

DOCTORAL THESIS

Cognitive bias in rhesus macaques (*Macaca mulatta*), a novel measure of animal welfare

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**Cognitive bias in rhesus macaques (*Macaca mulatta*),
a novel measure of animal welfare**

By

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**A thesis submitted in partial fulfilment of the requirements for the
degree of PhD**

School of Human & Life Sciences

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Abstract

This thesis presents the development and application of methods to assess cognitive markers of emotion and psychological wellbeing in a species of non-human primate, the rhesus macaque (*Macaca mulatta*). In humans, vulnerability to emotional disorders such as anxiety and depression is characterized by particular cognitive profiles, known as cognitive biases. For example, anxious people automatically attend to threat-relevant information, interpret ambiguous information negatively, and have negative expectations of future events. In this thesis, I first describe two treatments that were used prior to cognitive testing to induce positive and negative shifts in inferred affective state in the monkeys (enrichment and a health-check, respectively) and discuss the impact of these treatments on the monkeys' behaviour and physiology (Chapters 2 and 3). In the first cognitive study (Chapter 4), I present a method that uses eye-gaze to assess the extent to which threatening (versus non-threatening) stimuli capture visual spatial attention when two stimuli are presented at different locations. In the second study (Chapter 5), I present a simple operant touch-screen task to assess the extent to which a threatening distractor stimulus captures attention and impairs performance on an ongoing task when presented at the same location as the task-relevant stimulus. In the third study (Chapter 6), I present a Go/NoGo touch-screen task to assess judgements about the reward value of ambiguous stimuli. In all of these studies, the two treatments led to different cognitive profiles in the monkeys. Monkeys showed a) automatic capture of attention by threatening stimuli, which was followed by avoidance following the health-check, but not Post-enrichment; b) impaired task performance when a threatening distractor stimulus was presented Post-health-check, and improved performance on these

trials Post-enrichment; and c) a more negative judgement about the reward value of ambiguous stimuli Post-health-check versus Post-enrichment. I discuss these cognitive biases in light of available data from humans, and recent work with non-human animals. These data indicate that furthering our understanding of primate and other animal psychological wellbeing, may be achieved through the development of measures of cognitive bias, such as those presented here.

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Chapter 1

1 Introduction

Accurate assessment of emotion states and psychological wellbeing is a central goal of animal welfare research (Dawkins, 1990; Mendl & Paul, 2004). Emotions are widely accepted as componential phenomena which evolved due to their survival function (LeDoux, 1996; Damasio, 2000; Rolls, 2000). They comprise, broadly, physical (behavioural, physiological) and psychological (cognitive and subjective) components. For humans, methods exist to measure each of these components, and it is the study of the cognitive and subjective components that is considered central to understanding and improving human psychological wellbeing (Gray, 1971; Mathews & MacLeod, 2002).

In non-human animals (from herein animals), welfare has been assessed using behavioural and physiological measures, i.e. the 'non-psychological' components of emotion. The subjective component of animal emotions is not accessible, however the cognitive component is. Developing cognitive measures of emotion in animals, it has been argued, will increase the accuracy of our assessment of animal emotions and provide critical measures of animal psychological wellbeing (Harding et al., 2004; Paul et al., 2005).

In humans, emotional disorders such as anxiety and depression are characterized by specific cognitive profiles (Eysenck et al., 2006); these profiles are known as cognitive biases. Cognitive tests have revealed emotion-congruent cognitive biases in the way people attend to, interpret and recall information about the world (Eysenck, 1992; Bar-Haim et al., 2007). For example, anxiety is characterized by

preferential attention to threat-relevant information, negative interpretation of ambiguous information and negative expectations of future events (Mathews et al., 1989b; Richards et al., 2002; Bar-Haim et al., 2007). Depression is characterized by the preferential recall of negative events from the past and expectation of a greater number of negative, and fewer positive, events in the future (Haas & Canli, 2008; Strunk & Adler, 2009). Optimistic individuals, by contrast, show a bias to expect a greater number of positive future events (Schweizer & Schneider, 1997; Cummins & Nistico, 2002). Many of the methods used to examine cognitive biases in humans are suitable for modification for use with animals due to their independence from assumptions of awareness (cognitive biases have been demonstrated for subliminally-presented stimuli: Öhman et al., 2000) and non-reliance on verbal report (many methods use button-press responses: Bar-Haim et al., 2007).

In the first study of its kind, Harding et al. (2004) adapted methods used to measure cognitive bias in humans for use with rats. These authors trained rats on an operant Go-NoGo lever-press task using two auditory tones, one high-pitched (4kHz) and one low pitched (2kHz). One tone signaled reward, and required a lever press ('Go' response) to gain that reward. The other tone signaled punishment, and required not pressing the lever ('NoGo' response) to avoid the punishment (a burst of white noise). Half of the rats were then housed in unpredictable housing, and half in normal housing; these housing conditions were assumed to induce higher and lower levels of stress in the rats, respectively. Rats were then presented with experimental trials in which presentations of the two training tones were randomly interspersed with presentations of three ambiguous

tones intermediate in frequency to the two training tones (2.5, 3 and 3.5 kHz). Proportion and latency of lever presses were recorded. Rats in unpredictable housing were slower to press the lever following ambiguous tones than were rats in predictable housing, suggesting that when under environmental stress rats judged the ambiguous tones more negatively than did their ‘non-stressed’ counterparts. These results are comparable to the negative expectations of outcomes, and judgements about ambiguous information, that anxious people make compared with non-anxious individuals (MacLeod & Byrne, 1996; Richards et al., 2002).

Since Harding et al. (2004), further research has adapted the original method and demonstrated cognitive bias in starlings (Bateson & Matheson, 2007) and dogs (Casey et al., 2008). These studies suggest that cognitive methods may be developed to assess psychological wellbeing in a range of taxonomic groups. In this thesis I review these recently developed cognitive methods for use with an, as yet, limited range of animals, and discuss their implications in light of more established methods for studying psychological wellbeing in humans. This sets the context for the studies presented in this thesis, the primary aims of which are a) to present the first development of methods to study the cognitive component of emotion in a species of non-human primate (from herein primate), based on the original work of Harding et al. (2004); and b) to extend this research by developing novel paradigms for investigating other aspects of the cognitive component of emotion in primates. In the rest of this Introduction chapter I present the arguments for the development of such methods.

1.1 Accurate assessment of emotions is a central goal of animal welfare research

The assumption that animals experience emotions, and may therefore suffer distress, lies at the core of animal welfare research (Dawkins, 1990 and 2006b; Mendl, 2001; Harding et al., 2004). The study of animal emotions has, however, been hampered by argument over the validity of emotions as subjects for scientific research. This is due in large part to the consideration of emotions as unobservable subjective states not suited to scientific study (Fraser, 2009). Increasing public concern for animal welfare has resulted in government-funded research directives to address this issue. For example, the UK government-commissioned Brambell report in 1965 led to the establishment of the 'five freedoms' of animal welfare: 1) freedom from thirst and hunger; 2) freedom from discomfort; 3) freedom from pain, injury and disease; 4) freedom to express normal behaviour; and 5) freedom from fear and distress (FAWC, 2009). These freedoms have been adopted globally as welfare markers for captive farm, laboratory and zoo animals (Veissier et al., 2008). One category of captive animals which are found widely in laboratory and zoo settings and for which particular welfare concerns have been voiced are the non-human primates (Rennie & Buchanan-Smith, 2006; NC3Rs, 2009).

Dedicated committees for improving the welfare of primates in captivity are found in a number of scientific societies (including the American Society of Primatologists, Animals Procedures Committee, Animal Welfare Institute, Association for the Study of Animal Behaviour, Association of Primate Veterinarians, The Boyd Group, European Federation for Primatology, European Marmoset Research Group, International Primatological Society, Laboratory

Animals Ltd, National Centre for the Replacement, Refinement and Reduction of Animals in Research, and the Primate Society of Great Britain). 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, 2009). The recognition that the welfare of primates requires dedicated attention, and that all animals should be allowed the fifth freedom - freedom from fear and distress - provides a strong case for the study of primate psychological wellbeing. To enable this requires development of methods allowing accurate assessment of the psychological (cognitive) component of emotions in these animals.

Work in animal welfare has relied on a range of measures to assess wellbeing. Behavioural indicators of emotion typically focus on species-typical ‘positive’ behaviours and stereotypical or abnormal ‘negative’ behaviours (Novak, 2003; Clubb & Mason, 2004; Dawkins, 2004; Balcombe, 2009). Physiological measures such as changes in circulating corticosteroid hormones have been widely used as indicators of stress and anxiety in animals (Sapolsky et al., 2000; Shutt et al., 2007), but are typically limited in explanatory power since they reflect emotional arousal and do not provide information about valence (Dawkins, 2006b).

One problem with the use of behavioural and physiological measures is that behavioural indicators of emotion may become dissociated from physiological

indicators of emotion (Higham et al., In Press). For example, four personality dimensions have been identified in rhesus macaques on the basis of behaviour: sociability, confidence, equability and excitability (Capitanio, 1999). Monkeys who engage in high levels of behaviours associated with excitability and high confidence demonstrate specific cortisol profiles. However, monkeys who engage in high levels of behaviours associated with sociability and equability have cortisol profiles indistinguishable from those of monkeys who have low scores for these traits (Capitanio et al., 2004).

Ruys et al. (2004) measured the blood plasma cortisol levels of rhesus macaques undergoing chair restraint training on each of six consecutive days. Monkeys showed a decline in both behavioural agitation and circulating cortisol levels over the course of the six days of restraint. These responses may be interpreted as both psychological and physiological habituation to chair restraint. However, a follow-up session conducted six months post-training revealed that while monkeys continued to show no behavioural agitation to chair restraint, the cortisol response had returned to initial levels. The authors interpreted these results as evidence that behaviour may be an unreliable indicator of underlying physiological (and psychological) processes. The reduced cortisol response to chair restraint over the six successive training sessions reflected physiological adaptation to sustained high levels of circulating cortisol during training (Ruys et al., 2004). The negative feedback loop created by sustained high levels of cortisol is the mechanism by which chronic exposure to stress may lead to abnormal hormonal profiles, such as the low cortisol levels seen in hypocortisolism in both humans and animals (Fries et al., 2005).

In addition to traditional behavioural and physiological measures, some researchers have used experimental approaches, such as choice tests, to address issues of captive animal welfare. Experimental paradigms that test motivation to perform behaviours or access resources under different conditions (e.g. Mason et al., 2001), provide means of determining what animals ‘want’, which is, according to Dawkins (2004), a key concern in animal welfare. Motivation to perform a behaviour may be an indicator of the rewarding value access to a particular resource confers (Dawkins, 1990; Mason et al., 2001). Whether choice tests provide a measure of true reward value or simply reflect a contrast effect is unclear. Hence behavioural indicators of motivation and emotion in the laboratory must be interpreted in light of ethological data (Dawkins, 2004). In addition, species differences in the nature of the stress response, makes generalizing results between species difficult (Mason & Mendl, 1997; Clubb & Mason, 2004). Nevertheless, choice tests and other paradigms that allow consideration of the economics of behavioural decisions, provide a valuable means of assessing changes in specific behaviours in response to particular experimental manipulations.

Some animal welfare researchers argue for an *a priori* assumption that animals are sentient beings with awareness of their own emotional states, and therefore have the capacity to suffer (Wemelsfelder, 1993 and 1997; Goodall, 1997; Gallup, 1998). Others argue against this position (e.g. Heyes, 1998; Wynne, 2004). Most researchers accept that while empirical evidence for subjective states in animals is lacking, the possibility that animals may experience subjective emotions provides a serious concern for welfare research (Dawkins, 1990; Harding et al., 2004;

Kendrick, 2006). Although the precise subjective nature of animals' experience cannot be known, the likelihood of its existence provides a strong argument for the scientific study of animal emotions so that we may better understand their welfare implications (Dawkins, 1990 and 2006a).

1.2 Emotions are componential and functional

The variation in methods used to measure emotions reflects the fact that they are componential phenomena, comprising behavioural, physiological, cognitive and subjective components (Paul et al., 2005; Mauss & Robinson, 2009). Combining methods that address these different components will provide the most comprehensive data on animal emotions. There are well established methods for measuring the behavioural and physiological components of emotion in humans and other animals. These are covered in more detail in Chapter 3. The cognitive and subjective components of emotion have almost exclusively been studied in humans, and are the focus of research into human psychological wellbeing. While cognitive components of emotion in humans may be assessed using objective methods, subjective states are only inferred, indirectly, from verbal report. It is the measurement of the cognitive component of emotion in animals, specifically rhesus macaques, and the validation of this approach using behavioural and physiological data, that is the aim of this thesis.

There is strong evidence that emotions are survival mechanisms which have played a key role in the survival and evolution of many taxonomic groups of animals (Darwin, 1965; LeDoux, 1995). Neuroscientific data show that many of the neural mechanisms involved in emotional processing (for example the

thalamo-amygdala pathway) involve brain structures that are shared across taxonomic groups (LeDoux, 1996; Panksepp, 1998; Bradley & Lang, 2000; Damasio, 2000; Rolls, 2000). This suggests a significant function of emotions throughout the evolution of a wide range of taxonomic groups. There is general agreement that emotions allow behavioural flexibility, enabling organisms to avoid danger and seek rewards within dynamic environments (Scherer & Wallbott, 1994; Panksepp, 1998; Aureli & Whiten, 2003). The consideration of flexibility in the interplay between internal states and ecological factors has also been introduced to modeling approaches of life-history theory (McNamara & Houston, 2008). Including differential states provides models with added power to consider how the internal state of an individual may mediate life-history trade-offs under variable environmental conditions, and the implications of such trade-offs for behaviour.

The definition of emotion used here, and widely accepted by many animal emotion researchers, is that emotions are short-lived responses to rewarding or punishing stimuli (LeDoux, 1996; Rolls, 2000; Paul et al., 2005). Emotions involve different components, which may vary in the extent to which they are expressed between species (Clubb & Mason, 2004; Belzung & Philippot, 2007), or between individuals of the same species (e.g. cultural differences in humans: Scherer & Wallbott, 1994). Emotions, according to this definition, do not require consciousness, that is, subjective awareness of underlying emotions. Conscious awareness of one's own emotions results in feeling states, but these are not a prerequisite for emotional processes to occur (LeDoux, 1995; Damasio, 2000; Öhman et al., 2000). In the human brain, emotional stimuli (such as a threatening

face) may be processed subconsciously, so that stimuli presented without a participant's knowledge may affect subsequent behaviour, as is also seen in patients with blindsight (de Gelder et al., 1999; Heywood & Kentridge, 2000; de Gelder et al., 2005). Following other authors in the field of cognition-emotion interaction in animals, in this thesis the term emotion is used interchangeably with the term 'affect'. Affect is a broad term which encompasses both short-lived emotional states, which are stimulus-bound, and longer-lasting phenomena such as mood and trait temperament (e.g. Paul et al., 2005). Cognition is defined in its broadest sense as information processing (Shettleworth, 2001). Cognition includes processes such as sensory inputs, appraisal, storage and retrieval of information and associated outputs.

The componential nature of emotions means we need methods that measure most, if not all, of the components to develop a full understanding of emotions and their implications for psychological wellbeing in animals. The definition of emotions as functional responses to rewards and punishers that may occur outside of awareness, together with evidence that emotional responses involve brain structures that are shared across animal taxa, means we can study emotions in animals without inferring human-like subjective feeling states. The question of whether animals do, in fact, have subjective feeling states is beyond the scope of this thesis.

1.3 Cognitive bias and psychological wellbeing in humans

The cognitive component of emotion is considered central to human psychological wellbeing (MacLeod et al., 1986; Mathews, 1990; Mogg &

Bradley, 1998). People process information about the world differently according to their emotional state, mood, or longer-lasting personality traits (Richards et al., 2002). This is known as cognitive bias. As briefly described earlier, studies of cognitive bias in humans have shown that affective disorders such as anxiety, depression and phobia are related to changes in cognitive processes including attention, appraisal, memory, expectation and learning (Mathews et al., 1989a; Eysenck, 1992; Mogg & Bradley, 1998; Bar-Haim et al., 2007).

The most important use of cognitive bias measures in human psychological welfare research is in the identification of individuals who have cognitive profiles which leave them vulnerable to the onset of affective disorders associated with poor psychological wellbeing (Mathews & MacLeod, 2002). Emotion-congruent changes in information processing generate subsequent emotional states which, in the case of negative biases and emotional states, may ultimately lead to the onset and exacerbation of affective disorders such as anxiety or depression (Haas & Canli, 2008). Therefore, not only do changes in affective state influence cognition, but changes in cognition feed into affect. Possibly one of the greatest values of cognitive bias measures is that an existing cognitive bias is a strong predictor of vulnerability to the onset of future affective disorders (Williams et al., 1996), and provides a reliable predictor of (self-reported) experienced distress (Pury, 2002). Understanding the bidirectional emotion-cognition interaction has proven critical to research into human psychological wellbeing.

There are many types of cognitive bias and many types of affective system that interact with these biases. Compared with normal controls, people high in anxiety

demonstrate a bias to attend to threatening information (attentional bias: e.g. Bar-Haim et al., 2007), interpret ambiguous stimuli more negatively (interpretive bias: e.g. Mathews et al., 1989b) and expect more negative events to occur in the future (expectancy bias: e.g. MacLeod & Byrne, 1996; Eysenck et al., 2006). Depressed individuals typically demonstrate a bias to recall more negative past events (memory bias: e.g. Bradley & Lang, 2000; Eysenck et al., 2006). Phobics show similar biases for phobia-related information (e.g. Heinrichs & Hofmann, 2001; Öhman & Mineka, 2001; Bar-Haim et al., 2007). Therapeutic approaches indicate cognitive biases are not fixed phenomena, but rely on the interplay between cognitive and affective processes. For example, following training-induced positive interpretive bias for ambiguous information, previously anxious people report reduced levels of state anxiety (Mathews & MacLeod, 2002). Therefore the link between cognition and emotion has been well established in humans.

Cognitive biases may be induced in humans using mood manipulation (e.g. presentation of a small gift: Nygren et al., 1996; mock filming procedure: Richards et al., 2002). Cognitive biases may also be compared between groups of people according to clinical diagnosis (MacLeod et al., 1986) or between groups identified according to scores on state and trait questionnaires for anxiety and depression (Richards et al., 2002; Holmes et al., 2009). Therefore cognitive biases may be revealed in both state and trait affect.

The implication of cognitive bias research for understanding psychological wellbeing in humans means similar methods may provide important information on the cognitive component of emotion in animals, and therefore animal

psychological wellbeing (Harding et al., 2004; Mendl et al., 2009). The application of induced processing biases in the treatment of human anxiety disorders (Mathews & MacLeod, 2002) suggests a potential future application of such approaches in the treatment of psychological disorders in animals. In this thesis I address three of the core cognitive components of emotion. For clarity, each component is discussed at the start of each of the three main experimental chapters: Chapter 4 (attentional bias, specifically spatial orienting of attention towards threatening stimuli); Chapter 5 (attentional bias, specifically the emotional evaluation of stimuli); and Chapter 6 (judgement bias, subsuming expectancy and, to a lesser degree, interpretive biases).

1.4 Cognitive bias and psychological wellbeing in animals

The method developed by Harding et al. (2004; see also Harding, 2002) provides a means of studying the cognitive component of emotion in animals and, consequently, provides a novel measure of animal psychological wellbeing. It combines the cognitive theory and methods applied to humans, with animal operant conditioning approaches. The method developed by Harding et al. (2004) measures 'judgement bias'. Judgement bias is a general term which reflects the fact that several different cognitive processes (e.g. attention, stimulus appraisal, expectation and recall) may feed into the differential patterns of responding seen in stressed versus non-stressed (and in some cases enriched) animals.

Judgement bias is the only form of cognitive bias that has begun to be studied systematically in animals (Mendl et al., 2009). No one has systematically investigated the underlying biases that may feed into judgement bias in animals.

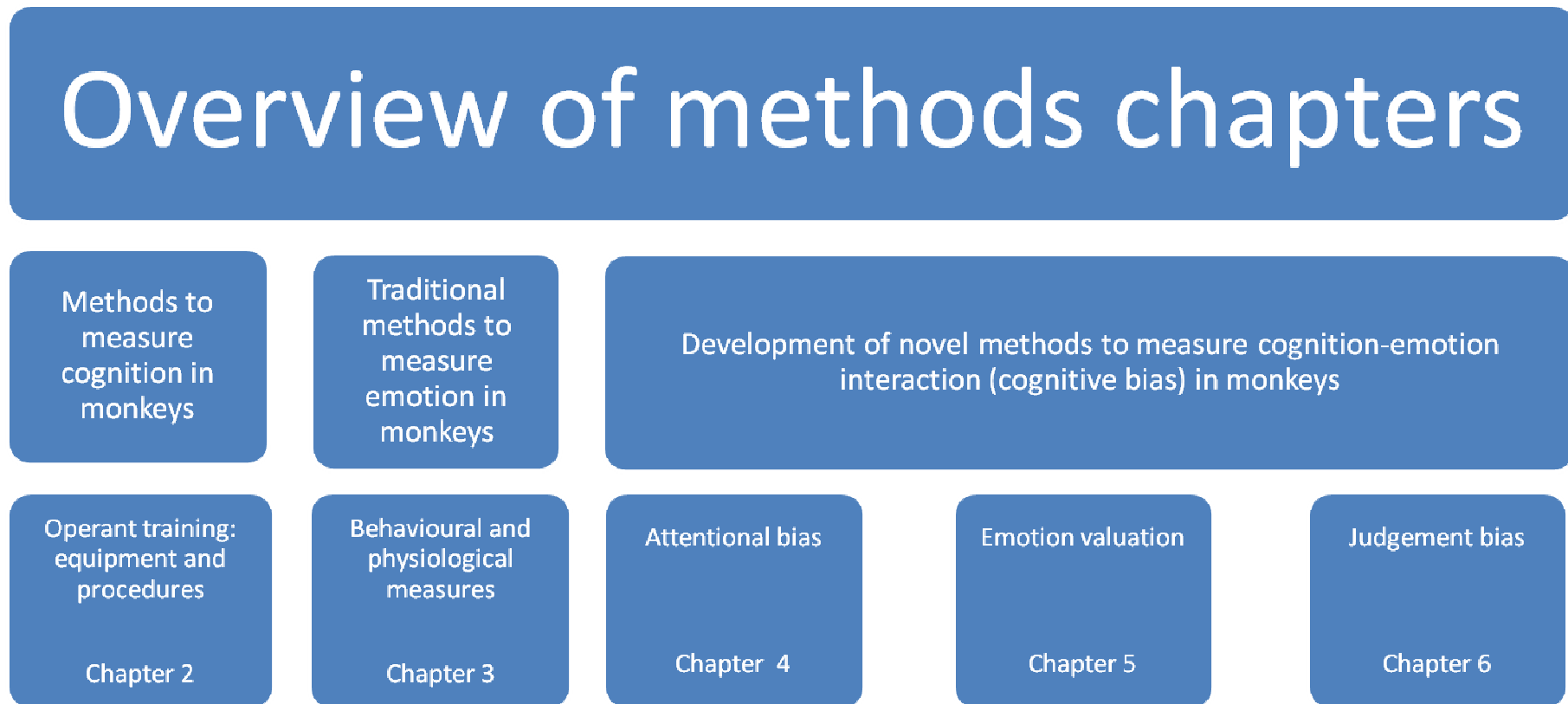
The Go-NoGo task developed by Harding et al. (2004) required an initial training phase which was found to be time-consuming (Mendl et al., 2009). Therefore, for the current research I undertook a series of studies which involved experimental procedures of increasing complexity to investigate firstly attentional bias, then bias in the appraisal of emotional information and, finally, judgement bias. The studies are presented in the order they were conducted.

1.5 Overview of the thesis

The main body of this thesis is composed of five Chapters which detail the development (Chapters 2 and 3) and application (Chapters 4-6) of methods to measure cognitive biases in rhesus macaques (Figure 1.1). In Chapter 2, I describe the equipment and operant procedures that were developed for measuring cognitive processes in rhesus macaques. The equipment for this research was purchased and constructed specifically for the purposes of the studies presented in this thesis. The monkeys who took part in the studies were naïve to working in a laboratory setting and so were trained prior to the start of the experiments. This initial pilot work formed the basis for the further development of the methods presented in Chapters 4-6.

In Chapter 3, I review traditional behavioural and physiological methods used to assess welfare in non-human primates and describe their application with the rhesus macaques who took part in the studies presented in Chapters 4-6. In particular, the behavioural and physiological measures were included to provide comparability with existing studies and provide additional data on the influence of two treatment conditions (a week of enrichment and restraint for injection and a

Figure 1.1 Overview of the experimental chapters presented in the thesis



health-check) used in the studies on other factors that may feed into affect, such as arousal. In each cognitive study, monkeys were tested following the two treatments: both following a week of environmental enrichment, and following a three-monthly health-check. The data presented in Chapter 3 are discussed in terms of the impact of these two treatments on the behaviour and physiology of the monkeys who took part in the cognitive studies.

Chapters 4-6 comprise the main experimental chapters. In Chapter 4, I review cognitive theories of the role of affect in mediating (spatial) attentional biases to emotional stimuli in humans. I then present the method developed to measure emotionally-mediated attentional biases for different facial expressions in rhesus macaques. In this chapter, I test specific hypotheses about early spatial orienting biases for threatening versus non-threatening faces, and the influence of affective state on these biases. This study was developed following evidence that anxious people are faster to detect threatening information than are non-anxious people. However, there are conflicting theories about the timing and the direction of attentional biases in humans. Some authors propose vigilance for threatening information (Seligman, 1971), with faster orienting of attention towards threatening versus neutral information by anxious versus non-anxious people (Eysenck, 1992). Other authors propose avoidance of threatening information in anxiety, with faster orienting away from threatening versus non-threatening information by socially phobic versus non-phobic people (Chen et al., 2002). Yet others propose a vigilant-avoidant strategy in anxiety whereby initial vigilance is followed by subsequent avoidance of threatening versus non-threatening information in anxiety (Garner et al., 2006b). The method presented in Chapter 4

was designed to test the competing hypotheses about the spatial orienting of attention towards or away from threatening information by monkeys when stressed versus when the same monkeys are not stressed. I then present the first data on attentional bias in rhesus macaques and discuss these in light of data from humans.

In Chapter 5, I review theories of emotion evaluation and response slowing in humans, and describe the development of a method to study these processes in rhesus macaques. This Chapter explores the effects of emotion on controlled aspects of attentional processing, and coexisting mechanisms such as behavioural approach-avoidance and freezing responses (Gray, 1971). This study was developed following evidence that anxious people demonstrate a more negative appraisal of threatening information than do non-anxious people, and also demonstrate an arousal-related slowing of responses to highly salient (arousing) stimuli versus non-arousing stimuli (Mogg et al., 2008). Response-slowing to threatening information has only recently been addressed in studies with humans, yet is important for an understanding of how arousal and emotion interact to influence latencies to respond to negative versus neutral or positive stimuli (Mogg et al., 2008). Further, the study is particularly important because of the implications of response-slowing for interpretation of studies where single stimuli that vary in valence are presented on each trial (for example, during emotional Stroop tasks: Mogg et al., 2008). In this chapter I present the first data on emotion-evaluation and response-slowing in rhesus macaques and discuss this in light of possible underlying mechanisms.

In Chapter 6 I review human and animal literature on judgement biases, and describe the development of the method to study judgement bias in rhesus macaques. This chapter builds on recent work conducted with animals which demonstrates that affect-mediated judgement biases may be measured in animals other than humans. I identify the various cognitive and affective processes that may feed into judgement bias, present the first data on affect-mediated judgement bias in rhesus macaques and discuss these data in the context of findings from both humans and animals.

Finally, in Chapter 7, I summarise the findings of the thesis, and discuss these in terms of how the three aspects of cognition-emotion interaction (attentional bias, emotion evaluation and judgement bias) may relate to one another, and to the behavioural and physiological data presented in Chapter 3. I then consider the implications of the methods developed for the measurement of psychological wellbeing in animals. To conclude, I review how the existing methods may be improved, and the ways in which they may be developed for future application to the study of psychological wellbeing in primates.

Chapter 2

2 Developing methods to study cognitive processes in primates

In this chapter, I describe the development of a method to study cognitive processes in adult male rhesus macaques. In Part A, I describe the equipment and operant procedures I developed for this purpose. In Part B, I describe the refinement of the operant procedures including the selection of appropriate stimuli for testing hypotheses about the effect of emotion-cognition interactions on the processing of social and non-social information. The main research for this thesis was conducted at the Sabana Seca Field Station of the Caribbean Primate Research Centre (CPRC), Puerto Rico. The procedures I present here were informed in large part by an initial period of training at the Psychology Department, Oxford University, in the laboratory of Dr Matthew Rushworth, and in the Department of Veterinary Sciences, Oxford University, with Dr Paul Honess. During this time, I learnt effective procedures for training monkeys to work in a cognitive laboratory, including how to train monkeys to enter a cage for transportation to a laboratory and how to train monkeys to touch stimuli presented on a touch-sensitive monitor in order to gain food rewards. All studies presented in this thesis adhered to the ethical guidelines of, and were approved by, Roehampton University ethics board and CPRC Institutional Animal Care and Use Committee (IACUC). Permissions are presented in Appendix 1.

The monkeys at the CPRC were naïve to any form of training or cognitive testing prior to the start of the study, and no cognitive laboratory of this kind had been established at the CPRC previously. The aims of the present chapter, therefore, are to describe the stages involved in establishing a laboratory for assessing primate cognition. These stages were broadly, i) habituating naïve monkeys to my presence (habituation phase), ii) training monkeys to enter a cage to be transported to the laboratory (cage-training phase) and, iii) training monkeys to respond to stimuli presented on a touch-sensitive monitor (laboratory-based operant training phase).

To begin, I present the rationale and training protocols I applied to establish a working laboratory with rhesus macaques and discuss the importance of habituation and positive reinforcement practices for effective and ethical training procedures. I then list the specific training aims. The subsequent sections detail the methods and training protocols employed, and present data on training success. At the end of each of these sections I summarise training success for the group and show the number of monkeys who reached the criterion to move onto the next stage of training. Individual training outcomes are also presented. Particular attention is paid to the effects of age, temperament, duration of single housing and task complexity on training success. Finally, I discuss the implications of the training procedures used, and the success rates obtained, for the establishment of a primate laboratory that would ultimately allow me to assess cognition in rhesus macaques.

Part A: Establishing the laboratory and basic operant procedures

2.1 Introduction

Training monkeys to work effectively and reliably in a laboratory setting involves several stages of animal learning and requires a species-specific protocol (Prescott et al., 2005). Initially, animals must habituate to the trainer's presence, and familiarise with daily working patterns (habituation and associative learning: Leussis & Bolivar, 2006). Secondly, animals must be given the opportunity to learn about the rewards (or punishers) that arise from given behaviours in response to specific stimuli (associative and operant learning: Prescott & Buchanan-Smith, 2003). Thirdly, animals must learn to perform particular experimental tasks according to the experimental paradigm being used (Prescott et al., 2005). With repeated experience under controlled conditions animals will learn to combine series of behaviours and perform even very complex tasks (such as visual discrimination and reversal learning tasks: Rudebeck et al., 2006). Finally, performance must be maintained so that animals work consistently at any given task, or series of tasks, under experimental conditions. Successful training and good working practices are contingent on the development of positive associations of the trainer and training procedures with reinforcers (resources that animals will work to secure, such as food). These can be used to train animals to engage voluntarily and reliably in a range of experimental procedures of increasing complexity.

2.1.1 Habituation

A preliminary goal of researchers studying animal behaviour is to habituate study subjects to human presence, so that researchers have a minimal impact on naturally occurring behaviours (Waite et al., 2002). For example, free-ranging chimpanzees visually monitor human observers, often interrupting other tasks to do so, and regulate distance from the observer accordingly (Bethell, 1998). Therefore, the presence of a human observer may influence an animal's behaviour, and cause the animal unnecessary stress (Prescott et al., 2005). This, in turn, may impair cognitive processes such as learning and memory that may affect experimental outcomes (Nishimura et al., 1999; Burman & Mendl, 2000; Belanoff et al., 2001)

Habituation, or 'desensitisation', is considered to have occurred when an animal ceases, or reduces, responses to an initially novel eliciting stimulus over repeated exposures (Leussis & Bolivar, 2006). Habituation is a process of learning that a stimulus (e.g. a human) is not associated with aversive outcomes. It occurs first and foremost at the neural level and may be expressed overtly at the behavioural level (Leussis & Bolivar, 2006). At the neural level it is a prerequisite for selective attention, whereby stimuli that are irrelevant to ongoing behaviour should be ignored, so that a greater majority of neural resources are available to attend to stimuli more relevant to an individual's goals. Evidence that threatening stimuli capture attention selectively and automatically (e.g. Macleod, 1991; Holmes et al., 2006) highlights the need for habituation to particularly salient stimuli, such as humans, by study animals (Rennie & Buchanan-Smith, 2006a). In

the case of cognitive studies where animals are required to attend to experimental stimuli, this is particularly important.

I habituated the participant monkeys for two purposes: to minimise my impact on their baseline behavioural and (possibly) neurophysiological arousal, and to develop a cooperative relationship, thereby maximising opportunity for learning during operant training. The point at which responses to a stimulus are judged to have changed sufficiently such that habituation can be said to have occurred is dependent on the methods and aims of the study (Prescott & Buchanan-Smith, 2003). Humans represent a highly salient environmental stimulus for captive animals, associated with the most positive and negative aspects of daily husbandry routines (Waite et al., 2002). It is therefore important to maximise the predictability of human-related events (such as husbandry procedures and training protocols), both in terms of their occurrence and outcomes (Rennie & Buchanan-Smith, 2006b).

One measure of habituation is a change in overt behavioural responses towards a human. Because behaviour may be observed easily, it provides a convenient measure of habituation, specifically behavioural habituation. Behavioural measures do not allow us to distinguish whether animals habituate at a physiological or neural level (Ruys et al., 2004). Therefore, the term 'habituation' is used here to refer to behavioural habituation specifically. Whether animals habituated at a neural or physiological level is not known. In the present study, degree of behavioural habituation was used to assess whether changes in behaviour towards a human influenced other training outcomes. Habituation data

were not used to determine which animals were suitable for further training. Therefore data on behavioural habituation are descriptive and had no influence on inclusion in further aspects of training.

2.1.2 Animal learning, positive reinforcement and operant training

Predictability of events and outcomes provides the information animals need to learn set environmental contingencies. An environment that is predictable both in terms of physical (Bassett & Buchanan-Smith, 2007) and social (Olsson & Westlund, 2007) parameters is less stressful than an uncertain environment (see also Harding, 2002). One essential environmental factor for animals to learn in the context of training is the association of a trainer with the delivery of positive reinforcers.

Positive reinforcement training (PRT) is a form of training which encourages animals to work to gain rewards (Prescott & Buchanan-Smith, 2003). Associating a trainer and training procedures (conditioned stimuli: CS) with positive reinforcers (unconditioned stimuli: UCS) reduces fear responses and increases opportunity for learning to occur (Reinhardt, 1997; Waite et al., 2002). Stressful husbandry procedures have been demonstrated to impair cognitive processes such as learning and memory (Mendl, 1999; Burman & Mendl, 2000). Classical conditioning (learning that a CS is associated with an UCS: Pavlov, 1927), when used with positive reinforcers, is likely to increase speed of learning since the trainer and training procedures become associated with positive outcomes. Consequently, attentional resources are less likely to be 'hijacked' by a fear response to the trainer. The impact of threatening distracting stimuli on ongoing

task performance is covered in detail in Chapter 5. In brief, work with humans has shown that highly stress-responsive individuals have a pronounced preconscious selective attention for threatening social information compared with less stress-responsive individuals, and this automatic capture of attention impairs performance on an ongoing task: there is a temporary freezing of ongoing processes (Roelofs et al., 2007). The interruption to ongoing cognitive processes by threatening stimuli (freezing) in humans may be comparable to the freezing responses seen in other animals, including rhesus macaques, when faced with threatening stimuli (Kalin et al., 1998). Unhabituated rhesus macaques who freeze in the presence of a human will very probably therefore be impaired in learning and performance of tasks in a laboratory environment.

Operant conditioning requires an animal to learn that performing certain behaviours leads to particular consequences (Reinhardt, 1997; Schapiro et al., 2003). Behaviours that result in positive outcomes (i.e. the delivery of a positive reinforcer) will, over time, come to be performed preferentially over behaviours that do not result in positive outcomes (Skinner, 1947). Behaviours that result in negative outcomes should come to be avoided. For the present research, Positive Reinforcement Training (PRT), in which animals work to gain a positive reinforcer, was chosen as the more scientifically and ethically sound means of training in the first instance. PRT provides animals with the option to work or not work during a given task. PRT therefore enables animals to learn at their own pace, without additional stress, and may also provide a form of enrichment in itself (Prescott & Buchanan-Smith, 2003; Manteuffel et al., 2009). This will improve the training conditions of animals (Schapiro et al., 2003), increase

cooperation with laboratory procedures (McKinley et al., 2003) and may produce a more conducive state for learning to occur (Scott et al., 2003). The most commonly used positive reinforcer is food (e.g. Vertein & Reinhardt, 1989), but other positive reinforcers such as access to social information may also be used (Deaner et al., 2005).

An alternative to PRT is Negative Reinforcement Training (NRT), in which animals work to avoid a negative reinforcer or punisher (Laule et al., 2003). NRT leaves the animal with little option but to work to avoid an otherwise inevitable negative event, and therefore may be a stressor in itself. During the present research NRT was only employed during the cage-training phase when PRT options had been exhausted. In such cases NRT was used to encourage the monkey to enter the testing cage where he could then be rewarded for doing so. The rationale was that, once a monkey was in the testing cage he would have the opportunity to learn that being in the testing cage was associated with receiving food. This would subsequently encourage a motivation to enter the testing cage on future occasions, when PRT alone could be reinstated.

The operant training protocol used in this study is detailed in Table 2-1. The protocol was adapted from Prescott et al. (2005) and Laule et al. (2003). These authors identify robust learning of the association between performing a behaviour and delivery of primary and secondary reinforcers to be a key factor for successful training. Primary reinforcers are usually small food items. Secondary reinforcers are always an otherwise neutral stimulus (such as a sound) which, over

Table 2-1 The series of stages for habituating and training naïve monkeys to enter a testing cage for transportation to a laboratory

Training Stage	Rational, aims and general procedures	Outcomes and Adapted Procedures used in this study
Identify goals	Identify sequential training stages required to encourage monkeys to cooperate in a laboratory setting.	A training protocol was developed to train singly-housed adult male <i>M. mulatta</i> in successive stages to enter a testing cage for transportation to a laboratory where they would subsequently engage in operant training with a touch-sensitive monitor.
Select primary reinforcer and effective ‘bridge’	Identify preferred foods for each monkey for primary reinforcement. Calculate calorie content and offset against daily food ration. A ‘bridge’ is a secondary reinforcing tone which sounds when an animal performs a desired behaviour. This helps animals learn which behaviour is being rewarded.	Preferred foods were identified for each monkey (there were persistent individual preferences and dislikes for apple, pear, kiwi, plum and fig). Reward rations were scaled to each monkey’s weight . A vocal click was identified as an effective bridge.
Habituate	Habituate monkeys to an unfamiliar human. Introduce basic reward contingencies. Short sessions repeated daily.	Monkeys learned to take food from my hand calmly, associate such cooperative behaviour with reward and appeared to be more relaxed in my presence.
Establish PRT contingencies	Train monkeys to associate vocal clicks with food.	Monkeys learned to sit calmly for longer periods of time between bridge and delivery of food.
Introduce the transport cage	Habituate monkeys to the sight, sound, smell and movement of the transport cage.	The transport cage was placed in front of each monkey’s cage and daily food ration placed inside or on top. Monkeys were left to enter, explore and feed in the transport cage with the door open.
Train to fully enter cage	Habituate monkeys to being inside the transport cage and the door closing. One short session conducted daily.	Monkeys entered and fed on the daily food ration inside the transport cage. Short sessions enhanced positive associations and avoided build up of stress or boredom.
Increase time in cage	Increase the duration for which the monkey is in the cage before being fed over successive days.	I left each monkey in the transport cage until he had eaten his daily food ration. Over successive days I reduced the size of the food reward until monkeys entered the cage for a small reward only.
Transport in the cage	Familiarise monkey with being transported in the cage and receiving a reward following transport to the laboratory.	On each day I moved the cage increased distances towards the laboratory before delivering the daily food ration, until monkeys were fed in the cage in the laboratory.

repeated pairings with the delivery of the primary reinforcer, comes to be associated with reward. Secondary reinforcers can be used to ‘bridge’ the gap between the moment an animal performs a desired behaviour and the delivery of the primary reinforcer, enabling the animal to identify more easily which behaviour is being reinforced. ‘Shaping’ is the process by which individual behaviours may be combined incrementally to result in animals learning complex behavioural sequences prior to being rewarded (Laule et al., 2003). Once reinforcement contingencies have been learnt, the general rule (behaviour leads to secondary reinforcer, which leads to primary reinforcer) may be applied to any number of behaviours and operant procedures (Prescott & Buchanan-Smith, 2007).

To date, PRT has been applied to a range of animal groups for husbandry and research procedures, such as presenting a limb for venipuncture (rhesus macaques: Reinhardt, 1997; tortoises: Weiss & Wilson, 2003), collection of urine samples (marmosets: McKinley et al., 2003), movement of animals between enclosures (rhesus macaques: Reinhardt, 1992), in-home-cage weighing (McKinley et al., 2003), as a behavioural management tool to increase affiliative behaviours (female rhesus macaques: Schapiro et al., 2001), and for research-related operant training (goats: Langbein et al., 2007; rhesus macaques: Rumbaugh et al., 1989). In each of the aforementioned studies, animal training proceeded through a series of reinforcement stages. Initially, small behaviours (such as approach towards a target) were rewarded. Once an animal reliably performed this initial behaviour, it was shaped so that, for instance, rewards were then only given when the animal both approached and touched the target. Such behaviour was further shaped by

rewarding the animal for additional behaviours such as staying for longer periods of time touching the target, or for touching another target simultaneously. Stages of training can be built up until an animal performs the final desired behavioural repertoire on cue.

2.1.3 Additional factors that may influence training success

In addition to describing the training protocol and presenting data on group training success, I also present data on the effects of age, duration of single housing, degree of habituation and temperament on training success. These have been indicated to be important considerations for the selection of animals to take part in training procedures, yet relevant data are sparse either due to a lack of studies (e.g. Capitanio et al., 2006), or exclusion of individual factors from the analyses (tamarin monkey: Banerjee et al., 2009; beagle: Nippak & Milgram, 2005)

Primate models of human aging have provided data that may be of use for the selection of monkeys for cognitive testing. For example, Bartus et al. (1979) examined visual discrimination and reversal learning in rhesus macaques between three and 18 years of age. There was a significant decline in reversal learning in the older animals, but no effect of age on learning the original visual discrimination task. Rapp (1990) found a significant effect of age on speed to learn an associative task, but not overall task success, suggesting that decline in attention with age affected speed to learn rather than ultimate ability to learn in older animals. Other studies have shown age-related decline in rhesus macaque memory (e.g. Presty et al., 1987). The above studies focused on computer-based

task performance. No published study has presented progressive data on the effects of age on ability to learn the range of tasks presented in this study (from simpler tasks such as entering a testing cage to more complex computer-based operant tasks). In the present study I therefore examined the effect of age on learning progressively more complex tasks.

Few studies, if any, present quantitative data on the effects of degree of habituation, duration of single housing, or temperament on computer-task performance. Capitanio et al. (2006) provide a summary of the factors that may influence the selection of primates for research purposes, but does not review studies addressing operant training procedures specifically.

Duration of single housing was defined in the present study as number of years a monkey had been in single housing, either at Sabana Seca field station, or at another location. Previous research suggests that separation from the social group and placement in single housing induces biobehavioural changes indicative of acute and chronic stress in rhesus macaques (Schapiro et al., 2000). There are no data directly addressing the impact of social- and housing-related stress on cognitive performance in learning operant tasks in rhesus macaques. In the present study, duration in single housing was identified as a possible factor that may influence training outcomes. The high rate of use of singly-housed animals in research laboratories where computer-based studies of cognitive performance are conducted, and the lack of published data on the effects of this significant social isolation factor necessitated exploration of the effects, if any, of duration of single housing on cognitive performance.

Habituation was measured as the increase in cooperative behaviours with increased familiarity with training procedures. Previous studies suggest that a cooperative working relationship between captive primates and humans enhances performance in a range of operant tasks related to husbandry procedures (Waitt et al., 2002). The impact of degree of behavioural habituation to a previously unfamiliar human was included as a factor that may impair performance in learning operant tasks.

Three categories of temperament were identified in the present study. These were based loosely on previously identified personality dimensions in rhesus macaques (Capitani, 1999). Due to difficulties in applying the same descriptors to singly-housed versus socially-housed animals, I applied my own terminology as follows: ‘aggressive’ (monkeys who respond to an unfamiliar human in an aggressive manner. This category is comparable to ‘confident’: Capitani, 1999), ‘submissive’ (monkeys who respond to an unfamiliar human in a submissive manner. This category is comparable to ‘excitable’: Capitani: 1999), and ‘cooperative’ (monkeys who respond to an unfamiliar human in an apparently calm manner. This category is comparable to ‘equable’: Capitani, 1999).

2.2 Aims and specific predictions:

The general aim of Part A of this chapter is to describe the initial habituation and training phases conducted to establish a primate laboratory with 12-20 adult male rhesus macaques trained to participate in studies using a touch-sensitive monitor. I present data on behavioural changes in monkeys during habituation to a previously unfamiliar human, training success for monkeys learning to enter a

testing-cage, and training success for learning to touch a stimulus presented on a touch-sensitive monitor. A secondary aim is to explore the influence of age, duration of single housing and degree of habituation on training success.

Aim 1: To habituate monkeys to a previously unfamiliar human and introduce to basic PRT procedures

Aim 2: To train monkeys to enter a testing cage for transportation to the laboratory

Aim 3: To train monkeys to perform operant responses to stimuli shown on a touch-sensitive monitor

Aim 1: Habituate monkeys and introduce PRT

2.3 Methods

2.3.1 Animals & Apparatus:

Twenty one adult male rhesus macaques (10.1 ± 6.6 years: mean \pm s.d. used throughout; range 3.6-24.7 yrs) housed at the Sabana Seca Field Station, Caribbean Primate Research Center (CPRC), Puerto Rico, participated in training to take part in a cognitive testing in a newly established cognitive laboratory. All animals had been born in captivity in the free-ranging colony at the Cayo Santiago Field Station, CPRC, (n=20) or in a semi-free-ranging corral at the Sabana Seca Field Station (n=1, monkey M232). Monkeys are captured yearly from the free-ranging breeding colony at Cayo Santiago, and transported to Sabana Seca for

breeding purposes. All animals are placed under quarantine in single cages for a minimum of 6 months before being rehoused in breeding colonies or in Specific Pathogen Free (SPF) research groups. Males surplus to breeding requirements and not classified as SPF are singly-housed for other research purposes. The animals who participated in this study comprised monkeys surplus to breeding requirements.

All monkeys had undergone normal weaning, which typically begins at around 16 weeks of age and is largely complete by 30 weeks as part of the free-ranging colony of the CPRC on Cayo Santiago (Rawlins & Kessler, 1986b). No monkey was removed from the natal troop prior to one year of age, and most much later. Young male rhesus macaques typically emigrate from the natal troop between 2.5 and 7 years of age (Berman, 1986; Colvin, 1986). The youngest monkey to take part in this research was 3.7 years at the start of the study. Male rhesus macaques reach puberty at around three years of age, reach sexual maturity around four years of age and reach adult size at around eight years (Cawthon Lang, 2005; Rawlins & Kessler, 1986b).

Throughout the study, monkeys were singly-housed in metal cages (0.9 x 0.9 x 1m) in an outdoor enclosure (10 x 20 x 3.5m). The enclosure was designed to provide shelter from direct sunlight and rain, yet allow natural daylight to reach the cages. The participant monkeys' cages were in two pens within the enclosure, while a breeding harem group (2 male:2 female) occupied the third pen. All participant monkeys had visual access to each other, the harem group, and to a large corral containing >100 rhesus macaques, situated 10m from the home

enclosure. All internal and external enclosure walls were constructed from thick wire mesh attached to an outer metal frame. The home caging was positioned against the outer fence of each pen. Each cage had a 4cm x 12cm aperture in the rear wall so that monkeys had visual access to the area surrounding the enclosure. The layout of the home enclosure was designed to allow every monkey maximal visual access to the outside of the enclosure, while minimising disturbance to the animals by outside events. The group had been housed in single caging for 4.1 ± 4.0 yrs (range one month: monkey 16P; to 15.5 yrs: monkey M232) prior to the start of training for this study, and had been housed in the outdoor enclosure where they resided during this study for 1.0 ± 1.1 years (range: 1 week to 13.5 years).

Training began in February 2006 (n=12) and July 2006 (n=9). Identical training protocols were used for both groups, and training data for all monkeys are presented together. Examination by the attending veterinarian revealed monkeys were in good health at the start of habituation. No food deprivation schedule was employed and animals had access to water *ad libitum* day and night while in the home cage.

2.3.2 Procedure:

Days 1-9: Assessment of behavioural habituation to the investigator

Training goals and outcomes are summarised in Table 2-1. On days 1-3, I fed the daily food ration to each of the monkeys in the presence of the enrichment officer, Josue Alejandro, with whom all animals were familiar. This process introduced

monkeys to my presence, and allowed the development of an association between my arrival and feeding time. Monkeys were fed in the same order each day. I placed ~10 monkey chow and 1/3 of a fruit (apple or pear) in the food well at the front of the first monkey's cage and then moved on to place food in the well of the adjacent cage. Once all animals had been fed, the enrichment officer and I left the enclosure.

During the initial three day feeding phase, I kept a record of monkeys' reactions to my approach to the cage. Reactions to approach and presentation of food were classified in three broad categories chosen to reflect fight or flight responses, or apparent absence of an overt fight or flight response. An assessment of each monkey's primary response was coded as 'aggressive' (fight: the monkey primarily responds with a threat face, shakes his cage or vocalises), 'submissive' (flight: the monkey primarily crouches, flees, startles, lip smacks, bares his teeth, presents his hind quarters, coos or engages in repetitive or self-directed behaviours) or 'cooperative' (the monkey makes no aggressive or submissive responses and takes the food offered). An increase in 'cooperative' responses and a corresponding decrease in 'aggressive' or 'submissive' responses were here taken as evidence of behavioural habituation occurring. Functionally defined, therefore, behavioural habituation reflects a shift from primarily responding to the presence of a human to responding to the presence of the food.

On days 4-9 of the habituation phase, animals were fed by hand in order to maintain the association of the investigator with food and introduce positive reinforcement for desired behaviours. A daily food ration of ~10 monkey chow

and a portion of preferred food (such as fruit, vegetables, seeds or flowers) were fed to each animal in turn. I approached each animal in his home cage in the same order whilst holding two monkey chow. I presented one chow through the hole in the front of the cage for one minute to encourage the animal to approach the front of the cage to collect it. I held the second chow in view at the front of the cage out of the monkey's reach. As the monkey took the pellet I gave a reinforcing verbal 'click' and offered the second chow. I withdrew the second chow if the monkey did not take it within two seconds. I then moved on to the next cage and repeated the procedure. Once I had offered each monkey two chow in this way I returned to the first cage and repeated the procedure for a total of six feeding trials per animal per day over six consecutive days. On each approach I made a record of the primary behavioural responses.

If a monkey did not take the first chow within the one minute time frame, the chow was withdrawn for 20 seconds and then offered again for one minute. If the monkey still did not take the chow, it was withdrawn and I moved on to the next cage and repeated the procedure. If the monkey took the first chow but did not take the second chow within the two-second time frame, the second chow was withdrawn and I moved on to the next cage. On each day animals were presented with monkey chow during trials 1-5, and preferred foods such as fruit slices, seed pods or flowers on trial 6. All monkeys received the remainder of the daily chow ration at the end of the training session irrespective of performance. The primary behavioural response shown over the six trials was recorded for each monkey on each day.

On each subsequent day of training to take food from hand I held food items at increasing distances from the front of the cage. On days 4-6 (i.e. the first three days on which food was offered by hand) I presented food items through the front of the cage. On day 7 I presented food items approximately 10cm from the front of the cage, approximately 20cm on day 8 and approximately 30cm on day 9. This encouraged animals to reach out of the cage to take food, a desired behaviour in later studies. The hole through which animals were encouraged to extend their arm was small (only one arm could be extended through it at one time), and was positioned towards the bottom of the cage (approximately 40cm from the floor). The position and size of the aperture prevented animals from being able to reach out to grab personnel at other times. In order to track ongoing behavioural habituation, data were also collected on days 20, 30 and 40, for comparison with data from days 1-3.

2.3.3 Data analysis

Habituation data were longitudinal, recorded as the number of monkeys who responded to a previously unfamiliar human with a particular behaviour (aggressive, submissive, cooperative or variable) on each day of the habituation phase. These data were assessed for normality using a Kolmogorov-Smirnov test, which showed all data were normally distributed, and were analysed using a Pearson's correlation. Longitudinal training latency data were also assessed using a Pearson's correlation, to assess change in speed of performance as learning progressed.

Approximate median splits were used to group monkeys for each of age (older: 10+ years, versus younger: <10 years) and duration of single housing (short-term: <2.5 years, versus longer-term: 2.5+years). Monkeys were also divided into two groups according to whether they showed evidence of behavioural habituation or not (habituated versus non-habituated).

Fisher's Exact tests were conducted to test whether age, duration of single housing or habituation influenced performance for learning to enter a testing cage and learning to perform operant responses to stimuli shown on a touch sensitive monitor. Data were categorical variables of group (e.g. older versus younger for age; long-term versus short term for duration of single housing; and habituated versus non-habituated for behavioural habituation) and performance (criterion met: yes/no). For each factor (age, housing, habituation), data were entered into a 2 x 2 contingency table, and a Fisher's Exact test was conducted. Fisher's Exact test is a conservative form of χ^2 which is calculated according to the difference of the observed cell values from the expected cell values for the null hypothesis. Fisher's Exact is a suitable alternative to χ^2 when cell values fall below 5, as is the case with a small sample size (Sokal & Rohlf, 1995).

Age-related and habituation-related differences in performance were assessed using one-tailed tests since previous studies indicate increased age is associated with impaired cognitive performance. A lack of habituation should logically result in impaired performance on a cognitive task, although there are no published data on this. The influence of duration of single housing on performance was assessed using two-tailed tests since these analyses were exploratory with regards to the

effects of short term (acute) stress versus longer term (chronic) stress. An unequal spread of data meant it was not possible to assess quantitative effects of temperament on performance. Therefore data on the influence of temperament on performance are descriptive only.

2.4 Results:

2.4.1 Habituation

The number of monkeys who responded aggressively, submissively or cooperatively on each of days 1-9 are shown in Figure 2.1. A Pearson's correlation revealed a significant increase in the number of monkeys who responded cooperatively to a previously unfamiliar human's approach to the cage from day 1 to day 9 of habituation ($r = 0.721$, $n = 9$, $P = 0.028$).

There was a significant decrease in the number of monkeys who responded aggressively from day 1 to day 9 of habituation ($r = -0.758$; $n = 9$; $P = 0.018$), but no significant change in the number of monkeys who responded submissively ($r = 0.333$; $n = 9$; $P = 0.382$).

The data collected on days 20, 30 and 40 following the start of habituation are also shown in Figure 2.1. The nine monkeys who responded with predominantly cooperative behaviour on two or more of days 20, 30 and 40 were deemed to have met the criterion for behavioural habituation to have occurred. The remaining 12 monkeys did not meet the criterion for behavioural habituation as defined here. Four of the 12 monkeys (C55, M232, 94E & 94K) showed varied response

patterns, responding cooperatively on one of the three days but not on the other two. Five monkeys (O22, X57, 45A, 92R & AI73) responded aggressively on all three days, and three monkeys (T52, 66S & 79S) responded submissively on all three days. These results indicate there are inter-individual differences in behavioural responses to the investigator's approach. These differences remained largely consistent throughout the research period (pers obs).

In summary, there was an increase in the number of monkeys who demonstrated predominantly cooperative behaviours when a previously unfamiliar human approached the cage from day 1 to day 9 of the habituation phase. This was accompanied by a reduction in the number of monkeys who responded with predominantly aggressive behaviours. Criterion for behavioural habituation was cooperative responses on two or more of days 20, 30 and 40 after the start of habituation. Nine monkeys met this criterion.

2.4.1.1 The effects of age, duration of single housing and temperament

The age, years spent in single housing and temperament for each monkey are shown in Table 2-2. There was no significant difference between age groups in the number of monkeys who met criterion for behavioural habituation (4/11 older monkeys versus 5/10 younger monkeys: Fisher's Exact, $P=0.670$). There was also no effect of duration of single housing on behavioural habituation (5/9 short-term versus 4/12 longer-term: Fisher's Exact, $P=0.28$).

Figure 2.1 The number of monkeys who responded aggressively, submissively or cooperatively on the first nine days of habituation and on days 20, 30 and 40.

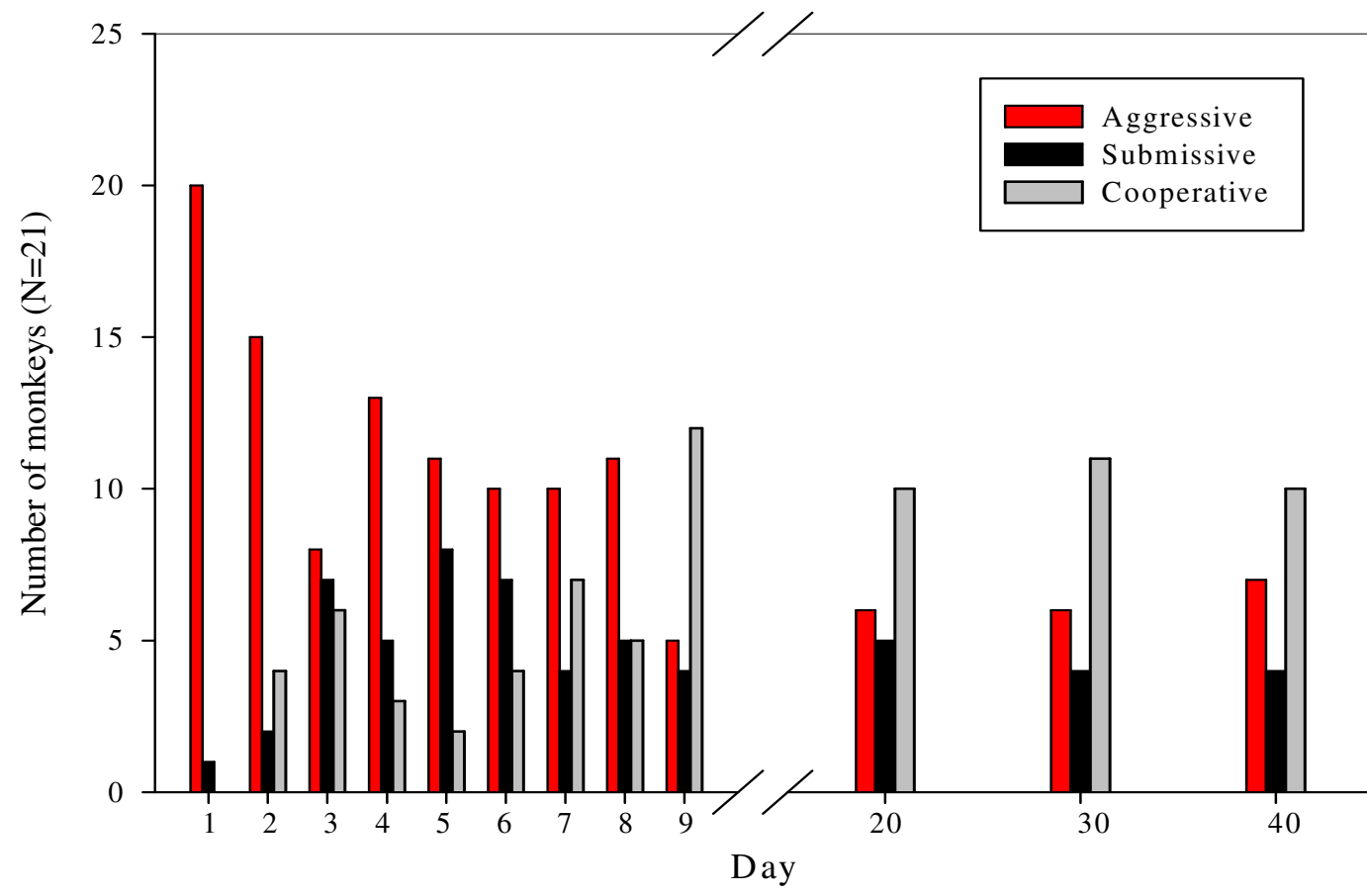


Table 2-2 Individual training outcomes for habituation, cage- and touchscreen training. ‘1’ indicates task was learnt to criterion. ‘0’ indicates task was not learnt to criterion. Median split for age shown by solid line. LT: long term confinement in single housing; ST: short-term single housing (dashed lines represent median split for confinement for either age group).

ID	Age (yrs)	Years captive	Temperament	Behavioural habituation	Cage Training	Touchscreen Training
C55	24.70	LT (3.9)	Variable	0~	1	1 ^[16]
L03	18.90	LT (11.9)	Agg	1	1 ^[NRT]	0~
O22	17.80	LT (11.0)	Agg	0	0~	-
M232	15.50	LT (13.5)	Sub	0~	0	-
X57	14.90	LT (3.9)	Agg	0	0	-
66D	11.33	LT (2.5)	Sub	1	1	0
94E	10.15	LT (3.9)	Agg	0~	1	1 ^[16]
G62	22.80	ST (1.9)	Agg	1	1	0
T52†	15.80	ST (1.8)	Coop	0	-	-
45A	13.20	ST (2.0)	Agg	0	0~	-
29C	12.05	ST (2.0)	Sub	1	1	1 ^[3]
94K	7.40	LT (2.5)	Coop	0~	1 ^[NRT]	1 ^[15]
86O	5.30	LT (3.0)	Coop	1	1	1 ^[12]
92R	4.75	LT (2.5)	Agg	0	1	1 ^[13]
79T	3.65	LT (2.5)	Coop	1	1	1 ^[4]
AI73	3.60	LT (2.5)	Agg	0	1	1 ^[4]
06H	9.90	ST (1.9)	Agg	1	1	1 ^[15]
16P	5.15	ST (0.1)	Agg	1	1	1 ^[9]
27S	4.66	ST (2.4)	Sub	1	1	1 ^[12]
66S	3.80	ST (1.6)	Sub	0	1	1 ^[5]
79S	3.70	ST (1.6)	Sub	0	1	1 ^[11]
Success rate	(%)			42.86	80.00	81.25

†Removed from study on medical grounds; ~ Showed some evidence of learning but failed to reach criterion; - Did not begin training; [x] the number of trials required to reach criterion; [NRT] Negative Reinforcement Training was required to reach criterion

Monkeys were scored for temperament on the basis of predominant behavioural responses to human approach on days 1-9 and days 20, 30 and 40. The most frequent behavioural response across the 12 days was taken as that most reflective of a given monkey's temperament. Monkeys were categorised as aggressive (n=10), submissive (n=6) and cooperative (n=4: Table 2-2). One monkey responded variably and did not have a predominant behavioural response (C55: 'variable').

In summary, a total of nine monkeys met criterion for behavioural habituation to a previously unfamiliar human. This was not affected by age or duration of single housing. A global examination of behavioural responses over days 1-9 and days 20, 30 and 40 identified monkeys who predominantly responded in an aggressive, submissive and cooperative manner respectively.

2.4.2 Introduction to Positive Reinforcement Training (PRT)

Twenty of the 21 monkeys learned to take chow from my hand within the 1 minute (first chow) and two-second (second chow) intervals, on each of days 4-9 during the habituation phase. On day 4 (the first day that food was offered by hand) the 20 monkeys took both chow from my hand on 70% of trials. Of these, nine monkeys took both chow on all trials, five monkeys took both chow on over 50% of trials, three monkeys took both chow on less than 50% of trials and four monkeys failed to collect both chow on any trial. On day 5 the 20 monkeys took both chow on a total of 81% of trials. On days 6-9, 20 monkeys took both chow on 100% of trials. One monkey never took food from my hand and was removed from the study at the end of habituation due to illness. In summary, 20 monkeys

learned to take food from my hand over a six day period and appeared familiar with the association of secondary reinforcing clicks and delivery of food. These 20 monkeys were considered suitable for PRT to enter a cage.

Aim 2: Training monkeys to enter a testing cage

2.5 Methods

2.5.1 Animals & Apparatus:

Twenty rhesus macaques participated in training to enter a transport cage for relocation to a testing area in later studies. The metal testing cage measured 0.6 x 0.8 x 0.6m. The sliding door to the testing cage was positioned so that it was level with the door of the home cage. Animals were tested in the same order on each day of training starting at 0700hrs, at least 30mins after the daily cleaning routine for the enclosure had been completed by a member of care-staff.

2.5.2 Procedure:

2.5.2.1 Days 10-24: Positive Reinforcement Training (PRT) to enter a testing cage

I used PRT to encourage monkeys to enter the testing cage and remain there to feed on the daily food ration. To do this, I positioned the testing cage in front of the home cage, placed two chow on top of the testing cage in view of the monkey, and stood behind the testing cage holding a stop watch and small food rewards (Figure 2.2a). Chow could not be pulled through the bars. However its presence encouraged monkeys to enter the cage.

PRT was used to shape monkeys' behaviour. Initially, if the monkey made any move towards the testing cage I gave a secondary reinforcing click and offered a chow through the aperture at the back of the testing cage. Using this approach, behaviour was shaped gradually so that only when the monkey touched, then partially entered and finally fully entered the cage was he rewarded with secondary and primary reinforcers.

On each day of training each monkey's progress was scored according to the degree to which he entered the cage. Entrance to the testing cage was scored as 0 (no part of the monkey passes through the testing cage door), 0.25 (head and shoulders pass through testing cage door but no limb passes through), 0.5 (head, shoulders and one or more arms pass through testing cage door: Figure 2.2b), 0.75 (head, shoulders, forearms and at least one hind limb enter testing cage) and 1.00 (animal enters testing cage fully so that the door may be closed: Figure 2.2c). On each subsequent day of training, the monkey was only rewarded for each approach that scored equal to or above the highest score for the previous day. The rate at which PRT progressed was adjusted for each individual according to their progress.

When a monkey fully entered the testing cage so that the door could be closed I immediately reinforced the behaviour with the delivery of the remainder of the daily food ration. While the animal was collecting the food I closed the cage door.

Figure 2.2 Cage training. a) the testing cage (right) is positioned in front of the home cage (left). Food on top of the cage encourages the monkey to enter. The door to the testing cage is pulled upwards to allow the monkey to enter. b) The monkey moves from the home cage to the testing cage. c) The monkey collects his reward while the testing cage door is closed.

a



b



c



On the first few days the cage was moved to the end of the enclosure, which allowed full view of a neighbouring corral of 100+ rhesus macaques. While in the new location the monkey was further rewarded with an additional preferred food (grapes, maize or juice in place of water). Animals were allowed to feed for 20 minutes, uninterrupted and observed from a distance. This was repeated until a monkey appeared relaxed on entering the cage and while being transported to the end of the enclosure.

Once a monkey appeared relaxed while being transported within the enclosure, on subsequent days the monkey was then transported in the cage to the laboratory, where each monkey was left to feed undisturbed in front of the operant training apparatus. The laboratory was a windowless room measuring 3 x 4 x 5m with a high degree of soundproofing. Chow rations were always adjusted where additional preferred foods were offered. A record was kept of the time taken for each monkey to enter the testing cage on each day. Criteria for successful training were identified as voluntary entry to the cage on three consecutive days for transportation to the laboratory. Each monkey was trained daily.

If a monkey did not fully enter the cage during the five minute PRT phase the daily food ration was placed inside the testing cage. The monkey was then left to feed, uninterrupted, for a further five minutes. The door was kept open during this time. No attempt was made to close the door if the monkey fully entered the testing cage after the daily food ration had been delivered.

2.5.2.2 Days 25+: Introduction of negative reinforcement training

Negative reinforcement training was employed for monkeys who failed to learn to enter the testing cage fully during the PRT phase. The testing cage was presented in front of the home cage as before. The monkey was allowed to enter the cage for one minute, and reinforced for fully entering the cage as above (door closed, full food ration given, positioned in view of social information). Where a monkey did not fully enter the testing cage within one minute the investigator and the enrichment officer used the squeeze-back to reduce the size of the home cage progressively and encourage the monkey to enter the testing cage. This is the method by which care staff at the Sabana Seca Field Station immobilise animals in the home cage for venipuncture prior to handling. Entry to the testing cage was immediately reinforced with the delivery of the daily food ration and additional preferred foods, the door was closed and the testing cage positioned in view of the neighbouring corral. This was repeated on three daily testing sessions. On the fourth session the testing cage was presented for 5 minutes as in the exploratory phase, with food items on show but out of reach. If an animal did not enter the cage fully during this time the squeeze-back was employed again as described.

NRT was continued until a monkey entered the testing cage without the need for negative reinforcement procedures. Where a monkey showed extreme resistance to the training procedures an assessment was made of progress and training was ceased where this was considered the most ethical move. Following three consecutive days on which the monkey entered the cage following NRT, PRT was reinstated to maintain performance on the task.

2.6 Results

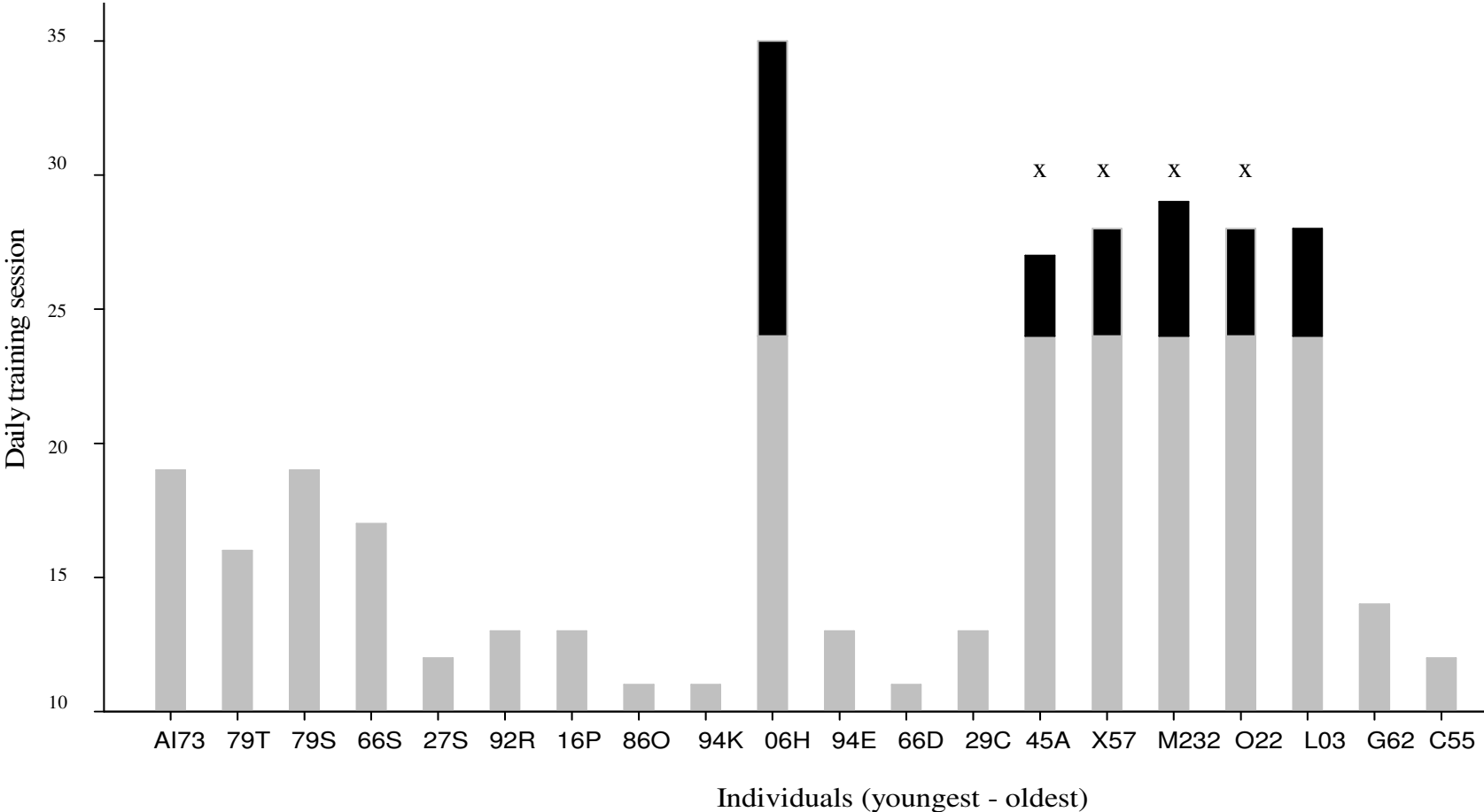
2.6.1 Cage-training success

Sixteen of the 20 monkeys learned to enter the testing cage for a food reward (Table 2-2). Fourteen monkeys learned to enter the testing cage for a food reward following PRT alone (3.7 ± 2.79 daily PRT training sessions, range: 1-9; Figure 2.3) and two monkeys learned to enter the cage consistently for a small food reward following both PRT and NRT (14 daily PRT training sessions followed by 11 [06H] and four [L03] daily NRT training sessions). Four monkeys failed to learn the task. 45A and O22 occasionally entered the cage for a small food reward following both PRT and NRT, but failed to reach criterion. X57 and M232 failed to enter the cage fully for a food reward during either PRT or NRT, usually keeping one limb in the home cage so the door could not be closed. The four monkeys who did not reach criterion were removed from the study.

2.6.1.1 The effects of age, duration of single housing and habituation

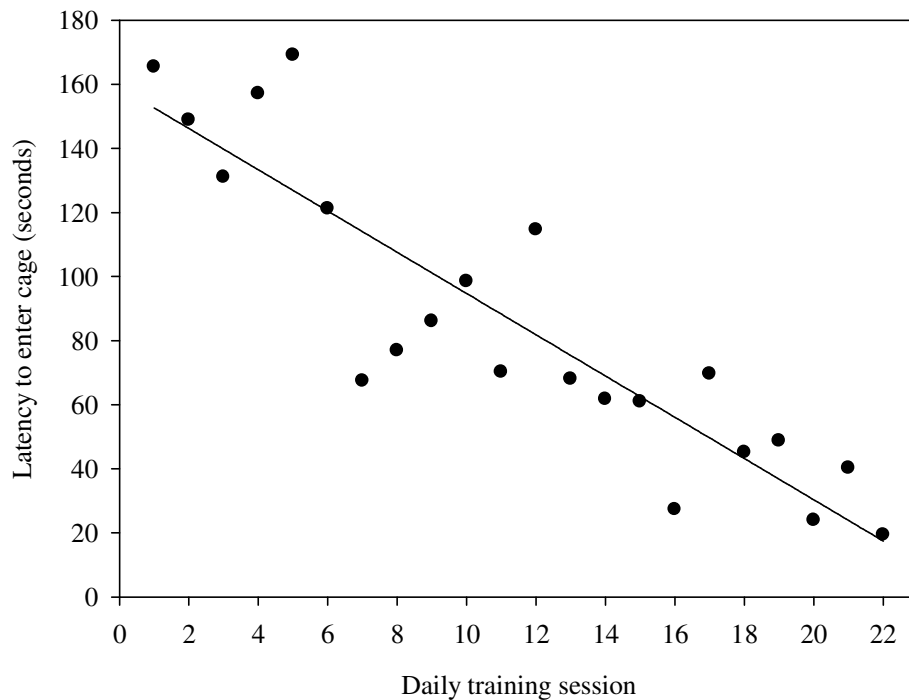
There was a significant difference between the younger and older age groups in training success (10/10 younger versus 6/10 older monkeys: Fisher's Exact, $P = 0.04$, one-tailed; Table 2-2). There was no effect of duration in single housing on training success (7/8 short-term versus 9/12 longer-term, Fisher's Exact, $P = 0.62$). There was a non-significant trend for monkeys who demonstrated behavioural habituation to have greater success in learning to enter the cage than did monkeys who did not show behavioural habituation (9/9 versus 7/11 respectively: Fisher's Exact, $P = 0.07$, one-tailed).

Figure 2.3 The number of daily training sessions required for each monkey to learn to enter the testing cage following PRT (grey bars – begun on day 10) and NRT (black bars – begun on day 25 for monkeys who did not learn using PRT within the time frame). x indicates the monkey failed to learn the task. Data represent the first of the three days on which the monkey met criterion.



A Pearson's correlation revealed a significant negative correlation between day of training and latency to enter the cage ($r = -0.895$, $n=22$, $P<0.001$: Figure 2.4). Latency to enter the testing cage decreased from a group average of 165 seconds on the first day on which each monkey fully entered the cage (169 ± 187 seconds older group; 162 ± 190 seconds younger group), to a group average of 19 seconds on the 22nd day of training (9 ± 7 seconds older group; 28 ± 35 seconds younger group).

Figure 2.4 Latency (seconds) to enter the testing cage over training sessions.



When grouped according to temperament, 7/10 aggressive, 4/6 submissive and 3/3 cooperative monkeys learned to enter the testing cage.

In summary, 16 monkeys learned to enter the testing cage. Fourteen of these learned after PRT alone. Younger monkeys were significantly more likely to learn to enter the testing-cage within the given time than were older monkeys, and there was a non-significant trend for monkeys who demonstrated behavioural habituation to be more likely to learn to enter the cage within the training phase than monkeys who did not show behavioural habituation. All monkeys became faster to enter the cage throughout the training phase.

