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Using preferred fluids and different reward schedules to motivate rhesus macaques
(*Macaca mulatta*) in cognitive tasks

Using preferred rewards to motivate macaques

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1 **Abstract**

2 Rhesus macaques (*Macaca mulatta*) used in behavioural neuroscience are often
3 required to complete cognitively complex tasks, for which a high level of motivation is
4 essential. To induce motivation, researchers may implement fluid restriction
5 protocols, whereby freely available water is limited, such that fluid can be used as a
6 reward in the laboratory. A variety of different rewards and schedules are used, but
7 there exists a lack of data assessing their effectiveness. In this study, we aimed to
8 quantify fluid preference in rhesus macaques and to use these preferences to compare
9 the motivational quality of different reward schedules: the monkey's previous reward
10 (i.e. the fluid used to reward them in past studies), their new preferred reward, a
11 variable schedule of previous and preferred reward, and a choice between the
12 previous and preferred rewards. We found that it may be possible to reduce the level
13 of restriction if an adequately motivating preferred reward is identified, but that this is
14 dependent on the animal. Each monkey responded differently to both the fluid
15 preference assessments and to the different reward schedules. As such, monkeys
16 should not be subject to "blanket" protocols but should be assessed individually to
17 maintain adequate scientific data collection at the least severe level of fluid restriction.

18 **Keywords**

19 Choice, variable schedule, refinement, fluid restriction

20 Macaques are widely used across biomedical sciences [1], and are often
21 selected as a model in behavioural neuroscience studies because of their similarities to
22 humans in brain structure and function, as well as their ability to perform complex
23 tasks [2,3]. In particularly cognitively challenging tasks, or where studies require large
24 numbers of trials to be undertaken, a high level of motivation is required. This may
25 lead researchers to choose to restrict the amount of freely available fluid in order to
26 motivate a monkey to perform a sufficient number of responses [4]. Using restriction
27 protocols has led to concerns about the impacts on the welfare of the animals [5,6],
28 though these may be largely unfounded [7].

29 An NC3Rs working group [4] emphasised that researchers should carefully
30 choose reward schedules and reward types to adequately motivate animals whilst
31 minimising the degree of restriction required. Many aspects of reward processing have
32 been investigated in macaques, such as reward uncertainty [8], differing reward sizes
33 [9], temporally varying reinforcement schedules [10] and the neuronal coding of
34 reward preference [11]. However, less work has been conducted into how to use this
35 knowledge of reward processing to refine laboratory procedures. Although there are
36 multiple ways in which motivation could potentially be increased, we chose to
37 investigate three aspects of reward that we thought may be effective at increasing
38 motivation to perform in tasks and allow restriction protocols to be relaxed. The first is

39 the use of preferred rewards, which can be more rewarding [12,13] and result in more
40 successful training of behaviour [14]. The second possibility is using a variety of
41 rewards, since animals may perform better when their rewards are varied throughout
42 a task, rather than using a single type of reward [15,16]. Finally, giving monkeys a
43 choice of reward may also enhance motivation [17–19]. Despite these possibilities,
44 there are currently no conclusive data to guide researchers to which method might be
45 the best way to motivate their animals in behavioural neuroscience tasks.

46 This study had three main aims: 1) to explore methods to efficiently quantify
47 fluid reward preference in rhesus macaques (*Macaca mulatta*); 2) to use schedules
48 involving previous rewards and new, preferred fluids to evaluate the motivational
49 abilities of different reward schedules; and 3) to establish if reward schedules involving
50 preferred fluids can keep macaques sufficiently motivated to perform cognitive tasks
51 when their fluid restriction was relaxed. We expected monkeys would be more
52 motivated when they were rewarded with schedules that included a preferred fluid,
53 and that increased motivation levels would enable animals to perform cognitive tasks
54 when fluid restriction was relaxed.

55

56 **Animals**

57 Experimental procedures complied with the European Union Directive 2010
58 (2010/63/EU), the National Institutes of Health (Guidelines for Care and Use of Animals
59 for Experimental Procedures), the Society for Neurosciences Policies on the Use of
60 Animals and Humans in Neuroscience Research, and the UK Animals Scientific
61 Procedures Act. The study was approved by the Animal Welfare Experimental Review
62 Board (AWERB) of Newcastle University.

63 Four male rhesus macaques weighing 8-13 Kg were used. Animal usage was
64 opportunistic, when individuals were not partaking in neuroscience studies (the
65 laboratory uses only male macaques). Each subject was housed with another male, in
66 cages (either 2.1x3.0x2.4m or 2.3x2.45x2.4m) and had visual, olfactory and auditory
67 contact with approximately 40 other macaques. Toys were given on a rotational basis
68 and dry food mix (Mazuri Primate Expanded, Old World Monkey Banana Chunks, Trio
69 Munch Rings and LP Forage Mix, *Special Diet Services*; Monkey Diet, *LabDiet*[®], *IPS Ltd*)
70 was added to floor shavings to encourage foraging. Cages were equipped with
71 perches, shelves, a 'balcony' and hoses. The facility had a 12:12 light/dark cycle (7 am
72 to 7 pm) and natural light from ceiling windows. The temperature and humidity were
73 approximately 20°C and 24%, respectively.

