Improving the Welfare of Laboratory-Housed Primates Through the Use of Positive Reinforcement Training: Practicalities of Implementation

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Output Related to the Thesis

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Presentations

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Abstract

Whilst there has been a recent increase in interest in using positive reinforcement training for laboratory-housed primates, there remains a reluctance to put into practice training programmes. Much of this reticence seems to stem from lack of expertise in the running of training programmes, and a perception that training requires a large time investment, with concurrent staff costs. The aim of this thesis was to provide practical recommendations for the use of training programmes in laboratories, providing primate users and carestaff with background information needed to successfully implement training programmes whilst improving the welfare of the animals in their care. Training was carried out with two species, cynomolgus macaques (*Macaca fascicularis*) and common marmosets (*Callithrix jacchus*) in three different research laboratories to ensure practicability was as wide ranging as possible.

Training success and the time investment required were closely related to the primates' temperament, most notably an individual's willingness to interact with humans, in both common marmosets and cynomolgus macaques. Age and sex however had no effect on an individual's trainability. The training of common marmosets was more successful than that with cynomolgus macaques, possibly due to differences in early experience and socialisation. Positive reinforcement training helped both species to cope with the stress of cage change or cleaning, with the monkeys showing less anxiety-related behaviour following the training programme than before.

Involving two trainers in the training process did not affect the speed at which common marmosets learned to cooperate with transport box training, but behavioural observations showed that initial training sessions with a new trainer led to animals experiencing some anxiety. This however was relatively transient. Whilst the training of common marmosets to cooperate with hand capture was possible, there seemed little benefit in doing so as the monkeys did not show a reduced behavioural or physiological stress response to trained capture as compared to hand capture prior to training. However strong evidence was found that following both training and positive human interactions the marmosets coped better with capture and stress was reduced.

It is recommended that an increased use of early socialisation would benefit laboratory-housed primates, and would also help improve the success of training. Further, the time investment required shows that training is practicable in the laboratory for both species, and that positive reinforcement training is an important way of improving their welfare likely through reducing boredom and fear.

CHAPTER 1

CAPTIVITY, ANIMAL WELFARE AND TRAINING

"Life is first boredom, then fear"

Philip Larkin, Dockery and Son (1964)

1.1 CAPTVITY

Humans keep large numbers of animals in captivity; predominantly animals are kept for food, with over 900 million animals farmed annually (FAWC, 2010), but also includes companion animals, zoo exhibits and those kept for research purposes. Animals in captivity face a number of challenges to their welfare; they are predominantly housed in confined environments, which are less complex and more predictable than those in which they are adapted to live, and even when species are domesticated this often leads to their welfare being compromised. Whilst in the wild animals are faced with predators, food shortages, changing weather and illness as well as an ever changing physical environment, in captivity, especially in intensive farming and laboratories, animals are thermally comfortable and have adequate nutrition (although both farm and laboratory-housed animals can be subjected to food restriction) but they experience structured and highly predictable lives (Webster, 1994, Wemelsfelder, 1998).

The relationship between predictability and animal welfare is complex. Most research has concentrated on the effect of the predictability of *events* such as electric shock and food delivery (reviewed in Bassett & Buchanan-Smith, 2007). Early work on the

subject by Weiss (1970) found that rats able to predict an aversive electric shock showed a less pronounced stress response than those unable to predict the shock, leading to the conclusion that predictability reduces stress. Positive events, such as appetitive events though, are recommended to occur on an unpredictable schedule as a highly predictable environment, lacking in the challenges animals are adapted to cope with, and leaving them without any meaningful activity may lead to them experiencing boredom (Morton, 1997, Wemelsfelder, 1990, 1998, 2005, Rennie & Buchanan-Smith, 2006b).

There has been a reluctance amongst many researchers to attribute the subjective feeling of boredom to animals, whilst other emotions such as fear and frustration are commonly accepted, although there seems little scientific basis for this (Wemelsfelder, 1990). Those scientists who argue that animals can experience boredom identify behaviours relating to increased passivity such as lying and 'non-behaving' as well as active behaviours such as redirected behaviour and stereotypies which are similar to those seen in humans asked to complete repetitive tasks which they report as being boring (Wemelsfelder, 1990, 2001). There seems to be sufficient evidence that animals can experience boredom, especially if it is thought of as psychological response rather than something more cognitively complex as proposed by Wemelsfelder (2005), to consider boredom as a serious welfare concern for many captive animals.

Whilst boredom in itself negatively impacts upon an individual's welfare, it has further consequences on how animals cope with their environment. Bored animals will often spend most of their time inactive, but when faced with an unusual or surprising event

they are likely to over react, showing an exaggerated fear response (Wemelsfelder, 2005). Whilst whether animals can experience boredom is still debated, the concept that animals can and do experience fear is more accepted, as it seems to be comparable to pain in being a physiological response (Morton, 1998), albeit with a subjective aspect. Humans are likely the greatest source of fear to captive animals, as alongside the control we have over their environments, our physical presence may also elicit fear responses. Fear of humans has been shown to affect basic functions such as growth rates and reproductive performance in farm animals (e.g. Barnett *et al*, 1994, Hemsworth *et al*, 1995), and in non-human primates (hereinafter primates) routine exposure to humans for extended periods has been associated with higher wounding rates (Lambeth *et al*, 1997) and increased heart rates (Manilow *et al*, 1974, Line *et al*, 1991), both suggestive of a fear response.

Primates are particularly likely to suffer from both boredom and fear in captivity, especially in the laboratory. Their housing, however good, is unlikely to allow primates to express the full extent of their complex cognitive and social abilities. They are then exposed to frequent interactions with humans, of whom they are naturally fearful (O'Neill, 1989), and who may perform painful or unpleasant scientific procedures on them, or even just reinforce their fear by catching them or performing other routine husbandry procedures which the animals find distressing. Additionally, rearing practices frequently used with primates such as early weaning and hand-rearing, where maternal deprivation occurs, lead to a reduced ability to cope with stress in later life (Dettling *et al*, 2002, Pryce *et al*, 2005), whilst peer-reared primates show greater fear responses to humans than those raised by their mothers (Novak *et al*, 2006).

Welfare standards are certainly improving within laboratories with large cage sizes and greater complexity of furnishings and other environmental enrichment. None-the-less there is always room for further improvement and it is critical to explore all ways to improve welfare. Positive reinforcement training is one way in which the relationship between primates and humans can be enhanced, and thus improvements made to laboratory-housed primate welfare. Providing animals with cognitive stimulation through PRT may reduce boredom, and increasing positive interactions with humans has been shown to decrease the fear and stress associated with aversive experiences (Reinhardt, 1992a).

1.2 ANIMAL WELFARE

The definition of welfare has been the subject of much academic debate, most notably in the late 1980s and 1990s, when many leading researchers attempted to provide working definitions of the concept of welfare (reviewed in Fraser, 2009). Three different approaches have been taken; biological functioning, subjective experience and the nature of animals. Interestingly, those most concerned with defining animal welfare have tended to be researchers of farm animal behaviour and welfare (e.g. Broom 1991, 1996, Fraser, 1999, 2009). The problem of defining the concept of welfare is one which seems to have troubled those concerned with laboratory animals less than those working with farm animals. Perhaps this is due to the nature of research with animals, which often involves compromising welfare, however it is defined. Further, it may be that the tighter legal framework surrounding the use of animals in the laboratory leaves less room for the interpretation of the concept of welfare.

The welfare of laboratory animals is predominantly based on the concept of refinement as initially defined in the framework of the 3Rs (replacement, reduction, and refinement) first proposed by Russell and Burch (1959). Russell and Burch used the term refinement to refer only to the minimisation of suffering during experimental procedures. However, the welfare of laboratory-housed primates is affected not only by research procedures, but also by their whole life experience from birth up to and including death (Buchanan-Smith *et al*, 2005, JWGR, 2009). This definition of refinement is now understood to include all aspects of the lifetime experience of animals including breeding, weaning, acquisition, transport, housing, husbandry and enrichment, and the fate of the animals at the end of the protocols, with all of these factors now being considered under the same framework (Buchanan-Smith *et al*, 2005). It refers to both minimisation of negative welfare states and also proactively aims to enhance positive welfare. In contrast, farm animal welfare is commonly determined in terms of the 5 Freedoms (freedom from hunger and thirst, freedom from discomfort, freedom from pain, injury and disease, freedom to express normal behaviour and freedom from fear and distress) established by the Farm Animal Welfare Council (1992), and this may also lead to differences in how welfare is perceived by various authors. However, similar to the historical perspective on welfare in laboratories, four of these freedoms are based on freedom from negative welfare states, and only one, freedom to express normal behaviour, focuses on promoting the positive. Changes in attitudes to animal welfare mean that more focus is now being placed on promotion of positive welfare (e.g. Napolitano *et al*, 2009, Westerath *et al*, 2009). The different approaches to animal welfare are described below, to provide a basis for the choice of animal welfare conception in this thesis. The approaches, whilst based of different principles, will often reach a similar conclusion, and need not be mutually exclusive.

1.2.1 Welfare in Terms of Biological Functioning

Defining welfare in terms of the biological functioning of the animal generally concerns what might be considered production factors, those such as disease, reproduction and growth rates, along with less apparent factors relating to the physiological coping of the individual in its environment, but which may lead to changes in the aforementioned indicators (Fraser & Broom, 1990, Duncan & Fraser, 1997). High welfare is associated good growth and reproduction, lack of injury and disease, longevity, and the animal coping easily with its environment, whilst poor welfare results from the failure to cope with the environment, which leads to injury, disease, poor growth and reproduction, and even death (Fraser & Broom, 1990, Broom, 1991, Duncan & Fraser, 1997). However, whilst it is generally agreed that conditions which lead to injury, severe disease and malnutrition lead to poor welfare (Wolfensohn & Lloyd, 1994, Fraser, 1995, Dawkins, 1998), the relationship between biological function and good welfare is more difficult.

Duncan and Fraser (1997) noted the influence on this approach to welfare of Seyle's (1950) work on stress, whereby he identified the relationship between activation of the hypothalamic-pituitary-adrenal (HPA) axis glucocorticoids and challenges such as cold and restraint. Further, Moberg (1985) identified stress as a risk to an animal's welfare. Welfare based on a biological functioning approach is relatively simple to measure objectively, and as such has formed the basis for much of the research into animal welfare (Duncan & Fraser, 1997). Hormonal and immunological responses to stress have been identified in primates (Line *et al*, 1991, Reinhardt, 1999, 2003, Lambeth *et al*, 2004, Honess *et al*, 2005b), and also other responses such as alopecia (Honess *et al*, 2005a), and together these form a basis for welfare research. Behaviour can be used to

assess biological functioning in animals, and impairment of physiological coping is commonly reflected by changes in behaviour (Mench & Mason, 1997).

Proponents of the biological functioning approach suggest that whilst subjective suffering matters, the measurement of this is not validated, and thus measurements of biological functioning are the only way to assess welfare (Gonyou, 1993). Others go even further, attaching little importance to the feelings of animals (McGlone, 1993). The biological approach to welfare is however limited in that whilst it may be argued that animals with poor welfare have higher incidences of disease, are malnourished, or fail to reproduce, the converse is not necessarily so; disease-free, well-fed, breeding animals cannot be assumed to have good welfare, especially if those animals have been specifically bred for high growth and reproduction rates. Further, there are considerable difficulties in identifying cut off points for changes in biological functioning which may affect welfare (Mendl, 1991, Mason & Mendl, 1993, Mench, 2003).

1.2.2 Welfare in Terms of the Nature of Animals

The definition of welfare in terms of the animal's nature is one which takes a slightly more philosophical approach. Rollin (1993) suggested that all animals have something he termed '*telos*', its genetically predisposed nature; for example it is in the *telos* of canaries to fly, pigs to root and cattle to ruminate (Duncan & Fraser, 1997). In order for an animal to have good welfare it must be kept in conditions which will satisfy its *telos* (Rollin, 1993), and allow it to perform its full repertoire of behaviours (Kiley-Worthington, 1989). The natural behavioural repertoire of wild animals however

includes behaviours related to adverse events (Dawkins, 1980), such as escape from predation, and this leads to a major criticism of this approach to defining welfare (Dawkins, 1980), as it cannot be said that animals suffering severe problems in nature have good welfare (Poole, 1996). This does not mean however that natural behaviour is not desirable; the performance of species-specific behaviours indicates that the captive environment is providing a similar environment to the one which the animal is physiologically and behaviourally adapted, and also that the behavioural needs of the animals are being met (Shepherdson, 1990).

1.2.3 Welfare in Terms of Subjective Experience

The subjective approach to animal welfare is based upon what the animal feels and experiences, and therefore emotional states, which most people accept animals can experience (Duncan & Fraser, 1997), such as pain, fear and happiness are important for this approach. Suffering, and the impact this has on welfare, has been identified as critical in the definition of animal welfare (Mason, 1991, Dawkins, 1998). Indeed Duncan and Petherick (1991) argue that how the animal feels, its subjective experience, is the only thing which matters in terms of its welfare. There is a debate about whether animals such as primates with complex cognitive abilities are likely to experience greater suffering, and therefore worse welfare (Bekoff, 1994, Mendl *et al*, 2001). Indeed Broom (2010) argues that cognitively more sophisticated animals may have the ability to cope with adversity better, but also be able to experience greater pleasure. Although measuring the subjective states of animals directly may not be possible, interpretation of physiological and behavioural in order to infer subjective feelings is frequently used (Duncan & Fraser, 1997).

Measures used to assess subjective experience of welfare include the use of preference testing, in which animals are given choices between two or more environments (e.g. Hughes & Black, 1973, Dawkins, 1977, Blom *et al*, 1992, 1993, Badihi, 2006), assuming that they prefer environments where they experience more comfort and less unpleasantness (Broom, 1988, Cooper & Mason, 2000, Mendl, 2001). Most of this research has been carried out in order to determine the preferred options from a range available. However this technique can also be used to assess animals' subjective experience of aversive stimuli, with the time spent in an unpleasant environment considered to be inversely related to its aversiveness, for example their aversion to different gases used for euthanasia or anaesthesia (Raj & Gregory 1995, Raj, 1999, Leach *et al*, 2002a, b). One criticism of this approach is that is does not measure the strength of the preference an animal shows (Duncan & Fraser, 1997). Another criticism is that it is not necessarily follow that by having to make do with a less preferred option means that the animal suffers (Dawkins, 1983). To avoid such criticisms, consumer demand theory is often used. Here the 'price' an animal pays for the commodity (e.g. the number of bar pushes it will perform, or the weight of a push door) can be used to determine how strong the preference is; a weak preference will be demonstrated by animals paying a lower 'price' than when preference is strong (Dawkins, 1990). However behaviour, and how an animal interacts with a resource has been shown to change dependent on its cost, highlighting the importance of looking further than merely time spent in a particular environment (Cooper & Mason, 2000).

Another recent theme of research has been that which considers the ability of people to interpret the emotional status of animals. Wemelsfelder and colleagues (e.g.

Wemelsfelder *et al*, 2001, 2009, Wemelsfelder & Farish, 2004, Rousing & Wemelsfelder, 2006) have validated a technique in a number of species which asks observers to make qualitative assessments of the welfare of animals using their expressive body language, whereby observers use self-generated qualitative terms, such as 'content' or 'anxious', to quantitatively describe individuals or groups of animals (Wemelsfelder & Farish, 2004). Results show that this qualitative assessment is in good agreement with quantitative assessment of behaviour (Rousing & Wemelsfelder, 2006). Further, qualitative assessments show good agreement with physiological measures such as heart rate and heart rate variability (unpublished data reported in Wemelsfelder, 2007, Farish, personal communication), perhaps overcoming a criticism of this approach that it is too anthropomorphic.

1.2.4 The Integrated Approach to Welfare Used in this Thesis

Laboratory-housed primates live in artificial environments which are likely to compromise their welfare, however it is defined. The measurement of welfare in this thesis is primarily done using behaviour, which can be interpreted in different ways,. Behaviour is the means by which an animal can respond to, and in some cases, control stressors, with cortisol being the hormone most closely related to this response. Therefore cortisol was chosen as a direct measure of physiological functioning, and response to a potentially stressful routine event (capture). The complexity and stimulation provided by a natural environment can rarely be recreated in captivity, especially in the laboratory, so it is likely that laboratory-housed primates will not perform their full behavioural repertoire. Whilst the promotion of natural behaviours is encouraged, it may be that the consequences of the performance of behaviours that are

more beneficial (Veasey *et al*, 1996), so perhaps the comparison between wild and captive behaviour is of limited use. Thus, few direct comparisons between behaviour in different environments are made, although in broad terms more 'natural' behaviour patterns are seen as indicators of positive welfare. Welfare in this thesis is considered to be predominantly a subjective feeling, in line with, for example Dawkins (1980, 1990) and Duncan (1996). Thus behaviour, in this thesis is chiefly interpreted as a measure of subjective feelings. Behaviours which are linked to relaxed activity are considered to reflect, if not good welfare, at least an absence of poor welfare, whilst passive inactivity is suggestive of feelings such as boredom, and increased vigilant behaviour as an indicator of perceived risk, anxiety and potentially of fear. Other species-specific measures of anxiety are also used, such as increased scent marking in common marmosets (*Callithrix jacchus*, Bassett *et al*, 2003). As the potential benefits of positive reinforcement training include providing predictability and control (to reduce fear), and cognitive challenge (to reduce boredom), these measures may be particularly pertinent to the assessment of welfare.

1.2.5 The Scientific Importance of Animal Welfare

There is an increasing awareness that the welfare of animals held for research purposes can impact upon the science being performed. Results of studies carried out on healthy animals who are not fearful are likely to be more consistent and meaningful than those carried out on animals with poor welfare such that behaviour and physiology are compromised (Poole, 1997, Reinhardt, 2004). In rodents it has been shown that standardized housing can impair the development of behaviour and brain function, which in turn impacts on the validity of the science for which they are used (Würbel,

2001). Thus, the drive towards environmental standardisation may have actually lead to greater variability in the science. Further, Garner (2005) suggests that animals exhibiting abnormal behaviours, such as stereotypy and self-mutilation are an indicator of poor welfare, may reduce scientific reliability and replicability, and thus improving welfare will improve science. However, much further research is required to establish how welfare and the quality of science interact across different species and scientific research.

1.2.6 The Ethical Importance of Animal Welfare

Much of the concern over, and study of, animal welfare is based on the assumption that humans have an ethical and moral responsibility for animals (Sandøe *et al*, 1997). Four main ethical standpoints have been identified in the ethical view of animals with whom we interact. A utilitarian view primarily assesses ethical importance of the individual, and their capacity to suffer, and compares that against the interests of the other parties concerned (Singer, 1975, Sandøe *et al*, 1997). This viewpoint informs much of the current legislature in the UK, with justification for animal experimentation requiring an analysis of the potential benefits of the research to be weighed against the cost in terms of suffering to the animals involved (Home Office, 1986a). However, others argue that the interests of one party should not be compromised for the benefit of another, and this animal rights view has been predominantly advocated by Regan (1984). Following an animal rights ethical perspective there is no justification for the use of animals in research or agriculture, or holding them in zoos (Regan, 1995). A third view concerns the integrity of the species, which not only considers the individual but also moral obligations to the species, for example by not letting species become extinct (Rolston,

1989, Sandøe *et al*, 1997). A final ethical viewpoint is the agent centred approach which considers how our treatment of others, including animals, affects us as humans, an approach taken by Kant (1989). Under this ethical framework 'cruelty' is considered to be morally bad, whilst caring is judged as morally good (Sandøe *et al*, 1997).

The agent centred approach to the ethical treatment of animals makes common sense, yet criticism of this approach is that it is too easy for us to justify our actions (Sandøe *et al*, 1997). This agent centred view of ethics might be particularly pertinent to those working with research animals, especially carestaff who are expected to care for them, but also to conduct potentially painful or unpleasant procedures. It could be argued that carestaff have the greatest capacity to be 'morally damaged' through animal testing and experimentation. A utilitarian approach to the ethics of animal use in research has been predominantly adopted in the UK, and within this framework the welfare of animals attains high importance. From this ethical standpoint humans involved with the care of research animals, and perhaps even the wider public, should work to minimise harms to the animal by maximising welfare, and ensuring the best results come from their use.

1.2.7 The Welfare of Laboratory-Housed Primates

Every single aspect of the welfare of primates in laboratories is influenced by humans. Humans determine factors such as how big the cages should be, what and when animals are fed, when they are weaned, who they live with. Whilst a lack of environmental complexity and stimulation might lead to primates experiencing boredom, it is likely that interactions with humans are a source of fear and stress. Indeed the competence of staff caring for laboratory-housed primates may be the greatest influence on their welfare (Scott, 1990, Rennie & Buchanan-Smith, 2006a). It is vital that staff interact

with primates positively, as there is good evidence that this improves health, welfare and the ability of the animals to cope with stress (Bayne *et al*, 1993, Bloomsmith *et al* 1997, 1999, Baker, 2004). The presence, and requirement to interact with, a human trainer is a big influence on their ability to successfully learn. Lack of fear of humans is therefore suggested as a major factor in the success of training programmes.

1.3 ANIMAL TRAINING

Many codes of practice and guidelines for the use of primates in research identify training as a means to refine primate welfare in the laboratory. The current UK Home Office code of practice identifies training animals to cooperate with routine procedures as the least distressing method of handling (Home Office, 1986b), whilst the U.S. Department of Agriculture (1999) goes further and specifically mentions the role of positive reinforcement training in reducing the stress animals experience. More recently the European Commission recognised the influence of positive training on the human-animal relationship in the laboratory, and the impact this has on the welfare of animals (European Commission, 2002). Other organisations such as the National Centre for the Replacement, Refinement and Reduction of Animals in Research (2006) and the International Primatological Society (2007) also recommend positive reinforcement training for primates in their guidelines.

Given this widespread recommendation of positive training, it is interesting that only two out of 15 surveyed institutions holding primates in the UK are using positive reinforcement training as the sole method of training their animals (Prescott & Buchanan-Smith, 2007). This suggests that the translation of theory to practice is not

being made. There may be a number of reasons for this, for example Prescott and Buchanan-Smith (2007) identified paucity of information on how to train as the primary reason as to why laboratories were not implementing training programmes. Of the institutes surveyed only six had a formal training programme in place with the remaining five institutes who trained their primates doing so on an *ad-hoc* basis. A lack of staff or the perceived time investment required for training has been identified by eight institutions as a further constraint on the uptake of training (Prescott & Buchanan-Smith, 2007), factors which could be addressed by the publication of data on time investment for common tasks, and identifying ways in which this time investment can be reduced.

1.3.1 The Terminology of Training

A number of types of animal training are discussed in this thesis, and whilst it is not my aim to expound these terms, it is important that they are defined for clarity in their subsequent use. Some terms, for example socialisation, may have wider meanings, but within this thesis the use of them is restricted to those definitions provided below (Table 1.1). Further, explanations of two of the basic types of training, target training and clicker training are provided along with a brief outline as to why these are often employed.

Term	Definition
Human Socialisation	The process of learning to interact successfully with humans
Habituation	The process by which response to a stimulus wanes as a result of repeated exposure (not through fatigue)
Desensitisation	The process of systematically pairing a positive reward with an aversive experience in order to reduce fear or anxiety
Negative reinforcement (NRT)	The process of increasing the frequency of a behaviour by removing something negative on its performance
Positive reinforcement (PRT)	The process of increasing the frequency of a behaviour by introducing something positive on its performance
Positive punishment	The process of reducing the frequency of a behaviour by introducing something negative on its performance
Negative punishment	The process of reducing the frequency of a behaviour by removing something positive on its performance

Table 1.1 Definitions of types of training used in this thesis. From McGreevy and Boakes (2007) Prescott *et al* (2005b) and Prescott and Buchanan-Smith (2007)

Target training

Target training is a behaviour which is commonly taught a first step in animal training. It has a two-fold benefit in that it provides a useful initial step in a number of other training tasks, and also provides a useful introduction to training for animals as they 'learn to learn'. It has been suggested than animals need to 'learn to learn' (Schapiro *et al*, 2005), in that they need to understand that, for perhaps the first time, their actions elicit a predictable and positive response from their caregivers. In a training paradigm animals need to be active in their behaviour in order to 'stumble' upon the desired

behaviour, or one which can be shaped into it, and using a secondary reinforcer can aid this. Initially animals are not just learning the task at hand, they are also learning about the consequences of their actions during training, and this process of 'learning to learn' is just as important as the actual outcome of the training task. As a result it is usual that the behaviour which is first trained is a relatively simple task but also one which has high utility. Target training fulfils this role well, in that it is simple for the animals to learn but also provides a useful basis for a number of other training tasks. The target can be any object, providing it is one which animals do not see regularly in another context, a pen or piece of laboratory equipment would not be suitable for example. Targets also need to be easily cleaned, easily replaced and available in distinct colours, or other variations, to enable individuals to identify their own specific target. The size, weight and shape of the target should be one that the animals can hold easily through the enclosure mesh/opening.

As noted above, training animals to touch and hold a specific target provides a basis for a number of other useful training tasks (Laule, 2010). Once an animal is target trained it can be moved easily around the cage, for example away from cagemates whilst they are being trained. They can also be moved into new areas, for example a transport box (Bowell *et al*, 2005), or onto weighing scales for body weight measurement (McKinley *et al*, 2003). Target training facilitates the selection of individuals from within a group, for example rather that catching an entire group the required individual can be requested to enter a transport box using his or her target, whilst other individuals can be kept away using their target. As such, target training has both a 'learn to learn' function and acts as a foundation to facilitate training in other tasks.

Clicker training

Whilst positive reinforcement training can be carried out without a secondary reinforcer, most animal training makes use of some form of intermediate reinforcer or signal between desired behaviour and reward. This secondary, or conditioned, reinforcer is something which initially has no meaning to the animal, but following repeated pairing with the primary reinforcer (usually food) becomes a reinforcer. The most commonly used type of secondary reinforcer in training is a device known as a clicker, though other means of producing a click (e.g. a retractable pen), whistles and voice commands are also used, and visual markers such as a light are occasionally used in specific situations, such as with deaf or very sound sensitive individuals. Clickers are commercially available small devices which, when a specific area is depressed, produce a distinct single click. The secondary reinforcer acts as a 'bridge' between the behaviour and reward. The real advantage of the secondary reinforce is that its precise timing allows training to be more flexible by marking the exact behaviour the animal is being rewarded for. This enables animals to be rewarded for behaviour which they perform away from the trainer, for example remaining at the back of the cage or interacting positively with a cage mate.

The animal being trained learns to associate the click noise (or other chosen secondary reinforcer) with a reward, then the click becomes associated with the reward itself, informing the animal performing the precise desired behaviour "Good, that is what I want" (Laule, 2010, p 208). The initial association between click and food is relatively easy to establish in animals which are confident around their trainer. He or she learns to associate getting a click, and subsequently a reward, with the specific behaviour being performed on hearing the click, leading to repeated performance of that behaviour.

Whilst this can be achieved using just the reward, the timing gap between the behaviour and reward can make it more difficult for the animal to understand exactly what it is being rewarded for, and thus slows training progress. With a click the trainee animal is able to make strong associations between behaviour and reward, and the trainer is able to indicate exactly which behaviour leads to reward. Further, the use of a secondary reinforcer enables greater precision in the shaping procedure, as subtle behaviours can be identified and rewarded. The use of a clicker therefore extends the utility of training to a wider range of situations and behaviours.

1.3.2 The Training of Primates in Laboratories

Whilst some training of primates has probably always occurred in the laboratory, it is only relatively recently that formal training has been identified as a means to refine husbandry and some data collection procedures. The training of primates in order to gather information on their cognitive abilities, in tasks such as discrimination tests, has occurred for longer, probably due to the necessity of training in order to undertake these tasks, and this will be described separately, but briefly as it is not the focus of this thesis. The other reason for training is to refine husbandry and data collection procedures, when there are less-welfare friendly alternatives.

Training necessary for data collection

Prescott and Buchanan-Smith (2007) reported that in the UK two institutions trained their macaques (*Macaca* spp.) for the generation of cognitive data through touch screen use, and one trained macaques for each of lever pressing, joystick use, eye-tracking and finger pressing. A further institution trained their marmosets for each of touch screen

use and lever pressing. No other institutions reported desire to start using training for such tasks, so it seems likely that this sort of specialised training is already used where needed. This does not mean however that refinements in techniques cannot be achieved, as noted by Scott *et al* (2003) who describe refinements in the collection of data. Both marmosets and macaques were trained to complete complex cognitive tasks in test chambers attached to the front of their cages, with no loss of performance (Crofts *et al*, 1999), whilst being able to move freely between their homecage and the test apparatus (in the case of marmosets) or move away into their homecage (in the case of macaques). Successful training has been carried out without the need for food or water restriction, with rewards either being additional to the daily diet of primate pellets (usually provided *ad libitum*), or a favoured part of the daily diet (e.g. fruits) (e.g. Anderson *et al*, 1996, Pearce *et al*, 1998, Crofts *et al*, 1999, Scott *et al*, 2003). However, food and fluid control is still used for some tasks that require extended periods of attention (JWGR, 2009) with the concomitant compromise in welfare this brings.

Training of cognitive tasks tends to be carried out in the absence of humans, with animals being monitored remotely via video links (e.g. Crofts *et al*, 1999), although this is not always the case (e.g. Anderson *et al*, 1996). Scott (1990) however recommends an increase in human-animal interactions in preference to computer controlled studies, suggesting this will further improve animal welfare and also aid data collection. Primates can learn complicated tasks using such paradigms, for example those skills learnt by individuals such as Ai the chimpanzee (*Pan troglodytes*) such as number recognition (Matsuzawa, 1985) and those to facilitate the study of memory and colour perception (e.g. Fujita & Matsuzawa, 1990, Matsuno *et al*, 2004, reviewed in Matsuzawa, 2003).

Training for cooperation with sample collection and dose administration

One of the main focuses of laboratory-housed primate training has been for the collection of samples, especially blood (Vertain & Reinhardt, 1989, Reinhardt, 1991, 1992a, Reinhardt & Cowley, 1992, Dettmer *et al*, 1996, Lambeth *et al*, 2004, Perlman *et al*, 2004, Coleman *et al*, 2008), for injection, (Reinhardt, 1992a, Bentson *et al*, 2003, Lambeth *et al*, 2004, Perlman *et al*, 2004, Videan *et al*, 2005, Schapiro *et al*, 2005), for blood pressure measurement (Mitchell *et al*, 1980, Turkkan, 1990) and other sample collection or drug administration (Kelley & Bramblett, 1981, Anzenberger & Gossweiler, 1993, McKinley *et al*, 2003, Lambeth *et al*, 2004, Perlman *et al*, 2004, Smith *et al*, 2004, Schapiro *et al*, 2005, Videan *et al*, 2005). A number of these studies combine the use of NRT and PRT, whereby the use of cage crushbacks and forced restraint (e.g. holding a limb until the monkey relaxes) is used alongside positive reinforcement (usually food) for performing the required behaviour (e.g. Vertain & Reinhardt, 1989, Reinhardt, 1991, 1992a, Reinhardt & Cowley, 1992, Dettmer *et al*, 1996). Such training is described by McKinley (2004) as 'engineered compliance', whereby whilst the animal is rewarded for the behaviour, but if it fails to perform it an aversive stimulus is applied, thus the individual does not really have a choice in whether it cooperates. In true positive reinforcement training the animal has a choice, and the consequence for not performing the behaviour is simply that they do not receive the reward. This engineered compliance however is preferable to traditional techniques where greater levels of 'manhandling', restraint and possibly chemical immobilisation are required. Indeed even when an element of negative reinforcement is used for the collection of blood, female rhesus macaques (*Macaca mulatta*) have significantly lower

plasma cortisol levels than those who have blood taken in more traditional methods, such as removal from the cage and physical restraint (Reinhardt *et al*, 1990).

Much of the more recent literature has looked at training primates using PRT alone, although the limited use of some negative reinforcement is recognised in some studies (Coleman *et al*, 2008). In rhesus macaques, blood collection is achieved by training the monkeys to place their arms into a specially designed Perspex sleeve and then holding onto a peg at the end, thus positioning the arm into a position where venipuncture can take place (Coleman *et al*, 2008). Chimpanzees were trained to present an arm or leg for anaesthetic injection in a seven step PRT programme (Videan *et al*, 2005); first they were rewarded for pressing their arm to the cage mesh, then allowing touch with a capped syringe, touch with a blunt needle, pressure with a blunt needle, a jab with a blunt needle without piercing the skin, a poke with a sharp needle, and finally injection.