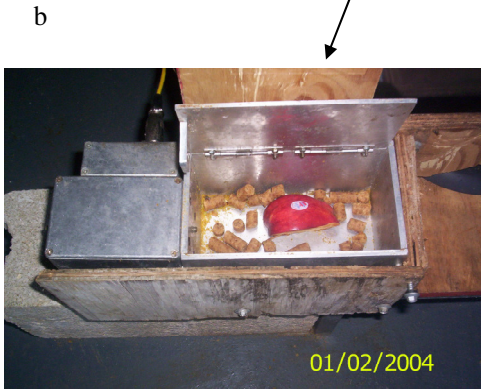
Aim 3: Training monkeys to perform operant responses to stimuli shown on a touch-sensitive monitor

2.7 Methods

2.7.1 Animals & Apparatus

The 16 male rhesus macaques who had been trained to enter a testing cage for transportation to the laboratory participated in training to respond to stimuli presented on a computer monitor (Figure 2.5). During the first phase of training, a 15inch training monitor was used to avoid risk of damage to the touch-sensitive monitor to be used in the later studies. The training monitor was placed in the wooden casing which would ultimately house the touch-sensitive monitor (Figure 2.5a). All other aspects of the apparatus were set up and functioning as they would be during the main experimental studies. These comprised a solenoid-operated lunch box for delivery of the daily food ration (purpose built for the study at the Department of Psychology, Oxford University), an automatic pellet dispenser (Biomed Associates Pedestal 45mg mount dispenser, ENV-203), a pellet tray for delivery of pellets, and a plastic chute from dispenser to tray (Figure 2.5b and c).

Figure 2.5 Laboratory apparatus. a) Touchscreen in wooden casing. b) Lunch box containing part of daily food ration. c) Pellet tray into which pellets are delivered from an automatic pellet dispenser via the plastic chute shown.



The training stimulus comprised a white square measuring 40mm x 40mm. Stimuli were presented at one of five locations on the training monitor using a Toshiba Satellite Pro A60 laptop computer. This laptop was also used to trigger the pellet dispenser and to remotely open the lunch box at the end of each session. During the initial training phase, I was positioned to the right of the apparatus where I could observe progress, yet was out of the view of the monkey.

2.7.2 Procedure

On the first day of training to use the apparatus I covered the computer monitor with honey and leaves, to encourage the monkey to reach out and touch the screen. The testing cage was positioned at a distance of 30cm from the monitor. I sat to the side of the cage with the laptop, which was used to trigger secondary reinforcing tones, and from where I could manually trigger the pellet dispenser to deliver one pellet down the chute for each screen press. Tones and pellets were delivered immediately after each touch to the screen.

Each training session lasted for a maximum of 20 minutes or until a monkey had performed 50 screen presses. At the end of the session a second tone was sounded to signal the end of the session and the lunch box was manually triggered to open to allow access to the daily food ration. Each monkey was then left to feed for 20 minutes in front of the screen, until the majority, or all, of the food ration had been consumed.

The number of leaves and amount of honey placed on the screen at the start of each session was reduced over consecutive days until no honey or leaves were

required. The rate of reduction was adjusted individually for each monkey according to speed of learning. Once a monkey was repeatedly touching the computer monitor in order to gain pellets, with no signs of frustration and without the need of initial prompts, an onscreen stimulus was introduced. The stimulus was a white square measuring 40mm x 40mm. Touches to the screen were now only reinforced with delivery of the tone and pellet when they occurred at the location of the stimulus. Criterion was reached when a monkey performed 30 correct responses within a 20 minute window.

Once a monkey had reached criterion and was working consistently and calmly with the training apparatus, the computer monitor was replaced with the touch-sensitive screen that would be used during the main experiments. Performance on the task was monitored for a further three days to ensure monkeys were working effectively and safely with the touch-sensitive screen. Once monkeys were working with the touch-sensitive monitor I observed progress from a video monitor in an adjacent room.

2.8 Results

Thirteen monkeys learned to touch a white square stimulus presented on a computer monitor in order to receive a small food reward (Table 2-2, final column). One monkey (G62) failed to touch the stimulus, one monkey (66D) touched the stimulus initially but failed to maintain performance. A third monkey (L03) was removed from the study due to poor health.

2.8.1 The effects of age, duration of single housing and habituation

There was a significant difference between the younger and older age groups in training success (10/10 younger versus 3/6 older monkeys: Fisher's Exact, $P = 0.04$, one-tailed; Table 2-2). There was no effect of duration in single housing on training success (6/7 short-term versus 7/9 longer-term, Fisher's Exact, $P=1.00$), nor was there an effect of habituation (6/7 habituated versus 7/9: Fisher's Exact, $P = 0.684$).

In summary, thirteen monkeys learned to touch a white square presented on a computer monitor in order to gain a pellet reward. Younger monkeys were significantly more likely to learn the task within the given time span than were older monkeys. There were no effects of duration of single housing or habituation on training success.

2.9 Discussion

In Part A of this chapter I presented the methods used to train adult male rhesus macaques to undertake operant procedures in a laboratory setting, along with training success for monkeys of different ages and captive histories. The general aim of the animal training procedures reported here was to establish a working laboratory of ~12 singly-housed adult male rhesus macaques. This aim was achieved. Of the 21 monkeys who participated in training, 13 learned all tasks up to, and including, touching a stimulus presented on a touch-sensitive monitor. These 13 monkeys were considered suitable to take part in experiments examining the effects of emotion state on cognition using computer-based methods. A further three monkeys (G62, L03 and 66D) learned to enter the testing cage and were

suitable for inclusion in studies which did not involve operant responses using the touch-screen. Here, I discuss the significance of the training stages employed to train adult male rhesus macaques to perform a range of tasks necessary for participation in the experiments reported in this thesis.

Nine rhesus macaques demonstrated behavioural habituation to the presence of a previously unfamiliar human, as defined by an increase in predominantly cooperative behaviours over nine days. This pattern remained consistent 20, 30 and 40 days later. There was a non-significant trend for monkeys who demonstrated behavioural habituation to demonstrate greater success in learning to enter a testing cage within the time span of the training phase used here. 'Habituated' monkeys were no more likely to learn to work with the touchscreen than monkeys who did not meet the criteria for behavioural habituation. The results of this study suggest that monkeys who predominantly respond to a human in a cooperative manner may demonstrate a tendency for enhanced ability, or speed, to learn tasks where a human is present, but may not demonstrate enhanced success in tasks where a human is not immediately present.

Twenty monkeys were categorised as aggressive (n=10), submissive (n=6) or cooperative (n=4: one of whom was removed from the study for health reasons) based on their predominant behavioural responses to a previously unfamiliar human. This finding supports previous work which suggests macaques exhibit stable personality traits. For example, Capitanio (1999) investigated persistent temperamental differences in male rhesus macaques. Monkeys rated as 'confident' (aggressive) in one environment were more likely to engage in

aggressive behaviours under a new set of environmental conditions. The uneven distribution of monkeys between the three classes of temperament identified in the current research made analyses unviable, therefore these data remained descriptive.

Learning success of the monkeys who took part in training to work in the laboratory was generally good. Twenty monkeys learned to associate the secondary reinforcer with food. Initially monkeys learned that on taking a food item from a human's hand a secondary reinforcer ('click') signalled that a primary reinforcer (a second food item) would be delivered shortly. The learnt association of secondary reinforcer with primary reinforcer was carried over to train monkeys successfully to enter the testing cage for transportation to the laboratory (n=16) and respond to stimuli presented on a touch sensitive monitor (n=13). The majority of monkeys responded to PRT alone. NRT was attempted with six monkeys who failed to enter the testing cage, and only two of these monkeys subsequently learnt the task. It is likely, therefore, that PRT alone may provide a successful means of training most rhesus macaques to perform tasks in a laboratory setting.

The training data presented here highlight the role of the processes of habituation, learning associations between primary reinforcers (unconditioned stimuli such as food), learning about secondary reinforcers (conditioned bridging stimuli such as the verbal 'click') and successive approximation (shaping) techniques, in training animals to perform complex tasks (Prescott, Bowell & Buchanan-Smith 2005; Laule, Bloomsmith & Schapiro, 2003). The animals who participated in this study

had never previously worked in a laboratory setting nor undergone behavioural training. Therefore, the procedures were new to all animals.

Age had a significant influence on some aspects of learning, in support of previously published data. Younger monkeys were more likely to learn tasks during the training phase than older monkeys. However, there was large inter-individual variation in learning success and both the oldest and youngest animals completed all stages of training. For example, age was a good predictor of cage-training success. All younger monkeys learned to enter the testing cage (10/10), while significantly fewer older monkeys (6/10) learned the same task. Age also predicted success in operant training. All younger monkeys (10/10) learned to touch a stimulus presented on a computer monitor, while significantly fewer older monkeys (3/6) learned the same task. The overall training success for younger monkeys was therefore 100% (10/10), while the overall training success for older monkeys was only 30% (3/10). The differences may be due to cognitive decline in older monkeys such that older monkeys are no longer capable of learning the tasks (Bartus et al., 1979), reduced speed of learning such that older monkeys required longer than the training phase used here to learn the tasks (Rapp, 1990), or covariates of age such as temperament, duration of single housing or pathologies not identified in the current studies.

Duration of single housing did not influence training success on any tasks. This is in contrast to previous studies which show that single housing leads to stress in non-human animals which, in turn, may impair cognition (e.g. Mendl, 1999; Schapiro et al., 2000). However, the data do not allow us to distinguish shorter-

term (acute) and longer-term (chronic) single-housing-related stress effects since both may lead to impaired learning. Further the division of animals according to a median split may not present an accurate categorization of 'short term' or 'long term' housing.

Speed of progression through successive approximations of behaviour varied between animals. Fast progression in one phase of training did not reliably indicate fast progression on the subsequent aspect of training. The rate of progression through each training stage was individually tailored according to each individual's apparent comfort in performing the current task and response to the incremental changes in task difficulty.

In summary, the protocols described here were effective for training experimentally naïve rhesus macaques aged between 3.6 and 24.7 years, to work in a laboratory environment on a range of tasks: from entering a testing-cage to responding to stimuli presented on a touch sensitive monitor. Older monkeys (>10 years) may be less suited to training than younger monkeys (<10 years). Individual differences may necessitate flexible training procedures tailored to the training needs of each monkey.

Part B: Development of stimuli and refinement of the operant procedures

2.10 Introduction

Here, I describe the development of stimuli suitable for testing cognitive processes in rhesus macaques, and describe the refinement of the operant training procedures specific to each of the experimental studies presented in Chapters 4-6 of the thesis. To begin, I give a brief summary of the primate visual system and the implications of morphological and functional similarity to the human visual system for the development of visual stimuli and paradigms.

2.10.1 Developing stimuli suitable for use with primates

Primates have been widely used in studies that use images presented on a computer monitor. In particular, pictures of conspecific faces have been validated for use in a range of laboratory studies with rhesus macaques (Perrett & Mistlin, 1990). Rhesus macaques will attend to pictures of conspecific faces on a computer screen, and may respond to face pictures with species-typical socioemotional behaviours (Perrett & Mistlin, 1990; Capitanio, 1999). These responses are species-specific so that animals respond differently to pictures of conspecific faces than they do to pictures of other species (Pascalis & Bachevalier, 1998; Humphrey, 1974; Dahl et al., 2009). Rhesus macaques look longer towards some conspecific faces than they do towards others (female rhesus macaque preference for male sexual skin coloration: Waitt et al., 2003), look preferentially towards conspecific faces than towards other areas of the body, with greatest interest in the

eye region (Nahm et al., 1997; Dahl et al., 2009), and demonstrate holistic face-processing in a comparable manner to humans, as evidenced by disrupted processing of inverted faces (Dahl et al., 2009). Further, there is evidence that some primates can distinguish between different facial expressions of emotion in pictures of conspecific faces in match-to-sample tests (chimpanzees: Parr, 2001; rhesus macaques: Parr & Heintz, 2009) and crossmodal identification looking paradigms (rhesus macaques: Ghazanfar & Logothetis, 2003).

The primate visual system is similar to the human visual system and rhesus macaques are a widely-used primate model of human visual system function (Calder, 2007; Rolls, 2007). Human and other old world anthropoids, such as rhesus macaques, have trichromatic colour vision, due to the presence of three types of colour-sensitive cones in the retina (Jacobs, 1996). Humans and other primates have direct magnocellular and parvocellular neural pathways from the retina to subcortical (magnocellular) and cortical (parvocellular) brain regions (Laycock et al., 2008). The magnocellular pathway incorporates the amygdala among other subcortical structures and allows rapid appraisal of the threatening value of salient stimuli that appear anywhere in the visual field (LeDoux, 1996). This rapid, low-level processing of visual information provides a ‘magnocellular advantage’ in the detection of threatening stimuli, such as threatening faces, for which there is evidence in both humans and primates (Laycock et al., 2008).

Evidence from fMRI studies with humans, and single-cell recordings from rhesus macaques, indicates that both humans and rhesus macaques have face-selective populations of neurons in the superior temporal sulcus (where information about

emotion expression, eye gaze and movement is coded: Rolls, 2007). These neurons pass on information about faces to other face-specific populations of neurons in the amygdala and orbitofrontal cortex (for further processing of identity, emotion expression and other aspects relevant to social communication and emotional behaviour. The orbitofrontal cortex also processes social feedback, such as changes in facial expression: Rolls, 2007). Data from split-brain monkeys with lesion to the corpus callosum demonstrate a right-hemispheric superiority for processing faces, as is also seen in humans (Vermeire et al., 1998).

The behavioural and neurophysiological data together demonstrate that colour photographs of conspecific faces presented on a computer monitor represent salient socio-emotional stimuli to rhesus macaques, which may be processed in a manner comparable to that seen in humans. (i.e. there is comparable colour cone value, face-specific brain regions that process socio-emotional information from faces, and neural pathways that preferentially link the left and right visual fields to the right and left hemispheres respectively). Therefore, the use of such stimuli is valid in a study of the effects of cognition-emotion interaction with respect to, for example, threatening versus non-threatening visual social information, such as that conveyed by facial expressions.

Face stimuli were used in two of the three main cognitive studies presented in this thesis (Chapter 4 and Chapter 5). I collected a separate stimulus set for either study so that all stimuli would be novel to each participant monkey at the start of each study. For each stimulus set I took digital photographs of male monkeys housed at the CPRC (from herein 'stimulus monkeys'). The stimulus monkeys

were housed in enclosures that were out of visual access to the monkeys who were to take part in the studies. Therefore, stimulus monkeys were unfamiliar to the participant monkeys. Photos were cropped, sized and adjusted for colour and brightness using Adobe Photoshop 7. Full details of the stimuli are given in the relevant chapters.

For the study presented in Chapter 4, a set of 10 threatening and 10 non-threatening face pictures was required for the testing sessions. These were to be presented in threatening versus non-threatening face pairs on two adjacent monitors so that monkeys had a choice of looking towards one of the two faces in the pair. I collected a set of 20 face stimuli, with one threatening (open-mouthed threat with forward gaze) and one neutral (closed mouth, forward gaze) face for each of 10 different stimulus monkeys. The staring open-mouthed face is used by rhesus macaques as a threat and a signal of attack (van Hooff, 1976; Chevalier-Skolnikoff, 1973). A neutral face does not signal threat. However, direct gaze is a signal of dominance in rhesus macaques (Chevalier-Skolnikoff, 1973; van Hooff, 1976). Neutral faces were therefore considered to be non-threatening insofar as they were less threatening when presented next to the staring open-mouthed face.

Prior to testing I needed to familiarize the monkeys with the testing apparatus and encourage them to look centrally between the screens in between trials and towards the screens when stimuli appeared. To do this, monkeys were shown colour images of female perinea (rumps) following evidence that male macaques visually attend to stimuli containing images of sexual skin (Waite et al., 2006). A series of training stimuli were compiled from colour photographs of 10 female

monkeys housed at CPRC, who were unknown to the participant monkeys (see Appendix 2). For each of the 10 female monkey identities, one picture with the rump in clear view was selected. Female pictures were trimmed so that the full body and rump were visible and superimposed on a grey background enclosed in a rectangular frame measuring 154mm x 164mm. Female rump stimuli were always presented in duplicate so that the same stimulus appeared, in mirror image, on both screens on each trial.

For the study presented in Chapter 5, I needed a second set of 10 threatening and 10 non-threatening face pictures. This study differed from the study in Chapter 4 since only one face was shown on each trial in Chapter 5, compared with paired stimuli in Chapter 4. Therefore, it was important when selecting faces that the non-threatening face, when viewed on its own, was unambiguous in its signal as a low-threat stimulus. I took digital photographs of a new set of 10 adult male stimulus monkeys and, for each stimulus monkey, selected one face picture with direct gaze (frontal face with neutral expression and direct gaze) as the threatening face and one face with averted gaze as the non-threatening face (profile face with neutral expression, looking away from the participant monkey). During the initial training phase, for which data are presented here, a grey square stimulus measuring 154mm x 164mm was used and no faces were presented.

For the study presented in Chapter 6, abstract stimuli were used, and do not require further discussion here.

2.11 Methods for the refinement of the operant procedures

Prior to running each experiment presented in Chapters 4-6, it was necessary to train monkeys to criteria in the specific protocols for each study. The order in which the studies presented in this thesis progressed was, in large part, determined by task difficulty. In this section I briefly describe the training protocol, and present training data, for each study, since each differed in terms of operant contingencies. Detailed descriptions of the procedures are given in each of chapters 4-6, so the following present a summary of the main features of each operant procedure. The aim of this section is to present training data and identify the processes behind selecting monkeys for inclusion in each of the main experimental studies. Where data allow, I explore the effects of age and duration of single housing on training success.

2.11.1 Monkey training for Chapter 4: Developing a method to study attentional bias in rhesus macaques using eye-gaze

The 16 monkeys who had learned to enter the testing cage (see Table 2-2) took part in training for the study. Training stimuli were matched pairs of photographs of female rumps, of the same dimensions as the face stimuli to be used during the final study (see Chapter 4 and Appendix 2). Stimuli were presented on two computer monitors and the monkey's gaze towards the stimuli was recorded by a video camera placed between the screens. Stimulus pairs were presented for 10 seconds on each trial. Monkeys were required to look centrally between the screens in between each trial and to look towards the screens during each trial. A pellet was delivered down a chute to a pellet tray in between the screens to encourage monkeys to look centrally. A daily training session consisted of 20

trials. Criterion was met if a monkey completed 20 trials within 40 minutes (i.e. looked centrally between the screens in between trials). This study presented monkeys with two stimuli on each trial and required no operant response.

2.11.2 Monkey training for Chapter 5: Developing a method to study emotion evaluation using basic operant responding

The 13 monkeys who learned to touch the white square presented on the touch-sensitive monitor (see Table 2-2) took part in training for the study. The training stimulus was a grey square, of the same dimensions as the stimuli used in the final study (Chapter 5). The grey square was presented for 60 seconds at one of three locations on the screen (left visual field: LVF; right visual field: RVF; or centrally). If the monkey touched the grey square within the 60 seconds he was rewarded with a pellet via the chute and the next trial began. Correct responses were eventually rewarded on only 40% of correct responses (reduced over trials from an initial 100% fixed reinforcement ratio: 100%FRR). A daily training session lasted 80 trials or 40 minutes, whichever occurred sooner. Criterion was met when a monkey made 80 correct responses within 40 minutes on three consecutive days. This procedure required monkeys to respond to one stimulus on each trial with one operant response.

2.11.3 Monkey training for Chapter 6: Developing a method to study judgement bias using a 'Go-NoGo' paradigm

The 13 monkeys who learned to touch the white square presented on the touch-sensitive monitor took part in training for this study. Monkeys were required to

learn that touching one stimulus (e.g. a long line) led to a pellet reward, and that touching another stimulus (e.g. a short line) led to a punisher (white noise and a delay until the onset of the next trial). Stimuli were presented for 2 seconds on each trial. Criterion was met when monkeys made correct responses on 80% of trials with >70% correct Go and NoGo responses respectively.

2.12 Results

Individual training data for task success (whether it was learnt or not) and number of daily training sessions to reach criteria are presented in Table 2-3.

2.12.1 Training success during the development of a method to study attentional bias presented in Chapter 4

Fifteen out of 16 monkeys looked towards pictures of female rumps presented on two monitors and looked centrally between the two monitors in between trials. These monkeys completed 20 trials within 40 minutes each and therefore met criterion for inclusion in the study. One monkey (66D) did not look towards the pictures presented on the screens nor did he look centrally in between the screens and was removed from the study.

Table 2-3 Training success for monkeys learning operant procedures used in the studies presented later in the thesis. ‘1’ indicates task was learnt to criterion. ‘0’ indicates task was not learnt to criterion.

ID	Age (yrs)	Cage Train	Attentional Bias (Chapter 4)	Emotional evaluation (Chapter 5)	Expectancy bias (Go/No Go) (Chapter 6)
		Enter transport cage	Look towards stimuli on two screens 2 stimuli: no response	Touch stimulus on screen. 1 stimulus: 1 response	Go/NoGo (touch or don't touch) stimuli on screen 2 stimuli: 2 response options
C55	24.70	1	1	1 ^[16]	0
G62	22.80	1	1	0	-
L03†	18.90	1	-	0	-
45a‡	13.20	0~	1	-	-
29C	12.05	1	1	1 ^[3]	0
66D	11.33	1	0	0	-
94E	10.15	1	1	1 ^[16]	0
06H	9.90	1	1	1 ^[13]	0
94K	7.40	1	1	1 ^[15]	1 ^[25]
86O	5.30	1	1	1 ^[12]	1 ^[25]
16P	5.15	1	1	1 ^[9]	1 ^[42]
92R	4.75	1	1	1 ^[13]	0
27S	4.66	1	1	1 ^[12]	0
66S	3.80	1	1	1 ^[5]	1 ^[29]
79S	3.70	1	1	1 ^[11]	1 ^[15]
79T	3.65	1	1	1 ^[4]	1 ^[17]
AI73	3.60	1	1	1 ^[4]	1 ^[30]
Success Rate (%)			93.8	81.25	53.85

†Removed from study on medical grounds;‡Monkey included in study despite poor performance in cage training; ~ Showed some evidence of learning but failed to reach criterion; - Did not begin training; [x] the number of trials required to reach criterion;

2.12.2 Training success during the development of a method to study emotion evaluation presented in Chapter 5

All 13 monkeys learned to touch a grey square stimulus presented on the touch-sensitive monitor for a pellet reward on 40% of 80 trials within 40 minutes, and all therefore met criterion for entry to the main study.

2.12.3 Training success during the development of a method to study judgment bias presented in Chapter 6

Seven out of 13 monkeys learned to perform the Go-NoGo task to criterion, performing at 80% accuracy with at least 70% correct responses on each of the ‘Go’ and ‘NoGo’ responses respectively. All seven monkeys who learnt the task were in the younger age group. None of the three monkeys in the older age group learned the task, and this difference between the groups was significant (Fisher’s Exact, $P=0.03$). Of the seven monkeys who learnt the task, there was no correlation between age and number of daily training sessions required to reach criterion ($r=0.227$, $n=7$, $P=0.624$).

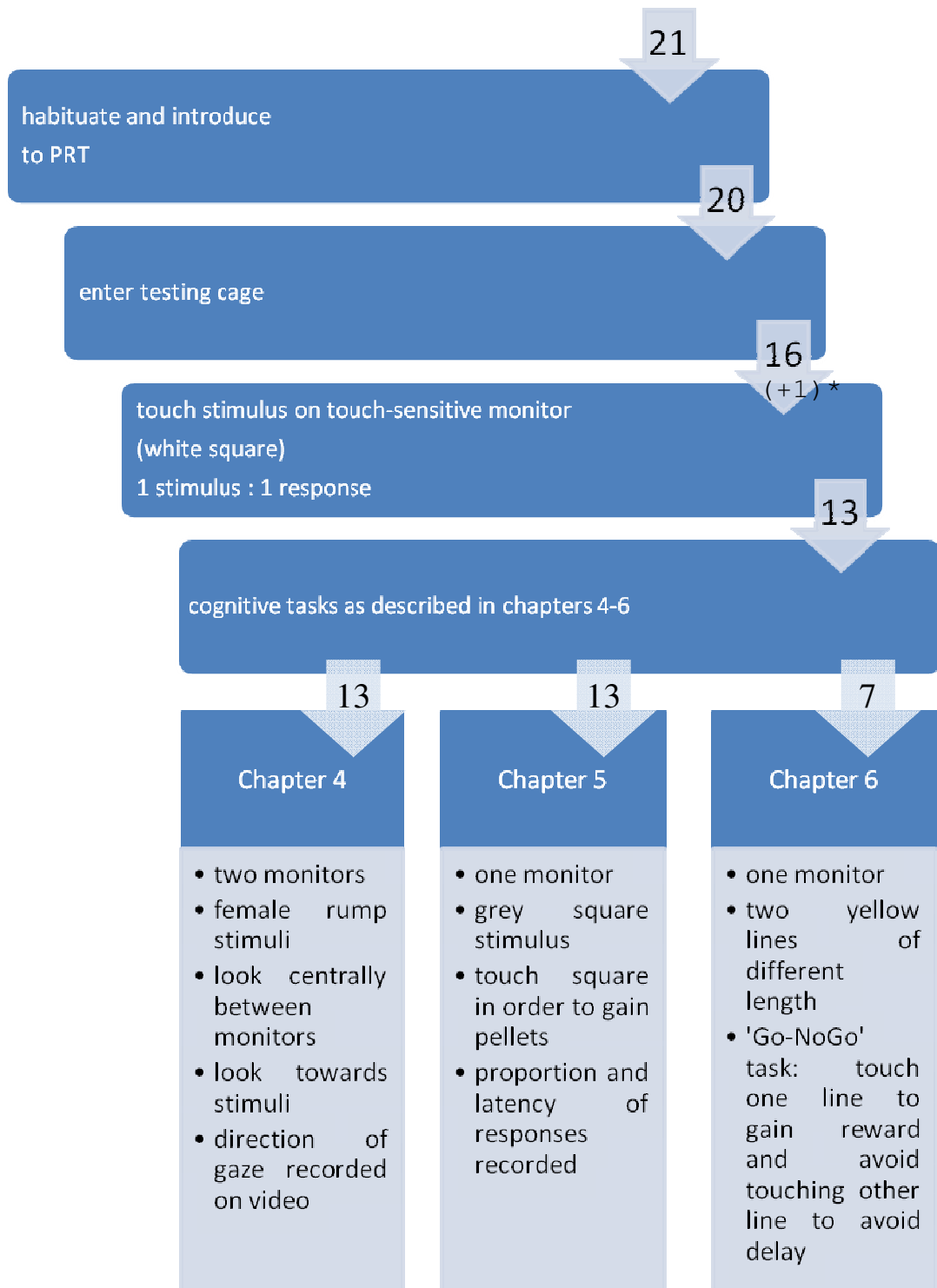
2.13 Discussion

Sixteen monkeys who had learned to enter a testing cage for transportation to the laboratory took part in a selection process for participation in a series of cognitive studies. (A further monkey, 45a, failed to regularly enter the cage, but did take part in the study presented in Chapter 4 on an ad hoc basis.) Fifteen out of the 16

monkeys learned to look towards pictures of female rumps presented on two computer monitors and met criteria to participate in the study of attentional bias presented in Chapter 4. Thirteen monkeys who had learned to touch a white square presented on a touch sensitive monitor undertook further operant training for selection to take part in a simple operant task (Chapter 5). All 13 monkeys learned this task. This is unsurprising given the similarity of the task to the original operant training task using the white square. The 13 monkeys then underwent training on a more complex Go-NoGo task (Chapter 6). Seven of the monkeys learned the task. Age was a significant factor in training success since all the successful monkeys were in the younger age group. Progression of training stages, and training success for the group, are summarized in Figure 2.6.

The data presented in this chapter indicate that rhesus macaques under the age of 10 years are the most suitable candidates for training to take part in cognitive testing. Younger monkeys had higher training success rates and also learnt tasks in anything between a few daily testing sessions and a month of training. Within the younger age group there were no linear effects of age on speed to train suggesting that individual differences may influence training speed more than age within the younger age group. Three older monkeys (C55, 29C and 94E) met criteria to take part in the simple operant task developed for use in Chapter 5, but did not reach criteria in the more complex Go-NoGo task developed for use in Chapter 6. This suggests that age is not an absolute predeterminant of training success, although it may preclude monkeys from learning more complex cognitive tasks within the time frame presented here.

Figure 2.6 Overall training outcomes for 21 monkeys who underwent training in the basic operant procedures involved in working in a cognitive laboratory. (+1)* signifies an additional monkey, 45a, who did not meet criterion for successful cage training but did enter the cage on occasion and consequently took part in the study presented in Chapter 4.



Chapter 3

3 General methods

In this chapter I describe two treatments that were used to manipulate affective state in the monkeys who took part in the studies presented in chapters 4-6. A key aim of these studies is to assess the relationship between affective state and cognition. Two treatments were identified that, it could be inferred, would induce positive and negative shifts in affective state, respectively, in male rhesus macaques. A week of enrichment was selected as a treatment likely to induce an increase in positive affect, and a veterinary health-check was selected as likely to induce an increase in negative affect. There are several measures that are widely used to assess shifts in inferred affective state in primates based on contextual cues. In this chapter I describe two traditional measures (behavioural and physiological) that were used to assess relative differences in inferred affective state in monkeys following the two treatments (a week of enrichment and a health-check).

Published data suggest that introducing environmental enrichment regimes may lead to physiological and behavioural changes in primates suggestive of reduced arousal and improved welfare (Schapiro et al., 1993; Honess & Marin, 2006b). Improved welfare is typically defined and assessed as a reduction in physiological and behavioural measures assumed to reflect stress (Honess & Marin, 2006b). A reduction in these stress-related measures is taken to indicate a positive shift in affective state, however it does not indicate positive affect *per se*. Measures of inferred positive affect in animals are less well understood, or studied, and it is unclear what a truly positive marker would be (Boissy et al., 2007). However,

based on available data (e.g. Honess & Marin, 2006b), a week of environmental enrichment was chosen as a treatment likely to induce a positive shift in affective state among singly-housed male rhesus macaques.

Published data indicate that invasive husbandry procedures, such as restraint and injection for veterinary purposes, lead to physiological and behavioural changes suggestive of increased levels of physiological arousal, and a negative shift in affective state (Bercovitch & Clarke, 1995; Ruys et al., 2004; Honess & Marin, 2006a). At the Caribbean Primate Research Centre (CPRC), where the present research was conducted, monkeys undergo a statutory health-check performed by the facility veterinarian, once every three months. During the health-check each monkey is restrained in the home cage and sedated with an injection of Ketamine Hydrochloride (KHCl). This procedure has been shown to act as a physiological stressor in captive primates (Ruys et al., 2004; Heistermann et al., 2006). There is evidence cortisol measures increase in rhesus macaques following physical restraint for injection with KHCl, but not following administration of KHCl to monkeys trained to extend an arm for venipuncture (Bentson et al., 2003). This suggests that changes in cortisol measures following restraint for injection reflect a response to restraint *per se*, rather than a physiological response induced by the presence of KHCl in the system (Fuller et al., 1984; Bentson et al., 2003). Therefore, the health-check was adopted as a husbandry treatment that could be incorporated into the current research as a treatment likely to induce a negative shift in affective state in the monkeys.

The aim of this chapter is to present data on the influence of two treatments (a week of enrichment versus a health-check) on the physiology and behaviour of captive male rhesus macaques. A further aim is to discuss what any observed differences in physiology and behaviour between the two treatments may tell us about possible differences in underlying affective state.

In Part A, I discuss the impact of the enrichment and health-check treatments on the physiology of a subset of the monkeys who participated in the cognitive studies presented in chapters 4-6. I report on methods used to collect and analyse a measure of physiological arousal: excreted metabolites of the corticosteroid hormone, cortisol. This is a widely used measure of physiological stress in primates, and cortisol levels may be assessed through the measurement of excreted faecal glucocorticoids, as has been done for a range of primate species (Abbott et al., 2003; Hodges & Heistermann, 2003a; Heistermann et al., 2006; Honess & Marin, 2006a; Lane, 2006). I then present data on the impact of the enrichment and health-check treatments on the levels of excreted glucocorticoid metabolites in the monkeys who took part in this study. I conclude Part A by discussing the extent to which the enrichment and health-check treatment conditions induced changes in excreted glucocorticoid metabolites indicative of the expected changes in affective arousal.

In Part B of this chapter, I discuss the impact of the enrichment and health-check treatments on rhesus macaque behaviour. I report on methods used to collect and analyse behavioural correlates of inferred affective state: namely self-directed, stereotypical and self-injurious behaviours. Self-directed behaviours are a widely

used measure of anxiety (a subcomponent of stress that occurs particularly in situations of uncertainty) in free-ranging primates (Maestripieri et al., 1992). Stereotypical and self-harm behaviours are abnormal behaviours seen only in captive animals. They are associated with psychological pathology arising from poor housing, rearing or other impaired socio-environmental factors, and are widely used by welfare researchers (Novak, 2003; Honess & Marin, 2006a; Reinhardt, 2008).

I conclude this chapter with a general discussion of the utility of the two treatments (a week of environmental enrichment and a health-check) for inducing behavioural and physiological changes indicative of changes in inferred affective states in captive male rhesus macaques.

Part A: Assessing physiological arousal

3.1 Introduction

When an animal encounters a stressor, the classic endocrine response is the release of glucocorticoids (GCs: Sapolsky et al., 2000). GCs are a class of steroid hormone that plays a role in the regulation of glucose metabolism, immune function and inflammatory responses. They are found in nearly all vertebrate tissues. Among primates, the physiological component of the stress response includes the release of the GC cortisol (Abbott et al., 2003; Lane, 2006). The exact functions of the cortisol stress response remain strongly debated (Sapolsky et al., 2000) and are beyond the scope of this review. However, there is general

agreement that, across vertebrates, the stress response is characterized, in part, by elevated levels of circulating GCs, and the immediate and pronounced availability of energy afforded by circulating GCs provides increased resources for an organism to respond to stimuli, including stressors, with a fight or flight response (Sapolsky et al., 2000; Carere et al., 2003; Young et al., 2004).

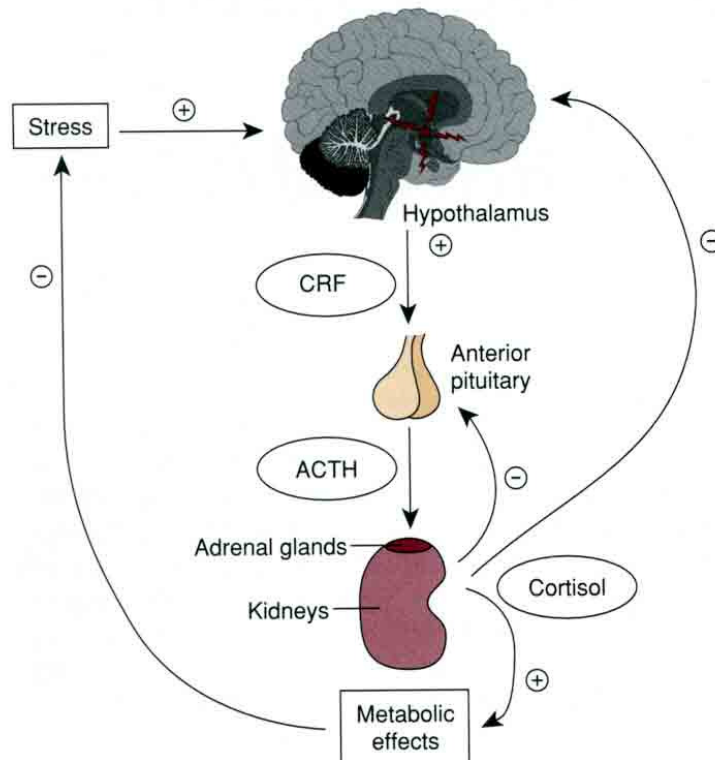
An important issue in the interpretation of GC measures is that they indicate physiological arousal, and are non-specific with respect to affective state. Therefore, while anxiety is, in part, characterized by an increase in GC levels, not all increases in GC levels reflect increased anxiety. Changes in GC levels have been associated with a range of energy-demanding activities not associated with stress, such as locomotion (Honeess & Marin, 2006a), seasonal reproductive activity (sifakas: Fichtel et al., 2007), environmental factors such as minimum ambient temperature (baboons: Weingrill et al., 2004), and life-history factors such as age (chimpanzees: Anestis et al., 2006). In addition, where changes in GCs are assumed to reflect a stress-related response, factors such as initial basal levels, speed and extent of response and time taken for GC levels to return to baseline are all factors that vary between individuals, for example as a function of dominance rank (Sapolsky, 1982).

Circulating GCs show marked circadian patterns of release over the 24 hour cycle with peak levels in the early morning and a nadir at night (humans: Stone et al., 2001; rhesus macaques: Jacoby et al., 1974). GC levels also change throughout the year with generally higher levels during colder months than warmer months (humans: Persson et al., 2008; baboons: Weingrill et al., 2004). Therefore, these

factors must be considered when assessing changes in GCs over days, months or years.

The general pathway of the endocrine response to stress, the hypothalamic-pituitary-adrenal axis, is shown in Figure 3.1. A physical or psychological stressor causes the hypothalamus to release corticotrophin-releasing factor (CRF). CRF binds to specific receptors on anterior pituitary cells, triggering the release of adrenocorticotrophic hormone (ACTH). ACTH is transported in the blood from the pituitary to the adrenal glands, stimulating the secretion of GCs from the adrenal cortex (Sapolsky et al., 2000). GCs (along with other hormones such as epinephrine, norepinephrine and glucagon) function to mobilise energy resources (glucose, amino acids and free fatty acids) from storage tissues.

Figure 3.1 The hypothalamic-pituitary-adrenal axis (Figure retrieved from <http://www.montana.edu/wwwai/imsd/alcohol/Vanessa/vwendocrine.htm>)



The increase in circulating cortisol levels initiates a series of metabolic effects aimed at alleviating the harmful effects of physiological stress through negative feedback to both the hypothalamus and the anterior pituitary. This negative feedback decreases the concentration of ACTH and cortisol in the blood once the state of stress subsides. Circulating plasma GCs are ultimately passed to the liver where they are converted to soluble, non-specific, metabolites and excreted in urine and faeces. A negative feedback mechanism functions to return circulating hormones to baseline levels once a stressor has passed (Sapolsky et al., 2000).

There are well-developed and validated techniques for analysing cortisol levels in primates. Circulating cortisol levels are typically measured directly from blood plasma (e.g. Clarke et al., 1988), or indirectly from saliva (e.g. Lutz et al., 2000). Circulating GCs show a rapid pattern of response, reflecting changes in GC levels over minutes or hours. Blood and salivary GC levels therefore provide valuable information about specific physiological responses to known stressors and may capture changes in GCs over short, as well as longer, periods of time. Such 'point sampling' techniques require stressful capture, training to present a limb for blood draw, or to chew on a swab so that saliva may be collected. These methods are invasive and prone to confounding results by inducing stress. Where changes in stress levels over hours, days, weeks or months are of interest, the use of non-invasive methods which detect more pervasive cortisol levels is more representative, ethical and applicable.

GC metabolites excreted in urine and faeces provide a non-invasive indirect measure of physiological arousal (namely the stress response) in a range of taxa

including birds, primates and carnivores (Carere et al., 2003; Young et al., 2004; Heistermann et al., 2006). Faeces, in particular, may be collected non-invasively with simple equipment, both from captive and free-ranging animals (Hodges & Heistermann, 2003b; Young et al., 2004; Dubuc et al., 2009). A single faecal sample contains GC metabolites (GCMs), which have been converted by the liver and excreted over many hours. Faecal samples therefore provide an indirect measure of circulating cortisol levels, reflecting cumulative stress of an animal over a period of time. These can therefore be used to detect stressful events. For example, Bergman et al. (2005) tracked the effects of socially significant events on the concentration of GCM in faeces ([fGCM]: [] used to signify concentration throughout) in free-ranging chacma baboons over a period of 14 months, using data derived from over 400 faecal samples. Arlet et al. (2009) collected a comparable number of faecal samples from male gray-cheeked mangabeys for a similar analysis over a period of 20 months. Large numbers of samples collected non-invasively over time therefore allow the time course of responses to chronic (longer lasting), or accumulative, stressors to be assessed.

The assay used here (5β -androstane- 3α - 11β -diol-17-one) to measure [fGCM] has been validated for use with rhesus macaques (Michael Heistermann, *pers. comm.*), and several other primate species, including two further species of macaque (Barbary macaque, *Macaca sylvanus*, and the longtailed macaque, *M. fascicularis*: Heistermann et al. 2006). Here, I describe the methods I applied to measure changes in [fGCM] in captive adult male rhesus macaque faeces in response to two treatments (following a week of enrichment and following the health-check).

3.2 Aims

The general aim of Part A is to assess physiological responses to two treatments (a week of environmental enrichment versus a health-check) which were used to manipulate inferred affective state in the male rhesus macaques that took part in the cognitive tests reported later in the thesis. In the health-check treatment condition, each monkey underwent the statutory three-monthly health-check which involved restraint for sedation with K_HCl. Data collected prior to, and following, restraint and sedation are labelled Pre- and Post-health-check, respectively. This treatment was predicted to induce physiological arousal indicative of a stress response. In the enrichment treatment condition, monkeys received a week of environmental enrichment. Data collected following the week of enrichment are labelled Post-enrichment (although enrichment was maintained until the end of all data collection for the enrichment phase). Due to time constraints it was not possible to collect samples prior to the start of enrichment, therefore a general baseline calculated from samples collected at other times was used. The enrichment treatment was expected to induce a reduction in physiological arousal. It was anticipated that the effectiveness of the two treatments would be reflected in an increase and a decrease in [fGCM], respectively.

3.3 Hypotheses

- 3.1. [fGCM] will increase Post-health-check relative to Pre-health-check levels.
- 3.2. [fGCM] will decrease Post-enrichment relative to general baseline levels.
- 3.3. [fGCM] will be significantly higher Post-health-check than Post-enrichment.

3.4 Methods

3.4.1 Animals and treatments

Faecal samples for analysis of [fGCM] were collected from eight monkeys (Monkeys: O22, 06H, 86O, 94K, 92R, 66S, 79S & 79T; \bar{X} age: 6yrs \pm 2.7) between July 2006 and February 2007. All monkeys were housed at the CPRC, as described in Chapter 2.

3.4.1.1 Health-check

The health check consisted of restraint for sedation by injection with Ketamine Hydrochloride (KHCl), followed by a routine veterinary inspection. Immediately prior to the veterinary health-check, the dedicated Veterinary Technologist (Carlos Pacheco, CP) entered the monkeys' enclosure with a trolley containing the necessary equipment. CP restrained the first monkey in his home cage using the squeeze-back mechanism and administered an intramuscular injection of KHCl (0.1cc/kg). Once sedated, the monkey was removed from the cage for the veterinary health-check (approximately 10 minutes duration per monkey). On return to the home cage each monkey was observed until he had recovered from the anaesthesia (approximately 15 minutes until mobile), and was then fed the daily food ration. The veterinary health-check consisted of weighing, inspection of nails, teeth and pelage and administration of a test for Tuberculosis bacteria. No further drugs were administered as routine. All monkeys on other medications were excluded from the study.

Veterinary health-checks were conducted according to the facility schedule which stipulates all monkeys must be examined at three-monthly intervals. All monkeys housed within the enclosure were assigned to groups according to the timing of their next veterinary health-checks. CP examined all monkeys within a group on the same day, at approximately midday (Figure 3.2).

3.4.1.2. Enrichment

The environmental enrichment treatment involved seven days of environmental enrichment in the home cage, followed by a further three days of maintenance enrichment concurrent with cognitive testing (Figure 3.3). The week of enrichment was used to induce a positive shift in affective state. The three day maintenance enrichment concurrent with cognitive testing was included to prevent any negative shift in affective state that might be induced by removing the enrichment prior to the end of testing. Throughout the thesis, 'Post-enrichment' is used to refer to the period immediately following the initial seven days of enrichment. Enrichments were provided throughout all Post-enrichment testing phases until testing had ceased.

On each day of enrichment monkeys were given between one and three enrichment devices. Enrichment devices were designed to deliver part of the standard daily food ration, and require maximal processing time. Enrichments included kong toys containing fruit and leaves frozen in ice, ice lollies of various sizes containing part, or all, of the fruit ration and fleece boards holding frozen fruit pieces and seeds (Reinhardt, 2008). During the enrichment treatment the daily chow ration was always placed on top of the home cage to increase foraging

time. Monkeys had to manipulate the chow, or chew them through the holes in order to reduce their size so that they would fit through the wire mesh. During the maintenance of enrichment concurrent with cognitive testing, devices were always delivered in the afternoon, after testing. In addition, extra care was taken to ensure the area around the animal housing was kept free of disturbances throughout the enrichment phase.

The timings of the environmental enrichment phases were set according to the date of the veterinary health-check. Therefore, the week of environmental enrichment was always conducted either 10 days before, or 10 days after, the date of the veterinary health-check. During the enrichment week, all monkeys housed in the enclosure were provided with environmental enrichment, regardless of whether they took part in that study or not. Where food enrichments were used, daily food rations were adjusted to maintain calorie intake. All procedures were approved by, Roehampton University ethics board and CPRC Institutional Animal Care and Use Committee, and faecal samples were shipped from Puerto to the UK under licence from DEFRA (Appendix 1).

3.4.2 Faecal sample collection

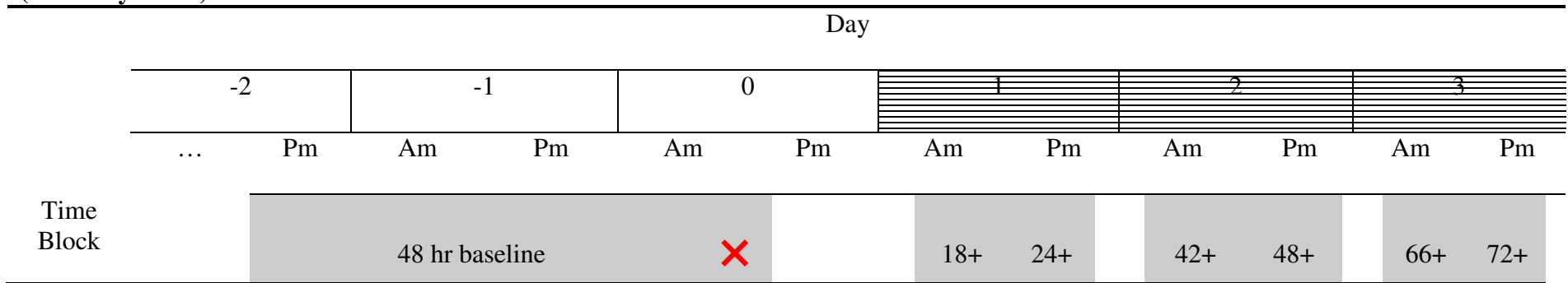
I collected faecal samples using a protocol adapted from Hodges and Heistermann (2003a). Samples were collected around three veterinary health-checks conducted between July 2006 and February 2007. Detailed collection protocol is given in Appendix 3.1. Cognitive testing was timetabled so that the first day of cognitive testing during the health-check treatment was always conducted on the day after


the veterinary health-check (Figure 3.2), and the first day of cognitive testing during the enrichment treatment was conducted on the day after seven days of enrichment had been completed (i.e. day 8: Figure 3.3).

I collected three sets of faecal samples for analysis. One set was collected to obtain data about general baseline [fGCM] when no experimental manipulations had been conducted. Two sets were collected to obtain data about changes in physiological stress in response to the two treatments: Pre- versus Post-health-check, and general baseline versus Post-enrichment. The number of monkeys from whom samples were collected at any one time was determined by time constraints imposed by other aspects of the project and the number of animals producing solid stools. A pilot study determined that samples could be collected efficiently from up to six monkeys on any single day.

Set 1 samples were collected from the eight monkeys according to the schedule for the health-check shown in Figure 3.2. Faecal samples were collected for 48 hours immediately prior to the administration of the stressor (i.e. from day -2 until 12 noon on day 0: Pre-health-check), and for 80 hours Post-health-check, concurrent with cognitive testing (days 1-3). Samples were collected for 80 hours Post-health-check aiming to capture the return to baseline [fGCM] following the anticipated peak increase Post-health-check (after Heistermann et al., 2006). On each day, samples were collected during one morning time block (6am–12 noon) and one afternoon time block (12noon–6pm). These data were used to assess changes in fGCM Post-health-check (an anticipated increase in [fGCM]: Hypothesis 3.1).

Figure 3.2 Faecal sample collection timetable during the health-check treatment. Samples were collected for two days (from day -2 pm to day 0 am) prior to the health check (conducted on day 0), and for three days Post-health-check, concurrent with any cognitive testing (from day 1 to 3).



 Time blocks for faecal sample collection

 Health check (12 noon day 0)


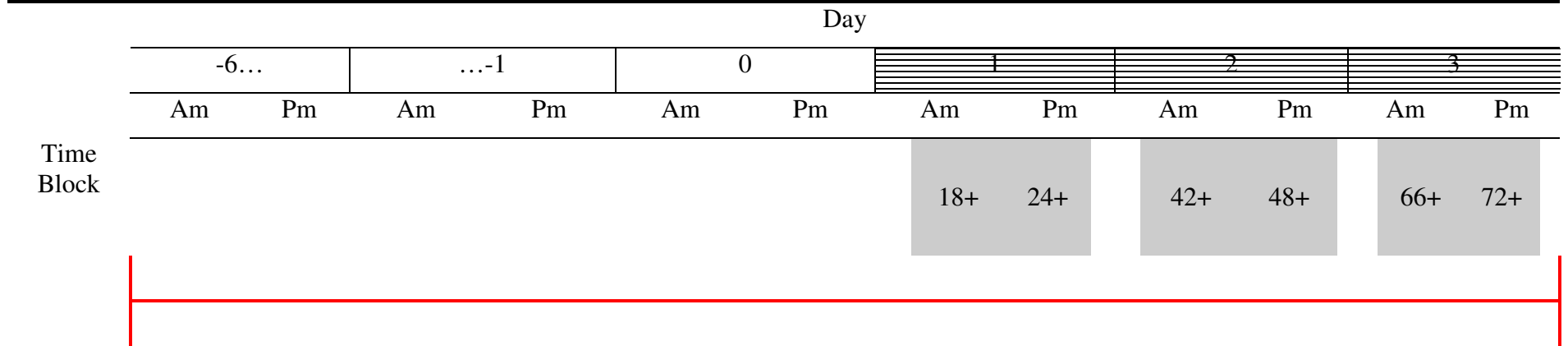


 Days on which cognitive testing was conducted

Figure 3.3 Faecal sample collection timetable used during enrichment treatment. Enrichments were provided for seven days (day -6 to 0, and maintained until day 3). Samples were collected for three days (days 1 to 3) concurrent with any cognitive testing (from day 1 to 3).



 Time blocks for faecal sample collection

 Days over which enrichment was provided

 Days on which cognitive testing was conducted

Set 2 samples were collected from the same eight monkeys according to the schedule for the Post-enrichment cognitive testing phases between July 2006 and February 2007 shown in Figure 3.3. During the Post-enrichment cognitive testing phase samples were collected during the same time windows (morning: 6am-12 noon; afternoon: 12 noon-6pm), concurrent with cognitive testing (days 1-3) for 80 hours. These data were used to calculate [fGCM] following a week of environmental enrichment, and to determine whether this differed significantly from general baseline [fGCM] (Hypothesis 3.2).

Set 3 'Baseline' samples were collected from the eight monkeys at intervals outside the cognitive testing periods between July 2006 and February 2007. Samples were collected during four-day collection blocks on days when no stressor or enrichment had been administered, when no experimental sessions were conducted, and at least three days (72hrs) after the end of any cognitive testing sessions. A 72 hour buffer period was selected to allow [fGCM] to return to baseline and stabilise following any response to the experimental manipulations (after Heistermann et al., 2006). These samples were used to determine individual baseline [fGCM] values against which changes in [fGCM] following the week of enrichment were compared (Hypothesis 3.2).

An additional eight samples were collected at random from four of the eight monkeys. These samples were used to measure the recovery rate of fGCMs from faeces using the laboratory hormone extraction procedure.

At the end of the research period all tubes containing frozen faecal samples were packed upright in foam-insulated shipping crates filled with ice packs for transportation to the UK for analysis. Crates were packed with ice-packs and shipped under a CITES licence for export of animal derivatives (licence number #06US126118/9) and a DEFRA licence for importation of animal pathogens (licence number AHZ/2537/2006/2: Appendix 1). On arrival in the UK samples were immediately placed in frozen storage at -20°C (February 2006) until the beginning of the extraction phase (March 2006).

3.4.3 Hormone metabolite extraction and measurement

To extract GCMs from solid faecal matter, I used a double extraction technique adapted from Ziegler et al. (2000). The procedure is given in full in Appendix 3.2. The efficiency of the extraction technique was validated by radioactive recovery. GCM extraction began within two weeks, and ended within six weeks, of sample transportation to the UK.

3.4.3.1 Recovering hormone metabolites from faeces

The efficacy of an assay must be validated by considering the degree of recovery of hormone or metabolite from a given substrate during the extraction procedure (Buchanan & Goldsmith, 2004). Eight faecal samples were treated with a radioactive label ($[6,7,^{-3}\text{H}]$ Oestradiol, 500dpm/50 μl) whilst still in the collection tubes, prior to running the extraction procedure. The quantity of radioactive label found in the resulting supernatant was measured in a beta-gamma scintillation counter (Beckham LS6500). Amount of label extracted into the supernatant

therefore gives a measure of the proportion of total GCM present in the original sample we may assume to be extracted using the extraction procedure described. The full procedure for recovery of labelled fGCM is given in Appendix 3.3.

The average percentage recovery across the eight samples was $104\% \pm 4\%$. A recovery rate of 100% of the GCMs from the faecal samples was therefore assumed in all calculations.

3.4.3.2. Enzymeimmunoassay

The enzymeimmunoassay (EIA: 5β -androstan- 3α - 11β -diol-17-one: Appendix 3.4.) used here has previously been validated for measurement of GCs in non-human primates (Heistermann et al., 2006) including rhesus macaques (M. Heistermann, unpublished data). Each sample was aliquoted, in duplicate, with standards, quality controls, reagents and buffers as described in Appendix 3.5.

3.4.3.3. Dilution factors and parallelism

Two microtitre dilution plates (see Appendix 3.6) were run to test for the most appropriate dilution of samples for the linear range of the assay standard curve, and to test for parallelism of sample performance to the standard curve. Six samples of extract obtained from three monkeys were used, each at five dilutions (1/10, 1/20, 1/40, 1/80 and 1/160). For each monkey, one sample expected to have a high [fGCM] and one sample expected to have a low [fGCM] were selected according to the testing conditions under which the samples were collected. A dilution factor of 1/80 produced Optical Density (OD) values that fell within the linear range of the standard curve for all samples.

To test for parallelism, OD readings were plotted against dilution factors for the sample dilutions and standard concentrations for the standard curve values. The dilution series samples mostly, but not all, were parallel to the standard dilution. It was therefore desirable that all samples were assayed at the same dilution factor, 1/80. Some samples (26/444) had to be measured at a dilution of 1/40 in order that this fell within the linear range.

3.4.3.4. Assay performance: Intra- and inter-assay variability and sensitivity

The EIA is sensitive to environmental factors, such as light and temperature, which may cause variability in readings across a single plate and between plates. To assess degree of intra-assay variation a test plate was run on which 17 high (H) and 16 low (L) concentration duplicates were measured (Appendix 3.7). Each pair of duplicates was averaged, resulting in 17 H concentration values and 16 L concentration values. The coefficient of variation (CV) was calculated as $CV = (SD / \bar{X}) * 100$ for the 17 mean H values and the 16 mean L values, separately. A predetermined value of 10% was used as the maximum acceptable level of intra-assay variability for a plate to be accepted as reliable. Intra-assay CVs for the test plate were H: 6.32% (n=17) and L: 6.46% (n=16).

To monitor inter-assay variability of the sample plates, four pairs of Quality Controls (QCs) were included on each plate (as shown in Appendix 3.5.): two pairs of a high concentration (QC_H) and two pairs of a low concentration (QC_L). The coefficient of variation (CV) was calculated for the two sets of QC_H and QC_L across all plates. The criterion level for CV was 15% variability across all plates. CVs were QC_H: 9.9% and QC_L: 14.9%. The assay was therefore considered to

have internal reliability. Sensitivity of the assay at 90% binding was 116pg/50µl. The linear range of the standard curve on plates was 120-1950pg GCM/50µl.

3.4.4 Calculations and presentation of hormonal data

3.4.4.1. Calculation of [fGCM]

The [fGCM] (ng fGCM/g faecal matter) for each sample was calculated using the following formula:

$$[\text{fGCM}] \text{ (ng/g)} = \frac{([\text{Sample}] \text{ pg/50}\mu\text{l} \times \text{Supernatant volume ml} \times \text{Dilution factor})}{(\text{Faecal weight g} \times \text{Recovery \%})}$$

Where:

[Sample]=output reading (pg/50µl).

Supernatant volume=total volume of sample supernatant (ml) following extraction.

Dilution factor=80 (where a dilution factor of 1/80 was used) or 40 (1/40).

Faecal weight=weight of dried faecal sample at the end of the drying stage (g).

Recovery=% recovery of hormone from the sample.

3.4.4.2. Data treatment and statistics

All [fGCM] are presented as ng fGCM/g faeces. Data to test hypothesis 3.1, that [fGGM] will increase Post-health-check relative to Pre-health-check, were [fGCM]s from Set 1 samples (samples collected during the 48hrs Pre-health-check and the 80 hrs Post-health-check, as shown in Figure 3.2). Criteria for inclusion in the analysis were that the -48hr to 0hr Pre-health-check baseline

[fGCM] for each monkey was derived from at least two samples, and that there was at least one sample for each of the 18+hr, 24+hr, 42+hr, 48+hr and 66+hr Post-health-check time blocks. Data for the 66+hr and (where present) 72+hr time blocks are shown for illustration of return to baseline, but were omitted from the analyses for standardisation.

Data to test hypothesis 3.2, that [fGGM] will decrease Post-enrichment relative to general baseline levels, were [fGCM]s from Set 2 and 3 samples (collected during the 80hrs Post-enrichment, as shown in Figure 3.3, and at intervals outside of testing and treatments for the general baseline). Criteria for inclusion in the analysis were that the general baseline [fGCM] for each monkey was calculated from at least two Set 3 samples, and that there was at least one sample for each of the 18+hr, 24+hr, 42+hr, 48+hr and 66+hr Post-enrichment time blocks.

Data to test hypothesis 3.3, that [fGCM] will be significantly higher Post-health-check than Post-enrichment, were [fGCM]s from Sets 1 and 2. Criteria for inclusion in the analysis were that data were available during a Post-health-check and Post-enrichment phase, per monkey, and that data were available for at least two of the 18+hr, 24+hr, 42+hr and 48+hr time blocks per condition per monkey.

There were three analyses for each hypothesis. The first analysis was a visual inspection of individual fGCM profiles in which the [fGCM] for every sample produced within an 80hr period Post-treatment (i.e. Post-health-check or Post-enrichment) was plotted along a time line (after Heistermann et al., 2006).

Baseline data were plotted as \bar{X} [fGCM] $\pm 2SD$ (shown at Time 0, after

Heistermann et al., 2006, although it is noted that Time 0 data in that paper represent values obtained from samples collected during different baseline periods). This allowed circadian patterns, peak [fGCM] and return to baseline to be identified per monkey. This informed data grouping for subsequent analyses.

The second analysis was a calculation of the maximum magnitude of increase in [fGCM] for each monkey. This was calculated as the ratio of the peak [fGCM] from 18-48hrs Post-treatment, divided by \bar{X} [fGCM] Pre-treatment (health-check treatment) or general baseline (enrichment treatment; after Heistermann et al., 2006). Heistermann et al. (2006) detected rises in [fGCM] following administration of ACTH or restraint for sedation with KHC1 in a range of primate species, in the order of 1.6–7.4 times the baseline level.

The third analysis involved grouping data for statistical analysis, for each individual, separately. To avoid confound of circadian patterns, data were separated into six-hour time blocks representing a morning (6am-12pm) and an afternoon (12pm-6pm) time block for each day. The Post-treatment time blocks are labelled as 18+hrs, 24+hrs, 42+hrs and 48+hrs. For example, the 18+hrs time block included all data from samples collected between 6am and 12pm on day 1 Post-treatment. Baseline data derived from samples collected between 6am and 12pm Pre-treatment (or during the general baseline phase for enrichment) were used for comparison with samples collected between 6am and 12pm Post-treatment (i.e. the 18+ and 42+ time blocks). Afternoon baseline data were compared with the afternoon Post-treatment data (24+ and 48+ time blocks). A series of t-tests was conducted for each monkey to investigate whether samples

produced within each Post-treatment time-block differed significantly from the equivalent (i.e. Am or Pm) baseline time block. Where data were available from more than one sample for both the baseline and Post-treatment time-block, and variance between both sets of data was equal (assessed using Levene's test), an independent t-test was conducted. Where variance was not equal the adjusted independent t-test, with equal variances not assumed, was used, as provided in SPSS. Where only one datum was available for either of the time blocks, a one-sample t-test was used, with the single value used as the test value. Bonferroni adjusted P values are used throughout. The number of samples within each time block is indicated in each of Tables 3-2 and 3-4.

3.5 Results

A total of 444 faecal samples were used in the analysis ($\bar{X}=56 \pm 39$ samples/monkey, range 14 – 137; Set 1=154 samples; Set 2=89 samples; Set 3=201 samples). The mean dried faecal weight of all samples was $0.43\text{g} \pm 0.21\text{g}$, range 0.1-1.6g. Measured [fGCM] ranged from 128–4063ng/g dried faeces.

3.5.1 Assessing physiological arousal Post-health-check

Individual [fGCM] profile plots are shown in Figure 3.4. Data from three monkeys (collected during four collection phases) met the criteria for inclusion in the analysis (monkey O22: n=13 samples; monkey 86O: n= 15 samples; monkey 66S: n=24 samples and n=24 samples collected during two different health-check phases and labelled as 'a' and 'b'). There was a general trend for a rise in [fGCM]

Figure 3.4 Individual [fGCM] profiles Post-health-check. Average 48 hour Pre-health-check baseline is shown at T0 (+2SD).

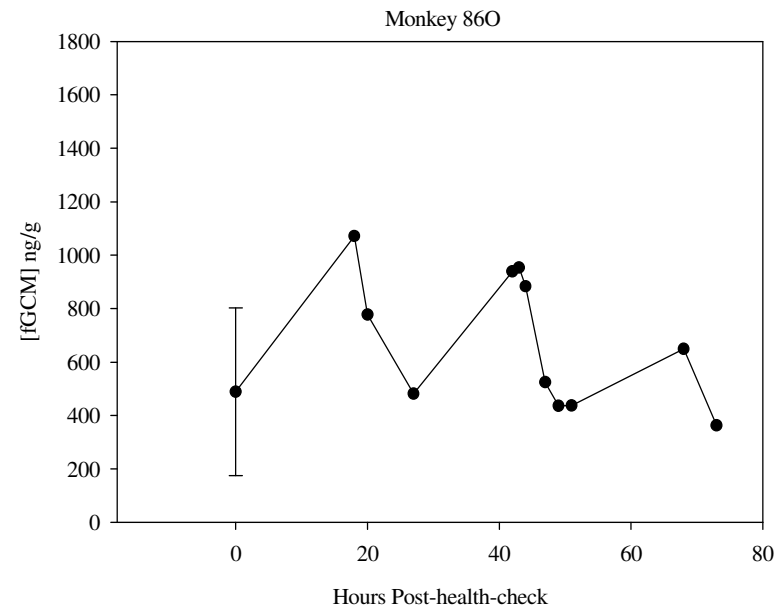
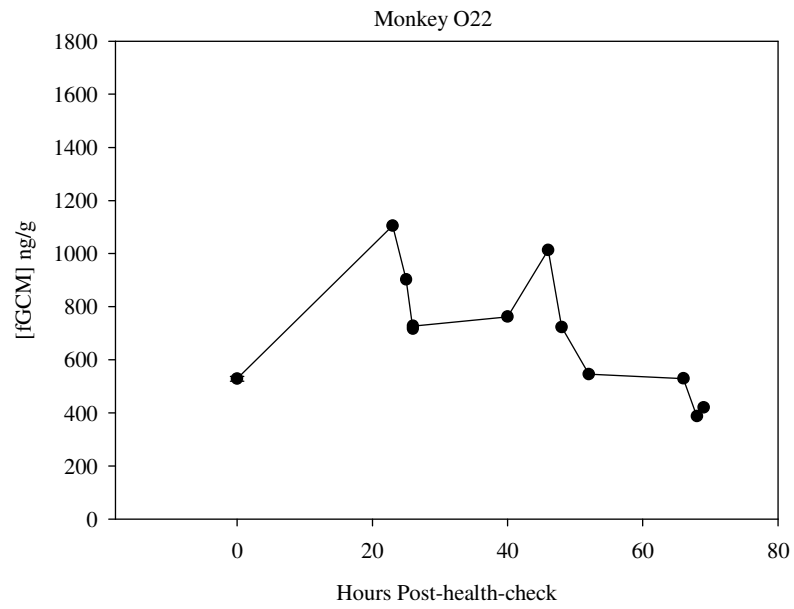
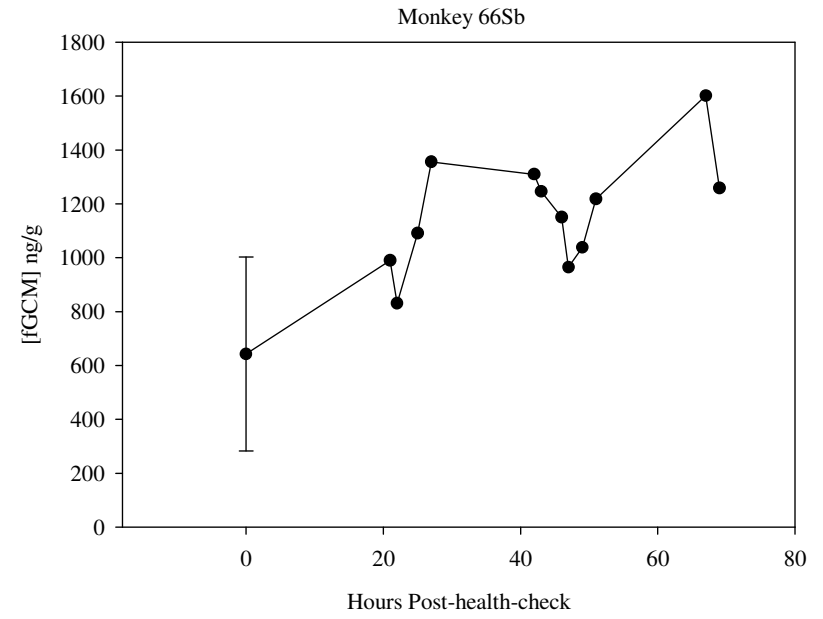
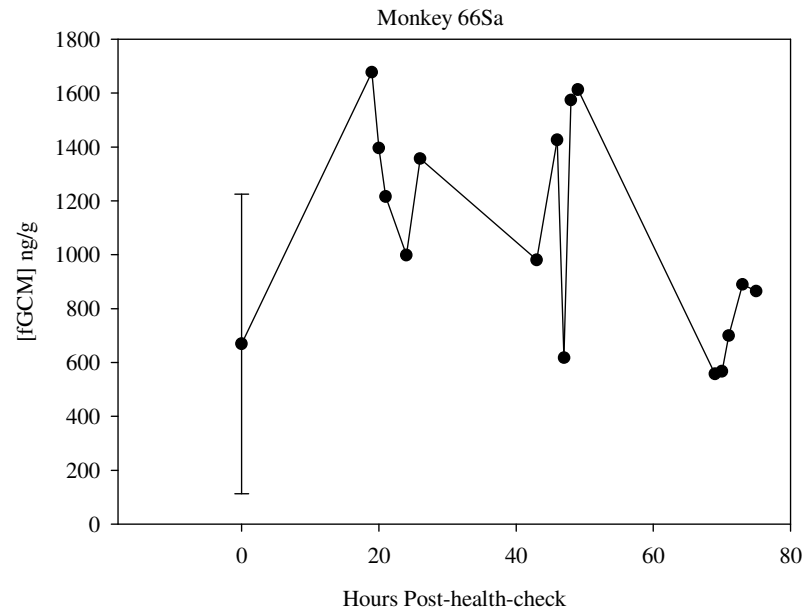


Figure 3.4 (Continued).



Post-health-check, with peaks above 2SDs of the Pre-health-check [fGCM] baseline level. For O22, 86O and 66S_a (but not 66S_b) [fGCM] showed an apparent return to baseline levels within 80 hours Post-health-check.

Data for the peak rise in [fGCM] are shown in Table 3-1. Magnitudes of increase ranged from 2.1 (monkeys O22 and 66S_b) to 2.5 (monkey 66S_a). There was no effect of baseline [fGCM] on magnitude of response ($r(4) = 0.511$, $P = 0.49$).

Table 3-1 Pre-health-check baseline [fGCM], Post-health-check (PHC) peak [fGCM], delay to peak and magnitude of maximum increase in [fGCM] in the 48 hours Post-health-check. (x) number of samples entered into analysis

ID	48Hr Baseline mean [fGCM] (ng/g)	Peak PHC [fGCM] (ng/g)	N hours PHC to peak [fGCM]	Magnitude of max [fGCM] increase
O22	529 (2)	1105 (11)	23	2.1
86O	489 (4)	1071 (7)	18	2.2
66S _a	669 (9)	1678 (9)	19	2.5
66S _b	643 (12)	1356 (8)	27	2.1

Excretion rates of GCMs into faeces were calculated from the timing of peak [fGCM] between 18-48hrs Post-health-check for monkeys O22, 86O and 66S_a. Data for monkey 66S_b were not included in the analysis since there was no return to baseline. Average delay to peak [fGCM] Post-health-check was 20hrs \pm 2.65.

Data were divided into six-hour (AM/PM) time blocks and t-tests (Bonferroni-corrected $P = 0.013$) were conducted to compare AM baseline versus AM Post-

treatment [fGCM] (i.e. 18hr+ and 42hr+ time blocks), and PM baseline versus PM Post-treatment [fGCM] (i.e. 24hr+ and 48hr+) time blocks ($\bar{X} = 2.3 \pm 0.93$ samples/time block/monkey: Table 3-2).

Table 3-2 Mean [fGCM] (ng/g) Pre-health-check, and at each of the time blocks between 18+ and 48+ hours Post-health-check. * P<0.05; ** P<0.013

ID	Pre-health-check		Post-health-check time block			
	[fGCM] (ng/g)		18+	24+	42+	48+
	(Am)	(Pm)	(Am)	(Pm)	(Am)	(Pm)
O22	529 (2)	-	1105** (1)	782 (3)	1013** (1)	635 (2)
86O	716 (1)	413 (3)	924 (2)	481 (1)	825 (4)	437 (2)
66S(a)	774 (6)	459 (3)	1430** (3)	1178** (2)	1008 (3)	1593** (2)
66S(b)	588 (6)	697 (6)	910 (2)	1223* (2)	1168** (4)	1128* (2)

The t-tests revealed no clear pattern of results. There was a significant increase in [fGCM] at 18+hrs from the AM baseline for two monkeys (O22: $t_1=92.38$, $P=0.007$; 66S_a: $t_7=3.386$, $P=0.012$; Table 3-2), a significant increase at 24+hrs for one monkey on one occasion (66S_a: $t_3=5.291$, $P=0.013$; note: 66S_b: $t_6=2.914$, $P=0.027$), and a significant increase at 42+hrs for two monkeys (O22: $t_1=77.75$, $P=0.008$; 66S_b: $t_8=7.083$, $P<0.001$). Monkey 86O showed no elevation in [fGCM] at any time-block between 18+ and 48+ hours Post-health-check.

In summary, these data indicate that the health-check did not lead to a consistent pattern of physiological response with respect to [fGCM] in male rhesus macaques. Three monkeys showed a significant rise in [fGCM] Post-treatment

compared with Pre-treatment levels, while one monkey showed no such response. The maximum magnitude of increase (≤ 2.5 times the Pre-health-check baseline) occurred, on average, 20hrs Post-health-check, and fell within the range of increase detected in other primate species (Heistermann et al 2006). These data indicate a tendency for excreted [fGCM] to rise following the health-check: for all four monkeys: [fGCM] rose above 2SD from baseline levels in the 48 hours Post-health-check, but on no occasion did they fall 2SD below the mean baseline.

3.5.2 Assessing physiological arousal Post-enrichment

Data from three monkeys met the criteria for inclusion in the analysis (monkey 94K: n=38 samples; monkey 66S: n=86 samples; monkey 79S; n=37 samples). Individual [fGCM] profiles are shown in Figure 3.5. Inspection of the individual plots revealed a general trend for Post-enrichment [fGCM] levels to fluctuate within 2SD from baseline \bar{X} [fGCM].

Data were inspected for the maximum nadir in [fGCM] Post-enrichment (Table 3-3). Magnitudes of decrease ranged from 1.4 to 2.6. To determine whether the decrease in Post-enrichment [fGCM] was significant for each individual, data were separated into six-hour time blocks as described previously ($\bar{X} = 7.5 \pm 14.2$ samples/time-block/monkey) and a series of t-tests conducted (Bonferroni $P=0.013$; Table 3-4). One monkey showed a significant decrease in Post-enrichment [fGCM] relative to general baseline (monkey 79S: 24+hrs: $t_8=4.105$, $P=0.003$; 48+hrs: $t_8=3.856$, $P=0.005$, and a trend at 18+hours). Two monkeys showed a trend for decreased [fGCM] at 48+hours Post-enrichment (monkey 66S:

Figure 3.5 Individual [fGCM] profiles Post-enrichment. General baseline from Set 3 samples is shown at T0 (+/-2SD).

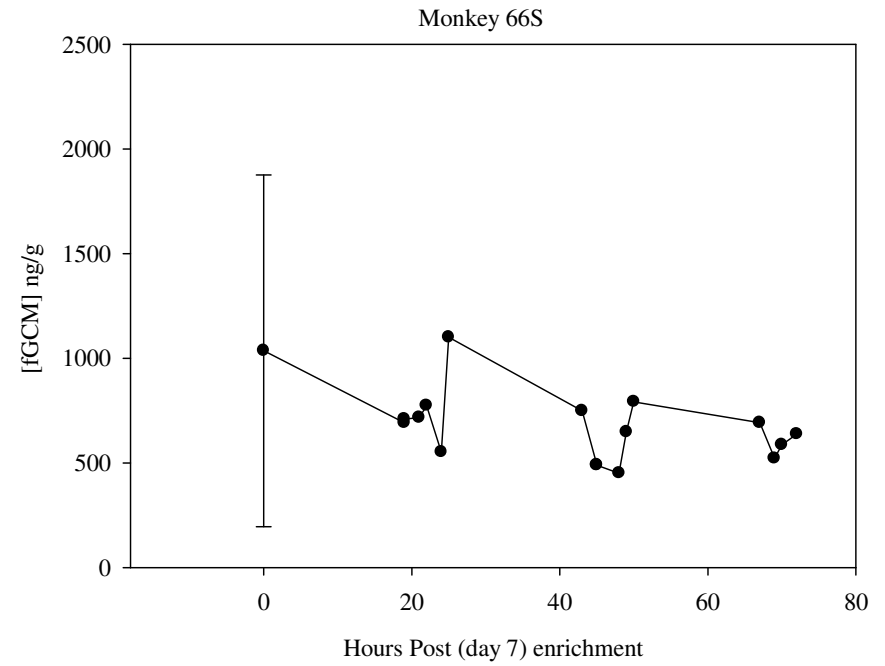
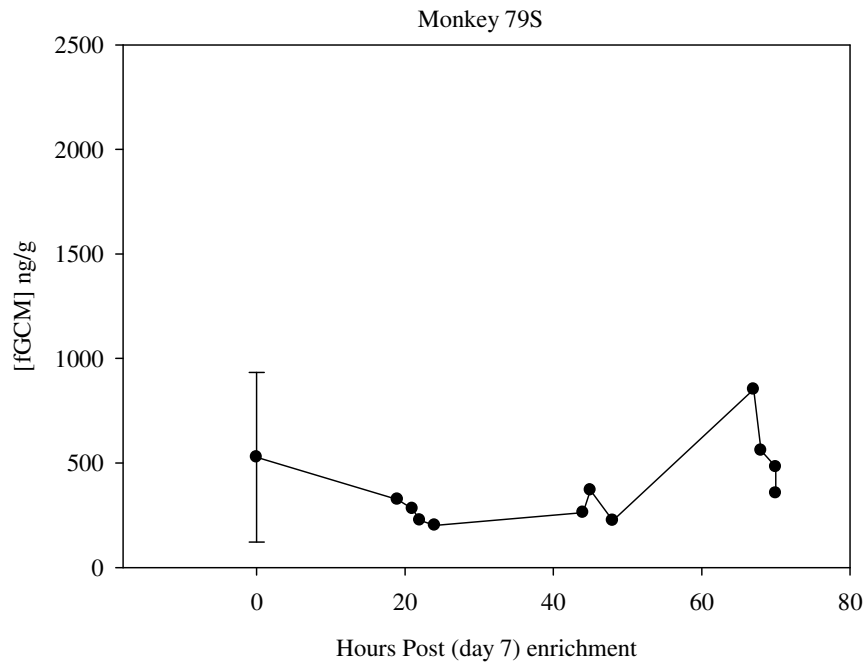
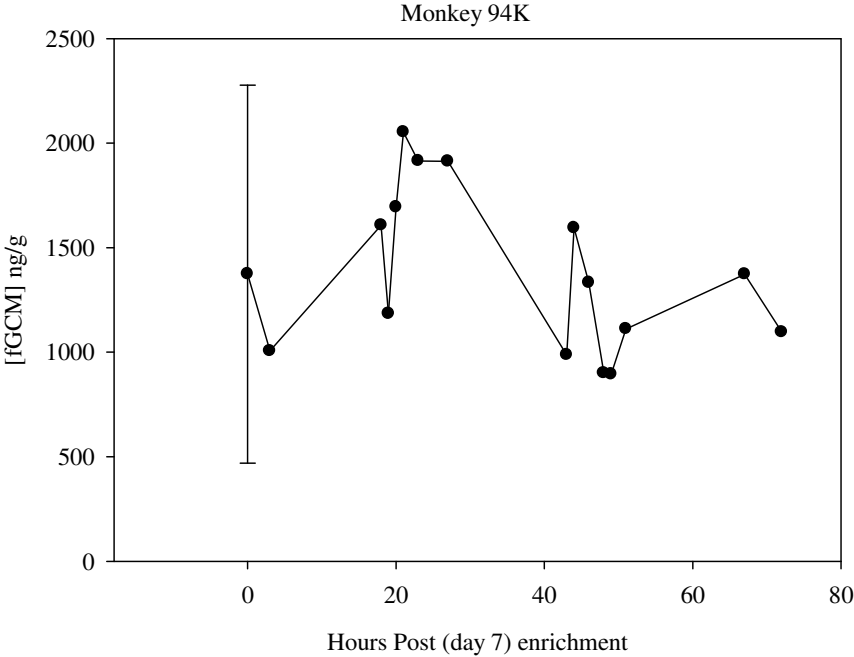


Figure 3.5 (Continued)



$t_{21}=3.210$, $P=0.04$; monkey 94K: $t_{11,9}=2.486$, $P=0.03$). Monkey 94K showed a significant increase in [fGCM] Post-enrichment relative to general baseline at 18+hrs ($t_{15}=4.248$, $P=0.001$).

