

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

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4 Caralyn Kemp¹, Harriet Thatcher¹, David Farningham², Claire Witham^{2,3}, Ann MacLarnon⁴, Amanda
5 Holmes⁴, Stuart Semple⁴, Emily J. Bethell¹

6
7 ¹Centre for Research in Brain and Behaviour, School of Natural Sciences and Psychology, Liverpool
8 John Moores University, L3 3AF, UK

9 ²Medical Research Council Harwell Unit, Centre for Macaques, Salisbury, Wiltshire, SP4 0JQ, UK

10 ³ Institute of Neuroscience, Newcastle University, Newcastle-upon-Tyne, NE1 7RU, UK

11 ⁴Centre for Research in Evolutionary, Social and Interdisciplinary Anthropology, University of
12 Roehampton, London, SW15 4JD, UK

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21 Corresponding author: Emily J. Bethell, School of Natural Sciences and Psychology, Liverpool John
22 Moores University, Byrom Street, Liverpool, L3 3AF. Email: E.J.Bethell@ljmu.ac.uk

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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 >30seconds. Males successfully trained on average within 30 minutes (2 training sessions); females trained on average in 1hr 52 minutes \pm 13mins (7.44 sessions), with rank (high, mid, low) affecting the number of sessions required. On average, dominant females trained in 1 hr 26 mins \pm 16mins (5.7 sessions), mid ranked females in 1hr 52 mins \pm 20mins (7.45 sessions), and subordinate females took 2hrs 44mins \pm 36 mins (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.

Keywords: 3Rs, group training, macaque, positive reinforcement training, primates, stationing

53 1. INTRODUCTION

54 With the increased recognition of the 3Rs in research (NC3Rs, 2006; Prescott, 2010; Russell & Birch
55 1959), training laboratory primates to cooperate with animal management and research procedures has
56 become a key welfare refinement (Bloomsmith *et al.*, 1998; Coleman *et al.*, 2008; LASA/MRC, 2004;
57 Laule *et al.*, 1996, 2003; NC3Rs, 2015; Perlman *et al.*, 2010, 2012; Prescott & Buchanan-Smith,
58 2007; Reinhardt, 1997; Schapiro *et al.*, 2003, 2005). However, emphasis on housing conditions that
59 fulfil animals' physical and social needs can result in perceived conflicts between colony management
60 practices and animal welfare (Prescott & Buchanan-Smith, 2007). It is therefore important to
61 document and share training protocols and outcomes from facilities embracing the 3Rs in their
62 management plans, so that means of best practice can be shared and developed further.

63
64 Training animals teaches them that their behaviour has consequences, and positive reinforcement
65 training (PRT) is particularly recommended from a welfare perspective because it encourages
66 voluntary participation for positive outcomes (Bassett & Buchanan-Smith, 2007; Prescott &
67 Buchanan-Smith, 2003, 2007; Westlund, 2015). The theory underlying PRT has been well described
68 elsewhere (e.g. Bloomsmith *et al.*, 2007; Laule & Whittaker, 2001, 2007; Schapiro *et al.*, 2005;
69 Westlund, 2015) and we give key terms and definitions in Table 1. There is widespread agreement
70 that opportunity for choice and control afforded by PRT not only has direct welfare benefits (Bassett
71 & Buchanan-Smith, 2007; Buchanan-Smith & Badihi, 2012) but may also improve the quality of
72 research data arising from use of animal models (e.g. Lambeth *et al.*, 2006; Prescott *et al.*, 2010).
73 Furthermore, PRT can provide a valuable colony management tool with time and money savings,
74 resulting from a cooperative relationship built on trust between trainer and trainee (Jennings *et al.*,
75 2009).

76
77 While PRT requires an initial time investment, evidence suggests this is small compared to the long
78 term time savings afforded by animals who calmly and efficiently participate in husbandry and
79 research procedures due to reduced stress, and faster and improved performance (Lambeth *et al.*,
80 2006; Perlman *et al.*, 2012; Reinhardt *et al.*, 1990; Westlund, 2015). Well trained animals are more

81 likely to participate in further, more advanced, training procedures, and may be more likely to
82 successfully participate in more cognitively demanding research protocols (Jennings *et al.*, 2009;
83 Westlund, 2015). Reduced stress levels contribute to improved health and reproductive outcomes (e.g.
84 Shively *et al.*, 2005; Capitanio *et al.*, 1998). We also suggest that implementing standardised group-
85 training protocols across facilities, and especially at breeding centres and in younger animals, may
86 provide a useful mechanism for minimising relocation stress in animals transferred between facilities
87 (e.g. Honess *et al.*, 2004). As animals are often transferred from breeding facilities to research centres,
88 training familiarity may help them adjust more readily to new environments with unfamiliar staff.

