# Cognitive bias in a non-human primate: husbandry procedures influence cognitive indicators of psychological wellbeing in captive rhesus macaques

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**Abstract**

The measurement of 'cognitive bias' has recently emerged as a powerful tool for assessing animal welfare. Cognitive bias was initially, and widely, studied in humans, and describes the way in which particular emotions are associated with biases in information processing. People suffering from clinical levels of anxiety or depression, for example, interpret ambiguous events more negatively than do non-anxious or non-depressed people. Development of methods **for use with** non-human animals has revealed similar biases in several species of mammals and birds, **and one invertebrate**. However, cognitive bias has not previously been explored in any species of non-human primate, despite specific concerns raised about the welfare of these animals in captivity. Here, we describe a touchscreen-based cognitive bias task developed for use with captive rhesus macaques. Monkeys were initially trained on a Go/No-Go operant task, in which they learned to **touch** one of two lines that differed in size **in order to receive a reward (food)**, and to desist from **touching** the other line **to avoid a mildly aversive stimulus (delay to the next trial and white noise).** In testing sessions, the monkeys were presented with lines of intermediate size. We measured whether touchscreen responses to these ambiguous stimuli were affected by husbandry procedures (environmental enrichment, and a statutory health check involving restraint and ketamine hydrochloride injection) presumed to induce positive and negative shifts in affective state respectively. Monkeys made fewer responses to ambiguous stimuli post-health-check compared to during the phase of enrichment **suggesting greater expectation of negative outcomes following the health check compared to during enrichment**. Shifts in affective state following standard husbandry procedures may therefore be associated with changes in information processing similar to those demonstrated in anxious and depressed humans, and in a number of other vertebrate taxa.

**Keywords**

animal welfare, **capture,** emotion, **enrichment, husbandry procedures,** rhesus macaque,

## Introduction

Improving methods used to assess the psychological wellbeing of animals in captivity is a key goal for animal welfare researchers (Dawkins 1990; Mendl & Paul 2004; Rennie & Buchanan-Smith 2006a, b; Veissier *et al* 2008; Broom 2010; Mason & Veassey 2010; **Mendl *et al* 2010a;** NC3Rs 2011). A particularly promising development in this area has been the emergence of 'cognitive bias' as an indicator of animal psychological wellbeing (Harding *et al* 2004; Mendl & Paul 2004; Paul *et al* 2005; **Mendl *et al* 2009, 2010a**). The cognitive bias model draws on work with humans which demonstrates a strong link between trait and state affect and cognitive processes (including attention, appraisal, expectation and memory: Eysenck *et al* 1991, 2006; MacLeod & Byrne 1996; Mathews & MacLeod 2002; Richards *et al* 2002; Bar-Haim *et al* 2007; Miranda & Mennin 2007). For example, people high in anxiety demonstrate a bias to judge ambiguous information as more negative, and report a greater expectation of negative future events, than do people who are low in anxiety (Eysenck *et al* 1991, 2006; Richards *et al* 2002; Blanchette *et al* 2007). Anxious people with co-morbid depression additionally demonstrate a reduced expectation of future positive events (MacLeod & Byrne 1996; Miranda & Mennin 2007). These emotion-mediated biases in the appraisal of the valence of stimuli, events and future outcomes are implicated in the onset and maintenance of clinical affective disorders in modern day human populations (Gray 1971; Mathews & MacLeod 2002). They are also reliable predictors of self-reported distress

experienced during stressful life events, and considered to be important markers of human psychological wellbeing (Mathews & MacLeod 2002; Pury 2002; Wilson *et al* 2006).

Recent work with rats (Harding *et al* 2004; Burman *et al* 2008a, 2009), starlings (Bateson & Matheson 2007), dogs (**Mendl *et al* 2010b**), sheep (Doyle *et al* 2010a), **honeybees (Bateson *et al* 2011) and chicks (Salmeto *et al* 2011),** has demonstrated that emotion-mediated cognitive biases in information processing are also evident in non-human animals (see **Mendl *et al* 2009** for a review). In these studies animals were tested using a species-specific variant of a 'Go/No-Go' task. Initially, animals were trained to make 'Go' responses (eg approach, or press a lever) to a rewarded stimulus and 'No-Go' responses (eg do not approach, or desist from pressing a lever) to an unrewarded or punished stimulus. Animals then underwent a manipulation presumed to induce a shift in underlying affective state, for example disrupted housing conditions to induce a negative shift (Harding *et al* 2004), or environmental enrichment to induce a positive shift (Bateson & Matheson 2007). During a subsequent testing phase, 'Go' and 'No-Go' trials were interspersed with test trials in which ambiguous probes (which possess characteristics intermediate to both the rewarded and non-rewarded/punished stimuli) were presented.