Table 3-3 The general baseline [fGCM], Post-enrichment nadir [fGCM], delay to nadir and magnitude of maximum decrease in [fGCM] in the 48 hours 'Post-enrichment'. (x) number of samples from which value was derived.

ID	Baseline mean [fGCM] (ng/g)	Nadir [fGCM] (ng/g)	N hours post- enrichment to nadir [fGCM]	Magnitude of max [fGCM] decrease
79S	528 (25)	202 (7)	24	2.6
66S	1036 (70)	489 (9)	45	2.1
94K	1374 (24)	987 (10)	43	1.4

Table 3-4 Mean [fGCM] (ng/g) for general baseline, and at each of the time blocks between 18+ and 48+ hours Post-enrichment. * $P<0.05$; ** $P<0.013$; **^H Post-enrichment time-block [fGCM] higher than baseline.

ID	Baseline		Time block			
	[fGCM] (ng/g)		18+	24+	42+	48+
	(Am)	(Pm)	(Am)	(Pm)	(Am)	(Pm)
79S	498 (16)	580 (9)	278* (3)	202** (1)	316 (2)	225** (1)
66S	995 (50)	1141 (20)	723 (4)	826 (2)	576 (3)	630* (3)
94K‡	961 (12)	1786 (12)	1691** ^H (5)	1913 (1)	1305 (4)	969* (1)

‡ Monkey 94K exhibited extreme signs of frustration on days 2 and 3 of the Post-enrichment testing phase. He repeatedly shook his cage and stopped working on the cognitive task on both days. Monkeys 66S & 79S showed no such behavioural signs of frustration during the equivalent testing phase.

These data suggest that for one monkey (79S) the enrichment treatment resulted in significantly lower [fGCM] relative to general baseline levels. For one monkey (66S)

there was no significant change, and for one monkey (94K) the enrichment treatment resulted in a significant rise in [fGCM].

In summary, these data indicate that a week of environmental enrichment did not result in a consistent pattern of physiological response with respect to [fGCM] in male rhesus macaques. One monkey exhibited significantly lower [fGCM] relative to a general baseline during the period for which samples were analysed. One monkey showed no change in [fGCM] and a third monkey exhibited a significant rise in [fGCM] relative to general baseline, following the enrichment treatment. These data suggest the need for a larger sample size and consideration of possible individual differences in responses to environmental enrichment in singly housed male rhesus macaques.

3.5.3 Assessing differences in physiological arousal Post-health-check versus Post-enrichment

Five monkeys met the criteria for inclusion in the analysis (06H, 94K, 66S, 79S & 79T). Data were available for each monkey from one Post-health-check and one Post-enrichment testing phase (Post-health-check: $n=67$ samples, $\bar{X}=2.5\pm 2.0$ samples/time block/per monkey; Post-enrichment: $n=60$ samples, $\bar{X}=2.3\pm 1.4$ samples/time block/monkey). Individual comparative [fGCM] profiles are shown in Figure 3.6. Inspection of the individual plots revealed a general trend for higher [fGCM] Post-health-check than Post-enrichment.

To determine whether [fGCM] differed significantly between Post-health-check and Post-enrichment phases, data were clumped into six-hour time blocks and a series of independent or one-sample t-tests conducted (Table 3-5: Bonferroni adjusted $P=0.013$). For each test, data were [fGCM] Post-health-check within a given time block versus [fGCM] Post-enrichment within a given time block, for each monkey, separately.

Two of the five monkeys showed significantly higher [fGCM] Post-health-check versus Post-enrichment, as predicted. Monkey 66S showed significantly higher [fGCM] Post-health-check versus Post-enrichment at two time blocks (18+hrs: $t_5=6.183$, $P<0.01$; 48+hrs: $t_3=7.504$, $P<0.01$). Monkey 79S showed significantly higher [fGCM] Post-health-check at 24+hrs ($t_1=54.74$, $P=0.01$). Two monkeys showed no difference in [fGCM] between the two treatments (monkeys O6H & 79T).. Monkey 94K showed the opposite pattern, with significantly higher [fGCM] Post-enrichment at 48+hrs ($t_3=5.413$, $P=0.01$), and a trend for higher [fGCM] Post-enrichment at 18+hrs ($t_4=3.87$, $P=0.02$).

Planned comparisons were conducted to examine group-level trends in [fGCM]. Missing data precluded a RMANOVA, therefore a permutations test was used (Mundry, 1999: see Chapter 4, p201). In brief, this test is suitable for data sets with small sample size, with missing cell values, where data may not be normally distributed and where the same individuals are used in the different pairwise comparisons. This revealed no significant group-level difference in [fGCM] at any time blocks between the two treatments (all pairwise comparisons $P>0.08$).

Figure 3.6 Individual [fGCM] profiles Post-health-check (●) and Post-enrichment (○).

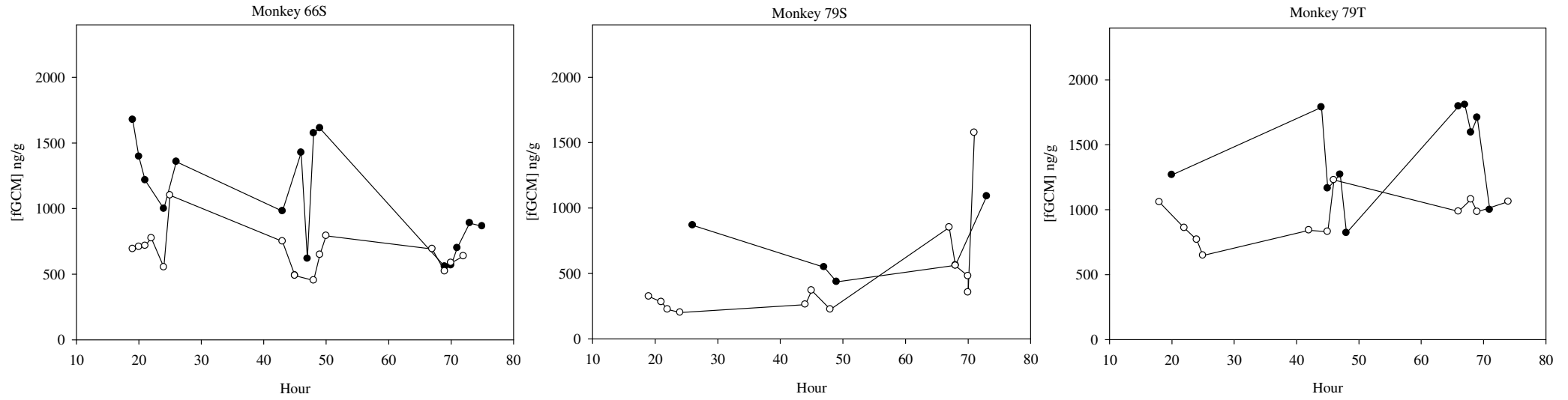


Figure 3.6 (Continued)

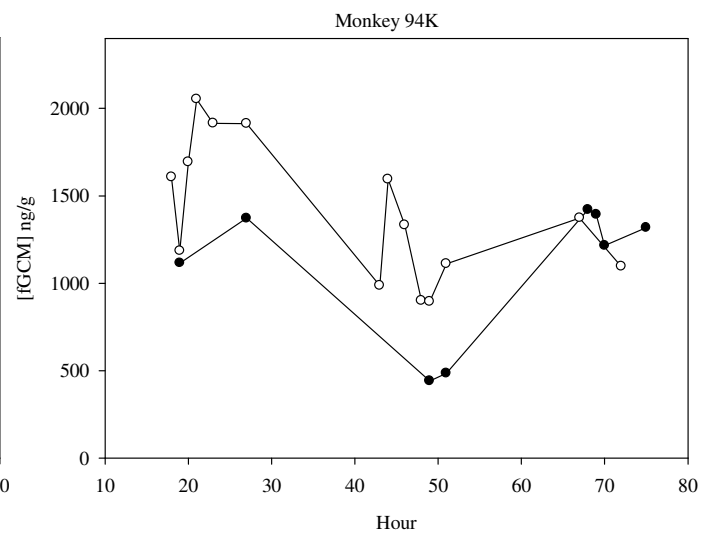
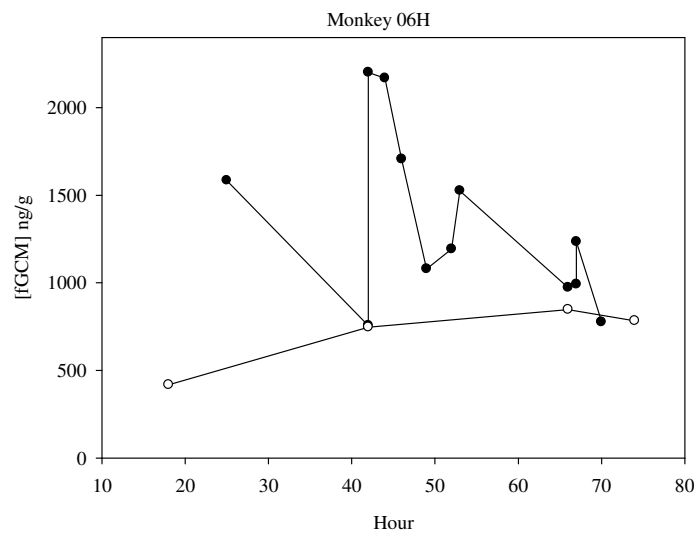


Table 3-5 Mean [fGCM] (ng/g) Post-health check (PHC) versus Post-enrichment (PE) at each time block. * P<0.05; ** P< 0.013; **^H Post-enrichment [fGCM] higher than Post-health-check [fGCM]

ID	Time block/ Treatment							
	18+		24+		42+		48+	
	PHC	PE	PHC	PE	PHC	PE	PHC	PE
66S	1430**	723	1178	826	1008	576	1593**	630
79S	-	303	869**	214	549	317	436	225
06H	-	418	1584	-	1708	747	1266	-
79T	1268	959	-	708	1408	967	822	-
94K	1115	1691* ^H	1372	1913	-	1305	463	969** ^H

In summary, these data indicate varied physiological profiles following the two treatments. For two monkeys, the health-check resulted in higher [fGCM] relative to the same time blocks during the enrichment treatment. For two monkeys there was no difference in [fGCM] between the two treatments, and for one monkey [fGCM] was higher Post-enrichment versus Post-health-check, contrary to the predicted pattern of response.

3.6 Discussion

The effect of two treatments on levels of excreted faecal glucocorticoid metabolites in male rhesus macaques was assessed. Overall, the data suggest increased [fGCM] following a health-check: 3/4 monkeys showed significantly

elevated [fGCM] Post-health-check compared with a Pre-health-check baseline, while a fourth monkey showed no such increase. There was no evidence for the predicted decrease in [fGCM] following a week of environmental enrichment: one each of three monkeys showed significantly elevated, significantly decreased, and no significant change in [fGCM]. When Post-health-check [fGCM] was compared against Post-enrichment [fGCM] at each of the time blocks two monkeys showed significantly higher [fGCM] Post-health-check, two monkeys showed no difference between the two treatments, and one monkey showed significantly higher [fGCM] in the enrichment treatment (versus Post-health-check). These data suggest the pattern of fGCM variation is not clear-cut, and must therefore be interpreted with caution, for example, due to individual differences in response.

The data presented here partially concur with previous studies which demonstrate increased indices of cortisol in primates following contexts assumed to be negative (Sapolsky et al. 2000; Bergman et al., 2005; Honess & Marin, 2006a). For example, restraint for injection has been shown to result in elevated levels of plasma cortisol in captive rhesus macaques (Fuller et al., 1984; Bentson et al., 2003; Ruys et al., 2004) and excreted metabolites of cortisol in faeces (Heistermann et al., 2006).

The data presented here do not concur with previous studies which found reduced indices of cortisol in primates following contexts assumed to be positive (insofar as those situations meet likely appetitive drives). For example, Shutt et al. (2007) measured [fGCM] among free-ranging female Barbary macaques (*M. sylvanus*) and found a negative correlation between [fGCM] and amount of grooming given,

suggesting that the giving of grooming may function to reduce stress in this species. Doyle et al. (2008) measured [fGCM] in singly housed male rhesus macaques Pre- versus Post-pair-housing. Monkeys showed a significant decrease in [fGCM] between the single-housing and the settled pair-housed condition, suggestive of a reduction of social-isolation related stress.

The findings here are in agreement with studies that report no reduction in plasma cortisol among rhesus macaques provided with environmental enrichment (Schapiro et al., 1993). The current findings are of particular interest since one criticism of Schapiro et al. (1993) is the use of blood draw to collect samples for analysis. The lack of evidence for reduced [GC] in both studies suggests environmental enrichment may not always lead to a reduction in physiological indicators of stress such as circulating, or excreted, glucocorticoids. Alternatively, environmental enrichments may lead to increased physiological markers of general arousal not associated with stress (eg increased motor activity).

Several issues in the use of non-invasive analysis of cortisol indicators arose during the study. Sample collection, storage, shipping and analysis were time-consuming and relatively expensive. There was some waste of samples, where too few samples were available to allow a cortisol profile to be established. There were limiting factors such as (possible) constipation, and diarrhoea, the latter particularly in response to acute stressors (for example, during a storm). These limitations resulted in a small sample size, and missing cell values in some analyses, both of which reduced statistical power. The large inter-individual variation and small sample size makes interpretation of the results difficult.

Monkeys showed a large degree of variation in the Pre-treatment [fGCM] levels. This may reflect true individual differences in baseline cortisol levels, or may be an artifact of the timing of the collection of baseline samples (e.g. following a stressor such as a storm). Monkeys also differed in the direction of change in cortisol Post-treatment. Again, this may reflect true differences in physiological stress-responsivity to a stressor (the health-check), reflect different psychological responses (eg appraisal of the treatments as more or less negative or positive), or may simply be an artifact of the differences in Pre-treatment baselines.

In summary, the analysis of excreted cortisol metabolites in faeces revealed no clear pattern of physiological response to two treatments, nor did it reveal a clear difference in the physiological profiles of the monkeys during the cognitive testing phase of each treatment. Analysis of the data was hampered by small sample sizes and missing data. A fuller interpretation of the effects of the treatments on the monkeys should rely on additional data such as those obtained from behavioural and cognitive approaches. However, although the current data are insufficient to clearly either rule in or rule out (in a statistical sense) a clear contrasting patterns of HPA function between the two treatments the small differences in physiological arousal found between the two treatments suggest a weak trend for increased arousal Post-health-check, and reduced physiological arousal following the week of enrichment. Differences in physiological arousal should therefore be considered when interpreting data from the cognitive studies presented in Chapters 4-6.

Part B: Assessing behavioural indicators of wellbeing and stress

3.7 Introduction

Behaviour is used widely to document changes in inferred affect in primates (Capitanio, 1999; Aureli et al., 2002; Novak, 2003; Honess & Marin, 2006a). The behaviour of free-ranging primates such as rhesus macaques, and the contexts in which these behaviours occur, have been well documented (Chevalier-Skolnikoff, 1973; van Hooff, 1976; Rawlins & Kessler, 1986a). Importantly for the current research, changes in captive rhesus macaque behaviour following certain experimental manipulations or husbandry routines have been used to document the stress-inducing or stress-abating effects of these procedures (Schapiro et al., 1993; Novak et al., 1998; Honess & Marin, 2006b; Bassett & Buchanan-Smith, 2007). Here, I introduce traditional behavioural measures used to assess the welfare of rhesus macaques housed in captivity, and present data on the extent to which two treatments (introduced previously: a week of enrichment and a health-check) induced changes in behaviour indicative of underlying changes in affect.

3.7.1 Behaviour as a measure of affect in primates

Behaviour is a widely used proxy measure of affective state in primates (Baker & Aureli, 1997; Novak, 2003). The behavioural displays of rhesus macaques have been well documented in free-ranging (Widdig, 2002; Maestriperi, 2007) and captive groups (Sackett et al., 1981; Augustsson & Hau, 1999; ILAR, 2008). We have reliable lists of natural patterns of rhesus macaque behaviour, and the contexts in which these behaviours occur are used to infer possible underlying

affective states associated with those behaviours. Behaviours that occur in contexts that are presumed to be positive are considered to reflect positive affect (e.g. play behaviour, allogrooming: Honess et al., 2004). Behaviours that occur in contexts assumed to be negative are considered to reflect negative affect (e.g. self-directed displacement activities such as scratching: Maestriperi et al., 1992; Baker & Aureli, 1997; Kutsukake, 2003).

In free-ranging primates, self-directed behaviours such as scratching and hair-pulling occur most frequently in situations of uncertainty, and are therefore considered to be associated with anxiety-like affective states (Maestriperi, 1993; Baker & Aureli, 1997; Kutsukake, 2003). These are context-dependent and subside relatively quickly (Baker & Aureli, 1997). However, the extent to which behaviours provide a direct measure of affect, or whether the same behaviour may have different affective underpinnings in different contexts is not known (e.g. Ruys et al., 2004). For example, Maestriperi (1993) suggests the increased vigilance and scratching seen in female rhesus macaques when their infants were in spatial proximity to the adult male or higher ranking females reflect differential components of anxiety. Maestriperi (1993) suggests increased vigilance reveals anticipation of danger, while scratching reveals uncertainty due to motivational conflict. Studies of scratching rates following aggression have shown that rate of scratching decreases faster when animals reconcile post-conflict than when they do not (Aureli et al., 1989). Furthermore, the presence of conspecifics may alleviate self-directed behavioural and physiological responses to aversive events (social buffering hypothesis: Kikusui et al., 2006).

Neuropharmacological data provide more direct evidence for behaviour-affect associations. Rhesus macaques are used as a primate model of human affective disorders such as anxiety (e.g.; Schino et al., 1996; Palit et al., 1998), post-traumatic syndromes and depression (e.g. Paul et al., 1996; Hugo et al., 2003). Anxiolytic drugs (drugs which reduce anxiety in humans) temporarily reduce the frequency of stereotypical behaviours in rhesus macaques (Schino et al., 1996; Hugo et al., 2003). Anxiogenic drugs (drugs which induce anxiety in humans) increase the frequency of stereotypical and self-directed behaviours in captive rhesus macaques (Schino et al., 1996; Palit et al., 1998). In both humans and primates, social stress is considered a primary contributing factor to the onset of depressive symptoms (Shively et al., 2009).

In captivity, primates may develop specific patterns of behaviour that are not seen in free-ranging populations. Stereotypical and self-injurious behaviours are associated with anxiety or pathologies associated with captivity, especially poor housing, rearing history and social isolation (Novak et al., 1998; Novak, 2003). In particular, early separation from the mother, and social isolation during infancy, have the most profound effects on the development of long term stereotypical and self-injurious behaviours in primates (Novak, 2003; Latham & Mason, 2008). By noting the conditions under which stereotypical and self-injurious behaviours arise, it is possible to identify conditions that reflect poor welfare for captive primates. In addition, self-directed behaviours may be used to identify anxiety-eliciting situations for primates in captivity. For example, self-scratching occurs at higher rates in poor housing, and may be abated with improved housing (Fontenot

et al., 2006) or may occur in response to temporary stressors such as high visitor numbers at zoos (lowland gorillas: Carder & Semple, 2008).

Together, ethological and neuropharmacological data suggest behaviours observed to occur in contexts assumed to be anxiety-eliciting, may arise from affective mechanisms also involved in human affective states, including anxiety. Data from captive primates suggest that in conditions of poor housing or social isolation, new sets of behaviours (i.e. behaviours not seen in free-ranging populations) may arise. The strong association of these behaviours with poor housing and social (particularly developmental) isolation, suggest a link with psychopathology and poor psychological welfare.

One difficulty with interpreting animal behaviour is that there is great inter- and intra-species variation in both the context in which a given behaviour may be performed, and the behaviours performed in a given context (e.g. Clarke et al., 1981; Sackett et al., 1981; de Waal & Luttrell, 1989). The extent to which behaviour can be used as a valid indicator of affect, therefore, is debated. The National Research Council states that there are very few direct behavioural correlates of stress, and even fewer, if any, for psychological distress in animals (ILAR, 2008). Recognising stress and distress in captive animals based on behavioural changes alone therefore remains a significant challenge, and indirect measures such as behaviour are typically used. Despite difficulty in interpreting indirect measures such as behaviour, the prevalence of behavioural data in the published literature, and the relatively non-invasive means by which such data

may be collected make them a useful proxy measure of welfare (Dawkins, 2004). Further, the consideration of behavioural indicators of affect may allow comparability between studies.

3.7.2 The influence of husbandry procedures on behaviour in captive rhesus macaques

Changes in behaviour following changes in physical or social environment have been used as indicators of improved or decreased wellbeing in captive animals. In Part A of this chapter I introduced two husbandry procedures conducted at CPRC that were used during the studies presented in chapters 4-6 in this thesis to induce changes in inferred affective state in adult male rhesus macaques. These were a week of enrichment and the three-monthly health check. Welfare studies have revealed that enrichment and husbandry interventions may have an impact on captive primate behaviour (Novak et al., 1998; Honess et al., 2004; Honess & Marin, 2006b).

Behaviours associated with negative affect in captive rhesus macaques have been revealed in studies in which aspects of housing, rearing history or husbandry procedures have been manipulated (Lutz et al., 2003b; Honess & Marin, 2006b). Paulk et al. (1977) monitored the behaviour of monkeys moved from standard to smaller caging. There was an increase in stereotypical behaviours, with individual variation in the types of stereotypies exhibited. In a similar study (Eaton et al., 1994), singly-housed rhesus macaques were moved to pair-housing, and behaviours compared against a single – pair – single-housed control group.

Controls engaged in more self-directed, self-injurious behaviour and stereotypical behaviours (including auto-grooming, nail-biting, hair-pulling and cage licking). It was suggested these behaviours were performed in the absence of the ability to perform social affiliative behaviours with a cage-mate. Controls also moved less than paired animals. Paired animals did not show a reduction in abnormal behaviours. Maintenance of abnormal behaviours (for >36 months) in paired animals was interpreted as evidence that social housing may not improve well-being for all monkeys. Data on reproductive and immunological factors indicated that singly-housed females had higher rates of infant mortality, but no significant reduction in health status (Eaton et al., 1994). Honess et al. (2004) recorded the Pre- and Post- air transportation behaviours of juvenile long-tailed macaques. They found an increase in inactivity and negative behaviours (especially hugging between cage mates, a behaviour associated with fearful contexts) immediately following transportation. Several weeks following transportation monkeys continued to show altered patterns of social interaction, with a reduction in allogrooming and an increase in play-fighting, which the authors argue reflect maintained behavioural or social disruption.

Monkeys raised in social, often sensory, isolation are at increased risk of developing stereotypical behaviour (Isolation syndrome: Sackett et al., 1978 and 1981). Stereotypical behaviours associated with isolation syndrome include stereotyped locomotion, rocking, repetitive, self-directed and aggressive behaviours. Lutz et al. (2007) monitored monkeys raised from birth to 10 months in a single cage with an artificial surrogate. These monkeys exhibited significantly

higher rates of self-biting behaviour than monkeys who were mother-reared or reared in peer groups of four monkeys for the first 10 months of life. Self-biters also engaged in fewer social interactions or contact. Reinhardt (2008) makes a distinction between self-biting and hair-pulling, which are forms of self-injurious behaviour, and stereotypies associated with anxiety and distress such as self-directed and stereotypical behaviours he believes not to be unequivocal indicators of stress (such as pacing and headflipping which may instead be coping mechanisms). The link between stereotypical behaviours and well-being is ambiguous, since their presence may reflect a proactive coping strategy that monkeys who do not exhibit the same behaviours lack. Data from a range of animals suggest that individuals with proactive stereotypical coping strategies suffer fewer stress-related illnesses, such as stomach ulcers, than their reactive coping counterparts (e.g. mice, rats and pigs: Koolhaas et al., 1999). Depressed posture is also associated with social isolation (Reinhardt, 2008).

Husbandry and research procedures may be particularly disruptive to captive animals (Waitt et al., 2002). Clarke et al. (1988) measured behavioural and blood cortisol responses to restraint, harnessing and transport in a cage. Rhesus macaques showed elevated levels of locomotion immediately following the stressor with a decrease over time, and corresponding increase in time spent inactive. Clarke et al. (1994) measured behavioural and heart-rate (HR) responses of three species of macaque (rhesus, bonnet and cynomolgus) to a novel environment (mild stressor) and physical restraint (acute stressor). Rhesus macaques had the lowest baseline HR, compared with bonnet or cynomolgus

macaques, and showed the fastest return to baseline levels following introduction to the novel environment, but HR remained high following physical restraint. HR was negatively correlated with rates of locomotion in the rhesus macaques, suggesting locomotion (and general motor activity) does not provide a reliable measure of arousal, and low levels may reflect tonic immobility (although the authors do not discuss learned helplessness as a possible explanation). HR was considered to provide a more reliable measure of psychological distress than behavioural measures in rhesus macaques. Comparison with other species suggests species-specific patterns of stress responsivity mean behaviours are not generalisable between even closely related species (Clarke et al., 1994).

These studies reveal that poor housing or rearing conditions or other negative contexts may lead to an increased incidence of self-directed, stereotypical and self-injurious behaviours. High rates of self-directed behaviours are associated with anxiety-inducing situations in both free-ranging and captive populations. Stereotypical behaviours are associated with social isolation (e.g. single-caging) although it is not known whether they provide a direct indicator of negative affect, or active coping strategies to reduce arousal under conditions of acute stress. Self-injurious behaviours are most common in monkeys with disrupted social development and dysregulation of the HPA axis. Self-injurious behaviours are considered an extreme pathological behaviour. There is converging evidence that self-injurious behaviours may function as a coping strategy to reduce arousal (Novak, 2003).

Changes in physical and social environment may also induce behavioural changes associated with positive affect, although in captive animals such changes arguably reflect reduced negative affect rather than ‘positive affect’ *per se* (Olsson & Westlund, 2007). Fontenot et al. (2006) measured behavioural responses of rhesus macaques following transfer from single indoor cages to single- or group- housing outdoors. There was a significant reduction in rates of self-biting, stereotypical, object-directed, yawning and scratching behaviour in both groups, and a reduction in pacing and autogrooming among group-housed monkeys only. There was no effect on self-injurious behaviour. These results were considered to reflect reduced anxiety following the improvements in the physical and social environments (daylight, larger enclosures, social housing), as well as opportunity to engage in more (‘positive’) behaviours due to the increase in space and opportunity for social contact (Fontenot et al., 2006). However, rates of scratching and autogrooming returned to baseline levels within 12 weeks.

In another study of social housing effects, Doyle et al. (2008) recorded behavioural and physiological (heart rate and faecal cortisol) responses of 80 singly-housed male rhesus macaques pre- and post-pairing. Faecal cortisol levels and heart rate declined post-pairing, as did ‘indices of psychological disturbance’ (body shake, scratch, autogroom, depressed posture, vigilance and yawning), time spent foraging and time inactive. An initial peak in foraging time immediately following introduction was suggested to be a displacement activity to relieve stress. There was no change in abnormal behaviour overall. This was suggested to reflect a response to accumulated time spent in caging.

In a study of the effects of visual barriers on the behaviour of captive rhesus macaques, addition of visual barriers was found to have no effect on overt behaviours such as aggressive threats or bared teeth displays, although there was an increase in the amount of time monkeys spent in close proximity (Basile et al., 2007). The low frequency of aggressive and bared-teeth displays made analysis problematic, highlighting the problem of monitoring changes in behaviours that may occur infrequently.

These studies reveal that, in primates, improved social or physical context may lead to a reduction in behaviours typically associated with negative contexts. It was therefore predicted that the enrichment treatment would lead to a reduction in behaviours assumed to reflect negative affect in monkeys: self-directed, stereotypical, and self-injurious behaviours. The health-check treatment was assumed to be negative and, therefore, it was predicted that an increase in self-directed, stereotypical, and self-injurious behaviours would occur Post-health-check.

3.7.3 Developing an ethogram for measuring captive male rhesus macaque behaviour

As a result of the literature review, it was identified that most available ethograms define rhesus macaque behaviour in the broad categories of self-maintenance behaviours (natural behaviours such as feeding, drinking, resting, sleeping and locomoting that are involved with day-to-day survival), affiliative behaviours (e.g. lip-smack and coo vocalisation), aggressive behaviours (e.g. staring threat face,

threat bark) and self-directed, stereotypical behaviours and self-injurious behaviour (e.g. scratching, hair-pulling, pacing, rocking).

Self-directed, stereotypical and self-injurious behaviours were identified as behaviours likely to increase Post-health-check, and subside Post-enrichment. Locomotion was not included as a behaviour of special interest since both increased and decreased levels of locomotion may be associated with negative contexts, and so the link between locomotion and affect may be highly dependent on context (cf. Clarke et al., 1988). Aggression has been used by some authors as a measure of negative affect. However, others view it as a natural (coping) response to threatening stimuli (e.g. Sackett et al., 1981). Sackett et al. (1981), in a study of social isolation effects on pigtailed, crab-eating and rhesus macaques, list aggression as a positive social behaviour, insofar as it is behaviour that animals with normal social functioning would be expected to exhibit. Further, there are varied definitions of aggression. For example, Honess and Marin (2006b) identify self-injury as a form of aggressive behaviour (redirected aggression), whereas Lutz et al. (2003a) argue self-injurious behaviour occurs in response to stressful situations, while aggression is mediated by social context.

The similarity between stereotypies and self-injurious behaviour seen in captive primates and those exhibited in some human psychopathologies is well documented (Hugo et al., 2003; Lewis et al., 1996; Lutz et al., 2003b). In addition, Kalin (1999) differentiates between offensive and defensive aggressive behaviours in primate models of human aggression. In this approach, offensive

aggression is an active coping strategy associated with reduced serotonergic activity, increased levels of testosterone, and lower levels of cortisol, while defensive aggression is linked to anxiety and is associated with extreme right frontal brain activity and high cortisol levels.

The ethogram I developed for the present research was designed to meet several criteria (after Bakeman & Gottman, 1997). Firstly, the ethogram was designed to provide an exhaustive list of behaviours suggested by previous research to be indicative of affect in rhesus macaques, with emphasis on non-social behaviours, since the subjects for the present study are monkeys in single housing. Secondly, the behaviours selected were required to demonstrate a clear distinction between behaviours associated with negatively-valenced affective state and those associated with positively-valenced affective states. Thirdly, the ethogram needed to allow all other (e.g. self-maintenance) behaviours to be coded.

Here, I present the development of the ethogram and present data from a preliminary study which was conducted to test the applicability of the ethogram developed during the initial literature review, and to test the software and analysis package. This development phase was conducted with the captive rhesus macaque colony at the Psychology Department of Oxford University, UK (2005). Having developed the ethogram and tested the software and analysis package in the UK, I then tested the reliability of the ethogram at the CPRC, Puerto Rico (2006). The finalised ethogram was then used to quantify changes in behavioural indicators of stress resulting from the husbandry manipulations conducted during 2006-2007.

3.8 Aims:

The general aim of this section is to assess the extent to which the two treatments (a week of enrichment and a health-check) lead to changes in behaviour indicative of changes in inferred affective state in captive rhesus macaques over three days during each treatment. To do this, I first developed an exhaustive and mutually exclusive ethogram of behaviours in captive male rhesus macaques. This was done at the Department of Experimental Psychology, Oxford University, UK. Secondly, I assessed the reliability of the ethogram as the degree of agreement between two observers in its application. This was conducted at the CPRC, Puerto Rico. Thirdly, I applied the ethogram to document behavioural changes Pre-versus Post-enrichment, Pre- versus Post-health-check, and to assess differences in behaviour between the two treatments: The hypotheses relate to the application of the ethogram.

3.9 Hypotheses:

3.4 Monkeys will spend an increased proportion of time engaged in stress-related behaviours in the 72 hours Post-health-check versus the 72 hours immediately Pre-health-check.

3.5 Monkeys will spend a decreased proportion of time engaged in stress-related behaviours during the last 72 hours of a 10 day enrichment phase versus the 72 hours immediately Pre-enrichment.

3.6. Monkeys will spend an increased proportion of time engaged in stress-related behaviours in the 72 hours Post-health-check versus the last 72 hours of a 10 day enrichment phase.

Aim 1: Developing the ethogram

3.10 Methods:

3.10.1 Animals & Apparatus:

Five adult male rhesus macaques (Hugh, Jamie, Jared, Jip and Shiny) housed in the Department of Experimental Psychology, Oxford University, UK, were observed during the development of the ethogram. Three monkeys (Hugh, Janie and Jip) were singly housed in metal cages (0.9 x 0.9 x 1m), one monkey (Shiny) was singly housed in the same size metal cage with access to two further cages (total area 2.7 x 0.9 x 1m), and one monkey (Jamie) was pair-housed with a more dominant male in a metal cage (0.9 x 0.9 x 1m) with adjoining wood and mesh enclosure (1.5 x 3 x 4m). Caging was positioned along two opposing walls in each of two rectangular animal housing rooms in the case of the four singly-housed individuals. Four monkeys (Hugh, Jared, Jip and Jamie) were housed in rooms that contained at least 10 other male rhesus macaques. Hugh, Jared and Jip were housed together throughout the study. Jamie was pair-housed in a second room. Shiny was housed in a room that contained just one other adult male rhesus macaque.

Behavioural data were recorded on an IBM ThinkPad 755CD notebook computer using JWatcher™ 0.9 software (JWatcher Animal Behaviour Laboratory Macquarie University, Australia, 2000). This software package was specifically designed for the continuous recording of behaviours, incorporating an event recorder that logs the moment at which a key (signifying a pre-specified behavioural code) is pressed. The software is flexible so that any given behaviour may be treated as both a state and event, and codes may be redefined as mutually exclusive, or not, during analysis. A 26-behaviour pilot ethogram constructed from published data on behaviours exhibited by rhesus macaques both in the wild and captivity (see Table 3.6), was used to guide the initial continuous behavioural coding. An additional category ‘out of view’ was included for animals that could not be seen clearly, for example when turned away or obscured by objects in the cage. All animals were in good health at the time of observation and were under regular veterinary supervision. No food deprivation schedule was employed and animals had access to water *ad libitum* day and night while in the home cage.

3.10.2 Procedure

Data were collected throughout June and July 2005. Monkeys were each observed for one morning and one evening session on randomly selected days over a total of five weeks. Morning observation sessions were conducted between 08:00-11:30 hours and afternoon observation sessions were conducted between 14:00-17:30 hours. Each of the five monkeys was selected for observation from a pseudo-randomised list so that each monkey was observed during a different observation window on each day. Animals were observed in their home cages.

Table 3.6 The ethogram used to measure behavioural changes reflective of underlying affective state in male rhesus macaques.

Behaviour	Definition	Paper
Self-directed		
Autogroom	Grooms self, includes masturbation.	e,g,i,k
Scratch	Scratches any part of body using hand or foot	a,b,e,i,k
Stereotypical		
Circle	Turns around repeatedly in a single location, or locomotes along a fixed repetitive (often circular, but can be linear) route two or more times in succession. Includes pacing back and forth along any fixed route.	a,e,i,k
Body shake	Shakes whole body vigorously	e,i
Head flip	Flips head backwards in a violent circular motion (often incorporated into circling or pacing repertoires).	e,i,k
Repetitive	Performs an action two or more times in succession. Includes all repetitive behaviours not otherwise listed	e,I
Rocking	Rocks body (forward-back or side to side) while standing, sitting or laying down.	a,e,i,k
Yawn	Yawns	i,k,
Self-injurious behaviour		
Self-injury	Bites self (including nails), pulls hair or causes harm to self by any other means (usually repetitive actions)	e,g,j,k
All other behaviours		
Vocalisation	All vocalisations	c,i
Aggressive threat	Aggressive behaviour directed towards another individual (monkey or human) including lunging with open mouth or vocalising, staring (with ears often forwards or flicking back and forth), lips protracted to reveal teeth, shaking cage	c,f,i,l
Bite cage	Bites or licks any part of cage that does not appear to have food or liquid on it.	e,g,i,k
Eat, drink, forage	Actively searching/foraging for food, holding food in hand and placing in mouth or chewing on it. Does not include instances where animal is simply chewing food stored in the cheek pouch	d,

Table 3.6 (continued)

Flee	Leaps/moves rapidly away from a stimulus that is potentially aversive. In the case of single housing, animals may flee towards the back of the cage away from the observer, or when another person enters the room. Often accompanied by threat or submissive behaviours.	e,
Inverted	Turns head upside down to view surroundings. Usually hangs from cage roof by forearms to do so.	e,i,k
Shake cage	Shakes cage using hands, feet or both	e,i,
Lip smack	Smacks lips	i,m
Movement	Moves from one part of cage to another. Includes fast and slow forms of quadrupedal and bipedal locomotion and brachiating.	c,g,h,i
Out of view	Animal is obscured from view	
Manipulate object	Manipulates object that is not food (although this may include food-related enrichments where no eating occurs), such as a toy or cage attachments, ropes etc.	e,i,k
Submissive present	Presents rear quarters towards another individual. Tail is raised, often looks back over or under shoulder towards the individual being presented to.	e,i
Bared-teeth display	Silent bared teeth display. Lips are protracted to reveal teeth and closed or partially open mouth.	e,f,l
Vigilant	Visually searches surrounding environment: eyes and/or head move continually with alert posture (sitting or standing upright, often leaning forward with ears pricked up). May also stare towards unspecified location but without 'Threat Face'	a,b,d,i,k
Crouch	Crouches down so that body is close to the floor, often appears alert, may be staring with ears pricked, vocalising or pulling a threatening facial expression with mouth open	e,
Inactive	Passive or sleeping. Includes apparently 'relaxed' and 'depressed' postures	c,d,i,k

a: Paulke et al. (1977).b: Maestripieri (1993).c: Clarke et al. (1988).d: Augustsson & Hau (1999).e: Sackett et al. (1978 and 1981).f: de Waal & Luttrell (1989).g: Eaton et al. (1994). h: Clarke et al. (1994).i: Doyle et al. (2008). j: Lutz et al. (2007).k: Fontenot et al. (2006).l: Basile et al. (2007). m: Chevalier-Skolnikoff (1973)

The narrowness of the animal housing rooms required the observer to sit at a distance of one metre from the front of the cage, facing the cage at an angle of 45⁰ and avoiding directly staring at the focal animal.

Five-minute morning and afternoon continuous-behavioural observation sessions were then conducted with JWatcher for each monkey on randomly selected days over the following five weeks. On entering the room, I allowed the monkeys five minutes to settle before I began coding. A focal observation session started once I was positioned in front of the focal monkey's cage and the timer on the notebook computer read at least five minutes from entry to the room. The behaviour being performed at the onset of the observation session was recorded and all subsequent changes in behaviour recorded by pressing the assigned keys on the notebook computer at the moment of onset of the behaviour. When a behaviour was observed that did not fit any of the previously assigned behaviours it was noted and added to the ethogram and assigned a code within JWatcher at the end of the session.

3.11 Results

The five monkeys were observed for a total of 430 minutes comprising 86 five-minute observation sessions ($\bar{X}=17.2 \pm 1.48$ sessions; range: 15-19 sessions). The pilot resulted in the ethogram shown in Table 3.6. Behaviours identified in the published literature were adopted with a few amendments specific to the current study. The behaviour 'depressed posture' was not included the ethogram. Although this category has been used by previous authors (e.g. Reinhardt et al., 1988), the differentiation

between 'resting' and 'depressed posture' was considered to be unreliable. Both codes could be used to describe monkeys who were motionless and not responding to surrounding events. Therefore, one gross category of 'inactive' was created to incorporate all instances when a monkey was not moving or engaging in any other activity, regardless of whether he appeared to be depressed or not. 'Head flip' was added as a conspicuous stereotypical behaviour often incorporated into circling displays. Vocalisations were combined into a single category (after Honess, Johnson and Wolfensohn, 2006). However, often it was not possible to identify calls or callers during real-time coding.

In summary, an ethogram of behaviours was created. Nine of the behaviours were identified as self-directed, stereotypical and self-injurious behaviours, of particular interest for the current research.

Aim 2. Testing the reliability of the ethogram

3.12 Methods:

3.12.1 Animals & Apparatus:

Ten monkeys housed at the Caribbean Primate Research Centre, Puerto Rico, took part in the study (Monkeys: 8, 11, 12, 13, 15, 23, 24, 25, 26 & 27; \bar{X} age=7.52 years \pm 6.54; range: 3.66 – 24.70). All monkeys were singly housed in the same enclosure, details of which were given in Chapter 2. Data were recorded using JWatcher software on two notebook computers, as described above.

3.12.2 Procedure:

Data were collected in April 2006. Initially, two observers (EB and a trainee veterinarian, Libette Romane: LR) observed and discussed the behaviours performed by different monkeys. Data were then collected to test inter-observer reliability. Ten monkeys were each observed for two-minutes per monkey, and behaviours recorded concurrently by the two observers. Monkeys were selected for observation randomly from a list. Continuous data were recorded by each observer concurrently on notebook computers with no conferring. Observation sessions were conducted between 6am and 6pm. Agreement between observers was calculated by dividing each two-minute observation session into 120 one-second time windows, and scoring agreement (1/0) for the behaviours coded within each window.

3.12.3 Statistics:

The number of agreements and disagreements for each behaviour were entered into a matrix and degree of reliability was calculated using Cohen's kappa (k) statistic (Bakeman & Gottmann, 1997). k is a statistical measure of inter-rater reliability. It provides a more robust measure than % agreement since it contains an adjustment for the level of agreement arising by chance alone. k values range from 0 (no agreement above chance) to 1 (complete agreement). Following Bakeman & Gottmann (1997) a predetermined kappa value of 0.70 was selected as the criterion level for good levels of agreement between the two coders.

3.13 Results:

Data for agreement between the two observers are presented in Table 3-7. A total of 1200 seconds of data were collected by each observer. During observation a new behaviour 'Jumping' was added to the ethogram as a conspicuous stereotypical behaviour that occurred during periods of high activity in the surrounding area, and which had not been identified during the literature review, nor seen during the pilot study at Oxford University. Crouching and bared teeth display were the only behaviours not observed during coding for reliability, although they were seen at other times. Overall agreement between observers was 75.25%, $k=0.72$. The ethogram was therefore considered to provide a mutually exclusive and exhaustive list of behaviours (with respect to the interests of the study) that may be applied reliably by two or more observers to document the behaviour of singly-housed male rhesus macaques.

Aim 3: Applying the ethogram

Changes in behaviour Pre- versus Post-health-check

3.14 Methods:

3.14.1 Animals & Apparatus:

Eleven monkeys housed at the Caribbean Primate Research Station, Puerto Rico, were observed to test each of the three hypotheses (Monkeys 06H, 29C, C55, 16P, 86O, 94K, AI73, 92R, 27S, 66S & 79S: \bar{X} age=8.1±4.5). Details of the enrichment intervention and the health-check are given in Part A.

Table 3-7 Agreement matrix for coding by two observers. Bold: number of agreements. Non-bold: number of non-agreements. Behaviours listed in abbreviated form in the same order as they are presented (in full) in the ethogram in Table 3-6 on page 129

Obs 1	Observer 2																								Total			
	Au	Scr	Cir	Bs	Hf	R	Rk	Y	Sh	V	Ag	L	Eat	Fl	Inv	Sh	Lp	M	J	Ov	Py	P	S	Vg		C	Ina	
Autogrm	42	3																								4	49	
Scratch	8	29																										37
Circle			15		3													8										26
B'shake				4																								4
Head flip			2		6																							8
Rep'tive						14													8									22
Rock							43																					43
Yawn								6																		1	7	
S-injury									7																	1	8	
Vocalise								5		3	3					8	2			12				3			70	
Aggress											12													6			18	
Lick cage												3	1														4	
Eat										1		2	56											4			63	
Flee														9				2			4						15	
Invert															6									8			14	
Shake cg				1						4	2					15											22	
Lipsmack																	2										2	
Move			3								5		9	4	2									9			164	
Jump						5												132	3								12	
Oov										5										89						21	115	
Play													2								15						17	
Present																						7					7	
SBT											1																1	
Vigilant														11					6					101	15		133	
Crouch																	2										2	
Inactive	9	4							1	7										32				31	253	337		
Total	59	36	20	5	9	19	48	7	10	51	23	5	68	24	8	23	4	148	17	133	19	7	0	162	4	291	1200	

3.14.2 Procedure:

Data were collected between April 2006 and February 2007. Monkeys were selected for observation from a pseudo-randomised list to provide equal numbers of morning and afternoon observation sessions per monkey. Observation sessions were conducted between 6am and 6pm on the three days immediately prior to the health-check and on the three days immediately following the health-check. Due to the fact that monkeys were sedated with KHC1 during the health-check no data were collected on the day of the health check itself. Each monkey was observed for one five minute morning observation session and one five minute afternoon observation session on each day. This resulted in six Pre-health-check and six Post-health-check observation sessions per monkey per health check. Data were collected during three veterinary examination periods at three-monthly intervals.

3.14.3 Statistics & data treatment:

Data on proportion of time engaged in behaviours were pooled for analysis. Firstly, the average number of seconds a monkey engaged in a given behaviour was calculated for each of the three morning observation sessions, and for each of the three afternoon observation sessions, for each of the Pre- and Post- treatment phases for each monkey. Averages were then calculated across the three days of morning and afternoon observation sessions, separately, for each of the Pre- and Post- treatment phases. The Pre-treatment morning and afternoon values were then averaged to produce a single Pre-treatment value per monkey. This was repeated for the Post-treatment morning and afternoon value for each monkey.

This resulted in a Pre- and a Post-treatment data set for comparison in the analysis. Where data were available from more than one health-check or enrichment phase, data were then averaged across the successive treatment phases for each monkey. Finally, data from the nine behaviours which comprised self-directed, stereotypical and self-injurious behaviour categories were pooled for each Pre- and Post-treatment phase. Wilcoxon signed ranks tests were conducted on paired proportion data (e.g. Pre-health-check versus Post-health-check) for both the pooled behavioural categories of self-directed, stereotypical and self-injurious behaviours, and for assessing changes in individual behaviours. Where table-wide values are examined, a Bonferroni adjusted P value is used. The Bonferroni adjusted P value for significance was $P < 0.002$. All data collected within each five-minute focal were included in the analyses.

3.15 Results:

A total of 128 observation sessions were conducted ($\bar{X} = 11.64 \pm 5.22$ observation sessions/monkey). There was no significant difference in the proportion of time monkeys spent engaged in self-directed, stereotypical and self-injurious behaviours Pre-health-check versus Post-health-check ($Z = 0.80$, $P = 0.42$).

Planned comparisons were performed to examine changes in behaviours, individually. There was no significant difference in proportion of time engaged in any single behaviour at the adjusted P level Pre- versus Post-health-check (Table 3-8). There was a trend for monkeys to vocalize more Post-health-check.

Table 3-8. Table of z-scores for proportion of time spent engaged in behaviours Pre- versus Post-treatments. -Z values signify a reduction from (e.g.) Pre- to Post treatment

Behaviour	Pre- v Post-health-check		Pre- v Post-enrichment		Post-enrichment v Post-health-check	
	Z	P	Z	P	Z	P
Autogroom	0.09	0.93	0.80	0.42	1.51	0.13
Scratch	1.69	0.09	-1.24	0.21	-0.62	0.53
Circle	-1.60	0.11	-2.20*	0.03	1.48	0.14
Body shake	0.42	0.67	-0.36	0.72	0.00	1.00
Head flip	1.60	0.11	1.34	0.18	-1.00	0.32
Repetitive	1.83	0.07	-0.67	0.50	0.00	1.00
Rock	-1.57	0.12	-0.73	0.46	0.13	0.89
Yawn	0.06	0.95	-0.71	0.48	0.05	0.96
Self-injury	1.60	0.11	0.67	0.50	-1.07	0.29
Vocalise	1.95*	0.05	-0.18	0.86	-0.09	0.93
Aggressive threat	0.37	0.72	-2.80**	0.005	0.00	1.00
Bite cage	-0.94	0.35	-0.56	0.58	0.37	0.72
Eat, drink, forage	1.26	0.21	0.80	0.42	-1.33	0.18
Flee	1.00	0.32	-1.34	0.18	1.00	0.32
Inverted	1.83	0.07	-0.77	0.44	0.74	0.46
Shake cage	0.31	0.75	-0.18	0.86	-0.13	0.89
Lip smack	-1.00	0.32	-0.51	0.61	-0.37	0.72
Move	0.62	0.53	0.18	0.86	-1.07	0.29
Jump	0.00	1.00	-0.73	0.46	-0.94	0.35
Out of view	1.57	0.12	-2.67**	0.007	1.84	0.07
Manipulate object	-0.18	0.86	2.58**	0.009	-0.80	0.42
Submissive present	1.00	0.32	0.00	1.00	-1.00	0.32
Bared teeth display	0.00	1.00	0.00	1.00	0.00	1.00
Vigilant	-0.53	0.59	-2.58**	0.009	-0.36	0.72
Crouch	0.00	1.00	1.41	0.16	0.00	1.00
Inactive	-0.09	0.93	2.04*	0.04	-1.16	0.25

* significant at unadjusted P level (P<0.05); ** (P<0.01), no changes in behaviour were significant at the Bonferroni adjusted P level of 0.002.

Changes in behaviour Pre- versus Post-enrichment

3.16 Methods:

The procedure was as described above. Data were collected on three consecutive days immediately preceding the week of enrichment, and on days 8-10 of the enrichment phase, during which monkeys were provided with enrichment on each of 10 consecutive days.

3.17 Results:

A total of 391 observation sessions were conducted ($\bar{X} = 37.27 \pm 19.39$ observation sessions/monkey, range 10 - 62). There was no significant difference in the proportion of time engaged in self-directed, stereotypical and self-injurious behaviour Pre-enrichment versus Post-enrichment ($Z=0.178$, $P=0.86$).

Planned comparisons were performed to examine changes in each behaviour, separately. There was no significant change in the proportion of time monkeys engaged in any behaviours Pre- versus Post-enrichment at the Bonferroni adjusted P level. Behaviours which showed a significant change at the unadjusted P level are indicated in Table 3-8. These were: reduced time spent engaged in circling, aggressive threat, vigilance, and inactivity; and increased time spent manipulating objects, and being out of view.

Changes in behaviour Post-enrichment versus Post-health-check

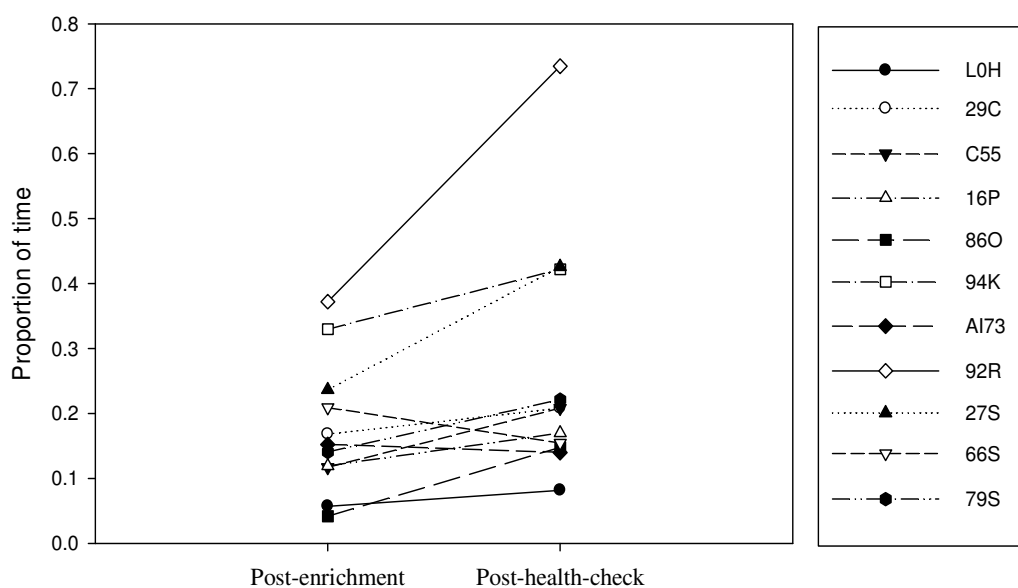
3.18 Methods:

A new set of data was collected from the same 11 monkeys. The procedure was as described above except that data were collected for three days Post-health-check and for three days Post-enrichment.

3.19 Results:

A total of 92 observation sessions were conducted (mean=8.36 ± 4.93 observation sessions/monkey). Monkeys spent significantly more time engaged in pooled self-directed, stereotypical and self-injurious behaviour Post-health-check versus Post-enrichment ($Z=2.401$, $P=0.016$; Figure 3.7).

Figure 3.7 Proportion of time monkeys engaged in self-directed, stereotypical and self-injurious behaviours (when data for these three categories were pooled together) Post-enrichment versus Post-health-check.



Planned comparisons for each behaviour separately revealed that no single behaviour was accountable for the significant difference in overall proportion of time engaged in self-directed, stereotypical and self-injurious behaviours between the two treatments (Table 3-8).

3.20 Discussion

An ethogram was developed for measuring changes in rhesus macaque behaviour following two routine husbandry procedures (a week of environmental enrichment and a health check) which, it was proposed, should lead to different affective states in captive rhesus macaques. Changes in behaviour associated with negative and 'positive' contexts were identified from the literature, namely a general observed increase in self-directed, stereotypical and self-injurious behaviours associated with negative contexts, and a reduction in these behaviours following an improvement in conditions. In the current study, monkeys spent significantly more time engaged in self-directed, stereotypical and self-injurious behaviours Post-health-check (when data for these three categories were pooled together), than they did Post-enrichment, supporting the hypothesis that the health-check should induce behaviours associated with negative context and the week of enrichment should decrease behaviours associated with negative context. It is likely the difference in behaviour following the two treatments arose from subtle shifts in behaviour following either treatment since there was no significant

change in behaviour between the Pre- and Post-health-check phases nor between the Pre- and Post-enrichment phases.

The finding that monkeys spent significantly more time engaged in self-directed, stereotypical and self-injurious behaviour Post-health-check versus Post-enrichment concurs with previous studies which show a similar pattern of change following ‘negative’ and ‘positive’ husbandry interventions (Capitano, 1999; Lutz et al., 2003b; Fontenot et al., 2006), although no published study has compared more aversive versus more positive husbandry procedures directly.

The pattern of results when either treatment (enrichment and health-check) is considered separately is unclear. Firstly, there was no significant change in the proportion of time monkeys spent engaged in self-directed, stereotypical and self-injurious behaviour Post- versus Pre-health-check. This suggests the health-check had no significant impact on the performance of these behaviours. This may reflect several possibilities: the health-check was not a negative treatment for the monkeys; the behavioural categories used do not reliably reflect increases in negative affect in the monkeys; the monkeys have behavioural coping strategies that do not incorporate all or some of the behaviours identified; individual differences in behavioural responses to stress mask any group-level effects; the sample size was too small to detect any general level trends in the data; there were too many small behavioural categories, resulting in too few data per category and a highly reduced Bonferroni P value; or, the three-day windows were not appropriate to capture treatment-related changes in behavioural measures. With

respect to the last point, analyses of data on the first day only revealed a similar pattern of results. The Z-scores presented in Table 3-8 suggest shifts in a few behaviours, but these did not reach significance at the Bonferroni-adjusted P level, and only the increase in vocalizations reached significance at the unadjusted level.

There was a trend for monkeys to spend less time engaged in circling, aggressive threat, vigilant and inactive behaviours, during the three days Post-enrichment versus the three days Pre-enrichment. There was also a trend for monkeys to spend more time manipulating objects and presenting submissively Post- versus Pre-enrichment. These trends were not significant at the adjusted P level, but were significant at the unadjusted P level. The increase in the proportion of time spent manipulating objects Post-enrichment versus Pre-enrichment may be explained by the provision of additional environmental enrichment devices in the Post-enrichment phase. Any reduction in the remaining behaviours may be an artifact of increased opportunity to manipulate objects, since behaviours were mutually exclusive categories, therefore an increase in time spent on one activity leaves less time available to engage in any other activities..

The trend for reduced circling Post-enrichment concurs with data from previous studies. However, there was no reduction in the other self-directed, stereotypical, and self-injurious behaviours that were identified as behavioural indicators of changes in inferred affective state. The reduction in aggressive threats and vigilance may reflect reduced arousal. There was an increase in the amount of time spent inactive Post- versus Pre-enrichment which would support this

interpretation. Maestriperi (1993) suggests vigilance levels reflect anxiety. An alternative interpretation may therefore be that reduced vigilance Post-enrichment reflects reduced anxiety, although there were no significant changes in other behaviours also believed to reflect anxiety-like states in rhesus macaques, such as rates of scratching (Maestriperi et al., 1992).

The reduced aggression Post-enrichment may reflect reduced negative affective states associated with fear and anger in humans. However, there was also an increase in submissive presentations Post-enrichment. The increase in submissive presentations may reflect not a shift in affective state, but simply a shift in the behavioural coping strategies employed to deal with potentially stressful situations.

In summary, the behavioural data are difficult to interpret with respect to inferred affective state of the monkeys following two treatments (a week of enrichment and a health-check). The clearest differences were found when comparing between the two treatments (Post-health-check versus Post-enrichment). However, there was a confound in the presence of extra enrichment devices in the cages Post-enrichment. Therefore, while these data suggest monkeys behaved differently following the two treatments, they show no clear patterns of change that may be interpreted easily in terms of underlying affective states.

3.21 General Discussion

Two treatments were introduced which were used in the main studies presented in Chapters 4-6 to induce changes in inferred affective state in captive male rhesus macaques: a week of environmental enrichment and a health check. Two traditional methods for assessing inferred affective state in primates were also introduced: a physiological measure (excreted cortisol metabolites) and behavioural measures (self-directed, stereotypical and self-injurious behaviours).

It was predicted that the health-check treatment presented a negative context, and would lead to an increase in physiological arousal and behaviours assumed to reflect negative affect in rhesus macaques. Conversely, it was predicted that the enrichment treatment presented a positive (or less negative) context, and would lead to a reduction in physiological arousal and a reduction in behaviours assumed to reflect negative affect in rhesus macaques (there were no behaviours identified as likely to reflect 'positive affect' *per se* in singly-housed rhesus macaques).

The data resulting from the application of the two traditional methods following the two treatments gave conflicting results. When comparing Pre- versus Post-treatment data, the data indicated a partial increase in physiological arousal Post-health-check compared to the Pre-health-check baseline, with no consistent decrease in arousal Post-enrichment compared with general baseline levels. The behavioural data suggested there was no change in behaviour Post-health-check from the Pre-health-check baseline, and no clear patterns of change Post-

enrichment from the Pre-enrichment baseline that could not be accounted for by the presence of enrichment devices.

The clearest patterns of data were evident when data from the Post-enrichment treatment were compared with data from the Post-health-check treatment. Monkeys were generally more physiologically aroused, and spent a greater proportion of their time engaged in self-directed, stereotypical and self-injurious behaviours, Post-health-check versus Post-enrichment. However, again data were difficult to interpret since the differences in physiological and behavioural data between the two treatments do not allow differentiation of the valence of inferred underlying affective state. The measures highlight differences, but do not provide information on whether the differences are qualitative (i.e. both high and low arousal may be associated with both positive and negative affect), or simply slight shifts in valence that are marginally significant in terms of wellbeing (i.e. highly negative versus fairly negative).

The faecal cortisol metabolite and behavioural data presented in this chapter are difficult to interpret in terms of whether the two treatments resulted in the predicted shifts in underlying affective state. Measures that tap into other components of affect, such as the cognitive component, may provide much needed information. A new (cognitive) angle will allow us to reconsider existing knowledge of physiological and behavioural measures of affect in primates in terms of underlying cognitive changes that, in humans at least, are considered a core component of emotion and psychological wellbeing.

Chapter 4

4 Attentional Bias

Attentional bias is a bias to attend preferentially to one type of information over another (MacLeod et al., 1986). Studies with humans have shown that affective state influences attentional bias (Bar-Haim et al., 2007). People high in anxiety, for example, demonstrate a bias to attend to threatening information. This may have important implications for the onset and maintenance of anxiety disorders and psychological wellbeing. If primates exhibit a similar cognitive capacity to attend preferentially to negative stimuli when stressed, this may also have implications for their psychological wellbeing.

In this chapter I review existing research on attentional bias (Part A), and present empirical data from the first study to investigate attentional bias in a species of primate (Part B). Part A is divided into three sections. In the first of these I discuss the theory and background for attentional bias research conducted with humans. This focuses on the development of theories and models that account for biases in initial orienting towards threatening information (early vigilance effects), maintenance of attention towards threatening information (sustained vigilance, or avoidance effects), and subsequent disengagement of attention from threatening information. Attentional bias, as it is defined here, has only been studied in humans, therefore the studies I review were conducted with humans unless otherwise specified. In the second section I review the methods used to study attentional bias in humans. In particular, I

describe the recent development of methods that use eye-gaze to measure overt attention. In the third section I discuss the implications of human-based research for the development of a novel method to study attentional bias in primates. I conclude this section with the main aims and alternative hypotheses that informed the design of the present study.

The study is detailed in Part B, which is also divided into three sections. In the first of these I describe the method I developed. In the second section I present the first data on attentional bias for threatening faces in a species of primate. In the third section I discuss the results of the study in light of the alternative hypotheses and available data from humans.

Part A: Literature Review

4.1 Introduction

4.1.1 Attentional bias in humans: theory and background

Attentional biases have been studied in humans using a wide range of experimental paradigms and participant populations. As such, there exists a range of theories regarding the role of affect in orienting attention towards or away from threatening stimuli (Bar-Haim et al., 2007). Data arising from different paradigms and

populations probably tap into different aspects of attention (i.e. attentional engagement, maintenance, and disengagement) and different aspects of affect (e.g. trait versus state anxiety, and comorbidity with depression). There is a clear consensus that affective state and attentional processes interact (Bar Haim et al., 2007). However, because of the different methods and populations employed, and the divergent results, there is general disagreement as to the functional significance of this interaction and its underlying mechanisms. In this section I review existing literature with specific emphasis on research which most directly relates to the current study, namely studies which focus on spatial orienting of attention relative to socioemotional stimuli (specifically faces) and those which consider state anxiety effects on attention. The following is a review of the main cognitive theories and empirical findings regarding the role of cognition in anxiety. This is organized according to the function of anxiety in directing attention towards or away from threat at different stages of processing (Table 4.1).

Early cognitive theories of the interaction between affect and cognition predicted a general bias in attention allocation towards threat, that is consistent throughout all levels of processing, and is enhanced in anxiety (e.g. schema theory: Beck, 1976; semantic network theory: Bower, 1981). Early cognitive theories provided the foundation on which more recent information processing models are based. Existing theories and models differ in the suggested direction of the attentional bias among anxious individuals (towards or away from threat), in the stages of processing at

which biases arise (i.e. early attentional engagement versus later attentional disengagement), and the differential roles of state and trait anxiety.

Vigilance theories predict rapid allocation of attention towards threat (Biological Preparedness: Seligman, 1971; Öhman, 2005; Information Processing model: Williams et al., 1988; Hypervigilance theory: Mathews, 1990; Eysenck, 1992; Cognitive model of Selective Processing: Mathews & Mackintosh, 1998; Cognitive-Motivational model: Mogg & Bradley, 1998, although it should be noted that the latter also incorporates later influences such as avoidance and maintenance). Vigilance models generally emphasise the importance of early attentional processes (rapid initial orienting to threat), and typically assume vigilance for threat to occur in both high and low anxiety, although it may be enhanced in the former.

Table 4.1 A summary of the main cognitive theories of the role of early and late stages of (human) cognition in directing visual attention towards, or away from, threatening information in anxiety.

Theory	Stage of processing	
	Early	Late
Early cognitive theories ^a	Towards	Towards
Vigilance	Towards	
Delayed disengagement		Towards
Avoidance	Away	Away
Vigilance-Avoidance	Towards	Away

a: Early cognitive theories considered processing biases to occur independently of affective state. All other theories listed consider the direction of attention for threatening information to be enhanced in anxious versus non-anxious individuals.

Delayed disengagement theories make no predictions about early attentional processes, but do predict that once a threat stimulus has been attended, attention is captured, resulting in a dwell of attention towards threat (Fox et al., 2001; Yiend & Mathews, 2001; Georgiou et al., 2005). As such, delayed disengagement reflects biases that arise at later stages of processing.

Avoidance theories predict rapid and sustained allocation of attention away from threat (Mansell et al., 1999; Garner et al., 2006b). Avoidance of threatening information may occur at both early and late processing stages, however, evidence for avoidance in the absence of initial vigilance effects is scarce.

Vigilance-avoidance theories predict that initial vigilance for threatening information is followed by rapid avoidance of that information (Williams et al., 1988; Mogg et al., 1987 and 1997; Mansell et al., 1999; Amir et al., 1998a). These are the only theories to consider differential patterns of response by early and later processing stages together.

Here, I discuss each of the competing theories regarding the spatial orienting of attention in humans with specific reference to the stage of processing (early versus late) and direction (towards versus away from threat). I conclude with an explanation of how the competing theories regarding attentional bias in humans informed the

hypotheses underlying the development of the first study to investigate attentional bias in a primate.

4.1.1.1 Early cognitive theories

Schema theory (Beck, 1976) proposed that the brain has processing biases for specific kinds of information and these biases are guided by schemas. In anxious individuals, schemas are particularly biased towards threat, therefore threat-related material is favoured over non-threat-related information, and this bias occurs at all stages of processing ('primal threat mode'). Because processing is directed by schemas, Beck and Clark (1997) consider the distinction between clinical and sub-clinical anxiety to be quantitative rather than qualitative. According to Schema theory, evolution has equipped the human brain with a processing 'schema' for threat-relevant information. This is a natural processing bias across animal species that arose during evolution because of its survival value in alerting an animal to potential danger. The extent to which the schema biases the processing of threat-relevant information in humans, and the correlation between this bias and the presence of real threat in the environment, distinguish between low- and high-, and sub-clinical and clinical, anxiety. Beck's schema theory considers both early automatic orienting of attention towards threat (vigilance) and later strategic processing of threat-relevant information (interpretation, recall) to play a central role in inducing and maintaining high (or clinical) levels of anxiety.

Schema theory led to the development of several network theories of the interplay between affect and attention, with specific reference to the role of attention in the development and maintenance of anxiety disorders. The first of these, **Semantic network theory** (Bower, 1981), proposed each emotional state is represented by a series of nodes which, when activated during a current mood state, bias information in a mood-congruent manner (see also Foa & Kozak, 1986). As with Schema theory, biases would be expected at all stages of processing. Subsequent studies identified mood-congruent attentional biases in anxiety (MacLeod et al., 1986; Broadbent & Broadbent, 1988) and phobia (Watts et al., 1986), and recall biases in depression (e.g. Mathews & Bradley, 1983), but not recall biases in anxiety (Mogg et al., 1987) nor early attentional biases in depression (MacLeod et al., 1986). These discrepant findings suggested that early and late stages of information processing may be governed by separate underlying mechanisms, contrary to schema and semantic network theories' suggestion that valence-specific schemas direct all stages of processing. This revised view is consistent with Posner and Peterson's (1990) account that visuo-spatial attention incorporates at least three distinct neural subsystems that co-ordinate the 1) shifting, 2) engagement and 3) disengagement of visual attention. Subsequent models therefore needed to allow for separate underlying mechanisms for early and late processing. These are summarized in the following sections, according to the predicted direction of the bias.

4.1.1.2 Vigilance theories

Vigilance theories (e.g. Seligman, 1971; Eysenck, 1992; Öhman, 2005; Williams et al., 2007) propose that anxious individuals are highly sensitive to threat-relevant information and attention is captured by threatening stimuli at very early, automatic stages of processing. Snake and spider-phobic individuals, for example, are faster to detect snake and spider stimuli respectively when these stimuli are presented in an array of competing non-threatening stimuli, regardless of stimulus location within the display (Öhman et al., 2001a). Similar effects have been found for schematic threatening faces amongst arrays of positive, neutral and other negative faces (children: Waters & Lipp, 2008; adults: Öhman et al., 2001b). An early form of vigilance theory, biological preparedness (Seligman, 1971; Mineka & Öhman, 2002), suggested that vigilance for threatening stimuli is an innate function of the mammalian brain which arises from a 'fear module', an evolutionarily-shaped behavioural system involving sub-cortical pathways via the amygdala (Öhman et al., 2007).

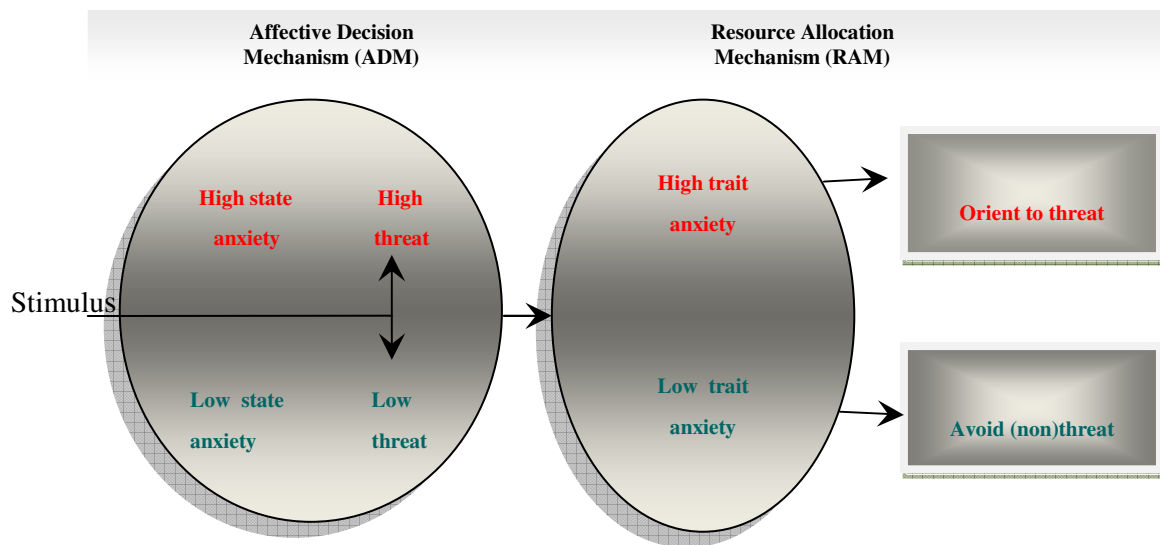
The most compelling evidence for an innate, automatic system for processing threat relevant information is provided by data on the role of the amygdala and thalamic pathways in the formation of selective associations during fear conditioning in a range of species including rats and humans (LeDoux et al., 1983; LeDoux, 1996; Öhman & Mineka, 2001). When the to-be-conditioned stimulus is threat-relevant (e.g. a threatening face), conditioning to an aversive unconditioned stimulus (e.g. shock) is facilitated, compared with conditioning to non-threat-relevant stimuli

(humans: Esteves et al., 1994). Similar selective association effects have also been demonstrated in rats (LeDoux, 1996) and rhesus macaques (Cook & Mineka, 1989 and 1990). LeDoux (1996) emphasises dissociable ‘high road/low road’ processing routes of the brain. The ‘low road’ processing route is proposed to occur via subcortical systems (amygdala and associated structures with direct magnocellular inputs from the retina) which allow rapid appraisal of the threat value of a stimulus presented foveally or peripherally, and is tuned to low spatial frequency inputs (Holmes et al., 2003; Laycock et al., 2008; see also Holmes et al., 2005). This coarse, rapid processing is accompanied by slower, top-down processing of more fine scale information (the ‘high road’), via a parvocellular processing route from the retina to cortical areas. This pathway is sensitive primarily to stimuli presented foveally (Holmes et al., 2006; Eimer & Holmes, 2007). Together, the two pathways lead to an appropriate psychophysiological or behavioural response (e.g. fear or freezing), according to the species and environmental context. LeDoux (1996) provided evidence for separate pathways directing early and later attentional processes, as well as appropriate behavioural responses to threat. These collective findings guided the formation of most, if not all, subsequent theories and models of information processing in anxiety.

The **information-processing model** proposed by Williams et al. (1988: Figure 4.1) makes specific predictions about the respective roles of early automatic and later strategic processing as well as differential effects of state and trait affect in information processing. According to the model, state anxiety interacts with an

Affective Decision Mechanism (ADM), which guides early pre-attentive processing of stimulus threat value. High state anxiety results in evaluation of high threat output from the ADM. Low state anxiety results in low threat output.

Figure 4.1 The information processing model (Williams et al., 1988) of attention for threat in anxiety suggests different mechanisms underlie early and later stages of information processing.



According to Williams et al.'s (1988) model, trait anxiety interacts with later attentional processes, which are guided by a Resource Allocation Mechanism (RAM). The RAM receives output from the ADM and symbolizes a more stable tendency for an individual to direct attention towards (vigilance in high anxiety) or away from (perceptual cognitive avoidance in low anxiety) threatening stimuli. Despite a later revision, a key problem with the model remains a lack of evidence for an avoidance

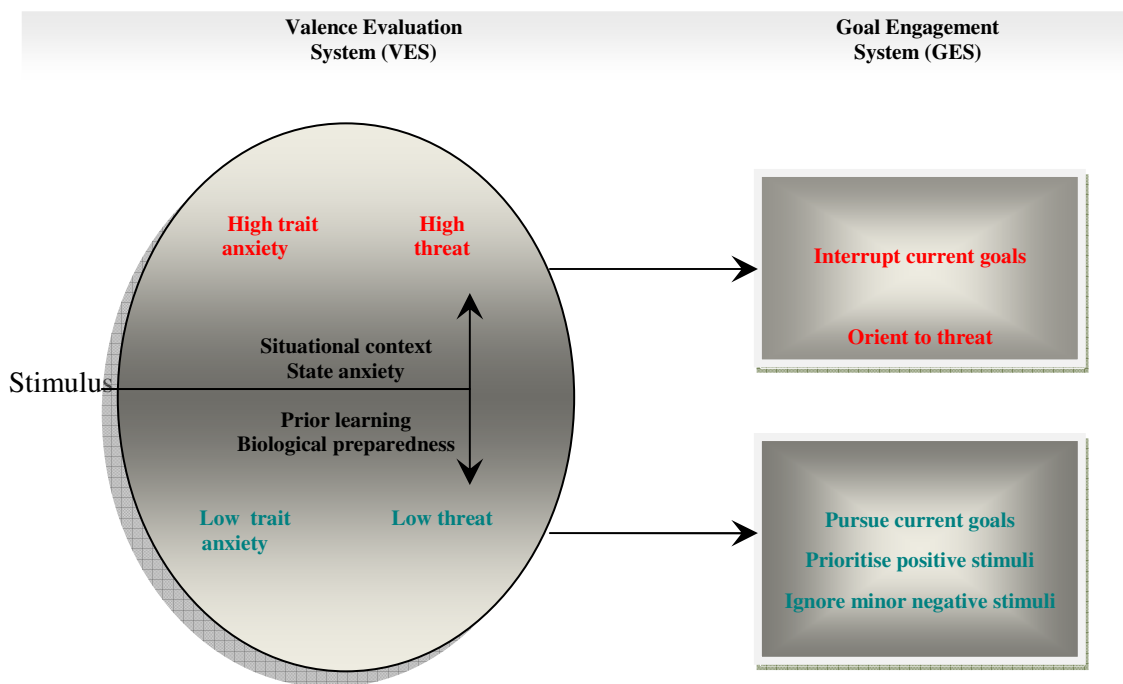
of threat-relevant stimuli by low anxious individuals (e.g. Mogg et al., 2000b) as well as conflicting results for the respective roles of state and trait anxiety on attention allocation.

An offshoot of Williams et al.'s (1988) information-processing model was Eysenck's hypervigilance theory of anxiety (Eysenck, 1992). This incorporated aspects of the biological preparedness hypothesis. According to hypervigilance theory, anxious individuals continuously scan the environment for threat and exhibit heightened selectivity and maintenance of attention towards threat-relevant stimuli. As well as early spatial orienting of attention towards threat, Eysenck (1992) considers 'secondary appraisal' processes to feed into the maintenance of anxiety and the hypervigilant attentional state.

