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Research Paper

A protocol for training group-housed rhesus macaques (*Macaca mulatta*) to cooperate with husbandry and research procedures using positive reinforcement

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ABSTRACT

There has been increased recognition of the 3Rs in laboratory animal management over the last decade, including improvements in animal handling and housing. For example, positive reinforcement is now more widely used to encourage primates to cooperate with husbandry procedures, and improved enclosure design allows housing in social groups with opportunity to escape and avoid other primates and humans. Both practices have become gold standards in captive primate care resulting in improved health and behavioural outcomes. However, training individuals and social housing may be perceived as incompatible, and so it is important to share protocols, their outcomes and suggestions for planning and improvements for future uptake. Here we present a protocol with link to video for training rhesus macaques (*Macaca mulatta)* housed in single-male – multi-female breeding groups to sit at individual stations in the social enclosure. Our aim was that the monkeys could take part in welfare-related cognitive assessments without the need for removal from the group or interference by group members. To do this we required most individuals in a group to sit by individual stations at the same time. Most of the training was conducted by a single trainer with occasional assistance from a second trainer depending on availability. We successfully trained 61/65 monkeys housed in groups of up to nine adults (plus infants and juveniles) to sit by their individual stationing tools for > 30 s. Males successfully trained on average within 30 min (2 training sessions); females trained on average in 1 h 52 min ± 13min (7.44 sessions), with rank (high, mid, low) affecting the number of sessions required. On average, dominant females trained in 1 h 26 min ± 16 min (5.7 sessions), mid ranked females in 1 h 52 min ± 20min (7.45 sessions), and subordinate females took 2 h 44 min ± 36 min (10.9 sessions). Age, group size, reproductive status, temperament, and early maternal separation did not influence the number of sessions a monkey required to reach criterion. We hope this protocol will be useful for facilities worldwide looking to house their animals in naturalistic social groups without impacting on animal husbandry and management.

1. Introduction

With the increased recognition of the 3Rs in research (NC3Rs, 2006; Prescott, 2010; Russell and Burch, 1959), training laboratory primates to cooperate with animal management and research procedures has become a key welfare refinement (Bloomsmith et al., 1998; Coleman et al., 2008; LASA/MRC, 2004; Laule et al., 1996, 2003; NC3Rs, 2015; Perlman et al., 2010, 2012; Prescott et al., 2007; Prescott and Buchanan-Smith, 2007; Reinhardt, 1997; Schapiro et al., 2003, 2005). However, emphasis on housing conditions that fulfil animals' physical and social needs can result in perceived conflicts between colony management practices and animal welfare (Prescott and Buchanan-Smith, 2007). It is therefore important to document and share training protocols and outcomes from facilities embracing the 3Rs in their management plans, so that means of best practice can be shared and developed further.

Training animals teaches them that their behaviour has consequences, and positive reinforcement training (PRT) is particularly

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Table 1
Glossary.

Term	Definition
Positive reinforcement	The occurrence of a behaviour is increased as it results in a reward (e.g. food)
Negative reinforcement	The occurrence of a behaviour is increased as it results in removal of an aversive stimulus (e.g. capture net)
Positive punishment	The occurrence of a behaviour is decreased as it results in the appearance of an aversive stimulus (e.g. verbal 'no')
Negative punishment	The occurrence of a behaviour is decreased as it results in removal of a reward (e.g. it results in a 'time out')
Shaping	also 'successive approximation'. A desired behaviour (such as 'hold target for 30 s') is broken down into successive stages (approach target, touch target, hold target, stay by target).
Bridge	A type of 'conditioned reinforcer "or 'secondary reinforcer". An initially unfamiliar stimulus (such as the "click" of a hand-held clicker or a verbal cue such as 'good') is repeatedly paired with a primary reinforcer so that it becomes a positive reinforcer through association. Specifically, a bridging stimulus can be produced exactly at the moment the animal performs a desired behaviour, therefore creating a bridge between performing the behaviour and receiving the primary reinforcer (e.g. food).

recommended from a welfare perspective because it encourages voluntary participation for positive outcomes (Bassett and Buchanan-Smith, 2007; Prescott and Buchanan-Smith, 2003, 2007; Westlund, 2015). The theory underlying PRT has been well described elsewhere (e.g. Bloomsmith et al., 2007; Laule and Whittaker, 2001, 2007; Schapiro et al., 2005; Westlund, 2015) and we give key terms and definitions in Table 1. There is widespread agreement that opportunity for choice and control afforded by PRT not only has direct welfare benefits (Bassett and Buchanan-Smith, 2007; Buchanan-Smith and Badihi, 2012) but may also improve the quality of research data arising from use of animal models (e.g. Lambeth et al., 2006; Prescott et al., 2010). Furthermore, PRT can provide a valuable colony management tool with time and money savings, resulting from a cooperative relationship built on trust between trainer and trainee (Jennings et al., 2009).