89

90 There are a number of published surveys of facility-wide practices and staff perceptions (e.g. Prescott
91 & Buchanan-Smith, 2007; Perlman *et al.* 2012) and some published protocols for training (e.g.
92 Westlund, 2015; Laule *et al.*, 2003). However, there are very few studies detailing group-level
93 training protocols together with data on training success rates. Of the published studies, descriptions
94 of training outcomes for primates typically involve relatively small numbers of individually trained
95 animals (e.g. Bloomsmith *et al.*, 1994; Reinhardt, 1997; Ward & Melfi, 2013), and animals in single
96 or pair housing (Clay *et al.*, 2009; Coleman *et al.*, 2008; Fernstrom *et al.*, 2009; Laule *et al.*, 1996;
97 Reinhardt, 1997; Reinhardt *et al.*, 1990). The training of primates in groups ($n > 3$) tends to cover
98 three categories of behaviour: collective behaviour, individual behaviour, and cooperative behaviour.
99 PRT of collective behaviour involves training a group to work together to achieve a goal, with all
100 group members performing the same behaviour, such as moving from one part of their enclosure to
101 another (e.g. Bloomsmith *et al.*, 1998; Veeder *et al.*, 2009). Individuals within a group can also be
102 trained, one at a time, to perform a task (e.g. Fagot *et al.*, 2014; Stone *et al.*, 1994) by simply
103 encouraging the target animal to one location of the enclosure and ignoring any other group members
104 who might approach to investigate. The training of cooperative behaviour is usually focused on group
105 management, such as cooperative feeding (Bloomsmith *et al.*, 1994; Schapiro *et al.*, 2001; Whittaker,
106 2005), in which dominant animals are reinforced for allowing lower-ranked conspecifics access to
107 desirable resources. Training animals in groups therefore requires staff to be sensitive to group
108 dynamics and it can be daunting for staff to initiate training efforts when the primates are not typical

109 research subjects (ie. training naïve) and live in large groups, such as in a breeding facility or
110 zoological institution (Westlund, 2015). The initiation and objective success of group training
111 programs with larger numbers of animals therefore requires greater documentation and validation
112 (Perlman *et al.*, 2012; Prescott & Buchanan-Smith, 2007), especially for animals in high-welfare
113 housing conditions where the opportunity to move freely may be perceived as a barrier to staff
114 initiating and maintain training.

115

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

131

132 **2. METHODS**

133 **2.1 Ethics**

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

137

138 **2.2 Animals and Housing**

139 Sixty-five adult rhesus macaques (65 female, 9 male; age range 29 – 220 months) housed as part of
140 the breeding stock at the Medical Research Council's Centre for Macaques (MRC-CFM) took part in
141 the training. The MRC-CFM is licenced by the Home Office to breed macaques for provision to UK
142 facilities. MRC-CFM works in strict accordance with the NC3Rs guidelines (NC3Rs, 2006). Images
143 of the facility's primate accommodation are available to view in the NC3Rs guidelines (NC3Rs, 2006)
144 and on the NC3Rs macaque website (NC3Rs, 2015) as examples of good practice in animal housing
145 and enrichment.

146 Monkeys were housed in 11 social groups, eight of which consisted of one adult male and breeding
147 females, with infants and juveniles, and three of which contained only adult females. Groups were
148 selected for training if they contained females who would later take part in a research study of
149 cognitive markers of wellbeing (Bethell *et al.*, *in prep.*). A number of life history variables were
150 recorded for each monkey including sex, age and group size. For females we additionally noted:
151 reproductive status (pregnant, dependent offspring, neither or both: these were obtained from visual
152 inspection and retrospectively by working back from timings of births); rank within the social
153 hierarchy (high, mid or low); temperament (ranging from affiliative to aggressive, described in more
154 detail below); and whether they had been removed from the mother earlier than 1 year of age (early
155 maternal separation as a proxy for early life stress).

156

157 Rhesus macaques have a linear hierarchy based on female relatedness and relationships (deWaal &
158 Luttrell, 1985; Jackson & Winnegrad, 1988). We determined the rank of each female within her group
159 through consultation with facility staff and through observation of displacements, direction of
160 aggression, and vigilance during the initial habituation phase. Two researchers (CK and HT)
161 conducted separate assessments and then compared for accordance, the result of which shows that the
162 hierarchal position of each female was clearly defined. Confidence and wariness were clear signals of
163 status, with dominant females typically approaching the trainer early in the process. Who was wary of

164 whom, as well as aggressive events between females, also helped determine rank. High ranking
165 females tended to dominate priority locations, especially near the breeding male, and would sit on the
166 middle level of the caging. Very low ranked females utilised the bottom level, stayed near hatchways,
167 and were quick to flee when more dominant animals approached them. Once the linear order of the
168 females for each group was determined, we calculated each animal's relative rank within their group.
169 Typically, we assigned the top 2-3 females as high ranking, the bottom 1-2 females as low ranking,
170 and all others as mid-ranked, and adjusted this according to the relative numbers in the group and
171 exertion of dominance by the top female.