Other cognitive models develop Williams et al.'s (1988) information processing model still further. Mogg & Bradley's (1998) **Cognitive-Motivational model** (Figure 4.2) emphasises the role of early stimulus appraisal processes in the development and maintenance of anxiety. According to Mogg & Bradley (1998), early stimulus appraisal by a Valence Evaluation System (VES) results in information output to a Goal Engagement System (GES). State and trait anxiety, biological preparedness, prior learning and situational contextual factors all feed into the VES, such that anxiety affects stimulus appraisal for threat, but not the allocation of resources, which should be stable across individuals according to whether threat is present or not. Where Williams et al. (1988) predict that high-trait anxious (HTA) and low-trait

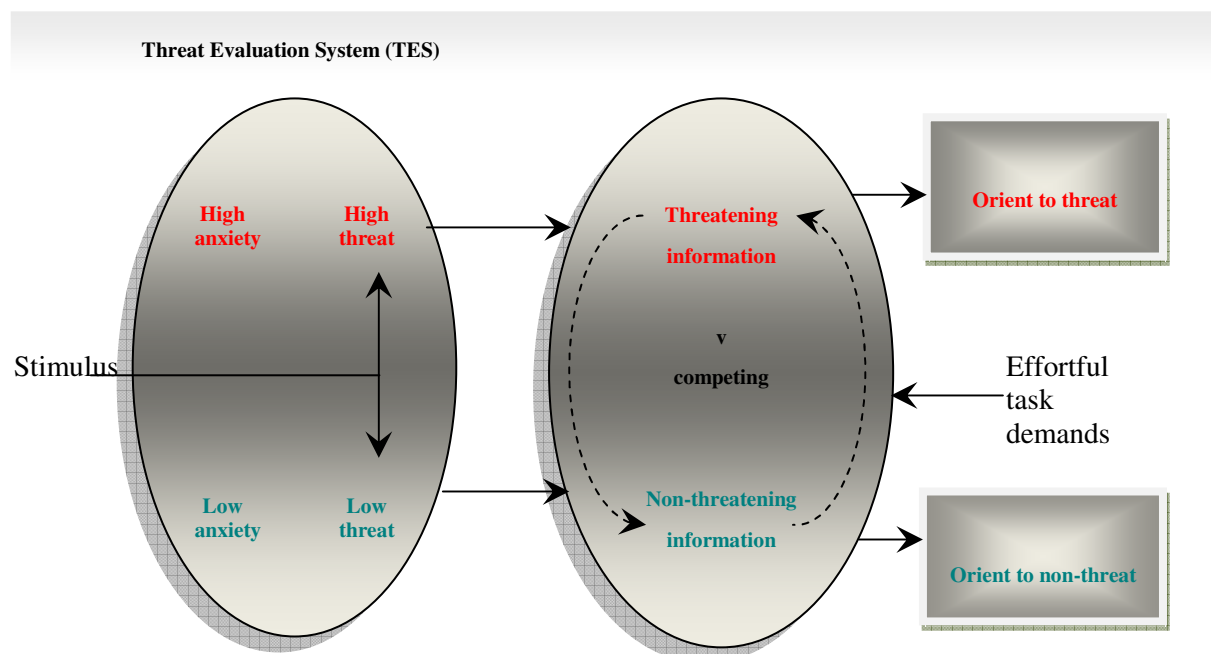
anxious (LTA) individuals should exhibit qualitatively different attentional responses to threat (vigilance versus avoidance, respectively), with state anxiety mediating stimulus appraisal for threat, Mogg and Bradley (1998) predict that high anxious and low anxious individuals should differ in their appraisal of the threatening value of a stimulus (more threatening or less threatening, respectively), but not in their attentional response (see also Mogg et al. 2000b). In the latter case, both should attend preferentially to highly threatening stimuli, irrespective of current goals, and attend to non-threatening stimuli only where these facilitate current or long-term goals.

Figure 4.2 The cognitive-motivational model (Mogg & Bradley, 1998) places emphasis on early stimulus appraisal



Mathews & Mackintosh (1998) put forward a **cognitive model of selective processing** in anxiety (Figure 4.3). This model, like that of Mogg & Bradley (1998), incorporates LeDoux's (1996) work on the role of automatic information processing via the amygdala-thalamic pathway, allowing anxiety-related interference from stimuli presented outside of awareness. According to this model the emotional valence of a stimulus is assessed in a Threat Evaluation System (TES), which utilizes the fast-acting amygdala-thalamic pathway. Anxiety level influences the degree to which the stimulus is appraised as threatening. The appraisal directly affects the prioritization given to such information over competing non-threat-related information, which is processed via a slower cortical pathway.

Figure 4.3 The cognitive model of selective processing (Mathews & Mackintosh, 1998) suggests early processing interacts with ongoing tasks demands



The extent of the bias is modulated by the relative dominance of top-down task-related conscious processes compared to bottom-up, automatic threat-related output from the TES. Anxiety state, distractor task demands, prior learning and stimulus type may all affect the dominance relationship between competing processing pathways. These factors are suggested to account for the disparate patterns of findings among different populations and between different experimental designs.

4.1.1.3 Delayed disengagement theories

Delayed disengagement/maintenance theories (Fox, 1994; Fox et al., 2001; Yiend & Matthews, 2001; Georgiou et al., 2005) propose anxiety has little effect on early attentional capture, but a greater effect on later processes that modulate the maintenance of attention towards threat. Anxiety therefore leads to a delay in disengagement from threatening information. The delay may be due to an inability to disengage attention from a stimulus presently being visually attended to (e.g. delay to detect the absence of a discrepant face among an array of angry faces in a visual search task: Fox et al. 2000; reduced ability to detect peripheral targets when a threatening stimulus is presented at fixation: Georgiou et al., 2005), or due to attentional ‘dwell’ on a previously visually attended stimulus (e.g. during exogenous cueing tasks: Fox et al., 2001; or attentional blink tasks: Fox et al., 2005).

4.1.1.4 Avoidance theories

Avoidance of emotional information has been demonstrated when emotional faces are paired with non-social stimuli (Mansell et al., 1999; e.g. pictures of household objects: Chen et al., 2002). For example, Chen et al. (2002) tested social phobic patients and matched controls on a dot-probe task in which positive (happy), neutral and negative (angry, sad, disgusted and fearful) faces were shown paired with pictures of household objects. Patients were faster to detect probes presented at the location of household objects versus both positive and negative emotional faces, while the matched controls were equally fast to detect probes at both locations. A similar study found evidence for attention away from emotional faces by a non-clinical sample with high social anxiety scores following a social stressor (Mansell et al., 1999). However, a more recent study failed to show avoidance of faces versus household objects (Garner et al., 2006b). It should be noted that evidence for complete avoidance of emotional faces has only been demonstrated when non-social alternative cues have been used.

4.1.1.5 Vigilance-Avoidance theories

Vigilance-avoidance theories focus on both early and later aspects of information processing (Mathews, 1990; Williams et al., 1996; Mogg et al., 1997; Heinrichs & Hofmann, 2001; Mogg et al., 2004; Garner et al., 2006b; Holmes et al., 2008). Specifically, early automatic attention towards threat is followed by later strategic avoidance. This attentional pattern allows early detection of threat (alerting the

individual to the presence and location of potential danger), followed by avoidance of threat (a possible mechanism to reduce anxiety arising from the presence of the threat-related stimulus itself: Derakshan et al., 2007). Mogg et al. (2004) presented high- and low-threat scenes to HTA and LTA, and blood-injury phobic, individuals during a dot-probe task. Pictures were presented for 500ms and 1500ms, to capture the early and later stages of attention allocation. HTA individuals were more vigilant for high-threat scenes at the shorter exposure, with no attentional bias evident for either anxiety group at the longer exposure. Blood-injury phobic individuals showed significant vigilance for high-threat scenes at the short duration and significant avoidance at the longer duration, suggesting a vigilant-avoidant pattern of attention allocation.

Vigilance-avoidance accounts incorporate a changing pattern of attention over time. Methods which map the temporal dynamic of the response are therefore more appropriate: namely electroencephalogram (EEG: Holmes et al., 2008) and eye-gaze measures (e.g. Garner et al., 2006b). Event-Related-Potential (ERP: a particular form of EEG) responses to centrally presented emotional faces suggest that HTA individuals demonstrate enhanced initial threat evaluation of fearful faces. This is followed by cognitive avoidance, as evidenced by attenuated threat processing among HTA individuals. While an enhanced initial threat evaluation was also evident in LTA individuals, no subsequent change in ERP response was evident (suggesting sustained attention towards fearful faces).

Derakshan et al. (2007) suggest the vigilant-avoidant pattern of attention allocation is particular to the repressive coping style. According to this model initial vigilance towards threatening information reflects early, rapid, automatic cognitive and physiological responses typical of HTA individuals. Initial vigilance is followed by controlled, strategic, top-down processing that disengages attention from the threatening stimulus as part of a coping strategy to reduce anxiety (e.g. Williams et al., 1996; Mogg & Bradley, 1998).

A **non-linear account** of the vigilant-avoidant processing style is provided by Garner et al. (2006b). They suggest that attentional biases subsume different processes that operate in parallel (i.e. when there is competition for attentional resources between two competing stimuli). These parallel processing pathways will be revealed most effectively where responses reveal defensive or socially submissive behaviours, e.g. where anxious individuals are provided with an opportunity to avoid social cues (such as shifts in gaze from social to non-social cues). This account is further supported by the findings of Calvo et al. (2006) who measured fixation latency and duration to emotional faces in a visual search task. They found speeded orientation, but reduced fixation duration, towards angry faces versus other-emotional and neutral faces presented for three seconds. However, Calvo et al. (2006) interpret the reduced duration of gaze towards angry faces in terms of enhanced processing efficiency of such faces, in contradiction to an avoidance of such stimuli. Further, anxiety

measures were not recorded to distinguish stress-related effects on attention allocation.

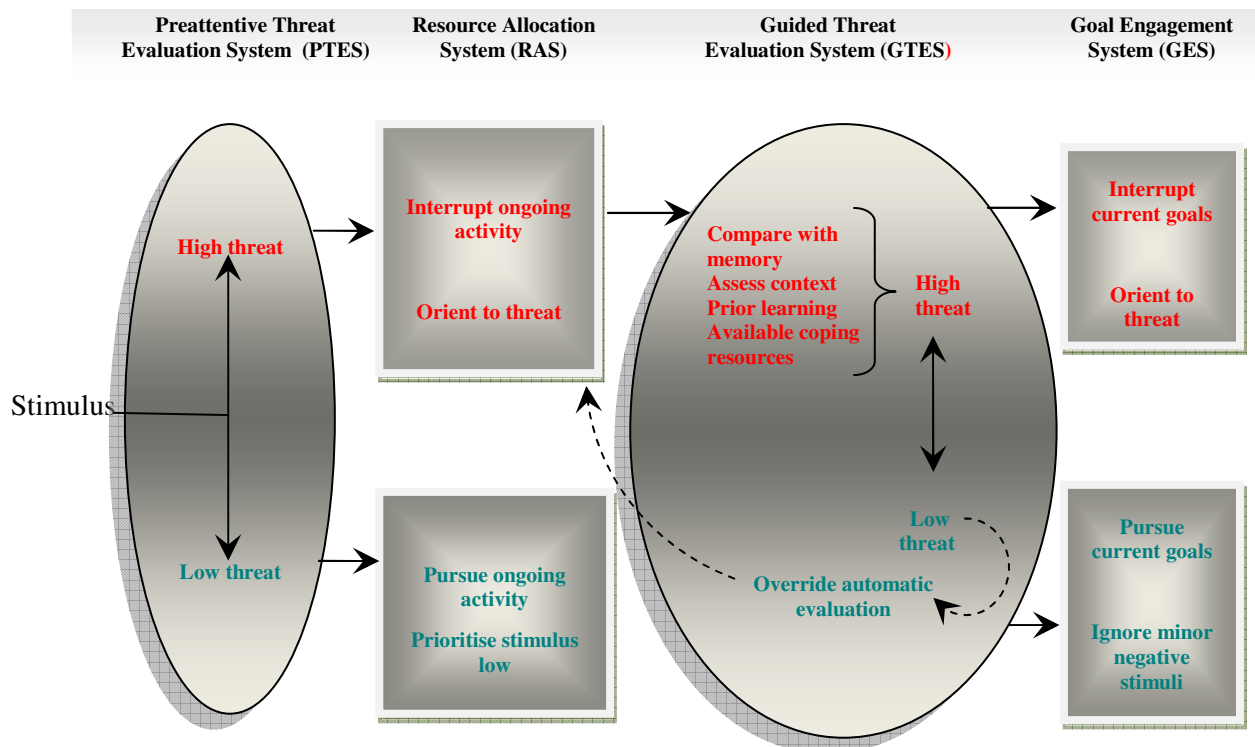
4.1.1.6 An Integrative model

A recent meta-analysis of all published research up to May 2005 has led to an integrative model of the cognitive mechanisms underlying threat processing (Bar-Haim et al., 2007; Figure 4.4). This model suggests vigilance towards threat in anxiety is a robust phenomenon, though one with low-to-medium effect size. The integrated model proposes four primary processing stages. The first two of these represent early automatic processing stages (Preattentive Threat Evaluation System, PTES, and a Resource Allocation System, RAS). The subsequent two represent strategic processing stages (Guided Threat Evaluation System, GTES, and Goal Engagement System, GES). Anxious individuals may exhibit processing biases at each and any of the four processing stages. The direction, strength and stage at which such biases in processing occur depends on the level of anxiety, stimulus intensity and relative influence of state and trait effects.

Bar-Haim et al. (2007) conclude there is strong evidence for a link between affect and attention, and for the role of this link in the development and maintenance of anxiety and other affective disorders. Underlying mechanisms may be broadly separated as early automatic and later strategic stages. Initial rapid preattentive threat evaluation

(directed by the PTES) via fast and direct magnocellular pathways to the amygdala and associated subcortical structures, directs early spatial orienting of attention towards threat, which is accompanied by an appropriate physiological response (directed by the RAS). Subsequent strategic evaluation of the threat (following retrieval of information from memory, assessment of context, prior learning effects and available coping strategies, directed by the GTES) combined with consideration of current goals (directed by the GES) allows strategic feedback and override or maintenance of the automatic orienting of attention and physiological response.

Figure 4.4 The integrated model (Bar-Haim et al., 2007) incorporates previous models to reconcile differential effects of early and later processing stages on attention to threat in anxiety.



In summary, several themes emerge from the existing human literature which informed the design of the current study. Firstly, there are conflicting data regarding the direction of attentional biases (towards, or away from, threatening stimuli). Most studies indicate a vigilant pattern of initial orienting of attention towards threat in anxiety (Bar-Haim et al., 2007), which occurs rapidly and automatically. Therefore, a design which measures direction of orienting towards, or away from, threatening versus non-threatening information would allow comparison of direction of attention with respect to threatening information in primates.

Secondly, there is a general consensus that both early automatic and later strategic processing stages influence allocation of attention to threat-related stimuli. The relative contribution of each, and the strength of feedforward/feedback pathways remain unresolved (cf Williams et al., 1988; Mathews & Mackintosh, 1998; Mogg & Bradley, 1998; Bar-Haim et al., 2007). It is clear that later strategic processing may be involved in attentional dwell and avoidance, although the conditions that elicit either tendency have yet to be fully elucidated. Given the evidence for face-processing by rhesus macaques reviewed in Chapter 2, a design which measures latency of initial orienting towards threatening versus non-threatening information, as well as latency to disengage, would allow comparison of early and later processing stages.

Thirdly, emotional faces are now widely used as threat- and non-threat-relevant stimuli in human studies, partly due to the automaticity of face processing in the

human brain, and the special salience of faces over other kinds of stimuli (e.g. Cresswell, 2008). Attentional biases may be mediated by valence differences between faces (e.g. enhanced vigilance for angry but not disgust faces in anxiety: Gilboa-Schechtman et al., 1999), be valence-non-specific (e.g. vigilance for both positive and negative emotional faces over neutral faces in anxiety: Garner et al., 2006b; Brosch et al., 2008), or may vary as a function of stimulus intensity (e.g. increased vigilance for high- versus medium-threat pictures in anxiety: Mogg et al., 2000b; Yuan et al., 2007). Therefore, a design using face stimuli which differ in threat value, e.g. aggressive versus neutral faces, would provide data comparable with that available from humans.

Typically, the different theories are supported by data from different experimental paradigms. It is therefore likely that these methods tap into different aspects of spatial attention, and that conflicting results reflect a complexity of competing parallel attentional processes, and limitations of current paradigms. In the next section I review the methods used to measure spatial attention in humans, and discuss their implications for the design of a suitable paradigm for measuring spatial attentional processes in nonhuman primates.

4.1.2 Current methods for studying attentional bias

The appropriate method for measuring attentional bias is determined by the stage of processing that is of interest (early engagement, ongoing maintenance or later

disengagement). A summary of studies, detailing methods used and findings with respect to direction of attentional biases is given in Table 4-2. Studies are organized according to the direction of attention with respect to threatening information implicated in each study. Only studies in which attention for threatening versus non-threatening faces are included.

The most common experimental paradigms used to measure attentional biases are those that tap into spatial attention. The **dot-probe** paradigm (MacLeod et al., 1986) is the most widely used method. Less frequently employed methods include **visual search** (e.g. Öhman et al., 2001b) and **exogenous cueing** tasks (e.g. Stormack et al., 1995; Fox et al., 2001). A recent development, of particular importance to the current research, is the additional mapping of **eye-gaze** during the aforementioned tasks (e.g. Rohner, 2002; Calvo & Averó, 2005). Eye-gaze and preferential looking measures are discussed in the final section with specific reference to their utility for use with primates (e.g. Waitt et al., 2003).

4.1.2.1 The dot-probe task

The dot-probe task (developed by MacLeod et al., 1986) is the most widely used paradigm for measuring spatial orienting to emotional stimuli. In its simplest form the task involves the simultaneous presentation of two stimuli, one neutral and one threat-related, equidistant from a central fixation point. After a predetermined time (e.g. 500ms) the stimuli are removed from the screen and a probe appears at the

Table 4-2 Published studies which measure spatial orienting to threatening versus non-threatening faces by humans

Method ¹	Threatening stimuli (TS)	Non-threatening stimuli (NS)	Anxiety measures ^{2,3}	Results	Reference
Papers supporting vigilance theories					
Dot-probe (s) 500ms (isi) 0ms (p) not given	High and mild threat scenes (photos)	Non-threat scenes	Non-clinical. High v Low TA (LTA v HTA: STAI)	Supports Cognitive-Motivational model, and stimulus intensity effects. HTA+LTA were faster to detect probes replacing high-threat compared with mild- or non-threat scenes. LTA were slower to detect probes replacing mild-threat versus non-threat scenes, suggesting avoidance, with no difference for high threat scenes. HTA were faster to detect probes replacing high-threat scenes versus non-threat scenes, suggesting vigilance.	Mogg et al. (2000b)
Dot probe (s)200ms (isi)50ms (p)6000ms	Angry faces (photos)	Happy and neutral faces	Non-clinical. High v Low SA and TA (STAI)	Supports emotionality effect. Vigilance for angry and happy faces in HA group (versus neutral faces). No delay to disengage, once data were adjusted for response-slowing	Mogg et al. (2008)
Dot probe + EM (s)500ms (isi)0ms (p) 1110ms	Angry faces (colour photos)	Happy, neutral, sad faces	Non-clinical. High v Medium v Low SA (STAI)	Supports threat-specificity effects. MSA and HSA were faster to detect probes replacing angry faces and made more initial eye shifts to angry faces than others.	Bradley et al. (2000)

¹ Where (s) = stimulus presentation time; (isi) = inter-stimulus/probe-interval; (soa) = stimulus onset asynchrony; and (p) signifies probe presentation time. EM signifies Eye Movement used as measure of (overt) attention. For visual search tasks values in brackets signify array size e.g. (2x2) signifies four pictures arranged in a 2 x 2 square.

² Only scales used in the classification of participants for the final analyses are given. STAI: Spielberger State-Trait Anxiety Inventory; RCMAS: Revised Child's Manifest Anxiety Scale; FNE: Fear of Negative Evaluation questionnaire; SADS: Social Avoidance and Distress Scale; POMS: Profile of Mood States questionnaire.

³ Data are from adults unless otherwise stated. TA: Trait Anxiety; SA: State Anxiety; SocA: Social Anxiety

Table 4.2 (continued)

Method	Threatening stimuli (TS)	Non-threatening stimuli (NS)	Anxiety measures	Results	Reference
Papers supporting vigilance theories (continued)					
Visual search + EM (1x7) (s) until response	Angry faces (photos)	Happy, surprised, disgusted, fearful, sad and neutral faces	-	Supports happy face superiority effect. The most visually salient expressions direct initial orienting to faces (in order: happy, surprised, disgusted, fearful, angry, sad), driven by bottom-up stimulus-bound featural processing (of the most salient features, i.e. the mouth region).	Calvo and Nummenmaa (2008)
Visual search (2x2 – 4x4) (s) 300ms and 800ms	Angry faces (schematic)	Happy, sad and neutral faces	-	Supports threat-specificity effect. Slower to detect absence of a discrepant face in all-angry displays versus all-happy or all-neutral displays. Faster to detect an angry face in an array of happy or neutral faces than a happy face in an array of angry or neutral faces. Effects of array size suggest serial processing and not ‘pop-out’.	Fox et al. (2000)
Visual search (3x4) (s) until response	Angry faces	Happy, disgust and neutral faces	Clinical. Generalised Social Phobics (GSPs) v non-anxious controls (NACs)	Supports threat-specificity effect. Faster to detect angry faces in an array of neutral or happy faces than happy faces among neutral or angry faces. GSPs were faster to detect angry faces than NACs, and faster to detect angry faces versus disgust faces (no effect in NACs)	Gilboa-Schechtman et al. (1999)
Visual search (2x2, 2x3, 2x4) (s) until response	Angry faces (schematic)	Happy, neutral and scrambled faces	Non-clinical. Children, High v Low TA (RCMAS)	Supports threat-specificity effect. Faster to detect angry faces among scrambled arrays than to detect happy or neutral faces. Effects of array size suggest serial processing and not ‘pop-out’.	Hadwin et al. (2003)

Table 4.2 (continued)

Method	Threatening stimuli (TS)	Non-threatening stimuli (NS)	Anxiety measures	Results	Reference
Papers supporting vigilance theories (continued)					
Visual search (2x2 – 5x5) (s) 1000ms or 2000ms	Angry faces (schematic)	Happy, neutral, sad faces	-	Supports threat/anger superiority effect. Angry faces were detected faster and with greater accuracy than other-emotion and neutral faces. Effects of array size suggest serial and parallel processing and not ‘pop-out’.	Öhman et al. (2001b)
Visual search with distractor task (2x2) (s) until response	Negative (sad) faces (schematic)	Happy and neutral faces	-	Supports negativity effect. Faster to detect negative faces in neutral or happy arrays. Slower to count the number of arcs in displays of negative faces than happy or neutral faces. Inversion effect negates interference effects.	Eastwood et al. (2003)
EM (s) until infant looked away for >4s	Angry and fearful faces (schematic and photos)	Neutral and Happy faces	Non-clinical. Infants of low and high social phobic mothers	Supports threat-specificity and emotional-intensity effects. Infants looked for longer towards high intensity angry faces than low intensity angry faces (10 wks). Same pattern for fearful faces (10 mos). No effect of maternal social phobia on infant.	Cresswell et al. (2008)
Papers supporting delayed-disengagement theories					
Spatial orienting task (s) 2000ms (soa) 600ms (p) 50ms	Fear faces (photos)	Happy, neutral faces	Non-clinical. High v Low TA (STAI)	HTA individuals were slower to detect probes presented concurrently with fear faces compared to trials with happy and neutral faces. There were no effects of face valence for LTA individuals.	Georgiou et al. (2005)

Table 4.2 (continued)

Method	Threatening stimuli (TS)	Non-threatening stimuli	Anxiety measures	Results	Reference
Papers supporting delayed-disengagement theories (continued)					
Exogenous cueing task (s)100ms/250ms (isi)50ms/200ms (p)2000ms	Angry faces (schematic and photos)	Happy, neutral and scrambled faces	Non-clinical. High v Low SA (STAI)	HSA individuals were slower to detect probes following (invalid) angry faces than either invalid happy or neutral faces. There were no effects of face valence on valid trials, of for LSA individuals.	Fox et al. (2001)
Papers supporting avoidance theories					
Dot-probe (s) 500ms (isi) not given (p) not given	Negative faces (colour photos)	Positive and neutral faces; Household objects	Clinical: Social phobia v matched controls	Supports non-specific bias away from emotional faces. Social phobic patients were significantly faster to detect probes occurring at the location of household objects versus emotional faces. Controls showed no bias.	Chen et al. (2002)
Dot-probe (s) 500ms (isi) not given (p) until response	Negative faces (anger, disgust, fear, sad: colour photos)	Household objects	Non-clinical. High v Low SocA (FNE). Bogus speech mood manipulation	Supports non-specific bias away from emotional faces. High socially anxious individuals under social evaluative stress were slower to detect probes at location of emotional faces, suggesting avoidance of emotional faces.	Mansell et al. (1999)
Papers supporting vigilance-avoidance theories					
EM: Electro-oculogram (EOG) (s)3000ms	Angry faces (B&W photos)	Happy and neutral faces (photographs)	Non-clinical. High v low TA (STAI)	Initial anxiety-independent vigilance towards angry faces, followed by subsequent avoidance by HTA but not LTA individuals	Rohner (2002)

Table 4.2 (continued)

Method	Threatening stimuli (TS)	Non-threatening stimuli	Anxiety measures	Results	Reference
Papers supporting vigilance-avoidance theories (continued)					
Dot probe + Eye movement (EM) (s)1500ms (isi)0ms (p) 10000ms	Angry faces (photos)	Happy and neutral faces.	Non-clinical Social Anxiety. High v Low (TA: FNE & SADS; SA): Public speech mood manipulation for SA	Supports non-specific bias for emotional faces mediated by SA, but not TA. HSA individuals were relatively faster to fixate ⁺ emotional faces than neutral faces, compared with LSA. However, LSA looked for longer towards ⁺ emotional faces than neutral faces (no bias for HSA).	Garner et al. (2006b)
Dot probe (s) 500ms or 1250ms	Angry faces (BW photos)	Neutral, happy and sad faces	Non-clinical. High v Low TA (POMS)	Inconclusive results. Early vigilance for threat in HTA versus LTA, revealed by faster RT on 500ms trials. On 1250ms trials vigilance was reduced in HTA and did not differ from LTA suggesting possible beginnings of avoidance, but evidence unclear.	Bradley et al. (1998)
Visual search with EM coded from video (2x2) (s) 150ms, 250ms and 3000ms	Angry faces (schematic)	Happy, sad and neutral faces	-	Supports threat-specificity effect. Faster to fixate, and preferential initial orienting towards angry versus other-emotional and neutral faces at 150ms exposure. Fewer, shorter, fixations towards angry faces during later processing, suggesting avoidance. Enhanced processing efficiency for threat stimuli suggested as mechanism.	Calvo et al. (2006)

location of one of the two previous stimulus locations. Participants are instructed to respond as fast as possible in identifying the location of the probe (e.g. left/right, up/down). Fast responses to a probe indicate attention was directed towards that location at probe onset, while slow responses suggest attention was located away from that location at probe onset. Changing the stimulus presentation times and stimulus onset asynchronies (SOAs) allows the time course of attention allocation to be mapped. For example, fast responses to the probe, following a subliminal (below threshold for awareness) or supraliminal (above threshold for awareness) presentation of a threat-relevant stimulus with a short SOA (<500ms) indicate rapid spatial orienting of attention towards the threatening stimulus (e.g. Mogg & Bradley, 1999; Holmes et al., 2005; Carlson & Reinke, 2008). Response latencies to the probe following supraliminal stimulus exposure, with a longer SOA (>500ms), reflect later processing stages, such as maintenance or disengagement effects.

In addition to temporal sensitivity, the dot-probe paradigm removes response bias effects (since the probe is always a neutral stimulus, such as a circle or cross) and arousal effects (since a threat-relevant stimulus appears on all response trials). Combination of dot-probe with ERP recordings has allowed precise measurement of the timings of differential attentional processes (e.g. Holmes et al. 2009), while combination with eye-tracking techniques has allowed dissociation of covert and overt attentional effects (e.g. Garner et al., 2006b)

4.1.2.2 Exogenous cueing task

The exogenous cueing task (Stormack et al., 1995; Fox et al. 2001; Georgiou et al., 2005) is an adapted version of Posner's (1980) spatial cueing paradigm. The procedure is similar to the dot-probe task, except that only one stimulus (either emotional or neutral) is presented on the screen on each trial (left/right location) and cues the location in which a target probe will appear on the majority of trials (valid trials). Stormack et al. (1995) suggest the speeding of response to valid emotional cues compared with valid neutral cues indicates attentional engagement, while the relative slowing of responses to invalid emotional cues (those where the probe appears at the opposite location to the cue) compared with invalid neutral cues indicates a delay to disengage attention from emotional stimuli. Koster et al. (2006) included a measure of trait anxiety in their emotional cueing task, along with manipulation of the stimulus presentation times to measure the time-course of allocation of attentional resources. Their data suggest that HTA enhances attention towards, and delays disengagement from, negative stimuli presented for 100ms. At longer stimulus presentations (200 and 500ms), faster responses to invalidly cued probes suggest attentional avoidance of negative stimuli among highly anxious individuals.

A restriction of the exogenous cueing task is that it lacks sensitivity to distinguish delayed disengagement due to attentional capture from arousal-related response slowing in the presence of threatening stimuli (Mogg et al., 2008). This method is increasingly considered less appropriate for measuring attentional shift than tasks such as the dot-probe. A variant of the exogenous cueing task (Georgiou et al., 2005) measures speed to orient attention away from an emotionally-valenced

stimulus, presented at fixation, towards an uncued, peripherally presented probe. Instead of cueing the likely position of the probe, the emotional stimulus in this case is a distractor, so that time taken to locate the probe provides a measure of the time required to disengage attention away from the stimulus. Angry and fearful faces presented at fixation delay detection of peripheral targets among anxious individuals (angry faces: Fox et al., 2001; fearful faces: Georgiou et al., 2005), while sad faces do not (Georgiou et al., 2005).

4.1.2.3 Visual search task

The visual search task requires participants to locate a target stimulus within an array of distractor stimuli (for example, a snake among flowers: Öhman et al., 2001a; an angry face in a crowd: Öhman et al., 2001b; Calvo et al., 2006). Studies have demonstrated an effect of trait anxiety on search times for negatively and positively valenced faces among arrays of neutral or emotional faces in both adults (enhanced detection of angry relative to happy faces in social phobics: Gilboa-Schechtman et al., 1999) and children (enhanced detection of angry relative to happy or neutral faces: Hadwin et al., 2003). The extent to which variation in search times can be attributed to early or late attentional processes is unclear. In a modification of Öhman et al.'s (2001a) experiment, Lipp et al. (2004) presented both fear- and non-fear-relevant animals among arrays of mushrooms and flowers. Faster detection times for all animals suggest that the original conclusion of an evolved fear module for detecting dangerous animals may be incorrect, and instead reflect constraints of using stimulus arrays, rather than an attentional process.

More consistent results have been provided by studies using face stimuli (the 'face in the crowd' effect: Hansen & Hansen, 1988). Fox et al. (2000) ran a series of visual search tests in which combinations of happy, angry and neutral schematic faces were shown on a screen. Angry faces were detected faster than happy or neutral faces, and angry faces were a distraction to identifying the presence of other-valenced faces. In another variant of the visual search task Eastwood et al. (2003) asked participants to count the number of arcs presented on a screen. The arcs were presented in configurations that formed upright or inverted schematic positive (happy), negative (sad) and neutral faces. The presence of negative schematic faces on the screen slowed response time relative to when happy or neutral faces were present, despite containing the same number of component parts. This suggests, that the presence of negative faces in an array captures attention, despite ongoing task demands (counting arcs).

Recent findings for a superiority effect of happy faces in a visual search task, bring previous threat- or negative-superiority theories into question. Calvo & Nummenmaa (2008) found faster detection for happy faces over all other emotional faces in visual search. They suggest enhanced detection of happy faces is due to increased saliency of the mouth region specifically, and attribute enhanced detection to stimulus-bound bottom-up featural processing which occurs independently of top-down appraisal of the emotional content of the face. Therefore the mechanism by which the 'face-in-the-crowd' effect arises, and the specificity of this effect, require further investigation.

4.1.2.4 Gaze measures

Eye movement and preferential looking measures have been used with both human adults (e.g. Bradley et al., 2000; Calvo & Avero, 2005; Garner et al., 2006b) and infants (e.g. Cresswell et al., 2008) in the context of attentional bias research. Fixation of one of two competing stimuli is interpreted as indicating a visual bias for the fixated stimulus. Bradley et al. (2000) used infra-red eye-tracking equipment to record direction of first eye movement towards emotional versus neutral faces presented for 500ms during a dot-probe task. Faster responses to threat-valid probes by high-state-anxious (HSA) and medium-state-anxious (MSA) individuals correlated positively with initial orienting of gaze towards threatening faces. Therefore direction of first look towards stimuli may provide a valid analogue measure of the spatial orienting of attention as revealed by reaction time data from dot-probe studies.

Following their finding, Bradley et al. (2000) suggested that the addition of duration of gaze may provide a valid measure for teasing apart early and later stages of attention allocation (initial orienting and later maintenance stages), a point Bradley made in a previous dot-probe study in which the limitations of the timings of stimulus presentations within a dot-probe paradigm failed either to reveal, or rule out, a vigilant-avoidant account (Bradley et al., 1998). This triggered a number of studies using gaze measures to map the time course of attentional bias (gaze direction and duration to threat scenes: Calvo & Avero, 2005; Nummenmaa et al. , 2006; Calvo & Nummenmaa, 2007; gaze towards faces: Calvo et al., 2006; Garner et al., 2006b; electro-oculographic, EOG, responses to faces: Rohner, 2002).

Garner et al. (2006b) measured latency and duration of gaze towards faces by high and low socially anxious individuals during a dot-probe study in which emotional versus non-emotional face pairs and face versus non-face pairs were shown. This was the first study to use duration of gaze as a measure of attention allocation towards emotional and non-emotional faces by individuals who differed in anxiety. Their data revealed effects of state and social anxiety on both latency and duration of gaze. Socially anxious individuals were relatively faster to gaze towards both positive and negative emotional versus non-emotional faces, compared with low-socially-anxious individuals, but spent less time overall looking towards emotional faces than did low-socially anxious individuals. This finding suggests a vigilant-avoidant pattern of attention allocation towards emotional faces by socially anxious individuals.

The Garner et al. (2006b) study demonstrates the value of gaze as a measure for revealing both early vigilance and later avoidant attentional strategies, which cannot be teased apart from reaction time data alone. Calvo et al. (2006) measured latency, duration and frequency of eye movements, coded from video, towards emotional faces in a visual search task. They found speeded detection of, but shorter gaze duration towards, angry faces, compared with other-emotion and neutral faces, suggesting eye movements coded from video provide a valid measure of both initial orienting and maintenance of attention with respect to threatening stimuli.

In addition to its value in mapping the direction of early and later stages of spatial attention within a single trial, eye movements also provide a suitable tool for measuring spatial attention in non-linguistic participants. Human infants, at 10 days of age, gaze for longer towards faces versus non-faces, at 10 weeks gaze for longer at high-intensity angry faces compared with low-intensity angry faces, and high versus low intensity fearful faces at 10 months (Cresswell et al., 2008). Increased fixation times are often discussed in terms of an attentional ‘preference’ for that stimulus but, depending on the design of the task, may also indicate a response to other factors such as novelty or expectation violation (e.g. Walden et al., 2004).

While no study has used eye gaze to investigate attentional biases for emotional versus non-emotional faces in a species of non-human primate, many studies have used eye-gaze to measure primate spatial attention, including attention towards conspecific faces. I discussed these studies in Chapter 2. Of particular relevance here, is validation of preferential looking paradigms for use with primates (e.g. preference for facial colouration in rhesus macaques: Waitt et al., 2003), and demonstrated reliability in coding primate eye gaze (chimpanzees: Bethell et al., 2007). These studies reveal that direction, duration and shifts in eye gaze may vary as a function of differences in social relevance of different faces, and these shifts may be reliably coded by human observers. However, there are few studies that document shifts in eye-gaze between two competing faces (cf. Waitt et al., 2003), and there are no studies that investigate shifts in eye-gaze between faces that differ in facial expression, nor between faces and non face stimuli, and the effects of different experimental treatments on these patterns.

4.1.3 Implications of human-based research for the development of a novel method to study attentional bias in primates

Several themes arose from the literature review that informed the design of the current study. Firstly, attention is componential. It comprises early attentional engagement, ongoing maintenance, and later disengagement stages. Therefore, a paradigm that measures each of these stages (such as eye-gaze) will provide a more complete picture of the attentional response than paradigms that capture only early or later stages (e.g. dot-probe versus exogenous cueing tasks respectively). Recent studies have argued for the importance of eye-gaze as a valid measure of preferential attention in attentional bias research (Bradley et al., 2000; Calvo & Avero, 2005; Garner, et al., 2006). Comparison of eye movement and reaction time data by Bradley et al. (2000) revealed that initial orienting of gaze predicted speeding of responses to threat-valid probes in a dot-probe task. Taken together with validation of gaze as a measure of preferential looking at faces in human infants (Farroni et al., 2002; Cresswell et al., 2008) and other species (monkeys: Waitt et al., 2004; sheep: da Costa et al., 2004), a preferential looking paradigm was selected for use in the current study.

Secondly, attentional biases may represent a bias to orient attention towards threatening versus non-threatening stimuli (vigilance), or away from threatening versus non-threatening stimuli (avoidance), in humans. Disagreement in the literature is partly due to the temporal specificity of the different paradigms used. Therefore, a test of alternative hypotheses with respect to the direction of any biases is most appropriate for a first investigation of attentional bias in another species.

Thirdly, attentional biases may be either threat-specific or emotion-general. The majority of studies point towards a threat-specific attentional bias in face processing among humans. Therefore, threatening versus non-threatening were selected for the stimuli, specifically aggressive versus neutral conspecific faces. I refer to these interchangeably as emotional versus non-emotional, threatening versus non-threatening and aggressive versus neutral to reflect data from humans.

Fourthly, attentional biases may be revealed in both trait and state anxiety, and both within, and between, human subjects. As a first investigation into the phenomenon in a species of primate, the two treatments described in Chapter 3 were considered applicable to this study. When working with small sample sizes, the within-subjects approach is more robust than between-subjects, allowing for tighter control of any extraneous factors which may influence the results (for example, trait characteristics). The design selected for the present study was therefore within-subjects, with experimental manipulations to increase physiological indicators of stress (Post-health-check), and reduce physiological indicators of stress (Post-enrichment).

In addition to the points listed here, laterality effects in attention have been revealed in humans (ERP: Holmes, et al., 2008; fMRI: Grand et al., 2003; dot-probe: Mogg & Bradley, 1999) and other animals (Tsao et al., 2008; Broad et al., 2000). A general right hemisphere (left visual field: LVF) superiority for processing faces has been shown in humans (Mogg & Bradley, 1999), including infants (Cresswell et al., 2008). Mogg & Bradley (1999) revealed a LVF advantage for processing faces presented subliminally, as revealed by faster

detection of probes occurring at the location of threatening faces presented to the LVF, compared with the right visual field (RVF), suggesting right hemisphere involvement (see also Calvo & Nummenmaa, 2007; Calvo & Avero, 2008). Comparable evidence for a right hemisphere advantage for processing faces has also been shown for rhesus macaques (fMRI: Tsao et al., 2008; split-brain: Vermeire et al., 1998), chimpanzees (tympanic membrane temperature: Parr & Hopkins, 2000), and sheep (Broad et al., 2000; Peirce et al., 2000), as discussed in more detail in Chapter 2. The possibility of a LVF processing bias was considered in the design of the current experiment.

4.2 Aims:

The general aim of this chapter is to describe the development of a method for measuring attentional bias in rhesus macaques. The method is applied to test competing hypotheses about the possible mechanisms underlying such biases, namely to distinguish early and late stages of processing and direction of the bias towards, or away from, threat. The following hypotheses relate to spatial orienting of attention to one of two stimuli when an aggressive face and a neutral face are presented concurrently within view. Differences in attention allocation to aggressive versus neutral faces are tested during two treatment conditions introduced in Chapter 3 (Post-health-check and Post-enrichment).

4.2.1 Alternative hypotheses and specific predictions:

4.1 Monkeys exhibit a **vigilant** pattern of attention allocation towards threatening (aggressive faces) versus non-threatening (neutral faces) stimuli, when both are shown together. This effect is mediated by affective state.

- a. Monkeys will **gaze first** towards an aggressive face when an aggressive-neutral face pair is shown.
- b. Monkeys will be **faster to gaze** towards an aggressive face versus a neutral face.
- c. Monkeys will be **slower to disengage** first gaze from an aggressive face versus a neutral face.
- d. Monkeys will gaze towards an aggressive face **for more time** than a neutral face.
- e. The above effects will be greater Post-health-check (i.e. after a stressor) versus Post-enrichment (i.e. following a period of low stress).

4.2 Monkeys exhibit an **avoidant** pattern of attention allocation with respect to threatening (aggressive faces) versus non-threatening (neutral faces) stimuli. This effect is mediated by affective state.

- a. Monkeys will **not gaze first** towards an aggressive face when an aggressive-neutral face pair is shown.
- b. Monkeys will be **slower to gaze** towards an aggressive face versus a neutral face.
- c. Monkeys will be **faster to disengage** first gaze from an aggressive face versus a neutral face.

d. Monkeys will gaze towards an aggressive face for less time than a neutral face.

e. All of the above effects will be greater Post-health-check (i.e. after a stressor) versus Post-enrichment (i.e. following a period of low stress).

4.3 Monkeys exhibit a **vigilant-avoidant** pattern of attention allocation towards threatening (an aggressive face) versus non-threatening (a neutral face) stimuli which is mediated by affective state.

a. Monkeys will **gaze first** towards an aggressive face when an aggressive-neutral face pair is shown.

b. Monkeys will be **faster to gaze** towards an aggressive face versus a neutral face.

c. Monkeys will be **faster to disengage** first gaze from an aggressive face versus a neutral face.

d. Monkeys will gaze towards an aggressive face **for less time** than a neutral face.

e. All of the above effects will be greater Post-health-check (i.e. after a stressor) versus Post-enrichment (i.e. following a period of low stress).

Part B: Development of the new method

In Part A, I presented the background and rationale for the development of a method to study attentional bias in rhesus macaques. In Part B, I detail the method developed, present data and discuss these in light of the human literature.

4.3 Method

4.3.1 Participants

Eight monkeys took part in the study (Monkeys 45A, 06H, 29C, G62, 86O, 94K, 92R, & 27S; range: 4.66 – 22.8 years old; average age: 10.0 ± 6.3 years). All monkeys had previously begun operant touchscreen training in the laboratory during the preceding six months. Five monkeys (29C, 86O, 94K, 92R and 27S) had responded well to previous training and worked in the laboratory on a daily basis. Three monkeys (45A, 06H & G62) had responded less well to earlier touchscreen training and worked infrequently in the laboratory (see Table 2-3, p71).

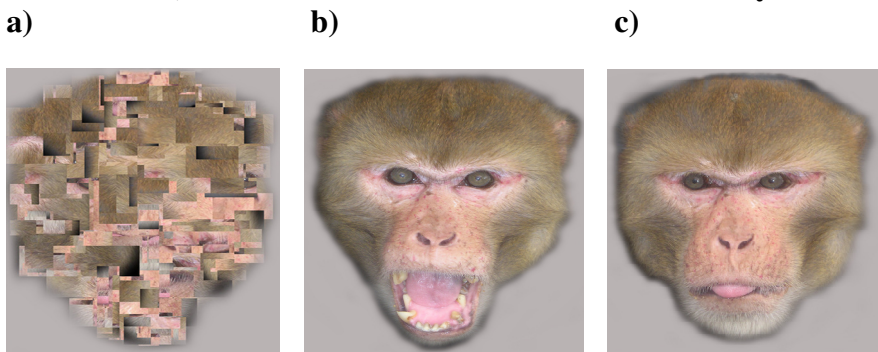
4.3.2 Stimuli and apparatus

The face stimuli consisted of 20 colour photographs of 10 male monkeys ('stimulus monkeys') housed at CPRC. The stimulus monkeys were unknown to the participant monkeys. For each stimulus monkey, one photograph showing a frontal view of the face with aggressive expression (from herein 'Aggressive face'), and one photograph showing a frontal view of the face with neutral

expression ('Neutral face') were selected. This resulted in one 'Aggressive face' and one 'Neutral face' per stimulus monkey (Figure 4.5a and b).

Face pictures were selected for a clear frontal view of the face with open eyes, and were trimmed so that only the head was visible. Face pictures were cropped around the face and matched for size before being superimposed on a grey background and enclosed in a rectangular frame measuring 154mm x 164mm, thereby subtending 14.71 x 15.66 degrees of visual angle when presented centrally on a computer monitor at a 60cm viewing distance (170 x 230 pixels on a 256 greylevel scale). The face stimuli were paired, according to stimulus monkey identity, to give 10 aggressive-neutral face pairs (Appendix 4).

Figure 4.5 Examples of the face stimuli used during the study. a) aggressive, b) neutral and c) scrambled face from one stimulus monkey.



Aggressive-neutral face pairs were assessed for equivalence of luminance (L) and contrast energy (C). This is standard practice in studies with humans (e.g. Holmes et al., 2008) and is of importance for studies with primates such as rhesus

macaques (Waite & Buchanan-Smith, 2006). L is a measure of brightness, and is measured in candela per square metre (cd/m^2) emitted by the stimulus on the screen. L values were not available for the stimuli, and so an indirect measure (luminosity: L_y) was used in the calculations here. The L_y function in Adobe gives a measure of the lightness of pixels (with white being lightest), and is based on the Red, Green and Blue (RGB) value for each pixel. L_y is a property that underlies L , therefore it is a suitable proxy measure for comparing equivalence between stimuli where absolute L values are not required.

C is a measure of the range of L_y within a given stimulus. It is important that two stimuli within any given pair do not vary significantly from one another in L_y or C . A difference in L_y or C would mean the two stimuli are perceptually different in terms of brightness. Differential gaze to such stimuli may therefore be accounted for by brightness effects rather than, for example, emotional content. The mean L_y value for each face stimulus was provided by the histogram function in Adobe Photoshop 7. The mean L_y for each face stimulus was entered into a paired-samples t-test to assess equivalence of L_y across aggressive-neutral face pairs. Aggressive and neutral faces were paired according to stimulus monkey identity. Aggressive-neutral face pairs did not differ in L_y ($t(9)=0.97$, $P=0.36$). C was calculated from the L_y values (minimum, maximum and mean, as provided by the histogram function in Adobe Photoshop 7) using the formula:

$$C = \frac{L_y \text{ max} - L_y \text{ min}}{L_y \text{ max} + L_y \text{ min}}$$

where $L_{y\max}$ and $L_{y\min}$ are the maximum and minimum L_y values of the stimulus. C ranges from 0 to 1.0 (MacIntyre & Cowan, 1992). The C value for each face was entered into a paired-samples t -test to assess equivalence of C within aggressive-neutral face pairs. Aggressive-neutral face pairs did not differ in C ($t(9)=2.197$, $P=0.92$). Therefore, the L_y and C of the aggressive faces were equivalent to that of the neutral faces with which they were paired. An additional check was run to examine the degree of variation between the different stimulus monkey face pairs. Mean L_y and C values for all face stimuli fell within 2SD of the mean L_y and C values for the entire set of 20 face stimuli. Therefore, variance in L_y and C was low across the entire stimulus set.

For each of the 10 neutral face stimuli a corresponding scrambled face stimulus was compiled (Figure 4.5c). Scrambled face stimuli were created using the batch process function in Adobe Photoshop 7. The marquee tool was used to randomly select a rectangular area of one of the neutral faces, and horizontally, or vertically, flip the selected area. This procedure was repeated for all areas of the face until the configuration of facial features was disrupted. Copies of the remaining nine neutral face stimuli were then batch processed using the same series of manipulations. This resulted in 10 identically processed scrambled face stimuli (Appendix 4). In total there were therefore 30 stimuli (10 each of neutral, aggressive and scrambled faces). Each stimulus monkey was assigned a number from 1-10. The 10 aggressive-neutral pairs were divided into two subsets, with aggressive-neutral face pairs numbered 1-5 in subset one, and aggressive-neutral

face pairs numbered 6-10 in subset two. The corresponding scrambled faces were then assigned to their matching stimulus subset.

Face stimuli were presented in pairs, matched for stimulus monkey identity, on two adjacent Sony 16in. computer monitors situated in the laboratory. The computer monitors were positioned to the left and right of a pellet tray which was connected via a chute to a (concealed) automatic pellet dispenser. The horizontal distance between the mid-points of the two screens was 45cm, so that the distance of each stimulus mid-point from the central line of fixation on each trial was 22.5cm. The screens were set at a resolution of 1024 x 768 pixels, and did not differ in illuminance readings when set to black screen, or when the same stimulus was shown on either screen (paired samples t-test: $t_{29}=0.151$, $P=0.880$).

The computer monitors in the laboratory were connected via a junction box with split-screen monitor (Figure 4.6a), to a Satellite Pro A60 laptop running Microsoft Office Powerpoint 2003 software. The junction box, split-screen monitor and laptop were all situated in an adjacent control room, from where the experimenter ran the observation. All sessions were filmed using a Samsung VP-L150 digital video camera placed centrally and behind the two monitors (Figure 4.6 b). The camera was positioned to film the monkey's direction of gaze. A live video feed to the control room allowed the experimenter to observe the monkey on a video monitor. Two small mirrors on the front of the cage revealed changes in light levels which allowed stimulus onset and offset to be detected on the video during later coding (Figure 4.6c).

Figure 4.6 The apparatus. a) The split screen monitor situated in the control room. This gives an indication of the size and location of the stimuli as they appeared on the two computer monitors in the laboratory. b) Monkey 94K gazes at an image on the computer monitor to his left. The inside edge of the monitor (black casing outlined in white dashed line) is visible to the right hand side of the image. The white pellet chute is visible on the left hand side of the image. c) The two mirrors on the front of the cage reflected light changes at stimulus onset and offset without allowing the stimuli to be viewed on the video.

a)



b)



c)



4.3.3 Design and Procedure

Face pairs were presented in a randomised order in the pair combinations: aggressive-neutral (experimental trials: Figure 4.7 top row); aggressive-aggressive, neutral-neutral, scrambled-scrambled and neutral-scrambled (control trials: Figure 4.7 bottom row). Experimental trials (aggressive-neutral face pairs) were included to test the experimental hypotheses about the allocation of attention towards emotional versus non-emotional faces. Control trials (aggressive-aggressive, neutral-neutral and scrambled-scrambled face pairs) were included to reveal any side-biases in viewing faces and abstract stimuli in general. Additional control trials (neutral-scrambled) were included to reveal any vigilance or avoidance effects for social versus non-social stimuli in general.

The procedure for a testing session was as follows. The Powerpoint presentation was opened so that the two screens showed the first black slide. The monkey was then transported to the laboratory in the testing cage, positioned in front of the apparatus and allowed to settle (this varied between monkeys: some settled instantly, others required several minutes). The experimenter immediately moved to the adjacent room, and set the video to record events. The monkey was encouraged to gaze centrally, between the two screens, by the delivery of a single primate pellet into the pellet tray. When the monkey gazed centrally between the screens the experimenter triggered the onset of the first trial (i.e. the next Powerpoint slide: Figure 4.8). On each trial a pair of faces was presented for 10 seconds. Stimulus offset was triggered automatically by the Powerpoint software at a latency of 10 seconds post-stimulus-onset. At stimulus offset, a plain black slide was shown until the monkey gazed centrally and the next trial began.

Figure 4.7 The three trial types. On experimental trials aggressive-neutral pairs were shown. On control trials aggressive-aggressive, neutral-neutral or scrambled-scrambled face pairs are shown

Experimental trials



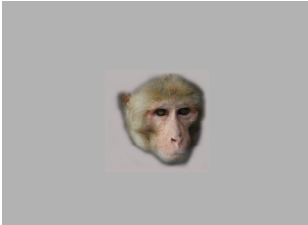
Aggressive-Neutral

Control Trials



Aggressive-Aggressive

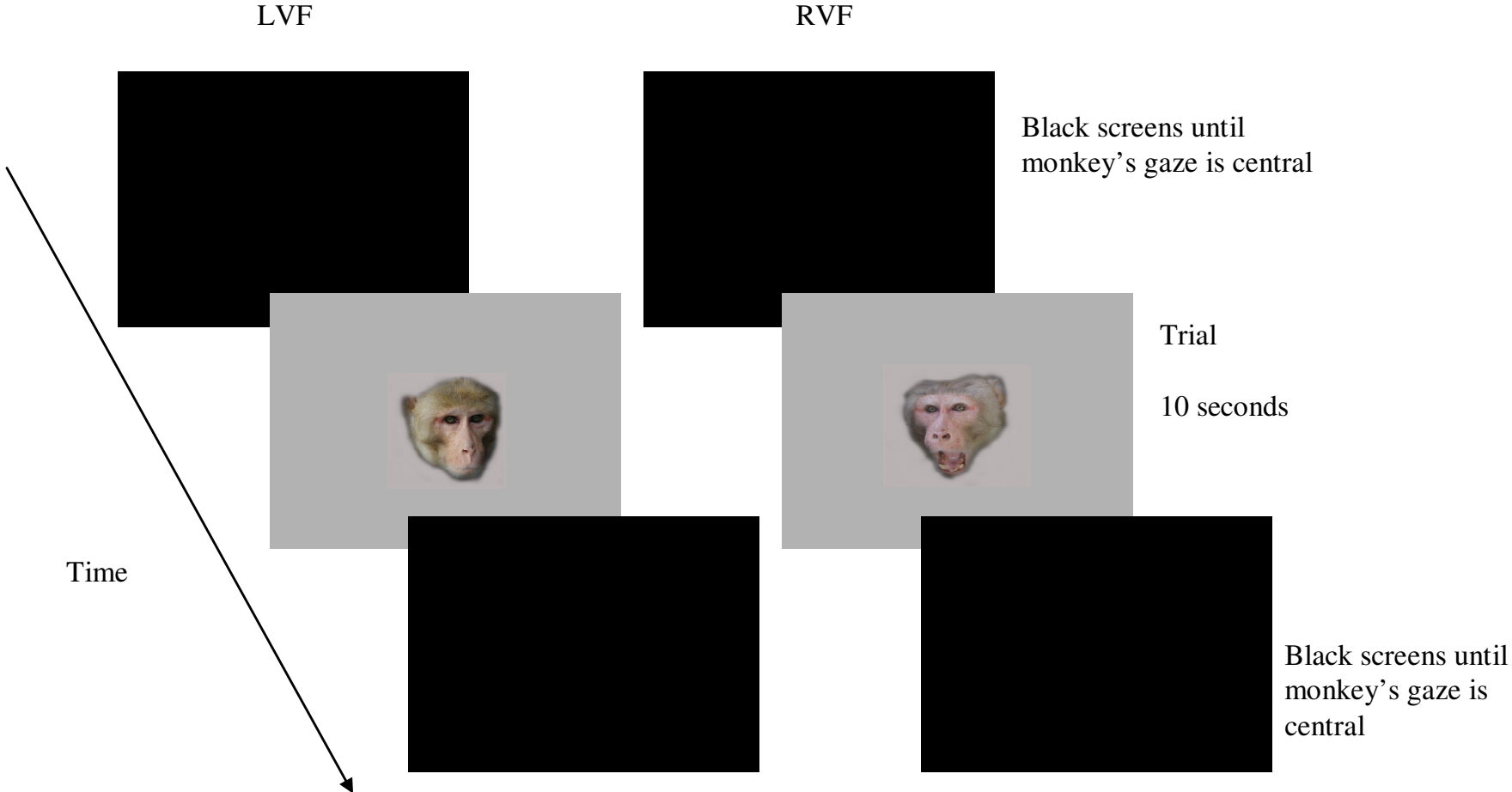
Neutral-Neutral



Scrambled-Scrambled

Neutral-Scrambled

Figure 4.8 The experimental procedure for presentation of face pairs on the two adjacent screens

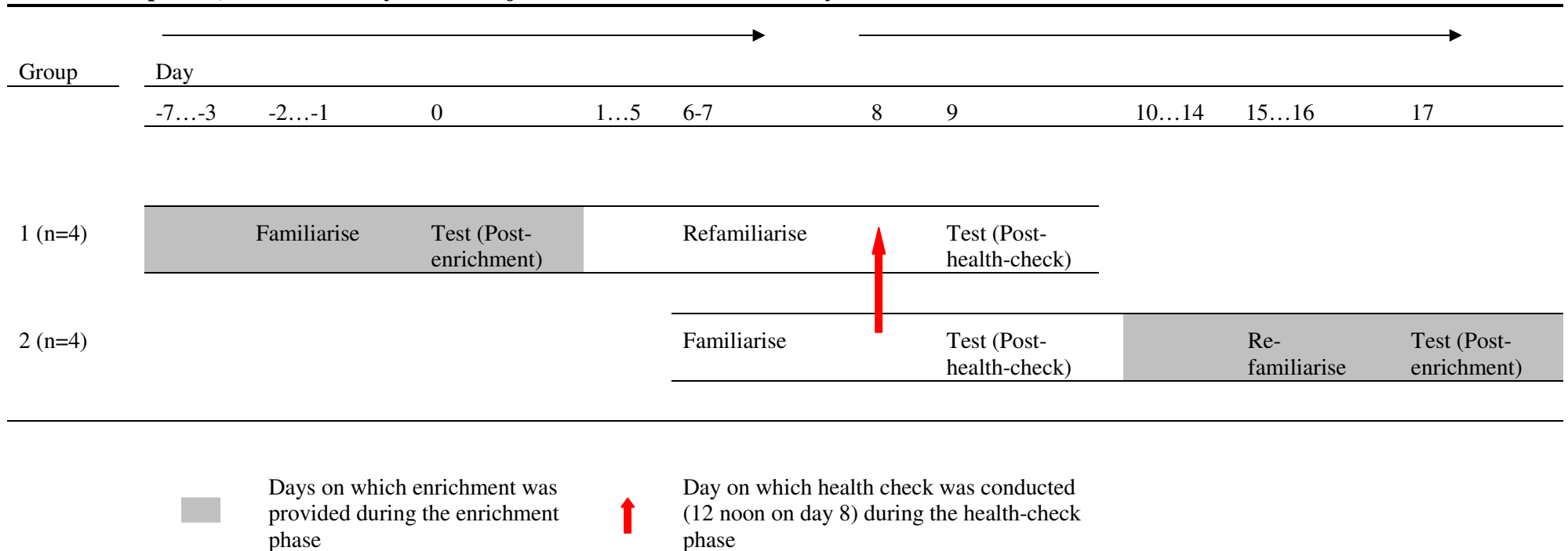


All monkeys were tested once Post-enrichment and once Post-health-check. The order of testing was counterbalanced across individuals so that four monkeys were first tested Post-enrichment (Group 1), and four were first tested Post-health-check (Group 2: Figure 4.9).

The order of events was as follows. On days -7 to -1, all monkeys housed in the enclosure received enrichment in the home cages, as described in Chapter 3. On days -2 and -1, Group 1 underwent familiarisation with the apparatus. During this phase each monkey in Group 1 was transported individually to the laboratory where he was shown the female rump stimuli and allowed to feed in front of the apparatus. On day 0, Group 1 took part in the Post-enrichment testing session, following the procedure described below. On days 1-5, all monkeys engaged in their normal daily routine (either participation in training for other laboratory tasks, or remaining in the home cage). On days 6 and 7, Groups 1 and 2 underwent a (re)familiarisation with the laboratory equipment, viewing female rumps on the screen and feeding in front of the apparatus. On day 8, all monkeys in the enclosure received their three-monthly health-check, conducted by the facility veterinarian. On day 9, Groups 1 and 2 took part in Post-health-check testing. On day 10, all monkeys in the enclosure began a second week of enrichment during which Group 2 underwent familiarisation on days 15 and 16. On day 17, Group 2 underwent Post-enrichment testing.

The experiment consisted of a series of 21 randomised trials within each testing session (Post-enrichment and Post-health-check: Table 4-3). There were six experimental trials during which the aggressive-neutral face pairs were presented,

Figure 4.9 The counterbalanced order of testing sessions conducted post-enrichment (p-e) and post-health (p-hc) check. Prior to each testing session monkeys underwent two days of familiarisation with the laboratory. All monkeys were provided with enrichment during the enrichment phases, and all monkeys were subjected to a health check on day 8.



counterbalanced for number of presentations of either face stimulus to the left and right visual fields LVF and RVF respectively. There were 15 control trials, composed of three each of aggressive-aggressive, neutral-neutral and scrambled-scrambled trials, and six neutral-scrambled trials. A record was kept of the order of trials, including the left/right orientation of the stimulus face-pairs, for later interpretation of gaze data coded from video.

Videos were later digitised and blind coded for direction of gaze on an AppleMac computer by the experimenter using iMovie HD version 6.0.3 software at Roehampton University. Video was scored for direction of gaze, on a frame by frame basis. Gaze was here defined as orientation of the eyes with respect to the two monitors. The codes were '**central**' (eyes directed forwards and between the two monitors), '**left**' (eyes directed towards the left-hand-side monitor), '**right**' (eyes oriented towards the right-hand-side monitor), '**away**' (head turned away so that both screens are outside of peripheral vision in any direction). When it was not possible to determine direction of gaze because either the head or eyes were out of view: '**out of view**' was scored. When the video quality was too poor to code: '**NA**' was scored. Reliability for coding was tested by two coders (EB and Magdalena Kobus), who had previously discussed and practiced scoring the gaze categories. A subset of previously uncoded video (10 trials) was coded by each on a frame-by-frame basis, and agreements and disagreement entered into an agreement matrix for analysis.

Table 4-3 The presentation of face-pairs to the left and right visual fields during experimental and control trials. Testing was conducted during one Post-enrichment session and one Post-health-check session.

	Visual field		Treatment	
	LVF	RVF	Post-enrichment	Post-health-check
Experimental trials				
	Aggressive	- Neutral	3	3
	Neutral	- Aggressive	3	3
Control trials				
	Aggressive	- Aggressive	3	3
	Neutral	- Neutral	3	3
	Scrambled	- Scrambled	3	3
	Scrambled	- Neutral	3	3
	Neutral	- Scrambled	3	3
Total N trials			21	21

4.3.4 Data analyses for experimental trials (aggressive-neutral face pairs)

Experimental trials (aggressive-neutral face pairs) were included to test the competing hypotheses regarding spatial orienting towards aggressive versus neutral faces. Separate analyses were conducted for each of the gaze measures. The measures were direction of first gaze, latency to first gaze, latency to disengage first gaze and total duration of gaze. In all of the subsequent analyses, order of testing (Post-enrichment first versus Post-health-check first) did not have an effect on the results, therefore this between-groups factor was removed from the analyses presented here. Groupings for order of testing are shown in all tables

for reference purposes. Due to evidence for hemispheric specialisation in the processing of emotional faces, visual field is included in those analyses where sufficient data were available.

All data were checked for a normal distribution using a one-sample Kolmogorov-Smirnov (K-S) test. Homogeneity of covariance was assessed with Levene's Test for equality of variance. All K-S and Levene's tests revealed data did not differ significantly from a normal distribution or equality of variance, unless stated otherwise in the text. Repeated Measures Anovas (RmAnova) were conducted to test interactions of treatment condition and stimulus type on gaze measures. Where there were missing cell values, which precluded a RmAnova, Mundry's (1999) permutation test for related samples with missing values was used, maintaining the position of missing values and running 10,000 permutations. This test is a variant of Friedman's One-way ANOVA, except that empty cells are retained in the analysis. Therefore Mundry's (1999) permutation test is appropriate for analyses of datasets with small sample sizes, with missing cell values, where data may not be normally distributed and where the same individuals are used in the different pairwise comparisons. All descriptive data are reported as mean \pm 1SE. Significant findings are presented in figures. Where there were missing cell values, data are presented in tables to identify the location of missing data. In all cases where data were collapsed across conditions, and there were missing cell values, only data which were represented in both conditions were included.

Direction of first gaze was measured as the proportion of trials on which monkeys gazed first towards an aggressive face on experimental trials. Only those trials on which each monkey was gazing centrally between the two screens at stimulus onset were included in the analysis.

Latency to first gaze was measured as the number of seconds monkeys took to direct their gaze towards the first stimulus to be gazed at. Only those trials on which each monkey was gazing centrally between the two screens at stimulus onset were included.

Latency to disengage first gaze was measured as the number of seconds monkeys gazed towards the first stimulus to be gazed at before disengaging gaze. All trials were included in the analysis, regardless of where the monkey was gazing at stimulus onset.

Duration of gaze was measured as the total number of seconds per 10 second trial that monkeys gazed towards aggressive faces and neutral faces on experimental trials. All trials were included in the analysis.

4.3.5 Data analyses for control trials

Control trials (aggressive-aggressive, neutral-neutral and scrambled-scrambled trials) were included to check for side biases in viewing faces in general. In addition, neutral-scramble control trials were included to examine biases for social versus non-social information. The consideration of side biases is important

for two reasons. Firstly, studies with humans have revealed visual field preferences for gazing towards emotional and non-emotional faces which are interpreted in terms of hemispheric specialisation for processing emotional faces. Aggressive-aggressive and neutral-neutral face pairs were included to examine laterality effects for emotional and non-emotional faces in the monkeys. Secondly, it is important to establish that each monkey has no intrinsic bias in the direction in which he gazes towards the two screens *per se*. Scrambled-scrambled trials were included to examine side biases in orientation towards non-face stimuli shown on the two screens. Following existing literature, only direction of first gaze and total duration of gaze were used in these analyses.

Following evidence that avoidance of social cues is evident when a non-social alternative cue is provided (Garner et al., 2006b), neutral-scrambled control trials were also included. These trials were designed to provide monkeys with competing social and non-social cues (after Mansell et al., 1999). Separate analyses were conducted for each of the four gaze measures, following the procedures outlined above.

4.4 Results

Seven of the eight monkeys completed the study. One monkey (monkey 27S) failed to settle in the testing cage Post-health-check. He did not gaze centrally between the screens and showed signs of agitation throughout. Video for this monkey was not scored and he was removed from the study. In total, 266 trials were recorded with good visibility and coded for analysis for gaze towards the

two screens. Reliability for coding was attained by two coders at $k=0.76$, which is considered a good level of reliability (Bakeman & Gottman, 1997).

4.4.1 Experimental trials (aggressive-neutral face pairs)

a) Direction of first gaze

A 2 x 2 RMANOVA was performed to determine whether treatment condition mediated direction of first gaze (alternative predictions ‘a’ and ‘e’) towards (vigilance), or away from (avoidance), emotional stimuli, and to investigate visual field effects. Data were proportion of experimental trials on which monkeys gazed first towards the aggressive face, with within-subjects factors of treatment (Post-enrichment versus Post-health-check) and visual field (LVF versus RVF: Table 4-4). There was a main effect of visual field which approached significance ($F_{1,4}=6.370$, $P=0.06$). There was no main effect of treatment ($F_{1,4}=0.00$, $P=1.00$), nor interaction between treatment and visual field ($F_{1,4}=2.67$, $P=0.18$).

Planned one-sample t-tests were conducted to investigate the main effect of visual field, separately for each treatment (Post-enrichment and Post-health-check: Table 4-4). The test value was 0.5. The proportion of trials on which monkeys gazed first towards aggressive faces presented to the RVF was significantly less than 0.5, both Post-enrichment ($t_6=5.28$, $P=0.002$) and Post-health-check ($t_5=3.16$, $P=0.02$). The proportion of trials on which monkeys gazed first towards aggressive faces presented to the LVF did not differ significantly from 0.5 (Post-enrichment: $t_5=1.69$, $P=0.15$; Post-health-check: $t_6=0.00$, $P=1.00$).

Table 4-4 The proportion of trials on which monkeys oriented first towards aggressive faces on experimental trials

ID	Initial treatment	Treatment			
		Post-enrichment		Post-health-check	
		LVF	RVF	LVF	RVF
06H		1	0	0.5	0
92R		1	0	1	0
94K	Post-enrichment	0.67	0	0	-
45A		0	0	0	0.5
29C		1	0	1	0
86O	Post-health-check	1	0.5	1	0.5
G62		-	0.33	0	0
Visual field bias		none	avoid**	none	avoid*

- missing cell values signify there were no valid trials for analysis, either because the monkey was not gazing centrally at the start of the trials, or because data were lost due to poor recording. A visual field bias is revealed when the proportion of trials on which monkeys oriented first towards the aggressive face differs significantly from 50%: avoid = a significant bias *not* to orient first towards aggressive faces presented to the specified visual field (*P=0.02 and **P<0.01); none = no significant bias.

A further one-sample t-test was performed to determine whether monkeys exhibited a bias in direction of first gaze towards, or away from, aggressive versus neutral faces. Data were proportion of trials on which monkeys gazed first towards the aggressive face in an aggressive-neutral face pair, collapsed for visual field and treatment. The proportion of trials on which monkeys gazed first towards aggressive faces did not differ significantly from 0.5 ($t_6=1.17$, $P=0.29$).

In summary, data for direction of first gaze provided no support for any of the alternative hypotheses (predictions 'a' and 'e'). There was some evidence for visual field effects in direction of first orientation towards aggressive-neutral face pairs which support an **avoidance** account of attention for emotional versus non-emotional faces: monkeys avoided gazing first towards aggressive faces presented to the RVF. This pattern was not influenced by treatment condition, and is contrary to previous studies which suggest a LVF bias in face processing in both humans and rhesus macaques. There was no evidence for a LVF bias for emotional faces in the present study.

b) Latency to first gaze

A 2 x 2 RMANOVA with within-subjects factors of stimulus type (aggressive v neutral face) and treatment (Post-enrichment v Post-health-check) was performed to determine whether treatment condition mediated latency to gaze towards aggressive versus neutral faces (alternative predictions 'b' and 'c'). Because monkeys avoided gazing first towards aggressive stimuli presented to the RVF, analyses could only be carried out for the LVF. There were no main effects of

treatment ($F_{1,3} = 0.47$, $P = 0.54$), nor stimulus type ($F_{1,3} = 2.12$, $P = 0.24$), and no interaction of treatment x stimulus type ($F_{1,3} = 0.24$, $P = 0.65$). However, data from only four monkeys were included in the analysis (missing cell values: Table 4-5).

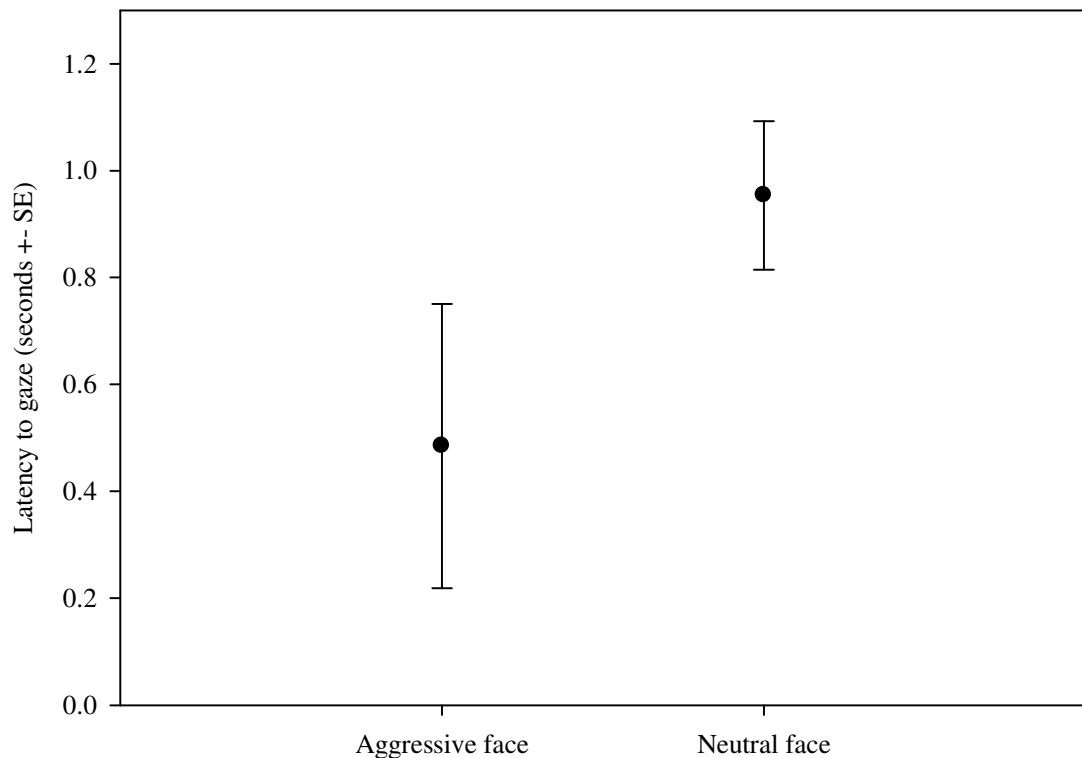
Table 4-5 Latency (seconds) to gaze first towards aggressive versus neutral faces, Post-enrichment and Post-health-check. - signifies the monkey never oriented first towards the stimulus.

ID	Initial treatment	Stimulus to which monkey first oriented			
		Aggressive		Neutral	
		Post-enrichment	Post-health-check	Post-enrichment	Post-health-check
06H		0.12	0.16	0.32	1.60
92R	Post-enrichment	0.46	0.52	2.12	0.06
94K		0.26	-	0.87	0.24
45A		-	0.16	0.59	0.48
29C	Post-health-check	4.06	0.04	3.12	0.04
86O		0.15	0.36	0.24	2.08
G62		0.04	-	0.52	1.08
Group mean		0.85	0.25	1.11	0.80
(SE)		(0.65)	(0.08)	(0.41)	(0.30)

To address the problem of missing cell values, a Post-Hoc Mundry permutations test (1999) was conducted to determine whether monkeys were faster or slower to gaze towards aggressive versus neutral faces, when these were the first to be gazed at (alternative predictions: 'b'). Latency data were collapsed across the two treatment conditions for each monkey, so that data from all seven monkeys could be included in the analysis. Monkeys were significantly faster to gaze towards

aggressive faces ($\bar{X}=0.48\pm0.27$ seconds) than neutral faces ($\bar{X}=0.95\pm0.14$ seconds: Mundry's ranked permutation test: $P=0.03$: Figure 4.10).

Figure 4.10 The latency (seconds) to first gaze towards aggressive and neutral faces when aggressive-neutral face pairs were shown.



In summary, these data support a **vigilance** (or a **vigilance-avoidance**) account of attention for emotional versus non-emotional faces. Monkeys were faster to gaze towards aggressive faces versus neutral faces when aggressive-neutral face pairs were shown. Treatment condition did not appear to influence this bias, although missing cell values meant only four monkeys were included in this analysis. Due to a general avoidance of aggressive faces presented to the RVF, it was not possible to test visual field effects.

c) Latency to disengage first gaze:

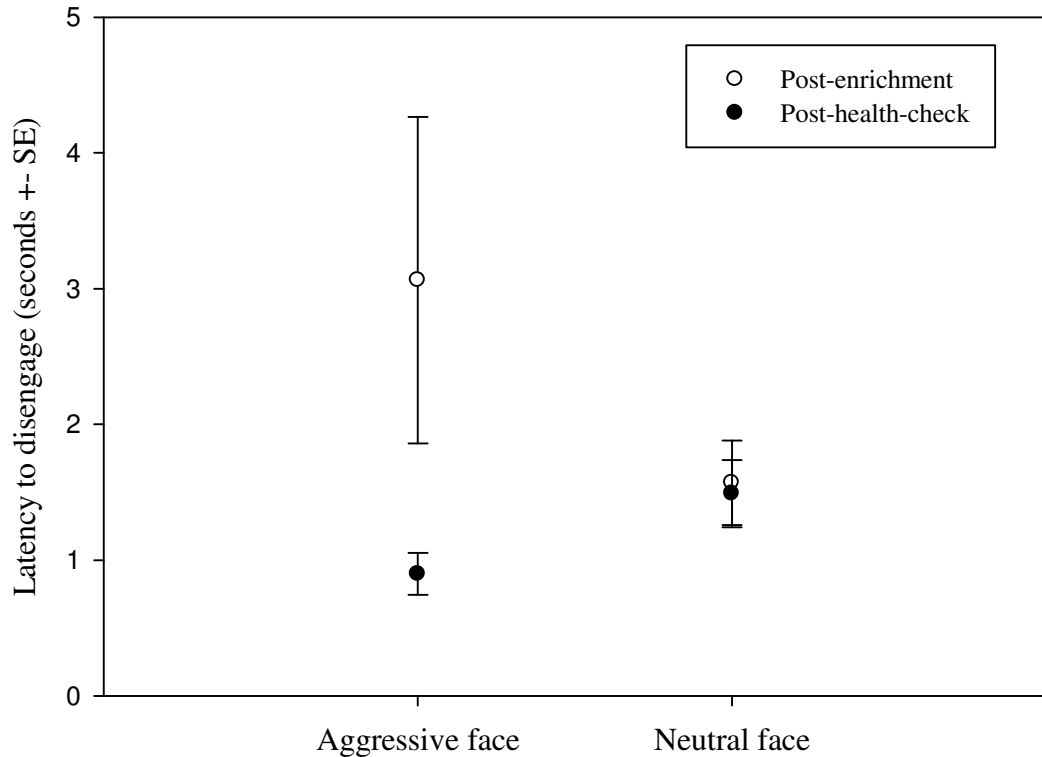
A 2 x 2 RMANOVA was performed to determine whether treatment mediated latency to disengage first gaze (alternative predictions 'c' and 'e'), with within-subjects factors of stimulus type (aggressive v neutral face) and treatment (Post-enrichment v Post-health-check). Visual field was excluded due to missing cell values for the RVF. There was a significant interaction of treatment x stimulus type ($F_{1,6}=5.39$, $P=0.05$; Figure 4.11). There were no main effects of either treatment condition ($F_{1,6}=2.56$, $P=0.16$) nor stimulus type ($F_{1,6}=0.64$, $P=0.45$).

To unpack the interaction between stimulus type and treatment condition, a Post-Hoc permutations test was conducted (Mundry, 1999). Pairwise comparisons revealed monkeys were faster to disengage gaze from aggressive faces Post-health-check versus Post-enrichment ($P=0.0004$). There was a trend for faster disengagement from aggressive versus neutral faces Post-health-check ($P=0.064$). There were no other significant differences or trends.

In summary, these data suggest that affective state may influence latency to disengage gaze from aggressive versus neutral faces. Post-health-check, all seven monkeys rapidly disengaged gaze from aggressive faces, and did so significantly faster than they disengaged gaze from the same aggressive faces Post-enrichment (although there was a greater degree of individual variation in latency to disengage gaze from aggressive faces Post-enrichment). There was also a trend for monkeys to disengage gaze more rapidly from aggressive faces than from neutral faces Post-health-check. These data support an **avoidance** (or a **vigilance-**

avoidance) account of attention for emotional versus non-emotional faces in the health-check treatment.