74 During the experiment, the macaques had controlled access to fluids. The fluid
75 restriction protocol consisted of 5 days of fluid restriction (minimum daily intake given

76 from Sunday to Thursday) with free access to water after completing work on Friday
77 and all day on Saturday. The minimum daily fluid intake was a volume of water which
78 sufficiently motivated the monkey to perform the laboratory task (based on being able
79 to perform approximately 1000 trials in a session) and is expressed as a percentage of
80 their consumption when given free access to water (Monkey 1 = 250ml, 24% or
81 25ml/Kg/day; Monkey 2 = 200ml, 25% or 13ml/Kg/day; Monkey 3 = 200ml, 22% or
82 16ml/Kg/day; Monkey 4 = 385ml, 30% or 23ml/Kg/day). Through participation in
83 experiments, individual monkeys were permitted to work for as much fluid as they
84 liked. On days where monkeys did not reach their daily fluid intake during the
85 experiment, they were supplemented with additional water. All monkeys had
86 previously performed in similar tasks in the laboratory set-up, and were familiar with
87 this type of experiment.

88 During the study, monkeys underwent daily checks by a technician or
89 veterinarian. In case of a health or welfare concern, technicians and the veterinarian
90 checked the animal several times per day. Fur condition, faeces, eyes, food intake and
91 activity levels were all visually assessed. Monkeys were sedated annually to assess
92 their general health (as per Felasa guidelines) and no microorganisms or parasites of
93 current concern for macaques were detected. Animals were kept for future studies at
94 the end of the current study.

95

96 **Methods**

97 *Establishing fluid preferences*

98 A fluid preference for each monkey was established by one of two methods.
99 The first used the experimental set-up, where animals had already been trained to
100 saccade to stimuli to access fluid rewards. We devised a simple saccade task, where
101 different fluid rewards were delivered by looking at visually distinct stimuli presented
102 on a screen, allowing animals to choose their reward. Fluid preferences for Monkey 1
103 and Monkey 2 was assessed in this way. Each monkey was seated in a primate chair
104 and stimuli were presented on a Sony GDM F500R computer monitor (85Hz,
105 1280x1024 pixels). Stimulus presentation, reward delivery and experimental timing
106 were controlled using Cortex (DOS-Version 5.95; IMH, <http://dally.nimh.nih.gov/>)
107 running on IBM-compatible PCs (situated outside of the monkey's room).

108 Animals performed a saccade choice task on each experimental day, consisting
109 of repeated trials until the animals stopped working. In each trial, they were required
110 to fixate on a central spot for 3000ms, after which three reward targets appeared. The
111 monkey had to saccade to any one of the three reward targets and fixate for 250ms to
112 complete a trial correctly and receive an associated fluid reward (~0.1ml fluid). Failure

113 to fixate on a stimulus for long enough terminated the trial and the animal was not
114 rewarded. The three reward targets (2 degrees of visual angle, dva) were located at
115 positions $(x=-6.0, y=6.0)$, $(x=0.0, y=-8.5)$ and $(x=6.0, y=6.0)$, equidistant from the
116 fixation spot $(x=0, y=0)$. To control for location bias, target location was pseudo-
117 randomised such that targets occupied the different locations for equal numbers of
118 trials. Each reward target was associated with a different fluid reward and was
119 distinguishable by colour for Monkey 1 (pink, red or blue) and by shape for Monkey 2
120 (circle, triangle and square).

121 We gave the monkeys the choice between water (which they had previously
122 received as a reward) and two fruit drinks, one nutritive and the other non-nutritive.
123 The nutritive fruit drink was Ribena (Lucozade Ribena Suntory Ltd; 40ml of undiluted
124 squash added to 210ml of water), which had been successfully used by other
125 researchers to motivate their animals. Fruit tea (a cranberry and raspberry tea bag
126 (Twining and Company Ltd; placed in 250ml of hot water for 5min, before being
127 allowed to cool) had the taste of fruit without any nutritive content.

128 The three fluids (water, Ribena and fruit tea) were delivered through a
129 mouthpiece, connected to three separate bottles by plastic tubing. The bottles were
130 calibrated to ensure that the same amount of reward was delivered from each. The

131 fluid preference task was run for six days for Monkey 1 and eight days for Monkey 2.
132 The additional two days for Monkey 2 were a result of a lack of consistent preference
133 in the first 6 days; two more days were added to see if any preference pattern
134 emerged (see further detail in the results section). The fluid with the highest number
135 of choices on more than 50% of the days was taken as the monkey's preference. These
136 preferences were then used to inform the design of the main part of the experiment,
137 which investigated the reward value of different motivational schedules.

138 Establishing fluid preferences using the laboratory set-up was time-consuming,
139 required additional apparatus and only allowed for three fluids to be tested. Given
140 that ideally fluid restriction refinements should be easy to implement, we decided to
141 test fluid preferences for Monkeys 3 and 4 using a second method in the home cage to
142 screen more fluids in a simpler and quicker task. The fruit tea was not used in the
143 home cage as it is not as viscous as the fruit juices and the bottles used in the cage
144 could not be calibrated to dispense equally like the bottles in the experimental set up.
145 By using juices of similar viscosity, we hoped to control for the amount that could be
146 consumed from the bottle.