It is the response to intermediate probes which is used to quantify cognitive bias. Animals that more often respond to the ambiguous probes with 'Go' responses are interpreted as having a heightened expectation of receiving a reward (they have a more positive cognitive bias). Fewer 'Go' responses to ambiguous probes signal a more negative cognitive bias. In all species studied to date, animals presumed to be in a relatively more negative affective state perform fewer 'Go' responses to at least one of the ambiguous probes than do animals

presumed to be in a more positive affective state. In other words, following a stressor, animals appear to develop a more negative outlook, while following a positive manipulation such as enrichment animals appear to develop a more positive outlook.

**The value of the cognitive bias approach is therefore that it captures directly aspects of the valence of affective state, something which behavioural and physiological measures do not do. For example, commonly used behavioural indicators of 'stress' such as self-directed, stereotypical and self-injurious behaviours have great inter- and intra-individual variation and may, in some contexts, better reflect coping strategies and developmental history (Maestripieri 2000; Novak 2003); cortisol, the widely measured 'stress' hormone may provide a better indicator of physiological arousal than (psychological) 'stress' *per se* (Honess & Marin 2006a). What cognitive bias studies do not currently show is whether an animal is in a categorically positive or negative emotional state, as opposed to simply in a relatively more positive or relatively more negative emotional state than the comparison condition (eg Boissy *et al* 2007; Mendl *et al* 2009). Distinguishing between absolute versus relative states remains a challenge for researchers, and it is likely that combination of the cognitive bias approach with neurophysiological data will help elucidate this issue in the future. What the current studies do show is that changes in an animal's environment influence how that animal processes information about, and responds to, ambiguous cues. Since environmental manipulations are common components of standard husbandry procedures used with all animals housed in captivity it is critical that we consider the psychological impact of such procedures.**

One group of animals **for which particular captive welfare issues have been raised** (Rennie & Buchanan-Smith 2006a, b; NC3Rs 2011), but for which cognitive bias has not yet been tested, is the non-human primates. The National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs) states that the use of primates in research is 'of particular concern…since, in the case of these animals, the potential for suffering is compounded because of their highly developed cognitive abilities and the inherent difficulties in meeting their complex social, behavioural and psychological needs in a laboratory environment' (NC3Rs 2011). The aim of the current study was to adapt the paradigm first developed to assess cognitive bias in rats (Harding *et al*. 2004) for use with rhesus macaques, *Macaca mulatta*. We used a repeated measures design, which allowed us specifically to address the effects of changes in emotion state within individuals. To induce shifts in emotion state we made use of two pre-existing husbandry procedures **that were familiar to the monkeys: restraint in the home cage for veterinary inspection, and addition of food- and object-based environmental enrichment. There is evidence that for rhesus macaques the former is putatively more negative than the latter (restraint: Heistermann *et al* 2006; enrichment: Honess & Marin 2006b).** We tested whether these two husbandry procedures influenced responses to ambiguous information characteristic of the cognitive biases implicated in psychological wellbeing in humans.

## Method

### *Study animals, housing and treatments*

Seven male rhesus macaques, *M. mulatta,* housed at the Caribbean Primate Research Centre, Puerto Rico took part in the study (average age: 4.5 years; range: 3.6 – 7.4 years). All animals were captive born and housed in an outdoor covered enclosure in single quarantine caging in

accordance with United States federal regulations. All animals had access to water *ad libitum* in the home cage **and were provisioned with food during morning and afternoon feeding rounds.** All aspects of the study conformed to the University of Puerto Rico's Institutional Animal Care and Use Committee (IACUC) guidelines (Protocol approval: A1850106) and were passed by the Ethics Committee of Roehampton University. All monkeys were naïve to operant training until six months prior to the start of the study, from which point they worked in the laboratory on a daily basis. **After the study had been completed** the monkeys were moved to pair-housing in larger purpose-built, floor-to-ceiling cages **for welfare purposes**.