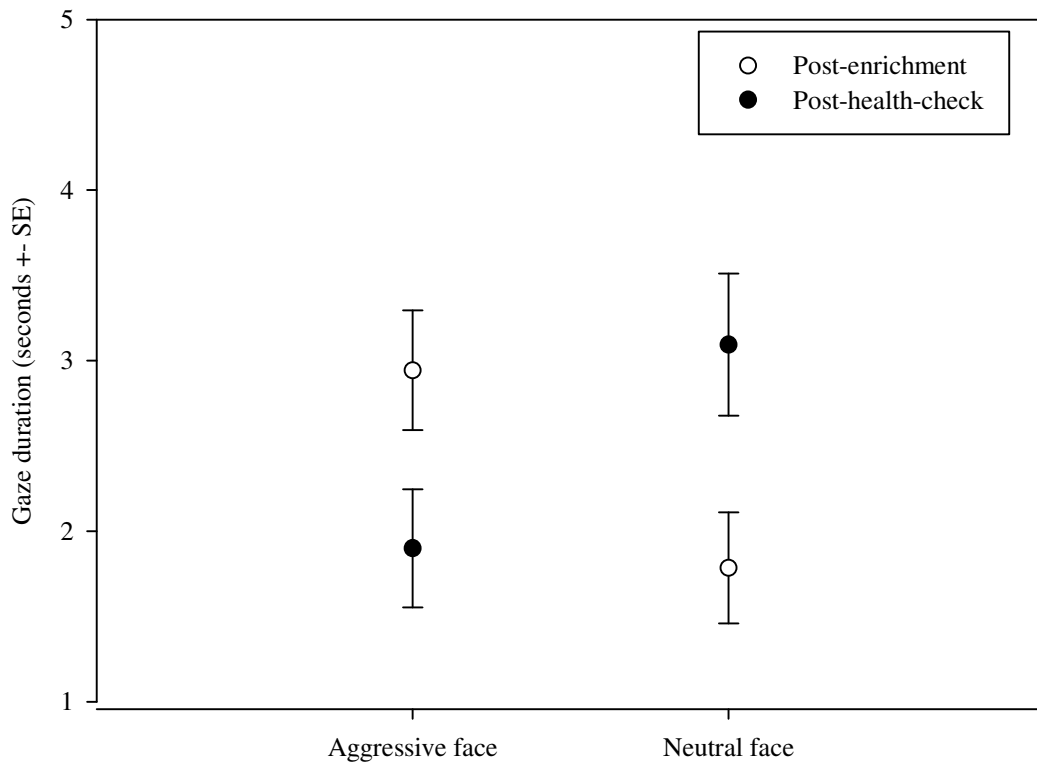
Figure 4.11 The latency (seconds) to disengage first gaze from aggressive and neutral faces.



d) Duration of gaze

A 2 x 2 x 2 RMANOVA was performed to determine whether treatment condition mediated duration of gaze towards emotional stimuli (alternative predictions ‘d’ and ‘e’), and to investigate visual field effects, with within-subjects factors of stimulus type (aggressive v neutral face), treatment condition (Post-enrichment v Post-health-check), and visual field (LVF v RVF). The interaction of treatment condition x stimulus type was significant ($F_{1,6}=5.89$, $P=0.05$: Figure 4.12). There were no main effects (treatment: $F_{1,6} = 0.26$, $P=0.62$; stimulus type: $F_{1,6} = 0.22$, $P=0.64$; visual field: $F_{1,6} = 0.27$, $P=0.61$) nor other interactions (all $P_s > 0.15$).

Figure 4.12 Mean duration of gaze (seconds) towards aggressive and neutral faces Post-enrichment and Post-health-check.



To examine the two-way interaction in more detail, data were collapsed for visual field, and a Post-Hoc permutations test was conducted as above. Pairwise comparisons revealed significant differences for duration gaze to the stimuli between the two treatments. Monkeys spent less time gazing towards aggressive faces Post-health-check versus Post-enrichment ($P=0.034$), and spent more time gazing towards neutral faces Post-health-check versus Post-enrichment ($P=0.021$). Post-health-check, monkeys showed a near-significant trend to spend less time gazing towards aggressive faces versus neutral faces ($P=0.057$). Post-enrichment, monkeys spent more time gazing towards aggressive faces versus neutral faces ($P=0.012$).

A further permutations test was conducted to determine whether monkeys gazed for more or less time towards aggressive versus neutral faces overall. Data were total number of seconds spent gazing towards each stimulus per 10-second trial, collapsed across the two treatments. Monkeys did not differ in duration of gaze towards aggressive versus neutral faces overall ($P=0.96$).

In summary, the data for duration of gaze support an **avoidance** account of attention for emotional versus non-emotional faces in monkeys, which is enhanced following the health-check. This is in line with patterns of bias seen in the **vigilant-avoidant** and **avoidant** accounts of attention for emotional faces seen in (socially) anxious humans. The finding that monkeys spent significantly more time gazing towards aggressive versus neutral faces Post-enrichment suggests a possible **vigilance** for emotional faces in this treatment. This is in line with data from humans that suggests a general vigilance for emotional faces across the population as a whole.

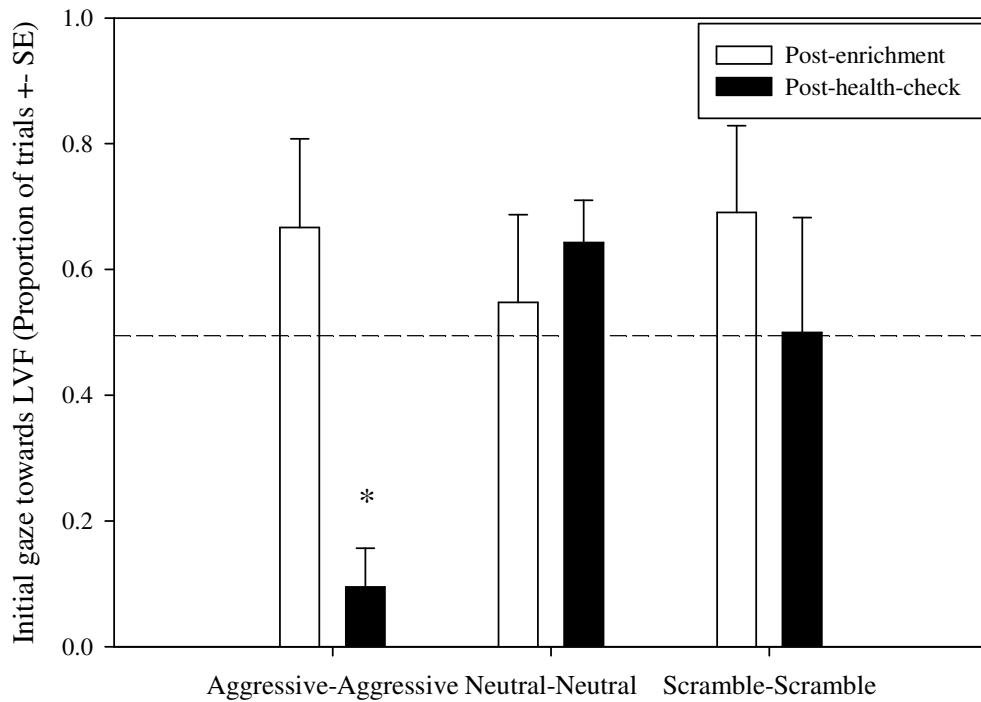
4.4.2 Control trials

a) Direction of first gaze

A 3 x 2 RMANOVA was performed on proportion of trials in which monkeys gazed first towards stimuli presented to the LVF during control trials, with within-subjects factors of stimulus pair (aggressive-aggressive, neutral-neutral and scrambled-scrambled) and treatment (Post-enrichment and Post-health-check). There was a significant interaction between stimulus type and treatment ($F_{2,5.57}$

=12.24, $P=0.01$, Greenhouse-Geisser adjusted; Figure 4.13). There were no main effects of either stimulus type or treatment.

Figure 4.13 The proportion of trials in which monkeys gazed first towards the stimulus presented to the left visual field (LVF) on control trials



Planned one-sample t-tests, with a test value of 0.5, revealed a significant bias to avoid gazing towards aggressive faces presented to the LVF when aggressive-aggressive face pairs were shown Post-health-check ($t_6=6.58$, $P<0.01$; Figure 4.13). Monkeys therefore demonstrated a significant bias to orient first towards aggressive faces presented to the RVF, when aggressive-aggressive face pairs were shown. There was a non-significant trend towards a LVF bias in first gaze towards neutral faces when neutral-neutral face pairs were shown Post-health-check ($t_6=2.14$, $P=0.07$). Monkeys showed no bias in direction of first gaze

towards scrambled faces when scrambled-scrambled face pairs were shown (Post-enrichment: $t_6=1.38$, $P=0.21$; Post-health-check, $t_5=0.00$, $P=1.00$).

In summary, visual-field effects were evident for monkeys viewing aggressive-aggressive face pairs and, to a lesser extent, neutral-neutral face pairs Post-health-check. Monkeys exhibited a significant RVF bias for viewing aggressive faces, and a trend towards a LVF bias for viewing neutral faces. Data for scrambled face pairs revealed monkeys exhibited no side bias for viewing non-face stimuli.

b) Duration of gaze

A $3 \times 2 \times 2$ RMANOVA was performed on mean duration of gaze, with within-subjects factors of stimulus pair (aggressive-aggressive, neutral-neutral and scrambled-scrambled), treatment (Post-enrichment, Post-health-check) and visual field (left, right). There were no interactions or main effects (for example, the interaction of stimulus \times treatment \times visual field was $F_{2,5}=1.74$, $P=0.27$; and treatment \times visual field was $F_{1,6}=2.5$, $P=0.16$). For all remaining tests, $P>0.28$. Therefore, there was no side bias for duration of viewing matched pairs of emotional faces, non-emotional faces, nor scrambled face stimuli in general.

4.4.3 Control trials (neutral-scrambled faces)

a) Direction of first gaze

A 2×2 RMANOVA was performed to determine whether treatment condition mediated direction of first gaze towards, or away from, faces versus scrambled-faces, and to investigate visual field effects. Data were proportion of trials in

which monkeys gazed first towards the neutral face, with within-subjects factors of treatment (Post-enrichment versus Post-health-check) and visual field (LVF versus RVF). There were no main effects of treatment ($F_{1,6} = 0.07$, $P=0.80$) nor stimulus type ($F_{1,6} = 1.14$, $P=0.33$), nor interaction of treatment x stimulus type ($F_{1,6} = 0.22$, $P=0.65$).

A one-sample t-test was performed to determine whether monkeys exhibited an overall bias in first gaze towards faces versus scrambled-faces. Data were proportion of trials on which monkeys gazed first towards the neutral face when a neutral-scrambled pair was shown, collapsed for visual field and treatment. The proportion of trials in which monkeys gazed first towards the neutral face did not differ significantly from 0.5 ($t_6=1.02$, $P=0.35$).

In summary, monkeys were equally likely to gaze first towards neutral faces and scrambled-faces when the two were presented together. There was no evidence for an influence of treatment condition on direction of first gaze.

b) Duration of gaze

A 2 x 2 x 2 RMANOVA was performed to determine whether treatment condition mediated duration of gaze towards faces versus non-faces, and to investigate visual field effects. Data were mean duration of gaze per 10-second trial, with within-subjects factors of stimulus type (neutral face v scrambled), treatment (Post-enrichment v Post-health-check), and visual field (LVF v RVF). There were no significant main effects or interactions (for example, the interaction of stimulus

x treatment x visual field was $F_{1,6}=0.18$, $P=0.69$). There was a main effect of treatment approaching significance ($F_{1,6}=4.55$, $P=0.08$). However, this was lost when data were collapsed for visual field. For all remaining tests, $P>0.40$).

A Mundry permutations test was conducted to determine whether monkeys gazed for more or less time towards faces versus scrambled-faces. Data were total number of seconds spent gazing towards each stimulus per 10-second trial, collapsed for visual field and treatment. Monkeys did not differ in duration of gaze towards neutral faces versus non-faces (Permutation test, $P=0.61$).

In summary, monkeys gazed towards neutral faces and scrambled-faces for equivalent amounts of time throughout the 10 second trial. There was no evidence for an effect of treatment condition on duration of gaze.

4.5 Discussion

Seven monkeys viewed pairs of emotional versus non-emotional conspecific faces and scrambled stimuli presented on two adjacent computer monitors, during two treatments: Post-enrichment and Post-health-check. Direction, latency and duration of gaze were reliably blind-coded by two observers, on a frame-by-frame basis from video. The experimental paradigm therefore provided a reliable means of measuring preferential gaze for faces by rhesus macaques.

The suggested patterns of attentional bias, as measured by gaze, are shown in Table 4-6. The data are partially in agreement with findings from human studies which suggest affective state has a modulating effect on attention to emotional social cues in humans. Monkeys demonstrated both vigilance for, and avoidance of, threatening faces and this pattern was, in part, mediated by treatment condition. This is the first study to reveal possible affect-related attentional bias for threatening information in a species of primate, using methods typically used with humans.

The main findings of this study were as follows. Monkeys were significantly faster to gaze towards aggressive versus neutral faces, irrespective of treatment condition. This suggests that rhesus macaques demonstrate a general vigilance for emotional faces: emotional faces engage attention and lead to an initial overt orienting response towards highly salient (possibly threatening) cues within the environment, and this occurs independently of affective state. This is in line with data from humans which suggest vigilance for threat (versus non-threat) may occur in both high and low anxiety (vigilance theories: Seligman, 1971; Eysenck, 1992; Öhman, 2005). Studies which suggest that vigilance is enhanced in high anxiety, tend to use the dot-probe paradigm (e.g. Bradley et al. 2000), which is able to tap into covert shifts in attention that may not be detected by video-coding of eye-gaze.

The second major finding of the present study was that treatment condition influenced the speed with which monkeys disengaged gaze from aggressive versus neutral faces. Monkeys were significantly faster to disengage gaze from

Table 4-6 Suggested patterns of attention allocation towards aggressive versus neutral faces Post-health-check and Post-enrichment as measured by eye-gaze in the current study.

Stimulus	Condition	Direction of first gaze	Latency to first gaze	Latency to disengage	Total duration of gaze	Attentional pattern
		'a'	'b'	'c'	'd'	
Aggressive face	Post-health-check	No bias (RVF avoidance)*	Faster*	Faster (trend only)	Shorter (trend only)	Vigilance-avoidance
	Post-enrichment			No bias (indiv diffs?)	Longer (trend only)	Vigilance
Neutral face	Post health check	No bias (LVF vigilance)*	Slower*	No bias	Longer (trend only)	Vigilance (delayed onset)
	Post-enrichment			No bias	Shorter (trend only)	Avoidance (ignore non-threat)

(and spent less time gazing towards) aggressive faces Post-health-check, versus Post-enrichment. Further, within the health-check treatment, monkeys showed a strong trend to disengage gaze faster from (and spend less time looking towards) aggressive faces, versus neutral faces. These findings suggest that, following the health-check, monkeys demonstrate an enhanced avoidance of emotional or threatening stimuli. These data are consistent with Garner et al. (2006b) who reported that, following a stressor, humans were faster to gaze towards emotional faces (both positive and negative) than neutral faces, and were also faster to disengage gaze from emotional faces. Further, Garner et al. (2006b) found that, following a stressor, socially anxious humans gazed towards emotional faces for less time than did low socially anxious controls, who gazed towards emotional faces for more time than neutral faces.

Garner et al. (2006b) discuss their findings in terms of avoidance of emotional information by anxious individuals, subsumed under a vigilant-avoidant pattern of attention allocation over the whole time course of the orienting response to emotional versus neutral faces. This is similar to the pattern of vigilance-avoidance for aggressive faces Post-health-check in the current study (Table 4-7, top row). During the enrichment treatment, initial general vigilance for aggressive faces was followed by a trend for longer duration of gaze towards aggressive versus neutral faces, suggesting sustained vigilance for threatening faces in this treatment (Table 4-6, second row).

While it is impossible to make direct comparisons with data from the human literature due to, among other things, differences in design (for example, within-

versus between-subjects designs), there are certain similarities in the shifts in patterns of attention between the two groups which indicate a need for further investigations of the influence of affective state on patterns of attention towards threatening social stimuli by rhesus macaques.

The current data may be considered in terms of alternative explanations which do not infer emotion-mediated attentional biases. The findings reported here are in partial agreement with previous work with humans which has shown enhanced processing efficiency of threatening faces. Calvo et al. (2006) claim that enhanced processing efficiency for threatening faces is revealed by shorter gaze duration towards angry than other-emotional and neutral faces. Shorter gaze towards aggressive faces may, therefore, reflect enhanced processing by the monkeys, rather than avoidance. However, this explanation is difficult to reconcile with the finding that monkeys gazed for longer towards aggressive faces versus neutral faces Post-enrichment. This suggests duration of gaze towards faces reflects attentional engagement and maintenance, rather than processing efficiency.

Whether the general early vigilance for aggressive faces is threat-specific or emotion-specific is unclear. The present study used only aggressive emotional faces. The reduced maintenance of attention towards emotional faces revealed by Garner et al. (2006b) was apparent for both angry-neutral and happy-neutral face pairs. The effect revealed in the present study may therefore reflect an emotion-general rather than threat-specific bias in attention. A necessary extension to the existing paradigm is inclusion of submissive-neutral face pairs. An appropriate stimulus to investigate this effect is frontal bared teeth expression (often termed a

fear-grin, or appeasement-gesture, due to its proposed function as a signal of benign intent: Parr et al., 2005).

During the matched-pairs control trials, monkeys gazed equally towards the three classes of stimuli. This finding suggests either that a) the monkeys did not find any of the three classes of stimuli more or less aversive than any of the others; or b) duration of looking behaviour indicates different underlying processes, such that the differential effects of preference, novelty, interest, hypervigilance, delay to disengage and so forth may be difficult to separate out using the current paradigm. Only when visual field and treatment were considered did differential looking patterns emerge for control trials. Monkeys gazed first towards aggressive faces presented to the RVF when aggressive-aggressive face pairs were shown, Post-health-check. Monkeys also showed a tendency to gaze first towards neutral faces presented to the LVF when neutral-neutral face pairs were shown, Post-health-check.

There was no effect of treatment condition on the amount of time that monkeys gazed toward each of the sets of control stimuli. This finding suggests that treatment condition does not influence general levels of interest in socially relevant information, or in stimuli presented on a monitor in general. Monkeys were therefore equally visually engaged with the stimuli during both treatments. Again, this experimental paradigm lacks the power to distinguish possible variation in the mechanisms underlying gaze towards stimuli presented on the screens (e.g. interest versus hypervigilance). Previous studies with cynomolgus macaques have demonstrated that, when viewing a single stimulus, both high and

low ranking males gaze towards a single primate or human face stimulus for more time than towards non-face stimuli, however, the behaviours accompanying the vigilance suggest different underlying motivational factors (Kyes et al., 1992).

Stimulus intensity may have an effect on attentional bias. Mogg et al. (2000b) showed high-threat versus non-threat, and mild-threat versus non-threat picture pairs to participants during a dot-probe task. Participants exhibited vigilance for high-threat scenes (responding faster to probes at the location of high-threat pictures relative to probes at the location of non-threat scenes), but no bias in vigilance towards mild-threat scenes. This bias was strongest in low-trait anxious individuals, and is comparable with the increased duration of gaze towards high-threat (aggressive) relative to lower-threat (neutral) stimuli by the monkeys Post-enrichment. Mogg et al. (2000b) also found a weak bias for high-threat stimuli among the high anxious group, contrary to the data reported here.

An assumption of the current study is that the aggressive faces were perceived by the monkeys to be more threatening than the neutral faces. Both stimulus types contained frontal images of faces with direct gaze, a signal of dominance among male rhesus macaques (Chevalier-Skolnikoff, 1973; van Hooff, 1976). The neutral faces used in this study are assumed to have been perceived by the monkeys as less threatening than the aggressive faces, and results have been interpreted accordingly. A proposed development to the existing design would be to incorporate faces without direct gaze (e.g. with eyes closed, averted gaze), to explore the effects of stimulus threat-value on vigilance patterns further.

Another assumption was that all monkeys viewed the same faces as equally threatening or non-threatening. Data from humans reveal dominance motivation may influence perception of facial expressions and therefore influence the extent to which attention is captured by different faces (Schultheiss et al., 2005; Schultheiss & Hale, 2007). For example, a smiling face may be a negative signal in a competitive context, and a sad or angry face may be a positive signal in a retributive context (Schultheiss et al., 2005). Power-motivated individuals (i.e. dominant individuals who seek to have an impact on others, and therefore find submissive facial expressions rewarding) show enhanced learning of a visuomotor task when low threat (e.g. same gender surprise) faces are shown, and impaired learning performance when high threat (e.g. same gender happy or angry) faces are shown (Schultheiss et al., 2005). Affiliation-motivated individuals (i.e. individuals who seek close affiliative relationships with others), on the other hand, show impaired learning when neutral faces are presented, but not when emotional, including angry, faces are presented. In a follow-up dot-probe study Schultheiss and Hale (2007) found that power-motivated individuals orient their attention towards faces signaling low dominance and away from faces signaling high dominance, while affiliation-motivated (possibly rejection-averse) individuals show vigilance for faces signaling rejection and a trend towards vigilance for faces signaling acceptance. These data were interpreted in terms of facial expressions as motivational incentives which may shape a perceiver's behaviour in accordance with that individual's motivational stance. The differential orienting to neutral versus aggressive faces in the current study may be partially explained in terms of the different socio-motivational stances of the monkeys Post-enrichment versus Post-health-check.

There was no evidence for a bias in first gaze towards aggressive versus neutral faces, as has been shown in humans. This may be due either to a) a lack of attentional capture by threatening emotional stimuli, or b) an artifact of the experimental design. Stimuli were presented on two computer monitors positioned 200mm apart, so that the stimuli themselves were separated by a distance of 450mm between midpoints. The inter-stimulus distance was selected to allow reliable discrimination of gaze direction when coding from video. The inter-stimulus distance (centre-centre), and therefore corresponding visual angle, used for supraliminal presentation of emotional faces in studies with humans is shorter (e.g. 115mm: Mogg & Bradley, 1999; 186mm: Garner et al., 2006b; 145mm/13.8°: Holmes et al., 2005). The paired stimuli were here presented peripherally, outside of the central field of vision. At peripheral locations, stimulus processing is degraded in both humans (Whiteside, 1976) and rhesus macaques (Fridman and Nadler, 2005). This may account for the lack of evidence for stimulus effects on direction of first gaze in the present study, although the presence of visual field effects for some stimulus combinations suggest that, even at peripheral locations, a degree of processing of stimuli may have occurred prior to detectable shifts in gaze.

The finding for visual field effects on direction of first gaze towards emotional versus non-emotional face stimuli is not in accordance with existing literature. Bradley (1999) revealed a LVF advantage in humans for processing threatening faces presented subliminally, suggesting that the right hemisphere processes information about emotional faces rapidly, and significantly faster than the left hemisphere. Calvo and Nummenmaa (2007) found that humans experience

enhanced affective priming by emotional stimuli presented parafoveally to the LVF, but not the RVF. In the current study, monkeys avoided gazing first towards aggressive (versus neutral) faces presented to the RVF, irrespective of treatment condition. However, during the aggressive-aggressive control trials, monkeys avoided orienting towards aggressive faces presented to the LVF. This may reflect enhanced processing of emotional faces by the right hemisphere in rhesus macaques, thereby negating the need to shift gaze overtly towards the LVF. The control trial data for aggressive-aggressive face pairs are also in accordance with previous studies with rhesus macaques which suggest laterality in face processing in this species (Vermiere & Hamilton, 1998; Tsao et al., 2008). Further tests would be needed to identify whether this effect is real, or an artifact of small sample size and number of trials.

In summary, this chapter presents the first data on emotion-mediated attentional bias for faces in a primate. The method developed here was successful in documenting rhesus macaque gaze towards either of two competing stimuli presented on separate computer monitors. Monkeys showed differential gaze towards aggressive versus neutral faces according to whether they had recently been exposed to a stressor (a veterinary examination: Post-health-check) or a week of enrichment (Post-enrichment). The data lend most support to vigilance and vigilance-avoidance accounts of attentional bias for threatening facial information. Further, differences in the patterns of attention allocation between the treatment conditions suggest attentional bias in rhesus macaques may be mediated by state factors, such as affect, as in seen in humans.

These findings are important for several reasons. Firstly, these are the first data on attentional bias in a species of primate. Secondly, the data reported here concur with recent data from humans, suggesting that, when using eye-gaze as a measure of attention, monkeys and humans exhibit similar patterns of attentional response to threatening and non-threatening information. This, in turn, suggests that attentional biases may involve evolutionarily old processing pathways in the primate brain. The visual field preferences in gaze suggest hemispheric specialisation for processing threatening faces specifically. Such specialisation would indicate emotional faces are an especially important source of socio-emotional information in macaque society, which is processed differently to other types of (non-emotional) social and non-social information. Finally, differential gaze towards threatening versus non-threatening information between the two treatments suggest attention in rhesus macaques may be mediated by internal factors in a comparable manner to humans.

Given the acceptance of attentional bias for threatening information as a marker for vulnerability to anxiety in humans, the data presented here may also present the first evidence of a cognitive marker of vulnerability to anxiety in a species of primate, other than humans. These data present a strong case for further investigation of attentional bias in primates, and the functional implications of these biases for the physical and psychological wellbeing of primates in captivity.

Chapter 5

Emotion evaluation and response-slowing

In Chapter 4 I reviewed literature and presented data on the effects of emotion state on the spatial orienting of attention (gaze) towards one of two competing stimuli that occur in different spatial locations. In the present chapter I review literature and present data on non-spatial attention, specifically the effects of emotion state on competition of attentional resources between two different types of information (emotional versus non-emotional) that are presented in the same location. People high in anxiety experience impaired (e.g. slower) performance on a non-emotional task when an emotional distractor component is introduced (MacLeod, 1991; Bar-Haim et al., 2007). The emotional component captures attention and interferes with the processing of, and/or behavioural response to, the task-related non-emotional information (Mathews and MacLeod, 1985). People who are low in anxiety, however, are typically unimpaired in performance on a non-emotional task when an emotional distractor component is introduced (Bar-Haim et al. 2007). For such individuals, the emotional component does not preferentially capture attention and so does not interfere with the processing of, and/or behavioural response to, the task-related non-emotional information. Increased anxiety is therefore associated with a slowing in task performance when emotional information is present.

As with the spatial orienting effects discussed in Chapter 4, the enhanced saliency of emotional versus non-emotional information among anxious humans may have severe implications for the onset and maintenance of anxiety disorders and

psychological wellbeing. If primates exhibit a similar cognitive capacity for enhanced processing of, particularly negative, emotional stimuli over neutral task-related stimuli, this may have implications for their psychological wellbeing also.

In this chapter I review existing research on the interference effects of emotional information on (non-spatial) task performance (Part A), and present empirical data from the first study to investigate emotional interference effects in a species of primate (Part B). Part A is divided into three sections. In the first of these I discuss the theory and background for emotion-evaluation and response-slowing research conducted with humans. In the second section I review the methods used to study emotion-evaluation and response-slowing in humans, and complementary approaches used with non-human participants. In the third section I discuss the implications of human-based research for the development of a novel method to study emotion-evaluation and response-slowing in primates. I conclude this section with the main aims and hypotheses that informed the design of the present study.

The present study is detailed in Part B, which is also divided into three sections. In the first of these I describe the method I developed. In the second section I present the first data on emotional evaluation and response-slowing to threatening versus non-threatening faces in a species of primate. In the third section I discuss the results of the study in light of the hypotheses and available data from humans.

Part A: Literature Review

5.1. Introduction

5.1.1. Emotion-evaluation and response-slowing in humans: theory and background

People are generally slower to perform an attentional task when an emotional distractor is introduced (Macleod, 1991). This effect is arguably enhanced for negative distractors, and among anxious populations (Williams et al., 1996; Bar-Haim et al., 2007). The methods used to measure the extent to which an emotional distractor impairs performance on an otherwise non-emotional task vary, as do the proposed underlying mechanisms.

The following is a review of the main cognitive and non-cognitive theories and empirical findings regarding the effects of emotion state on slowing of responses on a cognitive task when an emotional element is introduced. This has not been studied in a non-human animal species. Therefore, I draw on three broad literatures (Figure 5.1). The first literature draws on studies in humans which demonstrate impairment on a cognitive task, or in valence judgements, when an emotional distractor component is presented. The second literature draws on more recent neuroscientific and computer modeling approaches to explore the interruption of ongoing cognitive processes caused by the onset of a threatening stimulus. The third literature draws on studies which measure responses to emotional stimuli irrespective of other cognitive demands. The latter literature incorporates studies with both humans and non-human animals.

Figure 5.1 Overview of the main approaches to studying the influence of affective state on the processing of emotional information

	Model				
	Information processing models	Attentional control models	Reinforcement sensitivity models		
Brief summary	Traditional approaches that measure effects of affective state and emotional information on cognitive task performance, or on emotional evaluations of ambiguous stimuli.	Bottom-up processing of highly salient stimuli acts as a ventral circuit-breaker which interrupts ongoing dorsal (top-down) task-relevant, goal-directed processes.	Behavioural Approach System (BAS): a system sensitive to rewards which underlies approach behaviours.	Freeze-Flight-Fight System (FFFS): a defense-withdrawal system associated with high arousal which underlies fear-related avoidance and defensive attack.	Behavioural Inhibition System (BIS): a system sensitive to punishers that inhibits ongoing behaviour to orient attention towards salient stimuli.
Reason for inclusion in the review	Places current study in the historical context of human research. The *eStroop paradigm partially informed design of the current study.	Provides a recent account of the mechanism by which task-relevant goals and motivation feed into attention and emotion-evaluation of negative stimuli.	System underlies reward-related approach behaviours. Traditional apparatus, i.e. the *WGTA, partially informed design of the current study.	System underlies physical withdrawal from aversive stimuli. May lead to attenuation of approach behaviours.	System underlies early attentional orienting to stimuli. Activated when BAS and FFFS are in conflict. May lead to attenuation of approach behaviours during conflict of response.
Methods	Computer-based cognitive tasks: impairment (RT) when emotional distractor is present (e.g. eStroop)	Neuroscientific and computer modelling techniques.	Behavioural (e.g. conditioned responses), physiological and neural responses to emotional information (human and animal studies).		

*eStroop: emotional Stroop task; *WGTA = Wisconsin General Testing Apparatus. Measures latency to approach a positive reinforcer (typically food) when a negative stimulus is introduced. Traditionally used to induce approach-withdrawal conflict in primates.

In the first case, I review data from human cognitive studies, which have traditionally focused on **Information processing models** of cognition using the emotional Stroop (eStroop) paradigm and, to a lesser extent, emotion-evaluation paradigms. These studies aim to investigate the interference effect of emotional information on an ongoing cognitive task, and on emotion-evaluation of ambiguous emotional information (emotion-evaluation paradigms). Information processing models of cognition focus on competition for attentional resources between two competing types of information, with emphasis on how the two types of information pass through the brain. Information processing explanations of emotion-evaluation and response-slowing assume that slowing of responses on threat-relevant trials reflects Stroop-like interference by one processing pathway (threat-relevant) over the other (task-relevant). Early models considered the relative speed of processing between two pathways to account for the difference in processing priorities (e.g. Cattell, 1886, reviewed in MacLeod, 1991). More recent models consider that the relative strength of processing pathways account for the effect (e.g. Cohen et al., 1990).

Having placed the human research in its historical context, I conclude with current opinion on the utility of cognitive studies for investigating responses to emotional stimuli. This leads on to the second literature, where more recent approaches combine neuroscientific and computer modeling techniques to map the interplay between emotional, attentional and goal-directed circuits in the human brain.

Attentional control models (Cohen et al., 1990; Corbetta & Shulman, 2002; Taylor & Fragopanagos, 2005) focus on the interplay between dorsal and ventral

processing circuits. Inhibitory action by the ventral circuit on the dorsal circuit acts as a circuit breaker, triggered by highly salient or novel stimuli. This feeds into, and is mediated by, motivation and arousal. Together, these components of the system act to interrupt ongoing, task-related, attentional processes, and redirect attention towards salient or novel stimuli for more focused processing.

The third literature I draw on focuses on **Reinforcement sensitivity models** of responses to emotional stimuli (e.g. McNaughton & Corr, 2004). I present each of the attentional-behavioural systems within this model as independent, for clarity. However, there is likely to be some interaction between the various systems, that is beyond the scope of this review. Each system receives input from an emotion-evaluation system in the amygdala which is implicit within the model. The Behavioural Approach System (**BAS**) is an attentional system, sensitive to signals of reward, which triggers approach behaviours. This system underlies positive emotions. The Freeze-Flight-Fight System (**FFFS**) is a defense-withdrawal system, sensitive to punishers, which triggers survival and escape behaviours. This system underlies fear. The Behavioural Inhibition System (**BIS**) is an attentional system sensitive to conflicting cues of punishment and reward. This system underlies anxiety and, in the presence of negative stimuli, triggers a (non-spatial) attentional orienting response towards potentially harmful stimuli. The system resolves response conflict between the BAS and FFFS via inhibition of approach/withdrawal behaviours, allowing elicitation of behaviours concerned with conflict resolution (e.g. further stimulus evaluation).

5.1.1.1. Information processing models

Traditionally, emotion-evaluation and response-slowness in humans has been studied using a single task, the eStroop. The eStroop was the first task developed to measure attentional bias for threatening information in humans (Mathews and MacLeod, 1985; Bar-Haim et al., 2007). The eStroop task is a modified form of the classic colour-naming Stroop interference paradigm (Stroop, 1935). The original Stroop effect describes the difference in performance in identifying one of two competing types of information within a stimulus. Traditionally this is the difference in colour-naming performance (measured as latency to name the colour) on congruent trials (e.g. the word *blue* written in blue ink) compared with non-congruent trials (e.g. the word *red* written in blue ink). The difference in latency to respond between the two types of trial is attributed to facilitation of information processing when there is semantic congruence between the two types of information (word and colour are matched), compared with interference between competing processing pathways when there is semantic incongruence between the two types of information (word and colour are not matched). Interference reflects non-symmetry in the strength of the two competing processing pathways.

The eStroop shares the same basic premise as the classic Stroop: that participants must respond to stimuli that vary in two dimensions, one of which they must ignore. In the eStroop the dimension to be ignored has either an emotional or non-emotional component (Williams et al., 1996). Interference occurs where processing of emotional information (which is to be ignored) is enhanced relative

to that of the task-relevant non-emotional information. Slowing of responses to emotional stimuli during eStroop studies has been demonstrated in both trait and state anxiety (Williams et al., 1996).

Early eStroop studies measured colour-naming performance for threat-related versus non-threat-related words (MacLeod, 1991; Williams et al., 1996). Anxious individuals are typically slower to name the colour of threat-related versus non-threat-related words. Later studies incorporated naturalistic emotional components, such as faces, to investigate automaticity of processing effects (e.g. Anes & Kruer, 2004; Mauer & Borkenau, 2007; Beall & Herbert, 2008). Some authors then pointed to the fact that the classic Stroop and eStroop tap into different underlying processes (Algom et al., 2004). Algom et al. (2004) discuss the problem of comparing studies in which semantic congruency of competing stimulus dimensions is manipulated (both physical colour of the word, and colour meaning, in the classic Stroop), with studies in which there is no semantic relationship between the two dimensions of the stimulus (there is no semantic relationship between valenced words/pictures and the colours in which they appear, in the eStroop). This led to a reframing of the eStroop effect in broader physiological and behavioural terms, such as arousal, orienting and freezing responses, and a renaming of the task in terms of emotion-evaluation and response-slowness (e.g. Mogg et al., 2008).

5.1.1.2. Attentional control models: dorsal and ventral circuits and the ‘circuit-breaker’ system

Neuroscientific (e.g. LeDoux, 1996; Corbetta & Shulman, 2002) and computer modeling (e.g. Cohen et al., 1990; Fragopanagos & Taylor, 2005; Taylor et al., 2005; Korsten et al., 2006) techniques have recently been combined (Taylor & Fragopanagos, 2005) to address the role of cognition in emotion, and specifically the effects of emotional information on ongoing task performance. Based on evidence from neuroimaging studies with humans, Corbetta and Shulman (2002) first proposed that the brain contains a ‘circuit-breaker’ system which functions as a ventral alerting system in the presence of highly salient sensory stimuli (i.e. stimuli of high behavioural importance). At the onset of a salient stimulus, typically one that is sudden or unexpected, the circuit-breaker functions to reorient attention (e.g. from an ongoing task) by interrupting ongoing processing in the dorsal circuit (dealing with ongoing goal- or task-directed motor and cognitive activities), facilitating attention to the source of interest. The circuit breaker system is proposed by Corbetta and Shulman (2002) to be strongly lateralized to the right hemisphere in humans.

Taylor and Fragopanagos (2005) placed the circuit-breaker concept in the context of previous work with humans and other species (e.g. LeDoux, 1996). LeDoux’s work, which is based largely on brain-lesioning studies with rats, revealed the classic ‘quick and dirty’ direct pathway by which stimuli are tagged for valence in the amygdala (emotion) circuit. This information is then passed forward to sensory (e.g. visual) and cortical (e.g. orbitofrontal cortex: OFC) areas for more

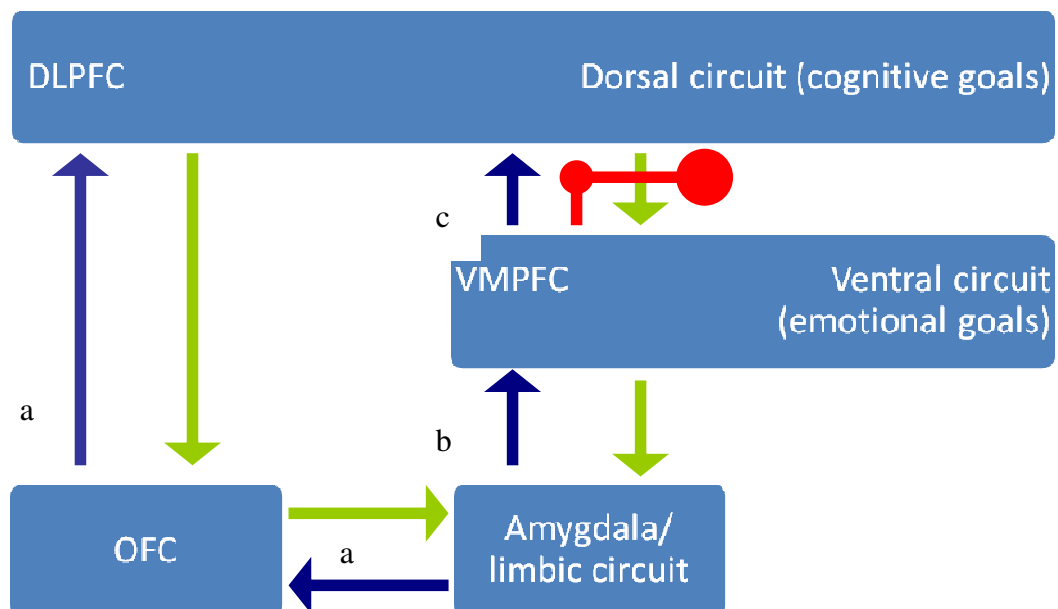
detailed processing and matching with task-related goal-directed process in the dorsolateral prefrontal cortex (DLPFC; LeDoux, 1996: Figure 5.2).

In addition to brain lesion studies, fMRI mapping studies have highlighted the inhibitory effect of the amygdala-OFC emotion circuit on the dorsal attentional circuit. Armony and Dolan (2002) mapped people's responses to negatively conditioned faces presented for 50ms during a dot probe task. Enhanced activity in the amygdala and OFC was associated with a significant delay to respond to probes following conditioned primes. The response-slowing to probes revealed interference in processing non-emotional task-related information following an emotional distractor, suggesting the amygdala-OFC emotion circuit may function automatically and preattentively to inhibit processing in the DLPFC. Conversely, studies of emotion regulation incorporating fMRI techniques have highlighted the relative strength of top-down inhibitory processes on the amygdala-OFC emotion circuit. Beauregard et al. (2001) presented people with erotic images and asked them to inhibit emotional responses to the images. Results suggested that the inhibitory effects between OFC and the dorsal attentional circuit are reciprocal, such that top-down control of emotional processes may be achieved via output from the DLPFC (Beauregard et al., 2001). Where emotional information is congruent with task demands, facilitation of the dorsal attentional circuit may occur (Taylor & Fragopanagos, 2005).

Taylor and Fragopanagos (2005) combined these various approaches to present a single model whereby two emotion circuits influence cognition in the human

brain (Figure 5.2). A direct bottom-up preattentive emotion circuit carries emotional (stimulus-bound) information from the amygdala to the DLPFC via OFC (Figure 5.2a blue arrows). Emotional information may inhibit DLPFC activity (goal-driven processes) or enhance it depending on the relevance of the emotional information to individual goals. Top-down effortful inhibition of emotional processing may occur via projections from the DLPFC to areas such as OFC (Figure 5.2a green downwards arrow). This is the route implicated in emotion regulation.

Figure 5.2 The two routes by which emotional information may influence cognition in the human brain. a) A direct bottom-up preattentive emotion circuit from the amygdala to the DLPFC via OFC. b) An indirect emotion circuit from the amygdala to the ventral attention circuit, where salient emotional information becomes attended. This is given priority of processing according to its relevance to emotional goals. c) Feedforward to the dorsal attention circuit may interrupt outputs from the dorsal attention circuits, resulting in a temporary circuit-breaker effect (red) on cognitive goal-directed behaviours. Adapted from Taylor and Fragopanagos (2005).



The second, indirect, pathway carries emotional information from the amygdala to the ventral attention circuit, situated in the ventro-medial prefrontal cortex (VMPFC: Figure 5.2b blue arrow). The VMPFC is involved in cognitive processing of emotional information and is where salient emotional information becomes attended. The VMPFC has onwards projections to the dorsolateral prefrontal cortex (DLPFC: Figure 5.2c, blue arrow) and therefore acts as a bridge between subcortical and higher cortical executive areas. This bridge may either facilitate processing of task-relevant emotional information by higher cortical areas, or interrupt ongoing task-relevant processing to allow reorientation of attention towards sudden or unexpected emotional stimuli, via the ‘circuit breaker’ (Figure 5.2c, in red). The DLPFC is concerned with executive functions such as goal-directed task-relevant processes. Selection of information by the DLPFC is guided by the relevance of that information to ongoing task demands, i.e. the relevance of the stimulus with respect to an individual’s goals, irrespective of its emotional content. This is given priority of processing according to its relevance to emotional goals. Feedforward to the dorsal attention circuit may interrupt outputs from the dorsal attention circuits, resulting in a temporary circuit-breaker effect on cognitive goal-directed outputs (c: green arrow).

5.1.1.3. Reinforcement sensitivity models

Gray (1971 and 1981) first proposed the reinforcement sensitivity theory as a neuropsychological framework for describing the mechanisms underlying behavioural outputs in response to emotional information, and the regulation of those outputs. Reinforcement sensitivity theory states that behavioural responses

to emotionally charged stimuli arise as a result of the relative strength of three motivational systems. The three systems are differentiated by their sensitivity to reward (appearance of a positive reinforcer, or omission of a negative reinforcer: BAS) or punishment (appearance of a negative reinforcer or termination of a positive reinforcer: FFFS) and reward-punishment conflict (e.g. when both BAS and FFFS are activated: BIS). The three motivational systems feed into emotional systems (BAS: positive emotions; FFFS: fear; BIS: anxiety; McNaughton & Corr, 2004).

Behavioural Approach System (BAS)

The BAS is a motivational system sensitive to signals of reward¹. It functions to direct behaviour towards positive reinforcers. High BAS sensitivity or activation is associated with approach behaviours ranging from optimism to fun-seeking.

¹ My definition of BAS contrasts with that of some other authors. This reflects the variety of definitions of BAS in the literature. I concur with authors who relate BAS to approach towards reward (Gray, 1981; McNaughton & Corr, 2004). However, others define BAS as a Behavioural Activation System, which activates goal-driven behaviour regardless of direction (approach or avoid: e.g. Amodio et al., 2008). Such authors consider BAS to incorporate, for example, angry approach (e.g. Harmon-Jones, 2003). Within this alternative framework, data from humans suggest lateralisation of approach and active withdrawal components of the BAS to the left and right hemispheres respectively (Harmon-Jones, 2003).

Overactivation of BAS has been associated with impulsivity, bipolar-disorder, attention-deficit/hyperactivity disorder and addictive behaviours for positive reinforcement (O'Connor et al., 2008). The system is governed mainly by the dopaminergic neurotransmitter system, which promotes goal-directed behaviour in response to rewards, particularly in the DLPFC, and is linked to approach motivation orientation (McNaughton & Corr, 2004; Rushworth & Behrens, 2008).

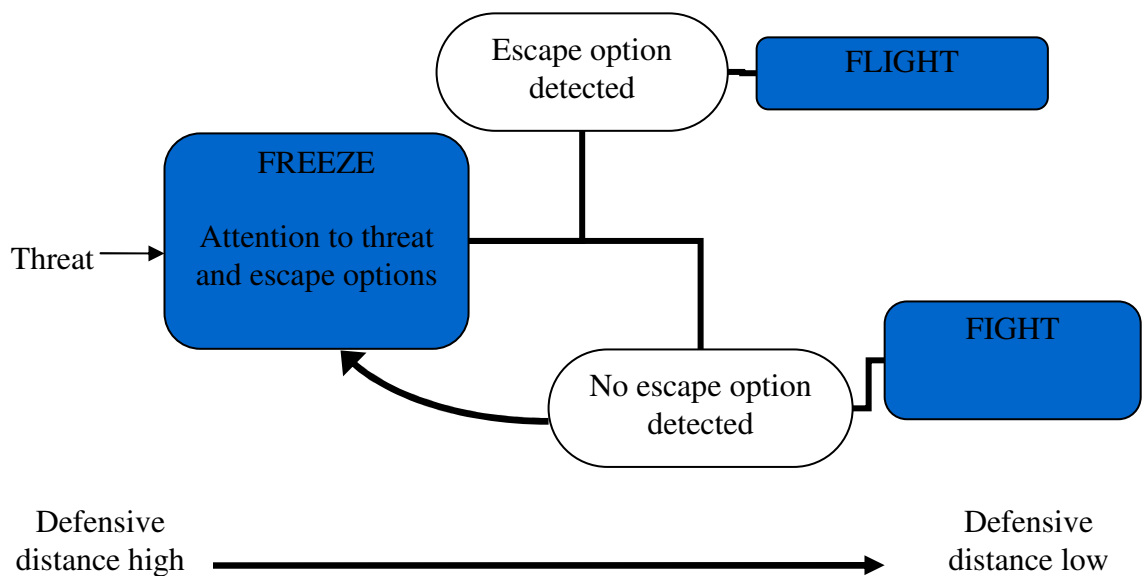
Data from humans suggest the BAS is lateralized to the left hemisphere (Harmon-Jones, 2003), although this effect may more truly reflect a reduction in activity in the right hemisphere (Coan & Allen, 2003). More recently, it has been argued that the BAS may comprise overlapping systems including early stage motivational factors and later stage emotional responsiveness to biological reinforcers (Smillie et al. 2006; Corr, 2008; O'Connor et al., 2008).

Freeze-Flight-or-Fight System (FFFS)

The FFFS (Freeze, Flight or Fight system) is a defense and withdrawal system that functions to move an animal away from danger. The FFFS has been mapped in terms of a temporal series of response options (freeze then flight then fight: Figure 5.3). Selection of a response is determined by defensive distance (i.e. threat far away versus threat near) and availability of escape options (Blanchard & Blanchard, 1988; McNaughton & Corr, 2004). Upon detection of a threat (e.g. a predator) the (prey) animal freezes, becoming hypervigilant to signals of threat and available escape or defense options. Depending on these factors an animal

will then usually, in the first instance, flee. When there are no flight options, the animal will remain frozen (e.g. where a predator moves away) or, when defensive distance falls below a critical threshold, engage in defensive fight behaviour. Finally, if physically restrained, the animal may become tonically immobile (Erhard et al., 1999). The FFFS underlies fear, and involves periaqueductal grey, hypothalamus, amygdala, Anterior Cingulate Cortex (ACC) and prefrontal areas in the computation of response options (with precise neural substrates often determined by defensive distance: McNaughton & Corr, 2004).

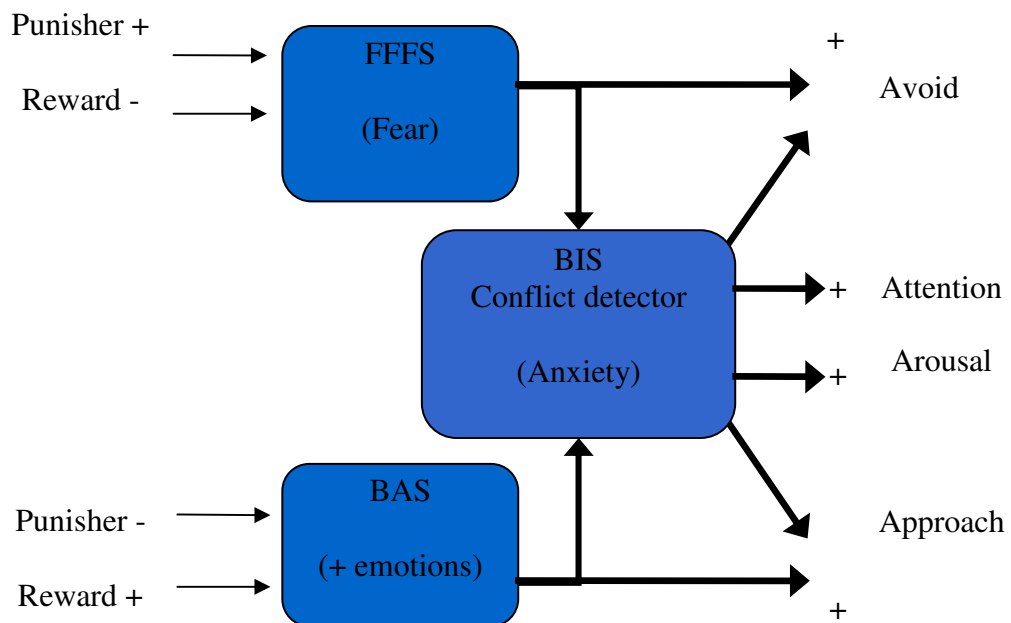
Figure 5.3 The stages of the FFFS. The typical series of events upon detecting a threat: freeze, flee or fight. Defensive distance may be loosely mapped from left to right, with detection of threat/greatest defensive distance to the left hand side and last-resort defensive fight/shortest defensive distance to the right hand side.



Behavioural Inhibition System (BIS)

The BIS (Behavioural Inhibition System) was originally conceptualized by Gray (1981) as a motivational system that inhibits behavior in the presence of novel or highly salient stimuli. Psychophysiological data have led to a reconceptualisation of the BIS as a bottom-up reflexive attentional orienting system, sensitive to cues of punishment, nonreward and novelty, especially where these signals conflict with positive cues from the BAS (McNaughton & Corr, 2004; Amodio et al., 2008; see also: Tsetsenis et al., 2007: Figure 5.4).

Figure 5.4 The Behavioural Inhibition System (BIS) is sensitive to signals of punishment, nonreward and novelty, especially when these signals conflict with outputs from the Behavioural Approach System (BAS). After McNaughton and Corr (2004)



It is now generally agreed that the BIS may be defined as an attentional system for monitoring response conflicts arising from outputs from the FFFS and BAS concurrently (the BIS is also implicated in approach-approach and avoid-avoid conflicts: Amodio et al., 2004)². The BIS functions to interrupt ongoing cognitive processes and enhance attention towards the threatening cues. This enhanced processing may then feed into later stages of the FFFS to be revealed in the overt behavioural response. The system is governed by monoamine neurotransmitter systems such as the noradrenergic and serotonergic systems and associated neural substrates, including the locus coeruleus in the brain stem and anterior cingulate cortex (ACC). The latter plays a key role in detecting expectancy violations (Rushworth & Behrens, 2008). High BIS sensitivity or activation is associated with enhanced attention, arousal, vigilance and anxiety (Gray, 1981; McNaughton & Corr, 2004). Overactivity of the BIS has been associated with anxiety-related disorders (Gray, 1981). Psychophysiological data suggest there is no lateralisation of the BIS in the brain (Coan & Allen, 2003). This is unsurprising given the multiple inputs to BIS from competing approach-withdrawal systems.

² My definition of BIS concurs with most authors, that the BIS as an attentional system for monitoring conflict within and between FFFS and BAS outputs. However, some authors continue to discuss the BIS in terms of an avoidance/withdrawal mechanism. In personality research BIS scales have traditionally been used as a measure of avoidance sensitivity, but are now generally reconceptualised in terms of anxiety sensitivity.

5.1.1.4. Other factors: Motivation, arousal, response speeding and slowing

The above systems are all mediated by additional factors, namely motivation and arousal (McNaughton & Corr, 2004). Motivation includes both top-down cognitive input (e.g. drive to perform a task) and bottom-up processes (e.g. implicit motivational systems such as thirst and hunger). Arousal describes the degree of psychophysiological activation of attentional and behavioural systems (McNaughton & Corr, 2004). Motivation and arousal have a largely quantitative effect on the processing of emotional evaluation (Gray, 1971). As such, they do not present competing theories, but factors that are a necessary component of the attentional and behavioural systems outlined above. The following is a brief overview of how motivation and arousal may influence attentional and behavioural responses to emotional stimuli, focusing on aspects that are most relevant to the current study.

A range of individual motivational factors have a quantitative effect on responses to emotional stimuli (e.g. the degree of slowing induced by the presence of an emotional distractor may be less among individuals highly motivated to perform the ongoing task). The motivation priming hypothesis (Lang et al., 1998), for example, describes the enhancement of the startle reflex in individuals during an aversive motivational state (e.g. in a state of fear), and attenuation of the startle reflex in individuals during an appetitive motivational state. Therefore, while the response is the same (startle), the magnitude of the response is modulated by motivational factors.

Motivational factors may also have a more qualitative effect on responses to emotional stimuli, particularly in humans (Lang et al., 1998). For example, motivation for social dominance influences people's performance on a learning task when task-irrelevant emotional faces are shown (Schultheiss et al., 2005). Schultheiss et al. (2005) presented participants with an abstract learning task during which three distinct visuomotor sequences were followed each by an emotional face (angry, happy, surprise), a neutral face, or no face. Participants were ranked according to their implicit motives, gained from questionnaire scores, on a scale from submissive (affiliation motivated) to dominant (power-motivated). Results revealed an interaction of viewed facial expression with the viewer's motivational need for dominance, influenced by whether the viewed face was of matched gender. For example, there was a negative relationship between task performance and dominance motivation for tasks followed by matched-gender happy faces (which impaired task accuracy) and both matched- and mismatched-gender happy faces (which impaired task speed). This result suggests that happy faces may be more aversive to individuals seeking to dominate others, while they may have a more positive connotation to less power motivated individuals with a greater drive for affiliation.

The equivalence of facial cues of dominance and emotion, and their interaction with individual motivational factors, is summarised in the Functional Equivalence Hypothesis (Hess et al., 2007). This states the signal value of facial expressions is determined not only by the expression itself, but is a function of an interaction of expression with facial morphology, further influenced by the relative genders of

the signaler and receiver (Hess et al., 2007). In the present study the use of neutral faces, and a post-hoc examination of dominance motivation were included to investigate possible effects of these factors.

Arousal may also influence responses to emotional stimuli. Stressed or anxious people have enhanced arousal relative to non-stressed or non-anxious people, hence differences in measures of arousal (e.g. galvanic skin responses) are used to distinguish stressed states from relaxed states (Howard & Hughes, 2008). Therefore, in studies where high-anxious versus low-anxious populations are studied, or where individuals undergo a stress manipulation, arousal levels typically differ between groups. This is reflective of the difference in arousal found in the monkeys in Chapter 3, Post-health-check versus Post-enrichment.

The effects of general arousal levels on speed of responding in humans has been studied in applied fields such as sports performance and driver safety, as well as human cognitive performance (Ozel et al., 2004). Speeding effects are summarized by the Yerkes-Dodson Law which states that task performance (in this example, speed to respond) increases with arousal up to a degree of arousal beyond which task performance falls off again (resulting in a performance curve: Yerkes & Dodson, 1908). Data from human studies using non-emotional stimuli indicate that small increases in arousal lead to speeded responding, and this speeding occurs at early information processing stages but not at later motor executive stages (Jepma et al., 2009). Arousal effects also account for differences in task performance where stimuli of varying degrees of associated arousal

(regardless of valence) are shown on separate trials (Mogg et al., 2008). Again, moderate increases in arousal lead to a speeding of motor responses (Bradley et al., 1992; Ozel et al., 2004). Bradley et al. (2006) found that increased arousal in humans speeded reaction times for recognizing both pleasant and unpleasant slides which had been shown previously. Arousal may therefore speed task performance with emotional stimuli irrespective of stimulus valence (negative/positive). Matching levels of arousal between negative and positive emotional states in human studies remains methodologically problematic, and differences are typically accounted for by including control trials (typically neutral faces) against which experimental (emotional face) trials are compared. To control for the effects of general motor-speeding of response when comparing between states associated with higher and lower levels of anxiety or stress, non-emotional control trials were incorporated in the current study.

Recent formulations have replaced theories of a non-specific stress response and general performance (arousal) effects with identification of components of the stress response that may act to influence cognition via different pathways (e.g. anxiety versus fear) and more clearly delineated definitions of the types of cognitive processes that may be affected (e.g. attention versus learning versus memory: Mendl, 1999). For example, in rats, prenatal stress increases emotionality and fear-related behaviour as reflected in impaired avoidance learning in adulthood (Lehmann et al., 2000b). Postnatal stress, on the other hand, enhances attentional processes which may lead to improved avoidance learning in adulthood (Lehmann et al., 2000b).

Where stimuli have arguably comparable levels of associated arousal but different valences (e.g. angry versus happy faces), arousal and valence factors may begin to be teased apart. Generic slowdown theories were developed to account for the relative slowing of responses on trials on which emotionally salient information is present relative to trials on which neutral information is present (e.g. MacLeod et al., 1986; Algom et al., 2004; Mogg et al., 2008). Delay to respond may result from a combination of acute physiological arousal, emotion-evaluation and attentional processes. Mogg et al. (2008) conducted a central cueing task during which either a neutral, angry or happy face was shown in the center of the screen. Participants were asked to respond by identifying the orientation of a subsequent probe. High anxious individuals were slower to respond to probes following angry and happy faces compared to neutral faces. Low anxious individuals were slower to respond to probes following happy (but not angry) versus neutral faces. These data suggest high anxious individuals demonstrate a slowing of response to all arousing stimuli, regardless of valence, while low anxious individuals show a slowing of response to arousing positive stimuli only.

In summary, it is likely that the way competing information is processed, the pathways through which it passes, the intrinsic salience of a stimulus (bottom-up stimulus processing), the emotional value attributed to it (bottom-up stimulus processing and top-down goal-related assessment), interaction with motivational systems, the behavioural system activated (approach/withdraw) and degree of arousal all contribute to emotion-evaluation and response-slowing.

5.1.2. Current methods for studying emotion-evaluation and response-slowing

The methods used to test information processing and emotion-evaluation models in humans are based on the eStroop task. These were discussed in section 5.1.1.1. More recent approaches incorporate data from neuroscientific and computer modeling techniques. These were discussed in section 5.1.1.2. In both cases methods have been applied using human participants.

The methods used to test reinforcement sensitivity theories of emotion-evaluation are more varied and have been applied using both human and non-human participants. These methods may be broadly divided into approaches that utilise conditioning procedures, versus those that focus on spontaneous responses to unconditioned (fear-relevant) stimuli.

No study to date has investigated the influence of affective state on emotion-evaluation and response-slowing in a non-human species using an adapted version of the computerized tasks typically used with humans. The following briefly discusses methods currently used with non-human animals to investigate comparable processes, with the aim of developing a unified approach, which is described in section 5.1.3.

5.1.2.1. Conditioning studies

Conditioning studies have played a key role in the development of our understanding of emotions (Gray, 1971). They have demonstrated that neutral stimuli may be conditioned to become fear-inducing (Pavlov, 1927; Gray, 1971) and that fear-relevant stimuli are more readily associated with an aversive unconditioned stimulus (UCS), and are more resistant to extinction, than are non-fear-relevant stimuli, in both humans and primates (rhesus macaques: Cook & Mineka, 1989 and 1990). The influence of affective state on the strength of these selective associations has only been studied in humans, particularly in individuals with phobias (Öhman, et al., 2001; Öhman & Mineka, 2001).

A test which has been used frequently with primates is the Wisconsin General Test Apparatus (WGTA: e.g. Cook et al., 1985; Rudebeck et al., 2006). During this task participants are required to reach their arm over a plexiglass box which contains a threatening object to reach a food reward. Appraisal of the threat value of the object placed within the plexiglass box is measured as a function of latency to reach towards the food. The presence of a snake in the plexiglass box has no effect on latency to reach in snake-naïve rhesus macaques. However, following observation of a conspecific responding fearfully to the snake (UCS), latency to reach over the WGTA in the presence of a snake slows significantly (Cook et al., 1985). Therefore latency to reach in the WGTA may reflect the outcome of competing approach and withdrawal processes in the presence of threatening stimuli. It should be noted that a comparable design has been used with humans whereby latency to pull a lever towards the body (approach) or push a lever away

from the body (withdraw) has been used to measure approach-withdrawal tendencies to emotional stimuli (faces) in socially anxious humans and non-anxious controls (Roelefs et al., 2009).

Brain lesioning and electrode implant studies have been combined with conditioning studies to determine the brain regions underlying the processing of emotional information (e.g. lesions in rats: LeDoux, 1996; single cell recording in macaques: Rolls, 2000). Again, while these studies have demonstrated differential responses to emotional (or emotionally-conditioned) stimuli, they have not tested the effects of preexisting emotional states on responses to such stimuli. Some preliminary work has been conducted with serotonin receptor knockout mice (Tsetsenis et al., 2007). The knockout mice showed enhanced fear conditioning (freezing) to emotionally-conditioned stimuli, relative to non-knock-out mice, when those stimuli were partial, but not perfect, conditioned cues. Freezing to perfect predictors did not differ between groups. Inhibition of neurons in the amygdala and hippocampus revealed a role of the hippocampus in the processing of ambiguous threatening cues and the amygdala in the processing of both ambiguous and unambiguous threat-relevant cues.

Conditioning approaches have been most valuable in identifying the types of stimuli which most easily become associated with positive and negative UCS, the speed with which positive and negative selective associations may be formed, their resistance to extinction following removal of previous reinforcement contingencies, and the mechanisms underlying these processes.

5.1.2.2. Non-conditioned responses to stimuli

Studies that focus on spontaneous responses to unconditioned (fear-relevant) stimuli have demonstrated the effects of these stimuli on approach and withdrawal behaviours. For example, Rodd et al. (1997) used duration of tonic immobility as a measure of attentional bias in domestic fowl chicks. Chicks were exposed to uncontrollable shock treatment which induced a state of learned helplessness (dissociation of behavior from outcomes – the antithesis of operant learning). Controls were exposed either to an escapable shock or no shock pretreatment. At 24 hours post-shock the chicks were exposed to threatening (artificial ‘predator’ eyes) and non-threatening (conspecifics present, predator eyes absent) external cues and handled by an experimenter to induce a state of tonic immobility. Number of attempts required to induce immobility and duration of immobility were measured. Chicks with learned-helplessness remained immobile for longer, and became immobile more readily than controls. However, the pattern of results with respect to the presence of external cues of threat was unclear. The presence of an experimenter may provide a more threatening cue than inanimate predator eyes, for example. It is therefore difficult to reconcile data from this approach with attentional processes. As with conditioning studies, the influence of preexisting affective factors on responses remains untested.

The startle reflex is a reflexive response to a sudden (startling) stimulus and has been measured in both humans (Kumari et al., 2001; Hess et al., 2007) and animals (rats: Lehmann et al., 2000a; rhesus macaques: Lutz et al., 2007). In humans, the startle reflex is most commonly measured in terms of latency and

amplitude of eye-blink responses to a startling auditory, visual or tactile stimulus (Lang et al., 1998). Patients with the anxiety disorder Obsessive-Compulsive Disorder (OCD), for example, show speeded onset and greater amplitude of eyeblinks to a startling acoustic stimulus compared with non-anxious controls (Kumari et al., 2001). Amplitude of eyeblinks is enhanced in both anxious and non-anxious individuals while watching a negative film (e.g. horror) versus a positive film (e.g. comedy: Kumari et al, 2001). Prepulse inhibition/facilitation techniques have been used to elucidate the effects of attentional modulation on the startle reflex (Davis et al., 2008). When a non-startling prepulse tone is sounded immediately before the startling tone, the magnitude of the startle reflex is reduced compared with responses to the startling tone alone (Lehmann et al., 2000a; Davis et al., 2008). This indicates reduced available attentional resources for automatic processing of the startling tone. In contrast, a longer interval between prepulse tone and startling tone elicits an enhanced startle reflex. This reflects the effects of increased and sustained arousal and attention on the startle response. Among humans with depressive symptoms, the startle reflex is attenuated to both positive and negative emotional stimuli (Mneimne et al., 2008). While the startle reflex is typically measured in humans in terms of magnitude of eyeblinks, in animals such as rhesus macaques and rats it is usually measured as whole-body movements (Gewirtz et al., 1998; Lehmann et al., 2000a; Lutz et al., 2007; Davis et al., 2008). Enhanced startle is attributed to hyperexcitability of the amygdala-based fear circuitry (Rosen & Schulkin, 1998).

5.1.3. Implications of human- and animal-based research for the development of a novel method to study emotion-evaluation and response-slowing in primates

Several themes arose from the literature review that informed the design of the current study. Firstly, emotion-evaluation processes and response-slowing reflect several underlying mechanisms that include approach (BAS) and withdrawal (FFFS) behaviours and resolution of conflict of these responses (BIS). There is a trade-off between stimulus saliency, emotional tagging, goal-directed motivation to perform the task, arousal, attention and consequent activation of approach or withdrawal behaviours. No study to date has directly adapted human paradigms to measure emotion-evaluation and response-slowing in non-human participants. Only a few previous studies have used a classical Stroop paradigm with monkeys (Japanese macaque: Lauwereyns et al., 2000; rhesus macaque: Washburn, 1994), but none of these considered the effects of affective state, nor used emotional stimuli. Therefore, a task based broadly on the cognitive paradigms used with humans (e.g. eStroop), incorporating aspects of the WGTA commonly used with primates, was developed.

Emotional stimulus content was selected for comparability with human studies, and a measure of task performance based on reaction time was selected. Aspects of the human paradigms which were adopted included non-emotional control trials to provide a measure of baseline task performance (in this case, tendency and latency to touch a grey square presented on a touch-sensitive monitor in order to receive a pellet reward), and experimental trials on which emotional distractor content was included. Faces were selected as appropriate emotional distractors in

line with recent work with humans (Bar-Haim et al., 2007; Mogg et al., 2008). Tendency and latency to respond by touching a stimulus presented on a touch-sensitive screen provides a measure of behavioural approach and withdrawal systems, comparable with reach measures during the WGTA. Such data will allow further hypotheses regarding underlying mechanisms to be formulated. For example, speeding of responses may reveal an activation of approach behaviours (BAS), possibly modulated by general arousal effects, while slowing of responses may reveal activation of withdrawal behaviours (FFFS), and conflict of responding (BIS).

Secondly, arousal may affect baseline task performance, speeding motor responses as arousal increases (Ozel et al., 2004). In order to account for arousal effects on baseline response tendency and latency, control trials are required (see Mogg et al., 2008). Control trials would also enable consideration and control of side preferences in baseline responding.

Thirdly, studies with humans demonstrate that emotional distractor content impairs ongoing task performance. The emotional content must be incorporated without changing the structure of the basic task. The inclusion of a conspecific face framed within the non-emotional control stimulus was selected, in line with studies with humans (Mogg et al., 2008). Highly salient emotional distractor content has been shown to impair task performance to a greater extent than less emotionally salient distractor content (Mogg et al., 2008). The inclusion of both types of emotional distractor content allows comparison of saliency/emotional content effects on task performance. For this first study to investigate emotion-

evaluation and response-slowing in rhesus macaques, conspecific faces with direct gaze (highly salient/threatening) and averted gaze (less salient/less threatening) were selected as appropriate emotional distractors³.

Finally, emotion-evaluation and response-slowing effects in humans have been revealed in both trait and state anxiety (Williams et al., 1996), both within the same individuals following a social anxiety stress manipulation (e.g. Roelofs et al., 2007 and 2009), and between individuals with different trait anxiety scores (e.g. Mogg et al., 2008). A within-subjects design with manipulation of state affect (i.e. using the Post-enrichment and Post-health-check conditions described in Chapter 3) was therefore considered appropriate for the present study.

In addition to the main points listed above, secondary themes were identified. Laterality effects in emotion-evaluation and response-slowing exist in humans (Davidson, 1992). Approach behaviours (BAS) are considered to be lateralized to the left hemisphere (Trevorthen, 1996). The right hemisphere has been implicated in enhanced circuit breaking (Corbetta & Shulman, 2002) and FFS responses (horses: Austin & Rogers, 2007; birds: Koboroff et al., 2008; primates: Rogers,

³ Studies with humans typically use facial expressions of emotion or gaze measures based on eye direction alone (direct versus averted). Due to the threatening value of direct gaze to rhesus macaques full frontal faces with direct eye-gaze were selected as threatening faces and profile faces (and hence averted gaze) were selected as non-threatening faces.

2009; dogs: Siniscalchi et al., 2008; humans: Thompson et al., 2009). It may therefore be possible that approach behaviours will be enhanced for stimuli presented to the left hemisphere (RVF, although this advantage is weak for stimuli presented in lateralised locations: Harmon-Jones, 2003), and withdrawal behaviours will be enhanced for stimuli presented to the right hemisphere (LVF). These effects may be further influenced by the right hemisphere advantage for processing faces in rhesus macaques (Vermeire et al., 1998; Tsao et al., 2008). Due to these laterality effects in humans, visual field was considered in the analyses presented here.

Priming and trait dominance effects also arose as factors that may influence response to emotional stimuli. Effects of the stimulus shown on the previous trial on response time on the current trial were investigated to ensure there were no significant carry-over effects between trials and to ensure the eight second inter-trial-interval (ITT) was sufficient to prevent this. Human dominance traits (specifically high dominance motivation) have been shown to slow responses to angry (threatening) faces in studies using a similar design to that used here (Schultheiss et al., 2005; Roelofs et al., 2007 and 2009). Therefore, monkeys were ranked for aggressive-approach tendency, based on data presented in Chapter 2, to test whether this had an effect on responses.

5.2. Aims:

The general aim of this chapter is to describe the development and application of a method for measuring emotion-evaluation and response-slowing to socio-emotional stimuli in rhesus macaques. A further aim is to examine the influence of two treatments, assumed to induce shifts in inferred affective state, on these processes. I test competing hypotheses about possible mechanisms underlying such biases, namely arousal and emotion evaluation. The paradigm is based on human cognitive studies which present an emotional distractor at the same location as a task-relevant stimulus, and measure slowing in responses to the task-relevant stimulus as a function of distractor saliency/emotionality.

5.3. Hypotheses and specific predictions:

1. Stress-related arousal increases baseline tendency and speed to respond (control trials)
 - a. Monkeys will **make more responses** to a non-emotional control stimulus when highly aroused (Post-health-check) than when less aroused (Post-enrichment).
 - b. Monkeys will **be faster** to touch a non-emotional control stimulus when highly aroused (Post-health-check) than when less aroused (Post-enrichment).

2. Emotional distractor content impairs task performance

- a. Monkeys will **make fewer responses** on trials with direct gaze faces than on trials with averted gaze faces.
- b. Monkeys will be **slower to respond** on trials with direct gaze faces than on trials with averted gaze faces.

3. Affective state mediates task impairment effects of emotional distractor content

- a. Monkeys will **make fewer** responses on trials when emotional distractor content is present (experimental trials) Post-health-check than Post-enrichment
- b. Monkeys will be **slower to respond** on trials when emotional distractor content is present (experimental trials) Post-health-check than Post-enrichment
- c. The above effects will be **enhanced** for faces with direct gaze compared with averted gaze.

Part B: Development of the new method

In Part A I presented the background and rationale for the development of a method to study approach and withdrawal responses to emotional stimuli in rhesus macaques and the influence of affective state on these processes. In Part B I detail the method developed, present data and discuss these in light of the human literature.

5.4. Method

5.4.1. Participants

Twelve monkeys took part in the study (Monkeys C55, 29C, 86O, 16P 79T, AI73, 06H, 94K, 92R, 27S, 66S and 79S; average age: 7.39 years; range: 3.60 – 24.7 years old). All monkeys had previously begun operant touch-screen training in the laboratory during the preceding nine months and worked in the laboratory on a daily basis.

5.4.2. Stimuli and apparatus

The face stimuli consisted of 20 colour photographs of 10 male monkeys ('stimulus monkeys') housed at CPRC. The stimulus monkeys were unknown to the participant monkeys. For each stimulus monkey, one photograph showing a frontal view of the face with neutral expression (hereafter 'Direct gaze'), and one photograph showing a profile view of the face with neutral expression ('Averted gaze') were selected. This resulted in one 'Direct gaze' and one 'Averted gaze'

photograph per stimulus monkey (Figure 5.5a and b). Face pictures were selected for use according to good resolution of image, availability of both frontal and profile images from the same individual, and neutral facial expression. Images were trimmed so that only the head was visible. Face pictures were superimposed on a grey background and enclosed in a rectangular frame measuring 154mm x 164mm, thereby subtending 14.71 x 15.66 degrees of visual angle when presented centrally on a computer monitor at a 60cm viewing. The face stimuli were paired, according to stimulus monkey identity, to give 10 direct-averted gaze face pairs (Appendix 5.1). A single stimulus consisting of the grey rectangular frame alone was also composed for training purposes and baseline control during testing (Figure 5.5c). The grey hue of the rectangular frame was selected for comparability with the hue of the monkeys' enclosures, against which monkeys viewed conspecifics in the home environment.

Direct-averted gaze face pairs were assessed for equivalence of luminosity (L_y) and contrast energy (C) using Adobe Photoshop 7, following the procedure described in Chapter 4. Direct gaze and averted gaze faces were paired according to stimulus monkey identity. The mean L_y for each face stimulus was entered into a paired-samples t-test to assess equivalence of L_y across direct gaze and averted gaze face pairs. Direct gaze and averted gaze faces did not differ in L_y ($t(9)=0.39$, $P=0.70$). The C value for each face was entered into a paired-samples t-test to assess equivalence of C within direct-averted gaze face pairs. Direct gaze and averted gaze faces did not differ in C ($t(9)=2.10$, $P=0.07$). Therefore, the luminance and contrast energy of direct gaze faces did not differ significantly

from that of the averted gaze faces with which they were paired. An additional check was run to examine the degree of variation between the different stimulus monkey face pairs. Mean L and C values for all face stimuli fell within 2SD of the mean L and C values for the entire set of 20 face stimuli. Therefore, variance in luminance and contrast energy was low across the entire stimulus set.

Figure 5.5 Examples of the stimuli used during the study: a) direct gaze and b) averted gaze from one stimulus monkey; c) a grey square was presented on control trials.

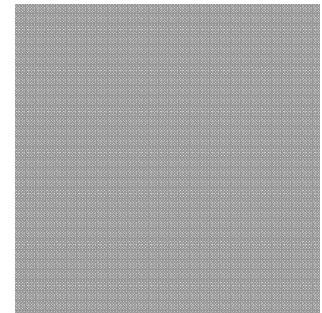
a)



b)



c)



Stimuli were divided into two subsets for counterbalanced order of presentation between individuals. Each stimulus monkey was assigned a number from 1-10. The 10 direct gaze-averted gaze pairs were divided into two subsets, with direct gaze-averted gaze pairs numbered 1-5 in subset one, and direct gaze-averted gaze pairs numbered 6-10 in subset two (Appendix 5.1).

One stimulus was presented on each trial. Stimuli were presented at one of three locations: left visual field (LVF), right visual field (RVF) and midpoint. Stimuli were presented on the screen so that the midpoints of each of the LVF and RVF locations were 100mm from the screen midpoint, along the horizontal midline. Averted gaze probes were presented on the screen in either a left or a right orientation so that the direction of gaze of the averted face was always directed away from the midpoint of the screen. Both orientations of averted gaze probes were presented at the central location. All stimuli were presented against a black background.

Stimuli were presented on a 15" Protouch Aspect TS17LBRAI001 touch-sensitive LCD monitor connected to a Toshiba Satellite Pro A60 laptop computer running EPrime experimenter-generator software, with all aspects of the equipment set-up as described in Chapter 2 (Figure 2.7). All sessions were filmed using two video cameras. One video camera (Samsung VP-L150 digital video camera) was positioned to one side of the apparatus to film responses made at the touch screen interface (capturing both the stimulus location, image identity and the 'touch' response). A second camera (JVC 700x digital zoom) was positioned above and behind the touch screen to film the monkey's behavioural responses in the cage. Cameras were placed out of direct view of the monkey. A live video feed to the control room allowed the experimenter to observe the monkey on a split-screen video monitor. All responses were rewarded with a secondary reinforcing tone, delivered via two speakers situated behind the apparatus, and automatic delivery

of a Noyes 190mg food pellet triggered by the pellet dispenser. Full details of reinforcement features and equipment are given in Chapter 2.

5.4.3. Design and Procedure

Main terminologies are given in Table 5-1. All monkeys initially underwent a series of training sessions to learn the basic task contingencies. During training only the grey square was presented, and monkeys learned to touch this in order to gain a pellet reward. Data for training sessions are presented in Chapter 2. The monkeys then took part in testing sessions during which control trials (grey square) were interspersed with experimental trials (grey square plus emotional distractor content: direct gaze or averted gaze face).

During testing, stimuli were presented in a randomised order. Experimental trials were those on which faces with direct-gaze and averted-gaze were presented (Figure 5.6a). Experimental trials were included to test the experimental hypotheses about the effects of emotional distractor content on task performance (measured as latency to touch the stimulus). Control trials were those on which the grey square was presented (Figure 5.6b) and were included to gain baseline response latencies.