Whilst the training of New World primates has lagged behind that for Old World primates and apes, the training of these species for the collection of urine is one area where this inequality is not so pronounced. Data are available on the training of chimpanzees (Stone *et al*, 1994, Lambeth *et al*, 2004) and vervet monkeys (*Cercopithicus aethiops*, Kelley & Bramblett, 1981) for the collection of urine, but a number of techniques for training New World monkeys to provide a urine sample are also reported. Anzenberger and Gossweiler (1993) describe the training of common marmosets to enter a specially designed apparatus on waking in the morning, in which they are temporarily contained in separate compartments, rewarded by the provision of mealworms, until they have urinated, following which they are released into the main cage. Smith *et al* (2004) describe how positive reinforcement of urination was carried

out in three species of New World monkey, whereby each time an individual urinated it was provided with a reward of grape, irrespective of where the urine was deposited, as the sample was then collected by pipette. McKinley *et al* (2003) further refined this and describe how urine samples can be collected by training marmosets to scent mark into a vial placed in a branch in the cage, using PRT alone. The authors note that those individuals target trained prior to scent mark training learn the scent marking task more slowly than when the order of tasks is reversed, possibly due to the initial fear and nervousness around people leading to increased levels of scent marking. These studies show the how the flexibility of training in New World primates has yet to be fully exploited for the refinement of laboratory practices with these species.

Although details are not provided, early work by Barrow *et al* (1966) describes how rhesus macaques can be trained using positive reinforcement and space restriction (though no particular negative reinforcement, although space restriction could be a negative reinforcer) to go to a restraint chair, put their head through the head hole and allow the neck plate to be secured. Further, rhesus macaques were trained to place their faces to a specially designed mask, individually made to fit, in order to obtain a reward of a fruit-flavoured drink. This apparatus allowed the tracing of eye movement using small cameras, and obviated the use of restraint chairs and sclera implants (Scott *et al*, 2003, Fairhall *et al*, 2006).

Training for management and husbandry procedures

Capturing primates without training may include the use of nets, which is often very stressful, or requires the use of anaesthesia (Rennie & Buchanan-Smith, 2006b).

Therefore, training for capture is highly desirable from a welfare point of view given the frequency with which capture is required in laboratories. In a survey of UK laboratories holding primates, nine institutes reported that they trained their animals for capture, and a further three would like to suggesting that this is of particular interest (Prescott & Buchanan-Smith, 2007). Indeed the training of primates, and in particular macaques, to cooperate with capture and movement has been one of the most commonly published husbandry training tasks. That the literature is so focussed on macaques and not marmosets probably reflects their greater size, and therefore the increased risks involved with their handling, as well as the greater numbers used, in comparison to marmosets. Training for capture and movement in macaques has predominantly involved three techniques; chute training, transport box training and pole-and-collar training.

Chute training is commonly used for group-housed animals and involves the entire group being trained to move down a chute, often to a smaller caging system where samples can be collected. Phillippi-Falkenstein and Clarke (1992) report the use of this technique in order to collect blood and faecal samples from corral-housed rhesus macaques, whereby technicians entered the corral with nets and PVC pipes, moved behind the group and used the pipes to bang on the metal support poles of the cages. In this way the macaques were chased into a chute system, where they could be separated and samples collected. In order for the macaques to enter the chute they must have found the entrance of the technicians aversive, as they were prepared to enter the confined space of the chute to get away from them. Macaques were moved within the chute system by 'nudging' them with the PVC pipes. However the authors reported no behavioural signs of fear or aggression, although they did not report which signs they

measured. The macaques were willing to take food rewards in the chute system, with the exception of when in the sampling cage, which the authors attributed to the animals' desire to be released back into the corral (the authors identify this as positive reinforcement in itself, however if escape from the sampling cage is considered to be the reinforcer, then this too is negative reinforcement). An alternative explanation of the refusal of food in the sample cage is that they were more fearful in the cage in which they experienced the aversive process of blood sampling. Luttrell *et al* (1994) report a similar method of capturing rhesus macaques, concluding it is an improvement on the previous method, net capture from a small indoor run, as the macaques no longer suffered from acute diarrhoea, rectal prolapse and lacerations. Obviously these are extremely severe indicators of distress, and their elimination is important for welfare, but this technique still employs negative reinforcement. As noted above, Phillippi-Falkenstein and Clarke (1992) did not identify which signs of fear or aggression they looked at. It is possible that if such gross indicators as those used by Luttrell *et al* (1994) were used, the macaques were still displaying fear, but that it was more subtle and therefore missed. The descriptions above should provide an indication that improved techniques for capturing primates, a very frequent occurrence in most laboratories, is required.

Similar techniques have been employed to facilitate cage cleaning and research procedures described by Knowles *et al* (1995), with the refinement of not using nets and carestaff remaining as still and quiet as possible. As with the training described above, food rewards were given on the completion of the task, and hence all these training techniques involved both negative and positive reinforcement. This type of training is possible however using solely positive reinforcement; Bloomsmith *et al*

(1998) report the success of training chimpanzees to enter their indoor housing using positive reinforcement only, whereby the animals were rewarded with favoured foods for coming in but there were no negative consequences for remaining outside (other than they did not receive the food). This technique could be used for any primate. However the chimpanzees were not subjected to research procedures once inside, which may impact upon the likelihood of them continuing to enter the indoor enclosure in future, and may have implications for the use of this technique in other situations. It was reported that subjectively, staff found this method of moving the chimpanzees less stressful when compared to previous methods combined negative and positive reinforcement techniques. This increased the frequency of capture indoors allowing more enrichment to take place (Bloomsmith *et al*, 1998), consequently bringing further benefits to the animals.

The second technique, transport box training, is more commonly used where animals are housed in cages rather than in larger corrals (e.g. Reinhardt, 1992b, Scott *et al*, 2003) although it can be used as an adjunct to chute training (e.g. Luttrell *et al*, 1994). Typically the transport box is attached to the cage and, if it does not enter spontaneously, the monkey is 'encouraged' into it by prodding it (e.g. Reinhardt, 1992b, Luttrell *et al*, 1994), by positioning staff above the level of the cage and using soft vocalisation (e.g. Scott *et al*, 2003) or by reducing space in the cage (e.g. Heath, 1989, Scott *et al*, 2003). However this method is not always successful; Heath (1989) describes how carestaff initially caught the macaques and placed them into the transport box until they were willing to enter on their own. The monkeys were rewarded with favoured, sweet foods for entering the transport box, so positive reinforcement was used, but techniques of negative reinforcement were also employed. Although little

published data on the capture of marmosets are available, it is common for them to be chased into a box, often their nest box (e.g. as described in Bassett *et al*, 2003), and then not rewarded, which not only employs entirely negative reinforcement, but also means that the marmosets may no longer view their nest box a safe place to rest (Buchanan-Smith, 2010). The training of common marmosets for capture in a transport box using PRT can be successfully achieved in the laboratory, and this has been described in detail in Prescott *et al* (2005a), although the current uptake rates of this methodology are unknown.

The third method of trained capture commonly used, especially for macaques and other larger primates such as baboons (*Papio hamadryas*, Marks *et al*, 2000) is pole-and-collar capture. The monkey wears a collar, which can then be attached to a pole, through which the movement of him or her can be controlled (Anderson & Houghton, 1983, Marks *et al*, 2000, Scott *et al*, 2003) and frequently this technique has been used to move animals to a restraint chair (e.g. Schmidt *et al*, 1989). Whilst little of the published literature gives details of training for pole-and-collar capture, Reinhardt (2008) provides the transcript of an online discussion giving an informal description of pole-and-collar training using positive and negative reinforcement, habituation and desensitisation. Whilst this method of capture is seen as preferable to the use of crushbacks and nets by some (e.g. Scott *et al*, 2003), Reinhardt *et al* (1995) have argued that this technique causes the monkeys distress, although no mention is made of this in a later publication on the subject (Reinhardt, 2008).

Most training carried out in UK laboratories employs both negative and positive reinforcement, with the most common positive reinforcement being verbal praise

followed by food. Praise however is not validated as a positive reinforcer (Prescott & Buchanan-Smith, 2007), and indeed even soft vocalisations seem to act as negative reinforcers, being used in the movement of macaques (e.g. Scott *et al*, 2003). The reinforcers used in UK laboratories follows the pattern outlined above as seen in the literature for capture training. Further, three laboratories reported that they only used negative reinforcement. It is therefore likely that training for capture involves a significant element of negative reinforcement. Training using negative reinforcement and positive punishment has been shown to not only be linked to behavioural problems, but also to be less effective than positive reinforcement in dogs (*Canis familiaris*, Hiby *et al*, 2004), and whilst this may not be directly applicable to laboratory-housed primates, it at least raises concerns as to the way capture training has been carried out. Additionally, training using positive reinforcement has been shown to improve the welfare of laboratory-housed primates, as discussed below (Section 1.3.3). The paucity of literature on the training for capture using PRT alone, alongside the interest in training for this task as evidenced by both the literature and the results of the survey of laboratories carried out by Prescott and Buchanan-Smith (2007), suggest that the investigation of methodologies for capture training laboratory-housed primates may be of value.

An area which has received less attention is the training of primates specifically to manipulate their social behaviour in some way. Bloomsmith *et al* (1994) report how a particularly dominant male laboratory-housed chimpanzee was trained to sit whilst other in the group fed, reducing aggression within the group at feeding time but this did not extend to a generalised reduction of aggression within the group. When rhesus macaques are trained to change the frequency of their affiliative interaction with

cagemates, low affiliators increase the level of affiliations outside of training sessions but not during training, whilst high affiliators decrease their interactions during training but not out with training (Schapiro *et al*, 2001). In this study the low affiliators were trained to groom a cagemate through target training, which then led to being able to move the monkey near the intended grooming partner and from this grooming was shaped. That such complex social behaviour can be trained using PRT and as compatible social housing is critical for good primate welfare, suggests that the scope for this type of training is great, and has yet to be fully exploited. Other studies have looked at training as an enrichment to reduce unwanted behaviours such as stereotypies, and these are discussed below.

1.3.3 The Benefits of Training

Training has been shown to be beneficial to the animals being trained, the staff involved in training and also to the science being undertaken. From an ethical perspective, that it benefits the animals is of primary importance, but the practicalities of laboratory-housing primates mean that benefits to staff and science also play a role in whether training is carried out. As briefly outlined above training can be used to directly manipulate the behaviour of primates to reduce aggression (Bloomsmith *et al*, 1994) and increase affiliative interactions (Schapiro *et al*, 2001), which have direct benefits to the welfare of the animals involved. Changes in the behaviour of primates are also observed when training is carried out as part of an enrichment programme. Bourgeois and Brent (2005) found that PRT reduced the amount of whole body stereotypical behaviour observed in baboons, and was more effective than inanimate forms of enrichment in doing so, and Coleman and Maier (2010) report a reduction in

stereotypies in rhesus macaques following a training programme. Further, Maier *et al* (2004) demonstrated that trained rhesus macaques also showed reduced levels of stereotypical behaviour, and Laule (1993) reported PRT reducing abnormal behaviour in a baboon, although these data were not quantified. Training has also been identified as a means of providing environmental enrichment to animals (e.g. Laule & Desmond, 1998, Bourgeois and Brent, 2005), as it provides them with mental stimulation, one of the aims of enrichment (Markowitz, 1982, Shepherdson, 1989, Markowitz & Aday, 1998, Poole, 1998), potentially reducing boredom. Data which demonstrates animals' willingness to work for food, even when it is freely available, a phenomenon known as contrafreeloading (Neuringer, 1969, Inglis *et al*, 1997), are often used to support the assertion that training is enriching, however little evidence is available to back this up. It is known that primates will work for food, even when the same food is available without the effort of foraging for it (Anderson & Chamove, 1984) suggesting that the challenges that training provides may be enriching. To support this suggestion, it has recently been shown that dwarf goats (*Capra hircus*) choose to obtain water in a learning task rather than from where it is freely available providing evidence for contrafreeloading when a greater cognitive challenge is involved (Langbein *et al*, 2009).

Certainly cooperating with either husbandry or research procedures is less stressful for the animals concerned. The training for capture in a chute as described above eliminated severe stress responses such as rectal prolapse and acute diarrhoea (Luttrell *et al*, 1994). When training is carried out for research procedures, significant differences in physiological responses are seen. Trained rhesus macaques showed reduced plasma cortisol when blood is collected cooperatively rather than by traditional

methods (Reinhardt *et al*, 1990, Reinhardt, 2003), suggestive of a reduced stress response. Further, chimpanzees trained to cooperate with anaesthesia had lower mean values of total white blood cells, segmented neutrophils, glucose, cholesterol, and systolic and diastolic blood pressure, and higher hematocrit levels than untrained animals, all indicators of a reduced stress response (Lambeth *et al*, 2004). These findings also indicate that the use of training is beneficial to the quality of science being performed, as reduced stress responses disrupt the normal functioning of physiological systems (Reinhardt *et al*, 1995, Hassimoto *et al*, 2004).

Training can change the behaviour of the animals outwith training sessions, as demonstrated by the changes in levels of affiliation described by Schapiro *et al* (2001), but whilst this change was seen in a behaviour which was directly being trained for, changes are also seen in behaviours not being trained for. Marmosets trained to provide a urine sample exhibited less inactive behaviour and self scratching in response to a stressor following training in comparison to before training (Bassett *et al*, 2003), indicating that the training process, even when it is for an unrelated task, helped them cope with the stress of capture. Evidence also suggests that training may decrease the performance of stereotypical behaviour in primates outwith training sessions (Laule, 1993, Maier *et al*, 2004, Bourgeois and Brent, 2005, Coleman & Maier, 2010) Further work however is required to validate this link.

Training can improve human-animal relationships, with the benefits that brings to both parties. Both Scott (1990) and Rennie and Buchanan-Smith (2006a) identify the selection and training of staff as being a key issue impacting upon the welfare of laboratory-housed primates. Reducing the stress to staff associated with routine

husbandry procedures such as capture can lead to an increase in frequency of environmental enrichment as greater access to enclosures is available (Bloomsmith *et al*, 1998). There are also further benefits in that whilst primates may instinctively perceive humans as predators or intruders, and so react fearfully or aggressively (O'Neill, 1989), positive human interactions can reduce these reactions (Bayne, 1989, Baker *et al*, 2003). Further, stump-tailed macaques (*M. arctoides*) who are considered to be friendly by staff, and engage in more affiliative behaviour with them, and are less disturbed by routine husbandry than those individuals considered to be unfriendly. It is interesting to note that whether an individual is considered to be friendly or unfriendly is relatively stable, even following increased positive interactions (Waitt *et al*, 2002).

From the above literature, it seems that training, especially positive reinforcement has an important role to play in addressing two of the predominant experiences laboratory-housed animals suffer, notably fear and boredom (Morton, 1997). Positive reinforcement training provides primates with mental stimulation, thus reducing boredom, and subsequently reducing exaggerated fear responses. Fear is also reduced by improving human-animal relationships and, on a more practical level, reducing the need for physical restraint, force and coercion (Laule, 2010). Further benefits to the welfare of laboratory-housed primates are provided by the use of PRT enabling animals to have a greater degree of choice and control over their environment, factors know to reduce stress responses in primates (Hanson *et al*, 1976, Mineka *et al*, 1986), and thus improve welfare (Badihi, 2006). Most of the data relating to training has been aimed at reducing negative experiences, however, PRT may also provide a basis for promoting positive experiences in laboratory-housed primates, and this remains a potential area for considerable future research. Indeed there is an increasing interest in the welfare

implications of coping successfully with appropriate challenges, and how this relates to positive emotions (e.g. Boissy *et al* 2007, Meehan & Mench, 2007, Puppe *et al* 2007).

1.4 AIMS OF THESIS

The aims of this study were twofold; first to assess the impact of the use of positive reinforcement training on the welfare of laboratory-housed primates, and second to address some of the issues relating to the practicalities of implementing PRT in busy research institutions. Two species of primate (common marmosets, *C. jacchus*, and cynomolgus macaques, *M. fascicularis*) were studied in three different research institutes, two pharmaceutical research establishments and one contract research organisation. In Chapters 2 and 3 comparable studies are made of the two species to investigate the relationship between temperament and trainability in order to establish not only success rates and time investment, but also if these factors are related. This might then enable some pre-selection to take place in order to improve success rates and/or decrease time investment. Further, the behavioural response of the animals to both the training programme and a routine stressor is examined to establish if PRT has an effect on the welfare of laboratory-housed primates. The effect of training being split between two different trainers on both training time investment and on marmoset welfare is investigated in Chapter 4, addressing the issue of multiple trainers which is common in practice but the effect of which is underreported in the literature. In Chapter 5 training marmosets to cooperate with a potentially aversive task, namely hand capture, is considered. Whilst macaques and apes are commonly trained to cooperate with aversive procedures, much of the training data on marmosets has focussed on training for neutral tasks, so the first aim of this study was to establish if this type of

training was possible with marmosets. Further, the behavioural and physiological (cortisol) responses to training and different methods of capture were determined in order to assess how the marmosets perceived these processes, and the impact upon their welfare that they had.

CHAPTER 2

THE INFLUENCE OF AGE, SEX AND TEMPERAMENT ON THE TRAINABILITY OF CYNOMOLGUS MACAQUES (*Macaca fascicularis*)

Whilst training is recommended for laboratory-housed primates, the success and time investment required for training behaviours has been little considered. These data are useful to those establishing a training programme as they may help to identify how animals will respond to training, and also how much time needs to be invested in it in order for it to be successful.

When 24 laboratory-housed cynomolgus macaques (*Macaca fascicularis*) were exposed to a training programme, the majority was successfully trained to cooperate with a simple training task of target training in 26 or fewer sessions. Not all macaques however reached criterion, but this was not influenced by age or sex. Macaque temperament, measured as a response to hand feeding, and as home cage behaviour, was a factor in reaching training criterion and also predicted how quickly individuals were trained. Shorter latencies to hand feed and higher rates of watchful behaviour exhibited in home cage baseline observations were predictors of faster training times. Cage cleaning negatively affected the behaviour and welfare of the monkeys, but the training programme impacted positively on their welfare. Training helped the macaques to deal better with a stressor and also decreased the amount of an undesirable behaviour, sitting alone, in their normal daily activity. Together, these results support recommendations that training has benefits, and provide new data on how to determine which individuals may be most suitable for training programmes.

2.1 INTRODUCTION

There is now a considerable body of data demonstrating that primates can be trained to cooperate with husbandry and scientific procedures in the laboratory. The majority of these data have come from studies with Old World primates, predominantly macaques, especially rhesus macaques (*Macaca mulatta*) and cynomolgus macaques (*Macaca fascicularis*) (e.g. Clarke *et al*, 1988b, Reinhardt *et al*, 1990, Reinhardt, 1992b, c) and chimpanzees (*Pan troglodytes*) (Schapiro, 2005, Videan *et al*, 2005). Recent studies have also provided data on the training of New World primates, primarily common marmosets (*Callithrix jacchus*) (see Chapter 3). Whilst these data are very valuable in terms of showing that training for a range of tasks is possible, there remains a considerable amount of data lacking with regards to which factors affect the success and time investment required for training behaviours. These data are of value to those embarking on a training programme as they may help to identify how much time will be required to train the animals and also how individual animals will respond to training.

A number of factors may affect how quickly, or indeed if, an individual primate will learn a task. The type of task being trained is clearly critical in determining the speed and success of training. Other factors are easy to quantify and assess, for example certain intrinsic factors such as the age of the animal or its sex. Further factors, such as social rank and temperament, may require more formal analysis whilst more complex extrinsic factors, such as previous experience are much more difficult to evaluate. Although understanding the role of previous experience may be interesting, the difficulties in measuring it and the limited potential impact in the selection of primates for training programmes mean that it not considered within the remit of this study. It is

for this reason that only the more simple factors of the primate's age, sex and temperament, along with factors relating to housing, are considered here. Laboratory staff will be able to easily and quickly identify or assess these factors, meaning that they do not become a barrier to the use of training in this setting.

2.1.1 Time Investment Required for Training

The uptake of training for primates in laboratories has been slow since it was first advocated widely in the scientific literature. In the UK just over half (6/11) of research and breeding establishments holding primates surveyed had a formal training programme (Prescott & Buchanan-Smith, 2007). The same survey revealed that one of the critical factors which limits the uptake of training was a perceived lack of time in which to carry it out. While reliable and relevant data on time investment continue to be unavailable to laboratory staff this perceived lack of time will continue to prove a barrier to the uptake of training. There are studies which do give information on time investment but often this is not a main finding of the study making it difficult to interpret, especially for busy laboratory staff. Quite often when training is mentioned in papers it is as part of an experimental procedure and not as the focus of the research. Where training is alluded to phrases such as "...they quickly learned this routine..." (Heath, 1989, p 17) or "...all animals had learned the proper behaviors after 25 days..." (Phillippi-Falkenstein & Clarke, 1992, p 85) are often found giving an indication that the time investment was at least considered but perhaps not recorded in detail. Bourgeois and Brent (2005), for example, used positive reinforcement training (PRT) as part of a study of the effects of environmental enrichment, training baboons for two 60-minute sessions per day but do not discuss what behaviours were being trained and why

as this was not within the remit of the study. Other research, such as Clarke *et al* (1988b) who discuss training three species of macaque (rhesus macaque, cynomolgus macaque and bonnet macaque, *M. radiata*) to enter a transport box, may give information on time investment but this is vague and requires interpretation by the reader. There are however studies which do give details of the time invested in training. A range of studies which provide good data on time investment for training are shown in Table 2.1.

Due to the range of species used in the listed studies and the variety of the tasks they were trained for, as well as factors such as differences in housing and trainer's experience, comparisons are somewhat limited. Comparisons between laboratory and zoo-housed primates are difficult due to the very different life experiences these two groups have; zoo-housed animals have larger enclosures into which they can retreat, tend to live in larger and more natural groups and have fewer, but generally more positive, interactions with humans. These factors are all likely to influence the outcome of training programmes. Nevertheless it is possible to identify patterns in the published data. With regards to the New World species, within the zoo-housed groups there is a wide variation in time required for animals to learn to hand feed, but much less variation in the time required to learn to touch the target. This suggests that there are species differences in time investment for training, most likely related to the animals' shyness/boldness, sociability or fear of humans as once this is overcome these differences become negligible. It may appear that target training zoo-housed callitrichids is much quicker than for laboratory-housed animals; however given that hand feeding must be achieved before target training commences, if hand feed is included in the time investment it takes four times as long for zoo-housed common

Reference	Species	Task	Reported Time investment	Calculated time investment per animal	Notes
McKinley *et al* 2003	*C. jacchus*	Provide urine sample	Mean of 52 minutes	26 minutes	Pair-housed
		Hold target	Mean of 64 minutes	32 minutes	
Savastano *et al* 2003 [a]	*C. geoffroyi*	Hand feed	1-2 x 10 minute sessions	5 minutes	Group size 2 and 4
	C. jacchus		60 x 10 minute sessions	120 minutes	Group size 5
	L. rosalia		60-150 x 10 minute sessions	30 minutes	Group size 2 and 5
	S. imperator		1 x 10 minute session	5 minutes	Group size 2
	C. geoffroyi	Touch target	2-3 x 10 minute sessions	7.5 – 10 minutes	Follows from 'Hand feed'
	C. jacchus		2 x 10 minute sessions	4 minutes	Zoo-housed
	L. rosalia		3 x 10 minute sessions	6 minutes	
	S. imperator		1 x 10 minute session	5 minutes	
Smith *et al* 2004	*C. geoffroyi*	Provide urine sample	6 x 30 minute sessions	30 minutes	5 in group Zoo housed
	L. rosalia		5 x 30 minute sessions	50 minutes	2 x 3 in group
	S. imperator		8 x 30 minute sessions	40 minutes	6 in group
Fernstrom *et al* 2009	*M. mulatta*	Touch target	Median of 9 x 5minute sessions (45 minutes)	45 minutes	Singly-housed and group-housed. 2-3 animals/group
Coleman *et al* 2009	*M. mulatta*	Allow venipuncture	Mean of 257.5 ± 30.9 minutes across 50.3 ± 5.1 sessions (mean 5.1 min)	257.5 minutes	Group-housed. 2-15 animals/group
	P. troglodytes	Provide blood sample	Mean of 219.0 ± 24.2 minutes across 31.0 ± 3.1 sessions (mean 7.3 min)	219 minutes	Individually housed
Laule 1996	*P. troglodytes*	Present arm for venipuncture	275 minutes	275 minutes	Group and singly-housed. 2-3 animals/group
Schapiro *et al* 2005	*P. troglodytes*	Present for sub-cutaneous injection	Mean of 98 minutes across 17 sessions	98 minutes	Group-housed. Group size not reported
		Present for intra-muscular injection	Mean of 86.9 minutes across 30.8 sessions	86.9 minutes	
		Males to ejaculate into artificial vagina	Mean of 197.5 minutes across 16.3 sessions	197.5 minutes	
Videan *et al* 2005	*P. troglodytes*	Provide blood sample	Mean of 121 ± 63.15 minutes across 35.13 ± 21.48 sessions	121 minutes	Group-housed. Group size not reported

Table 2.1 Reported and calculated time investment for training primates in captivity. [a] Paper reports time investment for 18 species of New World primate and seven behaviours, but only a sample reported in table.

marmosets to learn the task than for the same species housed in a laboratory (laboratory, McKinley *et al*, 2003, zoo, Savastano *et al*, 2003), although in the study with laboratory-housed animals, at least one monkey in each pair was hand feeding prior to the commencement of training. Similarly zoo-housed New World primates take up to twice as long to train to provide a urine sample than those housed in a laboratory, although species differences again seem to be important with zoo-housed golden lion tamarins (*Leontopithecus rosalia*) taking twice the time of laboratory-housed common marmosets, but zoo-housed Geoffroy's tufted-ear marmosets (*Callithrix geoffroyi*) and laboratory-housed common marmosets taking very similar time investments for this task (laboratory, McKinley *et al*, 2003, zoo, Smith *et al*, 2004). These data show the importance of considering each species in various housing conditions in relation to time investment of training.

There is a paucity of data on the time investment to train macaques using entirely PRT as much of the published work on training these species has involved negative reinforcement training (NRT). Where data are available however it provides a good comparison between rhesus macaques and common marmosets as both were laboratory-housed. Common marmosets learn to touch the target more quickly than rhesus macaques, with marmosets taking approximately two-thirds of the time required to train macaques (McKinley *et al*, 2003, Fernström *et al*, 2009). There is clear evidence to show that for rhesus macaques the time taken to learn a neutral task (target) is less than time investment for a more aversive task (venipuncture), which is unsurprising, but useful in that it provides evidence that rhesus macaque can be trained to cooperate with aversive procedures using predominantly PRT (Coleman *et al*, 2008). Time investment for training rhesus macaques to cooperate with venipuncture using NRT and PRT has

been shown to be an average of 31 minutes (Vertein & Reinhardt, 1989), approximately one-sixth the time investment using PRT. Using predominantly PRT however is the more humane technique, as it gives the monkey choice and control over the procedure.

Rhesus macaques take longer to learn to present a limb for venipuncture (using predominantly PRT) than chimpanzees, but this difference was not found to be significant in the study which compared the two species, possibly due to sample size and/or that chimpanzees individuals had previous experience of being trained whereas rhesus macaques were naive animals (Coleman *et al*, 2008). Training chimpanzees to accept injection took more time than training for the neutral target touch but not as long as for venipuncture, whilst training for semen collection in males took much longer. This is slightly surprising given that providing semen is probably highly rewarding for the individual performing it, so may reflect the difficultly of capturing and shaping this behaviour (Schapiro *et al*, 2005). There is considerable variation across studies in the time investment required to train chimpanzees for blood collection, with a reported time investment in one study (Coleman *et al*, 2008) of nearly double that reported in an earlier study (Videan *et al*, 2005). There is to date very little evidence that there are consistent differences in trainability between Old World and New World monkeys and apes, and neither size nor genera predicts how well an individual will learn a task. This perhaps suggests that factors beyond species and housing play an important factor in time investment. These may include unreported factors such as trainer skill and experience, training protocol, session length and frequency, and the animals' prior experience.

2.1.2 Characteristics of an Easily Trained Animal

There is considerable variation in the time investment required to train different individuals across all species, so identifying those individual animals who may prove easier or faster to train is a useful tool for those working in laboratories. An element of pre-selection of animals for projects in which training may be used could save time or increase success rates. If factors can be identified which make training more successful or require less time to undertake then the uptake of training may be more widespread. There may be a collection of factors which influence an individual's 'trainability', that is to say the chances of its success in learning a task and the time investment required in order for it to do so. Trainability will depend on the species; some species will learn some tasks more easily than others depending on factors such as their normal behavioural repertoire. For example, it is easier to train a dog to retrieve than a horse as dogs will naturally pick up items and carry them whereas this is unusual in horses, most likely due to differences in their feeding ecology. It is also easier for dogs to carry items in their mouths due to their dentition which allows objects to be held behind large canine teeth and gripped with sharp teeth, whereas horses do not have this physical advantage (McGreevy & Boakes, 2007).

Primates species have, broadly speaking, similar physical capabilities. Certainly those species commonly kept in laboratories are all physically able to climb and move easily around their cages and manipulate objects with their hands, and therefore are physically capable of performing the vast majority of tasks commonly trained. There are however differences between species in the speed at which primates learn tasks (see Table 2.1)

which may reflect temperament at a species level (Clarke & Lindberg, 1993, Clarke & Boinski, 1995, Coleman *et al*, 2008).

As there has been little research into what characteristics make a primate trainable, with the exception of Coleman *et al* (2005) (discussed below) the more extensive literature relating to the trainability of dogs will be discussed. Serpell and Hsu (2005 p 197) define trainability in dogs as "the ability and motivation to attend and respond in a positive way to human cues or signals". Motivation is not clearly defined, and could be for a reward or for the interaction itself, which may be rewarding. Reward motivation is critical in PRT and will affect trainability where rewards (usually food) are used. It is important that the animal is keen to receive the food and will therefore be willing to work for it. Motivation may also be akin to 'drive' that is to say the desire to do something, or perhaps to learn, which is not related to the reward offered. It has been shown that in goats learning has an intrinsic value, that is to say they seem to enjoy the process of learning and will do so even if the reward is available without having to partake in a learning task (Langbien *et al*, 2009). This could be due an internal drive to work, and comparable to contrafreeloading paradigms. Similarly a desire for work has been noted as being important in drug detection dogs (Maejima *et al*, 2007) alongside a lack of distractibility, although these two factors seem to be very closely related – if an animal has a high desire to work it is unlikely to be easily distracted. Consistency of behaviour across time also features as a factor in the trainability of search dogs (Rooney *et al*, 2007a) which again seems to be related to drive and lack of distractibility, perhaps with an added element of good concentration. A further important factor identified by Serpell and Hsu (2005) is a positive response to human cues.

Whilst sensitivity to the cues is important, a positive response to humans is more important in an individual's trainability (Serpell & Hsu, 2005). This is even more relevant in the laboratory where animals are subjected to, by humans, experiences which are potentially painful, frightening and stressful, so may be more closely related to fear. Sociability towards humans, that is to say their willingness to interact with us, was found to be related to training success in working dogs (Svartberg, 2002), as were playfulness, chase-proneness and curiosity/fearfulness. These factors were found to relate to the same higher-order dimension of temperament named as the shyness-boldness dimension by Svartberg (2002), suggesting that the element of boldness is critical to trainability across a number of activities (e.g. tracking, searching, delivering messages and personal protection), and that dogs who are most bold are more successful in learning and learn more quickly.

In rhesus macaques inhibited-exploratory dimension behaviour, that is to say how willing an individual is to explore and interact with its environment, has been found to relate to training success (Coleman *et al*, 2005). This seems to be very closely related to the shyness-boldness dimension discussed above. Indeed it is likely that they are measuring the same behavioural dimension and that the difference between shyness-boldness and inhibited-exploratory is purely semantic, although without further analysis it is not possible to ascertain this. It is also noted that rhesus macaques who were scored as moderate on the inhibited-exploratory dimension test took the least amount of time to learn the task, and Coleman *et al* (2005) suggest that this may be down to these animals being able to maintain their interest in learning over a longer period of time than the most exploratory individuals who became distracted or less motivated to learn. Further,

animals who are generally more anxious are likely to be more fearful of humans, and this may inhibit learning during training.

How an individual responds to a particular stressful experience may also influence their trainability. Trait anxiety (Eysenck, 1985) has been shown to affect cognitive function and this may also be reflected in a training paradigm. It is reasonable to suggest that less anxious individuals may learn more quickly than highly anxious individuals. Less anxious individuals may be less distracted by outside noises or movements which may disturb high anxiety animals, and low anxiety individuals are likely to recover more quickly from these disturbances and therefore be better able to concentrate on learning.