147 In the home cage, the monkeys could not be fluid restricted (under the
148 conditions of the Home Office licence), and so had free access to water during

149 preference tests. Initial tests consisted of a range of four different juices (apple,
150 pineapple, tropical and orange; all Tesco Stores Ltd) presented in a choice paradigm to
151 narrow the options down to two preferred juices. Each monkey was separated from
152 his cagemate and offered pairs of juices via 10ml syringes. An initial, randomly
153 selected pair of juices was presented to the monkey to sample in turn (5ml of each)
154 before both juices were offered simultaneously (5ml of each). Using only 5ml at a time
155 meant that we did not risk the monkeys becoming satiated. The juices were offered in
156 the same location simultaneously as they had been separately, so that the location of
157 the syringe signalled to the monkey which juice was which. The preferred juice (noted
158 by which syringe he chose to drink from) was then refilled and presented alongside a
159 new, randomly selected juice. This was continued until all combinations of juice had
160 been presented (a total of 6 pairs). The two juices chosen the most often were used in
161 the experimental stage, along with the fluid with which the monkey had been
162 previously rewarded in cognitive tasks.

163 The two preferred fluids and the monkey's previous fluid reward were
164 presented in 1L bottles attached to the cage in three positions: left, middle and right.
165 The monkey had 5-minutes access to the bottles, and the volumes consumed were
166 recorded. The 5-minute test was carried out at the same time each day (09:00-10:00)
167 for six days. Each day, the bottles were spatially arranged in a unique way that

168 allowed every combination of fluids and positions to be presented once. The fluid
169 chosen consistently over the 6 days (defined as chosen on 50% or more of the days)
170 was used as the preferred reward when assessing the reward schedules in the
171 laboratory. We note that each monkey drank from all three bottles on all testing days
172 (i.e. did not simply try one bottle without sampling the others).

173

174 *Assessing the motivational value of different fluid reward schedules*

175 Once fluid preferences were established, each monkey performed a familiar
176 task whilst fluid restricted at their normal level (established in previous studies).
177 Although the tasks differed between monkeys, the nature of the task was not relevant;
178 it was only important that a monkey was familiar with a task and could consistently
179 perform it to measure the effectiveness of the different reward schedules.

180 The monkeys performed their task on different days where they received one
181 of four different reward schedules. Upon completion of a correct trial, monkeys
182 received either the reward given to them in previous studies (previous reward), their
183 preferred reward established from the preference tests (preferred reward), a 50%
184 chance of receiving either the previous or preferred reward (variable schedule), or a
185 choice between previous and preferred rewards (choice schedule). In the choice

186 schedule, the monkeys chose their reward by fixating for 250ms on one of two stimuli:
187 a cross-shaped stimulus represented the previous reward and a circle represented
188 their preferred reward (presented at $(x=-6.0, y=0.0)$ or $(x=6.0, y=0.0)$ dva). The reward
189 schedules were carried out in four blocks of four days, with schedules randomised
190 within blocks, such that each block lasted for four days and only one schedule was
191 used on any given day. The number of correct trials performed was recorded on each
192 day as a measurement of the monkeys' motivation to work.

193 To assess whether the monkeys' performances on their respective tasks could
194 be maintained under less restrictive fluid restriction conditions, the daily fluid intake of
195 the monkeys was increased by 100ml, and the blocks of reward schedules repeated.
196 This increase was deemed suitable for all animals given that no monkey drank 100ml
197 over his minimum volume on a daily basis during the experiment.

198 **Statistics**

199 All data were checked for normality and equal variances, and analysed using
200 appropriate parametric or non-parametric tests in SPSS (v21, SPSS Inc, Chicago, USA).
201 All pairwise tests were corrected for multiple comparisons using false discovery rate
202 (FDR) *post-hoc* tests [20] and reported using a corrected alpha value (termed the q-

203 value). Details of individual tests are reported alongside their corresponding statistics
204 in the Results section below.

205 **Results**

206 *Establishing fluid preferences*

207 To establish fluid preferences, a one-way ANOVA (Monkeys 1, 2 and 3) or Kruskal
208 Wallis test (Monkey 4) was used to compare the number of choices for each fluid in
209 the laboratory (Monkeys 1 and 2), or the amount of each fluid consumed in the home
210 cage (Monkeys 3 and 4). In the laboratory set-up, a clear fluid preference could only
211 be established for Monkey 1. Monkey 1 differentially chose the three fluids (ANOVA,
212 $F_{(2,15)}=48.62, p<0.001$; Figure 1a), preferring Ribena to both fruit tea ($t_{(10)}=6.78,$
213 $q<0.05$) and water ($t_{(10)}=9.64, q<0.05$) and preferring fruit tea over water ($t_{(10)}=3.0,$
214 $q<0.05$). This pattern was consistent on every day, with the highest number of choices
215 always for Ribena, followed by fruit tea and the lowest number of choices always being
216 for water. Whilst Monkey 2 also varied in the number of choices for each fluid
217 ($F_{(2,21)}=3.89, p=0.037$; Figure 1b), this was not consistent across days and was biased
218 by a high intake of fruit tea in the first three days of testing (Figure 1c). We therefore
219 added an additional two days of testing to examine whether any preference pattern
220 emerged (a total of 8 days). However, the inconsistency remained, and Monkey 2 was

221 not continued in the experiment as we could not establish a preference or be sure that
222 the monkey understood the task.