The procedure for a testing session was as follows: the EPrime program was opened and participant monkey details entered into the initial information screen (i.e. Monkey ID and session number). The monkey was then transported to the

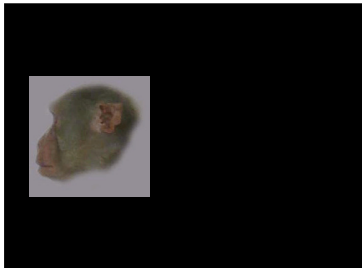
Table 5-1 Terminologies used in the study

Term	Definition
Training session	Sessions conducted prior to testing during which monkeys learnt to perform a basic task (touch a grey square to gain a food reward).
Testing session	Sessions on which experimental trials were conducted and experimental data were collected. Each monkey took part in two daily testing sessions following one week of enrichment (Post-enrichment) and two daily testing sessions over the two days immediately following the veterinary inspection (Post-health-check).
Control trial	A trial on which the grey square was shown.
Experimental trial	A trial on which a face stimulus was shown.
Emotional distractor content	Face stimuli (direct gaze and averted gaze faces superimposed upon the grey square used during control trials) were designed to contain emotional information which may capture attention and impair task performance.
Treatment	Testing was conducted in two treatment conditions for each monkey: Post-enrichment and Post-health-check.
Correct response	A correct response was defined as a touch to any stimulus shown on the screen within the 60000ms presentation time. If the monkey failed to touch the stimulus within the 60000ms presentation time this was recorded as an incorrect response.

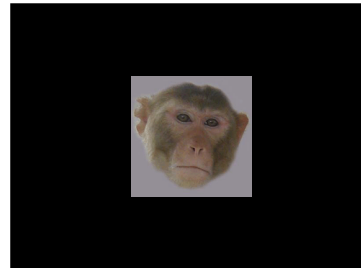
Figure 5.6 Examples of stimuli as they appeared at the three screen locations for a) Experimental trials and b) Control trials.

a)

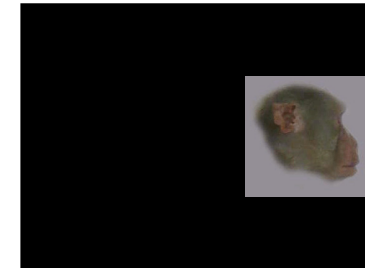
Experimental trials



Averted gaze
(LVF orientation)



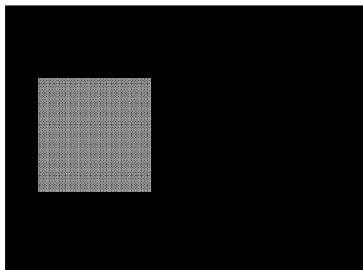
Direct Gaze
(Central presentation)



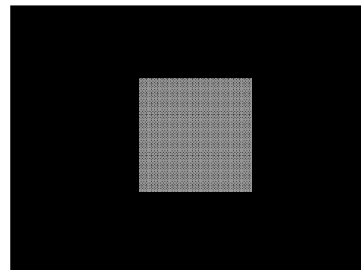
Averted Gaze
(RVF orientation)

b)

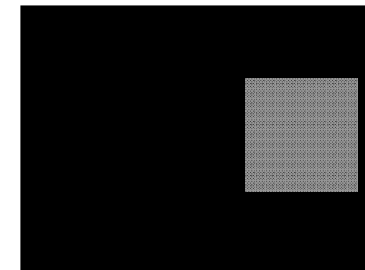
Control Trials



Grey square
(LVF presentation)



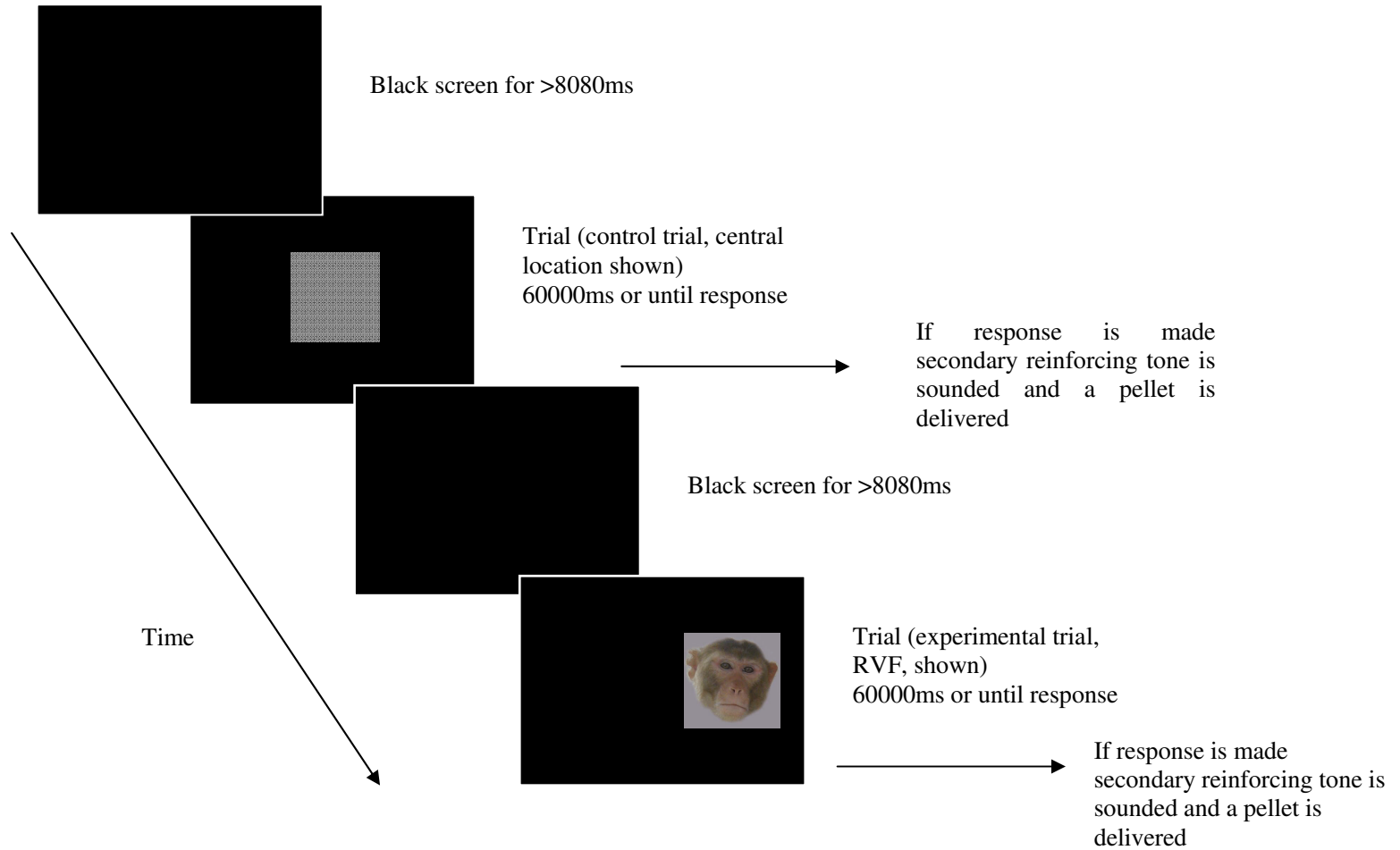
Grey Square
(Central presentation)



Grey Square
(RVF presentation)

Figure 5.6 (Continued) c) Example of the experimental procedure.

c)


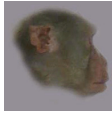



laboratory in the testing cage, positioned in front of the apparatus at a viewing distance of approximately 60 cm, and allowed to settle. The experimenter immediately moved to the adjacent room, and set the video to record events. The experimenter triggered the onset of the experiment by pressing the return key on the keyboard. On each trial a single stimulus was presented for 60 seconds, or until the monkey touched the stimulus: Figure 5.6c). Stimulus onset was triggered automatically by the EPrime software. Stimulus offset was triggered either by the monkey touching the stimulus, or automatically if the monkey did not touch the stimulus during the 60 second trial. All touches to stimuli were rewarded by immediate delivery of a pellet and reinforcing tone at a 100% fixed reinforcement ratio. At stimulus offset, a plain black screen was shown until the onset of the next trial.

Inter-trial interval (ITI) was set to a minimum of 8080ms. If the monkey touched the screen during the inter-trial interval the Eprime program automatically reset the inbuilt interval counter to 0ms. This ensured that stimulus onset only occurred when the monkey was not currently touching the screen, and had not touched the screen in the preceding 8080ms. The ITI allowed monkeys time to collect and eat pellets before the onset of the next trial. Further, extending the inter-trial interval reduces carry-over effects between trials. All responses (stimulus shown and latency to respond) were recorded by the computer.

Testing sessions consisted of an initial block of three practice trials during which the control stimulus was presented once at each of the three locations on the

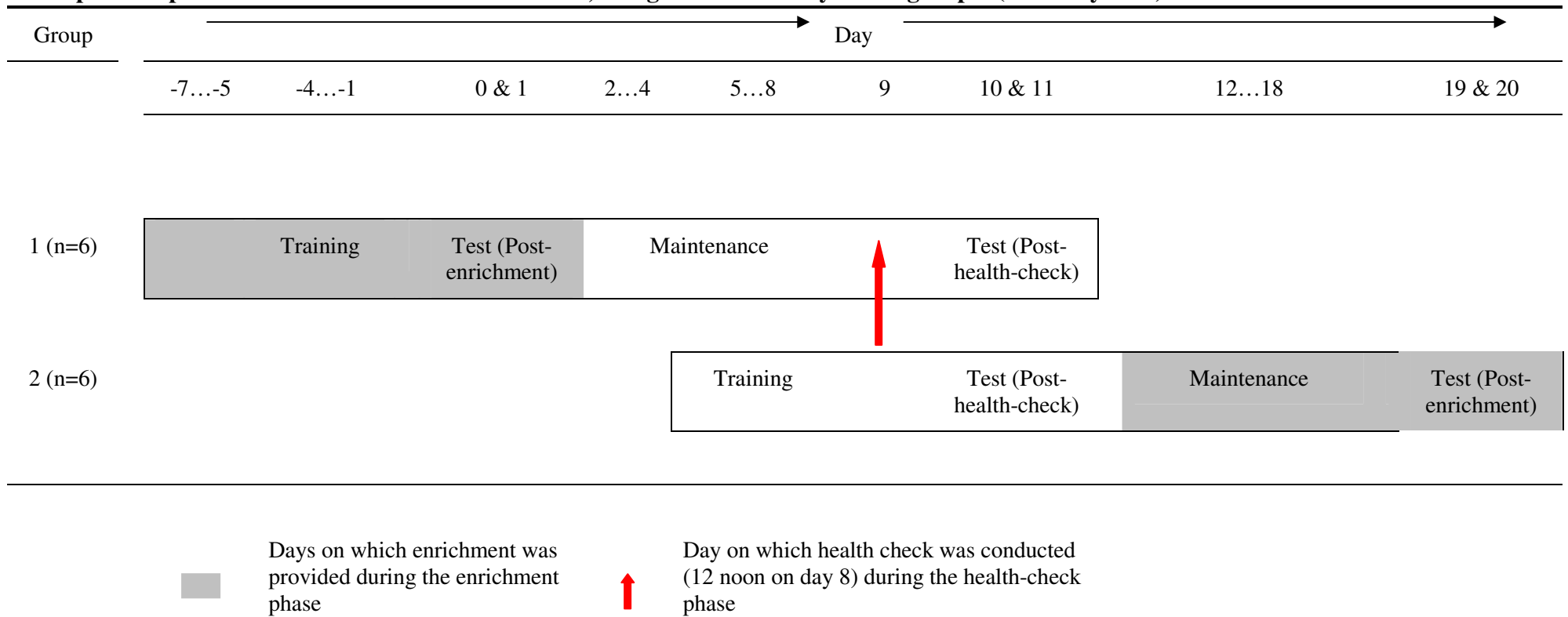
Table 5-2 The number of experimental and control trials on which stimuli were presented to the left, central and right visual fields during one daily testing session (left hand columns). Right hand columns show total number of experimental and control trials over all testing sessions for each monkey.

	One daily testing session (n trials)			All daily testing sessions (n trials)	
	Visual field			Treatment	
	LVF	CVF	RVF	Post-enrichment	Post-health-check
Experimental trials					
Direct Gaze					
	10	10	10	60 (30+30)	60 (30+30)
Averted Gaze					
	10	10	10	60 (30+30)	60 (30+30)
Control trials					
Grey square					
	5	5	5	30 (15 +15)	30 (15+15)
Total N trials	75			150	150

screen Figure 5.6b). This was included to remind monkeys of the operant task and to ensure each monkey was working prior to the onset of the first experimental trial in each daily testing session. The practice block was followed by one experimental block of 75 trials (Table 5.2). The experimental block comprised 60 experimental trials and 15 control trials, presented in a randomized order. During the experimental block, the 60 experimental trials comprised stimuli from either subset one, or subset two (each subset comprising 10 faces: five x direct gaze; five x averted gaze). Each of the 10 faces was presented twice at each of the three locations (LVF, center, RVF). Control trials occurred five times at each of the three locations.

All monkeys were tested on two daily testing sessions Post-enrichment and on two daily testing sessions Post-health-check. The order of testing was counterbalanced across individuals so that six monkeys were first tested Post-enrichment (Group 1), and six were first tested Post-health-check (Group 2: Figure 5.7). The order of events was as follows. Group 1 monkeys (Post-enrichment first) received four days of contingency training concurrent with enhanced enrichment in the home enclosure. This was followed by two daily testing sessions (Post-enrichment), then seven daily maintenance sessions, the health-check, and finally two daily testing sessions (Post-health-check), Group 2 monkeys followed a similar procedure which ran in the following order: training – health check – Post-health-check testing – maintenance during the week of enrichment – Post enrichment testing.

Figure 5.7 The counterbalanced order of testing with group 1 taking part in the enrichment treatment first (from day-7 until day 1). Group 2 took part in the health-check treatment first, alongside the monkeys from group 1 (from day 5-11).



The full series of events was as follows, and as summarized in Figure 5.7. On days -7 to -1, all monkeys housed in the enclosure received enrichment in the home cages, as described in Chapter 3. On days -4, -3, -2 and -1, Group 1 underwent training with the apparatus. On each day each monkey in Group 1 was transported to the laboratory where he was presented with a three-trial practice block followed by a 75 trial training block. Across all trials during a training session the control stimulus was presented 25 times at each of the LVF, central and RVF locations, in a randomized order (total 75 trials). As with testing sessions, the control stimulus was presented once at each of the three locations during the initial practice block. All correct responses were rewarded with a secondary reinforcing tone and a pellet. The training criterion was predetermined at 80% correct responses to the control stimulus at each of the three locations (i.e. 20 responses at each of the LVF, central and RVF locations). To assess feeding motivation, the number of pellets left in the pellet tray and the number of monkey chow left in the lunch box at the end of each session, were recorded.

On days 0 and 1, Group 1 took part in two consecutive daily Post-enrichment testing sessions (Figure 5.7), following the procedure described. On days 2-8 the six monkeys in Group 1 engaged in daily sessions during which the control stimulus only was shown for a total of 78 trials, as during days -4 to -1. On days 5, 6, 7 and 8, the six monkeys in Group 2 took part in three daily training sessions. On day 9, all monkeys in the enclosure received their three-monthly health-check, conducted by the facility veterinarian. On days 10 and 11, all 12 monkeys in Groups 1 and 2 took part in Post-health-check testing. On day 12, all monkeys in

the enclosure began a second week of enrichment during which the six monkeys in Group 2 engaged in daily maintenance sessions during which the control stimulus only was shown for a total of 78 trials, as during days 5 to 8. On days 19 and 20, Group 2 underwent Post-enrichment testing.

Throughout the study, care was taken to maintain a regular daily working routine. Staff access to the area in and around the animal housing was restricted and all non-essential husbandry procedures postponed until the end of the study. During the training and enrichment phases monkeys were provided with regular enrichments as described in Chapter 3, with daily food rations adjusted accordingly for calorie intake.

5.4.4. Data selection and treatment

Criteria for inclusion of each monkey in the analyses were a) performance to the 80% criterion for control trials on at least one daily testing session in each testing condition; and b) during test sessions, the monkey ate a comparable proportion of pellets and daily food ration to that consumed during training sessions. The latter were assessed according to the number of pellets left in the pellet tray, and the number of chow pellets and fruit slices left in the lunch box, at the end of each session. Daily testing sessions which met these criteria were considered valid and included in the analyses. Daily testing sessions that failed to meet these criteria were discarded from the analyses.

For analysis of proportion of responses made, frequency data are presented as

$$\frac{n \text{ (correct responses)}}{n \text{ (trials)}}$$

for each of control trials, direct gaze experimental trials, and averted gaze experimental trials. It is signified in the text whether data originate from one or two sessions within a given testing condition (n=60 or n=120 experimental trials, and n=15 or n=30, control trials, respectively).

For analysis of reaction times, latency data for all monkeys who met the above criteria were treated in line with human studies (e.g. Mauer & Borkenau, 2007; Beall & Herbert, 2008). All trials in which no response was made were discarded. All trials in which responses were made faster than 400ms post-stimulus onset were also discarded. Four hundred ms was selected as the minimum cut-off based on the assumption that this was the approximate minimal perception-reaction time. This was calculated as (perception time: ~140ms) + (motor signal time: ~160ms) + reach time (~100ms). Latency data for the remaining trials were then normalized using a Log₁₀ transformation.

In line with the human literature, ratio scores were calculated for each monkey by dividing experimental trial reaction time (RT) by control trial RT.

$$\text{Ratio score} = \frac{\text{mean log}_{10} \text{ experimental trial RT}}{\text{mean log}_{10} \text{ control trial RT}}$$

A positive ratio score indicates a slowing of responses on experimental trials compared with control trials.

In all of the subsequent analyses, order of testing (Post-enrichment first versus Post-health-check first) did not have an effect on the results, therefore this between-groups factor was removed from the analyses presented here. Groupings for order of testing are shown in all tables for reference purposes. Due to evidence for hemispheric specialisation in the processing of emotional faces and gaze, visual field is included in all analyses. All data were checked for a normal distribution using a one-sample Kolmogorov-Smirnov (K-S) test. All K-S tests revealed data did not differ significantly from a normal distribution, therefore parametric tests are used throughout. For t-tests, a Levene's test of equality of variance was also conducted. These were all non-significant. All descriptive data are reported as mean \pm 1SE. Significant findings are presented in figures. Where there were missing cell values, data are presented in tables to identify the location of missing data.

5.4.5. Data analyses for control trials (grey square)

Control trials (grey square) were included to collect baseline performance data, investigate the effects of stress-related arousal on task performance, to check for side biases in responding, and to distinguish valid from invalid testing sessions.

Invalid testing sessions were defined as sessions during which performance on control trials fell below 80% (i.e. fewer than 12 out of 15 correct responses).

To test the effects of stress-related arousal on tendency to respond (Hypothesis 1), a repeated measures ANOVA (RMANOVA) was conducted. Data were mean frequency of responses Post-enrichment versus mean frequency of responses Post-health-check at each of the three screen locations. To test whether stress-related arousal affects latency to respond, a second RMANOVA was conducted. Data were mean latency to respond Post-enrichment versus mean latency to respond Post-health-check, at each of the three screen locations.

5.4.6. Data analyses for experimental trials (direct- and averted-gaze faces)

Experimental trials were included to investigate the effect of emotional distractor content and affective state on task performance. The measures were frequency of responses and latency to respond. Frequency data were included to measure effects of emotional distractor content (direct gaze and averted gaze faces) and affective state on tendency to respond. Latency data were included to measure the effects of emotional distractor content and affective state on speed to respond. Screen location (as a proxy measure of visual field) was included to test for hemispheric differences in processing of emotional stimuli.

To test the two hypotheses that the presence of emotional distractor content impairs task performance (Hypothesis 2), and that this may be mediated by

affective state (Hypothesis 3), frequency data were entered into one RMANOVA. Data were frequency of responses on direct gaze and averted gaze trials, at each of the three screen locations, during the Post-enrichment and Post-health-check testing conditions. To test the hypothesis that the presence of emotional distractor content slows the latency to respond, and this may be mediated by affective state, latency data were entered into one RMANOVA. Data were ratio scores (\log_{10} transformed latency to respond on experimental trials divided by \log_{10} transformed latency to respond on control trials), for direct gaze and averted gaze faces separately at each of the three screen locations, during the Post-enrichment and Post-health-check testing conditions. All main effects and interactions arising from the higher-order RMANOVAS were then examined using appropriate lower-order tests.

Post-hoc Bonferroni corrected paired samples t-tests were conducted to address possible confounding factors and explore the data. Following evidence that the emotional distractor content presented on the previous trial may affect performance on the current trial (Algom et al., 2004), and that anxious individuals are more sensitive to such priming (Richards et al., Unpublished data), an investigation of possible priming effects of the previous trial was conducted. The aim of this analysis was to ensure that tendency and latency to respond on any given trial was not affected by the valence of the previous trial (control, direct gaze, averted gaze). A bivariate correlation was conducted to explore approach-motivation characteristics, since data from humans suggest dominance motivation may influence responses to emotional faces (e.g. Schultheiss, et al., 2005).

5.5. Results

Seven of the 12 monkeys completed all phases of the experiment and met all criteria for inclusion in the analysis (Table 5-3). These seven monkeys responded on $\geq 80\%$ of control trials during one or more daily testing sessions in each of the Post-enrichment and Post-health-check testing conditions. The monkeys collected a comparable number of reward pellets from the pellet tray during the Post-enrichment and Post-health-check testing sessions to that collected during training. The seven monkeys also collected the daily food ration from the lunch box. Data on criteria measures are presented first.

Performance data for experimental sessions are presented in Table 5-3. Seven of the 12 monkeys who took part in experimental sessions completed the study (four from Group 1 and three from Group 2). Three monkeys in each of Groups 1 (29C, C55 and 16P) and 2 (92R, 27S and 66S) reached criterion (80% responses to the grey rectangle) on all four testing sessions. One monkey (AI73: Group 1) reached criterion for just one daily testing session during either testing condition. A total of 1950 trials, from 26 testing sessions spread across seven monkeys therefore met the criteria for entry into the analysis.

Five of the 12 monkeys who took part in experimental sessions failed to reach criterion (86O and 79T in group 1; 94K, 06H and 79S in group 2). Three of the five monkeys failed to reach criterion for responses during both sessions in a single testing condition (79T, 06H: 11% and 40% accuracy Post-enrichment; 79S:

Table 5-3 Seven monkeys reached criteria for inclusion in the analyses. * signifies criterion was not met. – indicates data not available.

	ID	Age (yrs)	N experimental sessions completed ($\geq 80\%$)		Proportion of pellets eaten			Daily food ration eaten			Criteria met? (yes/no)
			Post-enr	Post-hc	Training	Post-enr	Post-hc	Training	Post-enr	Post-hc	
Group 1	C55	24.70	2	2	0.99	0.95	0.99	✓	✓	✓	✓
	29C	12.05	2	2	0.98	0.99	1.00	✓	✓	✓	✓
	86O	5.30	2	0*	1.00	1.00	-	✓	✓	✓	x
	16P	5.15	2	2	1.00	1.00	1.00	✓	✓	✓	✓
	79T	3.65	0*	-	0.92	-	-	✓	✓	-	x
	AI73	3.60	1	1	0.85	0.91	0.89	✓	✓	✓	✓
Mean	n=6	9.08 ± 3.38	1.50 ± 0.34	1.40 ± 0.40	0.96 ± 0.02	0.97 ± 0.02	0.97 ± 0.03				n = 4
Group 2	06H	9.90	0*	2	1.00	-	1.00	✓	✓	✓	x
	94K	7.40	0*	1	0.52	0.60	0.73	✓	✓	✓	x
	92R	4.75	2	2	1.00	1.00	1.00	✓	✓	✓	✓
	27S	4.66	2	2	1.00	0.82	1.00	✓	✓	✓	✓
	66S	3.80	2	2	1.00	0.65	1.00	✓	✓	✓	✓
	79S	3.70	2	0*	1.00	1.00	-	✓	✓	✓	x
Mean	n=6	5.70 ± 1.00	1.50 ± 0.34	1.40 ± 0.40	0.92 ± 0.08	0.81 ± 0.08	0.95 ± 0.05				n = 3
Total	n=12	7.39 ± 1.75	1.50 ± 0.23	1.40 ± 0.27	0.94 ± 0.04	0.89 ± 0.05	0.96 ± 0.03				n = 7

33% accuracy Post-health-check). One of the five monkeys (94K) showed signs of agitation during testing sessions, frequently shaking his cage and failing to look at the screen. A fifth monkey (86O) failed to reach criterion due to external interruptions on both days of Post-health-check testing. Data for these five monkeys were removed from the analyses.

A 1 x 3 RMANOVA was performed to examine whether motivation to work, measured as proportion of pellets eaten, varied with affective state. Data were proportion of pellets consumed with within-subjects factor of training/testing condition (training, Post-enrichment testing and Post-health-check testing) for the seven monkeys who passed all criteria to be included in the final analyses. The proportion of pellets eaten did not differ significantly between training and the two testing conditions ($F_{2,12} = 2.204$, $P=0.203$). Planned t-tests revealed there was no significant difference in pellet consumption between the testing conditions (Post-enrichment versus Post-health-check: $t(6)=1.55$, $P=0.17$) nor between either of the testing conditions and training (Post-enrichment versus training: $t(6)=1.32$, $P=0.24$; Post-health-check versus training: $t(6)=1.44$, $P=0.20$). All monkeys consumed the full daily food ration while in the laboratory after each training and testing session.

These results suggest there was no significant reduction in appetite or motivation to work, as measured by consumption of pellets and daily food ration, between the training and Post-enrichment and Post-health-check testing sessions. The seven monkeys consumed pellets at comparably high rates during both training and

testing phases. It is therefore likely that motivation to work on the task was maintained throughout the study. An increase in appetite or motivation would be difficult to assess, since these may be masked by a ceiling effect in the data.

Data from the 1950 trials entered into the analysis were treated as follows. For frequency data all 1950 trials were included. For latency data, trials on which responses were made earlier than 400ms were removed (n=173 trials: 67 Post-enrichment; 106 Post-health-check), as were trials on which the monkey did not respond (n=44 trials: 30 Post-enrichment; 14 Post-health-check). A total of 217 trials were therefore removed, resulting in 1733 valid trials for inclusion in the following analyses of latency to respond. The number of invalid trials removed for each monkey is shown in Appendix 5.2. An exploratory repeated measures t-test was performed to compare number of discarded trials Post-enrichment (n=97) versus Post-health check (n=120). There was no significant difference in the number of trials discarded between the two testing phases ($t(6) = 1.04, P=0.34$).

5.5.1. Does stress-related arousal improve task performance? (Control trials)

a) Frequency to respond

A 2 x 3 RMANOVA was performed to determine whether stress-related arousal improves task performance (frequency of responses) when non-emotional control stimuli are shown (hypothesis 1, prediction a). Data were proportion of responses on control trials, with within-subjects factors of testing condition (Post-enrichment versus Post-health-check) and screen location (left, central, right:

Table 5-4). There were no main effects of either testing condition ($F_{1,6} = 2.273$, $P=0.182$), nor screen location ($F_{2,12} = 0.000$, $P=1.00$), and a trend towards an interaction of location x testing condition ($F_{2,12} = 2.909$, $P=0.09$).

Table 5-4 Proportion of responses made on control trials presented to the left, central and right visual fields

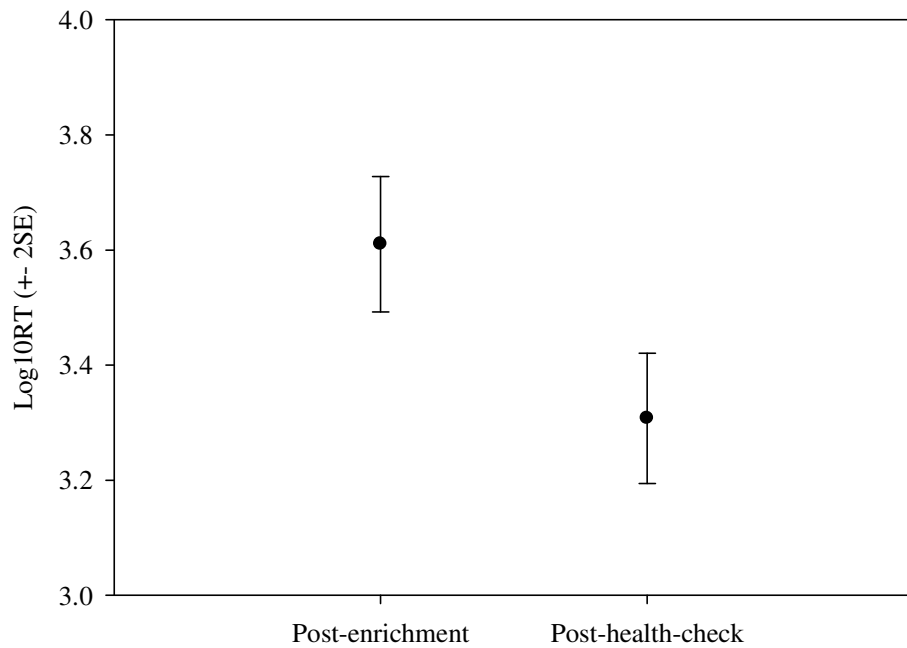
ID	Post-enrichment			Post-health-check		
	Left	Central	Right	Left	Central	Right
C55	1.0	1.0	1.0	1.0	1.0	1.0
29C	1.0	1.0	1.0	1.0	1.0	1.0
16P	1.0	1.0	1.0	1.0	1.0	1.0
AI73	0.9	0.9	0.9	1.0	0.9	1.0
92R	1.0	1.0	1.0	1.0	1.0	1.0
27S	0.9	1.0	1.0	1.0	0.9	1.0
66S	0.9	1.0	0.8	1.0	1.0	1.0
Group \bar{X}	0.96 (± 0.02)	0.99 (± 0.01)	0.96 (± 0.03)	1.00 (± 0.00)	0.98 (± 0.01)	1.00 (± 0.00)

b) Latency to respond

A 2 x 3 RMANOVA was conducted to test the hypothesis that stress-related arousal improves task performance (enhances speed to respond) when non-emotional control stimuli are shown (hypothesis 1, prediction b). Data were $\text{Log}_{10}\text{RTs}$ on control trials, with within subjects factors of testing condition (Post-enrichment versus Post-health-check) and screen location (left, central and right). There was a main effect of testing condition ($F_{1,6} = 17.611$, $P=0.006$). Monkeys were faster to

respond to non-emotional control stimuli Post-health-check than Post-enrichment (Figure 5.8). There was no main effect of screen location ($F_{2,12} = 0.307$, $P=0.741$), and no interaction of testing condition x screen location ($F_{2,12} = 1.561$, $P=0.250$). This suggests there were no side biases in speed to respond. To give an indication of the range of response latencies, median untransformed RTs are shown in Table 5-5.

Figure 5.8 Log₁₀RT for control trials Post-enrichment and Post-health-check



In summary, monkeys were faster to respond to non-emotional control stimuli Post-health-check than they were Post-enrichment. This supports the prediction (hypothesis 1, prediction b) that stress-related arousal may speed responses to non-

emotional stimuli (see Figure 5.8). There was no difference in frequency of responses Post-enrichment versus Post-health-check (hypothesis 1, prediction a).

These data suggest that stress-related arousal speeds baseline performance but does not affect tendency to respond. Any latency effects on experimental trials are therefore unlikely to be influenced by a speed-accuracy trade-off. Equivalence in frequency to respond may reflect a ceiling effect afforded by the long trial durations. There were no side biases in either frequency or speed of responding. As such, any differences in responses to stimuli at the three screen locations in the subsequent analyses are considered to be a reliable indicator of visual field preferences for emotional stimulus content.

Table 5-5 Median latencies (msecs) to respond to stimuli

ID	Post-health-check			Post-enrichment		
	Control	Direct Gaze	Averted Gaze	Control	Direct Gaze	Averted Gaze
29C	2803	2470	3031	8327	4000	6202
C55	1409	5800	3513	4121	3876	3518
16P	874	1313	1399	1817	1910	2195
AI73	6973	10113	3147	16953	4927	13080
92R	2513	1180	2153	2447	1500	2160
27S	2607	1887	2120	4073	2593	5880
66S	1144	1237	1303	5901	1946	3543
\bar{X}	2618	3429	2381	6234	2964	5225

5.5.2. Does emotional distractor content impair task performance, and is this mediated by affective state? Experimental trials (Direct gaze and Averted gaze faces)

Hypotheses 2 and 3 (that emotional distractor content impairs task performance, and this may be mediated by affective state) were tested by running a higher-order RMANOVA separately for each data set (frequency and ratio latency data respectively). Specific predictions were tested with subsequent analyses.

To test the effects of emotional distractor content and affective state on frequency of responses on experimental trials (hypotheses 2 and 3, prediction a in both cases), proportion data were entered into a 2 x 2 x 3 RMANOVA. The within subjects factors were testing condition (Post-enrichment versus Post-health-check), trial type (direct gaze and averted gaze) and visual field (LVF, CVF, RVF). There were no main effects of testing condition ($F_{1,6} = 2.006$, $P=0.206$), trial ($F_{1,6} = 0.607$, $P=0.561$), nor visual field ($F_{2,12} = 0.538$, $P=0.598$). There were no interactions (all $P_s > 0.3$). There was therefore no support for either of predictions 2a or 3a.

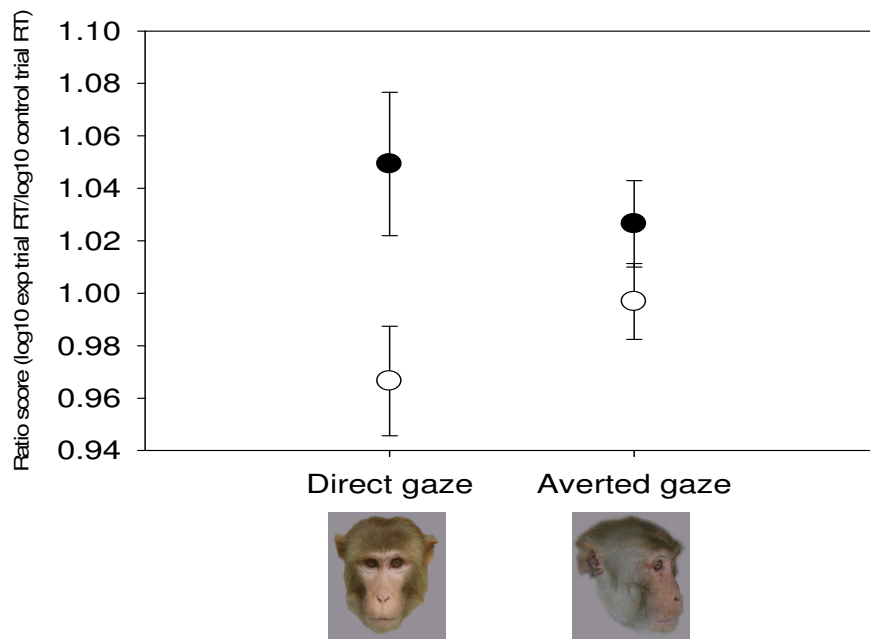
A 2 x 2 x 3 RMANOVA was performed to determine whether emotional distractor content and affective state influence latency to respond to emotional stimuli (hypotheses 2 and 3, prediction b in both cases, and hypothesis 3, prediction c). Data were ratio scores, with within-subjects factors of testing condition (Post-enrichment versus Post-health-check), trial type (direct gaze and averted gaze) and visual field (LVF, CVF and RVF). There was a significant effect of testing

condition ($F_{1,6} = 11.749$, $P=0.01$). Ratio scores were greater than 1 Post-health-check, suggesting slowing of responses on experimental trials relative to control trials, and were less than 1 Post-enrichment, suggesting speeding of responses on experimental trials relative to control trials. This supports hypothesis 3, prediction b, which states that monkeys will be slower to respond on trials when emotional distractor content is present Post-health-check than Post-enrichment. There was a significant interaction between testing condition and trial type ($F_{1,6}=6.68$, $P=0.04$; Fig 5.9). There were no other main effects (trial: $F_{1,6} =1.79$, $P=0.23$; VF: $F_{2,12}=0.25$, $P=0.78$) nor other interactions (all $P_s >0.40$). The absence of a main effect for trial type provides a lack of evidence for Hypothesis 2, prediction b, which states that monkeys should be slower to respond on trials with direct gaze faces than on trials with averted faces.

To examine the two-way interaction in more detail, and to test hypothesis 3, prediction c (that slowing of responses to direct gaze faces should be enhanced Post-health-check versus Post-enrichment), data were collapsed for visual field, and planned pairwise comparisons were conducted. These compared ratio scores Post-enrichment versus Post-health-check, separately for each of direct-gaze and averted-gaze trials. For direct gaze trials shown Post-health-check, ratio scores were above 1. This indicates monkeys were slower to respond on direct gaze experimental trials relative to control trials Post-health-check. Conversely, for direct gaze trials shown Post-enrichment, ratio scores were below 1. This indicates monkeys were faster to respond on direct gaze experimental trials relative to control trials Post-enrichment. A paired samples t-test (Bonferroni

adjusted $P=0.025$), with within-subjects factor testing condition (Post-enrichment versus Post-health-check) revealed a significant difference in the ratio scores for direct-gaze trials between the two treatments ($t(6)=3.41$, $P=0.01$; Figure 5.9). This is in partial support of Hypothesis 3, prediction c. There was no effect of treatment condition on ratio scores for averted gaze experimental trials ($t(6)=1.82$, $P=0.12$), also in partial support of hypothesis 3c (that slowing effects should be enhanced for direct gaze faces relative to averted gaze faces).

Figure 5.9 Ratio scores for latency to respond on experimental trials with direct gaze and averted gaze emotional distractor content. ● = Post-health-check; ○ = Post-enrichment



In summary, ratio scores revealed trends towards slowing of responses to direct gaze faces (relative to control trials) Post-health-check, and a speeding of responses to averted gaze faces (relative to control trials) Post-enrichment. This bidirectional effect (relative slowing Post-health-check versus relative speeding Post-enrichment) resulted in a significant difference between the ratio scores for monkeys responding to direct gaze faces Post-health-check versus Post-enrichment (but not for averted gaze faces). These data suggest emotional distractor content and affective state interact to influence latency to respond on a manual task when emotional distractor content is introduced. There were no visual field effects, nor were there effects on proportion of responses made.

5.5.3. Additional Post-hoc analyses

Two sets of post-hoc analyses were conducted to check for possible priming effects of previous trial shown, and to test possible effects of individual trait characteristics (i.e. approach motivation as measured by aggressiveness) on results.

a) Priming effects of previous trial

A 3 x 2 x 3 RMANOVA was performed to examine whether the stimulus presented on the previous trial influenced latency to respond on the current trial. Data were log₁₀RT, with within-subjects factors current trial (control, direct gaze, averted gaze), testing condition (Post-enrichment and Post-health-check) and previous trial (control, direct gaze, averted gaze). There was no main effect of previous trial

on response latency on the current trial ($F_{2,8}=2.435$, $P=0.149$), nor were there any interactions (all $P_s>0.5$).

In summary, the stimulus shown on the previous trial did not affect response latency on the current trial. This suggests that the variable ITT of 8080ms was sufficient to negate inter-trial priming effects.

b) Trait approach motivation

A bivariate correlation was conducted to test the effects of trait approach motivation on response-slowing to threatening faces. This analysis was conducted following evidence from humans that high approach motivation (which is associated with characteristics such as dominance, higher basal testosterone levels, and heightened BAS activation) slows responses to threatening faces (e.g. Passamonti et al., 2008). For each monkey, an approach motivation score was calculated as the sum of aggression scores from the habituation data reported in Chapter 2, for days 1-9 and 20, 30 and 40 of the habituation phase. Therefore, each monkey had a BAS motivation score on a scale of 0-12, with 0 indicating low approach motivation (no aggressive responses towards the investigator on approach to the cage on any of the 12 days) and 12 indicating high approach motivation (predominantly aggressive responses on each of the 12 days). Data were latency ratio scores ($\log_{10}RT$ experimental trials / $\log_{10}RT$ control trials) separately for the Post-enrichment and Post-health-check conditions. There was no correlation between approach motivation and ratio response latencies for direct

gaze trials either Post-enrichment ($r(7) = -0.167$, $P=0.36$) or Post-health-check ($r(7)=-0.052$, $P=0.456$).

In summary, these data suggest that approach motivation, as measured by tendency to act aggressively towards a human on approach to the home cage, did not correlate with response-slowing to direct gaze faces in this study.

5.6. Discussion

Seven monkeys learned an operant task during which they were required to touch a non-emotional control stimulus (a grey square) presented on a touch-sensitive monitor, in order to gain pellet rewards. The monkeys were then presented with the same task with the addition of experimental trials in which emotional distractor content was included (conspecific faces with either direct or averted gaze) during two testing conditions: Post-enrichment and Post-health-check. Frequency and latency of responses were recorded by the computer. Arousal affected baseline response speed (control trials). Affective state (as inferred by the Post-enrichment and Post-health-check treatment conditions) and emotional distractor content (direct gaze or averted gaze) influenced latency to respond on experimental trials. There were no effects for frequency of responses. The experimental paradigm therefore provided a reliable means of measuring the effects of inferred affective state, independent of arousal, on speed to respond on a non-emotional task when emotional distractor content is introduced.

The latency data presented here concur with evidence from humans that stress-related arousal may speed performance on non-emotional tasks (e.g. Ozel et al., 2004). I first predicted that increased arousal would lead to faster performance on control trials Post-health-check compared with Post-enrichment (hypothesis 1, prediction b). Data from control trials revealed that monkeys were, as predicted, faster to perform on control trials Post-health-check than Post-enrichment. This finding highlights the necessity of controlling for arousal when comparing between treatments.

To remove the effects of arousal-related speeding of responses between the two treatments, ratio scores were calculated for experimental trials. For each monkey, latency to respond on experimental trials was divided by latency to respond on control trials, for each treatment, separately. This is equivalent to the process of calculating ratio or difference scores with human participants (Bar-Haim et al., 2007; Mauer & Borke, 2007; Beall & Herbert, 2008). Ratio scores were chosen as preferable to difference scores (where subtraction is used instead of division) due to the small sample size and variability between monkeys in response latencies. Studies with humans use larger sample sizes and participants are instructed to respond as quickly as possible which reduces variability in latencies between individuals.

Ratio scores were used to test the predictions for hypotheses 2 and 3 regarding the effect of emotional distractor content and affective state on task performance. These predictions were formulated following evidence from humans that highly

salient or threatening faces slow performance to a greater extent than low salience or non-threatening faces and this slowing in task performance is enhanced among anxious individuals (e.g. Eastwood et al., 2003; Algom et al., 2004; Bar-Haim et al., 2007; Mauer & Borkenau, 2007; Mogg et al., 2008). Ratio data revealed a significant interaction of testing condition and trial type. Monkeys were relatively slower to respond to experimental trials (i.e. those with emotional distractor content) Post-health-check versus Post-enrichment, and this was enhanced for the more highly salient, threatening faces (direct gaze), but not for the less salient non-threatening faces (averted gaze).

The implications of the data for existing models of the influence of affective state on the processing of, and responses to, emotional information are given in Table 5-6. The data are consistent with findings from human studies which indicate that affective state modulates the extent to which non-task-relevant emotional information may capture attention and impair an individual's performance on an ongoing non-emotional task (Mogg et al., 2008). The data presented here provide the first evidence for emotion-mediated emotion-evaluation and response-slowing effects in a non-human. Monkeys were relatively slower to respond to direct gaze faces Post-health-check, and were relatively faster to respond to the same faces, Post-enrichment. This finding suggests direct gaze distractors capture attention and slow task performance when monkeys are in an increased state of stress. A non-significant trend for relatively slower responses to averted gaze faces Post-health-check versus Post-enrichment was also evident. This suggests that response-slowing effects are enhanced for more highly salient threatening (direct-

Table 5-6 Implications of the main findings for existing models of the influence of affective state on emotional information processing.

Model	Information processing models	Attentional control models	Reinforcement sensitivity models
Key aspects of model	<p>Competition between two types of information leads to slowing in the processing of one of them. ‘Relative speed of processing’ (RSpP): threatening information travels through the brain faster than non-threatening information. ‘Relative strength of processing’ (RStP): strengthened pathways for threatening information mean it is processed preferentially over non-threatening information.</p>	<p>Onset of a highly salient or threatening stimulus acts as a circuit-breaker which inhibits ongoing goal-directed processes. This allows orientation of attentional resources towards the threatening stimulus, revealed in a slowing in ongoing task performance.</p>	<p>Competition between three motivational systems sensitive to positive and negative reinforcers leads to approach (BAS) withdrawal (FFFS) and resolution of approach-withdrawal (and possibly approach-approach and withdrawal-withdrawal) conflict of response (BIS).</p>
Support from current research?	<p>✗ RSpP: Monkeys exhibited both slowing and speeding of responses on DG trials relative to baseline.</p> <p>? RStP: short-term strengthening of pathways for threatening information following a stressor may be revealed by relative slowing of responses on DG trials PHC v PE (Hypothesis 3b+c).</p>	<p>✓ Relative slowing of responses on experimental trials PHC v PE support a possible trade-off of bottom-up stimulus-bound and top-down goal-directed processes, modulated by stimulus saliency (DG = high, AG = low), arousal and motivational factors.</p>	<p>✓ BAS: motivation to gain pellet reward revealed in maintained task performance</p> <p>✓ FFFS and BIS: slowing of responses on DG trials versus AG trials, which is enhanced PHC, may be revealed by fear-related defense/withdrawal (FFFS, e.g. freezing), or anxiety-related approach-withdrawal conflict and ambiguity resolution (BAS-FFFS conflict with resolution by the BIS).</p>

PHC: Post-health-check; PE: Post-enrichment; DG: Direct Gaze experimental trials; AG: Averted gaze experimental trials
 BAS: Behavioural Approach System; FFFS: Fear-Flight-Fight System; BIS: Behavioural Inhibition System

gaze) versus less salient or non-threatening (averted gaze) faces. These findings are in line with data from humans.

Mogg et al. (2008) tested the response-slowing effects of threatening and non-threatening faces using a manual spatial-cueing task with humans. They found a response-slowing effect of threatening faces for high (state/trait) anxious individuals, but not low (state/trait) anxious individuals. These data are in accordance with the baseline-corrected data presented here for monkeys responding on direct gaze (threatening) trials Post-health-check versus Post-enrichment. The current data therefore support recent data from humans for response-slowing to threatening stimuli mediated by anxiety state. Mogg et al. (2008) emphasize the implications of response-slowing effects for the interpretation of attentional bias data from humans. Failure to consider response-slowing to arousing or threatening stimuli may lead to misinterpretation of attentional processes where single stimuli that vary in valence are shown on each trial. An important output of the current study is therefore the knowledge that response-slowing to emotional stimuli occurs in rhesus macaques, and this needs to be considered in the design of future studies that use emotional stimuli with primates.

Mogg et al. (2008; see also Algom et al., 2004) discuss the response-slowing effects on threatening trials in terms of a freezing response. This is consistent with Gray's (1981) reinforcement sensitivity model which suggests that threat should have a slowing effect on responses (freezing), and empirical data which highlight

the generic slowdown effect of threat on motor responses (Algom et al., 2004). The current results therefore lend support for attentional control and reinforcement sensitivity models of the influence of affective state and emotional distractor content on the processing of emotional information.

The current experimental paradigm does not allow differentiation of the underlying mechanisms. For example, relative slowing of responses to threatening stimuli Post-health-check may be due to circuit-breaking, freezing, flight or BIS activation, or any combination of these. However, the current study does provide the first demonstration that experimental paradigms currently being used with humans to investigate these processes may be adapted for use with rhesus macaques. Further, the current results suggest the data from such studies will be comparable to those from humans which is useful, both for furthering our understanding of the evolution of the psychological components of wellbeing, and for informing the adaptation of human psychological tools for use with other species.

The data lend little support to information processing models, due to the differential pattern of responses to direct gaze faces, namely slowing Post-health-check versus speeding Post-enrichment. This differential pattern of responding suggests the results cannot be attributed to relative speed of processing threatening information (RSpP) *per se*, since responses to direct gaze faces were slower Post-health-check. Interpretation of the current results in terms of relative strength of information processing models (RStP) is problematic since the current

study does not strictly use a Stroop paradigm, and therefore does not directly test interference in the parallel processing of two competing types of information. However, the finding that responses to threatening stimuli were faster Post-enrichment, and slower Post-health-check, is highly suggestive that factors other than strength of processing effects alone account for the difference in latencies to respond.

The current study makes an important contribution to existing knowledge in several ways. Firstly, it identifies the importance of controlling for arousal effects in cognitive studies of monkeys where manipulation of affective state and reaction time data are used. Secondly, it provides the first evidence for emotion-evaluation-related response-slowness in a non-human species, a particularly important methodological consideration in the design of future studies in which stimuli of different arousal value are shown on separate trials. Thirdly, this study presents the first data on the mediating role of affective state on impairment on an ongoing task in the presence of emotional distractor content in rhesus macaques. Fourthly, these affective state-mediated effects are in line with data from humans, suggesting the possibility of some homology in the underlying mechanisms. Finally, taken together, these points provide a strong argument for the further development of methods to investigate the cognitive component of emotion in non-human species, and demonstrate the possible future utility of such an approach for measuring psychological wellbeing in other species.

The experimental design presented here improves on existing methods used with humans. The use of a within-subjects design removes the potentially confounding factors associated with comparing between groups, especially where different patient populations are involved (Bar-Haim et al., 2007). The majority of studies conducted with humans use a between-subjects design. A few studies of the therapeutic benefits of cognitive training use a pre- and post- training measure of cognitive bias (typically a Stroop-like task: Mogg et al., 1995; Nay et al., 2004). A particular issue with human studies is the difference in baseline motor response latencies between such groups (which is typically measured using responses to neutral face stimuli, and may therefore in itself be confounded by emotional relevance of neutral faces). While the mechanisms underlying differences in generic speed of responding, and the direction and size of the effect, may vary with task and group, the effects are often pervasive and pose a problem for drawing meaningful between-group comparisons (as highlighted by Algom et al., 2004).

Data presented here revealed a generic speeding of responses for control trials Post-health-check. The within-subjects design identifies that this difference in generic responses is tied to changes in state characteristics; the speeding of the response, together with the cortisol data presented in Chapter 3, suggest the speeding is likely to be tied to increased arousal in monkeys Post-health-check relative to Post-enrichment. The use of ratio scores in the present study, which control for this arousal-related speeding removed the effects of generic condition-related arousal, so that any remaining changes in response latency must be due to

other factors related to the processing of and motor responses to emotional distractor content.

There are several ways in which the current paradigm may be improved. It is beneficial for all monkeys, when confronted with a threatening face, to identify the face as threatening. It is therefore likely that the direct-gaze face was perceived as threatening (at a categorical level) in both treatments. The finding that monkeys showed a relative speeding of responses to direct gaze faces Post-enrichment may reflect an aspect of the design, whereby touching the stimulus ended the trial and the stimulus was removed from the screen. The trend towards speeding of responses in this case may reflect animals' learning of this fact (termination of a negative reinforcer functions as a positive reinforcer: Rolls, 2000; McNaughton & Corr, 2004). Lower physiological arousal levels (see Chapter 3), and possibly reduced stimulus-bound arousal/freezing effects (Algom et al., 2004; Mogg et al., 2008), may result in preferential activation of approach mechanisms, such as BAS, resulting ultimately in a form of pro-active coping strategy.

To untangle the possibly confounding effects of the response-dependent stimulus-offset, a design with shorter trial durations, and a not-response-dependent stimulus offset, is required. For example, Mogg et al. (2000a) discuss the discrepancy in results in their study comparable with those of a previous study by Dawkins & Furnham (1989), both of which used an emotional Stroop paradigm with emotionally valenced words. Mogg et al. (2000a) suggest the speeding of

responses on threatening trials in their own study may have arisen due to the fact that a direct effect of making a fast response was the early offset of the threatening stimulus from the screen. Thus, an avoidant attentional style, may be evidenced by slower reaction times in a study where response has no effect on stimulus presentation time, yet may result in a speeding of response in a study where responding reduces the stimulus presentation time. In the current study touching the stimulus resulted in its removal from the screen. Therefore, over time, monkeys may have learned to touch the threatening stimulus in order to remove it from view, therefore 'avoiding' further exposure to it. Additionally, designs that incorporate face stimuli morphed for intensity and valence of facial expression (e.g. neutral–angry, or happy–angry) would allow us to explore the influence of affective state on sensitivity to emotional distractors of different salencies (e.g. Richards et al., Unpublished data).

The possible future directions for this approach are many. For example, the effects of emotional distractors on attention may be investigated further using a more faithful replication of the eStroop task. The influence of emotional distractors on an ongoing cognitive task (one more cognitively challenging than the simple 'go' response used here: e.g. Go-NoGo or match-to-sample) would provide data which would be more directly comparable with data from human studies.

In line with recent developments in the field, inclusion of a pressure-sensitive lever that requires responses by moving the lever either towards or away from stimuli, would allow a direct measure of approach and withdrawal tendencies, and

conflict between them (after Rotteveel & Phaf, 2004). The use of apparatus that directly tests motor approach and avoidance tendencies to affective stimuli is relatively new in the human literature. However, it has provided what some argue to be a direct measure and link between evaluative processes and motor responses in terms of approach-withdrawal models (Heuer et al., 2007; Roelofs et al., 2007 and 2009). Published studies reveal rhesus macaques may be trained successfully to use a joystick for computer-based cognitive studies (e.g. Parr & Heintz, 2009).

In summary, the findings reported in this chapter are important for several reasons. Firstly, they represent the first data on emotion-evaluation and response-slowing in a species of primate. Secondly, these data concur with recent data from humans. This suggests that monkeys and humans exhibit similar patterns of attentional and behavioural responses to emotional distractors, and that these responses are similarly mediated by affective state. This, in turn, suggests that these attentional systems and their corresponding behavioural outcomes may involve evolutionarily old processing pathways in the primate brain. Finally, given the implications of emotion-evaluation studies for understanding the cognitive component of emotion and psychological wellbeing in humans, such approaches may further our understanding of the mechanisms underlying psychological wellbeing in other species. Given the comparability of the results to findings from humans, the current data provide a strong case for the further development of this experimental paradigm to provide a measure of psychological wellbeing in primates.

Chapter 6

Judgement bias

Judgement bias is a bias in the assessment of the positive or negative significance of information that is otherwise ambiguous in nature (Eysenck et al., 1991). Studies in humans have shown that affective state influences judgement bias (e.g. Richards et al., 2002 and 2007). People high in anxiety, for example, demonstrate a bias to interpret ambiguous information as more negative than do people low in anxiety (Richards et al., 2007). This bias in the appraisal of the threatening value of objects or events has been implicated in the onset and maintenance of anxiety disorders and therefore may lead to reduced psychological wellbeing (Mathews & MacLeod, 2002). Negative judgement bias in humans has been shown to be a reliable predictor of experienced distress during stressful life events (Pury, 2002).

Recent work with rats (Harding et al., 2004) and starlings (Bateson & Matheson, 2007) has demonstrated that similar affect-mediated biases in information processing are evident in animals. The aim of the current chapter is to investigate whether the patterns of judgement bias evidenced in humans, rats and starlings are also evident in a primate, the rhesus macaque.

In this chapter I review existing research on judgement bias in humans and other animals (Part A), and present data from the first study to investigate judgement bias in a species of primate (Part B). Part A is divided into three sections. In the first of these I discuss the theory and background for judgement bias research conducted with humans. This focuses on the development of theories and models

that account for affect-mediated biases in the judgements made about ambiguous information. In the second section I review the recent development of methods to study judgement bias in animals. In the third section I discuss the implications of human and animal-based research for the development of a novel method to study judgement bias in primates. I conclude this section with the main aims and alternative hypotheses that informed the design of the present study.

The present study is detailed in Part B, which is also divided into three sections. In the first of these I describe the method I developed. In the second section I present the first data on judgement bias in a species of primate. In the third section I discuss the results of the study in light of the available data from humans and other animals.

Part A: Literature Review

6.1. Introduction

6.1.1. Judgement bias in humans: theory and background

Judgement bias is a general term that describes a bias in the processing of, and response to, a stimulus. It incorporates cognitive (attention, appraisal and action selection) and behavioural (motor output) components. Work with humans has shown that people's judgements about a given stimulus or scenario are influenced by affective state. For example, when asked about future expectations anxious people report a greater expectation of negative future events than do non-anxious

controls, while anxious people who also suffer from depression additionally demonstrate a reduced expectation of future positive events (MacLeod & Byrne, 1996; Garner et al., 2006a). When asked *a posteriori* to report the frequency with which angry, happy and neutral faces were shown during a cognitive task, socially anxious people reported that angry faces occurred more often in a series of faces than did non-socially anxious people (Garner et al., 2006a). Because judgement bias is a general term which describes the outcome of a range of underlying mechanisms, I will focus on the types of bias that are subsumed under the term, and then discuss the methods used to investigate them. The list is not exhaustive (for example memory bias is not included, but may equally feed into judgements about stimuli), but focuses on those aspects which are of greatest relevance to the current study.

Expectancy bias is generally measured as an *a priori* expectation of positive or negative events occurring in the future (e.g. MacLeod & Byrne, 1996). Expectancy biases are measured in humans using personal-future tasks and questionnaires. Personal-future tasks have demonstrated an increased expectation of negative future events in anxious people compared with non-anxious controls (MacLeod & Byrne, 1996), and in depressed people versus non-depressed controls (Strunk & Adler, 2009).

Evidence for a specific role for affect in the mediation of expectancy biases is inconclusive and varies with experimental design. Studies using biologically-relevant stimuli (e.g. angry faces or snakes and spiders) show that both anxious and non-anxious, or phobic and non-phobic, individuals show an *a priori*

expectancy bias for a selective association between negative stimuli and aversive outcomes (e.g. an aversive picture or a shock: Tomarken et al., 1989; Garner et al., 2006a). Therefore the expectancy bias for selective associations between fear-relevant stimuli and negative outcomes may operate at a level of automaticity which is generally unaffected by affective state. *A priori* expectations may be mediated by the relevance of the unconditioned stimulus (UCS) to the conditioned stimulus (CS). Selective associations are more readily assumed between predatory fear-relevant animals and a pain-relevant UCS (e.g. shock), and non-predatory fear-relevant animals with a disgust-relevant UCS respectively (Davey et al., 2003). During cognitive tasks, ‘on-line’ expectancy biases (expectancies reported on a trial-by-trial basis) have been shown to change over trials, demonstrating that expectancy biases may vary dynamically with (learning) context. Both socially anxious and non-socially-anxious people with an initial expectancy bias for a negative UCS (aversive pictures) following presentation of angry faces, showed no such expectancy bias during later stages of an illusory correlation task (described below), when asked to report expected outcomes on a trial-by-trial basis (Garner et al., 2006a).

Covariation bias is a bias in the *a posteriori* covariation estimate of the occurrence of negative outcomes following a given stimulus. Tomarken et al. (1989) developed the illusory correlation paradigm during which fear-relevant and non-fear-relevant stimuli were presented, each equally likely to be followed by an aversive UCS (shock). Spider phobics over-estimated the occurrence of a shock following the presentation of slides showing pictures of spiders, while non-phobics did not. The spider phobic group demonstrated a skew in their associative

learning that did not reflect true task contingencies. Garner et al. (2006a) reported no difference in selective associations developed by anxious and non-anxious individuals when viewing emotional faces (CS) and aversive pictures (UCS). These differential findings may reflect the nature of the CS and UCS used in each study. However, Garner et al. (2006a) did find that socially anxious people reported retrospectively a greater frequency of negative social cues (angry faces) to have been presented than did non socially anxious individuals. While illusory-correlation paradigms have only been used with humans, it is likely they tap into the same mechanisms that underlie the tendency to develop selective associations between biologically-fear-relevant stimuli and aversive outcomes demonstrated during conditioning studies conducted with rhesus macaques (Cook et al., 1985; Cook & Mineka, 1989).

Interpretive bias is a bias in the appraisal of a stimulus as being positive or negative. In studies with humans, interpretive biases are studied using biologically-relevant stimuli, including valenced, ambiguous and neutral words and scenarios (e.g. Eysenck et al., 1991; Richards & French, 1992). A recent trend in research has been to compare valence ratings of faces morphed between negative and positive expressions (e.g. Blanchette et al., 2007). Results from such studies point to an increase in the negative interpretation of these ambiguous faces with increased anxiety. Blanchette et al. (2007) presented pictures of morphed facial expressions to one group of participants under a mock filming procedure (to induce high state anxiety) and to a control group, under no filming procedure (to encourage low state anxiety). Face morphs were presented on a screen simultaneously with contextual cues comprising positive, negative or neutral

words. Participants were asked to judge faces as negative or positive. High-state-anxious individuals were influenced to a greater extent by contextual cues when rating the valence of ambiguous faces than were the low-state-anxious group, despite the fact there was no overall bias among the high anxious group to interpret faces as negative. In a second study, high-trait-anxious and low-trait-anxious participants were shown face morphs against positive and negative background pictures. High-trait-anxious participants rated morphed faces as negative significantly more often than did low-trait-anxious individuals, and this effect was independent of context. This study suggests an increase in trait anxiety leads people to make more negative judgements about ambiguous information (a negative interpretive bias) while an increase in state anxiety leads people to recruit additional sources of information to help resolve the ambiguity in meaning. The application of training-induced interpretive biases for the treatment of anxiety disorders, demonstrates the bidirectional (cognitive-affective) nature of this bias in information processing (Mathews & MacLeod, 2002). However, whether training outcomes are due to changes in perceptual sensitivity or induced response bias is unclear (e.g. Allan et al., 2007).

In summary, there is range of processing biases that feed into judgement biases in humans. These reflect different underlying mechanisms such as anticipation (expectancy bias), appraisal (interpretive bias) and associative learning and selective association (covariation bias). Next I present a review of the methods used to measure judgement biases in humans and then review the initial work that has been conducted with animals before discussing the implications of these for the current study.

6.1.2. Methods for studying judgement bias in humans

The appropriate method for measuring judgement bias is determined by the particular aspect that is of interest (prior expectancy bias, ongoing ‘online’¹ interpretive bias and online and *a posteriori* covariation bias). These are presented here in order to place the animal studies discussed later in the context of the longer-established field of study with humans, and also to highlight the utility of some approaches for the development of the methods presented here.

6.1.2.1. Expectancy bias: ‘thought’ tasks

Expectancy biases are typically measured in humans using the thought experiment. During the thought experiment participants are asked to report what they think should happen given a particular experimental scenario. Davey et al. (2003) conducted a thought experiment to examine people’s expectation of a fear-relevant UCS (shock) and a disgust-relevant UCS (vomit-inducing drink) occurring after images of fear-relevant and disease-relevant animal pictures. Overall, participants had an increased expectation of shock occurring after pictures of fear-relevant animals and of bad tasting juice being administered after pictures of disease-relevant animals. These results suggest a survival function of expectancy biases when applied to threatening biologically-relevant stimuli. Evidence from depressed people suggests that depressed mood is associated with a more pessimistic expectation of future events, including social evaluation

¹ Online biases are those that are measured on each trial, and may change on a trial-by-trial basis

(Strunk & Adler, 2009). Expectancy ratings may also be obtained from participants at the start of conditioning tasks simply by asking participants to rate the likelihood of each UCS occurring after each type of CS prior to exposure (e.g. Kennedy et al., 1997; Garner et al., 2006a).

Garner et al. (2006a) measured online as well as *a priori* expectancy ratings. They asked socially anxious and non-socially anxious participants to indicate whether they expected a pleasant, unpleasant, neutral or no outcome to follow a picture of an emotional face on a trial-by-trial basis. This method allowed the authors to monitor changes in expectancy of outcomes over the course of the experiment. It is mentioned here because it is, in part, analogous to the Go-NoGo responses that have been adopted in the animal studies presented later. Broadly, where correct 'Go' responses indicate the expectation of a reward following a positively reinforced conditioned stimulus (CS+), they also signify the expectation of a reward following an ambiguous cue. Similarly, where correct 'NoGo' responses indicate no expectation of reward following a negatively reinforced conditioned stimulus (CS-), they also signify no expectation of reward following an ambiguous cue.

6.1.2.2. Covariation bias: Illusory correlation and contingency judgement paradigms

In the study mentioned previously, Garner et al. (2006a) applied an illusory correlation paradigm to measure high and low socially anxious individuals' covariation estimates between emotional faces (angry, happy and neutral) and

aversive, pleasant and neutral outcomes (pictures). On each trial participants were shown an emotional face, followed by a picture. Pictures were presented in a pseudorandomised manner so that each emotional face was succeeded by an equal number of aversive, pleasant and neutral pictures. At the end of the session participants were asked to report the percentage of trials on which each face was followed by each outcome. The high socially anxious group reported significantly more aversive outcomes following angry faces than did the low socially anxious group. The illusory correlation paradigm has also been used to examine phobics' covariation estimates of shock following fear-relevant stimuli (Kennedy et al., 1997). This study found a strong covariation bias in phobic individuals for shock following phobia-related slides.

The contingency judgement task is an operant version of the illusory correlation paradigm. It is used to measure people's perceptions of the level of control their actions exert over certain outcomes (Allan et al., 2007). Participants are presented with, for example, a button and a light. At the start of each trial the participant may choose whether or not to press the button. At the end of each trial the light illuminates at a set probability, irrespective of whether a button press occurred or not. At the end of the session participants are asked to report the level of control their button presses had on the light illuminating. Non-depressed individuals typically over-estimate their level of control over the light illuminating, while depressed people make more realistic contingency judgements. This phenomenon is known as 'depressive realism' (Alloy & Abramson, 1979). There is ongoing debate as to whether differences in contingency judgements reflect depressive realism versus nondepressive optimism (Alloy & Abramson, 1979; Dykman et al.,

1989) or depressive pessimism versus nondepressive realism (including processes such as negative response and judgement biases: Beck et al., 1987; Allan et al., 2007; Strunk & Adler, 2009).

6.1.2.3. Interpretive bias: Lexical decision and judgement tasks

Interpretive biases are measured using lexical decision and judgement tasks. During lexical decision tasks participants listen to homophones (e.g. die/dye or pain/pane) or homographs (e.g. growth) and are asked to report the meaning, either verbally or by writing down the spelling of the word (e.g. Eysenck et al., 1987; Mathews et al., 1989b; Rusting, 1999). Eysenck et al. (1987) found that the number of negative-meaning spellings for each homophone correlated significantly with trait anxiety scores. A later study (Eysenck et al., 1991) elaborated on the lexical decision task by presenting clinically-anxious, recovered clinically-anxious and non-anxious controls, with recordings of whole sentences which were ambiguous in meaning. Participants then underwent a recognition-memory test in which disambiguous variants of the test sentences were presented on a computer screen. Currently-anxious participants recognized more of the negative variants as sentences which they had heard earlier, while non-anxious controls and recovered-anxious individuals recognized more of the neutral variants as sentences which they had heard earlier. There are several variants of the lexical decision task, namely story and scenario completion tasks (e.g. Amir et al., 1998b; Rusting, 1999).

Judgement tasks require participants to make a positive or negative judgement of an ambiguous stimulus, typically a facial expression (Blanchette et al., 2007). For example, Richards et al. (2002) asked high- and low-trait anxious people to report verbally the emotion most prevalent in each of a series of morphed faces. High-trait-anxious individuals were more sensitive to fear in faces morphed between fear and another emotion than were low-trait-anxious individuals. Following a mood induction, high-state-anxious individuals were more sensitive to anger in morphed faces that contained this emotion than were low-state-anxious individuals.

Given the evidence reviewed in Chapter 5 for selective associations of threat-relevant stimuli with a negative UCS in rhesus macaques (Cook & Mineka, 1989), it is likely that positive stimuli may be equally selectively associated with a positive UCS. This has yet to be tested, but the use of such selective associations, learned task contingencies (e.g. Go/NoGo and match-to-sample paradigms), inclusion of ambiguous stimuli and measures or manipulations of affective state, may provide the tools for the development of a measure of judgement bias in primates.

6.1.2.4. Gaze measures (attentional, interpretive and expectancy bias)

Eye-tracking techniques have been used to measure differences in eye-fixations among people reading sentences predictive of negative or neutral outcomes. Calvo and Avero (2002) measured high and low trait anxious individuals' eye fixations when reading sentences predicting threatening or non-threatening events,

followed by sentences containing target words that represented either the predictable event or an inconsistent event. High anxious people were faster to read post-target words following a predicted threatening target than were low anxious people. Conversely, high anxious people were slower to read post-target words when a sentence predictive of threat was followed by a mismatched neutral target. Calvo and Avero (2002) interpret these results as reflecting selective prediction of threat in high anxiety which facilitates reading when subsequent information is congruent with predicted meaning, and interference of processing where subsequent information is incongruent with the predicted negative meaning. Since eye-gaze has only been used in reading tasks of judgement bias, it does not present a suitable measure for use with non-linguistic subjects.

6.1.2.5. Optimal discounting approaches

Optimal discounting approaches have been used to investigate the effects of contextual and motivational factors on judgements and decision making processes. These approaches do not explicitly measure affect-mediated cognitive bias, but they do focus on the role of environmental and internal factors on the assessment of the positive and negative values of stimuli, and consequences of different types of behaviour (Wilson & Daly, 2004). Wilson and Daly (2004) applied an optimal discounting approach to determine whether situational context affected men's propensity to discount future rewards over immediate rewards. Male participants first completed a discounting task during which they were asked to choose between a series of paired monetary rewards, for example \$20 to be paid tomorrow, versus \$50 to be paid up to 236 days later. From these choices individual discount parameters were calculated. The participants were then asked

to give attractiveness ratings for pictures of either attractive or unattractive women and then presented with a second discounting task. The change in individual discounting parameters between the two tasks revealed that men who rated attractive women (but not those who rated unattractive women) showed a significant change in discounting. Men who had rated attractive women were more likely to shift from choosing larger, delayed, rewards to choosing smaller, next-day, rewards. A similar, non-significant, trend was evident for females viewing male faces. Wilson and Daly (2004) implicate, but do not fully discuss, the role of each of arousal, motivation and affective systems in generating the pattern of results in their study. Studies such as this demonstrate the utility of optimal discounting approaches for tapping into decision-making and appraisal processes in the assessment of the positive or negative value of information. However, they have not been applied explicitly to the study of the influence of affective state on such processes.

The human literature demonstrates that different methods are used to tap into different components of judgement biases, and both state and trait anxiety effects have been demonstrated. Rusting (1999) used a positive and a negative mood manipulation (music and imagery techniques) to induce changes in state affect. The mood manipulations induced interpretive biases for homophones and story completion tasks in the predicted directions, which were enhanced in individuals with trait characteristics complimentary to the mood induction (see also Rusting, 1998; Richards et al., 2002). Therefore, state affect has been demonstrated to influence judgement biases in humans (although this may not necessarily be independent of trait characteristics) and may also, therefore, influence judgement

biases in primates. It is also noted that the absence of a state effect does not preclude the existence of trait effects. For example, Blanchette et al. (2007) did not find a state-anxiety related bias for rating morphed facial expressions but did find a trait-anxiety related interpretive bias for morphed faces.

6.1.3. Methods for studying judgement biases in animals

Emotion mediated judgement bias was first studied in animals by Harding et al. (2004), who measured the influence of affective state on cognitive bias for ambiguous tones in captive rats. Rats were trained on a Go-NoGo task during which they learned to press a lever on hearing a tone of particular frequency in order to receive a reward ('Go' response), and to refrain from pressing the same lever on hearing a different tone in order to avoid a punishing burst of white noise ('NoGo' response). Prior to testing, rats were housed either in predictable or unpredictable housing conditions which was assumed to result in high-stressed and low-stressed animals respectively. During testing rats underwent the same Go-NoGo task they had learnt previously with the addition that 'Go' and 'NoGo' trials were randomly interspersed with probe trials on which one of three ambiguous tones intermediate to the positive/'Go' and negative/'NoGo' tones was played. Frequency and latency to respond on ambiguous probe trials were recorded and compared between the rats in the two housing conditions. Rats in unpredictable housing were significantly slower to respond to the food tone and the ambiguous probe tone closest to it compared to rats housed in predictable housing. Rats housed in unpredictable housing also tended to respond less

frequently to the food tone and closest ambiguous tone compared with rats housed in predictable housing.

Harding et al. (2004) were the first group to present the development of a non-linguistic judgement bias task for use with animals. Since the publication of that paper additional studies have extended the method to investigate cognitive biases in rats (Burman et al., 2008a; Burman et al., 2009), dogs (Casey et al., 2008) and starlings (Bateson & Matheson, 2007; Matheson et al., 2008). In this section I review the development of the original method with specific reference to the implications for the development of a method for use with rhesus macaques.

6.1.3.1. The current state of judgement bias research with animals

Table 6-1 lists published studies that have addressed emotion mediated judgement biases in animals. Criteria for inclusion in the table were that each study has been published in a forum where full methods and results are available (i.e. peer-reviewed journals and completed theses, but not conference abstracts), and that each study compared responses to stimuli that were to some degree ambiguous, by groups or participants that differed in measures of affect. The table demonstrates that few studies have addressed judgement biases in animals, and of those, the majority have focused on judgement biases following the paradigm developed by Harding et al. (2004).

Table 6-1 is divided into three parts, to reflect the distinction I make between judgement bias, interpretive and expectancy bias. Most studies fall under the

general heading ‘judgement bias’. These studies measure the behavioural outcomes of judgement processes, but are difficult to interpret in terms of underlying mechanisms (i.e. do responses to ambiguous stimuli arise from biases in perceptual sensitivity, information acquisition, evaluation and interpretation, response tendencies, or other factors such as motivation, arousal, learning and memory effects).

Some additional animal studies have addressed particular biases that may feed into judgement bias, specifically ‘expectancy’ and ‘interpretive’ biases. These are given separate headings in Table 6-1. Studies of expectancy biases are identified as those which investigate the influence of inferred affective state on behaviours indicative of expectation of reward. These use responses to stimuli that animals have learned indicate reward, as a measure of expectation. Studies of interpretive biases are identified as those which use biologically relevant stimuli and which therefore are likely to tap into processes involved in stimulus encoding. These are not absolute categorizations, rather an attempt to indicate the different approaches and their utility in addressing the many aspects of cognitive bias discussed in this thesis. Two studies from this thesis are presented in the Table to identify where they fit with existing studies. The study presented in this chapter is listed under the heading Judgement bias, since this study was largely based on the original method developed by Harding et al. (2004). The study presented in Chapter 5 of this thesis is listed under the heading Interpretive bias. While that study did not address Interpretive bias directly, it is likely that evaluation of the emotional significance of the faces to the monkeys contributed to the difference in responses between the two treatments.

Table 6-1 Published studies that investigate the influence of affective states or traits on cognitive biases in animals. CS: conditioned stimulus. P+: probe most similar to CS+; Pi: probe equidistant from CS+ and CS-; P-: probe most similar to CS-

Species	Method	Cues	Response	Design	Affect manipulation	Bias found?	Paper
Judgement bias							
Rat (n=7)	Go+/NoGo- (gain food/ avoid noise)	Auditory CS (2 x tone frequency). 3 ambiguous (intermediate: AmbI) frequency tones	lever press (Prop, latency)	Between groups	Predictable housing (P) v unpredictable housing (U)	Yes. U rats slower to press lever to Go+ tone and P+	Harding, et al. (2004)
Rat (n=24)	Go+/NoGo- (gain food/ avoid no food)	Spatial CS (location of food/ non-food trays). 3 AmbI spatial locations	Run to location (latency)	Between groups	Enriched (E) v unenriched (U) housing	Yes. U rats slower to arrive at location of P-	Burman et al. (2008a)
Rat (n=24)	Go+/NoGo- (gain food/ avoid quinine soaked food)	Spatial CS (location of food/ non-food trays). 3 AmbI spatial locations	Run to location (latency)	Between groups	Change in light intensity from training. High-Low (HL), Low-High (LH) (HH and LL controls)	Yes. LH rats were significantly slower to approach all AmbI locations	Burman et al. (2009)

Go+/NoGo-: a task in which participants learned to respond with one action (e.g. press lever: 'Go') to gain a reward and to perform another action (e.g. don't press lever: 'NoGo') to avoid a punisher; AmbI: Ambiguous or Intermediate probe stimuli; CS: Conditioned stimulus; Prop: Proportion of responses; P+: Ambiguous probe closest to the rewarded stimulus; Pi: central probe; P-: Ambiguous probe closest to the non-rewarded stimulus.