Other factors may also be relevant to the trainability of primates, or indeed other species. The willingness to try new behaviours and be flexible in their behaviour may be important, and this behavioural plasticity, an important element of which is innovation, and is something primates are noted for (Kummer & Goodall, 1985, Reader & Laland, 2001). Reader and Laland (2001, p 788) define innovation as "the discovery of novel information, the creation of new behavior patterns, or the performance of established behaviour patterns in a novel context". Innovation may therefore be an important factor in the trainability of an individual when the individual is required to offer new behaviours. Whilst a particular species may be noted for its behavioural plasticity, certain individuals within that species are likely to exhibit higher levels of this than others. These may be the most trainable individuals as they are offering more behaviours, which can then be shaped to the desired end behaviour. However where simple tasks are being trained for, as is the case with much of the training carried out in the laboratory, this behavioural plasticity may be less relevant.

The review indicates that the following temperament related factors may be relevant in assessing the trainability of primates

- Motivation for food
- Motivation or drive to work
- Distractibility/concentration
- Sociability with/attention to humans/cues
- Shyness/boldness
- Inhibition/exploration (if different from shyness/boldness)
- Anxiety/relaxation
- Behavioural plasticity/levels of innovation

Along with these factors, species, as discussed above, is likely to play a part in trainability but as species choice is severely limited in a laboratory setting this is perhaps less relevant than individual characteristics. Other factors such as age and sex may also influence trainability and these are discussed below.

2.1.3 The Influence of Age and Sex on Ability to Learn in Primates

The age and sex of a range of primates has been shown to affect their behaviour in a variety of respects. Behaviour changes as animals mature, with, for example, infant chimpanzees playing more than adults, and females engaging in more contact behaviour than males even in a laboratory-housed population (Brent & Veira, 2002). However, perhaps more pertinently to this study, across all primate species males show more innovation than females, and adults more than sub-adults (Reader & Laland, 2001).

Innovation is seen where an individual or group develops a new way of dealing with an environment, with perhaps the most famous example of this being the washing of potatoes by Japanese macaques (*Macaca fuscata*, Kawai, 1965). Innovation may be particularly relevant to training as discussed above, and as innovation has been found to vary with age and sex, there may be related differences in trainability. In laboratory-based studies sex differences in innovation and foraging however tend to be identified when animals are housed in mixed sex pairs, and predominantly in callitrichid species. This may suggest that social factors, such as males deferring to females, override any actual cognitive differences (common marmosets, Box, 1997). This is further substantiated by the review by Reader and Laland (2001) which identifies low ranking individuals as being greater innovators than higher ranked animals, which the authors propose is due to these individuals needing to take opportunities when they arise to a greater extent than high ranking individuals. This highlights the importance of the social setting of the study in interpretation of the results.

Learning ability does not however seem to be affected by sex or age, unless the animals are particularly old or young. For example old primates have difficulty in learning tasks, although there is considerable individual variation (rhesus macaques, Voytko, 1999), indicating that there may be some cognitive decline in older primates. Laboratories are unlikely to use such aged animals routinely but this may need to be considered if training of older animals is undertaken. The literature however suggests that males and females do not differ in their ability to learn, and that whilst juveniles may show a slightly better performance than adults the reverse may be the case in training paradigms. Table 2.2 provides a description of age and sex differences in relevant studies.

Reference	Species	Task	Effect of Age in Study	Effect of Sex in Study	Notes
Box, 1997	*C. jacchus*	Food retrieval	Not reported	Females more successful than males	Male-female pairs
Cameron & Rogers, 1999	*C. jacchus*	Food retrieval	Older marmosets interact for longer with task Older marmosets gain more food rewards No effect on latency to interact with apparatus	No differences between males and females	Single-sex groups
McKinley *et al*, 2003	*C. jacchus*	Training for weighing	Not reported	No differences between males and females	Male-female pairs
Yamamoto *et al*, 2004	*C. jacchus*	Food retrieval	Females perform better than males	Females more successful than males	Male-female pairs
Anderson *et al*, 1996	*M. arctoides*	Visual discrimination task	No difference between older and younger groups	Not reported	Mixed sex groups
Watson *et al*, 1999	*M. fascicularis*	Puzzle feeder maze problem solving	Younger animals solved more complex maze tasks than old individuals	Not reported	Not reported
Toxopeus *et al*, 2005	*M. fascicularis*	Discrimination reversal task	Younger animals took fewer trials than older animals	Not reported	Mixed sex groups
Reinhardt, 1992c	*M. mulatta*	Training to enter transport box	Juveniles more difficult to train than adults	Not reported	Female-female pairs
Drea, 1998	*M. mulatta*	Food retrieval	Sub-adults more successful than adults	No differences between males and females	Mixed sex group
Voytko, 1999	*M. mulatta*	Discrimination reversal task	Old adults learn less well than middle-aged adults	Not reported	Singly-housed
Videan *et al*, 2005	*P. troglodytes*	Training for injection	Not reported	No differences between males and females	Mixed sex groups

Table 2.2 Literature describing differences, or lack thereof, in performance of training and learning tasks in primates attributed to age and sex

2.1.4 Temperament Testing

A variety of challenges have been used to assess temperament across a wide range of species. Given the characteristics which appear to be relevant to trainability, it is desirable to assess different aspects of temperament; notably shyness-boldness (inhibition-exploration), human sociability and food motivation. Tests of temperament have been used in a range of species both in their own right to assess temperament and personality and as predictors of future performance. These two terms, temperament and personality, are often used interchangeably in the literature (Diederich & Giffroy, 2006); more so in the animal behaviour literature than the psychology literature. The former term is used here. Working animals such as dogs (*Canis familiaris*) and horses (*Equus caballus*) are particularly commonly the subject of these types of temperament tests as it is important for humans to predict future performance in fields such as military search dogs (Rooney *et al*, 2007a), guide dogs for the blind (Goddard & Beilharz, 1986) or suitability for new homes in dogs in rescue shelters (Vanderborg *et al*, 1991). The future success of young horses destined for a show-jumping career can also be predicted to some extent with temperament tests (Visser *et al*, 2003).

The choice of temperament tests used in this study was somewhat dictated by practical constraints. Tests needed to be simple to carry out and the results easy to interpret by laboratory staff. Questionnaires and other subjective measurements of temperament have proved successful in assessing temperament particularly in working dogs (e.g. Goddard & Beilharz, 1986, Rooney *et al*, 2007a), but would be inappropriate in this study. Carestaff are unlikely to have the detailed knowledge of individuals required as they may take part in caring for in excess of 100 individuals. Additionally, subjective

assessments of temperament often require sophisticated analysis, which is not feasible for care staff. As shyness-boldness (or inhibition-exploration), motivation, human sociability, trait anxiety and food motivation were identified as important factors in trainability, temperament tests which may show aspects of these factors were selected. Three tests were selected; firstly a novel object/ food motivation test, secondly human interaction test also involving food and thirdly an assessment of response to a stressor. Although there was some overlap, all tests were selected due to their relevance to trainability and simplicity of administering and interpreting. The choice of these tests is discussed below, but briefly the tests aim to assess elements of the following; the novel object test to assess shyness-boldness, food motivation, motivation to work, behavioural plasticity and distractibility, the human interaction test to measure food motivation, human sociability, motivation to work and shyness/boldness, and the response to a stressor to measure anxiety/relaxation and distractibility/concentration.

Response to a novel object and problem solving

Novelty has been shown to be useful in the characterisation of temperament/personality in a range of species. Human children who are behaviourally inhibited show less interaction with a novel object than those who have been identified as more outgoing (Kagan *et al*, 1987), and infant rhesus macaques identified as inhibited also explore a novel environment less than their less fearful peers (Suomi, 1991). The two most common forms of novelty animals are exposed to are a totally new environment (e.g. Suomi, 1991, Cameron & Rogers, 1999, Kilgour *et al*, 2006) and a novel object in their home or familiar environment (e.g. Rouff *et al*, 2005, Gibbons *et al*, 2009). In this case a novel object in the homecage was chosen as being an easier test to implement and

assess, and also as being less stressful for the animals involved and more pertinent to training carried out in the homecage. The response of an individual to a novel object is a widely used temperament test, and has been used in a range of species including pigs (*Sus scrofa*, e.g. Lawrence *et al*, 1991, Brown *et al*, 2009), cattle (*Bos taurus*, e.g. Kilgour *et al*, 2006, Gibbons *et al*, 2009), horses (e.g. Visser *et al*, 2003) and dogs (e.g. King *et al*, 2003), as well as in bush babies (*Otolemur garnettii*, Watson & Ward, 1996), macaques (rhesus, Coleman *et al*, 2005, lion-tailed, *M. silenus*, Rouff *et al*, 2005) and common marmosets (Cameron & Rogers, 1999). Common marmosets will interact more quickly with a novel object when it is at or above the mid height of their cage than when it is at the bottom of the cage (Majolo *et al*, 2003a) suggesting that the position of the novel object is important in a temperament test in this species. The purpose of the novel object test is primarily to assess an individual's reaction to something unusual and is used to measure boldness and exploratory type temperament dimensions (Coleman *et al*, 2005).

Adding food to a novel object may affect the result of the test as it now includes an element of food motivation, possibly leading to different results. Tests where an individual is required to solve a problem or manipulate a piece of equipment in order to obtain a food reward are less frequently used, but have been used in cattle (Randle, 1998), bushbabies (Watson & Ward, 1996), marmosets (Majolo *et al*, 2003a) and cynomolgus macaques (Watson *et al*, 1999). In common marmosets, novel objects were explored more quickly when they contained food than when they were empty (Majolo *et al*, 2003a) suggesting that the presence of food influences an animal's response to a novel object, although this result mirrored results with the same objects without food. Adding food to the novel object may therefore give the same results in terms of

interaction rates as if the novel object did not contain food, but more quickly. Response to a novel food object has been shown to be related to training success in rhesus macaques (Coleman *et al*, 2005) with 75% of those animals classified as 'exploratory' learning to touch a target while only 25% of those classified as 'inhibited' learning the task, suggesting this is important in the trainability of macaques.

Response to human interaction

Human interaction forms the basis of most training programmes, so it is important to assess if this influences the animals' learning of the task. Human interaction tests have been used to assess temperament in a range of species including pigs and cattle (e.g. Hemsworth *et al*, 1996, Brown *et al*, 2009). However a number of these studies focus on the response of the animal to a human approach, rather than allowing the individual to approach the human, a subtle but important difference when looking at PRT. In PRT the animal is asked to approach the human of its own accord, so whilst an individual might be willing to let a human approach to within a certain distance of itself (its flight distance), this may not be representative of its willingness to approach the human. Baker and Springer (2006) demonstrated that, in a number of species of monkey, but primarily rhesus macaques, being hand fed by familiar caregivers increased their likelihood of taking a food treat from an unfamiliar person, and in this test the monkey was allowed to approach the human. This test did differentiate between individuals, with just over 50% of tested monkeys taking the treat from a stranger, however it was not used as a measure of temperament, rather as a measure of the success of a feeding enrichment programme. Similarly, Itoh (2001) found individual differences in macaques' flight-avoidance distance (how close to a human observer they were willing

to go to get a raisin reward), suggesting that this type of test has potential to be a good measure of temperament in primates.

Response to a stressor

An individual's response to a stressful experience has been shown to be related to its ability to learn. This seems to work in two ways. First, those individuals who show the greatest response to a stressor - that is to say exhibit 'trait anxiety' (Eysenck, 1985) - have reduced cognitive function in comparison to those who don't (in infant rhesus macaques, Schneider *et al*, 1991, in adult cynomolgus macaques, Toxopeus *et al*, 2005, in rodents, Ohl *et al*, 2002, 2003). Second, individuals who are more stressed by the experience of the test do less well than those who are more relaxed in the test situation, as stress interferes with the processing of information, at least in humans (McNaughton, 1997). This suggests that an animal's response to stress may be related to how well it learns. For ethical reasons, and also for ease and practicality of use in a commercial laboratory, a separate stressor was not used. Instead a routine stressor was chosen. Bassett *et al* (2003) demonstrated that routine removal from the homecage followed by weighing as part of general husbandry procedures was enough of a stressor to elicit increased stress related behaviours in marmosets, and Line *et al* (1989a) demonstrated a stress response to cage cleaning in rhesus macaques, so this was chosen as the stressor in this study.

Relationship between the tests

The temperament tests used in this study aimed to assess different factors which may affect how well an individual macaque will learn a task. Whether the tests are

measuring different aspects of temperament however, is an interesting and important result in itself. If the tests seem to be measuring just one aspect of temperament, for example boldness, and they prove to have value in predicting success in the training task, then the test with the highest predictive value can be used and the remainder dispensed with as repetition. If however there are differences between the tests it can be said that they measure different dimensions of temperament (Gosling *et al*, 2003). No correlation was found between a human interaction test (without food) and novel object test in pigs (also without food) (Brown *et al*, 2009) and between human interaction and willingness to approach novel object in cattle (Kilgour *et al*, 2006), suggesting that human interaction and a novel object do measure different aspects of temperament and behaviour, at least in non-primate species.

2.1.5 Practical Limitations to Pre-selection

There are limitations to pre-selection; a bias towards one sex, a particular age group or temperament type may skew the results of a scientific study and therefore may not be useful in the laboratory. There may also not be sufficient numbers of animals available to allow a good choice of individuals. Pre-selection may however have a use in identifying those individuals who may prove very slow to train so they can be excluded, or perhaps allow some pre-training socialisation to be carried out. Excluding these less trainable individuals will make the process more time efficient and more likely to be used again. It is also more likely that staff will carry out a training programme if they have more success with it and therefore are more enthusiastic about training.

2.1.6 Effect of Positive Interactions with Humans

A final aim of this study was to assess whether positive interactions with humans affect the behaviour of the macaques out with the training sessions. There is some evidence that positive interactions with humans, including positive reinforcement training, have a benefit to the primates involved which extends beyond the interaction (discussed in 1.3.3). Trained laboratory-housed marmosets exhibited lower levels of stress-related behaviours than untrained marmosets in response to a stressor (Bassett *et al*, 2003) suggesting that training had a positive effect and helped them cope better with laboratory routines. In rhesus macaques desensitisation training (or counter-conditioning) to specific potentially aversive stimuli resulted in a significant reduction in fearful behaviour outside of the training sessions and in response to stressful stimuli (Clay *et al*, 2009), and baboons trained as part of an environmental enrichment programme exhibited reduced stereotypical behaviour (Laule, 1993, Maier *et al*, 2004, Bourgeois & Brent, 2005, Coleman & Maier, 2010).

2.2 AIMS OF THE STUDY

The aim of this study was to target train cynomolgus macaques and then to use this behaviour to facilitate training them to enter and remain calm in a transport box. However, it became clear from very early on in the study that this may be difficult to achieve in the time frame available before the macaques went onto a research study. The aims were therefore modified to simply target training the macaques. Further to this, the aim was also to establish if the age or sex affected if or how quickly macaques learned the task, and if temperament, as measured by simple, easy to administer tests,

could predict if or how quickly macaques learned the task. Finally the study was designed to assess if training had any effect on how macaques cope with a stressor.

2.3 METHODS

This study was carried out at contract research organisation laboratory, Covance UK. Covance carries out research on a range of species, but primates are housed separately to other species. In 2006 when this research was undertaken the primate unit held around 500-800 macaques at any one time, predominantly cynomolgus macaques but occasionally rhesus macaques are also used. All animals were housed in single sex gang cages on arrival at the unit moving to group pens housing between three and six individuals, depending on their size and study requirements approximately four weeks prior to the commencement of research. All of the macaques used in this study had been purpose-bred overseas.

2.3.1 Housing

The cynomolgus macaques used in this study were housed in a single room in groups of three animals. Trios of males were housed on the left-hand side of the room and trios of females on the right, with a gap between the sides of 1.5m. Four cages were located on each side of the room, each measuring 1.5m x 1.5m x 2.0m (Figure 2.1). All cages had opaque solid partitions between them, except from the middle to the front of the top half of the cage (0.75m from the back wall, 1.0m from the floor) which was translucent and through which individuals could have visual contact with their neighbours. All cages also had a wooden-floored veranda at the top right of the cage and three slatted wooden

platforms, running side to side at 0.5m and 1.5m and front to back on the left of the cage at 1.0m. The cage floors were covered with an approximately 1.5cm layer of sawdust as a substrate for foraging. Cage fronts were solid to 0.3m then constructed of horizontal metal bars except for the bottom left hand side of the front which has vertical bars. Two water bottles were available, one at 0.4m and another at 1.3m. Cages had a walk-in door on the right of the cage and a smaller 'pop door' at 1.3m on the left hand side (Figure 2.2, 2.3). Temperature within the room was maintained at 18 -24°C and humidity at 30 – 80%. Lighting was provided by fluorescent tubes on a 12h light/dark cycle, coming on at 0600h and off at 1800h. Air conditioning provided a minimum of 10 air changes per hour.

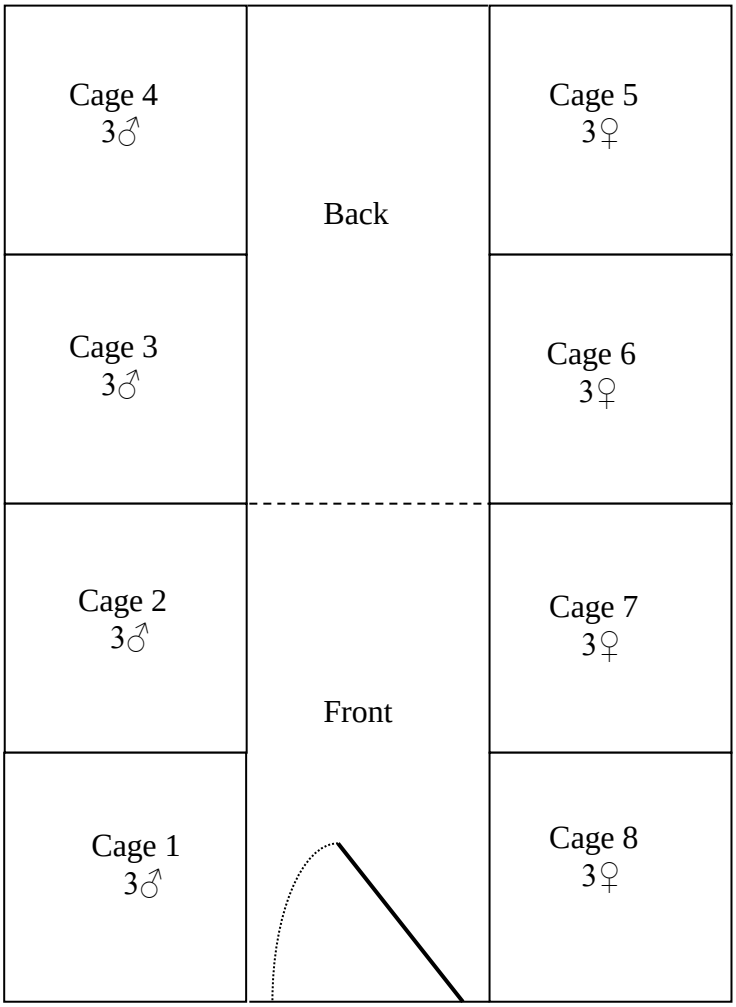

Figure 2.1 Schematic diagram of macaque colony room indicating cage position, numbers and occupants along with front/back split (not to scale)

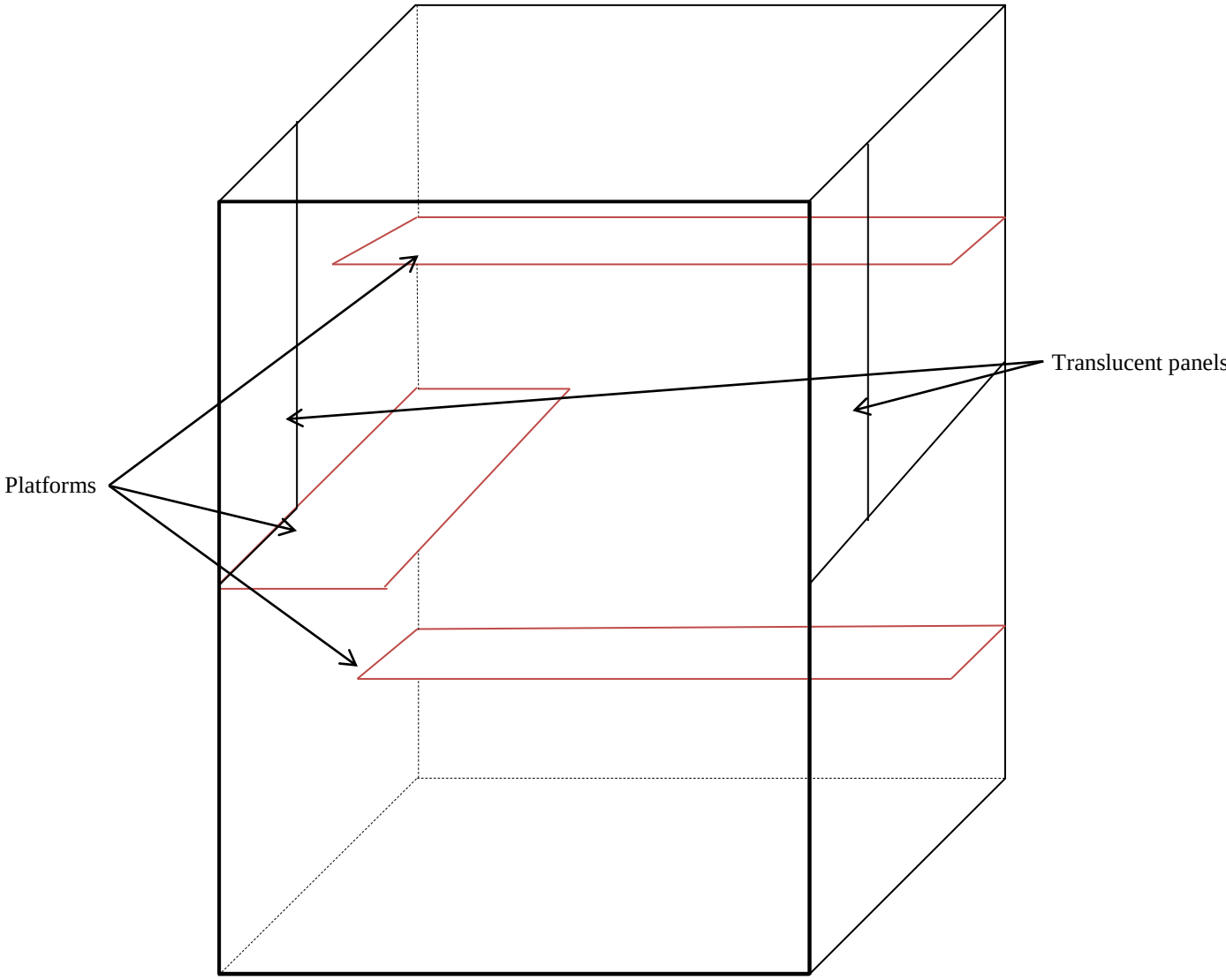

Figure 2.2 Schematic diagram of interior of macaque cage (not to scale)

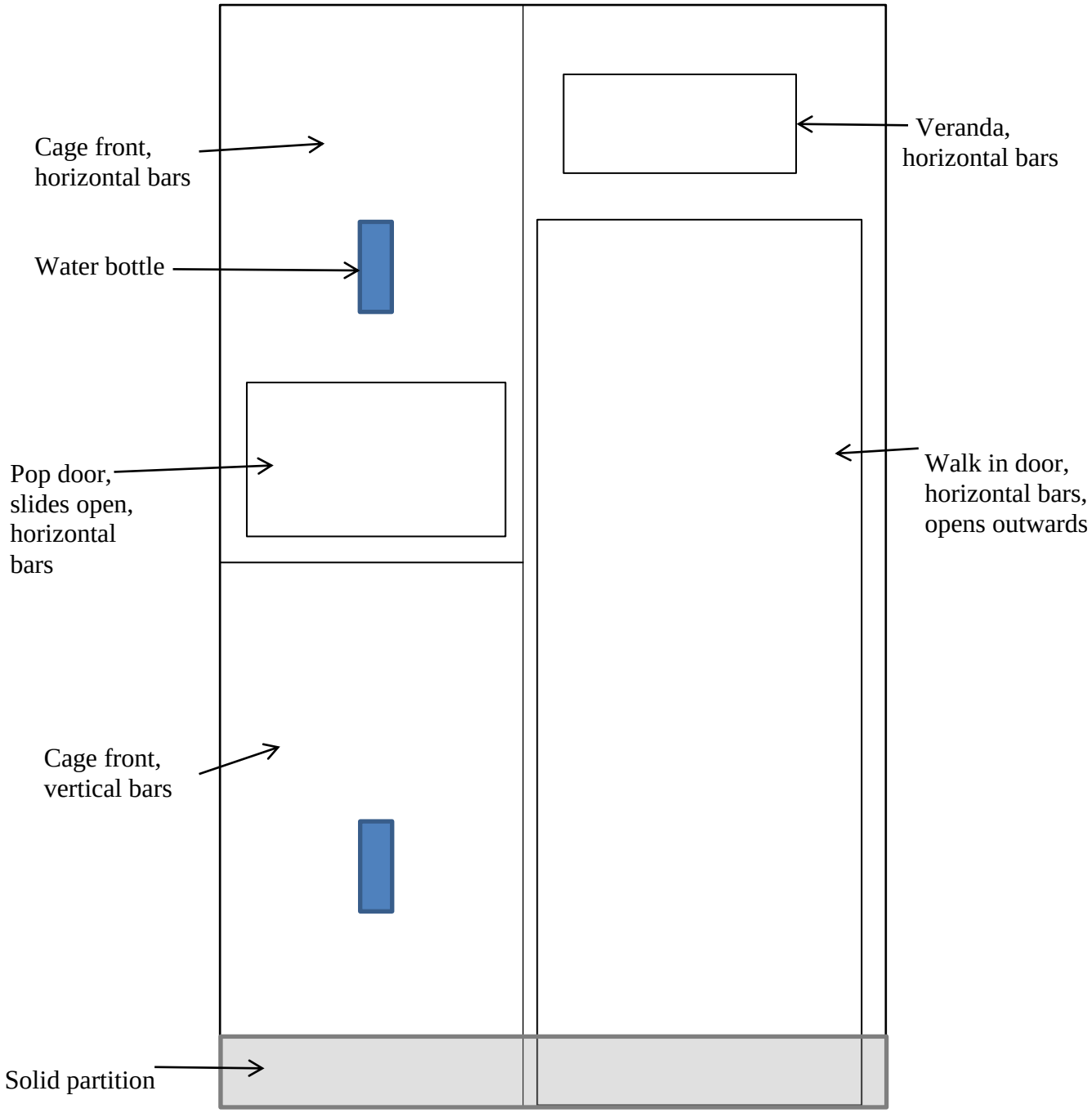

Figure 2.3 Schematic diagram of front of macaque cage (not to scale)

2.3.2 Husbandry

All macaques had access to mains water *ad libitum* from two bottles on the front of the cage, which were replaced with a clean bottle of fresh water twice daily. They were also provided with a supplement of diluted blackcurrant juice daily. They were fed three times daily, firstly receiving a 25g bonio biscuit (Spillers) at 0900h – 0930h then receiving their main ration of approximately 100g per animal of SQC Mazuir Primate Diet (Special Diet Services, Whitham) scatter fed throughout the cage at 1100h – 1130h. A final scatter feed was provided at approximately 1500h, consisting of fresh fruit, fresh vegetables, forage mix, peanuts, raisins or sunflower seeds. Macaques were also provided with a small selection of plastic dog and primate toys, at random, as enrichment.

Aisles in each room were cleaned daily by hosing and scraping, and cages were cleaned weekly. When cages were due to be cleaned the occupants were caught in a transport box and removed to a cage in a different room which was exactly the same as their home cage except that the floor had no sawdust but did house a plastic children's paddling pool filled with water. A small scatter feed of raisins was provided to encourage the use of the pool. Other plastic toys were also provided in this cage. Their home cages and room were then fully cleaned and disinfected, and once dry, fresh sawdust was put down. Each cage of macaques was then either released from its temporary cage and encouraged to run to its home cage or caught in the transport box and returned to the home cage. Any required physical checks on the macaques were also carried out at this time. Visual checks for health and welfare were carried out on

the macaques six times per day. No animals in this study were being used in any other research for the duration of this study.

2.4 EXPERIMENTAL DESIGN

2.4.1 Study Animals

Twenty-four cynomolgus macaques (*Macaca fascicularis*) were used in this study, 12 males and 12 females. All macaques were housed in same sex groups of three individuals as described above. At the start of the study macaques ranged in age from 13 to 36 months, with a mean age of 20.5 ± 0.75 months. The age of the males and females was not significantly different (t-test, males = 21.1 ± 1.5 months, females 19.9 ± 0.3 months, df = 12, t = 0.78, p = 0.45, NS). None of the study macaques had previously experienced training of any kind, nor had they had any special human interaction. They had all been housed in the laboratory for four to 12 months prior to the start of this study. Individuals were identified by individual characteristics such as face shape and colouring, but were also marked with a non-toxic coloured spray in one of three colours to ensure correct identification. Macaques were familiarised to my presence for two hours a day for two weeks (10 days in total). I entered the room quietly, and stood in the centre of the room for five minutes. I then spoke softly to the macaques for a further five minutes before sitting and standing in various places around the room for the remainder of the familiarisation period. At the end of the period, whilst the macaques were very familiar with me, habituation was not complete and the animals still spent significant amounts of time watching me. It was decided however to go ahead

with the study and to include a behavioural category which would measure the amount of time they spent "watching the observer".

The relative ranks of the individual macaques were determined during habituation. All instances of food stealing, either from the hand or the mouth, were recorded for approximately 30 minutes per pen on three occasions. Instances of food being stolen and of food stealing were recorded for each individual, and a score of food taken minus food lost calculated for each macaque. The macaque with the highest score was considered the highest ranked with the macaque with the lowest score being the lowest ranked. Results were confirmed through discussion with care staff who were asked to identify any anomalies they saw in the rankings. Overall agreement was good between these two methods, so although it was a rough guide it was considered to be valid for the purposes of this study.

2.4.2 Time Investment

Training was carried out daily, on weekdays only, at approximately the same time for each group, and groups were trained in the same order. Each training session lasted for a maximum of 15 minutes per group. This was split as equally as possible between the individuals in the group so each individual had five minutes of training per session. If not all macaques in the group were willing to cooperate with training, session length was reduced accordingly. Training sessions were also terminated if one of the macaques earned the maximum amount of rewards (15), and this was recorded. Care was taken to share training between the monkeys in the group as evenly as possible whilst still maintaining progress.

2.4.3 Temperament Tests

Three measures of temperament were recorded for all macaques. Testing and training were carried out in the following order:

1. Novel object test
2. Human interaction test
3. Habituation to experimenter
4. Baseline behavioural measurements (PRE TRAIN/BASE)
5. Response to a stressor behavioural measurements (PRE TRAIN/STRESS)
6. Training
7. Post-training baseline behavioural measurements (POST TRAIN/BASE)
8. Response to a stressor in trained animals - behavioural measurements (POST TRAIN/STRESS).

As it was desirable to test naive animals, no habituation was carried out prior to the novel object and human interaction tests. Habituation was necessary prior to the assessment of response to a stressor in order to obtain relatively normal behaviour at baseline levels, so this was carried out after the novel object and human interaction tests had been performed. The novel object test was performed first so macaques were not influenced by the experimenter who had previously given them food. The human interaction test was then performed followed by a period of habituation and the behavioural recordings to assess response to a stressor.

Response to a novel object and problem solving

A red plastic 'molecule ball' dog toy (Canac) measuring 15cm x 15cm x 12cm was filled with a mixture of peanuts and raisins, mixed with sawdust to slow food retrieval, and placed in the centre of the cage floor. A food with a stronger aroma such as banana would have been preferred but the toy proved difficult to clean so this limited the choice of foods. The object was presented at around 1000h – 1045h after macaques had received their bonio biscuit but prior to their main food being provided. There was however often a small amount of their main diet still on the cage floor suggesting that the macaques were not very hungry at the time of the test. Once the novel object had been placed in the cage, and the door shut, a stopwatch was started. I stood in front of an adjacent cage approximately 1m away from the test cage, and avoided staring directly at the macaques in the test cage which can be threatening to them. Latency from when the cage door was shut after the novel object was put in to when each individual first touched the novel object and when each individual first accessed the food inside was recorded. Time was limited to 5 minutes per test as pilot data showed that if macaques did not touch the novel object within this time they were unlikely to do so.