172

173 Temperament was classified by CK based on three categories of observations (Table 2): focal animal
174 behavioural observation in the social group; behavioural responses towards and eagerness to approach
175 trainers during habituation and training sessions (ie., confident to approach and cooperate or wary and
176 uncooperative); and behavioural interactions with group members during habituation and training (i.e.
177 willingness to let others receive rewards, how closely subordinates were allowed to sit, aggressive and
178 submissive behaviours). From these observations, we were interested in consistent characteristics that
179 indicated whether an animal was predominantly (more than 60% of the time) 'affiliative and
180 cooperative', 'aggressive and uncooperative' or 'predominantly neither' (that is, fitting into neither
181 category clearly).

182

183 Each group had access to a free-roaming room (3.35m x 8.04m x 2.8m) and an adjacent cage area
184 (1.5m x 6.12m x 2.8m), accessible through hatches, with a minimum total space of 3.5m³/breeding
185 animal in the largest groups. Each free-roaming room had a large bay window at one end facing
186 outdoors and allowing a natural day-night cycle. At the other end of each room was an internal
187 window into the hallway used by staff. Internal windows were fitted with movable mirrors so that
188 monkeys could manipulate the mirrors to view activities along the corridor. Rooms were furnished
189 with wooden platforms and poles (horizontal, vertical, diagonal), fire hose, ladders, plastic horse
190 jumps and saddle racks, PVC piping, plastic barrels and balls, and small plastic blocks attached to

191 structures or walls. The floor was covered with a deep layer of straw and shavings. All rooms were
192 temperature controlled ($20^{\circ}\text{C} \pm 5$) with humidity at $55\% \pm 10$.

193

194 Animals were free to move between the room and cage area at all times during training and at no
195 point were the hatches used to retain animals. Adjacent groups were able to see and hear each other
196 from the cage area, but there was no possibility for physical contact. All training took place in the
197 cage area, with open access to the free-roaming room at all times.

198

199 The macaques were fed twice daily by scatter feed, morning and afternoon, with sufficient food to last
200 for a 24 hr period. The diet varied daily and included a dried forage mix (cereal, peas, beans, lentils
201 etc.), a range of fruit and vegetables, bread and boiled eggs. Water was available *ad libitum* in both
202 the room and cage area.

203

204 **2.3 Training Protocol**

205 The training protocol is shown in Figure 1. Video of group target training may be viewed at:

206 <https://www.mrc.ac.uk/research/facilities-and-resources-for-researchers/mrc-centre-for->

207 [macaques/habituation-and-training/](https://www.mrc.ac.uk/research/facilities-and-resources-for-researchers/mrc-centre-for-macaques/habituation-and-training/). The key aims of training were to a) establish clear and consistent
208 signals for rewards and b) develop a relationship of trust that the trainer will behave consistently.

209

210 **2.3.1 Habituation**

211 Prior to training, all groups went through a period of habituation to familiarise them with the trainers
212 and the clicker device which used as a secondary reinforcer to ‘bridge’ between the moment of the
213 desired behaviour and reward (see Table 1). CK and HT were not members of care staff at the facility,
214 and monkeys therefore first needed to be habituated to their presence (Figure 1, Step 1). At the start of
215 the study, three habituation-only sessions (5-10 mins in length) were conducted for each group once
216 on each of three separate days within a 1 week window. During a habituation session, small pieces of
217 preferred food treats (peanuts and raisins) were offered in the caged area (Figure 1, Step 1.1). Treats

218 were small to prevent satiation and over-feeding. All monkeys were offered and encouraged to take
219 treats from the trainer's hand. When there was reluctance to do so the treats were placed on the cage
220 bars to entice the monkey to move forward. If a monkey was particularly nervous, the trainer would
221 initially step back when the monkey approached the front of the cage to encourage confidence to
222 move forward for treats. A clicker device was sounded at the moment when the monkey took a treat,
223 accompanied with the verbal reinforcement of "good boy/girl *name*". Verbal commands were given to
224 assist the monkeys in developing a positive association for trainers and researchers saying their name,
225 and the use of verbal and clicker cues together was considered to enhance opportunity for learning
226 (e.g. Westlund, 2015; Fernström *et al.*, 2009).

227

228 Once one or two monkeys were comfortable coming forward for treats and staying at the front of the
229 cage to feed (Figure 1, Step 1.2), training sessions began (Figure 1, Step 2). Training the food-
230 dominating monkeys to station first allowed us to manage the group most effectively. By stationing
231 these animals first, they learned to cooperate and this allowed us to then focus on other group
232 members, encourage them to come forward and train them individually in the group setting.

233

234 ***2.3.2 Training the first individual***

235 All training sessions were kept to a maximum of 15 mins. One training session was conducted per
236 day, as this had previously been found to be the most efficient frequency for the successful training of
237 macaques (Fernström *et al.*, 2009). Training was conducted with a focus on using positive
238 reinforcement for desired behaviours: in this case holding onto a target for stationing. The clicker was
239 used as a secondary reinforcer, or "bridge", with treats (peanut or raisin pieces) as the primary
240 reinforcer. As the monkeys became more comfortable with the presence of the researchers and taking
241 treats by hand, the clicker was used as a bridge, and activated prior to or instead of the treat.
242 Generally, peanuts and raisins were given out on different days but some monkeys had a preference
243 and would not cooperate for the other treat, and so efforts were made to adapt to individual
244 preferences.