During the initial training phase and subsequent enrichment treatment phase monkeys were provided with regular **familiar** additional enrichments (**juice** ice lollies, toys, twigs and preferred foods in **Kong toys**) **all frozen into equivalent sized ice blocks**, with daily food rations adjusted accordingly for calorie intake. Published data suggest such enrichments may lead to physiological and behavioural changes in primates suggestive of improved welfare (Honess & Marin 2006a, b). **Juice and food items in ice blocks were most often used in the current study because they were largely composed of water (0 calories), all animals who took part engaged with the blocks, spent prolonged periods of time manipulating them, fed on blocks preferentially over freely available chow in the home cage, would often actively take the block from the caretaker's hand when presented and, once the blocks melted, they left no debris in the home cage. Food rations were adjusted directly so that each animal received the same quantity of chow and fruit in a day, but a proportion of this would be provisioned in enrichment form during the enrichment phase.** During the health check treatment, monkeys were individually restrained in the home cage and sedated with an injection of Ketamine Hydrochloride (KHCl) before being removed

for a physical examination by the veterinarian. This procedure has been shown to act as a physiological stressor in captive primates (Ruys *et al* 2004; Heistermann *et al* 2006).

***Cognitive bias experiment***

The design of the cognitive bias experiment was a visual analogue of the 'Go/No-Go' paradigm developed by Harding *et al* (2004). Training stimuli were two yellow lines (Figure 1a). One line was long (70 x 13 mm), and one was short (16mm x 11mm), subtending 7.15 x 1.24 and 1.62 x 1.05 degrees of visual angle respectively when presented centrally on a computer monitor at a 60 cm viewing distance. These were used during training on the initial Go/No-Go task and for control trials during testing. The assignment of long and short line control trial stimuli to rewarded (S+) and unrewarded (S-) conditions was counterbalanced across monkeys (see below for details). Ambiguous probes were three intermediate-sized yellow lines (ambiguous probe trials: Figure 1b). One probe (P*i*) was intermediate in size between the two training/control stimuli **(33 x 12 mm),** and two probes (P+ and P-) were intermediate in size between P*i* and each of the training/control stimuli (S+/S-) respectively **(shorter probe: 22.5 x 11.5 mm; longer probe: 49.5 x 12.5 mm).**

Single stimuli were presented centrally on a 15" Protouch Aspect TS17LBRAI001 touch-sensitive LCD monitor connected to a Toshiba Satellite Pro A60 laptop computer running EPrime v1.0 experimenter-generator software. Touchscreen responses were recorded automatically by the computer. Correct responses were rewarded with delivery of **190mg** primate pellets **(P.J. Noyes, Lancaster, New Hampshire, USA)** from an automatic dispenser (Biomed Associates Pedestal 45 mg mount dispenser, ENV-203). **At the end of a daily session monkeys were rewarded with half of the daily chow ration and an item of**

**preferred fruit delivered via a purpose-built solenoid-operated lunch box**. All sessions were video recorded.

During training, animals learned to perform a Go/No-Go task during which only control trials were presented (S+ and S-: Figure 1a). Each line stimulus appeared on the screen until the monkey touched the stimulus, or until **2 sec** had elapsed if no touch had occurred by this time. **A 2 sec presentation time was selected based on the typical working speed of the animals during previous tasks: it allowed enough time for animals to respond on Go trials, whilst also allowing for a large number of trials to be run in each daily session.** Correct 'Go' (touch S+) responses were rewarded with a secondary reinforcing tone **(Microsoft Windows media file 'ding.wav', 11 kHz, 70 dB at 1 m, 0.6 sec**) a feedback screen showing the rewarded stimulus for **1 sec**, and two **primate** pellets which were delivered on 40% of trials on a variable reinforcement ratio (40%VRR). The reinforcement ratio was maintained at 40%VRR during the testing phase for 'Go' trials. **The trial was then followed by an inter-trial interval (ITI) during which a plain black screen was shown (variable duration of 5-6 sec), as were all other trial types.** Correct 'No-Go' (do not touch S-) responses were not rewarded **and were followed instantly by the ITI.** If the monkey incorrectly touched S-, a blue feedback screen immediately appeared for **16 sec** and a burst of white noise **(71 dB at 1 m, 2 sec)** sounded.