Response to human interaction

I approached the macaques' home cage slowly and from an angle of approximately 45°. I then stood approximately 40cm from the front of the cage at a slight angle so that I was not facing straight into the cage. I then offered the macaques a chocolate covered peanut or raisin, at random, at a height of approximately 1.3m on the left hand side of the cage, so macaques were able to approach comfortably along the shelf. The latency

of each macaque to take the reward was recorded up to a maximum of three minutes. Pilot data indicated that if the macaques had not taken food by this point they were unlikely to do so. If one macaque remained at the front of the cage and took more than four food items he or she was distracted by throwing food onto the floor at the front of the cage, whilst treats were offered to the second and/or third macaque in the original higher position. Only one food item was thrown at once so the other monkeys in the cage were not distracted. This ensured that all macaques in the group had the opportunity to perform the task and the food source was not dominated by one macaque.

Response to a stressor

Routine events in the lives of laboratory-housed primates, such as capture for cage changing, have been shown to cause stress (Bassett *et al*, 2003). The process of cage cleaning is described above, but briefly, macaques are caught, removed to an alternative cage and returned to their home cage once cleaned. This necessary routine occurrence provides a good opportunity to assess how individuals deal with a stressor without specifically stressing the animals for the sole purpose of this study. All macaques were captured within approximately 20 minutes, between 1000h and 1045h, and moved to the alternative cage. Once their home room was cleaned the macaques were returned, one pen at a time, from approximately 1130h, to allow behavioural observations to be carried out. No physical examinations were carried out on any of the macaques on recording days. Details of behavioural observations are described below.

2.4.4 Behavioural Observations

Following habituation, and prior to the commencement of any special human interaction or training, the behaviour of each of the macaques in the study was recorded using THE OBSERVER V5.0 via the handheld Workabout computer to provide a baseline behaviour measurement for the day prior to cage change. The use of the Workabout enabled both behavioural states to be recorded along with events, and for data to be gathered efficiently with minimal time spent looking away from the animals. The behaviour of each individual was recorded for 5 minutes, with cage mates being recorded consecutively. All observations were carried out between 1130h and 1430h Behavioural categories recorded are described below (Table 2.3).

Behaviour category	**Behaviour**	**Recorded as behavioural state (S) or event(E)**	**Description**
Locomotion	Locomotion	S	Normal relaxed walking, running, climbing
	Agitated locomotion	S	Quick, sudden running or climbing, usually upwards in direction
Sitting	Sit	S	Remain on haunches, still, relaxed, in one location, not actively watching anything
	Contact sit	S	Remain on haunches, still, relaxed, in contact or within 10cm of cagemate
	Huddle	S	Remain on haunches, still in tight bodily contact with cagemate, usually with limbs round each other
Vigilance	Watch	S	Actively observing either experimenter or other person/event/monkey outside cage
Foraging	Forage	S	Manipulate substrate to find food, manipulate food or eat food
Self directed	Urinate	E	Elimination of urine
	Defecate	E	Elimination of faeces
	Food Steal	E	Take food from hand or mouth of cagemate
	Self groom	E	Brush, pick through or part own hair with hands
	Self scratch	E	Repetitive raking of the skin and fur
Social	Allogroom	E	Brush, pick through or part cage mates' fur with hands, or be recipient of brushing, picking through or parting of own hair by cagemate
	Yawn	E	Gaping of mouth with stretched lips, sometimes retracted over teeth
	Lip smack	E	Lips pursed and lower jaw moved up and down rapidly and rhythmically making audible sound. May be accompanied by scalp retraction and flattened ears
	Mounts	E	Macaque stands and allows cage mate to climb onto their hindquarters in an approximation of copulation, or macaque climbs onto hindquarters of cagemate in an approximation of copulation
	Aggression	E	Receive or perform a physical threat including facial threats, chasing, hitting, grabbing and biting
Vocalisation	Coo vocalisation	E	Soft, low, longer duration vocalisation emitted with rounded lips
	Grunt vocalisation	E	Uh-uh-uh vocalisation, usually soft but sometimes harsher usually emitted in series
	Chirp vocalisation	E	Higher pitched repeated vocalisation

Table 2.3 Description of behaviours recorded, adapted from Thierry *et al* (2000)

Four behavioural observations were taken of each macaque, two before training (PRE) and two after the training programme was completed (POST). One pre-training observation was carried out during a normal day (BASE) and a second immediately after return to the home cage after cage cleaning (STRESS). The aim was to establish if either 'stressor level' or 'training level' had an effect on behaviour. For summary see Table 2.4.

		Stressor Level	
		Baseline (BASE)	Post-stressor (STRESS)
Training Level	Pre-training (PRE TRAIN)	PRE TRAIN/BASE	PRE TRAIN/STRESS
	Post-training (POST TRAIN)	POST TRAIN/BASE	POST TRAIN/STRESS

Table 2.4 Summary of terminology used to describe points at which behavioural observations were carried out.

Behaviour was recorded for five minutes per individual and time of day was matched as closely as possible to the expected time of return to the cage following the cage cleaning to be carried out the next day. As all animals were group-housed, and all animals in the group were returned at the same time, behaviour of one monkey was recorded between 0 and 5 minutes, the next monkey between 5 and 10 minutes and the third 10 to 15 minutes post return to the cage. The order of the individuals observed was random for the first observations but then kept the same across remaining observations.

2.4.5 Training Protocol

All training was carried out using positive reinforcement, whereby performance of the desired behaviour was increased by rewarding it with something positive, in this case a

food reward. A secondary reinforcer, a click from a clicker was used to mark the desired behaviour, and this was followed by a reward of either a chocolate coated raisin, peanut or honey-coated banana chip depending on the individual's preference. Any aggression between cage mates resulted in a 'time out' whereby the trainer turned their back on the cage and moved one to two paces away for 30 seconds. Macaques could receive a maximum of 15 rewards each per session, which lasted 15 minutes per pen, split as equally as possible between the three macaques in the cage. Once an individual macaque had obtained its full quotient of rewards it was encouraged to stay away from the other macaques by gently throwing raisins onto the cage floor for him or her to retrieve. Whilst not an aim of training, some of the macaques did learn to remain in the lower part of the pen once their specific training session had ended. In all cases the macaque was considered to be reliable in performing a desired behaviour if he or she performed it on nine out of ten occasions it was requested. If at any point a macaque failed to perform at a particular level the trainer went back and repeated the last level. Training was carried out once per day on weekdays between 1130h and 1430h. This was after the macaques had received their main pelleted food ration of the day and had chance to eat, but before they received their supplemental feed of more preferred food.

Initially all macaques were hand fed food rewards. Different rewards were offered to try to identify each individual's preference, but macaques tended to like all of the food rewards offered, therefore these were subsequently offered at random during training. Once the macaques were taking food reliably the bridge was paired with the food reward, in this case a commercially available clicker was used and food given immediately after (less than 1 second later). Once the macaque was expecting the food reward on hearing the bridge, as indicated by looking at the trainer or trainer's hand on

hearing it, the target was introduced. The target is an object which the macaques are trained to hold that can then be used to move them around the cage or into a new area (for example a transport box) and keep dominant animals away from submissive animals during training to enable more submissive individuals to be trained. In this case long plastic shoe horns were used, with one individual being trained to hold a grey shoehorn, a second a yellow shoehorn and the third a red shoehorn. Macaques have trichromatic colour vision so they were easily able to distinguish between the three targets.

Food rewards were held at the front of the cage behind the target so that the macaques had to reach past the target to get the food. This resulted in the macaques touching the target and receiving the bridge and reward. As the macaques made the association between touching the target and the bridge the food reward was moved away from the target, until the macaques touched the target without luring with food. Further shaping occurred as the macaques were subsequently rewarded for grasping the target and then holding it for gradually longer periods. Due to the layout of the cage the easiest place for the macaques to interact with the trainer was on the shelf which ran front-to-back at approximately 1m from the floor, so most of the initial training was carried out here for all individuals. However once the macaques were reliably holding the target they were asked to move around the cage and hold the target in different positions in the cage. There was a bias however with approximately half of the requests being moved to the bottom right of the cage for the first trained macaque, and the bottom left for the second trained macaque. If the third macaque reached this level of training his or her requests were spread randomly about the cage with approximately one-third being in the original training position of the middle shelf.

These positions were selected for a number of reasons, primarily as they were easiest for the trainer when managing three animals. However there were additional benefits of using these positions in that being on the cage floor below the height of the trainer is the most vulnerable for the macaques, so if they were willing to work there this showed they were comfortable with the training procedure. Being at floor level also allowed less confident macaques to keep more dominant individuals in visual range when interacting with the trainer and left them a clear vertical escape route away from them. A final benefit was that the macaques were able to sit on the floor rather than cling to the bars, which was more comfortable for them. Macaques were considered trained when they were willing to hold their target for 20 seconds in four different positions in the cage and hold their own target in their station position while a cage mate interacted with the trainer.

2.4.6 Statistical Analysis

For all analysis the level of significance was set at 0.05. This is despite multiple analyses being carried out, where it is recommended that corrections are used. This was done as despite a risk of Type I errors (false positives), it reduces Type II errors (false negatives). The corrections needed would have led to significance being set at such a high level that the risk of Type II errors increased. As it is desirable to look for biologically relevant patterns in the data, a significance level of 0.05 was used, but caution taken in interpreting the results. However, Bonferroni corrections were applied to the data post-hoc, and where results remained significant, this was highlighted. Data were checked for normality using Kolmogorov-Smirnov tests. If normal, parametric statistics were used, and where means are reported, standard errors are also provided.

Non-normally distributed data are analysed using non-parametric statistics. Variability for all parametric results is stated as plus or minus the standard error of the mean, whilst those for non-parametric results are stated as plus or minus the interquartile range. Ceiling values (a value greater than the maximum data collected) are used in some non-parametric tests.

Training

Training data were analysed per individual, as although the progress of an individual may have affected that of his or her cages mates, the aim of the study was to identify individual differences. Analysing means would therefore have rendered this meaningless.

Temperament Tests

Temperament tests were all analysed for each individual macaque. Relationships between data sets were identified using Pearson's Correlations. Further to this differences between means were identified with one-way ANOVAs and planned post-hoc Tukey tests.

Behavioural Data

Behavioural data were analysed in two ways. In order to assess if behaviour at either PRE TRAIN/BASE or PRE TRAIN/STRESS was related to training success, correlations between training success and duration of behaviour were carried out for individual macaques. Cage means were then calculated for all behaviours, and these

were used for analysis of the effect of the stressor and training. Although this meant that the number of subjects was reduced, the behaviour of one animal is likely to influence that of his or her cage mates, so these would not be independent, therefore mean values were used, essentially treating each cage as an individual subject.

Behavioural data were collapsed across 'stressor level', and also across 'training level' and analysis carried out on these data. Both sets of state behaviours (locomotion, agitated locomotion, sitting, contact sitting, huddling, watchful behaviour and foraging) were normally distributed so repeated measures ANOVA were carried out on these data with 'stressor level' and 'training level' as factors along with the interaction between these data.

Variable	**Between/Within Subjects**	**Levels**
Stressor level	Within	BASE STRESS
Training level	Within	PRE POST

Table 2.5 Variables analysed for state behaviours

Event behaviours were not normally distributed, and transformation did not create normality so non-parametric tests were used. Wilcoxon signed rank tests were carried out on each of the event behaviours for both 'stressor level' and 'training level'. Medians are reported with interquartile ranges.

2.5 RESULTS

2.5.1 Success Rates and Time Investment

Of the 24 cynomolgus macaques included in this study, 15 were successfully trained to touch a target within the set limit of 30 five-minute sessions (180 minutes). This equates to a 62.5% success rate in training, with the remaining 37.5% failing to reach criterion (holding the target for 20 seconds in four different locations). The mean number of sessions required for the trained macaques to reach criterion in this task was 18.5 ± 1.2, or 92.7 ± 6.1 minutes, with the fastest individual reaching criterion in nine sessions (45 minutes) and the last to learn requiring 26 sessions (130 minutes). The macaques could earn a maximum of 450 rewards. Across both trained and untrained macaques the mean percentage of rewards obtained per animal was 51.3% ± 5.7%, with trained macaques obtaining 71.2% ± 2.5% and untrained macaques receiving 18.1% ± 2.9% of available rewards. Most of the untrained macaques would touch the target but failed to reliably hold it, but some individuals never reliably approached to hand feed.

2.5.2 The Effect of Age, Sex, Housing and Rank on Success Rates and Time Investment

No correlation was found between the age in months of the macaques and the number of sessions required to reach criterion (Pearson correlation, df = 13, r = -0.047, p = 0.87, NS), and this was still the case when all macaques were included in the analysis by allocating ceiling values (equal to 30 sessions, the maximum number of sessions available) to those animals who were not successfully trained, (Spearman rank correlation, df = 22, r = 0.008, p = 0.97, NS). Seventy-five per cent (9) of the males and

50% (6) of the females were successfully trained, with no significant difference between these success rates (df = 1, X^2 = , p = 0.206, NS). There was no significant difference in the number of sessions trained males and females required to reach criterion (t-test, males, n = 9, mean 17.6 ± 1.6 sessions; females, n = 6, mean 20.0 ± 1.8 sessions; df = 11, t = -1.01, p = 0.33, NS), and no significant difference when data from all macaques were used in the analysis (Mann-Whitney, males, n = 12, median =19, Q1 = 15.25, Q3 = 29.0, females, n = 12, median = 27.0, Q1 = 20.5, Q3 = 30.0, W = 122.0, p = 0.11, NS). When rank within the group was considered, 87.5% (7/8) of top ranked, 62.5% (5/8) middle ranked and 37.5% (3/8) of bottom ranked macaques were successfully trained. The mean number of sessions required to reach criterion was 23.7 ± 1.2 sessions for third ranked macaques (n = 3), 17.4 ± 1.5 sessions for second ranked (n = 5), and 17.1 ± 1.9 sessions for the top ranked individuals (n = 7).

With the inclusion of data from all macaques (allocating a ceiling value of 30 to those macaques not successfully trained), analysis showed that there was a significant difference in the number of sessions required to reach criterion dependent upon rank within the group (Kruskal-Wallis, df = 2, top ranked, median = 18.5. Q1 = 13.5, Q3 = 23.0, middle ranked, median = 21.0, Q1 = 15.25, Q3 = 30.0, bottom ranked, median = 30.0, Q1 = 23.75, Q3 = 30.0, H = 7.04, p = 0.03). The position of the cage within the room in which individual macaques were housed may have affected the training success, 100% of macaques in the 2 most accessible cages (cages 1 and 2, Figure 2.1) were successfully trained, 50% in cages 7 & 8, 50% in cages 3 & 4, and 33% in cages 5 & 6, but analysis was not possible due to low numbers in some groups. When the time investment required to reach criterion was analysed in relation to whether animals were housed in cages at the front of the room (cages 1, 2, 7 and 8) or at the back (cages 3,4,5

and 6), no differences were seen when data from the subset of successfully trained macaques were analysed (t-test, front, n = 9, mean 18.8 ± 1.8 sessions; back n = 6, mean 18.2 ± 1.6 sessions; df = 12, t = 0.26, p = 0.80, NS), or when data from all macaques were included (Mann-Whitney, front, n = 12, median = 21.0, Q1 = 14.25, Q3 = 29.0, back, n = 12, median = 27.0, Q1 = 19.25, Q3 = 30.3, W = 125.5, p = 0.17, NS).

2.5.3 Relationship Between Temperament Tests and Training Success and Time Investment

Novel Object Test and Problem Solving Test

Twenty three of the 24 macaques (95.8%) touched the novel object within the allocated time of 300 seconds, and 17 out of the 24 (70.8%) accessed the food inside. Of the 23 macaques who touched the novel object, 15 reached criterion in training, whilst 12 of the individuals who accessed the food reached criterion, with five who did access the food failing to reach criterion (Table 2.6). The mean time to touch the novel object was 45.61 ± 8.14 s, and the mean time to access the food was 154.1 ± 17.4 s. No correlation was seen between the latency to touch the novel object or access the food and the number of sessions required to reach criterion during training either when data from the subset of successfully trained macaques were used in the analysis (Pearson correlation, touch, df = 13, r = 0.30, p = 0.27, NS; food, df = 10, r = -0.46, p = 0.14, NS), or when the entire data set was used (ceiling values of 30 used for sessions and 300 seconds for the tests; Spearman-rank correlation, touch, df = 22, r = 0.28, p = 0.18, NS; food, df = 22, r = 0.29, p = 0.17, NS).

	No touch	Touch only	Touch and food	Total
Trained	0	3	12	15
Untrained	1	3	5	9
Total	1	6	17	24

Table 2.6 Breakdown of numbers of macaques and their interaction with the novel object by training success.

Human Interaction Test

Fifteen of the 24 macaques in this study took food from the trainer in the human interaction test within the 180 second time limit, equating to 62.5% of the animals studied. Of these 15 macaques only 1 male remained untrained, and only 1 male who did not take food in this test was successfully trained (Table 2.7). The mean latency to take the food was 70.8 ± 11.6 s. There was a good correlation between the latency to take the food and the number of sessions required by an individual to reach criterion (Pearson correlation, $df = 12$, $r = 0.57$, $p = 0.03$) (Figure 2.4). When data from all of the macaques were used in this analysis (ceiling values of 30 sessions and 180 seconds allocated to those individuals who failed to reach criterion in either training or the test), this correlation was still seen, actually becoming a stronger correlation (Spearman-rank correlation, $df = 22$, $r = 0.72$, $p < 0.001$).

	No food	Food	Total
Trained	1	14	15
Untrained	8	1	9
Total	9	15	24

Table 2.7 Breakdown of numbers of macaques and their human interaction by training success.

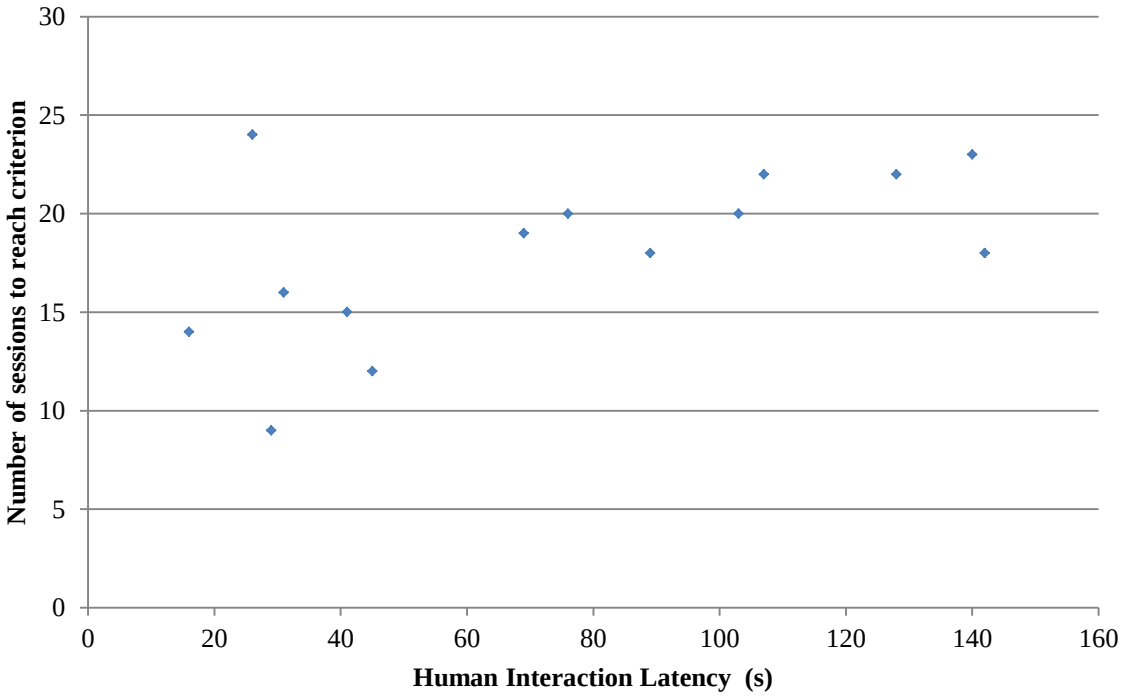

Figure 2.4 Correlation between latency (seconds) to hand feed in human interaction test and number of training sessions required to reach criterion for macaques who reached criterion in training

Response to a Stressor

Watchful behaviour at PRE TRAIN/BASE was positively correlated with the number of sessions required to reach criterion (Pearson correlation, df = 13, r = 0.73 p = 0.002) (Fig. 2.5), but no other behaviours in this observation were significantly correlated (Table 2.6). No correlations were found between behaviour at PRE TRAIN/STRESS and the number of sessions required to meet criterion (Table 2.8). The correlation between number of sessions and watchful behaviour at PRE TRAIN/BASE remained significant following a Bonferonni correction whereby significance was set at 0.003.

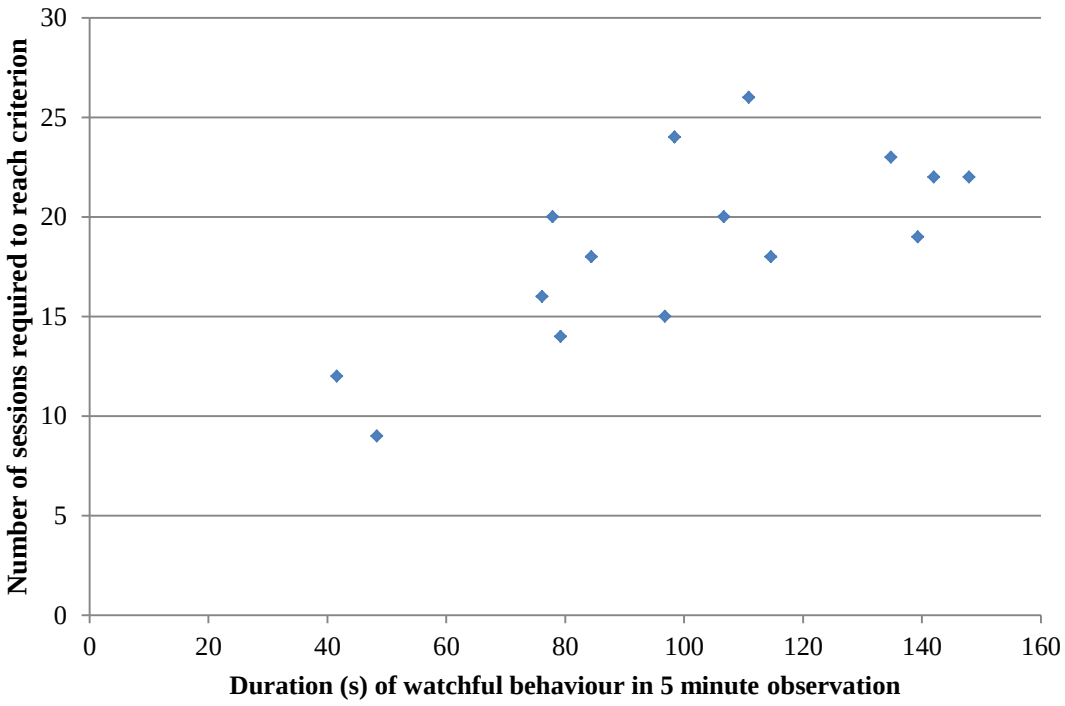

Figure 2.5 Correlation between watchful behaviour at PRE TRAIN/BASE and number of training sessions required to meet criterion for macaques who reached criterion in training

Behaviour		locomotion	agitated locomotion	sit	contact sit	huddle	watch	forage
PRE TRAIN/ BASE	r	0.02	0.06	0.14	0.23	0.10	0.73	0.07
	p	NS (0.95)	NS (0.83)	NS (0.63)	NS (0.41)	NS (0.72)	< 0.01 (0.002)	NS (0.81)
PRE TRAIN/ STRESS	r	-0.05	-0.40	0.02	0.42	0.30	-0.48	0.14
	p	NS (0.87)	NS (0.14)	NS (0.96)	NS (0.12)	NS (0.28)	NS (0.07)	NS (0.49)

Table 2.8 Results of Pearson correlations carried out between recorded behaviours at PRE TRAIN BASE and PRE TRAIN/STRESS and the number of training sessions required to reach criterion for macaques who reached criterion in training

Analysis of data from all 24 macaques (allocating a ceiling value of 30 sessions to those who failed to reach criterion) still showed a significant correlation between sessions to reach criterion and watchful behaviour at PRE TRAIN/BASE, and also watchful behaviour at PRE TRAIN/STRESS. In this analysis a correlation was also seen between sessions to reach criterion and agitated locomotion at PRE TRAIN/STRESS. Following a Bonferroni correction for multiple comparisons, with significance set at 0.003, only watchful behaviour at PRE TRAIN/BASE remained significant (Table 2.9).

Behaviour		locomotion	agitated locomotion	sit	contact sit	huddle	watch	forage
PRE TRAIN/ BASE	r	0.18	0.007	0.16	0.15	0.16	0.61	-0.14
	p	NS (0.39)	NS (0.98)	NS (0.47)	NS (0.48)	NS (0.45)	0.002	NS (0.81)
PRE TRAIN/ STRESS	r	0.09	0.49	0.10	-0.25	0.20	-0.49	0.15
	p	NS (0.68)	0.02	NS (0.63)	NS (0.24)	NS (0.35)	0.02	NS (0.48)

Table 2.9 Results of Spearman-rank correlations carried out between recorded behaviours at PRE TRAIN BASE and PRE TRAIN/STRESS and the number of training sessions required to reach criterion for all macaques

2.5.4 The Effect of Age, Sex, Housing and Rank on Response to the Temperament Tests

When the subset of data from macaques who successfully completed each temperament test were analysed, latency to take food in the human interaction test, latency to touch the novel object and latency to access the food in the novel object were not related to the age of the macaques (Pearson correlations, human, df = 13, r = 0.21, p = 0.66, NS; touch, df = 21, r = 0.12, p = 0.59, NS; food, df = 15, r = 0.34, p = 0.18, NS). Similarly, no differences were seen between males and females in the latency to take food in the human interaction test (t-test, males n = 9, mean = 61.2 ± 14.8 s, females n = 6, mean= 85.2 ± 18.7 s; df = 10, t = -1.00, p = 0.34, NS), latency to touch the novel object (t-test, males, n = 12, mean = 30.0 ± 9.6 s, females n = 11, mean = 62.6 ± 21.7 s; df = 12, t = -2.1, p = 0.06, NS) and latency to access the food (t-test, males, n = 10, mean = 173.1 ±

24.6 s , females n = 7, mean = 126.9 ± 21.5 s; df = 14, t = 1.42, p = 0.18, NS). Latency to take the food in the human interaction test was 71.9 ± 16.1 s (n = 7) for top ranked macaques, 58.4 ± 20.0 s (n = 5) for second ranked macaques and 89.0 ± 35.8 s (n = 3) for bottom ranked individuals, but analysis of these data was not possible due to low numbers of bottom ranked individuals taking the food. There was however no difference in the latency to touch the novel object across the dominance rankings (ANOVA, top, n = 8, mean = 38.9 ± 9.7 s, second, n = 8, mean = 33.8 ± 4.7 s, bottom, n = 7 mean = 66.9 ± 23.1 s; df = 2, F = 1.6, p = 0.22, NS) whilst analysis of latency to access the food was also not possible due to small sample size (top ranked, n = 5, mean = 145.4 ± 43.2 s, second ranked, n = 7, mean = 152.6 ± 25.7 s, bottom ranked, n = 3 mean = 164.8 ± 28.8 s).

With the inclusion of all macaques in the analysis, allocating ceiling values to those animals not successfully trained (30 sessions) or not successful in the temperament tests (300 seconds for touching the novel object and for accessing the food, 180 seconds for taking food from the experimenter), no correlations were seen between age and latency to touch the novel object (df = 22, r = 0.11, p = 0.61, NS) latency to access the food (df = 22, r = 0.12, p =0.58, NS) or latency to hand feed (df = 22, r = 0.01, p = 0.98, NS). In this analysis males were significantly faster to touch the novel object (Mann-Whitney, males, n = 12, median = 25.5s, Q1 = 15.25s, Q3 = 40.0s, females, n = 12, median = 51.0s, Q1 = 34.5s, Q3 = 114.0s, W = 112, p = 0.03) and to take food from the experimenter (males, median = 82.5s, Q1 = 29.5s, Q3 = 170.0s, females, median = 193.0s, Q1 = 77.5s, Q3= 180.0s, W = 106, p = 0.012), but there was no difference between the males and females in latency to access the food (males, median = 206.0s, Q1 = 125.8s, Q3 = 271.0s, females, median = 193.0s, Q1 = 101.2s, Q3 = 300.0s, W =

143.5, p = 0.73, NS). There remained no effect of rank on latencies to complete the temperament tests (Kruskal-Wallis; touch, df = 2, top, median = 35.0s, Q1 = 10.75s, Q3 = 66.25s, middle, median = 36.0s, Q1 = 20.0s, Q3 = 46.25s, bottom, median = 53.0s, Q1 = 22.0s, Q3 =163.5s, H = 2.01, p = 0.37, NS; food, df = 2, top, median = 249.0s, Q1 = 85.2s, Q3 = 300.0s, bottom, median = 226.0s, Q1 = 143.5s, Q3 = 300.0s, H = 1.04, p = 0.60; hand, df = 2, top, median = 79.0s, Q1 = 33.0s, Q3 = 132.3s, middle, median = 102.0s, Q1 = 33.5s, Q3 = 180.0s, bottom, median = 180s, Q1 = 115.2s, Q3 = 180.0s, H = 3.44, p = 0.18, NS).

2.5.5 Relationships Between the Temperament Tests

There were no relationships between the latency to take food in the human interaction tests and latency to touch the novel object (Pearson correlation, df = 13, r = 0.51, p = 0.06, NS) nor latency to access the food within (Pearson correlation, df = 9, r = -0.55, p = 0.06, NS) and no correlation between latency to touch the novel object and latency to access the food (Pearson correlation, df = 15, r = 0.22, p = 0.40, NS) when data were analysed only for those macaques who were successful in the respective temperament tests. Analysis of the data for all macaques showed no correlation between latency to touch the novel object and latency to hand feed (Spearman-rank correlation, df = 22, r = 0.19, p = 0.37, NS) or latency to access the food and latency to hand feed (Spearman-rank correlation, df = 22, r = 0.10, p = 0.63, NS), but a correlation between latency to touch the novel object and access the food within was seen (Spearman-rank correlation, df = 22, r = 0.48, p = 0.02). however when significance is corrected for multiple comparisons and set at 0.017, this was no longer significant. The macaques were significantly slower to access the food in the novel object than to touch the novel object

or take the food in the human interaction test, but no differences in the latencies to take food in the human interaction test and to touch the novel object were seen, when only data for those macaques who were successful in the respective temperament tests were used (repeated measures ANOVA, human interaction n = 15, mean = 70.8 ± 11.6 s, touch, n = 23, 45.6 ± 8.1 s, food, n = 17, mean = 154.1 ± 17.4 s; df = 2, F = 21.5, $p < 0.001$) (Fig. 2.6). Analysis of data from all macaques, allocating ceiling values of the maximum time allowed for the test to those macaques who did not successfully complete the temperament test, showed a significant difference between latencies for the tree tests (Kruskal-Wallis, touch, median = 38.0, Q1 = 48.3s, Q3 = 59.2s, food, median= 206.0s, Q1 = 120.3s, Q3 = 300.0s, hand, median = 117.5s, Q1 = 42.0s, Q3 = 180.0s, H = 29.3, $p < 0.001$).

