 1.4). Implementation of the Working Group's recommendations requires support from all involved in the care and use of macaques on food and fluid control protocols.

Evaluating food and fluid control protocols

1. Many, but not all, cognitive and behavioural tests performed with macaques require the use of food and fluid control in order to obtain the large number of trials for modern neuroscientific investigations. Wherever possible, avoid control of food or fluid intake and use alternative methods of reward (e.g. treats, social stimuli) instead (Grade D).
2. When considering or evaluating food and fluid control protocols, conduct searches of subject-specific literature databases and seek expert advice from multiple sources about: the need for, and necessary level of, food or fluid control; the implications of such control for animal health and psychological well-being; and the opportunities for refinement (Grade D).
3. Where control protocols are necessary, give careful consideration to the choice of food or fluid control, taking both animal welfare and experimental considerations into account (Grade D for macaques; Grade A for rodents; Hamilton and Flaherty, 1973, I; Gillette-Bellingham et al., 1986, I; Heiderstadt et al., 2000, I; Tucci et al., 2006, I).
4. When reporting studies involving the use of food and fluid control, include full descriptions of the protocols employed (Grade D; Taffe, 2004, IV).
5. Regularly review and refine all husbandry practices and scientific procedures associated with the use of food and fluid control protocols (Grade D).

General principles for refinement of food and fluid control protocols

6. Before choosing rewards, consult the recent literature on the species and seek advice from the veterinarian, animal care staff and experienced colleagues about rewards tried and discarded and the preferences of individual animals, because the desirability of the reward/s used in training and testing will influence the animals' performance and hence the requirement for, or level of, food or fluid control (Grade D).
7. Explore measures that might increase the rate at which the animals gain reward, given that this will mitigate somewhat the effect of controlled access to food/fluid (Grade D).
8. Introduce food or fluid control protocols gradually such that the animal has time to recognize and anticipate the limited availability of food/fluid and adapt its patterns of food/fluid intake. In the case of fluid control, this will help to prevent rapid changes in serum chemistry and to allow the kidneys and other systems to accommodate to low levels of fluid (Grade A; Wood et al., 1982, I+).
9. Always consider alternative explanations for poor performance before assuming that greater food or fluid control will increase motivation and improve performance (Grade D).
10. Keep the level of food and fluid control for each individual animal to the minimum required for achieving the scientific objective whilst maintaining an acceptable standard of animal welfare. Where restriction is significant, make regular attempts to manipulate the reward amount upwards to boost total food or fluid intake, consistent with achieving the required behavioural data (Grade D).
11. Maintain records of food intake (for food and fluid control regimens), fluid intake (for fluid control regimens) and body weight in a form that is easily interpreted and is acceptable to the regulatory authority, veterinarian and animal care staff. Such records should be readily accessible to all those involved with the welfare of the animals (Grade D).
12. Monitor food or fluid controlled animals as necessary to evaluate the health, condition and psychological well-being of each individual animal. Include observation of the animal's behaviour as well as physiological criteria (Grade C; Smith et al., 2006, III).
13. The measures and monitoring regimen to be used and the intervention points should be specified in the protocol and agreed with the veterinarian, animal welfare officer and ERP/IACUC (Grade D).

Use of food control

14. Whenever an animal obtains any portion of its food requirement through food reward, ensure that the sum of the nutritional value of the food earned through reward and the food provided "free" is sufficient to maintain the animal in a healthy state. There should be constant access to water in the home enclosure (Grade D).
15. For young/growing monkeys, ensure the feeding regimen is sufficient for each individual animal to maintain normal growth whilst continuing to perform the behavioural task effectively. Baseline food intake should be re-established periodically as the animal ages (Grade B; Hamilton, 1972, II).
16. If quantitative measurement of food consumption is necessary, use prudent husbandry to balance this requirement against the needs of the animal for social contact (Grade D).
17. Monitor body weight frequently (at least weekly) throughout all stages of the procedure and during breaks in the regimen (Grade D).
18. Compare body weight records against normal growth curves to ensure animals are receiving adequate nutrition, are not overweight or underweight, and that young animals are growing normally (Grade D).
19. Optimise motivation to work by identifying preferred food rewards for each animal. Where appropriate, maintain motivation by switching reward types within the experiment and providing a bonus when the animal works to the end of the session (Grade D).
20. Assess the need for breaks in the regimen with the veterinarian and animal welfare officer, weighing the advantages and disadvantages for animal welfare. Sudden changes in the amount and pattern of feeding (e.g. the number of feeds per day, timing and location of feeds) should be avoided (Grade D).

Use of fluid control

21. Whenever an animal obtains any portion of fluid requirement through fluid reward, ensure that the sum of the fluid earned through the reward and any supplemental fluid and fruit or vegetables provided is sufficient to maintain the animal in a healthy state (Grade D).
22. Establish baseline fluid intake individually for each animal over a period of several days when the animal has a stable profile or behaviour and physiology. For young/growing animals, this baseline should re-established periodically, and for mature animals, on the advice of the veterinarian (Grade B; Hamilton, 1972, II; Wakita, 2004, III).
23. Use prudent husbandry to balance the requirement for quantitative measurement of fluid consumption against the needs of the animal for social contact (Grade D).