XXXFigure 1XXX

**Each monkey took part in one training session per day, seven days per week, with each session consisting** of 62 control trials, presented in randomized order with the first and last

trials always S+ ‘Go’ trials (rewarded with 2 pellets on 100% fixed ratio). There were never more than three consecutive presentations of the same trial type. Criteria for learning the Go/No-Go task **during the training phase** were ≥80% correct responses over the 62 trial training block, with ≥70% accuracy for each of the ‘Go’ and the ‘No-Go’ trials respectively. **All seven monkeys reached training criterion (range = 19 - 43 daily training sessions).** Response accuracy at criteri**on** ranged from 70-100% for ‘Go’ trials **(all monkeys correctly responded on at least 70% of the ‘Go’ trials),** and 87%-100% for ‘No Go’ trials **(all monkeys correctly withheld from responding on at least 87% of the ‘No Go’ trials)**. The number of daily training sessions which monkeys **completed following achievement of criterion and before** the start of testing ranged from 5-11. All monkeys were required to perform to criteri**on** on three consecutive daily training sessions before commencing testing.

Following training, each monkey underwent six testing sessions during which control trials (S+ and S-) were randomly interspersed with ambiguous probe trials (P+, P*i*, P-). **Testing sessions were held daily at 24 hours, 48 hours and 72 hours after the statutory health check, and on the eight, ninth and tenth days of a 10 day enrichment phase (Figure 2). Control trials continued to be randomized and reinforced with 2 pellets at the 40%VRR for correct ‘Go’ trials, or delay and white noise for incorrect responses on ‘No Go’ trials.** Ambiguous probe trials were not reinforced. Each testing session consisted of three blocks. Within each block the first and last trials were always S+ ‘Go’ trials. Block 1 contained 12 control trials only: six S+ ‘Go’ trials and six S- ‘No-Go’ trials, presented in random order. Block 1 was included to ensure monkeys were working to criterion prior to the start of the experimental block. Monkeys were required to score 9 (75%) correct responses during block 1, with ≥4 correct responses for each of the ‘Go’ and ‘No-Go’ trials in order to

move onto block 2. Block 2 contained 48 control trials (24 x S+ 'Go' trials, and 24 x S– 'No-Go' trials), which were randomly interspersed with 18 (non-reinforced) ambiguous probe trials (6 x P+; 6 x P*i* and 6 x P-). Data were collected on frequency and latency of responses to control and ambiguous probe trials. Block 3 contained 20 control trials (10 x S+ 'Go' trials: 10 x S– 'No-Go' trials). This block was included to reinstate the reinforcement contingencies for control trials following the presentation of the ambiguous probes in block 2. Monkeys were required to perform ≥ 14 correct responses, with ≥ 7 correct responses for each of S+ and S- trials in block 3. After block 3, each monkey received the adjusted chow ration. Feeding motivation was assessed by the number of **primate** pellets left in the pellet tray and the amount of monkey chow left in the 'lunch box' at the end of each daily session. The order of testing (post-health-check versus enrichment treatment first) and allocation of control trial stimuli (long line or short line for S+) were counterbalanced across individuals so that three monkeys were first tested during the feeding enrichment phase (S+ long line, n = 1; **S+ short line n = 2**), and four monkeys were first tested post-health-check (S+ long line, n = 2; **S+ short line n = 2**).

XXX Figure 2 here XXX

***Data analysis***

**To assess whether performance during each testing session reached criterion for inclusion in the study, individual-level analyses were conducted initially.** For each daily testing session for each monkey, it was assessed whether correct responses were made on at least 80% of control trials in block 2 (≥ 70% S- and 70% S+, separately), and feeding motivation was assessed by a 1 x 3 Repeated Measures ANOVA on proportion of pellets

consumed during training and testing sessions (post-health-check, enrichment). **Five monkeys reached response criterion on all six testing sessions. Two monkeys failed to respond to the S+ to criterion on the day following the health check, and one of these also failed to respond to the S+ on the second day following the health check. For these two monkeys only data from testing days (two and three) for which data were available from both treatments were entered into the analysis.** Therefore, out of 42 testing sessions, six were discarded, resulting in 2376 trials, from 36 testing sessions included in the analyses.