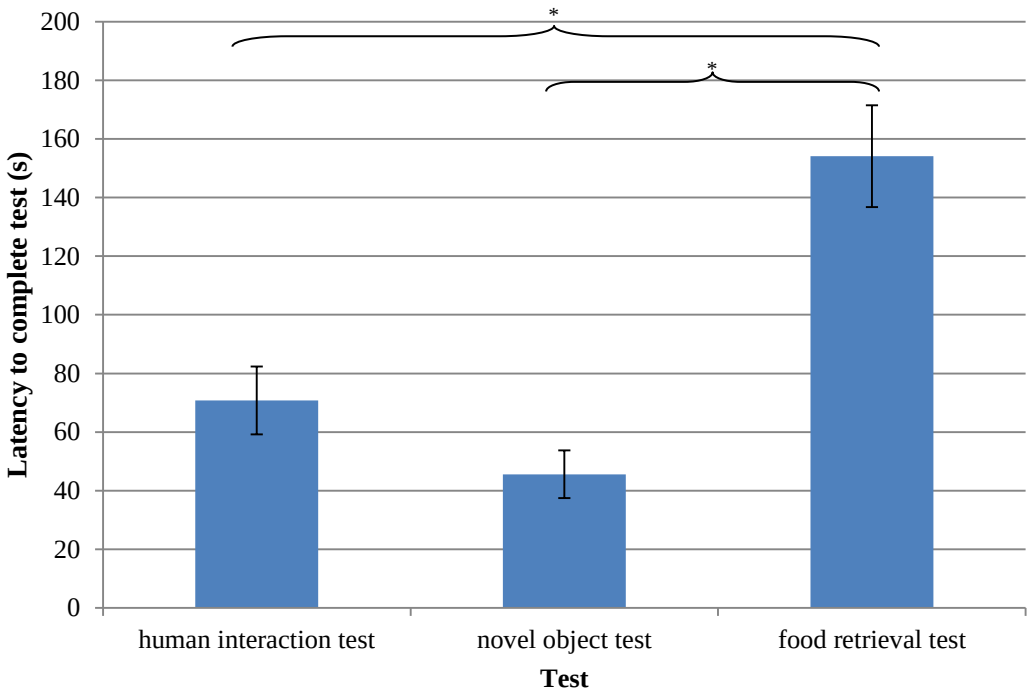

Figure 2.6 Comparison among the mean latencies to undertake the three temperament tests for individuals who were successful in the respective temperament tests. Error bars represent standard error of the mean.

2.5.6 Behaviour in Relation to the Stressor

Behavioural time budgets differed across 'stressor level'. Durations of normal locomotion, agitated locomotion, contact sitting and watchful behaviour were all significantly higher, as tested by repeated measures ANOVA, following the stressor than at baseline, whilst durations of huddling and foraging were lower after the stressor. Only sitting did not differ significantly across the observations (Fig. 2.7, Table 2.10).

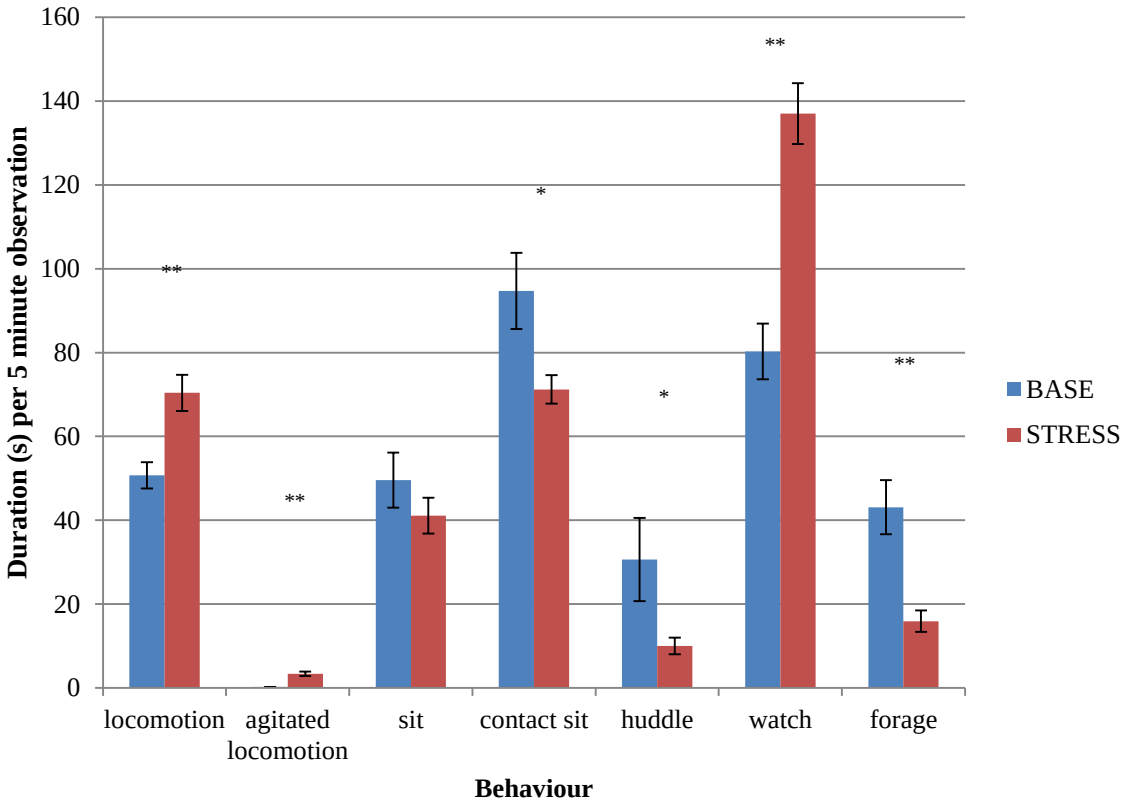

Figure 2.7 Mean durations of behaviours (seconds) for 'stressor level' collapsed across 'training level' (* p <0.05, ** p < 0.01) **Error bars represent standard error of the mean.**

Behaviour	$F_{1,21}$	p
locomotion	16.3	0.001
agitated locomotion	56.5	<0.001
sit	1.4	NS (0.25)
contact sit	1.1	NS (0.30)
huddle	4.9	<0.05 (0.038)
watch	32.5	<0.001
forage	22.2	<0.001

Table 2.10 Results of repeated measures ANOVA for 'stressor level'

Following a Bonferroni correction, whereby the significance level was set at 0.007, only the behaviour of huddling failed to reach significance where previously significance had been reported.

Significantly more self scratching, yawning, coo vocalisations and aggression was seen in STRESS observations than BASE observations, while self-grooming, allogrooming lip-smacking, mounting behaviour and grunt and chirp vocalisations were not significantly different across these observations, as tested by Wilcoxon signed rank tests (Fig 2.8, Table 2.11). Following a Bonferroni correction, whereby significance was set at 0.005, self scratching and coo vocalisations both showed significant increases following the stressor.

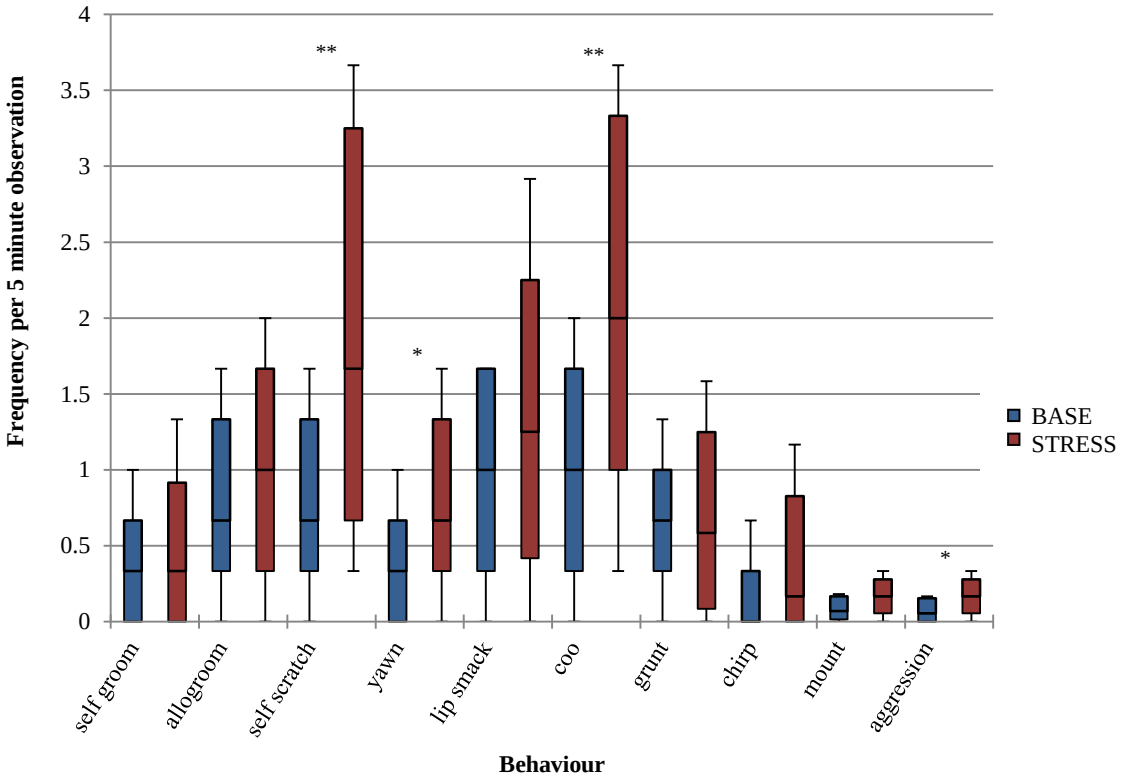

Figure 2.8 Median frequencies of behaviours for 'stressor level' collapsed across 'training level' (* p <0.05, ** p < 0.01)**. Whiskers represent minimum and maximum values.**

Behaviour	n	W	p
self groom	10	17	NS (0.31)
allogroom	10	13.5	NS (0.17)
self scratch	14	3.5	< 0.01 (0.002)
yawn	9	2.0	<0.05 (0.018)
lip smack	14	21.5	NS (0.06)
coo	14	0.0	0.001
grunt	11	12.0	NS (0.07)
chirp	11	18.0	NS (0.20)
mount	11	12.5	NS (0.08)
aggression	11	7.5	<0.05 (0.026)

Table 2.11 Results of Wilcoxon signed rank tests for 'stressor level'

2.5.7 Behaviour in Relation to Training

Training also affected behaviour in the home cage with durations of normal locomotion, agitated locomotion and sitting all being significantly lower, as tested by repeated measures ANOVA, after training had been carried out, and the duration of foraging higher post-training than prior to training (Fig 2.9, Table 2.12). Following a Bonferroni correction for multiple comparisons, with significance set at 0.007, a significant decrease in agitated locomotion and a significant increase in foraging were still seen.

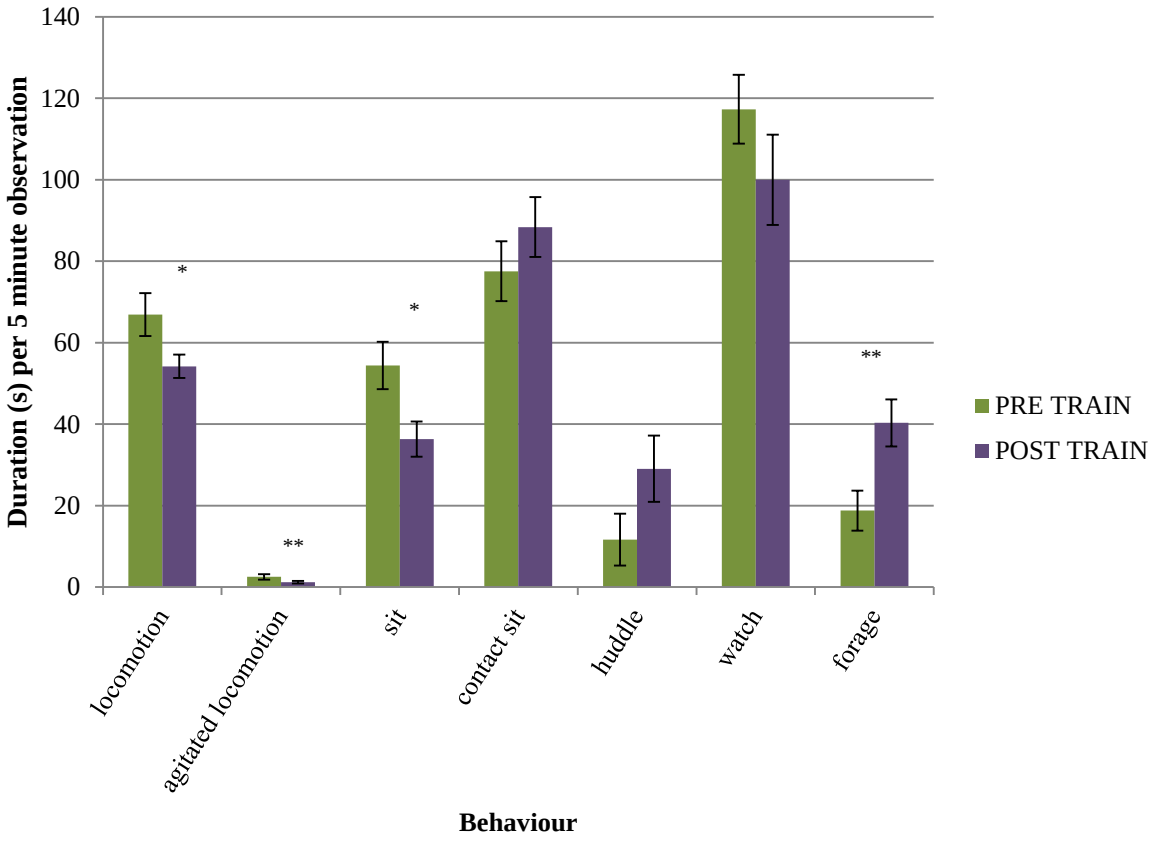

Figure 2.9 Mean durations of behaviours for 'training level' collapsed across 'stressor level' (* p <0.05, ** p < 0.01). **Error bars represent standard error of the mean.**

Behaviour	**$F_{1,21}$**	**p**
locomotion	6.8	< 0.05 (0.02)
agitated locomotion	9.8	< 0.01 (0.005)
sit	6.3	< 0.05 (0.02)
contact sit	1.13	NS (0.30)
huddle	3.5	NS (0.08)
watch	3.0	NS (0.10)
forage	14.0	0.001

Table 2.12 Results of repeated measures ANOVA for 'training level'

None of the measured event behaviours was seen at significantly different frequencies at PRE versus POST training observations (Wilcoxon signed rank test, Fig. 2.10, Table 2.13).

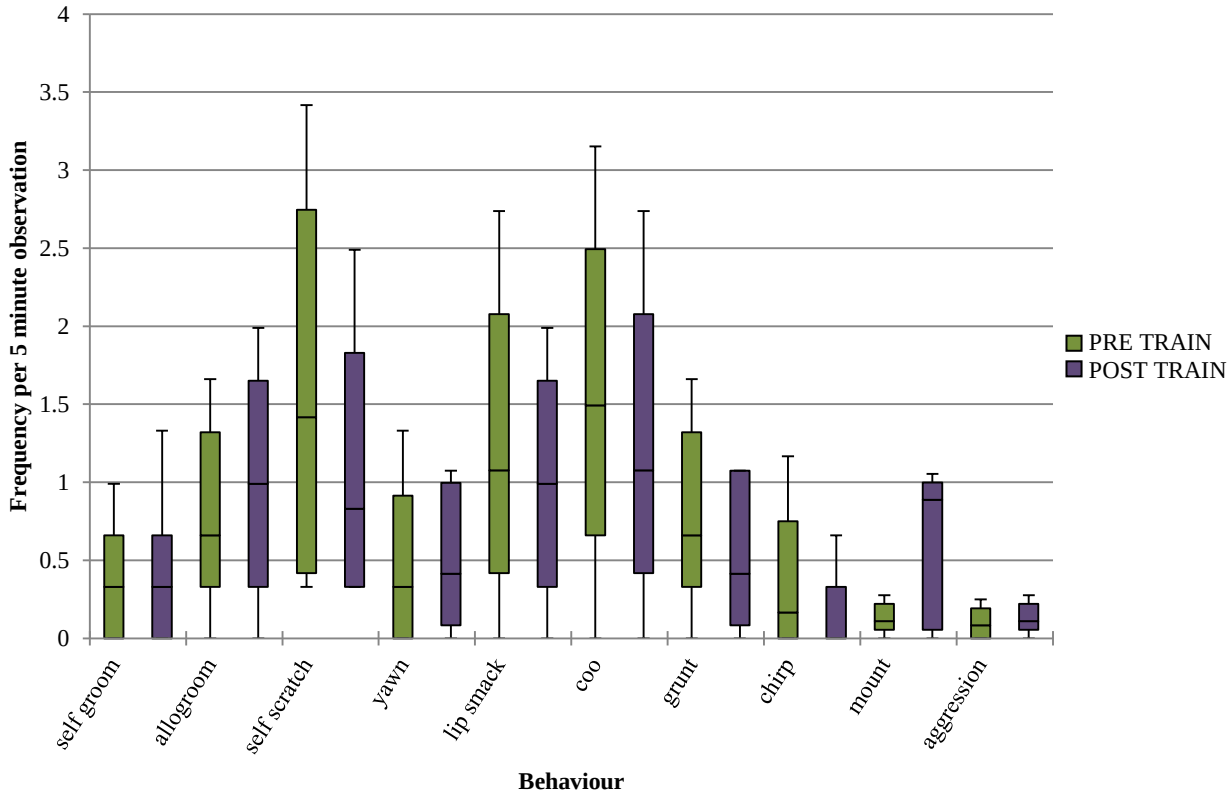

Figure 2.10 Median frequencies of behaviours for 'training level' collapsed across 'stressor level' (* $p < 0.05$, ** $p < 0.01$).**Whiskers represent minimum and maximum observed values.**

Behaviour	n	W	p
self groom	12	32.0	NS (0.61)
allogroom	13	32.0	NS (0.36)
self scratch	13	72.0	NS (0.07)
yawn	8	18.0	NS (1.00)
lip smack	11	55.5	NS (0.05)
coo	13	54.0	NS (0.58)
grunt	10	30.0	NS (0.83)
chirp	11	44.0	NS (0.35)
mount	10	28.0	NS (1.00)
aggression	12	26.0	NS (0.32)

Table 2.13 Results of Wilcoxon signed rank test for 'training level'

2.5.8 Behaviour in Relation to Interactions Between the Stressor and Training

Interactions were seen between BASE v STRESS and PRE v POST in two behaviours; agitated locomotion and sitting (repeated measures ANOVA, Figures 2.11, 2.12, Table 2.14).

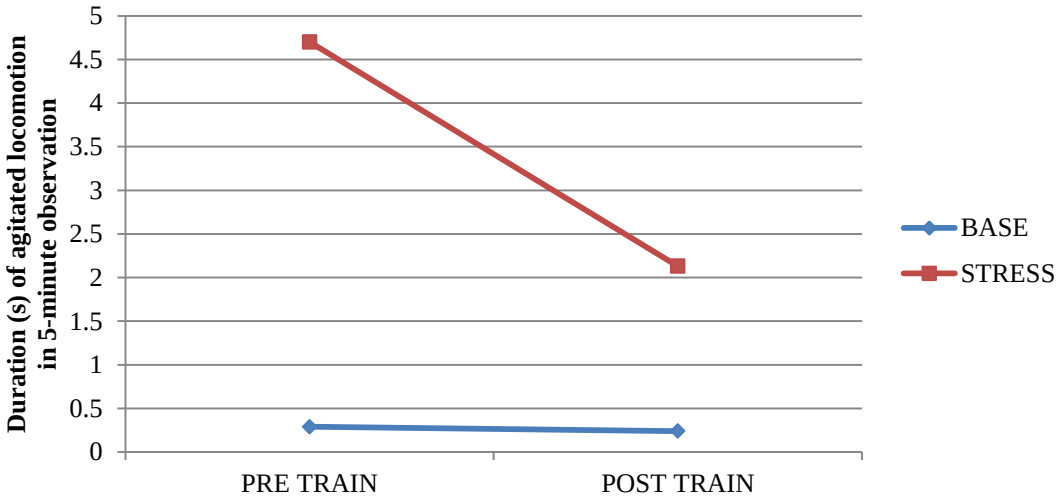

Figure 2.11 Interaction between 'stressor level' and 'training level' for agitated locomotion

Low levels of agitated locomotion were seen in BASE observations in both PRE TRAIN and POST TRAIN, and although an increase in agitated locomotion was seen at POST TRAIN/STRESS, levels were still lower than during PRE TRAIN/STRESS observations. Levels of agitated locomotion at PRE TRAIN/STRESS were approximately twice those seen at POST TRAIN/STRESS

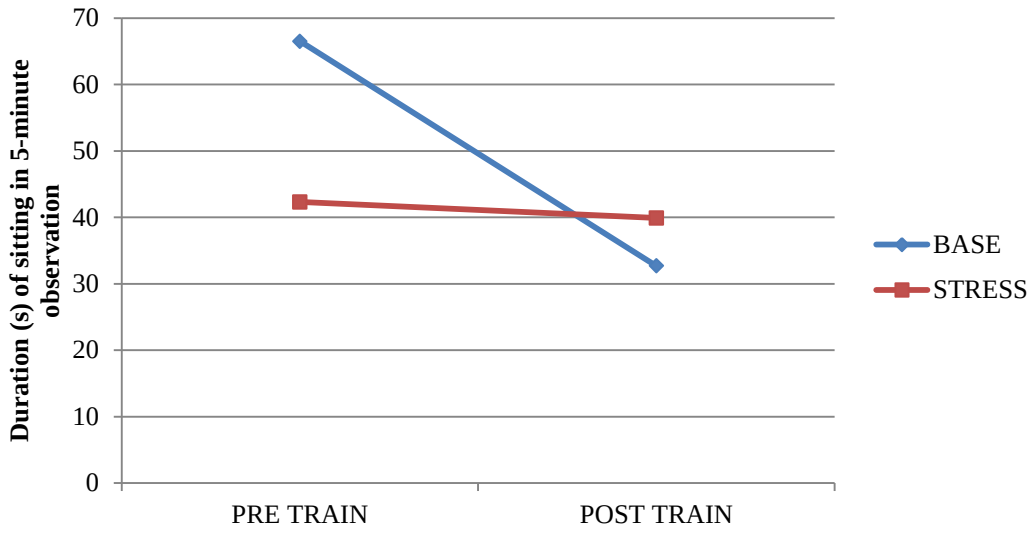

Figure 2.12 Interaction between 'stressor level' and 'training level' for sitting

Behaviour	$F_{1,21}$	p
locomotion	1.1	NS (0.32)
agitated locomotion	9.1	< 0.01 (0.007)
sit	4.7	< 0.05 (0.042)
contact sit	0.0	NS (0.96)
huddle	3.4	NS (0.08)
watch	1.9	NS (0.18)
forage	0.5	NS (0.49)

Table 2.14 Results of repeated measures ANOVA for interaction between 'stressor level' and 'training level'

Sitting was performed at the same level for both PRE TRAIN/STRESS and POST TRAIN/STRESS observations, but levels at PRE TRAIN/BASE were higher than those at POST TRAIN/BASE, where less sitting was seen than in either 'stress level' observation. This interaction however was only just significant ($p = 0.042$) so care needs to be taken in interpreting this result. Indeed, following a Bonferroni correction, whereby significance was set at 0.007, the interaction for sitting failed to reach

significance and the interaction for agitated locomotion was equal to the α value, meaning that significance has not been reached.

2.6 DISCUSSION

2.6.1 Success Rate and Time Investment

The success rate of training in this study was relatively low, with only 62.5% of the macaques successfully trained. This is comparable with that reported in a similar study with rhesus macaques (Coleman *et al*, 2005) where 60% were trained to complete a similar task. However in a more recent study a much higher success rate of over 96% was reported (Fernström *et al*, 2009) for target training in rhesus macaques. In the Coleman *et al* (2005) study particular behavioural profiles were selected to allow comparison; that is to say individuals classified as exploratory, moderate and inhibited were selected, and a disproportionately high number of inhibited and moderate individuals were selected as compared to the sampled population. Of the total sampled population 62% (n = 37) were classified as exploratory, 25% (n = 15) as inhibited and 13% (n = 8) as moderate, whereas in the training group 35% were classified as exploratory, 45% inhibited and 20% moderate. It was found that the training of exploratory and moderate individuals was more successful (85% and 75% success rates respectively) than for inhibited animals (22% success rate) (Coleman *et al*, 2005), and therefore the overrepresentation of inhibited animals in the training group could have lead to lower success rates. If a more representative sample of the population was selected training would likely have been more successful. Time investment (mean =

92.7 ± 6.1 minutes) was also greater than that reported in other studies; approximately twice that reported for rhesus macaques trained to touch a target (Fernström *et al*, 2009) and similar to that reported in chimpanzees trained to present a limb for sub cutaneous injection, a much more aversive task (Schapiro *et al*, 2005).

There is some evidence that rhesus macaques are bolder than cynomolgus macaques (Clarke & Mason, 1998) and had a greater cortisol response to confinement in a transport box (Clarke *et al*, 1998b). This species difference may be a factor in the success rates and time investment, as boldness/timidity may be an important factor in the trainability of primates at both species and individual level, but there may also be further differences between the studies. Differences in success rates and time investment could be due to the competencies of the trainers or the training protocol, for instance how quickly training was carried out within a session. It is possible that trainer competency was a factor in the differences seen, although in all studies trainers were experienced, but differences in training protocols or speed may also have played a part; overall cynomolgus macaques in this study received only 51.3% of rewards, with trained individuals taking 71.2% of rewards, suggesting training was quite slow within the session.

Although the studies were carried out in comparable environments and animals had similar backgrounds, that is to say laboratories with large numbers of animals and imported animals, small differences within the laboratory could affect how easily animals are trained. A culture of training was present in laboratories where comparable research had been carried out, evidenced by a number of papers and conference

proceedings on the subject being published (e.g. Schapiro *et al*, 2005, Coleman *et al*, 2005, 2008, Fernström *et al*, 2009) and this may affect how animals were treated prior to training as an awareness may be present of how the care staff interaction with the monkeys affects their welfare. It has been widely reported that the quality of stockmanship has great potential to affect the welfare of animals in their care (see Hemsworth, 2003 for review), and it is likely that this also applies to the laboratory.

2.6.2 The Effect of Age, Sex, Housing and Rank on Success Rates and Time Investment

The mean age of the macaques in this study was just 20.5 months at the start of training, meaning that these were juvenile macaques. Younger animals are used in regulatory toxicology as they are smaller and therefore easier to physically handle. Whilst there was no effect of age on the success or time investment in training in this study, only a small age range was represented with individuals ranging from 13 to 34 months old (the 34 month old individual was an outlier, with the next oldest being 24 months old) at the start of the study. In other studies rhesus macaques aged from five to seven years (Coleman *et al*, 2005) and 3 years old (Fernström *et al*, 2009) were trained, and in one of these studies with more success than the current study (Fernström *et al*, 2009). It has been shown that juvenile rhesus macaques are harder to train to enter a transport box than adults (Reinhardt, 1992c), and therefore it is likely that the fact that the cynomolgus macaques in this study were juveniles also played a part in the disparity in training success and time investment between this study and that of Fernström *et al* (2009).

Male cynomolgus macaques were no more likely to reach criterion in the training task than females, and there was no significant differences in time investment between the sexes. The studies by Coleman *et al* (2005) and Fernström *et al* (2009) both involved female macaques only, so there are no directly comparable studies involving both male and female macaques. The fact that males required no more or less time to reach criterion suggests that there are no differences between the sexes in their ability to learn in a training paradigm, and this is supported by studies of other primate species where no differences were seen between males and females (McKinley, 2004). Cage tier has been investigated in relation to welfare in primates, where it has been suggested that animals housed in more accessible upper tier cages may receive more positive associations with humans than those housed in lower tiers (Reinhardt & Reinhardt, 2000, Schapiro & Bloomsmith, 2001), but cage position within a room has not been studied. Cage position within the room did not affect trainability however so this is unlikely to be an important factor.

The rank of the macaque within the cage affected trainability. The top ranked and middle ranked macaques may have dominated training sessions, although care was taken to avoid this. The bottom ranked macaque may have been more hesitant to come forward to interact with the trainer due to a fear of aggression from the higher ranked animals or due to a voluntary inhibition of the behaviour, and evidence suggests that lower ranking individuals will perform less well in learning tasks in the presence of high ranking conspecifics, even if they are able to perform the behaviour (Drea & Wallen, 1999), but it is not possible to determine if this occurred in this study.

2.6.3 The Effect of Age, Sex, Housing and Rank on Response to the Temperament Tests

The age of the macaque was not related to their response to the novel object nor their response to the human interaction test. Again this may be due to the restricted age range available for testing, but there is no suggestion in the literature that age affects latency to interact with a novel object (Box & Smith, 1998, Kendal *et al*, 2005). Female macaques were slower to take food from the trainer in the human interaction test and also slower to touch the novel object than males, with these differences being found to be significant when data from all macaques were analysed, though the difference in time to touch the novel object was not robust enough to retain its significance following a Bonferroni correction. This finding is in line with that of Drea (1998) who found no differences between male and female rhesus macaques in a food retrieval task, but the difference between males and females in latency to hand feed is difficult to explain, with no relevant literature in primates, and this result having no parallel in other species of primate (Chapter 3).

Rank within the cage had no influence on the latency to touch the novel object, access the food within, or take food from the experimenter, in agreement with Itoh (2001) who found that an individual rhesus macaque's willingness to approach a person did not reflect their dominance rank in a group. These results are useful in that they show that higher ranked individuals were not monopolising the test, so these temperament tests are equally valid for all macaques.

2.6.4 Relationships Between the Temperament Tests and Training Success and Time Investment

Novel Object Test

The cynomolgus macaques in this study touched the novel object in a mean time of 45.6 seconds, whereas rhesus macaques touched a similar novel object in a mean of 13.2 seconds (Kinnally *et al*, 2008). Although there were methodological differences in these studies, in the type of novel object and the way it was presented (inside the cage in this study and outside the cage in Kinnally *et al*, 2008), this may give further weight to the argument that rhesus are bolder than cynomolgus macaques and that differences in their trainability may be due to species differences. Most of the macaques however were willing to touch the novel object in the current study and all of those who were trained had done so.

There was no difference between the latency to touch the novel object between those macaques who were successfully trained and those who were not, supporting this suggestion. Kinnally *et al*, (2008) argue that a macaques' response to a novel object can be separated into how cautious they are as reflected by the latency to interact with it, and their interest in the novelty as reflected by the duration of interaction with it. Whilst overall interaction duration was not recorded in this study it may be that the latency to get the food (if indeed they did get it) might reflect this duration. Although the duration to access the food was planned as a test of an individual's food motivation and problem solving ability, it may also reflect their interest in novelty. Animals with a high interest in novelty may have spent more time interacting with the object and also manipulated it

more so increasingly the likelihood of them finding and extracting the food as opposed to a less interested individual who investigated the item less thoroughly and therefore did not access the food. There was, however, no difference in the latency to get the food between trained and untrained individuals suggesting that this was not a predictor of trainability. The lack of significant results may suggest that a novel object test is not a useful predictor of trainability, but others have found that this kind of test does predict trainability (Coleman *et al*, 2005) and the latency to obtain the food within the novel object has been linked to social engagement in a group (Kinnally *et al*, 2008), those obtaining food more quickly being more integrated.

It may therefore be that the novel object was not challenging enough to be truly novel and differentiate between individuals. The selection of the novel object was limited by practical considerations in that the research was carried out in a laboratory where toxicology research was performed, so care had to be taken in the selection of objects. The macaques were familiar with similar types of toys which were used for enrichment so perhaps the novel object was not as novel as desired, as objects with more novelty, such as children's toys or items which moved or made noise as used in other studies (Majolo *et al*, 2003a, Kinnally *et al*, 2008), would have been. Other tests with novel objects have presented the objects outside of the cage (Kinnally *et al*, 2008) requiring animals to reach out of the cage in order to interact with them, this may add another dimension of novelty and possibly require an extra element of boldness which may have led to greater differentiation between individuals.

Human Interaction Test

Taking food from a human is an integral and key aspect of a training programme, so an individual's response to a human and their willingness to interact with them crucial in a training programme. Only 62.5% of the macaques took food from the trainer in the human interaction test, a relatively low proportion of the animals tested. Whether an individual took food was strongly related to their later success in reaching criterion in training with 93.3% of those taking food in this test being successfully trained and 88.9% of those who did not take the food remaining untrained. This highlights the importance of an individual's willingness to take food from a human in a training programme. It may also account for some of the discrepancy between the success rates in this study and that of Fernström *et al* (2009), as all their rhesus macaques were hand feeding prior to the start of training.

The success rate in this study, when only individuals hand feeding prior to training were considered, much more closely resembled that reported in this study of rhesus macaques. There was also a good correlation between latency to take the food and the number of sessions required to reach criterion which indicates that this is a sensitive predictor of trainability. Hesitancy to approach the trainer can lead to 'wasted' time within a session, for example in this study trained macaques took a mean of 71.2% of rewards, equating to approximately 11 out of the maximum 15 rewards per session. If these are spread evenly across the five minute session this equates to a reward approximately every 30 seconds. If actually doing the requested task takes less than 10 seconds, 20 seconds are therefore spent with the individual deciding whether to respond. More hesitant animals are likely to receive fewer rewards per session and

therefore require more sessions to reach criterion than their less hesitant conspecifics, even if they learn in the same number of overall 'training efforts'. This again highlights the importance of hand feeding and the benefits of ensuring all animals entering a training programme will not only hand feed from the trainer, but will do so with a minimum amount of hesitation.