223 In the home cage, fluid preferences were established for both Monkey 3
224 ($F_{(2,15)}=5.83, p=0.013$; Figure 1d) and Monkey 4 (Kruskal-Wallis, $H_2=11.43, p=0.003$;
225 Figure 1e). Monkey 3 preferred tropical juice to both his previous reward of Ribena
226 ($t_{(10)}=2.89, q<0.033$) and to orange juice ($t_{(10)}=3.42, q<0.033$), with no difference
227 between the orange juice and Ribena ($t_{(10)}=0.091, q>0.033$). Monkey 4 preferred both
228 new juices over his previous reward of water (apple: $U=2.93, q<0.033$; pineapple:
229 $U=2.93, q<0.033$), with no difference between apple and pineapple juice ($U=0,$
230 $q>0.033$). Apple juice was chosen to be carried forward as his preference as there was
231 a slightly more pronounced choice for this (median consumption: Apple 255ml,
232 Pineapple 245ml).

233 *Assessing the motivational value of different fluid reward schedules*

234 Motivation was assessed individually for each monkey by comparing the number of
235 trials completed at each fluid restriction level. Monkey 1 received Ribena as his
236 preferred reward alongside water (his previous reward). At normal fluid intake,
237 Monkey 1's performance varied across the four reward schedules (Kruskal Wallis,
238 $H_3=12.40, p=0.006$; Figure 2a). His highest performances (defined as the number of

239 trials completed correctly) were for his preferred reward or a variable reward
240 schedule, which he performed equally well for (Mann Whitney, $U=0.15$, $q>0.017$). His
241 motivation was lower for the previous reward compared to both of these schedules
242 (Ribena: $U=2.82$, $q<0.017$; Variable: $U=2.97$, $q<0.017$). Despite a trend towards
243 decreased performance when given a choice of reward, the number of trials was not
244 significantly different from the preferred reward ($U=1.78$, $q>0.017$), the variable
245 schedule ($U=1.93$, $q>0.017$) or the previous reward ($U=0.30$, $q>0.017$). Unlike Monkey
246 1, Monkeys 3 and 4 did not differ in their task performance for different fluid reward
247 schedules (Monkey 3: $H_3=7.22$; Monkey 4: $F_{(3,12)}=1.61$; $p>0.05$ for both; Figure 2b and
248 c), demonstrating that, for these two monkeys, the schedules were equally motivating
249 at a normal restriction level.

250 1000 daily trials are considered a minimum level of task performance in the
251 laboratory, given the recording requirements of our experimental approaches. When
252 rewarded with his previous reward, Monkey 1's performance did not differ from 1000
253 trials (one sample t test: $t_{(3)}=0.57$, $q>0.0375$), and all other reward schedules elicited
254 performance of over 1000 trials ($t_{(3)}<6.89$, $q<0.0375$ for all), showing all reward
255 schedules to be sufficiently motivating. These results suggest that the inclusion of a
256 preferred reward increased motivation to beyond that of water. Monkey 3's
257 performance was no different from 1000 trials when rewarded with his previous

258 reward, preferred reward or a variable schedule ($t_{(3)} < 2.41$, $q > 0.0125$ for all) but
259 dropped to lower than 1000 trials when he was given a choice ($t_{(3)} = 19.84$, $q < 0.0125$).
260 Monkey 4's performance did not differ from 1000 trials for any schedule ($t_{(3)} < 2.49$,
261 $q > 0.0125$ for all), suggesting that all schedules were sufficiently motivating at the
262 normal fluid restriction level.

263 The monkeys' minimum daily fluid intakes were then increased by 100 ml to assess
264 whether the same reward schedules remained motivating to the monkeys when the
265 fluid restriction was relaxed. At this increased fluid allowance, both Monkeys 1 and 3
266 performed differently for the different schedules (Monkey 1: $H_3 = 8.70$, $p = 0.034$;
267 Monkey 3: $F_{(3,12)} = 3.72$, $p = 0.042$; Figure 2a and b). However, Monkey 4 continued to
268 perform a similar number of trials for each reward schedule ($F_{(3,12)} = 0.17$, $p > 0.05$; Figure
269 2c). For Monkey 1, the use of his previous reward alone produced similar performance
270 to the variable schedule ($U = 1.93$, $q > 0.0083$) and the choice schedule ($U = 2.08$,
271 $q > 0.0083$) but the previous reward resulted in a lower level of work than that for his
272 preferred reward ($U = 2.82$, $q < 0.0083$). There was no difference in performance
273 between the variable, choice and preferred reward schedules (Table 1). Monkey 3's
274 performance when given a choice of reward was lower than when he was rewarded
275 either with his preferred reward ($t_{(6)} = 4.28$, $q < 0.017$) or variably rewarded ($t_{(6)} = 7.53$,

276 $q < 0.017$), but there was no difference between any of the other reward schedules
277 (Table 2).