**To treat data** for analysis of proportion of responses **made by each monkey per daily testing session, per treatment,** frequency data were calculated as (P = n 'Go' responses / n trials) for each of the control trials (S+ and S-), and the ambiguous probe trials (P+, P*i* and P), separately. **To treat data for** analysis of latency to respond, **individual** latency data were trimmed to remove responses faster than 400ms, as these were likely to reflect errors (i.e. **responses that occurred too quickly to reflect the monkey's perception-reaction time, given the distance of reach to the screen, probably due to the monkey having his hand on the screen at stimulus onset, or being already in the process of reaching to touch the screen before the stimulus had been presented)**. Mean latency to respond was calculated for each stimulus and probe, per monkey, per testing session, per treatment, including non-responses as **2 sec**. Exploratory analyses were conducted to assess possible effects of testing day on proportion or latency of responses. **A 3 x 5 (day x trial type) Repeated Measures ANOVA was conducted for each treatment, separately (including only monkeys for whom data were available on all three days). Analyses revealed no effect of testing day on proportion of responses made in either treatment (post-health-check: $F_{2,8}$ = 1.30, $P$ = 0.32; Enrichment: $F_{2,8}$ = 0.89, $P$ = 0.45), with a similar pattern for latency to respond**

**(both Ps > 0.38) so for all analyses data were collapsed across the three (or equivalent) testing sessions for each monkey within each treatment (post-health-check, enrichment).**

**Group – level analysis of data was performed** using Repeated Measures ANOVA. Data were first checked for the underlying assumptions of normality using the Shapiro-Wilk test and for homogeneity of variance using Mauchley's Sphericity Test. Data met the assumptions of normality without need for transformation. Greenhouse-Geisser corrected values were used where assumptions of sphericity were not met. Higher order 2 x 5 (treatment, trial type) Repeated Measures ANOVAs were conducted to assess within-subjects factors of treatment (post-health-check, enrichment) and trial type (S+, P+, Pi, P- and S-) for proportion of responses and latency to respond, separately. Significant main effects and interactions were examined using paired samples t-tests. Due to the small sample size it was not possible to include order of testing (post-health-check versus enrichment treatment first) in the higher order ANOVA. This was addressed separately in appropriate non-parametric Mann-Whitney U tests **to compare performance of the three animals that were tested in the enrichment treatment first with performance of the four animals that were tested after the health check first (see Figure 2:** Non-parametric tests were selected due to the inclusion of only three and four individuals respectively in the two groups, **and are interpreted with caution due to the low Power afforded by the small sample size). Two Mann-Whitney U tests were conducted per treatment, one each for proportion and latency data.** All descriptive data are reported as mean ± 1 SE.

**Although we carry out a number of statistical tests here, for three reasons we do not make adjustment for multiple testing. Firstly, these approaches greatly inflate the risk**

**of type II error (Nakagawa 2004); as our sample sizes are already low, this point is particularly relevant to our analyses. Secondly, such adjustments have been heavily criticized due to the inconsistency in their application (Moran 2003). Finally, reporting uncorrected *P* values is arguably the most transparent approach, allowing independent assessment of the validity of results.**

## Results

**All animals consumed equivalent proportions of primate pellets during training and the two treatments ($F_{2,12}$ = 1.40, *P* = 0.28) and were observed to collect the full daily food ration on all occasions.**

For proportion of responses, there was a significant interaction of treatment x trial type ($F_{4,24}$ = 2.74, ***P*** = 0.05) and a main effect of both treatment ($F_{1,6}$ = 7.93, ***P*** = 0.03) and trial type ($F_{4,24}$ = 59.16, ***P*** < 0.01: Figure **3**). Pairwise comparisons for each of the three probes revealed monkeys made fewer responses post-health-check versus during enrichment to the ambiguous probes P+ ($t_6$ = 2.53, ***P*** = 0.05) and P*i* ($t_6$ = 2.55, ***P*** = 0.04), but not to P- ($t_6$ = 1.50, ***P*** = 0.18). For control trials**,** there was no difference in responses to S+ post-health-check versus during enrichment ($t_6$ = 1.86, ***P*** = 0.11), and no difference in the proportion of responses to S- ($t_6$ = 0.60, ***P*** = 0.57). Mann-Whitney U tests revealed no effect of order of testing on proportion of responses across the five trial types in either treatment (all ***P* value**s > 0.16).