Response to a stressor

Watchful behaviour at PRE TRAIN/BASE was positively correlated with the number of sessions required for an individual to reach criterion, but none of the other recorded behaviours was similarly related to trainability when the data for successfully trained macaques were analysed, however when data from all macaques were analysed watchful behaviour and agitated locomotion at PRE TRAIN/STRESS were also significantly correlated with the number of sessions. The PRE TRAIN/STRESS results however were not robust enough to remain significant following a Bonferroni correction, suggesting that the relationship between watchful behaviour at PRE TRAIN/BASE is a particularly good indicator of trainability. That this behaviour was only related to trainability at baseline and not following a stressor is interesting in that trait anxiety, and its relationship to cognitive function is usually measured following a stressor (Eysenck, 1985, Toxopeus *et al*, 2005). However following a stressor all animals are likely to exhibit higher levels of watchful behaviour meaning that small differences may not be so apparent and the relationship with trainability to fail to reach significance. Nevertheless it appears that more vigilant monkeys, who are likely to be more anxious and possibly more fearful are less successful in training paradigms.

2.6.5 Relationships Between the Temperament Tests

The tests administered did not prove to be related; no correlations were seen between the latencies to interact with a person, touch the novel object or access the food inside, except when data from all macaques were considered, when a correlation between latency to touch the novel object and latency to access the food within was seen. That there was no relationship between the latency to touch the novel object and latency to access the food inside, when just animals who accessed the food were considered, is unsurprising given that it was necessary to touch the novel object prior to accessing the food.

The macaques took a similar amount of time to interact with the trainer and the novel object, suggesting that those who interacted did not view the human as more threatening, frightening or indeed pleasant than the novel object. They were however significantly slower to obtain the food inside the novel object indicating that they found this the most challenging aspect of the tests. This may be due to the position of the object, and the monkeys reluctance to go to the cage floor, or possibly a lack of interest in the food.

2.6.6 Behaviour in Relation to the Stressor and Training

In response to the stressor behaviours associated with anxiety increased, with a concurrent decrease in more positive behaviours, in response to the stressor. This indicates that the event chosen as the stressor, cage cleaning, was in fact stressful for the macaques. Following the training programme the reverse is seen, although the pattern is not as strong. Durations of behavioural indicators of good welfare increase

and indicators of reduced welfare decrease. Table 2.14 shows how behaviours respond to the stressor and the training programme. Exposure to the stressor leads to an increase in locomotion, agitated locomotion, watchful behaviour, self scratching, yawning, coo vocalisations and aggression, and a decrease in contact sitting, huddling and foraging. Following the training programme the macaques exhibited less locomotion, agitated locomotion and sitting, but more foraging.

Behaviour	POST STRESS (v PRE STRESS)	POST TRAIN (v PRE TRAIN)
locomotion	↑	↓
agitated locomotion	↑	↓
sit	=	↓
contact sit	↓	=
huddle	↓	=
watch	↑	=
forage	↓	↑
self groom	=	=
allogroom	=	=
self scratch	↑	=
yawn	↑	=
lip smack	=	=
coo	↑	=
grunt	=	=
chirp	=	=
mount	=	=
aggression	↑	=

Table 2.14 Summary of behavioural changes seen in response to the stressor and to training↑ indicates an increase in response to the treatment, ↓ indicates a decrease and = indicates no significant difference

How these individual changes are interpreted is discussed below, but the overall pattern of behavioural change indicates that whilst the stressor leads to reduced welfare in

macaques, the training programme has a positive effect on macaque behaviour and therefore is likely to improve the welfare of laboratory-housed macaques. As all macaques were not trained, the positive effects may be less obvious. Socialisation however, which all macaques in the study received, is likely to also have a positive impact on welfare.

Locomotion

Locomotion and agitated locomotion in this study were found to significantly increase following the macaques' return to their home cage after cleaning and decrease significantly following the training programme, and even when multiple comparisons were corrected for a significant increase in locomotion following a stressor was still seen. The use of locomotion as a measure of welfare can be complex, as in some circumstances increasing the levels of locomotion may be desirable, such as when individuals are apathetic or overweight (Laule & Desmond, 1998) as seen in orang-utans (*Pongo pygmaeus*) (Tripp, 1985), and can be an indicator of improved welfare (Bayne, 1989), but conversely increased levels of locomotion can be interpreted as an indicator of reduced welfare (Chamove, 1989, Chamove & Anderson, 1989). Marmosets exposed to a stressor exhibited increased locomotion (Bassett *et al*, 2003), and those exposed to a taxidermised predator also showed increased levels of locomotion which were subsequently reduced by the administration of an anxiolytic drug (Barros *et al*, 2000, 2001). These results give rise to the conclusion that the increase in locomotion was an indicator of stress. This is further supported by work by Coe *et al* (1982) where squirrel monkeys (*Saimiri sciurius*) showed increased levels of locomotion which were reflected by increases in cortisol response in response to a fear

stimulus. Increased levels of locomotion are also seen in primates in response to other stressors such as maternal separation (Laudenslager *et al*, 1990), social isolation (Levine, 1993), and novelty (Hennessy *et al*, 1995).

Increases in locomotion considered to be positive tend to be seen in response to positive changes in the animals' physical environment, for example increasing cage size (Badihi, 2006) or improving environmental enrichment (Tripp, 1985, Platt & Novak, 1997) rather than in response to improvements in psychological welfare such as those potentially provided by positive reinforcement training. That training lead to a reduction in the level of locomotion in this study suggests that it has a positive impact on the welfare of the macaques.

Although agitated locomotion accounts for a very small proportion of the daily activity budget it may have value in being a better indicator of welfare than overall locomotion, which as discussed above, can be open to interpretation. Agitated locomotion has been reported to be shown at lower levels in marmosets housed in larger cages than in smaller cages, and outdoors versus indoors, whilst the reverse is true of normal calm locomotion (Badihi, 2006) suggesting that perhaps this is a simpler measure of welfare than normal locomotion. Agitated locomotion decreased significantly following training. This indicates that training has positive welfare benefits for primates, especially if it is taken that this behaviour represents a better measure of anxiety or stress than levels of normal locomotion as discussed above.

Whilst no interaction was seen in normal locomotion between the stress conditions and training, agitated locomotion was affected by this interaction. Levels were

approximately similar, and low, at both baseline observations however increased levels were observed following the stressor, and this increase was much greater before training than after. This result is interesting for two reasons, firstly and most importantly it indicates that training reduces the macaques' stress response to an aversive event. This has been found in other studies (Bassett *et al*, 2003), and is one of the key benefits of PRT. However it is also interesting that this difference was seen in agitated locomotion but not in normal locomotion. In similar studies training did not affect locomotion in marmosets following a stressor (Bassett *et al*, 2003), but agitated locomotion was not recorded separately. As overall levels of agitated locomotion are relatively low they are unlikely to alter the results of the analysis on normal locomotion, so these subtle differences may be missed. This again emphasises the importance of recording these qualitatively different types of behaviours. That agitated locomotion was still seen to be significantly lower following a training programme, and significantly increased following a stressor when a much stricter α level of significance was used further highlights how robust this behaviour is as a measure of well-being in macaques.

Sitting

Inactive sitting when the macaque was not in contact with his or her cage mates did not differ significantly in this study between the baseline observations and observations following the stressor. Stressors have been shown to lead to decreases in inactivity in marmosets (Bassett *et al*, 2003). Changes were however seen in contact sitting and huddling behaviours, both of which were observed at significantly reduced levels following the stressor compared with baseline observations. Given that contact sitting

and huddling represent affiliative behaviour (Das *et al*, 1998, Thierry *et al*, 2000, 2004) which sitting alone does not it might have been expected that these behaviours may increase in response to a stressful experience as macaques look to their cagemates for reassurance. Anecdotal observations suggest that macaques often sleep when huddling, and this is likely to be a behaviour particularly affected by stress. Huddling may therefore be a more sensitive measure of stress than other types of inactivity in macaques. This again emphasises the importance of not just looking at the overall duration of a categorised behaviour but also looking at qualitative differences in the way the behaviour is expressed. Following corrections for multiple corrections, sitting behaviours failed to show significant differences across observations, which may suggest that they are not as robust as other behaviours as indicators of welfare.

Sitting was found to decrease following the training programme, and whilst levels of both contact sitting and huddling were higher after training this did not reach significance. That the different types of inactivity followed this pattern is interesting given the discussion above relating to the importance of the qualitative nature of this behaviour. It may also help to explain why there is confusion over the desirability of decreasing this behaviour.

No interactions between the 'stressor level' and whether the macaques had undergone a training programme were seen for either contact sitting or huddling. There was however an interaction for sitting alone. Whilst sitting was not significantly different following the stressor irrespective of training, PRE TRAIN sitting at baseline was significantly higher than POST TRAIN sitting at baseline, suggesting that PRT can improve welfare of macaques not only in response to a stressor, but also in their normal daily activity.

Vigilance behaviour

Watching the observer may be an indicator of the macaques' fear of the observer (i.e. watching for threats), or as a reflection of a more positive interest in them. It is therefore a difficult behaviour to interpret. Time spent watching the observer and other people and events outside the room increased significantly following the stressor in this study, even when corrections were made for multiple comparisons. Stressful events may prime the macaques to look for further threats, and so be more watchful. Once again this would argue for identification of qualitative differences between vigilance borne of fear, and that exhibited as a result of a positive interest. It would then be desirable to reduce fear-related watchfulness but the presence of positive interest may be beneficial to welfare providing it does not displace other beneficial behaviours, such as social interactions with cage mates.

Watchful behaviour was exhibited at lower levels following the training programme than before, but this did not reach significance. It would be expected that if habituation was the reason for the changes in behaviour following training, watching the observer would be one of the behaviours affected most as the macaques learned to ignore the observer. Bassett (2003) found that trained marmosets spent more time than untrained marmosets watching the observer, and explained this as trained animals watching the observer in expectation of a food reward. This is akin to the positive type of watching discussed above, rather than a fear motivated watching. This did not happen with the macaques in this study, perhaps due to species differences. It is possible that the lack of significant change in vigilance behaviour reflected a qualitative change rather than a quantitative change, in that fear motivated watching decreased but interest motivated

watching increased, creating no net change in this behaviour. It may prove difficult to identify qualitative differences in watchful behaviour but it would be interesting to do so to see how these two types of vigilance change in response to human interactions.

Foraging

Foraging behaviour decreased significantly following the stressor and this may reflect an unwillingness to move to the cage floor where they may have felt more vulnerable and remain higher up in the cage to gain a better vantage point. If this was the case it suggests that the increase in vigilance discussed above was exhibited as a result of fear rather than increasing positive interest. Foraging behaviour was found to increase following the training programme. This may be due to the macaques' being more willing to go to the floor of the cage in the presence of humans following positive interactions with them. Foraging is a behaviour which environmental enrichment programmes aim to increase as it is seen as a positive indicator of welfare (e.g. Anderson & Chamove, 1984, Bryant *et al*, 1998). The increase in this behaviour following training suggests that training improves the welfare of laboratory-housed macaques. Both the decrease in foraging following the stressor and the increase following training were robust enough to retain significance following correction for multiple comparisons, suggesting that this is a particularly useful behaviour for measuring the welfare of laboratory-housed macaques.

Social behaviours

Most social behaviours did not show any differences between baseline observations and post-stressor observations, however aggression rose following the stressor. The rise in

aggression could have reflected increased tensions within the groups as a result of increased individual stress levels, but the increase failed to reach significance when significance level was corrected for multiple comparisons. Affiliative behaviours such as allogrooming would perhaps not be expected to change immediately as a result of a stressor, and this was found in this study. Allogrooming has been found to reduce tension, particularly in social situations, and increased levels are seen in socially stressful situations (Schino *et al*, 1988), however as the stressor chosen was not a social stressor it is not surprising that allogrooming did not change immediately after the stressor. Mounting behaviour is also an affiliative behaviour (Thierry *et al*, 2004) so, in a similar way to allogrooming, perhaps it is not surprising that it did not change in response to the stressor.

Social behaviours did not differ between PRE TRAIN and POST TRAIN observations. This is unsurprising as affiliative behaviours are generally weaker indicators of stress due to them being rather more secondary indicators than direct indicators such as displacement activity (e.g. self scratching). Social behaviours such as allogrooming are often considered positive indicators of primate welfare (Honess *et al*, 2004), so an increase in these types of behaviours might have been expected if training improved welfare. The median level of allogrooming was higher following the training programme than before this however this did not reach significance, possibly due to low frequency of performance alongside high inter-group variability. Interesting, although again this result failed to reach significance, median levels of mounting behaviour were higher following training than before. Mounting in macaques is an affiliative behaviour (Thierry *et al*, 2004), so this may be an indicator of improvements to their welfare. Aggression and the related threat and fear behaviours, and lip smacking, showed no

change in response to training. These behaviours were seen at quite low levels, so perhaps this is related to this, but there is no evidence of disrupted social orders within the group as evidenced by increases in these behaviours.

Self-directed behaviours

Of the self-directed behaviours recorded self scratching and yawning increased following the stressor as opposed to baseline, whilst the other behaviours were not affected. Self scratching has been widely reported to increase in response to stressful situations (Schino *et al*, 1988, Maestripieri *et al*, 1992, Baker & Aureli, 1997), so the increase in this behaviour in this study validates that both the proposed stressor is actually stressful, and that other behaviours which may be difficult to interpret, such as increased locomotion, are indicators of stress. The statistical significance in the increase of self scratching following the stressor was still seen even when corrections were made for multiple comparisons. Yawning is often seen in response to behavioural arousal, particularly social stress, as well as an indicator of tiredness (Thierry *et al*, 2000). It is unlikely however that tiredness could account for this increase in yawning, given that observations were carried out in the middle of the day. Therefore this increase in yawning may reflect an increased level of stress perhaps indirectly as a result of increased aggression within the group rather than the stressor *per se*, however this result was not robust enough to retain significance when a more stringent significance level was set. Self grooming did not change following the stressor, and whilst this may be due to high variability and low frequency of performance of this behaviour, it seems to indicate that this behaviour does not reflect the level of anxiety an individual is experiencing. The training programme had no effect on the performance of self-directed

behaviours. Median levels of self scratching following training were just over half those seen before training, although this again failed to reach significance, again probably due to variation between groups.

Vocalisations

Of the vocalisations recorded only coo vocalisations were affected by the stressor, and the increase in these calls following the stressor was robust enough to remain significant following correction for multiple comparisons. Cynomolgus macaques emit a range of coo calls, although these were not distinguished in this study. Some of these calls are performed when macaques are calm and seem to be contact calls (Palombit, 1992), however a separate class of coo vocalisations known as "high-extended modulated calls" which are produced by mildly aroused individuals and "whimper" coos emitted generally by distressed infants and juveniles have been identified (Palombit, 1992). Given the overall picture described by the results of this study, and the fact that the animals studied were juveniles it seems reasonable to suggest that the majority of coo calls were whimper coos, and therefore this indicates a stress response. Further to this, pigtail macaque (*M. nemestrina*) infants emitted more coo vocalisations following maternal separation than when with their mothers and this corresponded to increased heart rates (Boccia *et al*, 1991), validating this result. Neither of the other two vocalisations recorded showed any change in response to the stressor. Without the aid of sonographic equipment it proved difficult to equate vocalisations heard in the laboratory with the many described by Palombit (1992), however Thierry *et al* (2004) identify grunts as affiliative calls in other macaque species, so if this follows for cynomolgus macaques this fits well with the pattern that affiliative behaviours seem

unaffected by the stressor. Similarly the calls labelled as chirp here seem akin to the chuckle identified by Masataka and Thierry (1993) and are mostly seen in agonistic interactions, and therefore remain constant across observations.

None of the vocalisations showed any change in their performance following the training programme. For reasons broadly similar to those discussed for self scratching above, whilst a decrease in coo vocalisations would have been a good indicator of improved welfare, this lack of change suggests that training has no negative impact on the macaques' welfare.

2.7 SUMMARY

Whilst training is recommended for laboratory-housed primates, there remains a considerable amount of data lacking with regards to which factors affect the success and time investment required for training behaviours. These data are of value to those embarking on a training programme as they may help to identify how much time will be required to train the animals and also how individual animals will respond to training.

The results show that the majority of laboratory-housed cynomolgus macaques can be successfully trained to cooperate with a simple training task of target training in 26 or fewer sessions. Training however was not completely successful as not all macaques reached criterion. Age and sex had no consistent effect. Temperament tests successfully predicted which macaques would reach criterion in training and also how quickly individuals were trained. The best predictors of faster training were quicker latencies to hand feed and higher rates of watchful behaviour exhibited in baseline observations. To investigate whether training impacted positively on behaviour following routine

husbandry, data were collected following cage cleaning both pre- and post- training. The behaviour of the macaques was significantly negatively affected following the cage cleaning process, but the training programme impacted positively on their welfare. Following a training programme macaques exhibited less of behaviours considered indicators of negative welfare and increased their performance of behaviours considered as indicators of positive welfare. Training helped the macaques to deal better with a stressor and also decreased the amount of an undesirable behaviour, sitting alone, in their normal daily activity. Together, these results support recommendations that training has benefits, and provide new data on how to determine which individuals may be most suitable for training programmes.

CHAPTER 3

INFLUENCE OF AGE, SEX AND TEMPERAMENT ON THE TRAINABILITY OF COMMON MARMOSETS (*Callithrix jacchus*)

Much of the literature on training primates has focussed on chimpanzees and macaques, but there is now an increasing amount of data published on the training of New World primates, most notably marmosets. However, little data have been published regarding the factors which may influence the success and speed of training in marmosets. Twenty-four laboratory-housed common marmosets (*Callithrix jacchus*) underwent a training programme whereby they were target trained, and then trained to remain calm in a transport box away from their home cage. Behaviour was recorded to assess the impact of training on the welfare of the marmosets.

All of the marmosets reached criterion in the training task within 17 sessions, with a mean time investment of just under eight sessions, equivalent to 40 minutes. The temperament tests predicted trainability; both willingness to interact with a novel object and latency to hand-feed predicting the time investment required to train an individual. Neither age nor sex of the marmosets predicted training success. Further to this, the behaviour of the marmosets prior to any training also predicted trainability, with those individuals who exhibited more self-scratching (an anxiety-related behaviour) taking longer to train than those who exhibited less self-scratching. The behaviour of the marmosets indicated that they experienced anxiety as a result of capture and return to a new clean cage, but experience of a training programme reduced this, thus helping the marmosets to cope with this stressful, but relatively common, husbandry event.

3.1 INTRODUCTION

Whilst the focus of many studies on training primates has been chimpanzees (*Pan troglodytes*) and macaques (*Macaca* spp), there is now a small body of literature on the training of New World primates, most notably marmosets. These studies have shown that it is possible to train common marmosets (*Callithrix jacchus*) to collect urine (Smith *et al*, 2004) and saliva (Cross *et al*, 2004) as well as to cooperate with weighing (McKinley *et al*, 2003) and veterinary inspections (Savastano *et al*, 2003). Much of the literature relating to the training of primates is discussed in Chapter 2, so only information relating directly to marmosets is discussed below.

3.1.1 Time Investment Required for Training

The implementation of training programmes for New World monkeys has lagged behind that for apes and Old World monkeys, as New World primates are small and, in comparison to larger primates, easy to physically restrain. Marmosets are relatively safe to handle, either with or without gauntlets and can be caught and restrained by hand with little risk to the caregiver (Buchanan-Smith, 2010). There is now an increasing interest in the use of training with New World primates as laboratories to explore ways to improve the welfare of their animals. As for other primate species, time investment required in a training programme will still be important, as lack of staff time is one of the greatest constraints within a laboratory. It may even be more crucial with smaller primates than larger more dangerous species as it is much easier to revert to 'traditional' methods of doing a task when the species in question poses little threat to the caregiver. If however it can be shown that tasks where the individuals are trained take less time and effort than the

same task with untrained animals, and the training process is not too onerous, and training improves the welfare of the animals concerned a positive reason for carrying out training can be promoted.

The time investment required to train common marmosets to hold a target has been reported as varying from 32 minutes per individual in laboratory-housed pairs up to 124 minutes per individual for zoo-housed groups (McKinley *et al*, 2003, Savastano *et al*, 2003). There may be many factors which affect this difference in time investment, including the type of establishment in which they are housed, group size, training protocol and criteria, and trainer competency. Different tasks also require different time investments, for example with training to provide a urine sample requiring less time investment in both laboratory-housed and zoo-housed individuals than training to hold a target in comparable housing establishments (See Table 2.1, Chapter 2).

Within some laboratories there seems to be a perception that New World primates are not as trainable as Old World primates, that they can't be trained for certain tasks and if they can be it will take longer due to their 'flightiness'. There however is little evidence to support this. Indeed it seems that training laboratory-housed common marmosets for the simple task of holding a target requires less time investment than training laboratory-housed rhesus macaques (*M. mulatta*) for the same task; marmosets requiring 32 minutes per individual and macaques 45 minutes per individual (common marmosets, McKinley *et al*, 2003, rhesus macaques, Fernström *et al*, 2009).

3.1.2 The Influence of Age, Sex and Housing on Ability to Learn in Primates

The age of a marmoset has been shown to affect its performance in a food retrieval task. Although there was no correlation between the latency to first interact with the test apparatus in a foraging task, and the age of the individuals, there were positive interactions between the time spent interacting with the apparatus and age, and the number of successful attempts to retrieve the food and age (Cameron & Rogers, 1999). This may suggest that marmosets become more cognitively capable as they age, but it is more probable that mature individuals are more patient and willing to work harder for longer than younger animals. Marmosets may therefore be more trainable as they age as they become more willing to persevere in a task, but there is no evidence specifically demonstrating this.

Female common marmosets were more successful in a foraging task than males (Box *et al*, 1995, Yamamoto *et al*, 2004), but this may be due to the males deferring to the females rather than an actual difference in their ability to perform the task. Further to this, no sex differences were seen in common marmosets in the time investment or success rate in a training situation (McKinley *et al*, 2003), and Blackwood *et al* (2004) reported no effect of age and sex on response to a novel object in the same species.

3.1.3 Temperament Testing

As discussed in Chapter 2, temperament tests have been used to predict aspects of behaviour such as working ability in dogs (e.g. Goddard & Beilharz 1986) and therefore may be of use in predicting trainability in primates. There is evidence that

the success of training in primates can be predicted by a simple temperament test which discriminates between exploratory and inhibited individuals (Coleman *et al*, 2005). Further to this, performance in temperament tests has also been shown to correlate with time investment in cynomolgus macaques (*M. fascicularis*, Chapter 2). As for laboratory-housed macaques, tests designed to be used with laboratory-housed marmosets need to be easy to administer and simple to interpret. Adapted versions of the tests used for macaques were devised, as these fulfilled both of these criteria. Using broadly similar tests also enabled a comparison between the species to be carried out.

Response to a novel object and problem solving

A number of papers have described the results of introducing novel objects to marmosets. They have been used to assess developmental differences (Menzel & Menzel, 1979), discover differences in foraging ability and handedness (Cameron & Rogers, 1999) and also to identify aspects of an enrichment device which encourage interaction (Majolo *et al*, 2003a). There is however little in the literature relating to the use of novel object to measure temperament in marmosets. Characteristics which may affect trainability are discussed in Chapter 2, but include boldness-timidity, motivation, distractibility and innovation. Some marmosets may be more inquisitive or bolder than others about novel objects, and this may correspond with being more able to learn new tasks. Similarly those marmosets that do not interact with a novel object or do not solve a problem solving task may be unsuited to training programmes.

It has been shown that marmosets are significantly slower to explore novel objects presented in the bottom section of their cage than the upper or middle parts, and spent less time exploring objects here (Majolo *et al*, 2003a) suggesting that the marmosets find going to and remaining at the bottom of the cage more challenging than remaining higher up. A novel object presented low down in the cage may therefore discriminate between bold and inhibited, or food orientated and less food orientated individuals better than an object presented higher up in the cage.

Response to human interaction

There is a paucity of evidence in the literature to suggest how marmosets and other New World primates respond to an interaction with a novel human. As discussed previously, willingness to interact with a human is vital to success in a training programme, so it may be important to identify individual differences in marmosets' willingness to interact in this way. In other primate species individuals have different and stable approach-avoidance distances (Itoh, 2001) which may relate to their willingness to interact with people.

Response to a stressor

Individuals who are generally more anxious, and are shown to have 'trait anxiety' (Eysenck, 1985), perform less well in cognitive tests (e.g. Toxopeus *et al*, 2005). Further to this, where humans are more stressed by the testing procedure they perform less well than more relaxed individuals (McNaughton, 1997). These results suggest that how an individual responds to stress may affect its ability to learn a task, so a test of stress response was identified as a possible way of classifying

trainability. As it was not desirable to stress the marmosets specifically for the purposes of this study, a routine event, cage cleaning, was chosen as the stressor as this has been shown to lead to a stress response in marmosets (Bassett *et al*, 2003).

Relationships Between the Tests

It is important to assess whether the temperament tests are identifying different aspects of temperament rather than just repeatedly measuring the same aspect, so tests should be compared, as discussed in Chapter 2. Measuring a range of temperament traits increased the likelihood of identifying those most closely related to trainability in marmosets.

3.1.4 Effect of Positive Interactions with Humans

Positive interactions with humans have been shown to reduce negative indicators and increase positive indicators of welfare in marmosets (Manciocco *et al*, 2009), so if training is perceived as positive by the marmosets then it is likely that a similar result will be seen following training. Indeed there is evidence to show that training does improve the welfare of marmosets, particularly in response to stressful events (Bassett *et al*, 2003). It is important to assess the impact of any type of intervention (such as enrichment) or interaction (such as training) on the welfare of the individuals involved to assess its efficacy and also to identify any impact on the welfare of those involved. Behaviour was therefore measured and analysed in order to identify the impact of training on the welfare of the marmosets.

3.2 AIMS OF THE STUDY

The aim of this study was to target train common marmosets, and then to use this behaviour to facilitate training them to enter and remain calm in a transport box. Further to this the aim was also to establish if the age or sex affected if or how quickly marmosets learned the task, and if temperament, as measured by simple easy to administer tests, could predict if or how quickly marmosets learned the task. It was considered important that these tests were easy to administer so that they were practical for laboratory staff to perform and analyse themselves. Finally the study was designed to assess if training had any effect on how marmosets cope with a stressor.

3.3 METHODS

The MRC Human Reproductive Sciences Unit, where this study was carried out, holds marmosets in a range of group sizes from family groups (approx 4-8 individuals) and single-sex and mixed-sex pairs. All animals in this study were housed in same-sex pairs, were bred in-house and had been parent-reared.

3.3.1 Housing

Pair-housed marmosets were housed in cages measuring 0.65m x 1.15m x 1.10m. Each cage formed part of a four-cage unit, and could therefore be an upper tier cage or a lower tier cage. Dividers could be removed from in between cages in order to create two full height cages (0.65m x 2.30m x 1.10m) or one family cage (1.3m x 2.30m x 1.10m), however all marmosets in this study were housed in single cages.

Marmosets from both upper tier and lower tier cages were included as it is suggested that primates housed in lower tier cages may have reduced welfare (Reinhardt & Reinhardt, 2000). Lower cages are darker, and monkeys housed in them may have less human interaction as it is inconvenient to reach lower tiers. Although marmosets housed in the lower tier show few behavioural differences to those housed in the upper tier (Badihi, 2006), there is a greater reluctance for those animals housed in the lower tier to approach food placed on the floor (Buchanan-Smith *et al*, 2002). Monkeys housed in the lower tier are unable to perform a vertical flee response to a height above the 'threat'; particularly important for highly arboreal species such as the common marmoset. A metal tray formed the bottom of each cage to facilitate easy cleaning, and this tray contained a deep layer of wood shavings. Also within the cage were 2-3 branches, a metal nest box and a rubber mesh shelf attached to the front of the cage on which the marmosets could comfortably sit when looking out of the cage. Branches or pieces of wood are an important element of cage furniture for marmosets as they provide an ideal substrate for the performance of behaviours such as chewing, climbing and in particular scent-marking. Being able to perform these behaviours is important for the welfare of captive both in terms of allowing them to express species-specific behaviours and maintain social relationships (JWGR, 2009). The mesh shelf allowed the marmosets to maintain a position at the front of the cage where they could see more without having to hang from the cage front. Further to this some cages also contained a suspended wire basket or a suspended rubber mesh tube (Figure 3.1)

All marmosets in this study were housed in one of two virtually identical rooms. Each room contained four cage units down each side and could therefore house up

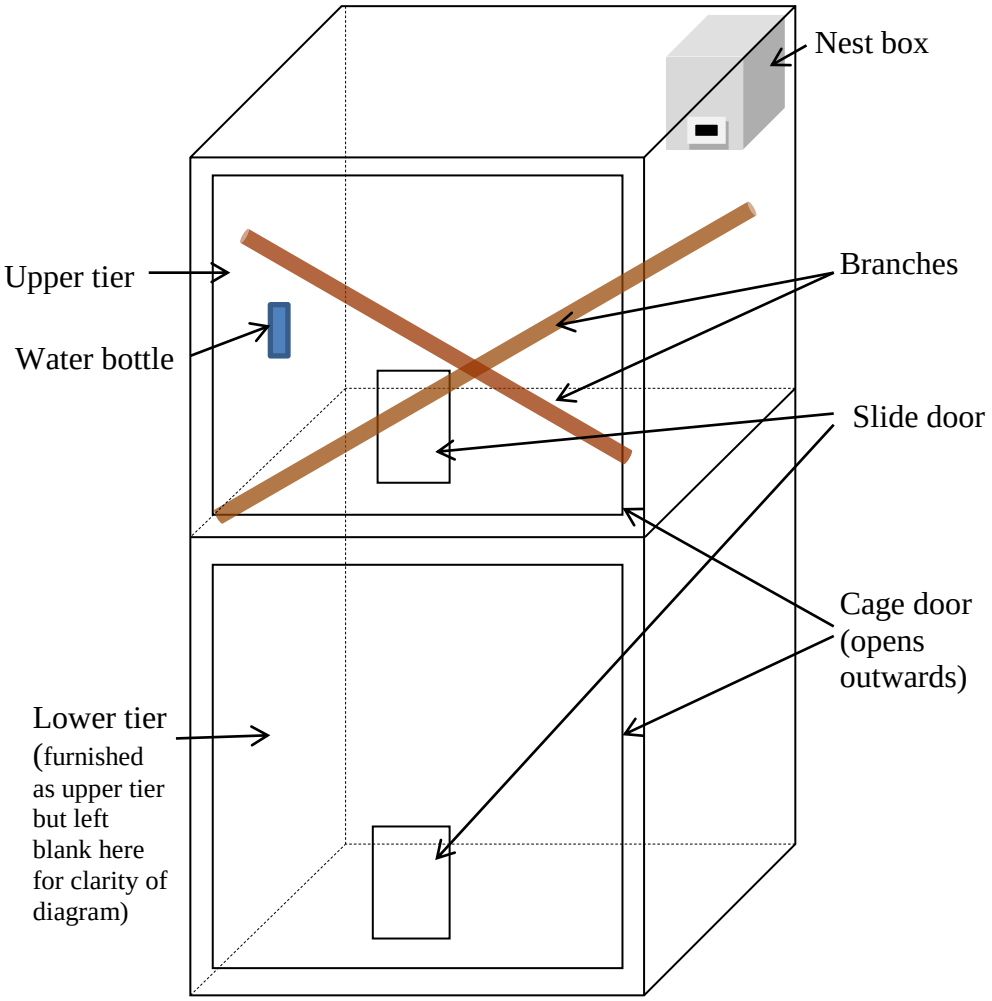

Figure 3.1 Schematic diagram of layout of marmoset cage (not to scale)

to sixty-four marmosets. The gap between the two rows of cages was approximately 2m, allowing clear visual contact whilst being large enough to limit territorial aggression between cages on opposite sides. The upper half of the door to the corridor contained a window through which the marmosets had some visual contact with any passing members of staff. Rooms were maintained at approximately 22-23°C and humidity at around 55%. A twelve hour light/dark cycle operated in all rooms, coming on at 0700h, around 30 minutes before staff arrived at the unit.

3.3.2 Husbandry

Marmosets had access to water *ad libitum* from a bottle on the front of the cage, which was replaced with a clean bottle of fresh water every day. They were fed once daily at around 1300h on a combination of New World Primate diet (Mazuri plc), chopped fresh and dried fruit (apples, oranges, pears, tomatoes, bananas, grapes, raisins, dates) seeds (sunflower and pumpkin) and three times a week a 'porridge' consisting of yoghurt, baby rice and protein supplement with added vitamins and minerals. A portion of food sufficient for two marmosets was prepared in paper dishes which were then placed on the floor of the cage.