Table 6-1 (Continued)

Species	Method	Cues	Response	Design	Affect manipulation	Bias found?	Paper
Judgement bias (Continued)							
Starling (n=6)	Active choice (gain immediate food/ gain delayed food)	Visual CS (light of 2s or 10s duration). 7 AmbI light durations	Peck at one of two coloured lights	Within subjects and between groups	Enriched (E) v unenriched (U) housing	Yes. E starlings responded more frequently to AmbI probes than U starlings	Matheson et al. (2008)
Starling (n=8)	Go+/NoGo- (gain tasty food/ avoid aversive food)	Visual CS (shades of grey). 3 AmbI shades of grey	Flip lid (Prop)	Within subjects and between groups	Enriched (E) v unenriched (U) housing	Yes. U starlings flipped fewer P+ (but only when housed in E first)	Bateson and Matheson (2007)
Mouse (n=20)*	Conditioning: perfect and partial (ambiguous) predictors of foot-shock	Visual and auditory CS (paired light and tone)	Freezing	Within subjects and between groups/ strains	Genetic (serotonin receptor knockout: KO) v wild-caught (W)	Yes. KO mice had a greater freeze response to ambiguous shock predictors versus perfect predictors	Tsetsenis et al. (2007)
Dog (n=24)	Go+/NoGo- (gain food/ avoid no food)	Spatial CS (location of food/ non-food trays). 3 AmbI locations	Run to location (latency)	Ranked	Separation related behaviour score (SRB)	Yes. High SRB dogs ran more slowly to Pi	Casey et al., (2008)

Table 6-1 (Continued)

Species	Method	Cues	Response	Design	Affect manipulation	Bias found?	Paper
Judgement bias (Continued)							
Rat (n=7)	Go+/NoGo- All Go responses rewarded with water. All NoGo responses punished with white noise (either none, mild or loud).	Visual CS (x 3 lights, each light with its own lever)	Lever press	Ranked	Trait anxiety measured using elevated plus maze and open arena	None found. Problem with trait measures.	Osher (2006, unpubl. thesis)
Expectancy bias							
Rat (n=48)*	Conditioning (reward only). Increased delay until delivery of reward 10mins after cue.	Visual and auditory CS	Activity during CS-US interval	Between groups	Standard housing (U) v enriched housing (E) v control (C: CS and US presented, but not paired)	Yes. U rats show more anticipa-tory behaviour in CS-US interval compared to E or C	van der Harst et al. (2003)
Rat (n=24)	Serial Negative Contrast (SNC) technique (reward reduction)	12 pellets or 1 pellet in tray during training. 1 pellet only during testing	Run to food bowl (latency)	Between groups	Enriched (E) v unenriched (U) housing	Yes. U rats showed a more prolonged SNC response than E rats.	Burman et al. (2008b)

Table 6-1 (Continued)

Species	Method	Cues	Response	Design	Affect manipulation	Bias found?	Paper
Interpretive bias							
Starling (n=32)	Distance, and changes in behaviour in the presence of, naturalistic and ambiguous stimuli	Visual US. Blank card (positive by association with food), eyespots, and ambiguous eyespots	Latency to approach food bowl	Between groups (affect) within-subjects (US)	Playback of conspecific threat call; predator call; conspecific alarm call; white noise prior to recording behaviour	No. Possible problem with ambiguous eyespot stimuli (but not the auditory stimuli)	Brilot et al. (2009)
Chicken (n=117)	Record tonic immobility (TI) to manual restraint in the presence and absence of naturalistic stimuli	Eyespots; Eyespots covered; Conspecifics present	TI (duration and n inductions required)	Between groups	Foot-shock without escape (learned helpless group: LH); foot-shock with escape (E); no-shock control C	? LH required fewer TI inductions in the presence of eyespots than E or C	Rodd et al (1997)
Rhesus macaque (n=7)	Performance on a non-emotional task when an emotional distractor is introduced	Visual CS (grey square). Distractor direct gaze (DG) and averted gaze (AG) faces	Frequency and latency to touch stimulus	Within subjects	Post-enrichment (E) versus Post-health-check (U)	Yes. Speed of responses to DG faces (relative to baseline) slower U versus E.	Bethell et al (2007) See Chapter 5

* In these studies additional animals were used as control groups.

The methods used in the studies presented in Table 6-1 vary according to the bias of interest. Nearly all published studies of judgement bias have used a modified version of the Go/NoGo paradigm first used by Harding et al. (2004). This approach involves operant training on the initial Go/NoGo task prior to the presentation of the ambiguous probes. The judgement bias tasks differ in the training cues used (auditory, visual or spatial), the response (lever press, latency to approach/touch), manipulation of inferred affect (enriched versus unenriched or poor housing, genetic strains, psychopharmacological manipulations, trait characteristics, veterinary inspection, playing predator vocalisations), and pattern of bias seen (e.g. changes in responses to the probe most similar to the rewarded stimulus [P+] are seen in some studies, while others find a change in responses to the probe most similar to the unrewarded or punished stimulus [P-], or the intermediate probe [P_i]). However, the tasks all share a learning stage during which the positive associations (all studies except for Tsetsenis et al., 2007) and negative associations (all studies) of one or more conditioned stimuli (CS) are established, and a testing stage during which responses to ambiguous stimuli are measured. Overall, the range of species studied and the general agreement between findings, suggest that this paradigm provides a robust method for further development.

The method currently used to study expectancy bias in animals involves conditioning with no operant training. This approach requires less training than the judgement bias tasks. For example, van der Harst et al. (2003) presented rats who were housed in either standard or enriched caging with a simple conditioning procedure. A paired light and tone (CS) was followed by the delivery of a sucrose

reward (US). The CS-US interval was increased over 32 trials to 10 minutes, after which it was maintained at 10 minutes until a total of 42 trials had been completed. During the 10 minute time interval anticipatory activity was recorded (including exploratory, locomotory, foraging, freezing and self-directed behaviours). Three control groups were included. Two control groups were included to investigate activity in the presence of the CS alone (CS, no US) for each of enriched and standard housing, and to account for the effects of sucrose consumption (unpaired CS and US). Results showed that rats kept in standard housing showed more anticipatory behaviours during the CS-US interval than did rats in enriched housing. Control groups showed this difference was neither due to housing condition, nor to sucrose consumption, or arousal in general. The differences in anticipatory activities were therefore attributed to an interaction between housing condition and anticipation of a reward, although the underlying mechanisms, for example positive-affect-mediated sensitivity to expected reward loss (Nygren et al., 1996) or stress-mediated sensitivity to actual reward gain (e.g. Ambroggi et al., 2009), are not known.

In another study, which partially taps into expectancy processes, Burman et al. (2008a) tested rats' responses to reward loss. Rats were housed in enriched cages for 12 weeks, following which the enrichment was removed for half of the rats (U) and maintained for the other half (E). Half of the rats in each housing treatment were then trained to run down a runway for 12 pellets, and half for one pellet. Following training, rats running for 12 pellets ran faster than rats running for one pellet, irrespective of housing treatment, suggesting a greater reward value of 12 versus one pellet. The number of rewards was then reduced to one pellet for

all animals, and latency to run was measured again. Rats that had recently undergone reward loss ran significantly more slowly than those who had not undergone loss of reward. However, U rats ran slowly for a greater number of trials than E rats following loss of reward. A partial explanation for the slower run times may be that U rats had a lower expectation of reward value returning to its original level than did E rats (Burman et al., 2008b). Such differences in expectancy may be mediated by affective processes such as frustration or disappointment (e.g. Mason et al., 2001; Brosnan & de Waal, 2003) that feed into sensitivity to reward loss (Nygren et al., 1996).

Table 6-1 includes the subheading Interpretive bias. Studies with humans have focussed on the positive or negative meaning attributed to homophones or ambiguous but biologically relevant stimuli such as faces (e.g. Blanchette et al., 2007). The use of biologically relevant stimuli has been incorporated into studies with starlings and chickens. Brilot et al. (2009) recorded starlings' latency to approach a food bowl in the presence of positive (colour card associated with food), negative (predator eyespot) and ambiguous (partial eyespot) stimuli. Prior to testing an auditory stimulus was sounded in order to alter the starlings' affective state (a conspecific call, a predator call, or a burst of white noise). The presence of eyespots slowed starlings' latency to approach the bowl, as did predator alarm calls and white noise. However, there was no interaction between visual and auditory stimuli, nor were there any effects for the ambiguous eyespots. The study therefore failed to find the expected bias (which may be due to design features such as stimulus saliency effects or relationship between auditory and visual stimuli: Brilot et al., 2009). The study does, however, highlight the

potential for combining ambiguous naturalistic stimuli with manipulations of affect and proxy measures of positive and negative ‘interpretation’ of those stimuli.

An earlier approach attempted to measure the influence of affective state (specifically individuals with learned helplessness versus normal controls) on defensive responses in the presence of a positive cue (conspecifics), a negative cue (eyespot) and a neutral cue (no eyespot; Rodd et al., 1997). I described the method previously in Chapter 5. Chickens in a state of learned helplessness were faster to enter a state of tonic immobility when handled in the presence of eyespots than were controls, and remained tonically immobile for longer than controls (although the latter also occurred in the absence of eyespots). The data are difficult to interpret in terms of cognitive bias, due to the design of the study. However, this study demonstrates the utility of measuring natural responses to naturalistic stimuli under different manipulations of affect, as a tool for investigating ‘interpretive’ processes in other species, hence its inclusion here.

There are no published studies investigating the influence of covariation bias in animals. In Chapters 4 and 5 I reviewed work which indicates rhesus macaques preferentially form selective associations between fear-relevant (e.g. snake) stimuli and a negative US (e.g. Cook & Mineka, 1990). The modification of illusory correlation paradigms used with humans (Kennedy et al., 1997; Garner et al., 2006a) may provide a useful tool to investigate covariation biases in other species.

Optimal discounting approaches have not yet been applied to study the role of affect (specifically) in decision making processes, in either humans or other animals. Some optimal discounting studies have investigated the effects of contextual cues (which may lead to changes in affective state) on decision making. Work with domestic fowl, *Gallus gallus domesticus*, (e.g. Abeyesinghe et al., 2005) and blue jays, *Cyanocitta cristata* (e.g. Stephens & Anderson, 2001) demonstrates methods are available for measuring optimal discounting in other species using the 'self control' paradigm. This paradigm presents animals with the choice between small immediate rewards and delayed larger rewards, which are accessed using an operant procedure. Results from such studies suggest domestic fowl show self-control, but only when a delayed reward is large enough (Abeyesinghe et al., 2005). Similarly, blue jays may sacrifice smaller immediate rewards for later larger rewards where resources are patchy (Stephens & Anderson, 2001). Other relevant aspects of decision-making include state-dependent-value-learning, whereby a range of animals from grasshoppers, fish and birds, to humans demonstrate a preference for stimuli associated with previous deprivation (Pompilio & Kacelnik, 2005; Pompilio et al., 2006; Aw et al., 2009; Woike et al., 2009). This effect arises from the increased reward value of mildly positive stimuli in poor conditions versus good conditions. It is therefore necessary to consider the interaction between motivational and affective states and reward learning and memory, as well as the enhancing effects of arousal (contextual cues: Woike et al., 2009) when assessing responses to stimuli using the judgment bias paradigm.

6.1.4. Implications for the development of a novel method to measure cognitive bias in primates

The current study was informed by Harding et al. (2004). The main aim was to modify the procedure used with rats for use with rhesus macaques and to provide comparable data on responses to ambiguous stimuli in monkeys in different affective states. In the first instance a Go/NoGo task that closely followed that developed by Harding et al. (2004) was selected as the most appropriate starting point. As with the previous studies reported in this thesis, visual stimuli were presented on a touch-screen monitor. Abstract stimuli were selected for use as being those which would be easiest to manipulate in terms of ambiguity (cf Brilot et al., 2009) and to allow comparison with Harding et al. (2004). A repeated measures affect manipulation was again selected as the most appropriate for maximizing sample size, given the demands of learning the Go/NoGo task.

6.1.5. Aims:

The general aim of this chapter is to describe the development of a method for measuring judgement bias in rhesus macaques. Specifically the aim of this chapter is to adapt the method developed by Harding et al. (2004) in order to test predictions about monkeys' responses to ambiguous probe stimuli following two treatments designed to induce changes in inferred affective state (a week of enrichment, suggested to induce a more positive affective state, and a health check, suggested to induce a more negative affective state).

6.1.6. Hypothesis and specific predictions:

Treatment condition affects responses to ambiguous probe stimuli (experimental trials) reflecting a more negative judgement about likely outcomes Post-health-check and a more positive judgement about likely outcomes Post-enrichment.

a) Monkeys will **make fewer** responses on trials when ambiguous probes are shown Post-health-check versus Post-enrichment

b) Monkeys will be **slower to respond** on trials when an ambiguous probe is shown Post-health-check versus Post-enrichment.

Part B: Development and outcomes of the new method

In Part A I presented the background and rationale for the development of a method to study judgement bias in rhesus macaques. In Part B I detail the method developed, present data and discuss these in light of judgement bias research with humans and animals to date.

6.2. Methods

6.2.1. Participants

Seven monkeys took part in the study (Monkeys 94K, 860, 16P, 66S, 79S, 79T, and AI73; average age: 4.46 years; range: 3.6 – 7.4 years old). All monkeys had

previously begun operant touchscreen training in the laboratory during the preceding six months and worked in the laboratory on a daily basis.

6.2.2. Stimuli and apparatus

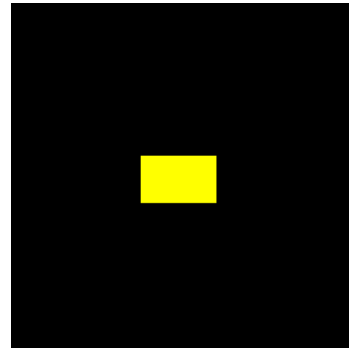
The training stimuli consisted of two yellow lines (control trials: Figure 6.1a). Yellow lines were selected on the basis they did not readily resemble any stimuli the monkeys were exposed to in previous or subsequent experiments and therefore had no previously learnt positive or negative associations within the context of stimuli presented on the touch sensitive monitor. One line was long, and one was short. The long line measured 70 x 13 mm and the short line measured 16mm x 11mm, thereby 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 60cm viewing distance.

Three probe stimuli were composed for testing purposes. Probes were constructed to be intermediate forms of the two test stimuli in length and width. Probes were identical to test stimuli in colour (and so contrast energy) and overall shape. Luminosity (L_y) and contrast energy (C) were calculated from data derived using Adobe Photoshop 7 as described in Chapter 4. The dimensions of the stimuli and probes are presented in Appendix 6 (Table A.6.1). The probes comprised three yellow lines, each of which was 50% larger in area than the next smallest probe (or short line in the case of the shortest probe). This resulted in one line probe which was intermediate in size between the two training stimuli (Experimental probe P_i), and two further line probes intermediate in size to either of the stimuli

Figure 6.1 a) The two stimuli used for training on the Go-NoGo task and for control trials during testing; b) The three intermediate probes used to test responses to ambiguous cues on experimental trials during testing. S+ Rewarded stimulus; S- Non-rewarded stimulus; P- probe most like unrewarded stimulus; Pi intermediate probe; P+ probe most like rewarded stimulus

a)

Control trials



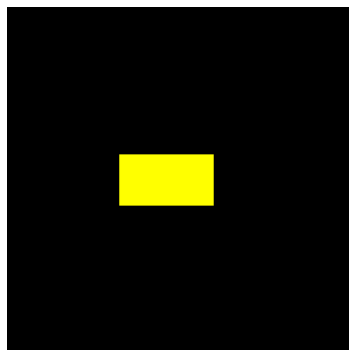
Short line (S+ or S-)



Long line (S+ or S-)

b)

Experimental trials



Short Probe (P+ or P-)



Middle Probe (Pi)



Long Probe (P+ or P-)

and intermediate probe (Experimental probe P+, and Experimental probe P-: Figure 6.1b). All probes were yellow.

One stimulus was presented on each trial. 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 experimenter-generator software, with all aspects of the equipment set-up as described in Chapter 2. All sessions were filmed using two video cameras as described previously.

6.2.3. Design and Procedure

Main terminologies are given in Table 6-2. All monkeys initially underwent a series of training sessions to learn the Go-NoGo task contingencies (Table 6-3). During training only control trials (long and short lines: Figure 6.1a) were presented. On each trial a single stimulus was presented for 2000ms, or until the monkey touched the stimulus. Stimulus onset was triggered automatically by the EPrime software. Stimulus offset was triggered either by the monkey touching the stimulus, or automatically if the monkey did not touch the stimulus during the 2000ms trial. Pellets were given as rewards for correct 'Go' responses at an initial one-pellet/100%FRR (one pellet delivered on 100% of correct 'Go' responses), reduced incrementally to two pellets/40%VRR (two pellets delivered on 40% of trials on a variable reinforcement ratio) over successive training sessions. The two pellet/40%VRR was maintained during testing.

Table 6-2 The main terminologies used in the study

Term	Definition
Training session	Sessions conducted prior to testing during which monkeys learnt to perform the Go-NoGo task
Testing session	Sessions on which experimental trials were presented and experimental data were collected. Each monkey took part in three daily testing sessions following one week of enrichment (Post-enrichment) and three daily testing sessions over the three days immediately following the veterinary inspection (Post-health-check).
Control trial	A trial on which the longest or shortest line was shown. Responses to control trials were recorded as 'correct' (S+ 'Go' and S- 'NoGo') or incorrect (S- 'Go' and S+ 'NoGo') and resulted in rewards (S+ 'Go') or punishers (S- 'Go').
Experimental trial	A trial on which an intermediate line probe was shown. Responses to experimental trials were non-reinforced, and there were neither correct nor incorrect responses.
Treatment	Testing was conducted during two treatment conditions for each monkey: Post-enrichment and Post-health-check.
Correct response	A correct response was defined as + 'Go' (positive stimulus/ Go response) or - 'NoGo' (negative stimulus/ NoGo response) on control trials.
Incorrect response	An incorrect response was defined as + 'NoGo' (positive stimulus/ NoGo response) or - 'Go' (negative stimulus/ Go response) on control trials.

6.2.3.1. Training

The procedure for S+ control trials is shown in Figure 6.2a. The correct response to S+ was 'Go' (Figure 6.2a: top three slides) and the incorrect response to S+ was NoGo (Figure 6.2a: bottom three slides). Each control trial began with a black screen presented for a variable duration of 5000-6000ms. The black slide was replaced with the S+, which was presented for 2000ms, or until the monkey touched it. If the monkey touched S+ (correct +'Go' response), the response was immediately rewarded with a reinforcing tone, feedback screen showing the rewarded stimulus for 1000ms, and immediate delivery of one or two pellets on 100%-40% of trials. At the offset of the feedback screen, a plain black screen was shown for a variable duration between 5000ms and 6000ms until the onset of the next trial. Incorrect responses on 'Go' trials (i.e. no Go response was made) were not rewarded, nor were they punished. If no response was made to the S+ within 2000ms, then the S+ was replaced by a black screen for 5000ms-6000ms until the onset of the next trial.

The procedure for S- control trials is shown in Figure 6.2b. The correct response to S- was 'NoGo' (Figure 6.2b: bottom three slides) and the incorrect response to S- was 'Go' (Figure 6.2b: top three slides). Correct 'NoGo' responses were not rewarded. The stimulus was presented for 2000ms and then replaced with a black screen for 5000-6000ms until the onset of the next stimulus slide. Incorrect responses on 'NoGo' trials (-'Go') were punished. On touching S-, it was removed from the screen and replaced by a blue feedback screen and a three second burst of white noise. The blue screen was shown for 16000ms. At the offset of the feedback screen a plain black screen was shown for 5000m-6000ms until the onset of the next stimulus slide.

Table 6-3 Reinforcement contingencies for the Go-NoGo task

Trial	Response	
	'Go'	'No-Go'
Control Trials		
S+	Correct, rewarded: 1 pellet delivered at a 100%FRR during training, incrementally reduced to 2 pellets rewarded at 40%VRR* at the end of training and during testing. Secondary reinforcing tone on all trials.	Incorrect, non-rewarded: Moves onto next trial with no feedback
S-	Incorrect, punished: White noise, blue screen, 16000ms delay to next trial	Correct, non-rewarded/non-punished: Moves onto next trial with no feedback
Experimental Trials		
P+	No reinforcement or other feedback	No reinforcement or other feedback
Pi		
P-		

S+ signifies the rewarded stimulus; S- signifies the punished stimulus.

Figure 6.2 a) The experimental procedure for ‘Go’ control trials; b) The experimental procedure for ‘NoGo’ control trials; c) The experimental procedure for experimental (probe) trials

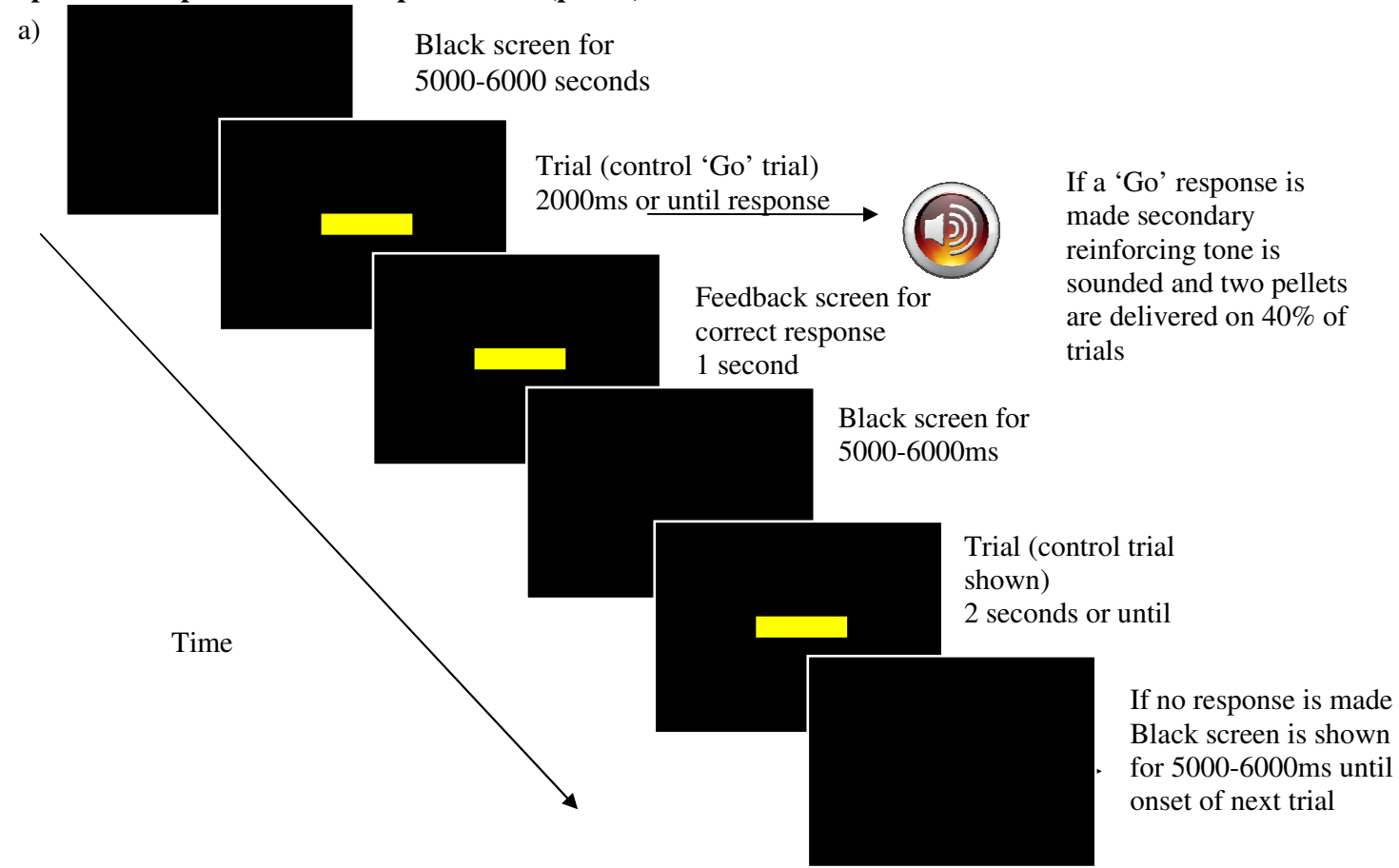


Figure 6.2 (Continued)

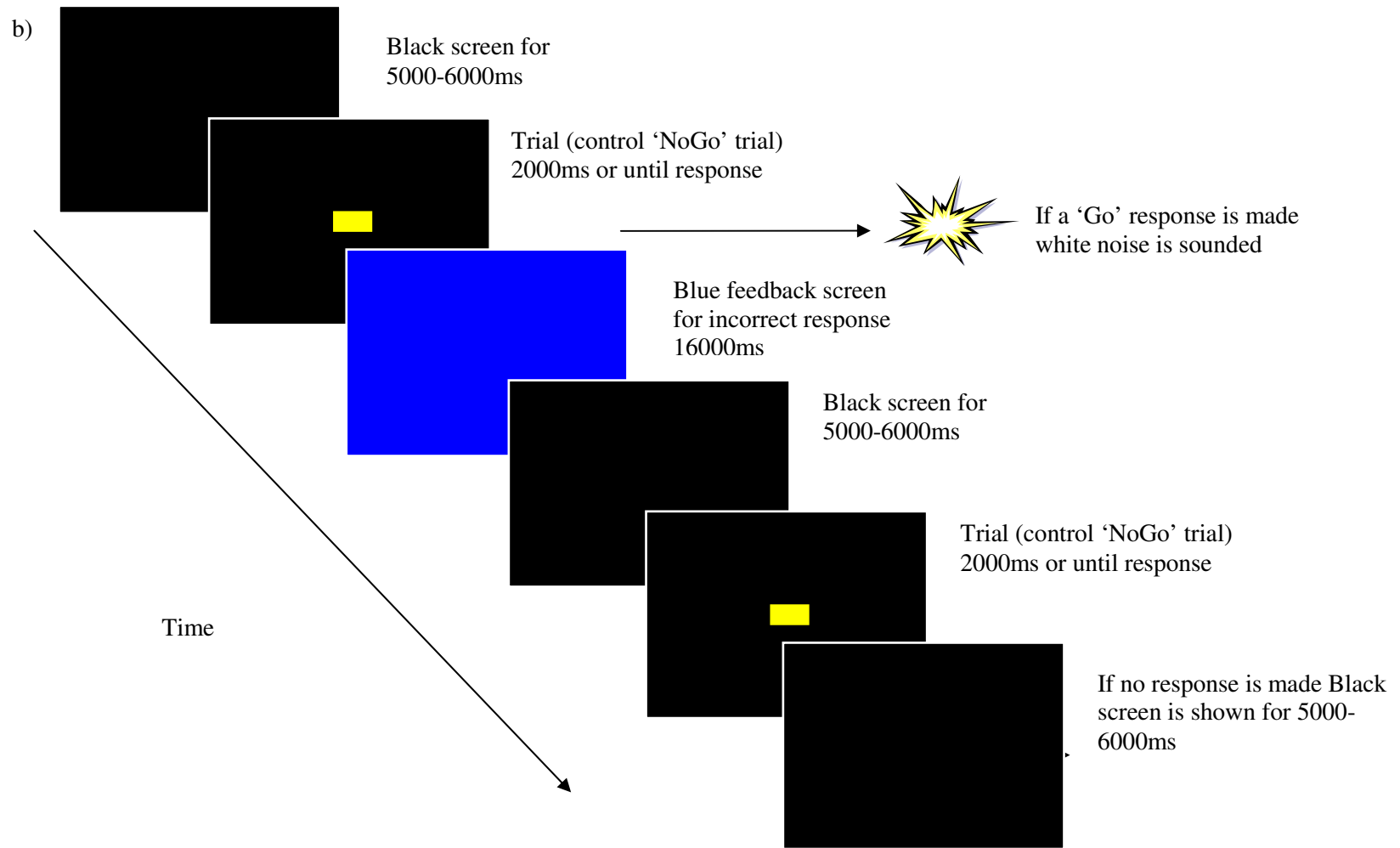
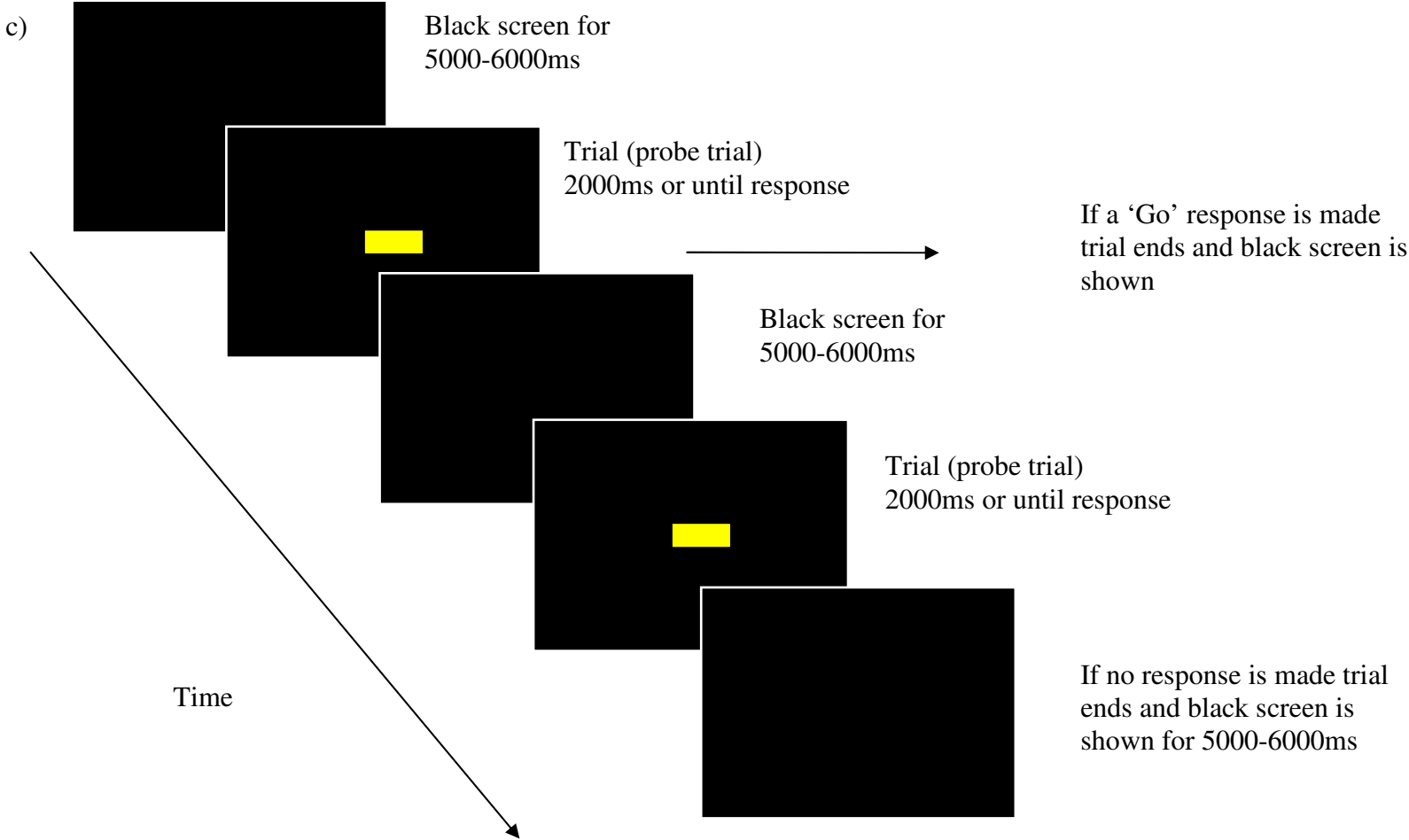


Figure 6.2 (Continued)



If the monkey touched the black screen during the inter-trial interval the Eprime program automatically reset the inbuilt interval counter to 0s and the black screen remained until the monkey removed his hand from the screen. Training sessions consisted of 60 control trials, with an additional two slides to ensure the first and last trials were always + 'Go' trials. Criteria for learning the Go-NoGo task were $\geq 80\%$ correct responses over the 60 trial training block, with $\geq 70\%$ accuracy for each of the 'Go' and the 'No-Go' trials respectively.

6.2.3.2. Testing

During testing sessions control trials were interspersed with experimental probe trials (P+, *Pi*, P-: Figure 6.1b). Trials were presented in a randomised order. Control trials were those on which S+ and S- (long and short lines) were presented. Experimental trials were those on which P+, *Pi* and P- (ambiguous probes) were presented. Control trials were included to gain baseline data on performance on the learned Go-NoGo task. Experimental trials were included to test the experimental hypotheses about the effects of the treatment conditions on responses to ambiguous stimuli.

The procedure for training and testing sessions was as follows. The EPrime program was opened and the participant monkey details entered into the initial information screen (i.e. Monkey ID and session number). The monkey was then transported to the laboratory in the testing cage, positioned in front of the apparatus at a viewing distance of approximately 60 cm, and allowed to settle. The experimenter immediately moved to the adjacent room, and set the video to record events. The experimenter triggered the onset of the experiment by pressing the return key on the keyboard. Presentation and

reinforcement contingencies for control trials were identical to those described for training sessions. On experimental trials a probe was presented for 2000ms or until the monkey touched the probe (Figure 6.2c). The inter-trial interval remained variable at 5000-6000ms. There was no reinforcement for 'Go' and 'NoGo' responses to probes.

Testing sessions consisted of three blocks. Within each block the first and last trials were always + 'Go' trials, as during training, with a series of intervening trials. The following numbers refer to the intervening trials. Block 1 contained 12 control trials only: six S+ 'Go' trials and six S- 'NoGo' trials, presented in random order. Block 1 was included to ensure monkeys were working to criterion prior to the start of the experimental block. A feedback score in the left hand corner of the screen allowed the experimenter to monitor performance. Monkeys were required to score ≥ 9 correct responses during block 1, with ≥ 4 correct responses for each of the 'Go' and 'NoGo' trials in order to move onto block 2. Where a monkey failed to reach criterion, Block 1 was repeated² until the monkey started working or the session was cancelled.

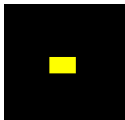

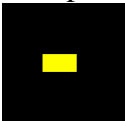

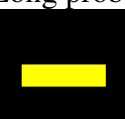
Block 2 contained 48 control trials (24 x S+ 'Go' trials, and 24 x S- 'NoGo' trials). These were randomly interspersed with 18 experimental trials (6 x P+; 6 x P_i and 6 x P-: Table 6-4: note this table shows number of trial from three daily testing sessions). This block was included to collect data on frequency and latency of responses on control and experimental trials in order to test the experimental hypotheses.

² Monkey 94K regularly required two runs of block 1 in order to start working. All other monkeys reached criterion on the first run on most sessions.

Block 3 contained 20 control trials (10 x S+ 'Go' trials: 10 x S- 'NoGo' trials). This block was included to reduce ambiguity in 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 during block 3. If a monkey failed to reach criterion, block 3 was run a second time only. At the end of the session each monkey received the daily food ration.

All monkeys were tested on three days Post-enrichment and three days Post-health-check. The order of testing was counterbalanced across individuals so that monkeys in Group 1 were first tested Post-enrichment, and monkeys in Group 2 were first tested Post-health-check (Figure 6.3). The order of events was as follows. All monkeys began training on the Go-NoGo task in mid November 2006, and those who learnt the task did so by mid January 2007. Following training, Group 1 monkeys (Post-enrichment first) undertook three consecutive days of maintenance sessions concurrent with enhanced enrichment in the home enclosure. This was followed by three daily testing sessions (Post-enrichment), then seven daily maintenance sessions, the health-check, and finally three daily testing sessions (Post-health-check), Group 2 monkeys followed a similar procedure which ran in the following order: training and maintenance sessions – health check – Post-health-check testing – maintenance and concurrent enrichment – Post enrichment testing.

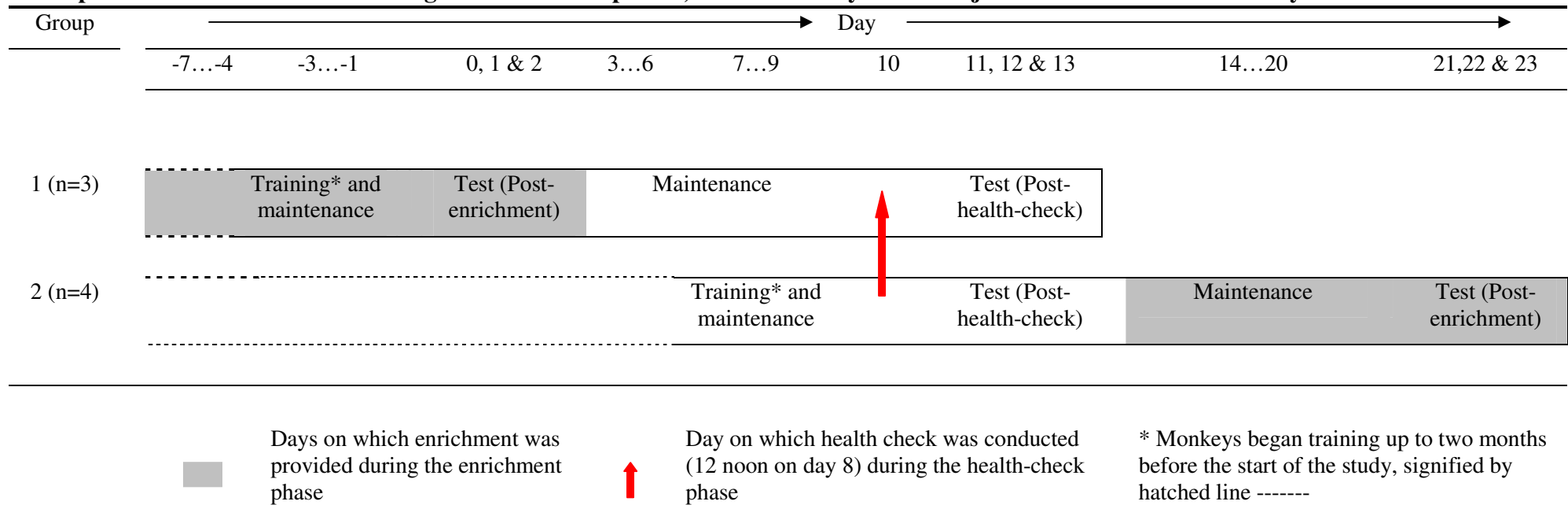
Table 6-4 The number of presentations of control trials (n=288) and experimental trials (n=108) conducted Post-enrichment and Post-health-check.

Trial	Trials per treatment condition (n)		Total (n)
	Post-enrichment (3 x daily testing sessions)	Post-health-check (3 x daily testing sessions)	
Control trials			
Short line 	72	72	288
Long line 	72	72	
Experimental trials			
Short probe 	18	18	108
Middle probe 	18	18	
Long probe 	18	18	
Total (n)	198	198	396

The full series of events was as follows, and as summarized in Figure 6.3. On days -7 to -1 all monkeys housed in the enclosure received enrichment in the home cages, as described in Chapter 3 and both Groups 1 and 2 underwent daily training on the 'Go-NoGo' task. On days -3, -2 and -1 Group 1 underwent maintenance training sessions during which they were required to work to criterion to qualify to take part in testing sessions on days 0, 1 and 2. On days -3, -2 and -1 the monkeys in Group 2 also worked on the 'Go-NoGo' task in the laboratory, as usual. On each of days -7 to -1 each monkey was transported to the laboratory where he was presented with a single-trial practice block followed by a 60-trial training block and a single (rewarded) end trial (total 62 trials). To assess feeding motivation, the number of pellets left in the pellet tray and the number of monkey chow left in the lunch box at the end of each session, were recorded.

On days 0, 1 and 2 Group 1 took part in three consecutive daily Post-enrichment testing sessions (Figure 6.3). Group 2 monkeys continued 'Go-NoGo' training on these days. On days 3-9 Groups 1 and 2 engaged in daily maintenance sessions on each of which the control stimuli were shown for 62 trials, as during days -3 to -1. On days 7, 8 and 9 monkeys in both groups were required to perform to criterion in order to qualify to take part in testing sessions on days 11, 12 and 13. On day 10 all monkeys in the enclosure received their three-monthly health-check. On days 11, 12 and 13 Groups 1 and 2 took part in Post-health-check testing. On day 14 all monkeys in the enclosure began a second week of enrichment during which Group 2 engaged in daily maintenance sessions on the 'Go-NoGo' task. On days 21, 22 and 23 Group 2 underwent Post-enrichment testing.

Figure 6.3 The counterbalanced order of testing sessions conducted post-enrichment (p-e) and post-health (p-hc) check. All monkeys were provided with enrichment during the enrichment phases, and all monkeys were subjected to a health check on day 10.



Throughout the study, care was taken to maintain a regular daily working routine. Staff access to the area in and around the animal housing was restricted and all nonessential husbandry procedures postponed until the end of the study. During the training and enrichment phases monkeys were provided with regular enrichments (ice lollies, toys, twigs and preferred foods), which were usually frozen inside ice blocks, with daily food rations adjusted accordingly for calorie intake.

6.2.4. Data selection and treatment

Criteria for inclusion of each monkey in the analyses were a) responses on at least 80% of control trials (and at least 70% of trials for each of the S- and S+ control stimuli separately) within a 28 trial window in block 2 on at least one daily testing session in each treatment; and b) the monkey ate a comparable proportion of pellets and daily food ration to that consumed during training sessions. The latter was assessed according to the number of pellets left in the pellet tray, and the number of chow pellets and fruit slices left in the lunch box, at the end of each session.

Performance data for experimental sessions are presented in Table 6-5. All seven monkeys who took part in testing met criteria for inclusion in the analyses. Five monkeys met criteria on all six of their testing sessions. For these monkeys data from all six testing sessions were included in the analyses. One monkey reached criteria during four testing sessions (monkey 94K) and one monkey reached criteria during five testing sessions (AI73). For these two monkeys only daily testing sessions for which data were available both Post-enrichment and Post-health-check were entered into the analysis

Table 6-5 Performance data for the seven monkeys who took part in testing sessions. All seven monkeys who took part in testing reached criteria for inclusion in the final analysis. (Values in brackets signify number of sessions where criterion was met within a 28-trial window only.) For all other sessions criterion was met over all trials.

	ID	Age (yrs)	N testing sessions completed to criterion		Proportion of pellets eaten			Daily food ration eaten			Criteria met? (yes/no)	
			Post- enrichment	Post- health- check	Training	Post- enrichment	Post- health- check	Training	Post- enrichment	Post- health- check		
Grp 1	94K	7.4	3 ⁽¹⁾	1 ⁽¹⁾	0.64	0.60	0.73	✓	✓	✓	✓	
	79S	3.7	3	3	1.00	1.00	1.00	✓	✓	✓	✓	
	79T	3.65	3 ⁽¹⁾	3	0.92	0.92	0.92	✓	✓	✓	✓	
											n = 3	
Grp 2	86O	5.3	3 ⁽¹⁾	3	1.00	1.00	1.00	✓	✓	✓	✓	
	16P	5.15	3 ⁽²⁾	3 ⁽²⁾	1.00	1.00	1.00	✓	✓	✓	✓	
	66S	3.8	3 ⁽¹⁾	3 ⁽¹⁾	1.00	0.92	1.00	✓	✓	✓	✓	
	AI73	3.6	3 ⁽¹⁾	2 ⁽¹⁾	0.89	1.00	1.00	✓	✓	✓	✓	
											n = 4	
Total	n=7	4.66 ±0.53										n = 7

(two and four sessions, respectively). A total of 2376 trials, from 36 testing sessions spread across seven monkeys therefore met the criteria for entry into the analysis.

For analysis of proportion of responses made, frequency data are presented as

$$\text{Proportion} = \frac{n(\text{'Go' responses})}{n \text{ trials}}$$

for each of the control trials (S+ and S-), and each of the experimental probe trials (P+, *Pi* and P-).

For analysis of latency to respond, latency data were treated following Harding et al. (2004), in order to maintain comparability of findings between the two studies. Mean latency to respond was calculated for each stimulus, in either condition, per monkey. Mean latency to respond was calculated from all trials, including non-responses which were included as 2000msec.

Due to small sample size it was not possible to include order of testing (Post-enrichment first versus Post-health-check first) or stimulus group (long line rewarded versus short line rewarded) in the analyses. The influence of these factors is addressed separately in Post-hoc tests.

Collapsed data were checked for a normal distribution using a one-sample Kolmogorov-Smirnov (K-S) test. All K-S tests revealed data did not differ significantly from a normal distribution, therefore parametric tests are used

throughout. For t-tests, a Levene's test of equality of variance was also conducted. All descriptive data are reported as mean \pm 1SE.

A 1 x 3 RMANOVA was performed to examine whether feeding motivation (a proxy measure of motivation to perform the task), measured as proportion of pellets eaten, varied with affective state. Data were proportion of pellets consumed with within-subjects factor of treatment condition (training, Post-enrichment testing and Post-health-check testing) for the seven monkeys who took part in testing sessions (Table 6.5). The proportion of pellets eaten did not differ significantly between training and the two treatments ($F_{2,12} = 1.399$, $P=0.28$). Planned t-tests revealed there was no significant difference in pellet consumption between the treatments (Post-enrichment versus Post-health-check: $t(6)=1.491$, $P=0.187$) nor between either of the treatments and training (Post-enrichment versus training: $t(6)=0.065$, $P=0.950$; Post-health-check versus training: $t(6)=1.538$, $P=0.175$). All monkeys consumed the full daily food ration while in the laboratory after each training and testing session.

These results suggest there was no significant reduction in appetite or motivation to work, as measured by consumption of pellets and daily food ration, between the training and Post-enrichment and Post-health-check testing sessions. The seven monkeys consumed pellets at comparably high rates during the training and testing phases. It is therefore likely that motivation to work on the task was maintained throughout the study. Increase in appetite would be masked by a ceiling effect in the data.

6.2.5. Data analyses for experimental trials (intermediate probes)

Experimental trials (P+, Pi and P-) were included to investigate the influence of treatment condition on responses to ambiguous stimuli. The measures were frequency of responses and latency to respond. Frequency data were included to measure the influence of affective state on tendency to respond. Latency data were included to measure the influence of affective state on speed to respond.

To test the hypothesis that treatment condition influences responses to ambiguous stimuli, frequency data were entered into a 2x5 RMANOVA. Data were frequency of responses with within-subject factors treatment (Post-enrichment versus Post-health-check) and trial type (S+, S-, P+, Pi and P-). Latency to respond to each of the S+, S- and the three probes Post-enrichment versus Post-health-check was also analysed using a RMANOVA. All main effects and interactions arising from the higher-order RMANOVAs were then examined using Bonferroni adjusted t-tests, or their non-parametric equivalents.

6.2.6. Data analyses for control trials (long line and short line)

Control trials (S+ and S-) were included to investigate the effects of treatment condition on performance on the learned Go-NoGo task, and to distinguish valid from invalid testing sessions, as defined in section 6.2.4.

To test the effects of treatment on tendency to respond, two paired samples t-tests were conducted. Data were mean frequency of responses Post-enrichment versus mean frequency of responses Post-health-check for each of the S+ and S-. To test

whether treatment affects latency to respond, two further paired samples t-tests were conducted. Data were mean latency to respond Post-enrichment versus mean latency to respond Post-health-check for each of the S+ and S-.

6.3. Results

The experimental hypothesis was tested by running two higher-order 2x5 RMANOVAs, separately for each of the frequency and latency data. The within subjects factors were treatment condition (Post-health-check versus Post-enrichment) and trial type (S+, P+, Pi, P- and S-). Specific predictions are tested with subsequent planned comparisons.

6.3.1. Frequency of responses

For frequency data, there was a significant interaction of treatment x stimulus ($F_{4,24} = 2.74, P=0.05$) and a main effect of both treatment ($F_{1,6} = 7.93, P=0.03$) and stimulus ($F_{4,24} = 59.16, P<0.01$; Figure 6.4). Monkeys tended to make more frequent responses Post-enrichment than they did Post-health-check, and tended to respond more frequently to S+, P+ and Pi compared with S- and P-. These data suggest that treatment condition and trial type mediate monkeys' frequency of responses on the Go-NoGo task. These effects are examined in more detail below.

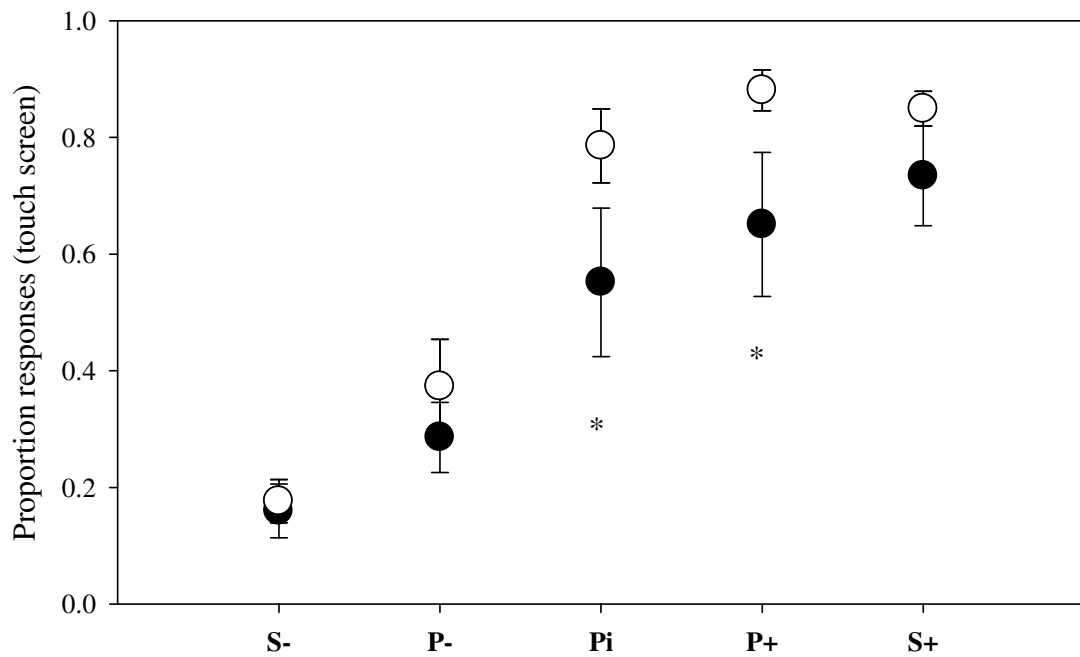
Regression equations were calculated per treatment per monkey. Data were entered into a paired samples t-test, separately for slope and intercept. There was a significant difference in slopes Post-enrichment versus Post-health-check

($t(6)=2.41$, $P=0.05$), indicating that monkeys made a greater number of responses Post-enrichment than they did Post-health-check. There was no difference in intercepts ($t(6)=1.58$, $P=0.16$), suggesting that the difference in slopes was driven by a difference in proportion of responses to any of S+, P+ and Pi, but not to S- and P-.

Planned pairwise comparisons were conducted to examine the two-way interaction, and difference in slopes between the two treatments, in more detail. Data were frequency of responses Post-enrichment versus Post-health-check, separately for each of P+, Pi and P-. Paired samples t-tests were used with the Bonferroni adjusted P value $P=0.017$. These analyses were conducted to test the prediction that monkeys will make fewer responses on trials when an ambiguous probe is shown Post-health-check versus Post-enrichment. There was a trend towards fewer responses Post-health-check versus Post-enrichment for each of P+ ($t(6) = 2.52$, $P=0.05$) and Pi ($t(6) = 2.54$, $P=0.04$)³. There was no difference in frequency of responses to P- Post-health-check versus Post-enrichment ($t(6) = 1.48$, $P=0.18$).

³ Levene's test for homogeneity of variance between Post-enrichment and Post-health-check data sets approached, but did not reach, significance for P+ ($P=0.08$) and Pi ($P=0.06$). Wilcoxon signed ranks tests supported the findings from the t-tests (P+: $Z=2.21$, $P=0.03$; Pi: $Z=1.99$, $P=0.05$).

Figure 6.4 The proportion of responses on control trials (S- and S+) and experimental probe trials (P-, *Pi* and P+) Post-enrichment (open circles) and Post-health-check (closed circles).



In summary, monkeys showed a trend towards the expected difference in frequency of responses to ambiguous probes Post-health-check versus Post-enrichment. This occurred for the intermediate probe *Pi*, and P+, but not for P-.

Two further planned comparisons were performed to determine whether treatment condition altered frequency of learned responses to stimuli of known reward value (S+ and S-). Data were proportion of responses Post-enrichment versus Post-health-check for each of S+ and S- respectively (Figure 6.4). Levene's test showed significant heterogeneity of variance for S+ ($P=0.02$). Bonferroni $P=0.025$ was used. There was a trend towards fewer responses to S+ Post-health-check

versus Post-enrichment ($Z=1.78$, $P=0.07$), and no difference in the proportion of responses to S- Post-health-check versus Post-enrichment ($t(6) = 0.63$, $P=0.55$).

In summary, there was no significant difference in monkeys' performance on control trials for the Go-NoGo task Post-enrichment versus Post-health-check. Monkeys were equally accurate in their responses in both conditions. These data suggest that treatment condition had no significant effect on monkey's baseline performance on the task. Therefore, factors such as learning, memory and motivation (which would be revealed by impaired task performance on control trials) are unlikely to account for the difference in frequency of responses to experimental trials.

6.3.2. Latency data

A 2×5 RMANOVA for mean latencies to respond revealed a main effect of stimulus ($F_{4,24} = 24.16$, $P<0.01$), and no effect of treatment nor interaction between the two (both $P_s >0.16$). The main effect of stimulus reflected the fact that monkeys were faster to respond to the S+ and probe closest to it, and were slower to respond to the S- and probe closest to it. There were also no other main effects or interactions when only data from the three probes were included in the same analysis.

Regression equations were calculated per treatment per monkey and data were entered into a paired samples t-test, separately for slope and intercept. There was

no significant difference between the slopes Post-enrichment versus Post-health-check for either slopes or intercepts (both $P_s > 0.3$).

In summary, treatment condition had no effect on latency to respond on either control or experimental trials.

6.4. Discussion

Seven monkeys learned to perform a Go/NoGo task during which they touched one CS (a long or short line) presented on a touch sensitive monitor to gain a pellet reward, and to refrain from touching another CS (a line of the opposite length: short or long) in order to avoid an aversive US (a burst of white noise and delay until the onset of the next trial). Monkeys were then presented with the Go/NoGo task during two treatments: Post-enrichment and Post-health-check. During testing, presentations of the CS were interspersed with ambiguous probe trials. Frequency and latency of responses to the ambiguous probes were recorded.

The proportion data from experimental trials provide strong supportive evidence for affect-mediated judgement bias in rhesus macaques, in line with patterns of results obtained with rats, starlings and dogs (Table 6-1) and humans (Eysenck et al., 1991; Richards et al., 2002 and 2007). Monkeys responded to the central probe (P_i) and the probe nearest the S+ (P_+) on significantly fewer trials Post-health-check than they did Post-enrichment. This is consistent with data from both animals and humans, which suggest that inferred negative affective state is associated with a reduction in frequency of responding to ambiguous cues associated with reward (Harding et al., 2004; Bateson & Matheson, 2007).

Alternatively, the change in responses to P+ and Pi may reflect a positive bias Post-enrichment (Matheson et al., 2008). There was no evidence for an influence of treatment on latency to respond to the CS and ambiguous probes. This in contrast to studies which found differences in latency measures to ambiguous probes as a function of affective state (e.g. Burman et al., 2008a; Harding et al., 2004). This may be due to a ceiling effect in the data, or arise from a speed-accuracy trade-off. For example, 'Go' responses to S- were incorrect responses, therefore reaction times to S- trials reflect errors only.

The data from control trials revealed treatment condition had no effect on either frequency or latency to respond to the CS. Monkeys responded as often and as quickly to the S+ Post-enrichment as Post-health-check, and this was also the case for the S-. There was therefore no significant change in task performance on control trials between the two treatment conditions. These data indicate there was no significant change in, or effect of, motivational or arousal factors on responses to the S+ and S- Post-enrichment versus Post-health-check, since monkeys were as likely to correctly perform both the 'Go' and 'NoGo' responses in either condition. Monkeys also consumed an equal amount of daily food ration, and rewarded pellets, Post-enrichment as they did Post-health-check. These findings are consistent with data from previous judgement bias studies that found no between-groups differences in responses to either of the CS during testing (e.g. Burman et al., 2008a). The present data build on this previous work by indicating that performance on a Go/NoGo task may also be maintained using a within-subjects design when testing the same individual under different treatment conditions that are assumed to induce changes in affective state.

The maintenance of task performance on control trials is important for the interpretation of data from experimental trials. It suggests that arousal, motivation and cognitive function (e.g. learning and memory) effects alone are unlikely to account for the change in frequency of responses to the ambiguous probes (Mendl et al., 2009). This is pertinent given the evidence for an influence of affect on such processes (e.g. attention and memory formation: Mendl, 1999; state-dependent-learning and reward sensitivity: Pompilio et al., 2006; van der Harst et al., 2003; Woike et al., 2009). Therefore, in the present study, changes in responses to ambiguous probes are likely to reflect factors associated with the processing of ambiguous stimuli rather than processes related to stress-related impairment in task performance. The current data add weight to the interpretation of studies that have found a reduction in performance on ‘Go’ trials among animals following a negative affect manipulation relative to those who have not undergone a negative affective manipulation (e.g. Harding et al., 2004).

There are many implications of these findings. Firstly, this is the first development of a method to measure judgement bias in a species of primate, indicating that the judgement bias task developed initially by Harding et al. (2004) is suitable for use with primates.

Secondly, the data presented here suggest that rhesus macaques may demonstrate affect-mediated judgement biases, and these may share features in common with judgement biases in humans and other animals (Garner et al., 2006a; Mendl et al., 2009). This finding is supportive of the argument that negative affective states

such as stress and anxiety result in more negative (and/or less positive) judgements about ambiguous stimuli (Eysenck et al., 1991; MacLeod & Byrne, 1996; Rusting, 1999; Richards et al., 2002; Harding et al., 2004; Garner et al., 2006a; Burman et al., 2008a; Casey et al., 2008; Matheson et al., 2008). Monkeys responded significantly less often to the central probe (P_i) and the probe nearest the rewarded stimulus (P_+) Post-health-check versus Post-enrichment. This suggests that the pattern of results may reflect mechanisms sensitive to reward rather than punishment. This is in line with findings from the majority of published studies of judgement bias in animals, which found a change in responses to P_+ (where three ambiguous probes were used: Harding et al., 2004; Bateson & Matheson, 2007), while one study has found a change in responses to P_- (Burman et al., 2008a). The findings may also relate to studies with humans that have found people who suffer from both anxiety and depression have a reduced expectation of future positive events (MacLeod & Byrne, 1996; Garner et al. 2006a), and that reward sensitivity may vary as a function of implicit motivational states (Woike et al., 2009).

Thirdly, this study provides valuable data towards furthering our understanding of the range of animal groups that demonstrate influences of inferred affect on judgement biases for ambiguous information (Mendl et al., 2009). Currently, this group includes rats, starlings and dogs (Mendl et al., 2009). The data presented in this thesis suggests rhesus macaques may also be added to this list.

Fourthly, the use of a within-subjects design indicates that changes in environmental factors (e.g. the introduction of husbandry procedures such as a

veterinary inspection of enrichment devices) can lead to changes in the way a given individual responds to the same information when that information is ambiguous in meaning. This concurs with data from starlings which show biases in responses to ambiguous stimuli that vary with environmental enrichment (Bateson & Matheson, 2007; and Matheson et al., 2008). No studies with humans have used a within-subjects design where the same participants are tested on the same task while in different moods (Richards, pers comm, 13th July 2009). Studies developing therapeutic methods in which people train to induce positive biases have been conducted (Mogg et al., 1995; Mathews & MacLeod, 2002). However, these address the effects of training individuals to attend to and interpret information differently, and therefore do not provide a comparable method to that used here. Between-group mood manipulations with human participants provide the closest comparison (Richards et al., 2002; Rusting, 1999). Richards et al. (2002) used a single mood manipulation to increase state anxiety. Rusting (1999) used a positive mood manipulation for one group and a negative mood manipulation for another group and found mood manipulation interacts with trait factors to influence information processing. Again, none of these studies with humans have tested the same participants on the same task under different conditions.

Finally, cognitive biases are considered to reflect vulnerability to clinical anxiety in humans (Mogg et al., 1995), and there is empirical evidence that cognitive biases provide a reliable predictor of experienced (self-reported) distress in humans that is more accurate than autonomic measures (van den Hout et al., 1994; Pury, 2002; Nay et al., 2004; Jansson & Najström, 2009). For example, Pury

(2002) measured interpretive bias for homophones in students during a period of low academic stress and found negative interpretive bias to be a reliable predictor of experienced negative affect during a later period of high academic stress. This provides an interesting avenue for the consideration of the possible judgement biases that might feed into subjectively experienced distress in animals, if such subjective states exist.

There are several areas where the method presented here may be improved. The use of two similar operant responses would provide a more robust response measure than the Go/NoGo format used here. For example, the active choice paradigm used by Matheson et al. (2008) required starlings to peck at one of two coloured lights following a CS. Following the S+, pecking the associated coloured light resulted in instant reward. Following the equivalent of an S-, pecking the associated (other) coloured light resulted in a reward, but only after a delay. Following the presentation of probe cues, pecks to either coloured light were recorded. In a similar manner, a design in which monkeys are required to touch the screen in different places (or, for example, move a lever in different directions) on each response would provide data that could be more easily compared between conditions. This would also negate the problem in analyzing the latency data in the current study whereby latencies reflected correct responses to the S+, but incorrect responses to the S-.

An important future development will be to incorporate facial expressions into the judgement bias task. Humans suffering from anxiety show heightened sensitivity to expressions of fear (high trait anxiety) and anger (high state anxiety) in

morphed faces, compared to non-anxious controls (Richards et al., 2002). Current research (Anne Richards, Mandy Holmes and Emily Bethell) is investigating the use of adaptation and oddball⁴ paradigms for measuring the effects of state and trait anxiety on interpretation of emotion in morphed faces, as well as neural correlates of these processes (ERP). The development of methods currently used with humans for use with primates would enable us to test hypotheses about the significance of cognitive biases for social stress among social animals housed in captivity. The inclusion of baseline trials for the ambiguous probes would allow direct tests of the relative influence of each treatment condition on responses to the probes. The current method only allows differentiation in responses between the two treatment conditions. It does not allow us to identify whether the difference in frequency of responses arises from an optimistic bias Post-enrichment or a negative bias Post-health-check.

In summary, the data presented here demonstrate that the judgement bias task originally developed by Harding et al. (2004) may be adapted for use with primates. Following a stress-inducing veterinary procedure, monkeys showed a reduction in the proportion of positive responses to ambiguous probes relative to proportion of responses made Post-enrichment. This effect was apparent for the intermediate probe and the probe closest to the rewarded stimulus. This suggests

⁴ The adaptation and oddball paradigms mentioned involve the repeated presentation of a prototype face (e.g. angry or fearful), followed by, or interspersed with, unexpected and infrequent presentations of ambiguous faces. Behavioural and ERP responses to the ambiguous faces are compared between groups that differ in anxiety measures.

that following a stressor, monkeys have a reduced expectation of positive outcomes following ambiguous cues, compared to expectations for the same ambiguous cues after a week of enrichment. This pattern of results is similar to that seen for other animal species (starlings: Bateson & Matheson, 2007; rats: Harding, Paul, & Mendl, 2004), and is suggestive of state effects in humans (Rusting, 1999; Richards et al. 2002). Given the evidence for the role of interpretive processes in the experience of negative affect in humans (Pury, 2002) Judgement bias tasks may, therefore, provide a reliable measure of psychological wellbeing in rhesus macaques.

Chapter 7

Discussion

The main argument of the thesis is that cognitive biases, which provide an indicator of psychological wellbeing in humans, may also provide a valuable measure of psychological wellbeing in animals. The general aim of this thesis was to develop and test methods that measure the cognitive component of emotion in rhesus macaques. The end goal is to contribute data that will inform discussion about the capacity of animals to suffer distress or experience positive psychological wellbeing. The general aim was met: methods were developed to measure several aspects of the cognitive component of emotion in rhesus macaques. Two measures (emotion-mediated attentional bias and emotion evaluation) have not been tested in animals previously. The third measure (emotion-mediated judgement bias) represents an adaptation of recently developed methods for use with animals.

In this general discussion I first summarise the main arguments for the study of cognition-emotion interactions as a measure of animal psychological wellbeing. These include ethical arguments, data from recent methodological developments in animal cognitive bias research, and evidence from neuropharmacological and neuropsychological studies. Secondly, I present an overview of the experimental chapters, summarising the methods developed and the implications of the data for theories of cognition-emotion interaction in animals. Thirdly, I conclude with discussion of the implication of these novel methods and findings in light of the end goals of the thesis and future directions of research into animal psychological wellbeing.

7.1. Arguments for the development of novel methods to study psychological wellbeing in animals

In Chapter 1, I presented the argument for measuring cognition-emotion interactions in animals as a novel measure of psychological wellbeing. The measurement of psychological wellbeing is a central goal of animal welfare research, yet we have few methods to assess psychological processes associated with positive and negative affect in animals (Paul et al., 2005). In humans, affective disorders such as anxiety and depression are characterized in part by specific cognitive profiles (MacLeod et al., 1986; Mogg & Bradley, 1998; Mathews, 1990). These cognitive profiles are known as cognitive biases. People in negative affective states broadly attend to, appraise, expect and recall negative information more than positive information (Williams et al., 1996; Mathews & Mackintosh, 1998; Mogg & Bradley, 1998; Bar-Haim et al., 2007). People in positive affective states show biases for positive information (Schweizer & Schneider, 1997). Negative cognitive biases feed into and maintain negative psychological states and are reliable predictors of experienced distress (Mogg et al., 1995; Mogg & Bradley, 1998; Pury, 2002; Jansson & Naström, 2009). The measurement of cognitive biases is consequently a valuable tool in the assessment of negative psychological wellbeing in humans. By contrast, positive biases are considered to be associated with positive psychological wellbeing, although this is less well studied (Cummins & Nistico, 2002).

If animals have the capacity to suffer psychological distress (a possibility that drives animal welfare research) then it is parsimonious to consider that this capacity may rest, in some part, on underlying mechanisms evolved from a common ancestor shared with other animal groups including humans (Byrne, 1999; Mendl & Paul, 2004). In other words, cognition-emotion interactions contribute largely to psychological wellbeing in humans, and therefore, by definition, psychological wellbeing in some animal taxa may also depend on cognition-emotion interactions to some degree (Paul et al., 2005). The study of emotion-mediated cognitive biases in animals therefore provides an important avenue of research for developing our understanding of animal psychological wellbeing.

The assertion that cognitive biases may provide a valuable measure of psychological wellbeing in primates is supported by the data presented in this thesis. Monkeys demonstrated differential patterns of response to threatening, non-threatening and ambiguous information, and these varied as a function of inferred affective state (Post-health-check versus Post-enrichment). Importantly, these biases in responding are comparable to the patterns of responding seen in affective-disordered humans versus normal controls. These data, together with data from recent studies with rats, dogs and starlings (Mendl et al., 2009) are highly suggestive that methods traditionally preserved for humans provide a valuable tool for the assessment of cognition-emotion interactions in animals as a novel measure of psychological wellbeing.

Cognition was defined as the mechanisms by which animals take in, process, retain and act on information from the environment (Shettleworth, 2001). Cognition involves processes such as sensory perception, attention, learning, decision making, memory and (where present) conscious thought (Gross, 1993). Emotions were defined as states elicited by rewards and punishers that drive organisms to seek fitness-enhancing stimuli and avoid aversive or harmful stimuli (Panksepp, 1998; Rolls, 2000). Emotions are therefore adaptations selected for their impact on survival and reproductive fitness (LeDoux, 1996; Damasio, 2000; Rolls, 2000). They involve behavioural, physiological, cognitive and subjective components (Paul et al., 2005). The extent to which different cognitions and emotions are expressed will necessarily vary between species due to different evolutionary histories and environmental pressures (e.g. Mason & Mendl, 1997; Clubb & Mason, 2004). However, there are core similarities, for example, the role of the amygdala in processing fear-relevant information in the brains of mammals, reptiles and birds (LeDoux, 1996). It is the similarities which are of greatest importance to this thesis since, by definition, our understanding of animal psychological wellbeing will necessarily be based on an understanding of human psychological wellbeing.

In each of chapters 4-6, I reviewed the background literature to cognition-emotion interactions in humans. In each of the reviews, it is clear that much of what is understood about the mechanisms underlying human cognition-emotion interactions comes from studies with animals. For example, theories of information processing in humans are based on data from rats regarding the role of the amygdala and thalamic pathways in rapid orienting and appraisal of

potentially threatening stimuli (LeDoux, 1996). LeDoux (1996) highlighted the role of direct magnocellular inputs from the retina to the amygdala (via the pulvinar and colliculus) in addition to less direct inputs from cortical sensory areas to the amygdala. Stimuli are tagged for valence in the amygdala and then passed forward to sensory and cortical areas for more detailed processing and matching with ongoing cognitive processes and motivations. The work of LeDoux (1996) is widely used and applied to models of attention in humans, including attentional bias (e.g. LeDoux is referenced strongly in vigilance theories of orienting to threat in anxiety: Mathews & Mackintosh, 1998; Mogg & Bradley, 1998) and the development of attentional control models (e.g. the circuit-breaker system which acts as a ventral alerting system to redirect attention to threat: Taylor & Fragopanagos, 2005). Work with rats also informed the development of reinforcement sensitivity models of the role of emotion-cognition interactions in directing behavioural responses to threat (Gray, 1971). Later amendments to these models have also relied heavily on data from animals (McNaughton & Gray, 2000; McNaughton & Corr, 2004). Our understanding of the emotions we identify as ‘fear-related’ (underlying the Freeze-Flight-Flight System: FFFS), ‘positive’ (underlying the Behavioural Approach System: BAS) and ‘anxiety-related’ (conflict between FFFS and BAS leads to activation of the Behavioural Inhibition System: BIS), is based on our understanding of these emotional-motivational systems in animals. Given the importance of animal studies in informing the development of theories and models of human cognition-emotion interactions, it is not surprising that the methods developed with humans may be suitable for adaptation for use with the animals who first informed those methods.

7.2. Overview of the experimental chapters

The experimental chapters met the general aim of the thesis: to develop and test methods that measure the cognitive component of emotion in rhesus macaques. The data arising from these methods supported the main argument of the thesis, that as cognitive biases provide a measure of psychological wellbeing in humans, so may this therefore provide a valuable measure of psychological wellbeing in animals. Data were gained on three aspects of cognition-emotion interaction in rhesus macaques. These were emotion-mediated biases in spatial attention (attentional bias: Chapter 4), emotion-mediated biases in appraisal and response processes (emotion evaluation and response-slowing: Chapter 5) and emotion-mediated biases in interpretive and expectancy processes (judgement bias: Chapter 6). I will discuss each of these in turn and relate these findings to the behavioural and physiological data presented in Chapter 3, as well as the technical aspects of establishing a laboratory and training animals presented in Chapter 2.

7.2.1. Developing a method to assess attentional bias in rhesus macaques

In Chapter 4, I presented data on emotion-mediated attentional bias in rhesus macaques. Monkeys demonstrated a vigilant-avoidant pattern of overt orienting to threatening versus non-threatening faces Post-health-check. Overall, monkeys tended to look faster towards the aggressive face in an aggressive-neutral face pair, regardless of treatment condition, and a tendency to spend less time looking at the aggressive face overall Post-health-check. These findings are consistent with data from humans which suggest anxious (stressed) individuals show an

initial vigilance for threatening versus non-threatening information, followed by a subsequent avoidance of threatening versus non-threatening information (Garner et al., 2006). This vigilant-avoidant pattern of attentional bias has been demonstrated in studies which measured eye-gaze in humans. This is also the method used in this thesis.

The data presented in chapter 4 are important for both the human and animal literatures. Firstly, these are the first data on emotion-mediated attentional bias for threatening versus non-threatening information in an animal species. They suggest that inferred affective state alters the way in which monkeys attend to conspecific faces. Further tests are required to unravel the underlying mechanisms and social implications of these biases for rhesus macaques and other species.

Secondly, the data presented here add novel and valuable information for the refinement of theories of attentional bias in humans. There is much debate in the human literature about the direction of attentional biases. Traditional theories of attention focused on vigilance for threat in anxiety (vigilance theories: Eysenck, 1992; Williams et al., 1996; Mathews & Mackintosh, 1998; Mogg & Bradley, 1998), while some authors argued for avoidance of threatening information in anxiety (avoidance theories: Mansell et al., 1999). More recent work has identified initial vigilance, followed by avoidance, of threatening faces by anxious people (vigilance-avoidance theories: Rohner, 2002; Calvo et al., 2006; Garner et al., 2006). The data presented in this thesis concur with the vigilance-avoidance account. This is the first evidence for affect-mediated attentional bias in a species of primate. Further, the data suggest that attentional biases in anxiety may

function in a similar manner in humans and primates. If it is found that the similar patterns between rhesus macaques and humans arise from common underlying mechanisms, this would have important implications for our understanding of the evolution of early attentional biases for threat in humans.

Thirdly, this study highlights the utility of gaze measures for assessing the time-course of the attentional response to threatening information in rhesus macaques, as has been shown for humans. Eye-gaze measures capture early and late processes, which are required for vigilance-avoidance accounts of spatial orienting of attention to threat in anxiety. Vigilance theories and avoidance theories have arisen from dot-probe and visual search studies. These are limited in the time-window of the attentional response recorded. In the case of dot probe studies this is determined by stimulus presentation time, which is usually ≤ 500 ms (e.g. Mansell et al., 1999; Bradley et al., 2000; Chen et al., 2002; Mogg et al., 2008; Holmes et al., 2009). Visual search tasks are also commonly used (e.g. Öhman et al., 2001; Eastwood et al., 2003) but these are only sensitive to initial latencies to detect a stimulus within an array and hence do not measure subsequent processes. Therefore, the use of eye-gaze to assess spatial orienting of attention to stimuli is a powerful method for use with both rhesus macaques and humans.

Fourthly, the data are important for highlighting the possible role of early and later attentional processes in the onset and maintenance of affective disorders in primates. In humans, vigilance for threatening faces is a predictor for vulnerability to anxiety (Mathews, 1990). Following stressful husbandry procedures, such as

the health-check used in the method presented here, monkeys may have a negative attentional bias, and therefore display patterns of attention that enhance ongoing detection of environmental threat and produce sustained negative affectivity. From a psychological welfare perspective this may leave animals vulnerable to deterioration in psychological wellbeing. There are also implications for the social interactions of both singly- and socially-housed captive primates alike, since overall avoidance of faces in anxiety impairs social interactions and may exacerbate social submissiveness (Chen et al., 2002).

In summary, the method developed to measure attentional bias in rhesus macaques was successful and produced data of importance for both the human and animal literatures. A few improvements to the method are however needed. Due to a slight delay between triggering the onset of each trial and the stimuli appearing on the screens, on some trials monkeys were no longer looking centrally between the screens at stimulus onset and these trials were lost from some of the analyses. Training monkeys to look towards a central cue prior to stimulus onset, as is done in human studies, should improve accuracy in measurement of direction and latency of first gaze. This would provide a greater number of valid trials in the final analysis, but would also require training and therefore take longer to execute. Promising future directions of this research would be to include non-aggressive emotional faces to test the emotion-specificity of the attentional response. Development of a dot-probe paradigm suitable for use with monkeys would provide a means of identifying the timings of vigilance and avoidance effects (e.g. Mogg et al., 2008)

7.2.2. Developing a method to measure emotion evaluation and response-slowing in rhesus macaques

In Chapter 5, I presented data on the effects of two treatments on threat evaluation and response-slowing in rhesus macaques. Monkeys showed differential patterns of impairment on a simple operant task in the presence of threatening versus non-threatening distractor faces Post-health-check versus Post-enrichment. Once differences in arousal between treatments were accounted for, the data were consistent with data from humans which suggest that the presence of a threatening distractor interrupts ongoing task-relevant cognitive processes and impairs (slows) task performance in anxious individuals but not in non-anxious controls (Bar-Haim et al., 2007). In this thesis monkeys were slower to touch stimuli containing direct gaze face distractors, relative to control trials, Post-health-check versus Post-enrichment. These data are consistent with models of attentional control in humans, whereby the onset of highly salient information acts as a circuit breaker which inhibits ongoing goal-directed processes (Taylor & Fragopanagos, 2005). This allows redirection of attentional resources towards the salient stimulus for further evaluation, resulting in a slowing in task performance (Algom et al., 2004; Mogg et al., 2008). The data are also consistent with reinforcement sensitivity models which propose that competition between three motivational systems in the brain results in different degrees of impairment on an ongoing cognitive task (Gray, 1971; McNaughton & Gray, 2000; McNaughton & Corr, 2004). Conflict between the system sensitive to positive reinforcers (BAS) that drives approach behaviours towards reward, and the system sensitive to negative reinforcers (FFFS) that drives avoidance behaviours away from threat, may result in a conflict of response (BIS) and subsequent impairment on an ongoing cognitive

task (McNaughton & Corr, 2004). Activation of any or all of the systems may underlie the pattern of results revealed in the current study (Algom et al., 2004; Mogg et al., 2008).

The method developed here was informed by recent advances in human eStroop paradigms (particularly the use of faces: Bar-Haim et al., 2007), advances in theories regarding underlying mechanisms of the task impairment effects of threatening distractors (sensitivity reinforcement models: McNaughton & Gray, 2000; attentional control models: Taylor & Fragopanagos, 2005), and recent consideration of arousal related response-slowing (Mogg et al., 2008). These different approaches were combined with methods that directly test approach and avoidance of threatening stimuli in primates and humans (Wisconsin General Task Apparatus used with primates: Cook et al., 1985; social approach-avoidance task used with humans: Roelofs et al., 2009). This produced a method that directly tests approach-avoidance tendencies in primates in different affective states (As inferred by treatment condition) with respect to differently-valenced stimuli. It also allowed tests of the effects of inferred affective state on task impairment in the presence of distracting emotional information.

The data presented in Chapter 5 are important for several reasons. Firstly, these are the first data on emotion evaluation and response-slowing in the presence of emotionally (or motivationally) relevant stimuli in an animal species. The data indicate that speed to perform a task changes when a threatening face is presented. Post-health-check, monkeys show a slowing of response to direct-gaze faces, but

no slowing of response to averted gaze faces. Post-enrichment, monkeys show a speeding of responses to direct-gaze faces but not averted gaze faces. Emotion evaluation, approach-avoidance, arousal and affective systems were discussed as possible contributory factors to the slowing of responses to threatening stimuli by monkeys Post-health-check. This paradigm requires further investigation in order to identify the underlying mechanisms and social implications of these findings in rhesus macaques and other species.

Secondly, the method has implications for arguments about the emotional saliency of face stimuli used in cognitive tests with humans. It has been argued that face images presented on a computer monitor are likely to convey less meaning to a perceiver than the facial expressions encountered during real-life interactions. The requirement for monkeys to touch the distractor stimuli directly, rather than press a remote button as in human studies, provides a novel means of assessing approach-avoidance behaviours, since it requires participants to touch the stimuli. This is likely to increase the tendency for both approach and avoidance behaviours. No study conducted with humans has required participants to touch stimuli directly. However, this may provide a valuable means of enhancing the emotional saliency of stimuli shown on a screen.

Thirdly, the trend for slowing of responses, relative to baseline, on direct-gaze trials Post-health-check, versus a trend for speeding of responses, relative to baseline, Post-enrichment, has implications for captive primate welfare. In anxious humans, threatening stimuli capture attention and limit attentional

resources available to perform other tasks (Taylor & Fragopanagos, 2005). This can lead to impairment in other areas of cognitive function from social communication to learning and memory in both humans and animals (Mendl, 1999). As with the bias for spatial orienting towards threatening information in anxiety revealed in Chapter 4, attentional capture by distracting threatening information presented during an ongoing task may also have implications for the onset and maintenance of negative affective states in primates.

Fourthly, the finding for arousal-related speeding of responses on control trials indicates that arousal affects speed to respond to non-emotional stimuli. This finding highlights the fact that control trials are therefore essential for studies where animals are likely to be in different states of arousal.