245

246 Training proceeded in the same manner for each individual in the group (Figure 1, Step 3). In the
247 breeding groups, training was always first conducted with the breeding male. Although they were not
248 tested as part of the overall research program, it was important to train them to station and keep out of
249 the way of the females who were taking part in the research. This discipline reduced the likelihood of
250 the male interrupting training and testing sessions with the females, in particular the lower ranked
251 females, in order to steal their treats. The males were also trained to sit when at their station (the
252 females tended to sit at their station automatically) using the verbal command “sit” and a
253 corresponding hand gesture. We observed that when trained to sit, males were less likely to move
254 away from their station.

255

256 Each monkey was assigned an individual stationing tool (Figure 2). Station tools were designed to be
257 strong, durable, safe and distinctive in appearance; we used durable dog toys attached to carabiners
258 and then clipped to the caging. The monkeys were given the opportunity to investigate the station
259 tool. When the male approached his assigned stationing tool, he was rewarded with a click, a treat,
260 and a verbal cue of “good boy *name*”. This behaviour was gradually shaped over time so that he was
261 only ever rewarded if he sat next to and was touching his station tool for progressively longer periods
262 of time. If the monkey had shown interest in the station tool and approached and received treats, but
263 moved away during the training, the trainer would walk over to the target monkey, point at them and
264 say their name, and then walk to the station tool, point at it and use the verbal cue of “station”. This
265 would be repeated as often as necessary within the limitations of the training session so that the
266 monkey would associate a particular station with themselves. If the monkey touched the stationing
267 tool (Figure 1, Step 2.2), they were also rewarded. If the monkey did not touch the stationing tool, we
268 would put food on the carabiner or push the carabiner in between the bars towards the monkey to
269 encourage exploration and we found that many macaques responded to this action by reaching out to
270 the carabiner if only to push it back out – this touching was always rewarded.

271

272 The aim of our training protocol was for the monkey to hold on to some aspect of the stationing tool
273 to encourage them to remain in one location and not follow the trainer (Figure 1. Step 2.4). It was

274 therefore necessary that touching became holding. To this end, the length of time the monkey had to
275 be in contact with the station before being rewarded steadily increased from a brief touch to up to 30s
276 (i.e. shaping, Laule *et al.*, 2003). The verbal cue “hold” was used. With the longer periods of holding,
277 we used the clicker to reinforce the behaviour but did not give a food reward until the target time
278 period had been achieved. Once an animal had reached the threshold of 30s of continuous holding, we
279 found that most macaques would continue holding throughout training and testing.

280

281 Some macaques would not touch their station at all, despite repeated efforts, but would remain at it.
282 This was fine for our testing needs, so long as the monkey consistently remained at its station (Figure
283 1. Step 2.4), and so we did not continue pushing these animals to touch the tool itself. However, we
284 found that some of these animals would much later (typically months after learning to sit by their
285 station) start exploring the stationing tool and touch it. This was then rewarded and encouraged as
286 described in Figure 1 Steps 2.2 – 2.4.

287

288 At the end of the training session, the verbal cue of “all done”, with a corresponding waving hand
289 gesture, was used before the station tool was removed. This cue was used to signify to the macaques
290 that the training session was over and that no more signals or rewards were coming. Although unique
291 cues signalling the start and end of training sessions have not been assessed within the literature, there
292 is debate amongst trainers regarding their usefulness (see Pryor, 2016). One thought is that they are
293 important for the animals to understand when they are in a ‘training’ context as opposed to other
294 contexts (e.g. cleaning or feeding). This may speed up the learning process, as it helps animals to
295 distinguish disruptions to training due to extraneous factors from the intended completion of a session.
296 We also did not test whether or not the signal was necessary. However, we felt it was useful, given the
297 large number of macaques per trainer, for an end signal to be used so that the animals would learn that
298 even when the trainer was not working directly with them, the session was continuing and therefore
299 they should remain at their station in order to receive a reward.

300

301 **2.3.3 Training the group**

302 Once the first animal, usually the breeding male when one was present, had learned to station for at
303 least 30s, we began training the next individual (Figure 1. Step 2.5), usually the dominant female. We
304 started a training session by stationing the first animal who had been trained. Once the first animal
305 had been stationed, the trainer moved away and attached a new stationing tool to the caging at a
306 distance at least out of arms' reach (Figure 1. Step 3.1). Through trial and error we learnt which
307 animals could be stationed near each other without aggression and which needed to be kept well apart;
308 we also utilised different heights in the cage area, and adapted to individuals' preferences for
309 positioning, especially for the larger groups. Low ranked animals, in particular, tended to prefer to be
310 in a position where they could view the breeding male (or more dominant females) but were not on
311 the same level and therefore had a quick escape route if necessary. It helped, in some groups, to insert
312 dividing panels into the caging to act as visual barriers between particular group members; however,
313 this method was used sparingly as use of dividing panels can signal multiple outcomes (including
314 negative events such as veterinary inspections), and it was necessary to spend time habituating the
315 animals to the panels being put in.