While PRT requires an initial time investment, evidence suggests this is small compared to the long term time savings afforded by animals who calmly and efficiently participate in husbandry and research procedures due to reduced stress, and faster and improved performance (Lambeth et al., 2006; Perlman et al., 2012; Reinhardt et al., 1990; Westlund, 2015). Well trained animals are more likely to participate in further, more advanced, training procedures, and may be more likely to successfully participate in more cognitively demanding research protocols (Jennings et al., 2009; Westlund, 2015). Reduced stress levels contribute to improved health and reproductive outcomes (e.g. Shively et al., 2005; Capitanio et al., 1998). We also suggest that implementing standardised group-training protocols across facilities, and especially at breeding centres and in younger animals, may provide a useful mechanism for minimising relocation stress in animals transferred between facilities (e.g. Honess et al., 2004). As animals are often transferred from breeding facilities to research centres, training familiarity may help them adjust more readily to new environments with unfamiliar staff.

There are a number of published surveys of facility-wide practices and staff perceptions (e.g. Prescott and Buchanan-Smith, 2007; Perlman et al., 2012) and some published protocols for training (e.g. Westlund, 2015; Laule et al., 2003). However, there are very few studies detailing group-level training protocols together with data on training success rates. Of the published studies, descriptions of training outcomes for primates typically involve relatively small numbers of individually trained animals (e.g. Bloomsmith et al., 1994; Reinhardt, 1997; Ward and Melfi, 2013), and animals in single or pair housing (Clay et al., 2009; Coleman et al., 2008; Fernstrom et al., 2009; Laule et al., 1996; Reinhardt, 1997; Reinhardt et al., 1990). The training of primates in groups ($n > 3$) tends to cover three categories of behaviour: collective behaviour, individual behaviour, and cooperative behaviour. PRT of collective behaviour involves training a group to work together to achieve a goal, with all group members performing the same behaviour, such as moving from one part of their enclosure to another (e.g. Bloomsmith et al., 1998; Veeder et al., 2009). Individuals within a group can also be trained, one at a time, to perform a task (e.g. Fagot et al., 2014; Stone et al., 1994) by simply encouraging the target animal to one location of the enclosure and ignoring any other group members who might approach to investigate. The training of cooperative behaviour is usually focused on group management, such as cooperative feeding (Bloomsmith et al., 1994; Schapiro et al., 2001; Whittaker, 2005), in which dominant animals are reinforced for allowing lower-ranked conspecifics access to desirable resources. Training animals in groups therefore requires staff to be sensitive to group dynamics and it can be daunting for staff to initiate training efforts when the primates are not typical research subjects (i.e. training naïve) and live in large groups, such as in a breeding facility or zoological institution (Westlund, 2015). The initiation and objective success of group training programs with larger numbers of animals therefore requires greater documentation and validation (Perlman et al., 2012; Prescott and Bucahanan-Smith, 2007), especially for animals in high-welfare housing conditions where the opportunity to move freely may be perceived as a barrier to staff initiating and maintain training.

Here we present the training protocol and training outcomes for group-housed rhesus macaques (*Macaca mulatta*) taking part in an NC3Rs-funded research project (NC/L000539/1) investigating cognitive measures of psychological wellbeing. Our research was conducted within a breeding facility where macaque group sizes ranged from two to 11 adults, plus infants and juveniles. The methodology for the research project required the adult female macaques to remain by a stationing tool so that they could be individually presented with stimuli, and their responses filmed by a fixed camera (Bethell et al., 2015; Szott, 2015; Thatcher, 2016). For both scientific and welfare purposes, it was important that the macaques remained within their social group during testing and that we minimised any actions that might cause stress. To this end, we planned to train all adults within each group to allow control over the group as a whole. The trainers (CK as primary trainer with later assistance from HT) had to divide their duties during the research stage and so it was essential that the monkeys could be managed as a group by one trainer. This paper details the training methods used and the outcomes, including best predictors of training success. We hope this will provide a useful protocol for other facilities to encourage training of animals to engage in routine procedures without the need for removal from the social group.

2. Methods

2.1. Ethics

The research program and training plans were formulated in discussion with the facility Home Office Inspector (Nov 2011) and subsequently approved by Roehampton University Ethics Committee (approval #LSC 14/113).