In summary, the method developed here was successful in measuring changes in speed to perform an operant task in the presence of threatening and non-threatening face distractors. The initial task requires some alterations in order to tease apart the likely underlying mechanisms of this change in latency to respond. As with the attentional bias task, a pre-training phase in which monkeys learn to gaze towards a central fixation cross would ensure monkeys were attending to the location of the stimulus at stimulus onset. This would reduce noise in the data. Further directions of this method are to increase operant task difficulty (for example, introduce a discrimination task in which correct and incorrect responses may be distinguished), and to investigate saliency versus valence effects of distractors (e.g. positive versus negative faces: Bradley et al., 1998).

7.2.3. Developing a method to measure judgement bias in rhesus macaques

In Chapter 6, I presented data on judgement bias in rhesus macaques. Following the original method developed by Harding et al. (2004), monkeys were trained on a Go-NoGo task in which they learned to touch one stimulus (a short or long line) to gain a reward and to avoid touching another stimulus (a long or short line) in order to avoid a punisher. Go-NoGo responses to previously unseen ambiguous probes (lines of intermediate length) were then measured Post-health-check and Post-enrichment for each monkey. Monkeys responded less often to the central probe and probe closest to the rewarded stimulus Post-health-check than they did Post-enrichment. The findings are consistent with data from rats, starlings, dogs and humans which suggest a more negative (or less positive) judgement and corresponding response to ambiguous stimuli by anxious (stressed) individuals compared with non-anxious (non-stressed) individuals (Mendl et al., 2009). The studies reviewed in Mendl et al. (2009) used variations of the Go-NoGo design on which the study presented in this thesis was also based.

There are several implications of the data presented in Chapter 6. Firstly, these are the first data on judgement bias in a species of primate. The method developed was suitable for recording monkeys' responses to stimuli of known and unknown reward or punishment value, when in different affective states. The method presented is therefore suitable for further development to assess in more depth the underlying mechanisms and conditions under which ambiguous information may be processed as more or less negative.

Secondly, the data add supportive evidence for existing animal data on judgement bias. The pattern of results is consistent with recently published findings with a range of animals (Mendl et al., 2009). This suggests that affective state may influence patterns of responding to ambiguous indicators of reward or punishment in a similar manner across species, including humans. Whether the similarities in the data arise from mechanistic or functional similarities of judgment biases remains to be assessed. This is especially important for interpretation of the data since similarly-valenced affective states are characterized by different cognitive profiles. For example, depression is characterized by a reduced expectation of positive events, while anxiety is characterized by an increased expectation of negative events.

Thirdly, these data add a new taxonomic group to the list of non-human animals for whom judgement biases have already been demonstrated. Previously, judgement bias using a Go-NoGo paradigm has been demonstrated in a rodent (rat), a carnivore (dog) and a bird (starling). Other paradigms have revealed comparable or suggestive findings for chickens and mice (Rodd et al., 1997; Tsetsenis et al., 2007). In this thesis I present data that indicate judgement biases exist in primates as well.

In summary, the development of the Go-NoGo task to measure judgement bias in rhesus macaques was successful and provided data that will contribute to ongoing work in animal cognition-emotion interaction research. In the context of the other

studies presented in the thesis, it was clear that there are many possible underlying mechanisms (e.g. spatial orienting of attention, priority of processing, emotion evaluation and activation of approach-avoidance systems) that may contribute to the differences seen in responding to ambiguous probes following different treatments (enrichment versus health-check). There are many avenues for refinement of the method, such as inclusion of approach-avoidance lever responses used with humans and training to fixate a cross prior to the onset of each trial. Consideration of trait factors, in addition to treatment-induced state factors, would allow investigation of the interplay of trait and state characteristics on responses to stimuli. Following recent advances in the human literature (e.g. Richards et al., 2002), the inclusion of ambiguous social stimuli, such as morphed facial expressions, would allow investigation of the effects of emotion state on interpretation of ambiguous social cues. Further, presentation of ambiguous stimuli in the presence of valenced contextual cues (Richards et al., 2007), would allow examination of the interplay between affect and contextual cues on perception of social signals in primates (e.g. Flack & de Waal, 2007).

7.3. Triangulation of measures of affect in rhesus macaques – what cognitive measures tell us that traditional measures cannot

All of the above experimental studies rely on the inference that the enrichment and health-check treatments altered monkeys' underlying affective states. The

experimental data from chapters 4-6 showed a significant change in the cognitive component of the affective response between the two treatments (Post-health-check versus Post-enrichment). Behavioural and physiological responses to the treatments were also measured to allow assessment of the treatments in terms of traditional methods for studying inferred affect in rhesus macaques.

Previous studies have shown that enrichment interventions influence the behaviour and physiology of captive primates in a way that is typically assumed to reflect an improved or positive affective state (Kalin et al., 1998; Dawkins et al., 2004; Honess & Marin, 2006). Invasive husbandry procedures typically lead to behavioural and physiological responses that are considered to reflect stress or negative affective states (Ruys et al., 2004). In the present study, changes in behaviour and levels of excreted faecal cortisol metabolites following to two treatments (a week of enrichment and a health-check) were recorded. These data were used to assess the effects of the two treatments on the behaviour and physiology of the monkeys, and to allow comparability with previously published studies that have used these traditional measures to assess the welfare of captive primates. However, the results were unclear. There was some evidence for differential behavioural and physiological responses following the two treatments, but interpretation of the data in terms of underlying affective states was difficult. It is only in light of the cognitive data that more substantial conclusions about valence differences in inferred affective states may be drawn.

The behavioural data revealed monkeys spent more time engaged in self-directed and stereotypical and self-harm behaviours Post-health-check than they did Post-

enrichment. This result is suggestive that monkeys were either in a more negative affective state, and/or that they were more physiologically aroused Post-health-check compared with Post-enrichment. However, the data lack the power to distinguish between these two possibilities (affect versus arousal). There are several reasons for this. Firstly, behavioural responses may become dissociated from physiological responses and are therefore not reliable indicators of affect (e.g. dissociation of behaviour and cortisol responses in dogs: Beerda et al., 2000; and primates: Capitanio, 1999; Ruys et al., 2004; Higham et al., 2009; and dissociation of behaviour and autonomic responses in humans with a repressive coping style: Derakshan et al., 2007). Secondly, the same behaviour may result from different underlying mechanisms. For example, self-directed behaviours such as scratching and autogrooming may be induced in primates with anxiogenic drugs and attenuated with anxiolytic drugs, and are therefore assumed to reflect anxiety-like states (Schino et al., 1996). This has led to the formulation of such behaviours as displacement activities that reflect anxiety in situations of uncertainty (chimpanzees: Baker & Aureli, 1997; rhesus macaques: Karere et al., 2009), but they are also self-maintenance behaviours which serve, for example, to remove ectoparasites under non-stressful conditions (Dunbar, 1988).

Stereotypical and self-harm behaviours may function as a coping mechanism to reduce arousal in animals who have limited response options to threat, providing a measure of coping style rather than a direct measure of underlying affect (Reinhardt, 2008; Higham et al., 2009). There are no accepted behavioural indicators of positive affect in singly-housed monkeys, so this dimension could not be examined. The behavioural data therefore indicated a difference in

monkeys' behaviour Post-health-check versus Post-enrichment but were inconclusive with respect to the positive or negative nature of the behaviours, whether they reflected underlying affective states such as anxiety, or active coping mechanisms.

The physiological data revealed that monkeys had higher levels of faecal cortisol metabolites Post-health-check than they did Post-enrichment. This finding suggests that monkeys had higher levels of arousal Post-health-check than Post-enrichment. The physiological data do not provide power to identify whether the difference in arousal was related to differently-valenced affective states or not. Therefore, it is possible that monkeys were in a negative affective state following both treatments, but this was simply enhanced Post-health-check.

The cognitive data provide potential explanatory power about the valence of affective states that the behavioural and physiological data in themselves do not. To begin, there was some agreement between the traditional and cognitive measures with respect to arousal. The physiological data suggested monkeys were more physiologically aroused Post-health-check than they were Post-enrichment. During the emotion-evaluation task presented in chapter 5 monkeys were faster to respond on control trials (to touch a grey square) Post-health-check than they were Post-enrichment. Control trials comprised non-emotional stimuli, and were included to measure baseline speed to respond (i.e. underlying physiological arousal) specifically.

With regard to the valence of the underlying affective states, each of the cognitive studies presented in this thesis provided data that could be interpreted in several ways. The data may be interpreted as indicating that monkeys were in a positive emotional state Post-enrichment and a negative emotional state Post-health-check. Data from humans demonstrate that optimistic people have an enhanced expectation of positive future outcomes (Schweizer & Schneider, 1997), depressed and anxious people have an enhanced expectation of negative future events, and depressed people have a reduced expectation of positive future events (Williams et al., 1996). In the judgement bias tasks presented in chapter 6, monkeys responded to the intermediate probe and the probe closest to the rewarded stimulus as often as they responded to the rewarded stimulus Post-enrichment. This may be interpreted as an enhanced expectation of reward – an optimistic bias. Post-health-check, monkeys responded to the previously mentioned two probes significantly less often than they did Post-enrichment. This may be interpreted as a reduced expectation of reward – a pessimistic bias associated with depression. Alternatively, it may be interpreted as reflecting ‘depressive realism’ (Alloy & Abramson, 1979) since monkeys still responded on at least half of the trials for either probe.

A second interpretation is that monkeys were in a negative emotional state Post-health-check and a less negative, or near-neutral, emotional state Post-enrichment. The physiological data indicated that monkeys were more physiologically aroused Post-health-check than they were Post-enrichment, but provide no indication of valence. In the attentional bias task presented in Chapter 4, monkeys demonstrated a vigilant-avoidant pattern of attention allocation to aggressive faces

when an aggressive-neutral face-pair was shown Post health-check. This is comparable to the vigilant-avoidant pattern of attention allocation to threatening versus non-threatening faces seen in anxious people (Rohner, 2002; Garner et al., 2006). Post-enrichment, monkeys were equally fast to orient towards threatening faces, but were slower to disengage their gaze from these faces. This is compatible with vigilance theories of attention for threat whereby threatening stimuli both draw and hold attention in anxious people (Eastwood et al., 2003). Therefore early orienting to threatening versus non-threatening stimuli may be revealed in negative affect, but increased physiological arousal and/or negative affect may lead to faster disengagement of attention away from threatening stimuli once engaged. This influence of arousal on the time course of attentional bias in negative affect has not been tested in humans. However, it is interesting to note that phobics, who are characterized as having extreme anxiety with regards to certain classes of stimuli, are vigilant for phobia-related information (e.g. faster detection of snakes and spiders in an array of competing non-threatening stimuli: Öhman et al., 2001) and also demonstrate subsequent avoidance of phobia-related stimuli (Amir et al., 1998; Chen et al., 2002).

A third interpretation is that the tests reveal nothing about valence of the affective states of the monkeys Post-enrichment versus Post-health and the data simply reveal differences in physiological arousal. However, this interpretation does not fit the data from any of the studies easily. In studies with humans, increased arousal speeds latency to respond on non-emotional cognitive tests (e.g. playing an auditory accessory stimulus prior to probe onset speeds latency to respond to probes: Jepma et al., 2009), but there is no evidence increased physiological

arousal alone can account for differential responses to stimuli of different valence. For example, startle probe studies have revealed that looking at highly arousing positive pictures attenuates the startle response in humans, while looking at highly arousing negative pictures potentiates this response. However, looking at low arousal positive and negative stimuli both lead to an attenuated startle response (Cuthbert et al., 1996). Further, it is not clear whether attentional bias in humans reflects automatic capture of attention by threatening stimuli as proposed in the threat-specificity hypothesis (e.g. Bradley et al., 2000) or automatic capture of attention by arousing salient (emotional) stimuli in general, as proposed by theories of general emotionality effects (e.g. Mogg et al., 2000).

In Chapter 4, there was no overall difference in speed to orient gaze towards, or disengage gaze from, faces Post-enrichment versus Post-health-check when aggressive-neutral face pairs were shown. If arousal was the primary factor underlying the results it would be expected that overall speed to gaze towards and away from stimuli would vary between the two treatment conditions. Monkeys gazed towards aggressive faces for more time Post-enrichment and towards neutral faces for more time Post-health-check, but there was no difference in total time spent looking towards faces overall between the two treatment conditions. Given that arousal typically influences speed to respond, while affective state influences type of response (e.g. engagement versus disengagement), these data are difficult to reconcile with an arousal-only interpretation.

In the emotion evaluation task presented in Chapter 5, arousal effects had a significant impact on speed to respond on control trials. Monkeys were faster to

respond to a grey square when it was presented on the screen in order to gain a pellet Post-health-check than they were Post-enrichment. This is compatible with an arousal-related speeding of responses to non-emotional stimuli seen in humans (Jepma et al., 2009). This study demonstrated the importance of considering both arousal and valence together in tests of cognition-emotion interaction.

Finally, in the judgement bias task presented in Chapter 6 monkeys made more responses Post-enrichment than they did Post-health-check, but there was no difference in speed to respond between the two treatments. If responses on the judgement bias task reflected physiological arousal alone, then the speeding of responses to non-emotional stimuli (as revealed on control trials in the emotion evaluation task discussed previously) would have resulted in the opposite pattern: faster, and possibly more, responses Post-health-check than Post-enrichment.

In summary, the data from the three cognitive studies, together with the behavioural and physiological data, suggest that monkeys were in a more negative affective state Post-health-check than they were Post-enrichment. The data raise interesting questions regarding the cognitive profiles expressed by monkeys following two different treatments (a health-check and a week of enrichment). There were parallels with the patterns of attentional, emotion evaluation and judgement biases seen in humans, and judgement biases in animals. The studies also raised important issues regarding controlling for physiological arousal when measuring latencies to respond following different treatments. It was not possible to distinguish negative affect from positive affect, although there was strong

evidence that Post-enrichment monkeys were in a less negative (more positive) affective state than they were Post-health-check.

7.4. Conclusion on the utility of cognitive measures of emotion in rhesus macaques

The cognitive laboratory used in this research was established for the purposes of the studies presented in this thesis. The monkeys used in the study had never taken part in any form of cognitive experiment previously. From the beginning of establishing the laboratory to the end of the cognitive testing took approximately two years. The first year was spent raising money, researching and acquiring equipment and piloting the use of stimuli and touchscreens. The second year was spent setting up the laboratory at the Caribbean Primate Research Centre, training the monkeys and running the cognitive experiments. The laboratory and procedures were successful in many aspects of measuring cognition in rhesus macaques which I have discussed above. Therefore, within a two-year time frame it is possible to establish a cognitive laboratory with rhesus macaques trained to respond to stimuli on a touch-screen using a range of experimental paradigms.

The methods developed in this thesis provide valuable data which should inform both the design and direction of future studies of cognition-emotion interactions in animals, and also discussion of the implications of these measures for animal psychological wellbeing. Each of the three cognitive studies presented represents the first development of a method to measure the aspect of cognition-emotion interaction of interest in a species of primate. Therefore, each method requires

replication and refinement, as discussed. The studies demonstrate that assessment of primate emotions, and understanding of primate and other animal psychological wellbeing, may be reliably achieved through the development of measures of cognitive bias, such as those presented here. Importantly, while cognitive bias measures have been discussed here as a measure of emotion state, an interesting future direction is to consider the use of cognitive bias as a measure of vulnerability to psychological distress. Given the reliability of cognitive bias as a predictive factor for experienced psychological distress in humans, methods that address cognitive bias in animals may provide new insights to the potential for non-human species to experience distress. This, undoubtedly, must be the greatest challenge for animal welfare research.

Appendices

Appendix 1

Permissions and ethical approvals

1.1. Ethical approval granted by Roehampton University

1.2. Ethical approval granted by the Institutional Animal Care and Use Committee, Medical Sciences Campus, University of Puerto Rico

1.3. Export licence for biological samples shipped out of Puerto Rico (CITES licence # 06US126118/9)

1.4. Import licence for biological samples shipped from Puerto Rico to UK (DEFRA licence #AHZ/2537/2005/2 and #AHZ/2537/2006/2)

1.1 Ethical approval granted by Roehampton University

RESEARCH OFFICE

Director of Research
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Roehampton
University 

Ref: LC/EB

Ms Emily Bethell
CREA
Whitelands College
Parkstead House
Roehampton
London SW15 4JD

19th July 2005

Dear Emily

Application for Ethical Approval
Title: Interpretative Bias in Rhesus Macaques

I am delighted to say that the Ethics Board has considered and approved your revised application.

However, the Board asked me to bring to your attention the new good practice report on research involving animals produced by the Nuffield Foundation. The report can be downloaded from:
<http://www.nuffieldbioethics.org/go/ourwork/animalresearch/introduction>

Please let me know if you have any queries.

Yours sincerely



Linda Clapham
Research Administrator

Cc: Professor Ann MacLarnon, Director of Studies
Dr Stuart Semple and Dr Amanda Holmes, Co-Supervisors
Paul Dickerson, School Ethics Sub Committee



1.2 Ethical approval granted by IACUC, UPR



UNIVERSITY OF PUERTO RICO, MEDICAL SCIENCES CAMPUS
UNIVERSIDAD DE PUERTO RICO, RECINTO DE CIENCIAS MEDICAS
PO BOX 365067, SAN JUAN PR 00936-5067 • TEL.: 787-758-2525 EXT. 1836
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INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE
COMITÉ INSTITUCIONAL PARA CUIDADO Y USO DE ANIMALES

UPR

March 13, 2006

Ms. Emily Bethell
School of Human & Life Sciences
Roehampton University
Parkstead House
Holybourne Avenue, Roehampton
London, SW 15 4JD, UK

Dear Ms. Bethell:

A1850106 – Cognitive Bias in Rhesus Macaques (*Macaca mulatta*)

The Medical Sciences Campus Institutional Animal Care and Use Committee (IACUC) evaluated your Initial Submission for the above protocol in its meeting on February 24, 2006.

This is to inform you that the Initial Submission is **approved**. You are granted permission to conduct the study as described immediately. The expiration date of the study is February 24, 2007, unless closed before that date.

Please note that any change to the approved protocol must be submitted and approved by the IACUC before initiating any activity. If you have any questions or require additional information, you may contact Mrs. Dorianne Santana, IACUC Administrator, at (787) 282-0031, before submitting the protocol.

Cordially,

A handwritten signature in black ink, appearing to read 'Elizabeth Rivera'.

Elizabeth Rivera, DVM
Chairperson

dfr

C: CPRC, Sabana Seca Field Station

1.3 Export license (CITES)

FORM 3-201A (1/97)		CONVENTION ON INTERNATIONAL TRADE IN ENDANGERED SPECIES OF WILD FAUNA AND FLORA		Page 1 of 1	
<input type="checkbox"/> EXPORT PERMIT <input type="checkbox"/> RE-EXPORT CERTIFICATE <input checked="" type="checkbox"/> OTHER CERTIFICATE (see block 9)		1. Original Permit/Certificate No. 06US126118/9		2. Valid until 04/04/2007	
3. Permittee (name and address, country) CARIBBEAN PRIMATE RESEARCH CENTER UNIVERSITY OF PUERTO RICO P.O. BOX 1053 OFFICE B-315 SABANA SECA, PR 00952-1053 U.S.A.			4. Consignee (name and address, country) MS. U. PARADES PHYSIOLOGY DEPT. UNIV. LIVERPOOL CROWN STREET LIVERPOOL L69 3GE UNITED KINGDOM		
5. Special Conditions MUST COMPLY WITH ENCLOSED GENERAL PERMIT CONDITIONS. EXCEPTION TO DESIGNATED PORT [50 CFR 14.32]: MAY USE San Juan, Puerto Rico; MUST SCHEDULE WITH USFWS INSP. AT 787-749-4338 AT LEAST 72 HRS. PRIOR TO USE.			5a. Purpose of Transaction M		5b. Security No. 074787
<i>For live animals, only valid if the transport conditions comply with the CITES Guidelines for Transport of Live Animals or, in the case of air transport, with IATA Live Animals Regulations.</i>			6. U.S. Management Authority DIVISION OF MANAGEMENT AUTHORITY U.S. FISH AND WILDLIFE SERVICE DEPARTMENT OF THE INTERIOR WASHINGTON, D.C. 20240 UNITED STATES OF AMERICA 10/05/2006 Issuing Date United States Management Authority AUTHORITY: Endangered Species Act of 1973 (16 USC 1531 et. seq.)		
7/8. Common Name and Scientific name (genus and species) of Animal or Plant		9. Description of Part or Derivative, including identifying marks or numbers (age/sex if live)		10. Appendix No. and Source	
A. Common Name RHESUS MACAQUE Scientific Name MACACA MULATTA		9. BRED-IN-CAPTIVITY: 12 mls blood samples.		10. 2 C 11. Quantity (including units) 12 ML 11a. Total Exported/Quota	
12. Country of Origin U.S.A.		Permit/Certificate No. 06US126118/9		Date of Issue 10/05/2006	
12a. Country of Last Re-export		Re-export Certificate No.		Date of Issue 12c. Pre-Convention: Date of Acquisition	
B. Common Name RHESUS MACAQUE Scientific Name MACACA MULATTA		9. BRED-IN-CAPTIVITY: 12 VIALS OF HAIR SAMPLES.		10. 2 C 11. Quantity (including units) 12 NO 11a. Total Exported/Quota	
12. Country of Origin U.S.A.		Permit/Certificate No. 06US126118/9		Date of Issue 10/05/2006	
12a. Country of Last Re-export		Re-export Certificate No.		Date of Issue 12c. Pre-Convention: Date of Acquisition	
13. Export / Re-export Endorsement: The official who inspects shipment upon exportation / re-exportation must enter the total quantities of specimens being exported / re-exported in this block.		14. Bill of Lading/Air Way-Bill Number Port of Exportation / Re-exportation San Juan, PR Total No. of Shipping Containers 12 NO		15. This document valid only with inspecting official's ORIGINAL stamp, signature and date in this block. 06US126118/9 9/14/07 Inspecting Official's Stamp, Signature and Date	

1.4 Import licences (DEFRA)

Licence No: AHZ/2537/2005/2

DEPARTMENT FOR ENVIRONMENT, FOOD AND RURAL AFFAIRS

ANIMAL HEALTH ACT 1981

IMPORTATION OF ANIMAL PATHOGENS ORDER 1980

IMPORT LICENCE

The Secretary of State for Environment, Food and Rural Affairs, by this licence issued under the terms of the Importation of Animal Pathogens Order 1980 authorises

Dr Stuart Semple
School of Human and Life Sciences
Roehampton University
Holy Bourne Avenue
London
SW15 4JD

**Name and Full
Address**

to land in England in accordance with the conditions set out below

Up to one hundred (100) 30ml bottles containing faeces derived from *Macaca mulatta* (macaque: non-human primates) in ethanol.

**Pathogen or
Carrier**

from

Cayo Santiago

**Country of
Origin**

at

London Heathrow, Gatwick or Stansted airport.

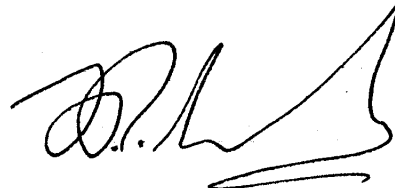
Port of Entry

unless revoked by the Secretary of State by notice to the person to whom it is issued.

This licence expires on 19 December 2007

Dated: 20 December 2005
Amended: 22 December 2005

cc: Chelmsford



Officer of the Secretary of State for
Environment, Food and Rural Affairs

CONDITIONS ATTACHED TO THE LICENCE

1. The material is to be taken direct to Dr Stuart Semple, School of Human and Life Sciences, Roehampton University, Holy Bourne Avenue, London, SW15 4JD.
2. The material must be packed in sealed containers.
3. All inner and outer packaging must be swabbed with suitable disinfectant before leaving Cayo Santiago, Puerto Rico.
4. All packages must be clearly labelled "Animal Pathogen - importation authorised by licence number AHZ 2537/2005/2 issued under the Importation of Animal Pathogens Order 1980."
5. Irrespective of the mode of transport, all specimens must be packaged so that they fully comply with the requirements of relevant Post Office or International Air Transport Association (IATA) regulations.
6. All outer wrappings and packaging must be incinerated on arrival.
7. Inner containers must be disinfected on arrival and autoclaved before disposal.
8. The material is to be used for laboratory work in vitro only, under the direct supervision of Dr Stuart Semple or persons supervised and nominated by Dr Semple.
9. Before being imported each sample must be suspended for at least 96 hours in not less than 9 times its own volume of ethanol (>95%). This volume may be reduced immediately before transport. After importation each sample must then be re- suspended in the same volume of fresh ethanol (>95%) for at least 48 hrs before work on the samples commences.
10. All manipulations of the material must be carried out in a Class I/II/III microbiological safety cabinet which conforms to BS EN 12469 2000.
11. On completion of laboratory procedures all contaminated or surplus material must be autoclaved or incinerated.
12. If the imported material is to be incinerated off-site, its removal and disposal must be undertaken by an operator licensed to do so under the Environmental Protection Act 1990.
13. This licence is valid for 1 consignment only which must be imported by 1 April 2006.
14. The imported material or cultures derived from it must be autoclaved or incinerated prior to the expiry of this licence or the licence must have been renewed.

Licence No: AHZ/2537/2006/2

DEPARTMENT FOR ENVIRONMENT, FOOD AND RURAL AFFAIRS

ANIMAL HEALTH ACT 1981

IMPORTATION OF ANIMAL PATHOGENS ORDER 1980

IMPORT LICENCE

The Secretary of State for Environment, Food and Rural Affairs, by this licence issued under the terms of the Importation of Animal Pathogens Order 1980 authorises

Emily Bethell
Center for Research in Evolutionary Anthropology
Whitelands College
Roehampton University, Roehampton
Holybourne Avenue
London
SW15 4JD

Name and Full Address

to land in England in accordance with the conditions set out below

Three boxes containing up to six hundred and fifty (650) samples in total of faeces and hair derived from the Rhesus macaque (*Macaca mulatta*).

Pathogen or Carrier

from

Puerto Rico

Country of Origin

at

London Heathrow, Gatwick or Stansted airport, or by International Postal Services.

Port of Entry

unless revoked by the Secretary of State by notice to the person to whom it is issued.

This licence expires on 7 June 2008

Dated: 8 June 2006

cc: DVM Reigate



Officer of the Secretary of State for
Environment, Food and Rural Affairs

CONDITIONS ATTACHED TO THE LICENCE

1. The material is to be taken direct to Emily Bethell, Center for Research in Evolutionary Anthropology, Whitelands College, Roehampton University, Roehampton, Holybourne Avenue, London, SW15 4JD.
2. The material must be packed in sealed containers.
3. All inner and outer packaging must be swabbed with suitable disinfectant before leaving the Caribbean Primate Research Center, University of Puerto Rico, Puerto Rico, 00949.
4. All packages must be clearly labelled "Animal Pathogen - importation authorised by licence number AHZ/2537/2006/2 issued under the Importation of Animal Pathogens Order 1980."
5. Irrespective of the mode of transport, all specimens must be packaged so that they fully comply with the requirements of relevant Post Office or International Air Transport Association (IATA) regulations.
6. All outer wrappings and packaging must be incinerated on arrival.
7. Inner containers must be disinfected on arrival and autoclaved before disposal.
8. The material is to be used for laboratory work in vitro only, under the direct supervision of Emily Bethell or persons supervised and nominated by Emily Bethell.
9. All manipulations of the fresh material (that which is not extracted in ethanol) must be carried out in a Class I/II/III microbiological safety cabinet which conforms to BS EN 12469 2000.
10. On completion of laboratory procedures all contaminated or surplus material must be autoclaved or incinerated.
11. If the imported material is to be incinerated off-site, its removal and disposal must be undertaken by an operator licensed to do so under the Environmental Protection Act 1990.
12. There must be no transfer of the imported material or its derivatives to any other person or laboratory without prior authority from the Secretary of State, apart from the transfer of the material off-site for incineration.
13. This licence is valid for 2 consignments only which must be imported by 31 March 2007.
14. The imported material or cultures derived from it must be autoclaved or incinerated prior to the expiry of this licence or the licence must have been renewed.

Appendix 2

Training stimuli for Attentional Bias study



Appendix 3

Detailed methodological sections and data not presented in Chapter 3

3.1. Full protocol for collection of faecal samples from captive *Macaca mulatta*.

3.2. Full protocol for the extraction of hormone metabolites from frozen faecal samples collected from *M. mulatta*.

3.3. Full protocol for the recovery of radiolabel from *M. mulatta* faeces

3.4. Materials and reagents for the 5 β -Androstan-3 α ,11 β -diol-17-one enzymeimmunoassay

3.5. Full protocol for the 5 β -Androstan-3 α ,11 β -diol-17-one enzymeimmunoassay

3.6. Protocol for running dilution plates

3.7. Protocol for calculating intra-assay variability

3.8. Additional behavioural data not shown in Chapter 3, Part B.

3.1. Protocol for faecal sample collection

On each day of collection a clean sheet of corrugated cardboard was placed under each animal's single cage at 6am, immediately following cage cleaning. I inspected the cage floor and the cardboard for faecal matter throughout each day at 10-30 minute intervals. Where faecal matter was present I removed the whole bolus, using a clean glass rod, onto a clean sheet of paper. If the sample was solid it was kept for analysis. Loose stools or diarrhoeic samples were disposed of as biohazard waste. Loose stools may indicate a reduced gut-passage time, affecting the time for absorption of fGCMs and therefore provide unreliable data.

Samples were examined for contamination (urine, water, food or any other non-faecal substrate which might affect [fGCM], or dried bolus weight). Contaminated samples were disposed of as biohazard waste. For accepted samples, a thumb-nail sized piece (~2g wet weight) of the bolus was separated and homogenised using the glass rod. All larger undigested matter (hair and other material that may affect dried sample weight) was removed and the homogenised sample placed in a 30ml screw top centrifuge tube (SarstedtTM). Two Toughtag® labels were attached to each tube: one marked in pencil and a second in marker pen. A third label was attached to the tube lid. Tubes were labeled with animal ID, date, time, and sample number. Tubes were sealed with parafilm to prevent leakage during storage and transport, and placed in a freezer at -20⁰C within 10 minutes of collection.

Following sample collection, all remaining faecal matter was removed from the cage floor. Any cardboard which had come into contact with faeces or urine was

replaced with a new sheet. The glass rod was cleaned with water and wiped dry with a clean paper towel. All disposable items that had come into contact with urine or faeces were disposed of as biohazard waste.

3.2. Full protocol for the extraction of hormone metabolites from frozen *Macaca mulatta* faecal samples

Collection tubes containing frozen faecal samples were removed from the freezer and allowed to defrost for 5-10 minutes, or until the faecal matter had thawed enough to allow thorough homogenisation.

A 10ml syringe was used to dispense 7.5ml of 70% ethanol into each collection tube and a glass rod was used to mash the faeces to produce a faecal suspension.

Collection tubes containing the faecal suspension were then covered with parafilm and stored in a fridge for 48 hours before further handling to reduce pathogen load to a minimal level.

On each day of extraction, 50-100 conical-based graduated centrifuge tubes (30ml) were labelled with numbered stickers on the tube and lid. For each sample collected, two sets of centrifuge tubes were labelled: **Set A** and **Set B**.

Each labelled **Set A** tube was weighed (without lid) on a calibrated Sartorius BL60S scale and the tube weight recorded (g to 3 decimal places, i.e. 0.001g).

Samples were processed in batches of eight (the capacity of the centrifuge).

Eight collection tubes containing faeces in ethanol were removed from the fridge. For each tube, the faecal suspension was poured carefully into each sample's corresponding pre-weighed and labelled centrifuge tube A. Any remaining compacted faecal matter in the collection tube was re-mashed in a small amount of methanol dispensed using a pasteur pipette. The remaining matter was poured into the centrifuge tube so that all faecal matter was transferred. A small amount of methanol was then used to rinse any remaining faeces off the rod into the centrifuge tube, ensuring that all faecal matter was transferred.

Set A centrifuge tubes were then shaken on a VWR VX2500 multi-tube vortexer for 15 minutes. Samples were checked after 30 seconds to ensure all faecal matter was suspended in the ethanol, and that maximal extraction could therefore occur.

The **Set A** centrifuge tubes were removed from the shaker. Any faecal matter remaining on the sides of the tube above the meniscus were dislodged using a small amount of 80% methanol dispensed from a pasteur pipette.

Set A tubes were then centrifuged on a Heraeus Labofuge 400R at 4500rpm for 12 minutes.

After centrifuging, the **Set A** tubes were inspected. Any faecal matter remaining on the sides of the tube, above the meniscus, was dislodged with a further small amount of methanol and the suspensions again centrifuged at the same setting for a further 12 minutes.

On removal from the centrifuge, the supernatant from each sample was poured into the corresponding **Set B** centrifuge tube. Care was taken to transfer as much of the liquid as possible (containing the suspended hormone) while not transferring any faecal matter into tube B.

7.5ml of methanol were then dispensed into the **Set A** centrifuge tube. The pellet was again homogenised using the glass rod and steps 8-12 repeated.

Supernatant from the second extraction phase was poured into centrifuge tube B containing the supernatant from the first extraction step. This resulted in two centrifuge tubes for each sample: tube A containing the faecal pellet, and tube B containing hormone suspended in 50:50 ethanol-methanol mix (>15ml).

Set B centrifuge tubes containing supernatant were capped, sealed with parafilm and stored at 4⁰C.

Protocol for measuring faecal weight:

The pre-weighed tubes (Set A: see Appendix GM1) containing the faeces were placed in a Griffin 1/200 drying oven at 40⁰C for 24 hours.

After 24 hours tubes were removed individually and weighed without lids.

Samples were weighed after a further 24 hours drying time, and reweighed until the dry weight stabilised over three consecutive weighing sessions.

The final weight (g to 3dp) of the dried faeces in tube A was recorded.

The original weight of the empty tube A was subtracted from this value to provide the calculated dry faecal weight.

3.3. Full protocol for the recovery of radiolabel from *M. mulatta* faeces

All procedures were conducted at the Roehampton radio-lab under the technical guidance and supervision of Professor Ann MacLarnon, Professor Jolanta Opacka Juffry and Mr Balbir Josen.

A Working dilution of [6, 7, -³H] Oestradiol was prepared (by Mr BJ) and dispensed at 50µl into each of eight faecal collection tubes containing faeces in 10ml ethanol.

The extraction procedure was performed as given in Appendix 3.2.

The final volume of dosed supernatant in each of the centrifuge tubes after extraction was noted.

A 50µl volume from the dosed supernatant was dispensed into a scintillation vial, and 5ml of scintillation cocktail added.

Step 4 was repeated. Duplicates were labelled #a and #b.

For the Total Count control a 50µl volume of working dilution radiation was dispensed into a scintillation vial, and 5ml of scintillation cocktail was added.

For the Background control, 5ml of scintillation cocktail was dispensed into a scintillation vial.

The Scintillation counter was used to count the samples, providing an initial count for each of samples #a and #b, a mean count of #a and #b, a total count, and a background count.

The percentage recovery was calculated.

3.4. Materials and reagents for the 5 β -Androstan-3 α ,11 β -diol-17-one enzymeimmunoassay

EIA Assay	Antibody Used For Coating
5 β -Androstan-3 α ,11 β -diol-17-one	Anti- Sheep IgG (whole molecule), developed in Rabbit
	Sigma Product No: S1265
	Preciptin analysis: 1ml of antiserum contains 1.8 – 2.5mg of specific antibody.

- 1) The Sigma Product No: S1265 ‘Anti- Sheep IgG (whole molecule), developed in Rabbit’ antibody solution may be stored frozen in working aliquots.
- 2) Repeated freezing and thawing is not recommended.
- 3) If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation.

Solutions

Carbonate Coating Buffer, pH 9.6 (Store at 4°C for up to 6 months)

For 0.5 Litre:

0.75g Na₂CO₃ (Sodium Carbonate anhydrous – BDH 102404H)

1.46g NaHCO₃ (Sodium Hydrogen Carbonate – BDH 102474V)

Dissolve in 0.5 Litre of distilled water, and adjust to pH 9.6 with HCL or NaOH.

Stock Assay Buffer (Store at 4° C for up to 1 month)

For 0.5 Litre:

2.98g Na₂HPO₄ (di-Sodium Hydrogen Orthophosphate, anhydrous –BDH 102494C)

4.25g NaCl (Sodium Chloride – BDH 102415K)

Dissolve in 0.5 Litre distilled water. This buffer has an approximate pH of 9.

Assay Buffer / 0.3% BSA, pH 7.2 (can keep for 1 week stored at room temperature)

For 100ml:

100ml stock assay buffer

0.3g BSA (Albumin, Bovine, Fraction V Sigma A4503)

Adjust the pH from approximately 8.7 to 7.2, using HCl.

Assay Buffer / 0.1% BSA, pH 7.2 (can keep for 1 week stored at room temperature)

For 100ml:

100ml stock assay buffer

0.1g BSA (Albumin, Bovine, Fraction V Sigma A4503)

Adjust the pH from approximately 8.7 to 7.2, using HCl.

Stock Phosphate Buffered Saline (x25), pH 7.2 (Store at 4°C for up to six weeks)

For 400ml:

79.49g NaCl (Sodium Chloride – BDH 102415K)

11.49g Na₂HPO₄ (di -Sodium Hydrogen Orthophosphate, anhy. –BDH 102494C)

2.0g KCl (Potassium Chloride – BDH 101984L)

2.0g KH₂PO₄ (Potassium Dihydrogen Orthophosphate – BDH 102034B)

Dissolve in 400ml of distilled water, and adjust to pH 7.2 with HCl or NaOH

Washing Solution – PBST (Store at 4°C for up to 1 week)

For 1 Litre:

40ml Stock PBS (x25)

500µl Tween 20 (0.05%w/v) [Polyoxyethylene (20) sorbitan monolaurate] –
Sigma Aldrich 437082Q

Add both solutions to a volumetric, and make up to 1 Litre with distilled water.

TMB Stock Solution (Store at room temp. in the dark for up to 1 week)

For 10ml:

Dissolve 125 mg of TMB (3, 3', 5, 5'-Tetramethylbenzidine –Sigma Aldrich T 2885) in 10ml of DMSO (Dimethyl Sulfoxide –Sigma Aldrich 154938). Store in a 30ml polyethylene amber bottle (VWR cat. no. 215-7544).

Stock Substrate Buffer (X5), pH 3.8 (Store at 4°C for up to six months)

For 250ml:

11.875g C₆H₈O₇•H₂O Citric Acid (BDH-100813M)

9.75g Na₂HPO₄ di- Sodium Hydrogen Orthophosphate, anhy. (BDH-102494C)

0.625g Hydrogen Peroxide Urea (Sigma U1753)
H₂NCONH₂•H₂O₂

Dissolve in 250ml of dissolved water, and adjust to pH 3.8 with HCl or NaOH.

Working Substrate Buffer solution, pH 3.8 (make fresh, store for 1 week at room temp.)

For 100ml:

20ml Stock Substrate Buffer

80ml Distilled water

Adjust pH to 3.8 if required.

TMB / Working Substrate Buffer Solution (make fresh, store for 1 week at room temp)

For 1 plate add the following into a glass beaker:

17 ml of working substrate buffer

250µl stock TMB solution

Once made, the beaker must be kept in the dark.

2M H₂SO₄ - This is used to stop the assay reaction.

Streptavidin-Peroxidase (Store for up to 1 year at -18°C)

From Streptomyces avidini lyophilized powder, 80 – 150 units/mg protein. Unit definition: one streptavidin unit will bind 1µg biotin.

For 2000ng/20µl (0.1mg/1000µl):

Dissolve 1mg of streptavidin-peroxidase (Sigma S5512) in 10ml of distilled water and vortex.

Dispense 1ml aliquots into nine 1.7ml eppendorf tubes.

Dispense the remainder as 20µl (2000ng/20µl) aliquots into 1.5ml eppendorfs.

Date, label and store these aliquots in the freezer at -18°C. When stocks are low, the 1ml aliquots can be thawed and dispensed into fifty 20µl volumes. One eppendorph containing 20µl is sufficient for one plate. This will need a further dilution when used on the day of the assay (20ng/150µl well i.e 20µl in 16ml assay buffer).

Preparation of Stock Standard from the Vendor

PdG

A 5mg vial of 5 β -Pregnane-3 α , 20- α -diol-glucuronide is bought from Sigma (Cat. no. P3635). An initial concentration of 1000 μ g/ml (1000, 000, 000pg /1000 μ l) is prepared in ethanol. The stock standard is to be prepared from this at 16,000pg/50 μ l:

$$16000\text{pg}/50\mu\text{l} = 320,000\text{pg}/1000\mu\text{l} = 0.320\mu\text{g}/1000\mu\text{l}$$

Table illustrating how standard is prepared

Final Concentration	PdG Added	Diluent Added	Final Dilution
a) 100 μ g/ml	100 μ l of initial concentration (1000 μ g/ml)	900 μ l ethanol	1/10
b) 10 μ g/ml	100 μ l of (a)	900 μ l ethanol	1/10
c) 1 μ g/ml	400 μ l of (b)	3600 μ l ethanol	1/10
d) 0.320 μ g/ml	2560 μ l of (c)	5440 μ l assay buffer	1/3.125

PdG at a concentration of 0.320µg/ml was dispensed at 150µl volumes into yellow eppendorf tubes and stored in freezer at temperature of -20°C. A volume of 1ml was dispensed into white eppendorf tubes and stored in freezer at -20°C.

The QCH and QCL samples are prepared from the 1µg/ml dilution shown in the table above at 0.004µg/ml (200pg/50µl) and 0.001µg/ml (50pg/50µl). This would be a dilution of 1/250 and 1/1000 respectively in assay buffer. QCH was dispensed at a volume of 250µl into pink eppendorf tubes, whilst QCL was dispensed likewise into blue eppendorf tubes. They were then stored in the freezer at -20°C.

Coating- This Takes 3 Days

Day 1

Thaw out working aliquot of IgG (assuming concentration to be 2.5mg/ml) from freezer.

Dose coating buffer with the antibody at 1µg/150µl.

Table A3.1. illustrates how much antibody should be added to the coating buffer, for Corning (Costar 3590) 96 well EIA/RIA Clear Flat Bottom Polystyrene High Bind Microplate(s) (Fisher Cat. No. DPS-110-080G) at 150µl volumes/well.

The mixture is dispensed using eppendorf Research Pro 1200 (8 channel, 50-1200µl).

The coated plate(s) are covered with clingfilm, and left overnight at 4°C.

Plates must not be stacked more than 3 high.

Table A.3.1. Table for calculation of coating buffer: antibody ratios.

No. of 96 Well Microtitre Plates to Be Prepared at 150µl/well						
Coating Buffer	1	2	3	4	5	10
Required	15ml	30ml	45ml	60ml	75ml	150ml
[Required]	1µg/150µl	1µg/150µl	1µg/150µl	1µg/150µl	1µg/150µl	1µg/150µl
[mg/ml]	0.1mg/15ml	0.2mg/30ml	0.3mg/45ml	0.4mg/60ml	0.5mg/75ml	1mg/150ml
[µg/µl]	100µg/15000µl	200µg/30000µl	300µg/45000µl	400µg/60000µl	500µg/75000µl	1000µg/150000µl
Antibody required (µl)	40µl	80µl	120µl	160µl	200µl	400µl

Day 2

Remove coated microtitre plate(s) from fridge, and pour off the IgG solution.

Bang the plate(s) dry with some paper towel on the bench.

Dispense 300µl of Assay Buffer / 0.3% BSA into each well.

Cover the plate(s) with clingfilm and store in the fridge, overnight at 4°C.

Day 3

Remove the plate(s) from the fridge.

Pour off the Assay Buffer / 0.3% BSA.

Bang the plate(s) dry with some paper towel on the bench.

Cover the plate(s) with clingfilm, and store at -20°C.

Addition of Reagents / Samples - This takes 2 days

Day 1

The following are removed from the fridge or -20°C freezer and then defrosted or brought to room temperature:

-Stock P.B.S (x25), if needed

-Assay buffer / 0.1%B.S.A

-Plate(s) coated with unspecific antibody (Sigma R9754)

-Aliquoted steroid-specific antibody (glass vial)

-Aliquoted biotin labelled steroid (glass vial)

-Standard (yellow eppendorf tube, PdG @ 16000pg/50µl)

-Quality Control High (pink eppendorf tube, PdG @ 200pg/50µl)

-Quality Control Low (blue eppendorf tube, PdG @ 50pg/50µl)

-Test sample(s)

The following reagents are prepared:

a) Dilution of Standard (yellow eppendorf tube)

The PdG standard curve is made up using assay buffer / 0.1% B.S.A as the diluent. It is prepared at the following concentrations: 1600, 800, 400, 200, 100, 50, 25, 12.5 and 6.25 pg / 50µl. Each dilution will be dispensed in duplicate.

b) Test Sample

The test sample is prepared at dilutions such as 1/5, 1/10, 1/20, 1/40 and 1/80 using assay buffer / 0.1% B.S.A as the diluent. Each dilution will be dispensed in duplicate.

c) Biotin Labelled Steroid (glass vial)

The glass vial containing aliquoted biotin labeled steroid is opened, and the appropriate amount of assay buffer / 0.1% B.S.A added. The vial is gently agitated to ensure proper mixing. Using the EDOS 5222, and a 5ml Combitip plus, the biotin labeled steroid will be dispensed into all the wells of the microtitre plate.

d) Steroid Specific Antibody (glass vial)

The glass vial containing aliquoted steroid specific antibody is opened, and the appropriate amount of assay buffer / 0.1% B.S.A added.

The vial is gently agitated to ensure proper mixing.

Using the EDOS 5222, and a 5ml Combitip plus, the steroid specific antibody will be dispensed into most of the wells of the microtitre plate.

The plate(s) are washed 4 times in plate washer with PBST and then dried by banging on a paper towel.

The reagents prepared above are pipetted into the microtitre plate(s) as shown in Table A3.2. below:

Table A.3.2. The order in which reagents are added, and the quantities dispensed, into wells

	Assay Buffer 1	1.1.1.1 <u>Standard</u> 2	1.1.1.2 <u>Sample</u> 3	Biotin Labelled Steroid 4	Steroid Specific Antibody 5
Blank	100µl	-----	-----	50µl	-----
Zero	50µl	-----	-----	50µl	50µl
Standard	-----	50µl	-----	50µl	50µl
Test Sample	-----	-----	50µl	50µl	50µl
Quality Control	-----	-----	50µl	50µl	50µl

Incubation

The microtitre plate is sealed with cling film, and the reagents mixed through circular movements on the bench top. It is then incubated **overnight** (12<20 hours) in the fridge at 4°C.

Quick Reference Guide to Use of the EDOS 5222:

Turn on the EDOS by pressing the ON / OFF key located on the main body.

A menu showing <Single Dispense> as the default is shown on the display.

Insert the appropriate dispenser attachment into the arm of the EDOS (see Table A.3.3.).

The sensor in the arm of the EDOS will automatically know which attachment has been inserted into it.

Table A.3.3. Reagents, dispenser attachments and volumes dispensed using the EDOS.

Liquid Dispensed	Dispenser Attachment Used	Volume Dispensed
Biotin Labelled Steroid	5ml Combitip Plus	50µl
Steroid Specific Rabbit Antibody	5ml Combitip Plus	50µl
Streptavidin-Peroxidase	10ml Combitip Plus	150µl
TMB / Working Substrate Buffer	Eppendorf Research Pro 1200	150µl
2M H ₂ SO ₂	Eppendorf Research Pro 1200	150µl

Press ENTER key on the main body.

To adjust the volume dispensed, press the solid black◀, ▶, ▲, or ▼ key on the main body.

Place the attachment into the vial containing the liquid to be dispensed.

Press the R key on the arm of the EDOS once to Re-set.

Press the hollow black \triangle key once to take the liquid into the attachment. It will automatically stop filling when full.

Press the hollow black ∇ key once to get rid of any bubbles. To dispense the liquid, press the down arrow on each occasion.

When finished, press the R key again to re-set.

To eject the attachment out of the EDOS arm, press the solid black \blacktriangledown key with a horizontal bar above it.

Day 2

Make sure the following reagents (see page 3) are made up:

- TMB Stock Solution
- Stock Substrate Buffer (X5), pH 3.8
- Working Substrate Buffer Solution, pH 3.8

Remove the following from fridge/freezer until they reach room temperature:

- Assay plate left overnight
- Eppendorf tube containing 2000ng / 20 μ l Streptavidin – Peroxidase
- Assay buffer / 0.1% B.S.A, pH 7.2

Wash the plate 4 times in plate washer with PBST and bang dry.

Aliquot 20 μ l of Streptavidin-Peroxidase from 1.5ml eppendorf tube into a beaker containing 16ml of assay buffer / 0.1%B.S.A. Rinse the tube with assay buffer several times. Using the EDOS 5222, dispense 150 μ l into each well. Seal the plate with clingfilm and shake for 30 minutes at room temperature.

Prepare TMB / Working Substrate Buffer solution.

Wash plate 4 times in plate washer with PBST and bang dry. Using the eppendorf Research Pro 1200, dispense 150µl of TMB / Working Substrate Buffer solution. Seal plate with clingfilm and shake at room temperature in the dark for about 45 minutes. Make sure to check colour formation after 10 minutes (zero should be the darkest blue).

To stop the reaction, use the eppendorf Research Pro 1200 to dispense 150µl of 2M H₂SO₄ and read the extinction on the plate reader at 450nm (reference filter 630nm).

The calculation of concentrations based on the optical density is done by the Ascent Software.

3.5. Full protocol for the 5 β -Androstan-3 α ,11 β -diol-17-one enzymeimmunoassay

Preparing the EIA (Day 1):

1. An assay plate, antibody solution and antigen solution were removed from the freezer and allowed to thaw away from any light source for 15 minutes.
2. The serially double-diluted standard curve solution was prepared from the stock solution (12500pg/50 μ l).
3. Faecal sample extracts were prepared at a 1/80 dilution.
4. The microtiter plate was washed 4 times with PBS washing buffer using the Wellwash 4MK2 (Thermo Labsystems) plate-washer. The plate was dried by shaking and banging on blotting on paper.
5. Assay buffer was dispensed into the duplicate Blank (100 μ l buffer in each of wells A1 and A2) and Zero-standard (50 μ l buffer in each of wells B1 and B2) wells (Figure A3.1).

Figure A3.1: The layout of the 96-well microtitre plate as used for measuring the concentration of [fGCM] in the samples used in the analyses presented in the thesis. All wells were designated in duplicate as described in the text.

	1	2	3	4	5	6	7	8	9	10	11	12
A	Blank	Blank	S1	S1	S6	S6	S14	S14	S20	S20	S26	S26
B	Zero	Zero	S2	S2	S7	S7	S15	S15	S21	S21	S27	S27
C	0.6	0.6	39	39	S8	S8	QCH1	QCH1	S22	S22	S28	S28
D	1.21	1.21	78	78	S9	S9	QCL1	QCL1	S23	S23	S29	S29
E	2.43	2.43	156	156	S10	S10	S16	S16	S24	S24	S30	S30
F	4.87	4.87	S3	S3	S11	S11	S17	S17	QCH2	QCH2	S31	S31
G	9.75	9.75	S4	S4	S12	S12	S18	S18	QCL2	QCL2	S32	S32
H	19.5	19.5	S5	S5	S13	S13	S19	S19	S25	S25	S33	S33

6. Standard curve solutions (0.6-156pg/50µl), sample dilutions (33 diluted sample hormone-metabolite extracts, 50µl) and quality controls (one of [high]: **QCH**; and one of [low]: **QCL**, 50µl) were pipetted into the designated wells of the microtiter plate.
7. Assay buffer (6ml) was added to 25µl biotin-labelled antigen in a glass vial with screw-tight lid and inverted gently to mix.
8. 50µl of biotin-labelled antigen solution was dispensed into every well.
9. Assay buffer (6ml) was added to 21µl antibody in a glass vial with screw-tight lid and inverted gently to mix.
10. 50µl of antibody solution was dispensed into all wells EXCEPT the Blank.
11. The plate was covered with clingfilm and placed on a plate shaker for five minutes to mix reagents.
12. The plate was then placed in a refrigerator at 4°C to incubate overnight (16 hours).

Continuation of EIA (Day 2):

1. The plate was removed from the fridge and allowed to warm to room temperature for five minutes.
2. The assay plate was washed four times with PBS washing buffer and blotted dry as before.
3. An aliquot of 20µl streptavidin-horseradish peroxidase was diluted in 20ml assay buffer in a glass beaker. The beaker was covered with parafilm and inverted several times to mix the solution. 150µl of the streptavidin-horseradish peroxidase solution was dispensed into all wells.

4. The plate was sealed with clingfilm and incubated for 30 minutes on the shaker at room temperature and in the dark.
5. After 30 minutes, the plate was washed 4 times with PBS washing buffer and blotted dry.
6. 295µl TMB stock solution was added to 20ml working substrate buffer in a glass beaker. This was covered with parafilm and inverted several times to mix the contents. The solute was kept out of the light since TMB is light reactive.
7. 150µl substrate solution was dispensed into every well.
8. The plate was immediately sealed with clingfilm and incubated on the shaker, at room temperature and in the dark, for 30-60 minutes.
9. The plate was checked after five minutes for appearance of blue colour in the wells. This indicates a reaction is occurring and the plate is developing.
10. The plate was checked again after 30 minutes and at regular intervals to monitor development.
11. Adequate plate development was assessed by eye. Zero-standards develop a darker blue colour (**OD** = 1). Low concentration standards show weaker colour development (**OD** <<1). A gradient of colour change will be visible throughout the standard curve (wells containing low concentrations of the stock standard solution will appear dark blue and wells containing high concentrations of the stock standard solution will appear pale blue).
12. Once colour development was complete the enzyme reaction was stopped by adding 50µl 2M H₂SO₄ to every well.
13. The optical densities of each well in the plate were measured using a Multiskan Ascent (Thermo Labsystems) plate photometer at a wavelength

of 450nm. This system provided data on the **OD** of every well on the plate, and was used to construct a standard curve for the serial standard dilutions, based on a four-parameter logistic (sigmoidal) function.

14. The linear range of the standard curve was used to determine appropriate sample dilutions (only ODs within the linear range of the standard curve were accepted), and sample hormone metabolite concentrations.

The criteria for acceptance of an assay plate as reliable were:

The Blank must measure <0.1 nm **OD**. The Blank provides a measure of background reactivity, and should be close to zero. A high blank value indicates an unacceptable level of background noise, possibly due to contamination.

The Zero must fall within an **OD** range $0.5 < 1.5$ nm. An optimal value for the Zero is 1.00nm. A value below 0.5nm indicates the plate has not developed sufficiently (e.g. due to incomplete binding during the final substrate stage). A value above 1.5nm indicates the plate has over-developed (e.g. the plate was left to incubate for too long during the final substrate phase, or the reaction was not stopped fully.)

The standard curve requires a correlation coefficient (r) close to 1, indicating it is close to the appropriate four parameter function. This provides a measure of the linear function of the serial dilutions of the standard. If the serial dilutions of the standard are poor then the values against which the samples are calibrated will be inaccurate, leading to unreliable data on hormone concentration of the samples.

A plate should contain no more than five poor duplicates. A poor duplicate is defined as one where the difference of the two **OD** values for a duplicate is greater than 10%. Poor duplicates reflect overall plate quality, and may be due to a number of factors. Multiple poor duplicates indicate poor reliability across the plate. Poor duplicates for the standard will provide an unreliable standard curve against which to compare sample values. A plate may be rejected if there is ≥ 1 poor duplicate in the standard curve.

The Coefficient of variation (Cv) between the two sets of QCH and two sets of QCL readings on a plate should be less than 10%. A Cv above 10% for either of the QCH or QCL indicates readings across a plate are not comparable. Post hoc to completion of all plates, QCH and QCL values are assessed for inter-assay variation. QCH and QCL values should each fall with 2SD of the mean inter-assay variation. QC values outside of 2SD of the mean inter-assay variation indicate that all readings from that plate are unlikely to be comparable with values measured on other plates. Once all plates have been run, plates for which the QCH or QCL fall outside of 2SD of the mean for all plates should be rerun, until all plates fall with 2SD of the mean.

Where a plate failed to reach these criteria it was discarded and a repeat plate prepared. Where a plate met all of the above criteria but there were some (≤ 5) poor duplicates, only the samples for which duplicates were poor were repeated on the next plate. In addition, samples for which the **OD** fell outside the linear range were tested again, at the same dilution factor for samples that fell outside, yet close to, the limits of the linear range, or at an adjusted dilution factor for samples that fell far outside the linear range.

3.6. Protocol for running dilution plates

Two microtitre plates, each containing serial dilutions of five samples, were used to determine an appropriate dilution factor for samples of both high and low [fGCM], and to assess degree of parallelism. The layout of the microtitre plates is shown in figure A3.2.

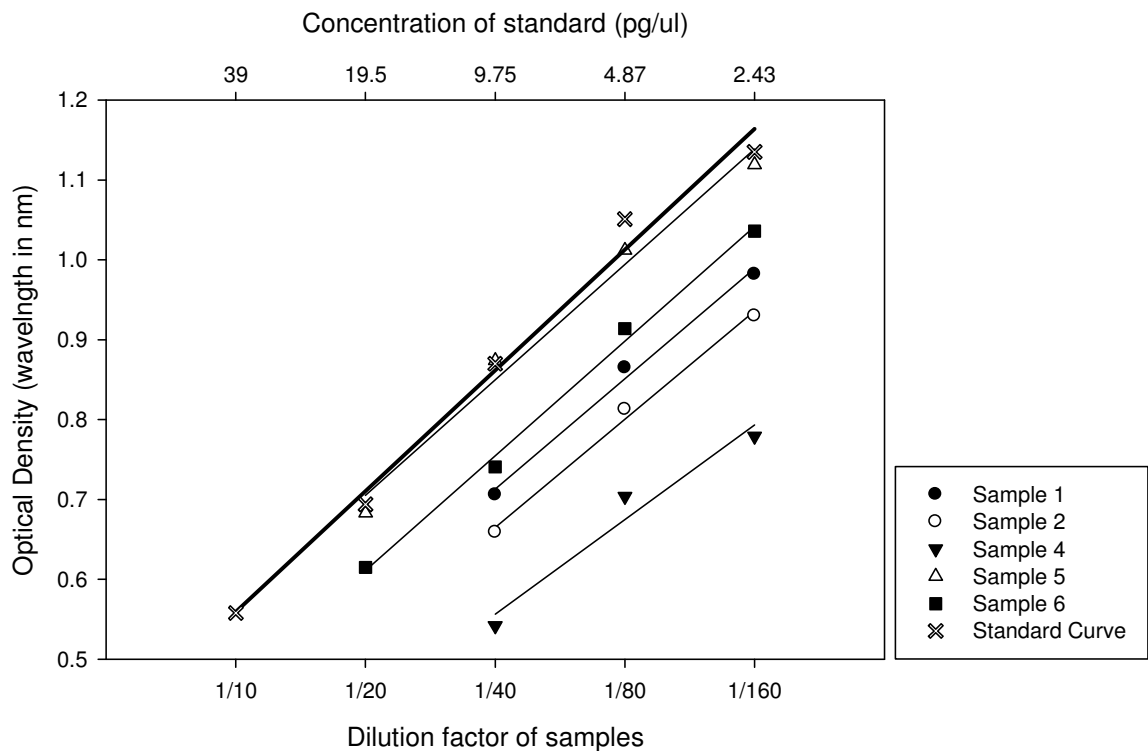
Samples were selected to provide [fGCM]s that were likely to be at the higher and lower ends of the likely range of all samples to be analysed to ensure that a dilution factor most appropriate for the maximal number of samples was selected. The six samples were each serially diluted at five concentrations of 1/10, 1/20, 1/40, 1/80 and 1/160 and dispensed onto a plate (Plate 1) following the scheme shown in Figure A5.1. The procedure was repeated using the same sample extracts on a second plate (Plate 2) with serial dilutions at 1/40, 1/80, 1/160, 1/320 and 1/640. For each plate the EIA was conducted as described in Appendix GM3.

Figure A3.2. The layout of the 96-well microtitre plate used to determine the appropriate dilution factor for samples.

	1	2	3	4	5	6	7	8	9	10	11	12
A	Blank	Blank	S1	1/10	S2	1/10	S3	1/80	S4	1/160	S6	1/10
B	Zero	Zero	S1	1/20	S2	1/20	S3	1/160	S5	1/10	S6	1/20
C	0.6	0.6	39	39	S2	1/40	QCH1	QCH1	S5	1/20	S6	1/40
D	1.21	1.21	78	78	S2	1/80	QCL1	QCL1	S5	1/40	S6	1/80
E	2.43	2.43	156	156	S2	1/160	S4	1/10	S5	1/80	S6	1/60
F	4.87	4.87	S1	1/40	S3	1/10	S4	1/20	QCH2	QCH2		
G	9.75	9.75	S1	1/80	S3	1/20	S4	1/40	QCL2	QCL2		
H	19.5	19.5	S1	1/160	S3	1/40	S4	1/80	S5	1/160		

Results from the inspection of optical density readings for the samples revealed that all samples fell within the linear range of the standard curve at a dilution of 1/80, and most fell within the linear range at 1/40. Serial dilutions for five samples on the second of the two dilution plates performed (Plate 2), which contained sample dilutions between 1/10 and 1/160, provided three or more optical density readings that fell within the linear range of the standard curve. Regression lines for the serial dilutions of the five samples and the standard curve are shown in Figure A3.3.

Figure A3.3: The optical density readings for the linear range of the standard (known concentrations between 39 – 2.43 pg/μl) and five extracts containing fGCM at dilutions between 1/10 and 1/160. Regression lines for each sample are shown. The regression line for the standard curve is shown in bold.



The standard curve represents the standard against which sample readings are calibrated in calculation of their respective OD readings. Therefore, samples which fail to dilute in parallel to the standard will provide inaccurate data on fGCM levels. Degree of parallelism was more accurate between sample dilutions than between the samples and the standard. Since it was the comparison of values between testing conditions for each monkey, and not absolute [fGCM] that was of interest, care was taken to include all samples from a given individual on as few plates as possible and, where possible, to assay all samples at the same dilution for that individual.

3.7. Protocol for calculating intra-assay variability

A series of six test plates were run to assess the level of intra-assay variability prior to conducting the EIA using the samples containing [fGCM]. Each test plate contained pairs of duplicate wells for each of the blank, zero, standard dilutions, QCHs and QCLs. Two samples of known high and low concentration (H and L) were pipetted, in duplicate, into the remaining wells as shown in Figure 3.4. The average concentration readings for the 17 pairs of H wells and the 16 pairs of L wells were used to calculate the coefficients of variation for each. A predetermined level of 15% was set as the maximum cut-off for intra-assay reliability to be accepted.

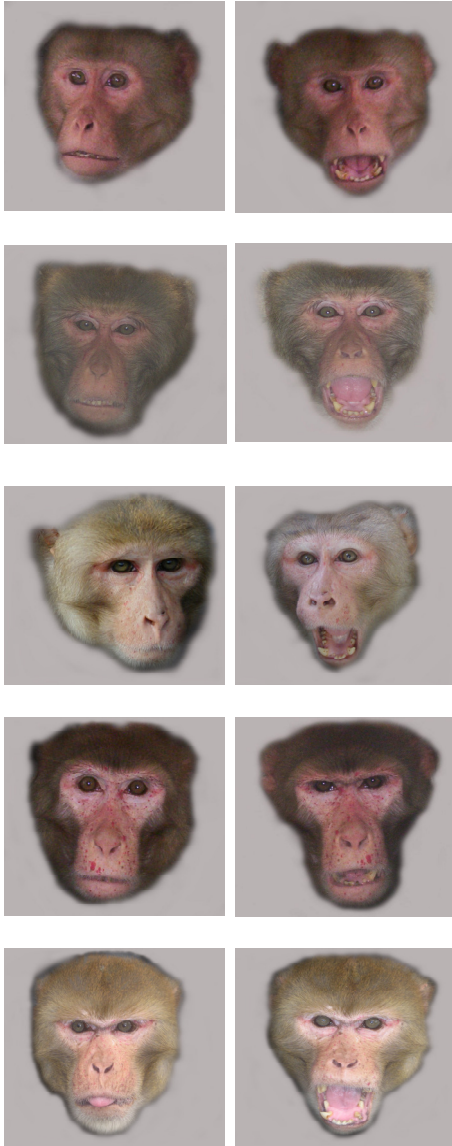
Figure A3.4. The layout of the 96-well microtitre plate as used for assessing the intra-assay reliability for samples of high and low concentration.

	1	2	3	4	5	6	7	8	9	10	11	12
A	Blank	Blank	H	H	L	L	H	H	L	L	H	H
B	Zero	Zero	L	L	L	L	H	H	L	L	H	H
C	0.6	0.6	39	39	L	L	QCH1	QCH1	L	L	H	H
D	1.21	1.21	78	78	L	L	QCL1	QCL1	L	L	H	H
E	2.43	2.43	156	156	L	L	H	H	L	L	H	H
F	4.87	4.87	H	H	L	L	H	H	QCH2	QCH2	H	H
G	9.75	9.75	L	L	L	L	H	H	QCL2	QCL2	H	H
H	19.5	19.5	H	H	L	L	H	H	L	L	H	H

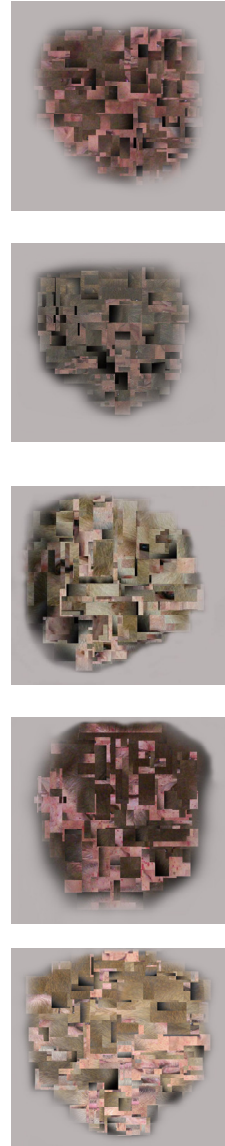
Appendix 4

Face stimuli used to study Attentional Bias (Chapter 4)

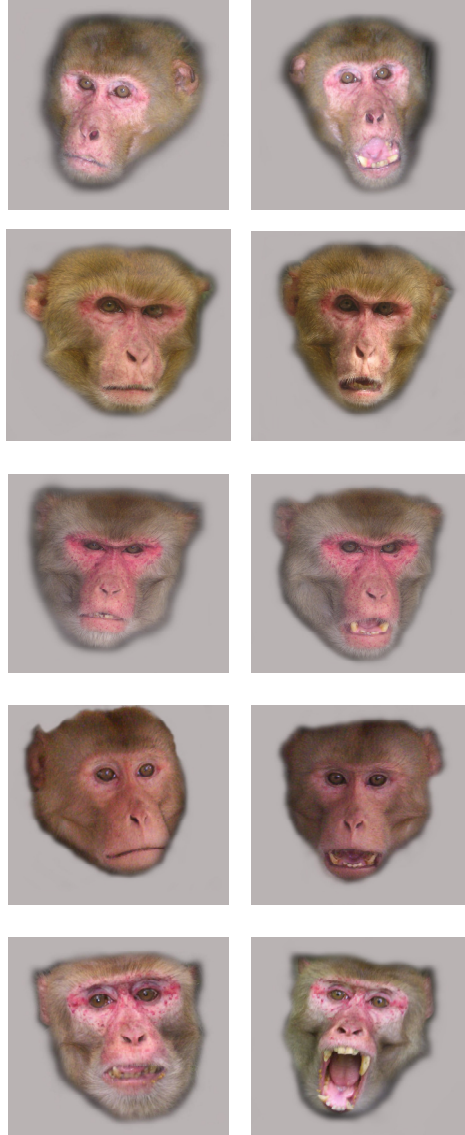
Set 1
Neutral and aggressive faces



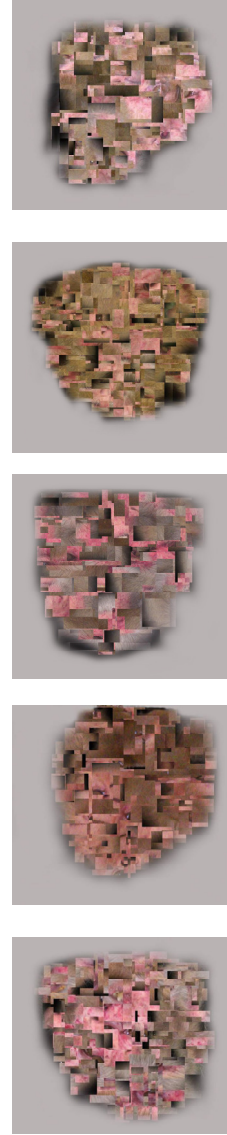
Set 1
Scrambled faces



Set 2
Neutral and aggressive faces



Set 2
Scrambled faces



Appendix 5

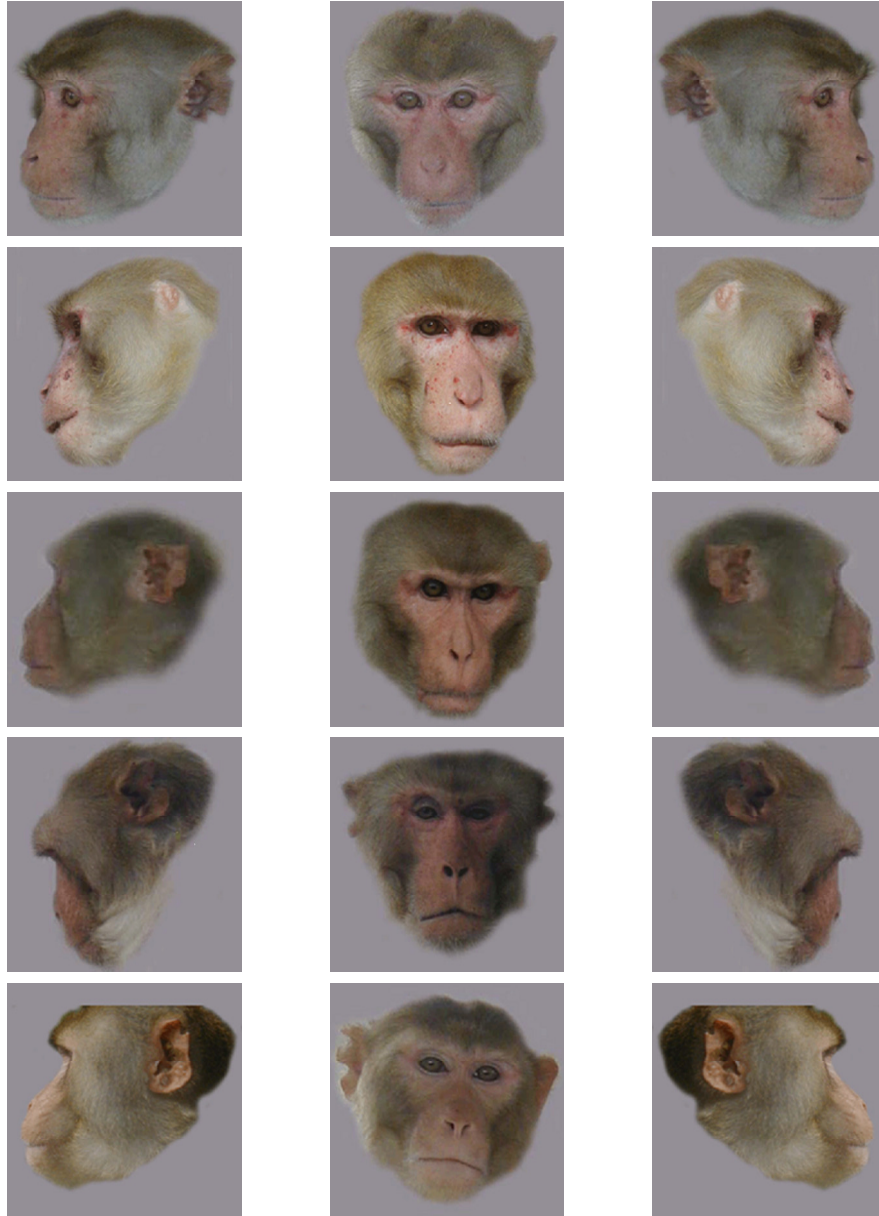
Additional information for emotion evaluation and response slowing (Chapter 5)

5.1. Face probes

5.2. Distribution of invalid trials

5.1. Face probes

Probe Set 1



Probe Set 2



5.2. Distribution of invalid trials

Table A.5.1. The distribution of invalid trials that were removed for each monkey

ID	Responses <400ms						Non-responses						Total
	Post enrichment			Post health check			Post enrichment			Post health check			
	Control	Direct gaze	Averted gaze	Control	Direct gaze	Averted gaze	Control	Direct gaze	Averted gaze	Control	Direct gaze	Averted gaze	
29C	-	-	1	-	6	-	-	-	-	-	-	-	7
C55	-	1	1	-	3	1	-	1	1	-	-	-	8
16P	1	1	3	1	3	6	-	-	-	-	-	-	15
AI73	2	2	-	1	-	1	3	11	9	1	7	3	40
92R	8	14	15	9	20	19	-	-	-	-	-	-	85
27S	2	3	1	4	7	3	1	-	-	1	1	-	23
66S	3	6	3	-	11	11	3	1	-	-	-	1	39
Total	67			106			30			14			217

Appendix 6

6.1 Table showing the dimensions of the stimuli and probes.

6.2 Table showing individual data sets for proportion of responses made to stimuli of learned reward value (S+ and S-) and three ambiguous probes (P+, Pi and P-).

6.3 Table showing the distribution of invalid trials.

6.4 Seven monkeys reached the training criterion for inclusion in the study. Three monkeys in Group 1 and four monkey in Group 2 learned to perform a ‘Go-NoGo’ task. Criteria were: correct responses on $\geq 80\%$ of trials overall, with $\geq 70\%$ accuracy for each of the ‘Go’ and ‘NoGo’ trials respectively, on three successive days of training. Six monkeys failed to meet these training criteria. * signifies criteria were not met.

6.1 Dimensions of the stimuli and probes

Table A. 6. 1 The dimensions of the two control stimuli (short line and long line) and three experimental probes

Stimulus/ Probe	Length (mm)	Width (mm)	Area (mm ²)	Luminosity ¹	Contrast Energy ²
Control (short line)	16	11	176	06.79	0.99
Experimental Probe 1	22.5	11.5	259	09.21	0.99
Experimental Probe 2	33	12	396	14.86	0.99
Experimental Probe 3	49.5	12.5	594	25.45	0.99
Control (long line)	70	13	910	37.83	0.99

1 Luminosity value obtained from Adobe Histogram function

2 Contrast energy calculated as differential of Lmax and Lmin, as described in Chapter 4

6.2 Individual proportion data

Table A. 6.2 The proportion of responses made on control trials (S+, S-) and experimental trials (P+, Pi and P-) by each monkey in each treatment

ID	Post-enrichment					Post-health-check				
	S+	P+	Pi	P-	S-	S+	P+	Pi	P-	S-
94K	0.75	0.72	0.67	0.11	0.06	0.38	0.00	0.17	0.00	0.00
79S	0.90	0.94	0.72	0.33	0.13	0.94	0.78	0.83	0.33	0.17
79T	0.97	0.94	0.83	0.72	0.22	0.86	0.94	0.83	0.33	0.07
86O	0.86	1.00	0.89	0.50	0.18	0.85	0.94	0.72	0.44	0.21
16P	0.85	0.89	1.00	0.50	0.28	0.79	0.72	0.67	0.44	0.35
66S	0.86	0.83	0.89	0.17	0.31	0.86	0.67	0.67	0.28	0.26
AI73	0.75	0.83	0.50	0.28	0.07	0.67	0.58	0.17	0.25	0.06
\bar{X}	0.85	0.88	0.79	0.37	0.18	0.73	0.65	0.55	0.29	0.16
SE	±0.03	±0.04	±0.06	±0.08	±0.04	±0.09	±0.12	±0.13	±0.06	±0.05

6.3 Distribution of invalid trials

Table A.6.3 The distribution of invalid trials removed from the analyses

ID	Responses <400ms										Non-responses										Total
	Post enrichment					Post health check					Post enrichment					Post health check					
	S+	P1	P2	P3	S-	S+	P1	P2	P3	S-	S+	P1	P2	P3	S-	S+	P1	P2	P3	S-	
94K	15	2	3	5	4	2	0	0	0	0	2	1	3	5	57	16	6	5	6	24	156
79S	11	3	1	0	1	6	2	1	0	2	7	1	5	12	63	4	4	3	12	60	198
79T	15	2	3	5	4	7	2	2	3	2	2	1	3	5	57	10	1	3	12	67	206
86O	4	3	1	1	5	7	2	1	4	6	10	0	2	9	59	11	1	5	10	57	198
16P	23	2	1	2	3	18	4	4	1	7	22	4	3	11	56	21	7	8	12	49	258
66S	27	6	6	1	21	19	5	7	4	12	10	3	2	15	50	9	5	6	13	53	274
AI73	2	0	1	0	1	0	0	0	0	0	18	3	9	13	67	15	5	10	9	42	195
Total	184					130					590					581					1485

Table A.6.4 Training data and individual response accuracy

	ID	Age (yrs)	N daily training sessions to reach criterion [or cessation of training]	N daily training sessions performed at criterion prior to start of testing	Response accuracy (%) [Go/NoGo]	Criteria met? (yes/no)
Group 1	C55	24.7	[5]	[0]	[42/54]*	×
	06H	9.9	[8]	[0]	[13/96]*	×
	94K	7.4	28	11	87/100	✓
	92R	4.8	[27]	[0]	[50/42]*	×
	27S	4.6	[40]	[2]	[80/25]*	×
	79S	3.7	20	6	93/93	✓
	79T	3.7	19	10	100/97	✓
Mean		8.4 ± 2.8				n=3
Group 2	29C	12.0	[4]	[0]	[0/100%]*	×
	94E	10.2	[1]	[0]	[0/100%]*	×
	86O	5.3	27	9	93/87	✓
	16P	5.2	43	5	87/87	✓
	66S	3.8	31	10	100/80	✓
	AI73	3.6	32	7	70/93	✓
Mean		6.7 ± 1.4				n = 4
Total	n=13					n = 7

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