278 Again, we compared the monkeys' performances to a 1000 trial threshold. Monkey
279 1's daily performance remained around 1000 trials when rewarded with the choice
280 and the variable schedules ($t_3 < 2.47$, $q > 0.025$), and he performed over 1000 trials when
281 rewarded with his preference ($t_3 = 6.67$, $q < 0.025$). However, his performance was not
282 sufficient (<1000 trials) when rewarded with his previous reward ($t_3 = 5.36$, $q < 0.025$);
283 suggesting that his previous reward had now decreased in value whilst the preferred
284 reward continued to be motivating. In contrast, Monkey 3's performance with his
285 previous reward remained at around 1000 trials ($t_3 = 2.51$, $q > 0.0375$) whereas
286 performance dropped below 1000 when he was given his preferred reward, the
287 variable schedule or a choice of rewards ($t_3 < 26.67$, $q < 0.0375$ for all), indicating that the
288 previous reward was the only motivating fluid at this restriction level. For Monkey 4,
289 trials completed for his previous reward, preferred reward and variable schedule did
290 not differ from 1000 ($t_3 < 3.19$, $q > 0.0125$ for all) but did fall below 1000 for the choice
291 schedule ($t_3 = 5.52$, $q < 0.0125$), demonstrating the lack of value this had at an increased
292 fluid intake.

293 Finally, we assessed changes in performance for each of the different reward
294 schedules from when fluid restriction was changed and carried out *t* tests for each
295 schedule to establish any change in the number of trials performed. Monkeys 1 and 4
296 showed no differences between their performances at the different fluid intakes for
297 any of the reward schedules. Monkey 3, however, had a significant decrease in trials
298 performed in the choice reward schedule when daily fluid intake was increased
299 ($t_{(6)}=3.80, q<0.0125$), but no change for any other schedule (Table 3).

300

301 **Discussion**

302 Our study shows that using a preferred fluid can help to increase motivation in
303 cognitive tasks and allow restriction protocols to be relaxed to achieve adequate
304 performance in the task. However, we only found the use of preferred fluids to be
305 effective when the fluid preference was established under fluid control in the
306 laboratory, and not when it was established in animals that were under free access to
307 fluids in the home cage. In addition, schedules where an animal could choose his
308 reward (Choice), or where the preferred reward arrived only 50% of the time
309 (Variable), did not appear to increase motivation beyond simply being rewarded all the
310 time with the preferred fluid. We discuss how our results can potentially contribute

311 towards establishing methodology to enhance motivation in laboratory macaques
312 performing behavioural neuroscience tasks, and enabling researchers to relax fluid
313 control protocols to address welfare concerns.

314 Our study provides the first empirical support for the idea that using preferred
315 fluids can enhance motivation and allow relaxation of fluid control for macaques used
316 in behavioural neuroscience tasks. Reward preference is evident at both neuronal
317 [11,21] and behavioural levels in macaques [22,23], however, using preferences to
318 refine fluid control may be dependent upon the method by which fluid preferences are
319 established, or it may be dependent on the individual. We established preferences
320 using one of two methods and were only able to establish a fluid preference that
321 enhanced motivation and task performance in one animal (Monkey 1), where
322 preference was established under fluid control in the laboratory. Interestingly, this
323 method was not always successful, as Monkey 2 did not show a consistent preference
324 using this method. It is unknown whether this was because Monkey 2 could not learn
325 to associate a symbol to a corresponding fluid to make a choice or due purely to a lack
326 of preference. Although we could establish fluid preferences for the two animals
327 tested unrestricted in their homecage (Monkeys 3 and 4), these preferences did not
328 translate to improved performance in the laboratory.

329 One possible reason for this difference is because the preferences were evaluated
330 under different fluid control protocols: Monkey 1's preference testing was conducted
331 under fluid control, whilst that of Monkeys 3 and 4 was done whilst the animals had
332 free access to water. Perhaps the preferences that monkeys have when satiated simply
333 do not transfer to preferences under fluid control, and consequently, preferred fluids
334 established under free access were less effective as rewards when the monkeys were
335 fluid restricted later in the study. An alternative explanation, which we currently
336 cannot rule out is that inter-individual differences were responsible for the results.

337 A further possibility is that the previous reward of each monkey impacted on the
338 efficacy of the new, preferred reward. Monkeys 1 and 4 had previously been
339 rewarded with water, and Monkey 3 with Ribena. Monkey 3 continued to perform
340 well for his previous reward at the increased fluid allowance, whereas Monkeys 1 and
341 4 decreased their performance to below 1000 trials. The monkeys are supplemented
342 with water if they have not reached their daily intake allowance via task performance
343 and thus for Monkey 3, it may be that Ribena remained motivating when he had
344 learned he would receive only water afterwards. Conversely, for Monkeys 1 and 4, it
345 was probably less motivating to be rewarded with water, as it could be received for
346 "free" after work, especially when motivation was lower and they could afford to wait
347 for their water. This would be an interesting aspect of reward motivation to be

348 investigated in the future, as it may provide a potential method to reduce fluid
349 restriction, by rewarding animals solely with fluids which they cannot receive away
350 from the experimental set-up.