316

317 Initially, the first monkey to be trained would typically follow the trainer as they started training the
318 second animal (Figure 1. Step 3.2) and so it was necessary to walk them back to their own station,
319 using the finger point hand gesture, starting from the animal (with the verbal cue of their name) and
320 moving to the station (with the verbal cue of "station"). Over time we would stop rewarding with food
321 when they returned to their station. At this point in training, only remaining at the station without
322 interruption for longer durations was rewarded. Ignoring an animal who had learnt this rule but still
323 left their station to follow the trainer would result in the monkey returning to their own station without
324 command. This would be rewarded with a click and verbal cue of "good girl/boy *name*" but no food.

325

326 It was necessary for the trainer to be aware of the group dynamics as the training progressed, rather
327 than remaining solely focused on any one particular individual. The trainer could only focus on each
328 new animal for a short period of time before it was necessary to reward the previously trained
329 animals. However, the time between rewards for the trained animals increased over time so that

330 attention could be paid to each new monkey being trained. This also meant that higher ranked
331 macaques learnt that they were only rewarded if they allowed lower ranked animals to receive their
332 treats first; this was essential to reduce aggression. Once trained, the dominant animals were given
333 larger rewards than the subordinates. In larger groups, it was helpful (although not essential) to have a
334 second trainer present so that one person could focus on maintaining the already trained animals in
335 position while the other trainer focused on a new trainee, or on training two new macaques
336 simultaneously while the first person reinforced the rest of the group together.

337

338 The process of training individuals within a social group was typically oriented around the hierarchy,
339 with the lowest ranked animals coming forward for training last in a group. It was important that, as
340 the number of trained animals increased, the trainer did not leave the animal being trained to reinforce
341 all the other monkeys who were waiting; this would be too long a disruption to the training. Instead,
342 the trainer would reward only two or three animals before returning to the trainee and then reward a
343 different two or three monkeys at the next opportunity. Importantly, the breeding male was rewarded
344 more often than the females, especially when he was known to be particularly food-oriented or
345 aggressive.

346

347 At the start of a training session with multiple trained animals, the trainer would always put the
348 stationing tools up in the same order, starting with the breeding male, the dominant female and then
349 working through animals down the hierarchy (typically in the order of training). At the end of the
350 session, the station tools were removed in the reverse order. Each animal was given the “all done” cue
351 individually. The criterion for successful training was defined as stationing for >30s while we worked
352 with other animals in the group. Once an animal reached criterion for successful training we viewed
353 subsequent sessions as ‘maintenance’ sessions. We had 60 days to train monkeys prior to the onset of
354 the cognitive study for which they were being trained to station.

355

356 ***2.3.4 Dealing with undesired behaviours***

357 Although our training focused on positive reinforcement methods, the trainer also gave some
358 indication when an undesired behaviour had occurred. PRT standards recommend ignoring the
359 behaviour by not providing a reward and encouraging extinction of the behaviour (Pryor, 1999).
360 However, in our protocol, we occasionally used the word “no” to indicate an unwanted behavioural
361 response from the monkeys and no click/treat was given. This was especially useful when two trainers
362 were present to coordinate between us. If a monkey persistently gave an undesired behaviour (such as
363 moving away from the stationing tool) and the use of the previously learned verbal or gestural cues
364 for the desired activity was ignored, the trainer would hold out their hands with palms open (to signal
365 no food), and then turn their back (i.e. a “time out”; Prescott *et al.*, 2005).

366

367 **2.5 Statistical analysis**

368 Data on training success are reported for all 65 monkeys. Tests for normality were conducted
369 using the Shapiro-Wilk test and normal Q-Q probability plots. We used the ‘lm’ function of the
370 ‘stats’ package in R (R Core Team, 2016) to fit linear regression models using an information-
371 theoretic approach on likelihood measures (AICc; Akaike, 1974) to identify the best predictors of
372 training success (number of sessions) and number of trainers (1 or 2) required. The former was
373 conducted for the females whose life history and behavioural data were available (n = 55). The
374 predictor variables were age (continuous variable), number of adults in the group (continuous variable
375 from 2 – 9), reproductive status (pregnant, dependent offspring, neither or both), dominance rank
376 (high, medium, low), temperament (affiliative/cooperative, aggressive/uncooperative, predominantly
377 neither), and early maternal separation (yes/no). We also included the null model in the analysis and
378 used the ‘model.sel’ function in R to compare model fits. Given the limited window of time available
379 for training (as few as 20 days for the more submissive females who were last to begin training), those
380 monkeys who showed clear evidence of learning but failed to reach ‘criterion’ due to the shorter time
381 available for them, were assigned a ceiling value of 50 sessions to retain them in the analysis (i.e. the
382 maximum number of training days available to females within the training phase; for examples of use,
383 see Ash & Buchanan-Smith, 2016; Held *et al.*, 2006). We justify this on the basis that three monkeys

384 who failed to reach criterion had performed well prior to the birth of their offspring midway through
385 training; we have no reason to assume (based on the success rates of the cohort overall) that these
386 monkeys would not have learnt the task otherwise.