The paper food dishes and any remaining food were removed every weekday morning between 0800h and 1000h, and the room floors were also cleaned at this time. Shaving trays were removed, emptied, wiped clean and refilled weekly. Full room cleaning occurred once a month when all marmosets were caught in their nest box and dirty cages were removed and replaced with clean cages. Dirty cages were

cleaned in a cage wash and room floors and lower walls were washed down with mild disinfectant. Before being returned to their clean cages marmosets were given a brief physical examination and the fit of identification collars worn by all adult marmosets was checked. If required, marmosets were weighed before being returned to their cage. Scientific procedures such as blood-sampling or dosing were routinely carried out in the mornings, but none of the study animals was being used for scientific procedures.

3.4 EXPERIMENTAL DESIGN

3.4.1 Study Animals

Fifteen same sex pairs of common marmosets (*C. jacchus*) were used in this study. These were made up of seven male pairs and eight female pairs. Four of the male pairs and three of the female pairs were housed in the upper tier of cages, whilst the remainder were housed in the lower tier. At the start of the study marmosets ranged in age from seven months to 72 months and there was no significant difference between the ages of the males (N = 14, mean = 25.6 ± 5.7 months) and the females (N = 16, mean = 23.25 ± 2.25 months t = -0.38 df = 16, p = 0.71, NS). None of the study marmosets had previously experienced training of any kind nor had they had any special human interaction. Individuals were identified by their collar tags and also by a small shaved notch about three-quarters of the way down the tail of one marmoset in each pair. Shaving a notch into the tail fur allowed quick, easy and reliable identification of an individual when their tag was not visible. None of the marmosets in this study was being used for any experimental work at the time of this study and had not been used for at least one month prior to the commencement

of the study. Marmosets were familiarised to my presence for two hours a day for two weeks (10 days in total). I entered the room quietly, and stood in the centre of the room for five minutes. I then spoke softly to the marmosets for a further five minutes before sitting and standing in various places around the room for the remainder of the familiarisation period. At the end of the period, whilst the marmosets were very familiar with me, habituation was not complete and the animals still spent significant amounts of time watching me. As other studies have found similar results with marmosets in laboratories being particularly difficult to habituate it was decided to go ahead with the study but to include a behavioural category which would measure the amount of time they spent "watching the observer".

3.4.2. Time Investment

Training was carried out daily, on weekdays only, at approximately the same time for each pair, and pairs were trained in the same order. Each training session lasted for a maximum of 10 minutes per pair. This was split as equally as possible between the individuals in the pair so each individual had five minutes of training per session. If only one marmoset in the pair was willing to cooperate with training, session length was reduced accordingly. Training sessions were also terminated if one of the marmosets earned the maximum amount of rewards (16 pieces of marshmallow), and this was recorded. Care was taken to share training between the two monkeys in the pair as evenly as possible whilst still maintaining progress, and as a result of this maximum rewards were only gained before the session finished in two instances.

3.4.3 Temperament Tests

Three measures of temperament were recorded for all marmosets. Testing and training were carried out in the following order

1. Novel object test
2. Human interaction test
3. Habituation to experimenter
4. Baseline behavioural measurements (PRE TRAIN/BASE)
5. Response to a stressor behavioural measurements (PRE TRAIN/STRESS)
6. Training
7. Response to a stressor in trained animals behavioural measurements (POST TRAIN/STRESS)

As it was desirable to test naive animals, no habituation was carried out prior to the novel object and human interaction tests. Habituation was necessary prior to the assessment of response to a stressor in order to obtain relatively normal behaviour at baseline levels, so this was carried out after the novel object and human interaction tests had been performed. The novel object test was performed first so marmosets were not influenced by any perception of the experimenter who had previously given them food. The human interaction test was then performed followed by a period of habituation and the behavioural recordings to assess response to a stressor.

Response to a novel object and problem solving

A translucent plastic film canister (diameter 3cm, height 5cm) was filled with six pieces of chopped banana (approximately 3g). Banana was chosen as it has a strong

aroma ensuring that the marmosets would be able to detect the presence of the food, and also because bananas are a favoured food (Caldwell *et al*, 2009), increasing the motivation of the animals to access the reward. The canister was then placed with the open end down on the floor of the home cage, in the position where the food tray was normally placed. There was no other food in the cage as tests were carried out between 1000h and 1200h so old food had been removed and that day's food had not yet been given. Once the canister had been placed in the cage, and the door shut, a stopwatch was started. The observer stood in front of an adjacent cage approximately 1m away from the test cage, and avoided staring directly at the test cage which can be threatening to marmosets. Latency from when the cage door was shut after the canister was put in to when each individual first touched the canister and when each individual first accessed the banana was recorded. Time was limited to 5 minutes per test as pilot data showed that if marmosets did not touch the canister within this time they were unlikely to do so.

Response to human interaction

I approached the marmoset's home cage slowly and from an angle of approximately 45°. I then stood, or for lower tier cages knelt, approximately 40cm from the front of the cage at a slight angle so that they were not facing straight into the cage. I then offered the marmosets a small piece of marshmallow, a highly favoured food (Caldwell *et al*, 2009), at a height approximately three-quarters of the way up the cage so it was above my head height. The latency of each marmoset to take the reward was recorded up to three minutes. Pilot data indicated that if the marmosets had not taken food by this point they were unlikely to do so. If one marmoset remained at the front of the cage and took more than four pieces of marshmallow he

or she was distracted by offering marshmallow lower in the cage, whilst marshmallow was offered to the second marmoset in the original higher position. This ensured that both marmosets in the pair had the opportunity to perform the task and the food source was not dominated by one marmoset.

Response to a stressor

The routine husbandry event of removal of marmosets from their home cage, weighing and subsequent return to their home cage is an event which the animals find stressful (Bassett *et al*, 2003). The marmosets are firstly chased into their nest box by a technician wearing gauntlets. The nest box is then removed and placed onto the floor of the room where the marmosets are unavoidably subjected to the noise and vibration of the cages being moved. They are then removed from the nest box by a technician wearing gauntlets, manipulated to check their health and collar fit before being returned to the new cage. The new cage contains no familiar scents, and although it remains in the same position in the room, this makes the new cage highly unfamiliar. This necessary routine occurrence provides a good opportunity to assess how individuals deal with a stressor without specifically inflicting stress upon the animals for the sole purpose of this study.

Once the marmosets were trained their behaviour was recorded following cage change as described above. This was done to establish whether training had any effect on how marmosets responded to the stressor. Observations were carried out during the first cage change following the completion of training. Depending on how quickly both marmosets in the pair learnt the task, and which room they were housed in, this ranged from three to 16 days following the end of training. Those

marmosets who were considered trained had however continued to receive some positive interaction from the experimenter in the form of a request to hold the target for which they were rewarded up until all marmosets were trained. This was to prevent frustration and resultant aggression which had been noted anecdotally if some trained monkeys missed their training session when others nearby were trained.

3.4.4 Behavioural Observations

Following habituation, and prior to the commencement of any special human interaction or training, the behaviour of each of the marmosets in the study was recorded using THE OBSERVER V5.0 via the handheld Workabout computer to provide a baseline behaviour measurement for the day prior to cage change. The use of the Workabout enabled both behavioural states to be recorded along with events, and for data to be gathered efficiently with minimal time spent looking away from the animals. The behaviour of each individual was recorded for 5 minutes, with cagemates being recorded consecutively. All observations were carried out between 10.00 and 13.30hrs Behavioural categories recorded are described below (Table 3.1).

Behaviour Class	**Behaviour**	**Recorded as behavioural state (S) or event(E)**	**Description**
Locomotion	Locomotion	S	Normal relaxed walking running, climbing
	Agitated locomotion	S	Quick running or climbing, usually upwards in direction
Sitting	Sit	S	Still, relaxed, in one location, not actively watching anything
	Contact sit	S	Still, relaxed, in contact or within 10cm of cagemate
Vigilance	Watch	S	Actively either observer or other person/event outside cage
Foraging	Forage	S	Manipulate substrate to find food, manipulate food or eat food
Other	Nest box	S	Out of sight in nest box
Social	Fight	E	Initiates aggressive physical encounter with cagemate
	Threat	E	Physical lunge towards cagemate or observer, or aggressive display
	Retreat	E	Move away from fight or threat from cagemate
	Ano-genital present	E	Present rear region with tail raised exposing genitals to cagemate or observer
	Play	E	Friendly, boisterous interaction between cagemates
	Scent mark	E	Rub ano-genital region on substrate
	Allogroom	E	Manipulation of fur or body parts of cagemate
Vocalisation	Tsik	E	Short, sharp, repeated mobbing calls
	Phee	E	Long, tonal, whistle, contact call
Self directed	Drink	E	Intake water from water bottle
	Self Scratch	E	Rapid, agitated touching or manipulation of single body area
	Groom	E	Calm manipulation of own body and fur
	Urinate	E	Elimination of urine
	Defecate	E	Elimination of faeces
	Object manipulate	E	Physical interaction with item in cage

Table 3.1 Description of behaviours recorded during data collection sessions, adapted from Stevenson & Poole (1976)

Three behavioural observations were taken of each marmoset, two before training (PRE TRAIN) and one after the training programme was completed (POST TRAIN). One pre-training observation was carried out during a normal day (PRE TRAIN/BASE) and a second immediately after return to the home cage after cage cleaning (PRE TRAIN/STRESS). Only one observation was carried out following training, and this was done following return to the home cage after cage cleaning (POST TRAIN/STRESS). The aim was to establish if the stressor had any effect on behaviour, and then if training affected behaviour in response to the stressor. For summary see Table 3.2.

		Stressor Level	
		Baseline (BASE)	Post-stressor (STRESS)
Training Level	Pre-training (PRE TRAIN)	PRE TRAIN/BASE	PRE TRAIN/STRESS
	Post-training (POST TRAIN)	~~POST TRAIN/BASE~~	POST TRAIN/STRESS

Table 3.2 Summary of terminology used to describe points at which behavioural observations were carried out

3.4.5 Training Protocol

The aim of the training programme was to train the study animals to enter a transport box in a relaxed manner and remain calm whilst inside it whilst it was moved out of visual and auditory contact with his or her room. All training was carried out using positive reinforcement, whereby performance of the desired behaviours was increased by introducing something positive, in this case a favoured food reward, when the behaviour was performed. A secondary reinforcer or 'bridge'

was used to mark the exact moment the behaviour was performed and to tell the marmosets when they had performed a behaviour which would earn them a reward. Rewards were small pieces of marshmallow ($^1/_8$ of a small marshmallow), and marmosets could only earn a maximum of 16 rewards (two small marshmallows) per session. Sessions lasted for up to 10 minutes per pair. In all cases the marmoset was considered to be reliable in performing a desired behaviour if it performed it on nine out of ten occasions it was requested. If at any point a marmoset failed to perform at a particular level the trainer went back and repeated the last level. Training was carried out once per day on weekdays only until both marmosets in the pair were trained. All training was carried out between 1000h and 1300h when no other food was present in the cage.

Initially all marmosets were hand fed food rewards. Once they were doing this reliably the bridge was paired with the food reward by in this case saying the word 'good' and giving the food immediately after (less than 1 second later). Once the marmoset was expecting the food reward on hearing the bridge, as indicated by looking at the trainer or trainer's hand on hearing it, the target was introduced. The target is an object which the marmosets are trained to hold that can then be used to move them around the cage or into a new area (for example the transport box) and keep dominant animals away from submissive animals during training to enable more submissive individuals to be trained. In this case plastic teaspoons were used, with one individual being trained to hold a white teaspoon and the other in the pair being trained to hold a dark blue teaspoon. Care was taken to select contrasting colours which were easily distinguished by the majority of marmosets who have dichromatic vision (all males and homozygous females) and those who have

trichromatic vision (heterozygous females) (Surridge *et al*, 2003, Jacobs, 2007). Food rewards were held at the front of the cage behind the target so that the marmosets had to reach past the target to get the food. This resulted in the marmosets touching the target and receiving the bridge and reward.

Shaping, the process of building up a desired behaviour through successive approximations of that behaviour, was then used to produce the required behaviour of holding the target. When the marmosets were holding their target reliably in different locations around the cage the transport box was introduced. The transport box was transparent plastic, measuring 33cm x 22cm x 18cm with a mesh front and a slide door on the back. The box was attached to the front of the home cage with the box slide door against the small slide door on the front of the home cage. The mesh front enabled the training interaction to continue when the marmosets were in the box as the spacing between the mesh was large enough for a marmoset to reach out and hold the target.

The transport box was placed on the front of the home cage and both home cage and transport box doors were opened allowing the marmosets to have access to the transport box to explore and familiarise themselves with the box for five minutes. They were then asked to come into the box by holding their target at the front of the transport box (Plate 1). If a marmoset was reluctant to enter the box their target was held by the box entrance and gradually brought further into the box. Where possible, rewards were given at the front of the transport box.

Once a marmoset would enter the transport box and hold the target reliably he or she was then required to remain holding their target for increasing periods of time up to 20 seconds. Once this was reliably established the marmoset was requested to hold their target whilst remaining in the transport box whilst the door was closed and immediately opened again, then as the door was closed and left closed for periods of up to one minute whilst the box remained attached to the home cage. Marmosets were not required to continually hold their target for the entire time they were shut in the transport box, but were requested to hold their targets when the door was closed and subsequently opened. This allowed the marmosets to remain in visual and auditory contact with his or her cagemate who remained in the home cage and remain in a familiar position in the room.

Once the marmoset was performing reliably at this level, and was calm when shut in the box, the box was removed from the front of the cage and placed on the floor of the home room for periods of up to five minutes. The marmosets were placed on the floor as there were no higher surfaces available to place them on. Whilst the floor was less than ideal the alternative was to bring in a trolley to put them on. Unfortunately the trolley was very strongly associated with being caught in the traditional manner and caused the whole room to become nervous and flighty, so gradual acclimation of the individual to spending time on the floor was preferred. Marmosets were asked to come and hold their targets at random points throughout this period in the transport box on the floor. When this was reliable, the transport box was removed from the room and placed on a trolley in a corridor out of visual and auditory contact with the home room. Marmosets were considered trained in

this task when they would return to the transport box within 30 seconds of exiting the box, and this could be repeated three times.

Plate 1. Marmoset holding target whilst in transport box

3.4.6 Statistical Analysis

For all analysis the level of significance was set at 0.05. This is despite multiple analyses being carried out, where it is recommended that corrections are used. This was done as despite a risk of Type I errors (false positives), it reduces Type II errors (false negatives). The corrections needed would have led to significance being set at such a high level that the risk of Type II errors increased. As it is desirable to look for biologically relevant patterns in the data, a significance level of 0.05 was used, but caution taken in interpreting the results. However Bonferroni corrections were subsequently applied to the results, and where data retained statistical significance, this was highlighted. Data were tested for normality using Kolmogorov-Smirnov tests and where normal parametric statistics were used, and means are reported with the standard error of the mean. Non-normally distributed data were analysed using non-parametric tests, and medians and inter-quartile ranges given. Ceiling values (a value greater than the maximum data collected) are used in some non-parametric tests.

Training

Training data were analysed per individual, as although the progress of an individual may have affected that of his or her cagemate, the aim of the study was to identify individual differences. Analysing means would therefore have rendered this meaningless.

Temperament Tests

The temperament tests were analysed using one way ANOVAs on mean latencies, and Pearson's correlations on latencies, recorded behaviours and number of sessions to reach criterion.

Behavioural Data

Behavioural data were analysed in two ways. In order to assess if behaviour at either PRE TRAIN/BASE or PRE TRAIN/STRESS was related to training success, correlations between training success and duration of behaviour were carried out for individual marmosets. The behaviour of a marmosets' cagemates is likely to influence the behaviour and responses of the individual animal being tested. For this reason data from each pair were pooled and a mean value for each cage for each behaviour was calculated, effectively reducing sample size from 30 to 15 for the analysis of the effects of the stressor and training. Repeated measures ANOVAs were then used to analyse the duration of behaviours across the three observations (Table 3.3).

Variable	**Between/Within Subjects**	**Levels**
Observation	Within	PRE TRAIN/BASE PRE TRAIN/STRESS POST TRAIN/STRESS

Table 3.3 Variables analysed for state behaviours

If event behaviours occurred at excessively low frequencies behaviours (i.e. median values were zero for all three observations) they were discounted from analysis as insufficient data were available. The frequencies of event behaviours were not normally distributed, and transforming the data did not provide normally distributed

data, so non-parametric tests were used. Wilcoxon signed rank tests were used to compare data from the three observations over three analyses, PRE TRAIN/BASE v PRE TRAIN/STRESS, PRE TRAIN/BASE v POST TRAIN/STRESS, and PRE TRAIN/STRESS v POST TRAIN/STRESS.

3.5 RESULTS

3.5.1 Success Rates and Time Investment

Training laboratory-housed marmosets to enter a transport box using positive reinforcement training proved to be possible. All 30 marmosets were successfully trained to enter the transport box, giving a 100% success rate for this task.

Although all the marmosets were trained, the time investment required varied greatly between individuals. When the data from all 30 marmosets were analysed, the mean number of five minute sessions required to train individual marmosets to reach criterion in this task was 7.9 ± 0.69. This equates to approximately 39.5 ± 3.44 minutes per marmoset. The fewest number of sessions required was four, and four marmosets were trained in this number of sessions, whilst the marmoset who took the longest took 17 sessions to learn to perform the task reliably.

3.5.2 The Effects of Age, Sex and Housing on Time Investment

There was no correlation between the age of the marmosets at the start of the study and the number of sessions required for them to learn the task (Pearson correlation, df = 28, r = -0.25, p = 0.24, NS). Female marmosets learnt the task in a mean of

8.13 ± 0.84 sessions, and the males in a mean of 7.64 ± 1.10 sessions. There was no significant effect of sex on the speed of training of the marmosets (t-test, males n = 14, mean = 7.64 ± 1.15, females n = 16, mean = 8.13 ± 0.84 sessions; df = 24, t = 0.34, p = 0.74, NS).

The younger marmoset in the pair required a mean of 8.73 ± 1.11 sessions to learn the task, whilst the older marmoset in the pair took 7.07 ± 0.79 sessions. There was no difference between the number of sessions required to train the younger marmoset in the pair than the older marmoset in the pair (t-test, younger n = 15 mean = 8.73 ± 1.1 sessions, older n = 15 mean = 7.07 ± 0.79; df = 14, t = 1.51, p = 0.12, NS). When those marmosets considered sub-adult (< 18 months old, n = 16) were compared to the rest of the marmosets, there were no differences in the number of sessions individuals required to learn the task (t-test, df =27, t = 0.94, p = 0.18, NS) although the sub-adults did take longer to learn (8.50 ± 0.99 sessions) than mature adults (7.21 ± 0.94 sessions).

When taken as pairs, the second marmoset took a mean of 3.00 sessions longer than the first marmoset in the pair to learn the task (first trained n = 15, 6.4 ± 0.70 sessions, second trained n = 15 mean = 9.4 ± 1.1 sessions). There was a correlation between the number of sessions the first marmoset required to learn the task and the sessions required by the second marmoset (Pearson correlation, df = 13, r = 0.57, p = 0.03) (Figure 3.2).

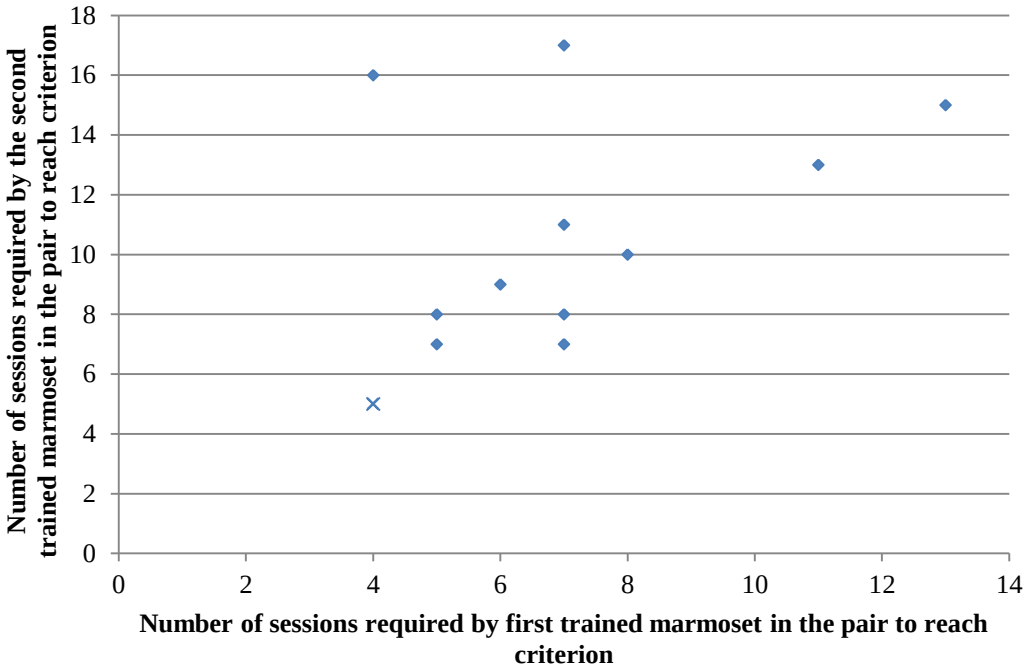

Figure 3.2 Correlation between the number of sessions required for first marmoset in the pair to learn the task and the second marmoset in the pair to learn the task. x marks four data points (analysis includes all marmosets)

3.5.3 Relationships Between Temperament Tests and Time Investment

Novel Object and Problem Solving Test

Twenty-four of the 30 marmosets (80%) touched the novel object within the 300 second time limit and 14 marmosets accessed the food reward (47%); this equates to 58% of those marmosets who touched the object. The mean time to touch the novel object, excluding those who did not touch it, was 64.2 ± 10.2 seconds, with the fastest to touch taking 9 seconds and the slowest 210 seconds. The marmosets who accessed the food took a mean of 128.6 ± 13.7 seconds to do so (range 35 - 215 seconds).

Those marmosets who touched the novel object and accessed the food inside took a mean of 6.57 ± 0.59 sessions, those who touched the object but did not access the food took a mean of 8.0 ± 1.16 sessions, whilst those who did not touch the object required a mean of 11.40 ± 2.38 sessions. One-way ANOVA showed that there was a significant difference between these groups (df = 2, F = 5.03, p = 0.02). Planned post-hoc Tukey tests showed that those marmosets who accessed the food took significantly fewer sessions to reach criterion than those individuals who did not touch the novel object (T = 3.14, p = 0.03), but there was no difference in the number of sessions required by those who touched the novel object and those who accessed the food (T = 0.67, p = 0.56, NS). There was also no difference in the number of sessions taken by marmosets who touched the novel object without accessing the food and those who did not touch the novel object (T = 2.42, p = 0.17, NS) (Figure 3.3). There was no correlation between latency to either touch the novel object or access the food and the number of sessions needed to learn the task, when data were analysed for just those marmosets who were successful in these tasks (Pearson correlation, touch df = 22, r = 0.22, p = 0.30, NS; food df = 12, r = 0.23, p = 0.43, NS). When ceiling values were used (the time limit for the test), and data for all individuals were analysed, no significant correlations were seen (Spearman rank correlation, latency to touch v number of sessions, df = 28, r = 0.22, p =0.24; latency to access food v number of sessions, df = 28, r = 0.23, p = 0.23, NS).

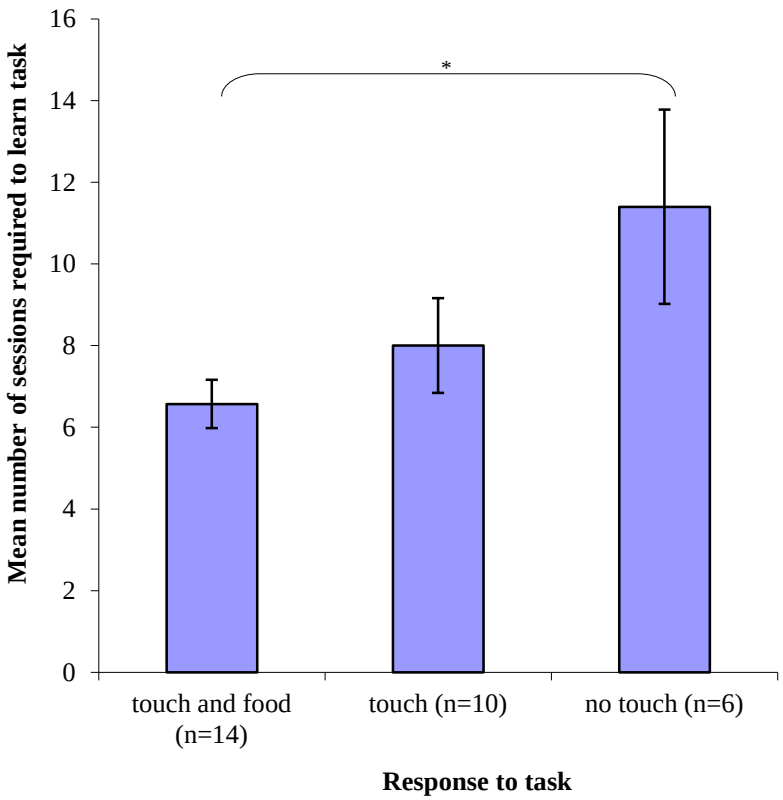

Figure 3.3 Number of 5 minute sessions required for marmosets who touched the object and accessed the food (touch and food), touched the object but did not access the food (touch) and did not touch (no touch) the object to learn the task (* $p < 0.05$). **Error bars show standard error of the mean. Error bars show standard error of the mean.**

Human Interaction Test

Nineteen out of the 30 marmosets (63%) approached the experimenter and took the food reward within the 180 second time limit. The mean time to take the food from the experimenter, excluding those who did not take the food, was 18.42 ± 4.2 seconds, with a minimum of 2 seconds and a maximum of 63 seconds.

There was a significant difference between the time taken to learn the task by those marmosets who took the food from the experimenter within the time limit (mean =

6.26 ± 0.42 sessions) and those who did not take the food (mean = 10.73 ± 1.4 sessions, t-test, df = 11, T = -3.09, P = 0.01, Figure 3.4).

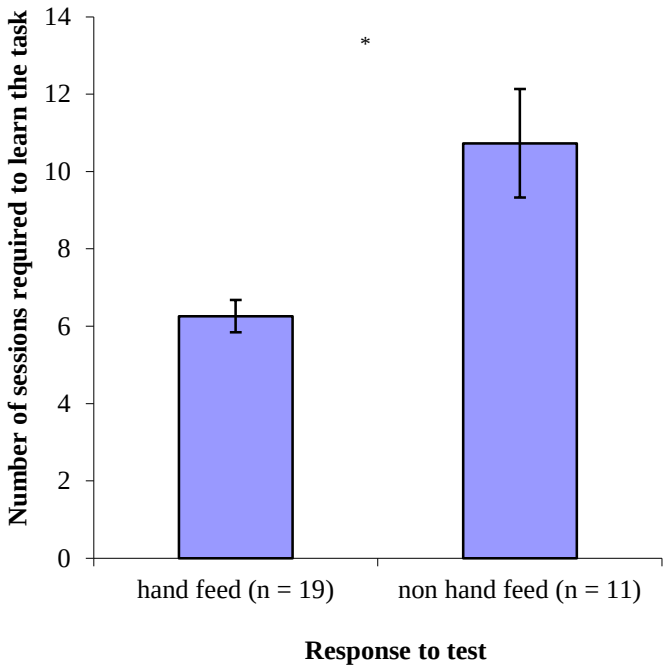

Figure 3.4 Number of 5 minute sessions required for marmosets who did (hand feed) and did not (non hand feed) take food from experimenter within time limit (* p <0.05). **Error bars show standard error of the mean. Error bars show standard error of the mean.**

There was a significant, although borderline, positive correlation between the latency to take the food and the number of sessions taken to learn the task when a subset of just marmosets who were successful in the test was used (Pearson correlation, df = 15, r = 0.47, P = 0.049, Figure 3.5). When ceiling values were used to include all marmosets in the analysis this correlation retains its significance (Spearman rank correlation, hand feed v sessions, df = 28, r = 0.43, p = 0.02).

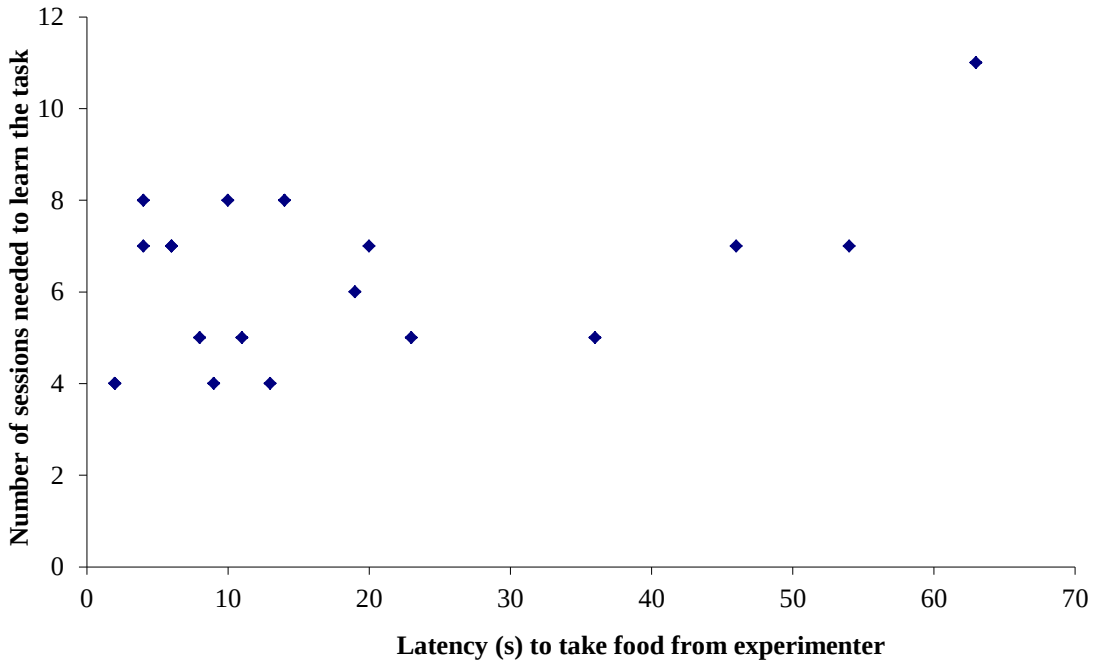

Figure 3.5 Correlation between the latency to take food from the experimenter (s) and the number of sessions needed to learn the task for marmosets successful in hand feed temperament test

Only 13% (2/15) of the marmosets who learnt the task first in their pair did not take the food from the experimenter, and 20% (3/15) of these first trained individuals did not touch the novel object. Within the 'first trained' subset of marmosets there was no correlation between latency to touch the novel object and the number of sessions needed to meet criterion (Pearson correlation, df = 13, r = 0.47, p = 0.08, NS) or latency to access the food and sessions (Pearson correlation, df = 5, r = -0.41, p = 0.36, NS), however latency to hand feed was still positively correlated with sessions to reach criterion (Pearson correlation, df = 11, r = 0.57, p = 0.04), however all of these results included just those individuals who had successfully completed the relevant temperament test. Due to the high numbers of marmosets in this subset who completed the temperament tests, or rather the low number who did not, further analysis was not possible. When ceiling values (set at the time limit for the

particular temperament test) were used so data for all marmosets were included in the analysis, no correlation was seen between number of sessions and latency to access the food (Spearman rank correlation, df = 28, r = 0.06, p = 0.84, NS) but the significant correlation between number of sessions and latency to hand feed remained (Spearman rank correlation, df = 28, r = 0.55, p = 0.035). As all of the first trained marmosets touched the novel object within the time limit no further analysis was necessary.

Response to a Stressor

No correlations were seen between the duration of any of the recorded state behaviours at PRE TRAIN/BASE and the number of sessions individuals required the reach criterion. Similarly no correlations were seen between durations of behaviours at PRE TRAIN/STRESS and sessions (Table 3.4).