351 We had predicted that other reward schedules containing a preferred fluid
352 (Variable and Choice) would enhance motivation, but this was not necessarily the case.
353 Although the variable schedule produced performance similar to that of the preferred
354 reward at the normal fluid intake, performance dropped for two of the monkeys when
355 the fluid restriction was relaxed. It is perhaps surprising that the variable schedule was
356 not more motivating given previous evidence: variability in reward size, and the
357 consequential unpredictability, results in dopamine release in macaques, particularly
358 when the chance of receiving a reward is at 50% [24]. In addition, for rats, using two
359 varied rewards throughout a task has been shown to reduce habituation and enhances
360 performance above that of a single reward alone [15,16,25]. However, we found no
361 evidence for enhanced performance using these schedules, and there would be no
362 advantage to their use beyond providing a preferred fluid alone. Our findings are more
363 in line with Bowman *et al.* [26], who showed that variable schedules are less
364 motivating to primates than they are to other species.

365 When considering the choice schedule, we expected that free choice may enhance
366 motivation [12,17,18], but we found a decrease in motivation. We speculate that low
367 levels of performance were likely due to the additional effort that was required. The
368 monkeys first had to perform the initial task correctly, before being offered a choice.
369 This meant that a trial took longer, and required additional cognitive steps. While
370 choice is often seen as potentially rewarding [17–19], our findings suggest that the
371 costs and benefits may balance out under these laboratory conditions.

372 Although we tested three aspects of reward, there are further avenues yet to be
373 fully explored. Firstly, occasional larger rewards (“jackpots”) have been advocated as
374 potentially rewarding for animals [27]. However, there is currently no evidence they
375 prove motivating to macaques performing in cognitive neuroscience studies. In
376 addition, recent work by Fischer and Wegener (in press) used a non-binary positive
377 reinforcement approach. They provided different volumes of fluid dependent on how
378 optimally the monkey has performed a task (e.g. larger volumes for successfully
379 performing a new step of the task and lower volumes for reverting to a previous
380 version of the task). This training technique warrants further implementation to assess
381 effectiveness in other laboratories.

382 **Conclusions**

383 Our study demonstrates that if an animal has a preferred fluid reward, it can be
384 possible to use it in cognitive tasks to relax their fluid restriction whilst keeping their
385 performance at a level to enable sufficient high-quality scientific data collection. This
386 has laboratory welfare implications, since relaxing fluid restriction protocols addresses
387 one of the key concerns around the use of primates in behavioural neuroscience [4].
388 Whilst we advocate establishing and using preferred fluids for primates, more research
389 may be required to find a more efficient method to identify preferred fluids that
390 increase motivation under laboratory conditions. The only method we found to be
391 effective was conducting preference tests in the laboratory, which can be time-
392 intensive. It thus increases the time animals have to engage in licenced protocols.
393 Whether this is justified in light of the limited impact of fluid control [7], remains to be
394 determined. Our data do not provide support for homepage preference testing, but we
395 would encourage further exploration of this issue.

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402 **Conflict of interest**