387

388 3. RESULTS

389 In total, 61 of the 65 monkeys who were approached for training, reached criterion for successful
390 training to sit by a stationing tool (Table 3: 9/9 males; 52/56 females). Of the four females who did
391 not train successfully, one we chose to discontinue training due to aggression towards her trainers and
392 is therefore not included further in our analyses (henceforth $n = 64$). The other 3 females gave birth
393 during training and failed to stay by their station for 30 seconds after 25 (6.25hrs), 35 (8.75hrs) and 40
394 (10hrs) training sessions, respectively. Training was stopped for these animals, due to time constraints
395 imposed by the start of the research programme and they were assigned a session value of 50 for
396 analysis. The successfully trained females reached criterion in an average of 7.4 training sessions
397 (range 1 – 24). All nine males reached criterion for successful station training in two training sessions
398 (and in addition they all learned to follow the command to “sit”).

399

400 Comparison of linear regression models (Table 4) revealed the only significant predictor of number of
401 sessions required to train females was dominance rank (lm: $F_{(2,53)} = 4.51$, $p = 0.038$). High ($n = 20$)
402 and mid ($n = 22$) ranking females reached criterion on average in 1hr 26 mins (5.7 ± 1.06 sessions)
403 and 1hr 52 mins (7.45 ± 1.25 sessions), respectively, while low ranking females ($n = 10$) took on
404 average 2hrs 44mins \pm 36 mins (10.9 ± 2.39 sessions). All other factors (age, group size, reproductive
405 status, temperament, and early maternal separation) failed to explain the data any better than the null
406 model.

407

408 Forty of the female monkeys were successfully trained by a single trainer working alone. Model
409 comparison showed that rank significantly explained the number of trainers required to successfully
410 train a monkey (lm: $F_{(2,49)} = 4.44$, $p = 0.01$). A second trainer was useful in the training of lower
411 ranked females, with 50% requiring 2 trainers present in order to reach success criteria; this was

412 significantly different from high ranked females ($t = 2.91$, $p = 0.005$), who only needed a second
413 trainer 5% of the time. Mid ranked females needed a second trainer in 27.27% of cases, which was not
414 significantly different from high ($t = 1.81$, $p = 0.08$) or low ($t = 1.49$, $p = 0.14$) ranked females. All but
415 one male were successfully trained with only one trainer present.

416

417 Four macaques, each from a separate group, would rattle their station tool so as to attract the trainer's
418 attention. We considered this to be an undesirable behaviour as it distracted the other monkeys which
419 would be problematic during the planned research. We initially ignored the behaviour but it
420 continued. When this behaviour occurred, we then ended the session for that animal and removed the
421 station. In all cases, rattling decreased substantially to a point where it did not happen, or happened so
422 infrequently that it was not deemed problematic, after two sessions.

423

424 **4. DISCUSSION**

425 We present a PRT protocol and data for training rhesus macaques in breeding groups of up to nine
426 adults (plus infants and juveniles) to approach and remain by individual stationing tools. We
427 successfully trained 61 (out of an original 65 animals who were approached) during daily 15-minute
428 training sessions spread over a 12 week period. Following this protocol, training staff at similar
429 facilities should expect to be able to train dominant male macaques within two daily training sessions;
430 dominant and mid-ranking females within eight daily training sessions (2 hours); and the lowest
431 ranking females within 3 weeks or a month of daily training sessions (3-5 hours). These results
432 compare favourably with some previously published data. For example, Schapiro *et al.* (2003)
433 successfully target trained 24/30 group housed adult rhesus macaques, reporting that the fastest
434 animals trained within 55 minutes and the majority within 4 hours. In that study, it was reported that
435 dominant animals leaving their stations to take rewards from lower ranking individuals created the
436 greatest time cost during training. In our study we tried to avoid this confound by targeting the
437 dominant animals first. Fernström *et al.* (2009) successfully target trained 32/33 macaques, housed in
438 groups of 2-3 individuals, in ~15 x 30min sessions. Our protocol for training macaques in social

439 groups of up to 9 adults has a very comparable success rate to studies where trainers can focus on a
440 couple of animals at a time.