XXXFigure 3 hereXXX

Analysis of latency data revealed a main effect of trial type ($F_{4,24}$ = 41.40, ***P*** < 0.001), but no effect of treatment ($F_{1,6}$ = 4.26, ***P*** = 0.08) and no significant interaction between the two ($F_{1.6,9.6}$ = 2.38, ***P*** = 0.15: Figure 4). The main effect of trial type was driven by the difference in response speed on control trials, with faster responses to S+ than to S- in both treatments (post-health-check: $t_6$ = 7.90, ***P*** < 0.001; enrichment: $t_6$ = 7.63, ***P*** < 0.001). Comparison between trial types adjacent to each other in the series revealed a significant difference between P*i* and P- (post-health-check: $t_6$ = 3.69, ***P*** = 0.01; enrichment: $t_6$ = 3.32, ***P*** = 0.02) and a difference between P- and S- in the enrichment treatment ($t_6$ = 4.66, ***P*** = 0.003). All other comparisons were non-significant (**all *P* values > 0.08**). Mann-Whitney U tests revealed no effect of order of testing on latency to respond across the five trial types in either treatment **(all *P* values > 0.16).**

XXXFigure 4 hereXXX

## Discussion

The data presented here suggest that differential shifts in emotion state following two standard husbandry procedures influence judgements about the positive or negative meaning of ambiguous information. Seven rhesus macaques were trained and tested on an adapted version of Harding *et al*'s (2004) cognitive bias Go/No-Go task. The likelihood of responding to ambiguous probes was influenced by treatment condition, while likelihood of responding to previously learned stimuli was not. Specifically, during a period of enrichment monkeys were more likely to touch ambiguous probes P+ (the probe closest to the rewarded stimulus) and P*i* (the probe intermediate between rewarded and non-rewarded stimuli) than they were

to touch the same probes **on the days** following a health check. This is the first evidence for emotion-mediated cognitive bias for ambiguous stimuli in a non-human primate.

The data presented here indicate that rhesus macaques demonstrate patterns of emotion-mediated cognitive biases comparable to those exhibited by humans and other animals (Eysenck *et al* 1991, 2006; MacLeod & Byrne 1996; Garner *et al* 2006; **Mendl *et al* 2009**). This finding supports the argument that such biases play a fundamentally similar role in directing the behavior of diverse mammalian and avian taxa (**Mendl *et al* 2009, 2010a**). In humans, different affective traits and states are associated with specific patterns of processing bias. For example, anxiety is associated with an increased expectation of negative events (Eysenck *et al* 1991, 2006) while depression is associated with both increased expectation of negative events and reduced expectation of positive events (MacLeod & Byrne 1996). **Our findings suggest that, with careful development of paradigms like the one presented here, we may have a powerful new tool to help us identify and differentiate between emotion states in non-human primates (Mendl *et al* 2009). A crucial step in this direction is manipulating the salience of the positive and negative events used during training. For example, by comparing responses to probes intermediate between positive and neutral, and between negative and neutral reinforcers, we may begin to test hypotheses about the extent to which animals show a changed expectation of negative events (as in anxiety in humans), positive events (as in depression), or both (as seen in depression with comorbid anxiety: see Bateson *et al* 2011; Salmeto *et al* 2011)**.

The picture emerging, as to whether non-human animals **demonstrate changes in expectation of positive or negative events following experimental manipulations of**