403 The authors declare that there is no conflict of interest

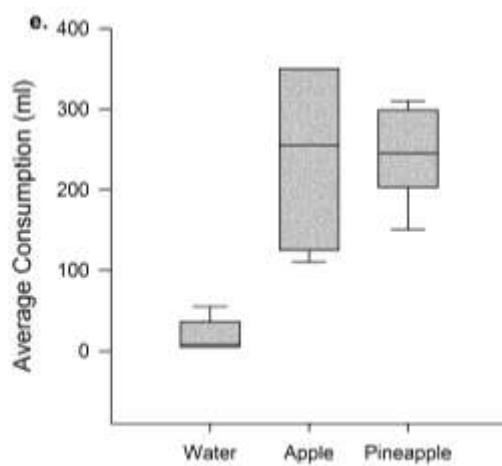
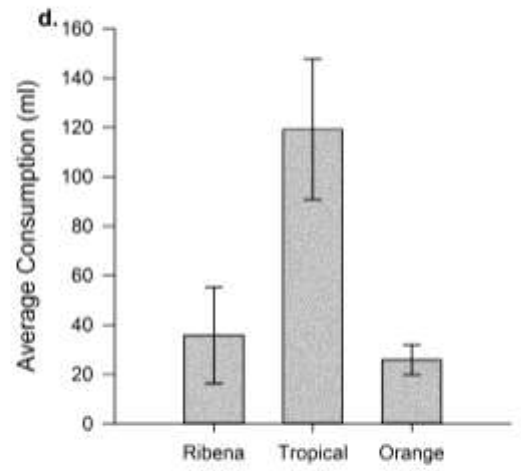
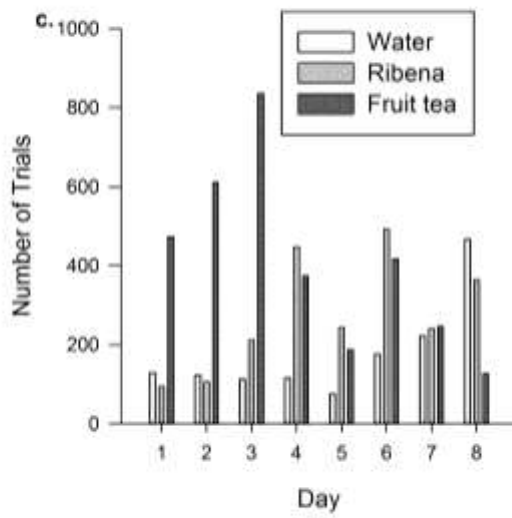
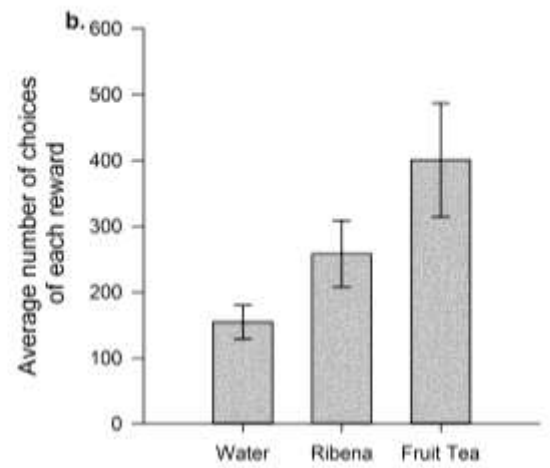
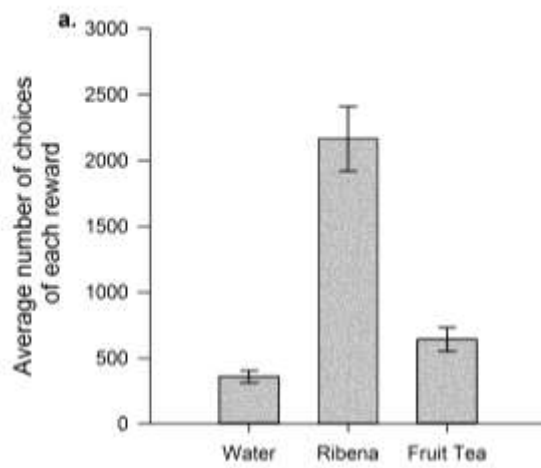
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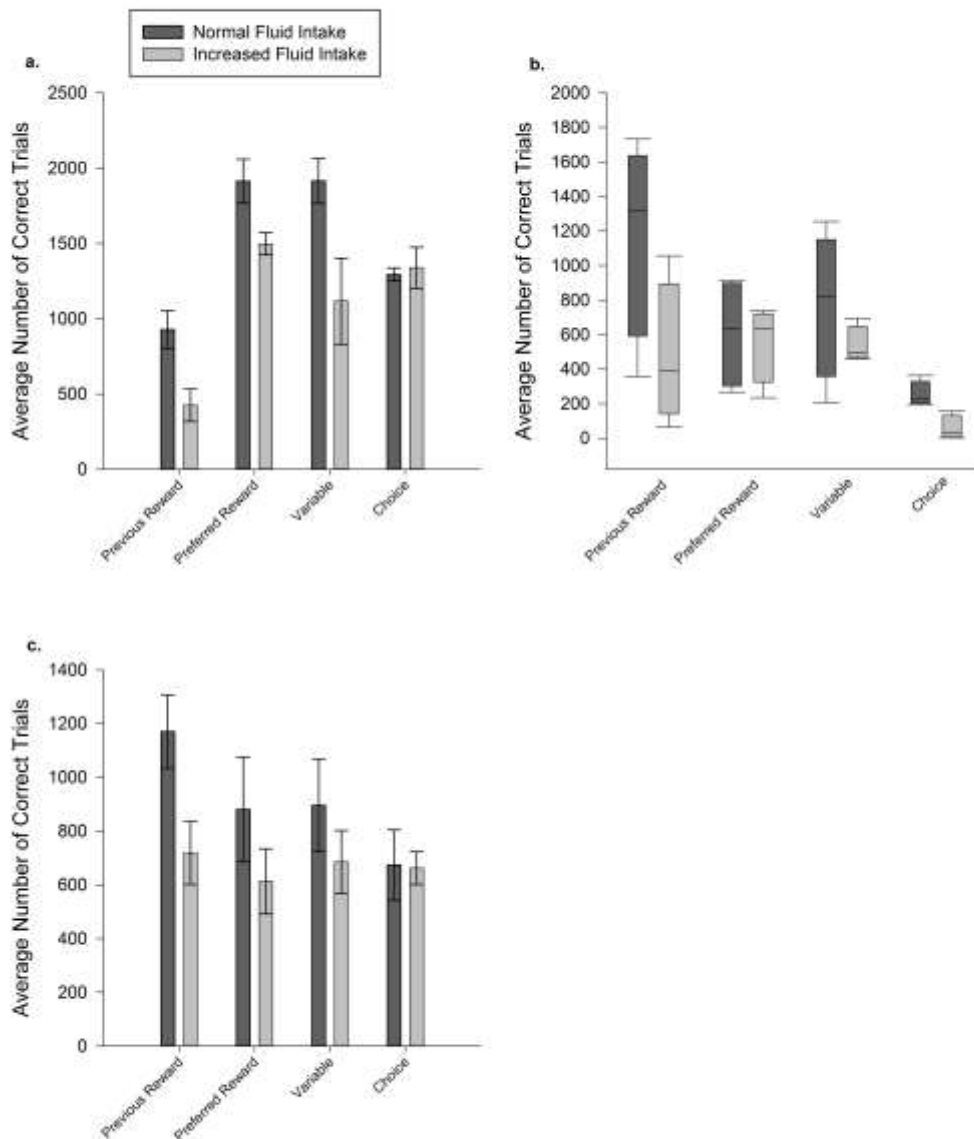
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474 Figure 1. Fluid preference testing in the laboratory (Monkeys 1 and 2) and in the home cage
475 (Monkeys 3 and 4). The average number of choices for rewards in the preference test for (a)
476 Monkey 1 and (b) Monkey 2. Monkey 2 was not continued in the experiment as his preference
477 was not stable across the 8 testing days (c). The average consumption of each reward in 5
478 minutes over 6 days for (d) Monkey 3 and (e) Monkey 4. Bar charts display mean (\pm SEM)
479 and box plots display medians. The previous reward of each monkey is always shown
480 as the left hand bar.