441

442 When we initiated this study, we predicted that due to the strict hierarchy, generally aggressive
443 temperament of rhesus macaques, and the presence of infants and juveniles, only the most dominant
444 animals of each group would be successfully trained and therefore available for voluntary
445 participation in the subsequent research program. However, we achieved a success rate far beyond our
446 original expectations and 61 station-trained animals went on to take part in cognitive studies while
447 freely moving in the social group. This demonstrates not only that the applied PRT methodology
448 works, but also that it is possible for a single trainer to train multiple animals simultaneously and
449 subsequently work with them during research procedures.

450

451 The only factor that predicted individual time to training success in our study was dominance rank.
452 This is not surprising given the initial focus of the protocol on dominant animals, but is also in
453 keeping with some previous studies which similarly found that lower ranked individuals take longer
454 to train (e.g. Veeder *et al.*, 2009; Wergård, 2016). This is most likely due to the fact that subordinate
455 animals tend to be more prone to attack by dominants, and are typically more timid in approaching
456 trainers or in remaining at their station, despite understanding the training contingencies. There was
457 no effect of age, group size, reproductive status, temperament, or early maternal separation on time to
458 train in our study. There were also no obvious predictors of failure to learn for the three females who
459 did not reach criterion for successful training: one was high ranked and two were low ranked, and
460 they were each housed in a different group (size range: 4-9 adults). The high ranked female (96
461 months, group size four adults) was particularly wary around people and showed little to no indication
462 that the attempt at training (40 sessions) had made much impact although she would come forward if
463 food was offered; the two low ranked females (131 months and 176 months, both in groups of 9
464 adults) were generally keen to train (35 and 40 sessions attempted, respectively) but were very wary
465 of their group members and became more nervous after the birth of their infants. While they learned

466 to approach their stations, they would not consistently remain at the station, holding or otherwise, for
467 30s and therefore could not be considered ‘successfully trained’.

468

469 Thirty seconds was found to be a suitable time benchmark for training success, since macaques
470 subsequently would remain at their station for longer periods during the later research phase. The
471 cognitive testing (not presented here) often required the monkeys to be cooperative for periods of just
472 over an hour, dependent on group size and willingness to work. Although the trained macaques did
473 not sit at their station consistently for that whole period, we can report anecdotally that diversions
474 from their stations were brief and animals could be encouraged quickly back to their stations if
475 required. The training did ensure that the animals that did wander away rarely disturbed other
476 macaques still at their station, which was our primary aim.

477

478 Throughout the sessions, the macaques were free to come and go as they chose; they were not
479 constrained to the caged area. Indeed, it appeared that most stayed to watch the training of others. It is
480 likely that this provided an opportunity for social learning (e.g. Perlman *et al.*, 2010), and some
481 monkeys appeared to show immediate understanding of the required behaviour at the start of their
482 training. It was, therefore, essential to ensure that the first few monkeys in each group were properly
483 trained and did not develop bad habits. We did find that a small number of macaques (n=4), after most
484 or all of their group had been trained, would rattle their station tool so as to attract the trainer’s
485 attention. Ignoring this behaviour typically had no effect and it became necessary to retrain these
486 animals to hold the stationing tool and wait for a reward. In some cases, it was necessary to end the
487 session for that animal and remove its station – we found that the rattling behaviour would decrease
488 substantially after two sessions in which this behaviour was ignored.

489

490 The biggest hindrance to training the females appeared to be the presence of newborn infants.

491 Anecdotally, we observed some females became less willing to participate in the days after giving

492 birth, but in some cases for up to several months afterwards. Mothers were often wary of the trainers

493 if they came too close to their infants and could become mildly aggressive. This usually died down

494 after a couple of sessions. We also had problems with older infants and juveniles snatching treats
495 when we were offering them to adults, which could elicit aggression. We were not authorised to train
496 the younger animals as some end users specify that they do not want previously trained animals. We
497 hope that coordinated training protocols across facilities (and between breeders and end users) will
498 eventually result, as the ease and benefits of working with animals in the social group are realised.

499

500 For some animals, it was helpful (although not essential) to have a second trainer present. This
501 allowed one trainer to focus on a new trainee while the other trainer maintained the already trained
502 animals. The methodology we have described here was suitable for one trainer to maintain, and we
503 had success with it, with the majority of animals ($n = 48$) requiring only one trainer. Group size was
504 not an explanatory variable, and we can report that, since this study, one trainer at the facility has
505 single-handedly trained a group of 11 adults to station individually. However, for us to better access
506 and attend to low-ranked females, a second trainer was useful, particularly during the early stages. By
507 keeping the more dominant animals occupied, it was possible to focus one trainer's attention on a
508 low-ranked female, allowing the macaque to develop confidence in joining in without retribution from
509 higher-ranked conspecifics. We recommend that a second trainer be used for this kind of training
510 when one or more animals is particularly submissive to conspecifics.