24. Optimise motivation to work by identifying preferred fluid rewards for each animal (Grade D).
25. Monitor the number of trials performed by the animal. If the animal reliably performs the number of trials required by the experiment and thus earns the full fluid allowance, then the amount of allowance should be gradually increased, while monitoring the performance. Where supplements are provided, these should be the maximum consistent with maintaining reliable performance (Grade D).
26. To encourage feeding and prevent weight loss, give food in close temporal proximity to the access to fluid. Provide a good quantity of high water content foods, such as fresh fruit and vegetables; the water content of such foods can be taken into account when calculating the allotted amount of fluid for each animal (Grade B; Kutscher, 1969, I; Natelson and Bonbright, 1978, II).
27. Record daily for each animal the amount of fluid earned in the task, any supplements, fruit and/or vegetables given, body weight and indices of hydration status, such as skin and faecal appearance. (Grade C; Smith et al., 2006, III).
28. To ensure animals are receiving adequate fluid, compare body weight records against normal growth curves and perform a daily check of health and psychological well-being via a scoring system agreed with the veterinarian and animal care staff. In addition, each animal should be inspected periodically (e.g. every 3 months) by a veterinarian (Grade C; Smith et al., 2006, III).
29. Increase fluid provision when an animal is exhibiting weight loss which is likely to compromise the animal's welfare, is young and is failing to gain a reasonable weight during the time when it should be growing, or is judged by the veterinarian to be compromised due to some other circumstance. Cease restriction if an animal is showing clinical signs of dehydration, is treated for disease, or if recommended by the veterinarian for other animal health reasons (Grade D).
30. Gradually return animals to *ad libitum* water before a procedure that requires general anaesthesia. Maintain the animals on *ad libitum* water until the veterinarian advises that fluid control can recommence (Grade D).
31. Assess the need for breaks in the regimen with the veterinarian and animal care staff, weighing the advantages and disadvantages for animal welfare. (Grade D).
32. When transitioning an animal to/from fluid control, gradually decrease/increase the amount of water freely available in the home enclosure over a period of several days or weeks and monitor the animal closely (Grade A; Wood et al., 1982, I+).

Future research

33. There is a clear need for further research and for sharing of data and experience between laboratories to facilitate refinement of the use of food or fluid control as a motivational tool for macaques used in behavioural neuroscience. The NC3Rs and other bioscience research funding bodies should increase their investment in refinement research in this area (Grade D).

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Appendix A. Glossary

- *Ad libitum*: Free access to unlimited food/fluid such that the animal can choose how much or how little to eat/drink.
- Allo-grooming: Grooming another animal.
- Appetitive rewards: Attractive (desired or sought after) rewards.
- Bloat: Distension of the gut with gas and fluid.
- Cognitive: Pertaining to cognition, the mental process of being aware, knowing, perceiving, thinking and remembering.
- Control: Control of access to food or fluid, or the amount of food or fluid provided, to motivate an animal to respond reliably to food or fluid reward in an experimental session.
- Deprivation: Total denial of food or fluid; typically described in terms of the interval during which food or fluid is withheld from an animal. Toth and Gardiner (2000) note that although the terms deprivation and restriction overlap conceptually and often used interchangeably, these procedures may affect the animal in different ways. In many restriction schedules, animals have the opportunity to eat or drink to satiation each day but the number of opportunities may be limited to one per day. Relatively few studies subject animals to multiple days of total deprivation.
- Foraging: Searching for, procuring, processing and consuming food.
- Haematocrit: The volume percentage of erythrocytes (red blood cells) in whole blood.
- Hypovolaemia: Abnormally decreased volume of circulating fluid (plasma) in the body. The term “clinically significant hypovolaemia” is used to communicate the concept that the volume is pathological or dangerous or stressful to the animal.
- Osmolality: The concentration of a solution in terms of osmoles of solute per kilogram of solvent. The osmole is a non-SI unit of measurement that defines the number of moles of a chemical compound that contribute to a solution's osmotic pressure. In laboratory reports, osmolality is expressed as “so many” milliosmoles per kilogram of water (mOsm/kg H_2O).
- Plasma osmolality: A measure of the concentration of substances such as sodium, chloride, potassium, urea, glucose and other ions in blood. Plasma osmolality is used to investigate fluid and electrolyte abnormalities.
- Polydipsia: Abnormally large intake of fluids by mouth; excessive water drinking.
- Restriction: Limitation on *ad libitum* intake, as opposed to total denial of food or fluid; typically achieved by limiting the amount of food or fluid provided on a daily basis or the amount of time each day that the animal is given access to food or fluid.
- Trial: A discrete set of events which may typically include an initiating behaviour or start signal, the presentation of stimuli and a response by the animal, culminating in a feedback signal which may be a reward. An experimental session may comprise any number of discrete trials.

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