481



482 Figure 2. The average number of correct trials performed by (a) Monkey 1, (b) Monkey

483 3 and (c) Monkey 4 when rewarded with their previous reward, preferred reward, a
484 variable schedule or a choice schedule at both their normal and increased fluid intakes.
485 Bar charts display mean (\pm SEM) and box plots display medians.

Table 1. Monkey 1. Pairwise comparisons for the numbers of trials performed after the daily fluid allowance had been increased by 100 ml. Results are controlled for multiple comparisons using False Discovery Rate (FDR) tests. “NS” indicates non-significance.

| Reward Schedule | Reward Schedule | Median Difference | U-value | P-value | FDR q-value | Significance After FDR corrections |
|-----------------|-----------------|-------------------|---------|---------|-------------|------------------------------------|
| Previous | Preferred | 1076 | 2.82 | 0.005 | 0.0083 | Significant |
| | Variable | 660 | 1.93 | 0.054 | 0.0083 | NS |
| | Choice | 864 | 2.079 | 0.038 | 0.0083 | NS |
| Preferred | Variable | 416 | 0.89 | 0.37 | 0.0083 | NS |
| | Choice | 212 | 0.74 | 0.46 | 0.0083 | NS |
| Variable | Choice | 204 | 0.15 | 0.88 | 0.0083 | NS |

Table 2. Monkey 3 Pairwise comparisons between the numbers of trials performed after the daily fluid allowance had been increased by 100 ml. Results are controlled for multiple comparisons using False Discovery Rate (FDR) tests. “NS” indicates non-significance.

| Reward Schedule | Reward Schedule | Mean Difference | Std Error of Difference | <i>t</i> -value | df | <i>p</i> -value | FDR <i>q</i> -value | Significance After FDR corrections |
|-----------------|-----------------|-----------------|-------------------------|-----------------|----|-----------------|---------------------|------------------------------------|
| Previous | Preferred | 83.25 | 236.79 | 0.35 | 6 | 0.74 | 0.017 | NS |
| | Variable | 61.25 | 215.30 | 0.28 | 6 | 0.79 | 0.017 | NS |
| | Choice | 419.75 | 211.61 | 1.98 | 6 | 0.095 | 0.017 | NS |
| Preferred | Variable | 22 | 123.98 | 0.18 | 6 | 0.87 | 0.017 | NS |
| | Choice | 503 | 117.46 | 4.28 | 6 | 0.005 | 0.017 | Significant |
| Variable | Choice | 481 | 63.88 | 7.53 | 6 | <0.001 | 0.017 | Significant |

Table 3. Difference in trial performance when the fluid allowance was increased. Results are controlled for multiple comparisons using False Discovery Rate (FDR) tests. “NS” indicates non-significance.

| | Reward Schedule | Mean Difference | Std Error of Difference | <i>t</i> -value | df | <i>p</i> -value | FDR <i>q</i> -value | Significance After FDR corrections |
|----------|-----------------|-----------------|-------------------------|-----------------|------|-----------------|---------------------|------------------------------------|
| Monkey 1 | Previous | 503.25 | 164.79 | 3.05 | 6 | 0.022 | 0.0125 | NS |
| | Preferred | 416.75 | 163.48 | 2.55 | 4.48 | 0.057 | 0.0125 | NS |
| | Variable | 799 | 322.67 | 2.48 | 4.51 | 0.062 | 0.0125 | NS |
| | Choice | 44.25 | 143.41 | 0.31 | 6 | 0.77 | 0.0125 | NS |
| Monkey 3 | Previous | 708.5 | 359.18 | 1.97 | 6 | 0.096 | 0.0125 | NS |
| | Preferred | 53.5 | 196.08 | 0.27 | 6 | 0.79 | 0.0125 | NS |
| | Variable | 240.50 | 222.33 | 1.08 | 6 | 0.32 | 0.0125 | NS |
| | Choice | 196.25 | 51.70 | 3.80 | 6 | 0.009 | 0.0125 | Significant |
| Monkey 4 | Previous | 451.25 | 179.28 | 2.52 | 6 | 0.045 | 0.0125 | NS |
| | Preferred | 268 | 229.30 | 1.17 | 6 | 0.29 | 0.0125 | NS |
| | Variable | 210.25 | 207.81 | 1.01 | 6 | 0.35 | 0.0125 | NS |
| | Choice | 11.75 | 144.40 | 0.08 | 6 | 0.94 | 0.0125 | NS |