511

512 The training presented here was successfully transferred to the subsequent cognitive testing phase of
513 this study. Stationing was used to situate animals within each group in particular locations around the
514 caging area, in order for one individual to be tested without other members of the group being able to
515 view the visual stimuli directly. The training was used to primarily keep the macaque taking part in
516 testing in the one location where we film performance, as well as keep other members of the group
517 from interfering. An additional spin-off was care staff initiating their own training of the macaques.
518 Stationing was an ideal starting point, given the small ratio of staff to macaques, and its usefulness for
519 inspecting injuries and newborns. However, there were some difficulties to this transfer due to the
520 monkeys' prior relationship with care staff and restricted habituation opportunities. We encouraged
521 further habituation sessions to help develop a more positive relationship and expanded these to

522 visiting veterinary staff. Veterinarians commented that they noticed an attitude change from the
523 macaques after two-three habituation sessions (Drs J Willshire and J Hemingway, personal
524 communication). This reflects previous evidence that positive reinforcement can improve
525 relationships between staff and animals (Bayne *et al.*, 1993; Bloomsmith *et al.*, 1997).

526

527 Throughout this paper, we use the term PRT to focus the reader's attention on desired behaviours and
528 their relationship to rewards. This is to avoid some of the misunderstandings that arise from common
529 misuse of the learning theory terminology. For example, both positive reinforcement and negative
530 punishment (see Table 1) were used in the protocol reported here. These terms relate to the
531 appearance ('positive') or removal ('negative') of reward to increase the performance of desired
532 behaviours ('reinforcement') or to decrease the performance of undesired behaviours ('punishment').
533 It is important to note that the main focus of our training method was positive reinforcement but
534 negative punishment was used in the case of the four females rattling their station tools (only 2
535 occurrences of this methodology were typically required to see a strong reduction in this behaviour).
536 The important take-home message here is that we only manipulated the amount and frequency of
537 *rewards* that animals received. Rewards activate dopamine systems in the primate brain and are linked
538 to appetitive learning and seeking behaviour (Panksepp & Moskal, 2011); as we found the macaques
539 to be highly food motivated, solving problems related to gaining access to food rewards should be,
540 overall, an enriching experience. We avoided using negative reinforcement or positive punishment
541 (Table 1), both of which use fear-eliciting stimuli to manipulate animals' behaviour and are therefore
542 likely to impact negatively on welfare (Laule & Whittaker, 2007; Prescott *et al.*, 2005). Furthermore,
543 our results here show that it is possible to train large numbers of group-housed macaques with
544 minimal staff using only PRT.

545

546 Station training is generally considered to be the basic standard upon which other training protocols
547 are built (Laule *et al.*, 2003). Although it is not always possible to train every animal in a facility to
548 cooperate in husbandry procedures, targeting a few key animals in each group should help to reduce
549 stress and improve welfare. We hope that the protocol and data presented here will add to the existing

550 literature and encourage others to take up PRT training of group-housed animals in facilities where
551 this is not yet standard practice.

552

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557

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680 **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 seconds') 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).

681

682

683 **Table 2.** Behavioural categories used to describe temperament as either affiliative/cooperative,
 684 aggressive/uncooperative, or mixed, when observations were not predominantly (>60%) one or the
 685 other.

Context for observation	Interaction with...	Temperament category	Description of observations
During habituation and station training sessions	Trainer	Affiliative/cooperative	Approaches training staff quickly when indicated. Allows other adult females to be trained. Does not snatch treats and run away. Remains in cage room consistently. Utilises dominant locations. Unfazed by presentation of stationing tools – quick to investigate (within 2 mins of first presentation).
		Aggressive/uncooperative	Threatening trainer during sessions. Snatches treats and runs away. Spends a lot of time in hatchway or play room. Utilises lower levels of caging area. Nervous about stationing tools – not quick to investigate (more than 2 mins or multiple sessions).
	Conspecifics	Affiliative/cooperative	Allows other adult females to receive treats without challenging. Allows at least one other adult female to sit within 1m on the same horizontal level.
Aggressive/uncooperative		Threatens adult females when they are offered treats.	
Focal observation of animals in the social group	Conspecifics	Affiliative/cooperative	Grooming other adult female in group. Sitting closely (bodily contact) with other adult female/s.
		Aggressive/uncooperative	Displacing an adult female. Attacking, biting, hitting, chasing other adult female/s.

686 **Table 3.** Group size, group composition (adults only) and training success.

Number of adults in group	Number of animals approached for training (M:F)	Number of animals successfully trained
1:8	1:8	1:7
1:8	1:8	1:7
1:8	1:6	1:6
1:9	1:6	1:5
1:5	1:5	1:5
1:5	1:5	1:5
1:7	1:4	1:4
2:3	2:3	2:3
0:6	0:5	0:5
0:9	0:4	0:3
0:2	0:2	0:2
9:70	9:56	9:52

687

688 **Table 4.** Model comparison revealed dominance rank was the best predictor of the number of sessions
689 to train.

Predictor variable	df	Log likelihood	AICc	delta	weight
Dominance rank	3	-208.98	424.4	0.00	0.52
Null model	2	-211.22	426.7	2.25	0.17

690