371 **affective state and as measured by changes in** response to ambiguous probes closer to the
372 rewarded or the unrewarded/**punished** stimuli, is varied. A number of studies, including the
373 current study, reveal changes in response to P+, the probe closest to the rewarded training
374 stimulus (rats in unpredictable housing: Harding *et al* 2004; starlings following removal of
375 enrichment: Bateson & Matheson 2007; **sheep following administration of a serotonin**
376 **antagonist: Doyle *et al* 2011; a chick model of depression: Salmeto *et al* 2011)**. **Such**
377 reduced responding to P+ is expected in depression (with or without co-morbid anxiety).
378 Reduced responding to the ambiguous probe P-, the probe nearest the unrewarded/punished
379 stimulus, is expected in anxiety (and depression if accompanied by reduced responding to
380 P+), and has been demonstrated in rats (following removal of enrichment: Burman *et al*
381 2008a; see also Mendl *et al* 2010b for a non-significant trend in dogs), a congenitally helpless
382 (rat) model of depression (Enkel *et al* 2009) **and chick models of anxiety and depression**
383 **(Salmeto *et al* 2011).** Other studies have found significant effects for P*i*, the intermediate
384 probe (dogs showing separation-related behaviour: Mendl *et al* 2010b; sheep following
385 physical restraint and release: Doyle *et al* 2010a). **A key issue in comparing findings across**
386 **these studies is the relative salience of the positive and negative events in each case, for**
387 **which meaningful comparison data are not currently available. Therefore, we**
388 **tentatively** suggest the significant change in frequency of responses to both P+ and P*i*, but
389 not to P-, in macaques following a health check relative to during a period of enrichment,
390 **may implicate** a role of mechanisms sensitive to reward **(specifically food pellets)** as
391 opposed to non-reward or punishment **(white noise and delay), but this requires further**
392 **exploration**.
393

Our finding that standard husbandry procedures can lead to changes in the way rhesus macaques respond to novel ambiguous cues has implications for the way we think about 'stressors' in a captive animal's environment. Although a given stimulus may not be stress-inducing *per se*, the stressfulness of a stimulus may be a function of its ambiguity and the emotional state of the animal. The strength of this effect may vary between species, as suggested by contrasting patterns of emotional responsiveness and cognitive bias across taxa. While most studies show a negative bias following a stressor or a more positive bias following enrichment, there are some exceptions. Bateson and Matheson (2007) found a negative shift in cognitive bias among starlings moved from enriched to standard cages, but no evidence for a positive shift in bias among birds moved from standard to enriched cages. Doyle *et al* (2010a**; see also Sanger *et al* 2011)** found a positive shift in cognitive bias in sheep following a restraint and isolation procedure, **compared to non-restrained control animals,** and interpreted this as reflecting relief following the termination of the stressor, resulting in a pattern of bias opposite to that which may have been expected. These variations suggest possible species differences in sensitivity of emotional response to experimental manipulations and highlight the possibility that manipulations do not always result in the shift in underlying affect that has been presumed, or that there may be a limited time-window for **detecting** this shift. **Interestingly, given that restraint was used as a stressor by both Doyle *et al* (2010a) and in the current study, the differential patterns of response (positive shift in bias immediately following release from restraint: Doyle *et al* 2010a; negative shift in bias 24 – 72 hours following release from restraint here) may reflect the influence of additional factors on emotional response to presumed stressors, such as the role of control versus learned helplessness (eg Rodd *et al* 1997). It is arguable that the repeated exposure to restraint over three days conducted by Doyle *et al* (2010a) prior to**

418 **testing provided animals with a reliable cue that resulted in a sense of control on**
419 **release. Sense of control is associated with robustness to stressors in humans (Seligman**
420 **1991, 1994). By comparison, the tri-monthly health-check conducted with the monkeys**
421 **in the current study occurred infrequently, and lacked predictable cues, which may**
422 **have resulted in a state more similar to learned helplessness. Learned helplessness is**
423 **associated with depression in humans (Seligman 1991; Ozment & Lester 2001).** An
424 additional finding in our study was the utility of the cognitive measure to assess the duration
425 of the psychological response to the health check. There was no effect of testing day (days 1-
426 3) on proportion or latency of responses to the control stimuli and ambiguous probes,
427 suggesting that the statutory three-monthly health check may present a psychological stressor
428 that has a persistent effect lasting several days or more. **Inclusion of baseline measures,**
429 **currently lacking from most studies in both the animal and human literature, will**
430 **enable further investigation of these contextual and temporal factors.**
431
432 There were several aspects of the current study that were designed to address specific
433 concerns raised about the paradigm first developed by Harding *et al* (2004; see also **Mendl *et***
434 ***al* 2009**). In their study, Harding *et al* (2004) compared two groups of rats, in one of which
435 depressive-like symptoms had been induced using unpredictable housing; they consequently
436 required an additional set of tests to check for arousal, motivation and cognitive function
437 differences between treatment groups. These checks are particularly pertinent given the
438 evidence for an influence of affect on processes such as attention and memory formation
439 (Mendl, 1999), state-dependent-learning and reward sensitivity (van der Harst *et al* 2003;
440 Pompilio *et al* 2006; Burman *et al* 2008b; Woike *et al* 2009; **Mendl *et al* 2009).** The within-
441 subjects repeated measures design in our study, along with the inclusion of the control trials

during all stages of training and testing, provided an inbuilt check for these factors, thereby removing the need for these extra tests. The use of the touchscreen with a variable reinforcement ratio also had the advantage that, once animals were trained, a large number of test trials could be run in a short space of time (typically < 8 sec per trial, **allowing each animal to be tested and allowed to feed at the apparatus within a ~40 minute window**). **The number of experimental trials we were able to run** in a daily testing session **(n = 66, of which 18 were probe trials) was large compared to those obtained using spatial orienting paradigms in which animals are required to move from a start location to the stimulus or probe location (typically in the range of 1 - 9 probe trials per day across species tested: eg Burman *et al* 2008a; Doyle *et al* 2010a; Mendl *et al* 2010b); this reduces the** need for **an** extended **number of days** of testing during which time learning might reduce the ambiguous meaning of the probes (see Doyle *et al* 2010b). The variable reinforcement ratio on control trials reduced the likelihood of animals learning that probe trials were not reinforced. The delivery of pellets via a concealed chute following correct 'Go' trials meant responses were not influenced by possible odour cues to the presence of food rewards during the trial.

Alternative explanations for our results, such as contrast effects **(the effect of previous experience on the perception of the current situation as negative, positive or neutral),** arousal, motivation and risk-taking behaviour must also be considered. In our study there was no evidence for an effect of order of testing on likelihood of responding to probes and stimuli, and no effect of treatment on latency to respond, indicating that contrast and arousal effects are unlikely to account for the observed patterns of change. There was also no effect

of treatment on proportion of responses to the control stimuli suggesting it is unlikely that feeding motivation or risk-taking behavior had a significant effect on the results.

Finally, cognitive biases are considered to reflect vulnerability to clinical affective disorders in humans (Mogg *et al* 1995), and there is empirical evidence that cognitive bias **measures** provide reliable predictors of experienced (self-reported) distress in humans that are more accurate than autonomic measures **such as skin conductance** (eg Pury 2002; Jansson & Najström 2009). For example, Pury (2002) measured biases in interpretation of homophones in students during a period of low academic stress and found negative bias in the interpretation of homophones to be a reliable predictor of consciously experienced negative affect during a later period of high academic stress. **Jansson and Najstrom (2009) found that cognitive biases were reliable predictors of self-reported emotional distress in response to a laboratory stressor, while skin conductance responses were less reliable predictors, requiring additional information, such as heart rate variability, for interpretation.** We lack methods to assess whether other species have any awareness of their emotional states (eg whether they can *feel* distressed). Given the predictive power of cognitive bias measures for determining experienced distress in humans, it is interesting to consider whether these measures may provide us a window into comparable psychological processes in other species. As such, we support the notion that the cognitive bias model may provide information about psychological processes in animals that is not accessible using other measures.

**Animal Welfare Implications**

Our results indicate that singly-housed rhesus macaques show a negative shift in cognitive bias following a health check relative to during a period of feeding enrichment. This **relative** negative bias in information processing, which in humans is associated with affective states such as anxiety and depression, may last for several days. This raises important issues about the frequency with which medical or research interventions that involve potentially stressful procedures, such as restraint in the home cage, should be made, the need to consider alternative methods (eg training to present a limb for injection), and raises points for consideration regarding animals recovering from such interventions (eg the potential for heightened sensitivity to psychological stressors, **and the potential duration of such heightened sensitivity**). **This approach may equally have value in identifying positive shifts in cognitive bias, and the duration of such shifts, which may indicate improvements in psychological wellbeing and assist in the identification of positive emotion states. In humans, experimental manipulations to induce positive shifts in cognitive biases have been used in therapeutic approaches to treat affective disorders (eg. Seligman 1991; Yiend *et al* 2005; Tran *et al* 2011) and it may be that, with further research, similar approaches could be applied with non-human animals.** Importantly our data highlight the need for further development and investigation of methods to measure cognitive bias and the psychological component of affect in non-human primates.

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