	Behaviour							
	Behaviour	locomotion	agitated locomotion	sit	contact sit	watch	nest box	forage
PRE TRAIN/ BASE	r	-0.25	-0.12	-0.09	0.04	0.18	0.11	0.14
	p	NS (0.18)	NS (0.54)	NS (0.64)	NS (0.83)	NS (0.34)	NS (0.57)	NS (0.46)
PRE TRAIN/ STRESS	r	-0.08	0.14	-0.04	0.29	-0.003	0.34	0.02
	p	NS (0.67)	NS (0.46)	NS (0.83)	NS (0.12)	NS (0.99)	NS (0.06)	NS (0.93)

Table 3.4 Results of correlations carried out between recorded behaviours in PRE TRAIN/BASE and PRE TRAIN/STRESS observations and the number of training sessions required to reach criterion (df = 28)

At PRE TRAIN/BASE levels of ano-genital present, scent mark, tsik vocalisations and phee vocalisations were not correlated to the number of sessions required to learn the task. The amount of self-scratching however in PRE TRAIN/BASE observations was correlated with the number of sessions (Spearman rank correlation, df = 28, r = 0.61, p < 0.001) (Figure 3.6). When subjected to a Bonferroni correction, giving a p value of 0.002, this result is robust enough to remain significant. There was no correlation found between the levels of ano-genital presents, scent mark, tsik vocalisations, phee vocalisations and self-scratching in the PRE TRAIN/STRESS observations and the number of sessions taken to learn the task (Table 3.5).

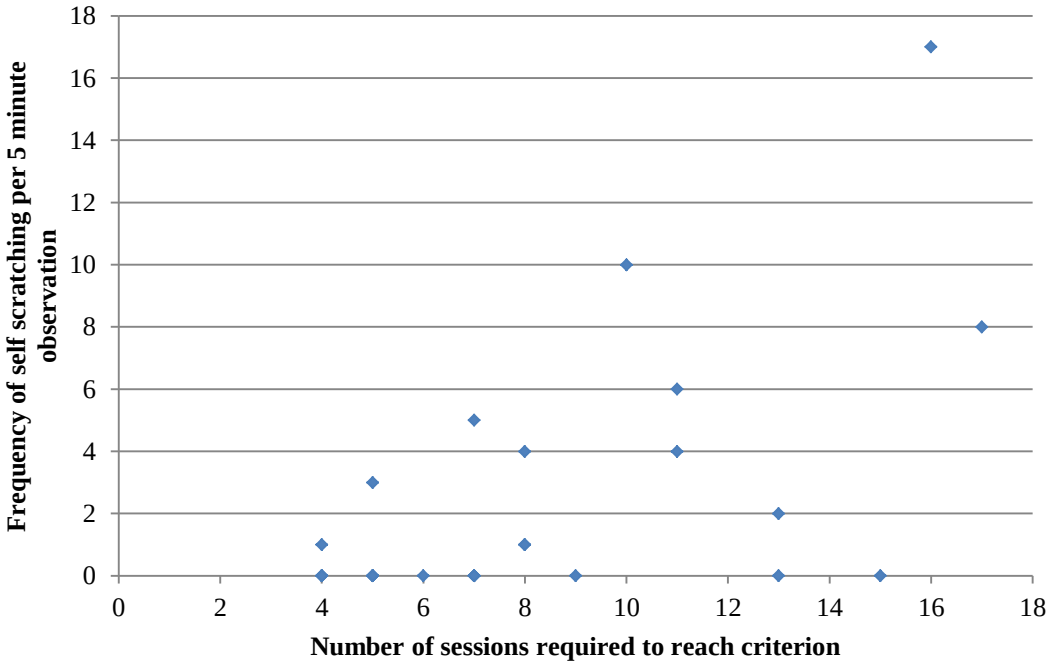

Figure 3.6 Correlation between the frequency of self scratching seen at PRE TRAIN/BASE and the number of sessions required to reach criterion for all marmosets

		ano-genital presentations	scent mark	tsik vocalisations	phee vocalisations	self scratch
PRE TRAIN/ BASE	r	-0.05	-0.12	0.22	-0.05	0.61
	p	NS (0.79)	NS (0.53)	NS (0.24)	NS (0.79)	<0.001
PRE TRAIN/ STRESS	r	0.07	0.05	-0.02	-0.31	0.14
	p	NS (0.71)	NS (0.79)	NS (0.92)	NS (0.10)	NS (0.46)

Table 3.5 Results of correlations carried out between recorded behaviours in PRE TRAIN/BASE and PRE TRAIN/STRESS and the number of training sessions required to reach criterion (df = 28)

3.5.4 The Effect of Age, Sex and Housing on Response to the Temperament Tests

When the subset of data including those marmosets who were successful in the temperament test was analysed, latency to touch the novel object, latency to access the food within and latency to take food in the human interaction test were not correlated to the age of the marmosets (Pearson correlations, touch, df = 22, r = 0.05, p = 0.82, NS; food, df = 12, r = -0.48, p = 0.08, NS, human, df = 17, r = -0.25, p = 0.30, NS). Analysis of the whole data set, attributing ceiling values (equal to the test time limit) to those marmosets who were not successful in the temperament tests, found no significant correlation between age and latency to touch the novel object (Spearman rank correlation, df = 28, r = -0.06, p = 0.74, NS), latency to access the food (Spearman rank correlation, df = 28, r = 0.13, p = 0.51, NS) nor hand feed (Spearman rank correlation, df = 28, r = -0.39, p = 0.06, NS).

In the subset of data including only those marmosets who were successful in the temperament tests, whilst there was no difference between males and females in the speed in which they touched the novel object (males n = 11, mean = 65.0 ± 11.5 s, females n = 13, mean = 63.6 ± 16.6 s; t-test, df = 20, t = -0.07, p = 0.94, NS), differences were seen between males and females in their latency to access the food and latency hand feed in the human interaction test. Males were significantly faster than females to access the food (males n = 6, mean = 98.2 ± 17.0 s, females n = 8, mean = 151.4 ± 16.7 s; t-test, df = 11, t = 2.23, p = 0.047) and also significantly faster to hand feed in the human interaction test (males n = 7, mean = 5.71 ± 1.3 s, females n = 12, mean = 25.8 ± 5.6 s; t-test, df = 12, t = 3.51, p = 0.004).

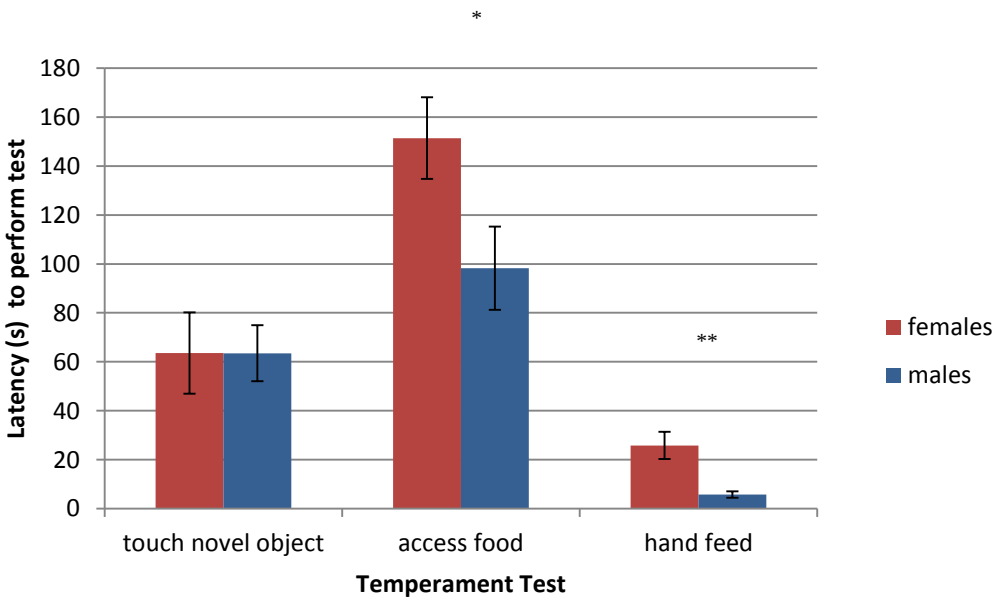

Figure 3.7 Comparisons between latencies of males and females to perform novel object test, food access test and human interaction tests. (* $p < 0.05$, * $p < 0.01$) **for marmosets successful in the temperament test. Error bars show standard error of the mean.**

However when all data were used, with ceiling values equal to the test time limit attributed to those marmosets who failed the tests, no differences were seen between males and females in the latency to touch the novel object (Mann-Whitney, females, n = 16, median = 64s, males, n = 14, median = 81.5s, W = 231.5, p = 0.50, NS), latency to access the food (females, n = 16, median = 257.5s, males n = 14, median = 300s, W = 254, p = 0.80, NS) or latency to hand feed (females, n =16, median = 29.5s, males, n = 14, median = 95.5s, W = 254.5, p = 0.79, NS).

The subset of data including just those individuals who were successful in the temperament tests showed that marmosets housed in the lower tier of took approximately twice as long to touch the novel object as those housed in the upper tier (lower tier n = 14, mean = 81.1 ± 14.6 s, upper tier n = 10, mean = 40.7 ± 10.0 s; t-test, df = 21, t = -2.28, p = 0.03), but housing tier did not affect latency to access the food in the novel object (lower tier n = 6, mean = 142.5 ± 24.5 s, upper tier n = 8, mean = 118.1 ± 15.9 s; t-test, df = 8, t = -0.84, p = 0.43, NS) or latency to take food in the human interaction test (lower tier n = 10, mean = 17.7 ± 6.5 s, upper tier n = 9, mean = 19.2 ± 5.53 s; t-test, df = 16, t = 0.18, p = 0.86, NS). When all data were analysed no differences in latencies to complete any of the temperament tests were seen (Mann-Whitney; touch, upper, n = 14, median = 36.5, lower, n = 16, median = 81.5, W = 169.5, p = 0.05, NS; food, upper, n = 14, median = 151.5s, lower, n = 16, median = 300s, W = 174, p = 0.06, NS; hand feed, upper, n = 14, median = 34.5, lower, n = 16, median = 28.0, W = 223, p = 0.81, NS).

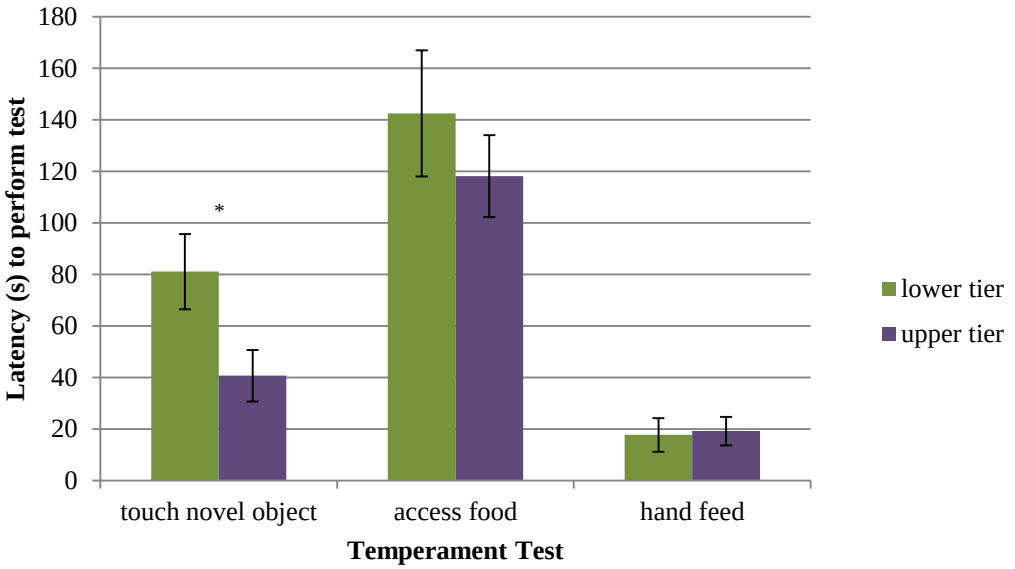

Figure 3.8 Comparisons between latencies of upper tier housed marmosets and lower tier housed marmosets to perform novel object test, food access test and human interaction tests. (* $p < 0.05$, * $p < 0.01$) **for marmosets successful in the temperament tests**. **Error bars show standard error of the mean.**

3.5.5 Relationships Between the Temperament Tests

With the subset of successful marmosets, a positive correlation was found between the latency of the marmosets to touch the novel object and their latency to access the food within (Pearson correlation, df = 15, r = 0.58, p = 0.015) (Figure 3.9). No correlations were found between the latency to touch the novel object and latency to hand feed in the human interaction test (df = 15, r = 0.24, p = 0.35, NS) nor between the latency to access the food and hand feed in the human interaction test (df = 8, r = 0.06, p = 0.87, NS). Similar results were found when all data were analysed, with ceiling values (of the test time limit) attributed to those animals who were not successful in the test. There was a correlation between latency to touch the novel

object and to access the food (Spearman rank correlations, r = 0.67, p < 0.001), but no correlation between latency to touch the novel object and latency to hand feed (r = 0.34, p = 0.06, NS) or between latency to access the food and latency to hand feed (r = 0.24, p = 0.20, NS).

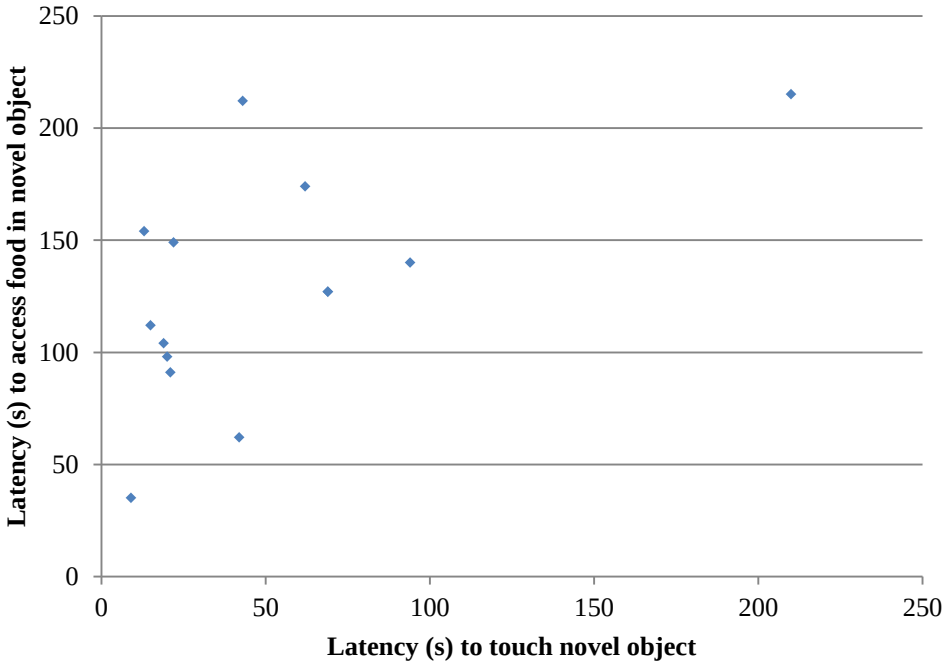

Figure 3.9 Correlation between latency (seconds) to touch novel object and latency (seconds) to access food in novel object for marmosets successful in the temperament tests

With a subset of data including only marmosets who were successful in the temperament tests, the mean latencies to perform the tests were significantly different (ANOVA, df = 2, F = 27.1, p < 0.001). Planned post-hoc Tukey tests showed that the marmosets were significantly faster to take food in the human interaction test than to either touch the novel object (T = 4.5, p = 0.007) or access the food (T = -3.5, p = 0.005), and significantly faster to touch the novel object than access the food (T = -7.4, p = 0.008). However, when all marmosets were included in the analysis (with test time limits allocated as ceiling values), although there was still a significant difference between the latencies to touch the novel object and

access the food inside (Wilcoxon signed rank tests, n = 30, W = 0.0, p < 0.001), and between latencies to access the food and to hand feed (n = 30, W = 450, p < 0.001), no significant difference was seen between latencies to touch the novel object and to hand feed (n = 30, W = 313, p = 0.10, NS).

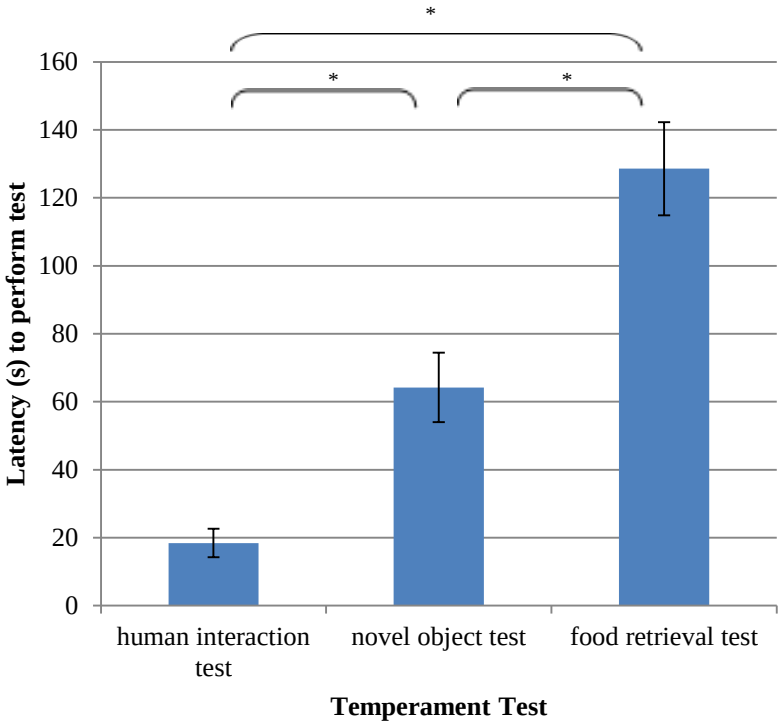

Figure 3.10 Comparisons between latency to touch novel object, latency to access food in novel object and latency to take food in human interaction test (* p < 0.05, ** p < 0.01) **for marmosets successful in hand feed temperament test**. **Error bars show standard error of the mean.**

3.5.6 Behavioural Observations

Behavioural time budgets differed across the three observations. Sitting, watchful behaviour, agitated locomotion and foraging were all exhibited for significantly different durations across the observations whilst time spent in normal locomotion, contact sitting and in the nest box did not differ (Figures 3.11, 3.12, Table 3.7). Planned post-hoc Tukey tests on behaviours compared PRE TRAIN/BASE and PRE TRAIN/STRESS to identify the effect of the stressor, and PRE TRAIN/STRESS and POST TRAIN/STRESS to identify the effect of training. Results are also presented of comparisons between PRE TRAIN/BASE and POST TRAIN/STRESS PRE TRAIN/BASE and POST TRAIN/STRESS for completeness, although none of these were found to differ significantly. Results of comparable Wilcoxon signed ranked test for event behaviours are also presented below.

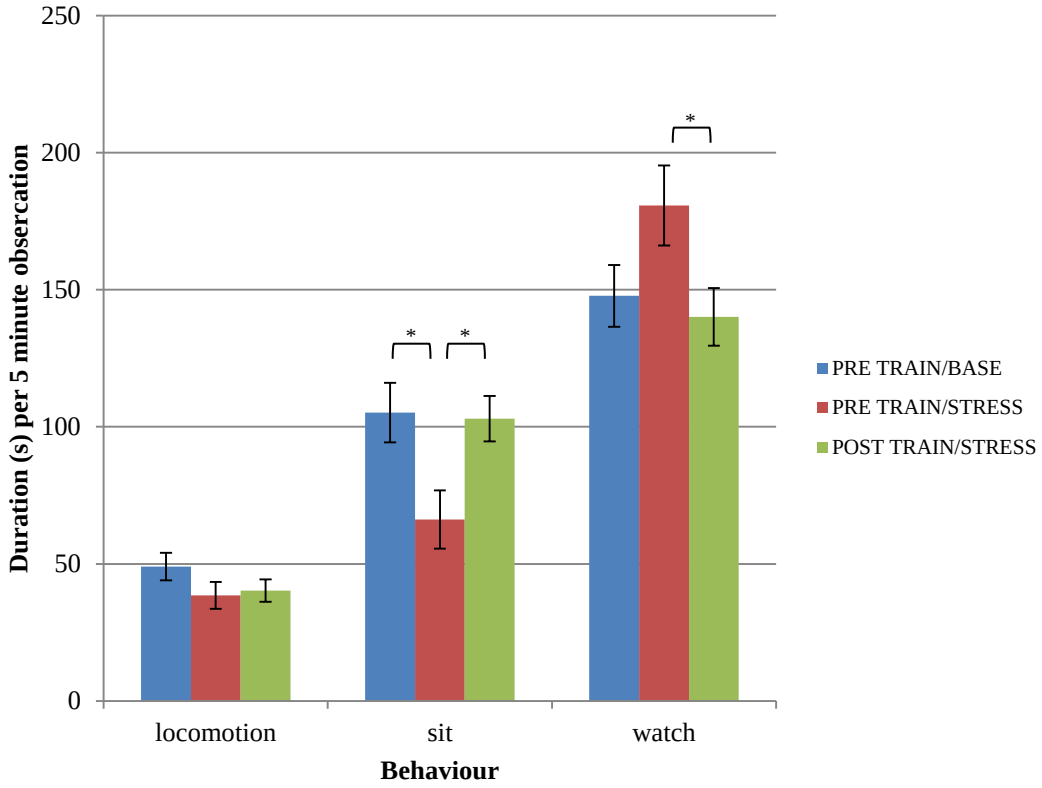

Figure 3.11 Mean durations (in seconds) of longer duration behaviours (> 20 s) per 5 minute observation, across three observations, PRE TRAIN/BASE, PRE TRAIN/STRESS, POST TRAIN/STRESS (* $p < 0.05$, ** $p < 0.01$). **Error bars show standard error of the mean.**

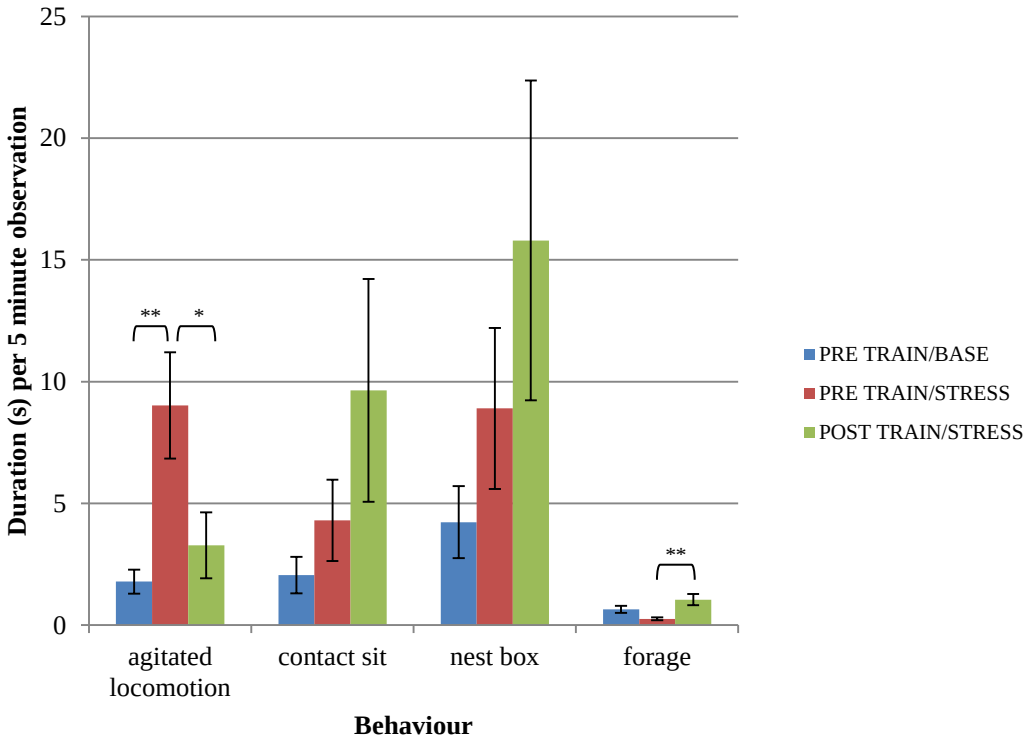

Figure 3.12 Mean durations (in seconds) of shorter duration behaviours (< 20 s) per 5 minute observation across three situations, PRE TRAIN/BASE, PRE TRAIN/STRESS, POST TRAIN/STRESS (* $p < 0.05$, ** $p < 0.01$). **Error bars show standard error of the mean.**

Behaviour	$F_{2,28}$	p
locomotion	1.5	NS (0.24)
agitated locomotion	6.16	< 0.01 (0.006)
sit	6.05	< 0.01 (0.007)
contact sit	2.05	NS (0.15)
watch	3.68	< 0.05 (0.038)
nest box	1.95	NS (0.16)
forage	5.22	< 0.05 (0.012)

Table 3.7 Results of repeated measures ANOVA on durations of recorded behaviours across the three observations

When significance level is corrected for multiple comparisons, and is set at 0.0072, both agitated locomotion and sitting continue to reach this level of significance.

3.5.7 Behaviour in Relation to the Stressor

When durations of behaviours at PRE TRAIN/BASE and PRE TRAIN/STRESS were compared, agitated locomotion was seen to increase significantly and sitting behaviour to decrease significantly, whilst watchful behaviour and foraging did not show any change (Figures 3.11, 3.12, Table 3.8). Following a Bonferroni correction, whereby significance is set at 0.0125, these remain significant.

Behaviour	T	p
agitated locomotion	3.352	< 0.01 (0.007)
sit	-3.096	< 0.05 (0.012)
watch	2.073	NS (0.11)
forage	-1.59	NS (0.31)

Table 3.8 Results of post-hoc Tukey tests, comparisons between PRE TRAIN/BASE and PRE TRAIN/STRESS for duration behaviours

None of the event behaviours, that is ano-genital presents, scent mark, tsik and phee vocalisations and self scratching, showed any significant difference in response to the stressor (Table 3.9).

Behaviour	n	W	p
ano-genital presentations	11	12.0	NS (0.08)
scent mark	11	18.0	NS (0.20)
tsik	10	17.0	NS (0.31)
phee	10	13.5	NS (0.17)
self scratch	14	21.5	NS (0.06)

Table 3.9 Results of Wilcoxon signed rank test for event behaviours for PRE TRAIN/BASE v PRE TRAIN/STRESS

3.5.8 Behaviour in Relation to Training

The behaviour of the marmosets in response to the stressor changed significantly following training. They exhibited significantly higher levels of sitting and foraging and significantly lower levels of agitated locomotion and watchful behaviour when PRE TRAIN/STRESS was compared to POST TRAIN/STRESS (Figures 3.11, 3.12, Table 3.10). When the p value was corrected for multiple comparisons to 0.0125, only forage continued to show significant difference.

Behaviour	T	p
agitated locomotion	-2.640	< 0.05 (0.035)
sit	2.919	< 0.05 (0.018)
watch	-2.550	< 0.05 (0.042)
forage	3.23	< 0.01 (0.009)

Table 3.10 Results of post-hoc Tukey tests, comparisons between PRE TRAIN/STRESS and POST TRAIN/STRESS for duration behaviours

There were however no differences in the frequencies of ano-genital presentations, scent mark, tsik and phee vocalisations and self scratching (Table 3.11).

Behaviour	n	W	p
ano-genital presentations	7	3.5	NS (0.09)
scent mark	11	12.0	NS (0.07)
tsik	15	34.0	NS (0.17)
phee	7	5.0	NS (0.37)
self scratch	12	34.0	NS (0.49)

Table 3.11 Results of Wilcoxon signed rank test for event behaviours for PRE TRAIN/STRESS v POST TRAIN/STRESS

No differences were seen in any of the behaviours, either those recorded as durations or as frequencies, between PRE TRAIN/BASE and POST TRAIN/STRESS (Figures 3.11, 3.12, Tables 3.12, 3.13).

Behaviour	T	p
agitated locomotion	0.685	NS (0.77)
sit	-0.177	NS (0.98)
watch	-0.477	NS (0.88)
forage	1.64	NS (0.25)

Table 3.12 Results of post-hoc Tukey tests, comparisons between PRE TRAIN/BASE and POST TRAIN/STRESS for duration behaviours

Behaviour	n	W	p
ano-genital presentations	8	20.0	NS (0.83)
scent mark	11	47.0	NS (0.23)
tsik	14	55.5	NS (0.88)
phee	7	18.0	NS (0.55)
self scratch	15	88.5	NS (0.11)

Table 3.13 Results of Wilcoxon signed rank test for event behaviours for PRE TRAIN/BASE v POST TRAIN/STRESS

3.6 DISCUSSION

3.6.1 Success Rate and Time Investment

All of the marmosets in this study were trained to both touch a target and remain calm when isolated in a transport box, a success rate which is a very encouraging success rate. McKinley *et al* (2003) also trained all their common marmosets to touch a target, Bassett *et al* (2003) had a 100% success rate with training common marmosets to provide a urine sample, and Fernström *et al* (2009) trained 97% of their rhesus macaques to touch a target. However others have had less success in training, with success rates of 44% being reported for rhesus macaques for touching a target (Coleman *et al*, 2005), and just 12% success for training to enter a transport box in the same species (Fernström *et al*, 2009). In Chapter 2 success rates for training cynomolgus macaques to touch a target are shown to be 62.5%, suggesting it may be easier to train common marmosets than macaques. There are a number of

differences in the way marmosets and macaques are sourced and used in the laboratory, and these may affect their trainability. The previous experience of macaques and marmosets, especially in relation to humans, is likely to be significantly different as marmosets are usually bred in-house (at least in the UK), whereas macaques, particularly those used by contract research organisations, are generally bred overseas and imported (Honess *et al*, 2004). Nevertheless it appears that success rates for common marmosets are particularly high which is encouraging for the implementation of training programmes in this species in the laboratory.

Overall time investment for the whole task was a mean of 39.5 minutes per monkey (approximately 8 x 5 minute sessions), with target training taking a mean of 22.2 minutes of that total (approximately 4.5 x 5 minute sessions). This compares favourably with other studies of the same species, where target training time investments of 32 minutes (McKinley *et al*, 2003) and 124 minutes (Savastano *et al*, 2003) per animal have been reported. Of this 124 minute time investment, only four minutes were actually needed to establish the behaviour once individuals were hand-feeding, and in another species of marmoset (Geoffroy's tufted-ear marmoset, *C. geoffroyi*), training to hold a target took 7.5-10 minutes, which suggests that the time investment found in this study falls within the bounds of that found in other studies, but that laboratory-housed callitrichids seem to take longer to learn this task once hand feeding than those housed in a zoo.

Other studies have shown that when common marmosets are trained to provide a urine sample by scent marking, a behaviour associated with stress, they learn this more quickly if they have not been target trained first (McKinley *et al*, 2003),

suggesting that this close interaction with humans is a source of stress to the animals. The large time investments seen in other studies to train monkeys to hand feed (Savastano *et al*, 2003) may be further evidence of this. This fear of humans may also play a large role in the success of a training programme, as if an animal cannot overcome this fear it will have difficulty in learning in a human-lead training programme. In this study, by chance, there were no pairs in which at least one of the monkeys would not hand feed in the human interaction test, and pairs where at least one monkey would hand feed were preselected by McKinley *et al* (2003), which likely influenced the time investment and success rate.

That training took less time in this study than in McKinley *et al* (2003) is interesting as both studies were carried out at the same laboratory, albeit over two years apart, where it would be expected that factors such as husbandry and the monkeys prior experience might be comparable. Differences in trainer competency may explain this but it is more likely to be due to slight differences in training protocol, for instance the levels set for meeting criterion in each stage, the number of rewards available or even the time taken between each reward being available (for example in this study marmosets were required to hold the target for a maximum of 10 seconds, whereas in McKinley *et al* this was 20 seconds).

The time investment required to train an individual to stay in the transport box in this study equates to just five minutes per day for eight days, and therefore is not very much, but has potential to become a large time investment for multiple animals. Whether this is feasible in the laboratory however depends on the number of animals involved and staff availability. This level of time investment is

considerably less than that required for the training of chimpanzees for more aversive tasks, which is regularly performed in laboratories in the USA (e.g. Schapiro *et al*, 2005).

Feasibility also depends on the time taken to carry out the task with untrained animals and that taken with trained animals. McKinley *et al* (2003) found that the time investment required to train common marmosets to cooperate with weighing was recouped in 8 - 20 sessions. No assessment of the time required to capture animals was made in this study, so time recouped or lost cannot be calculated. The opinions of the carestaff were divided as to the practicality of this method of capture; some reported that it was easier and less stressful for them to capture some of the marmosets in this way than in their traditional manner, whilst others thought that the traditional method was easier and less stressful. A training programme will only be successful if staff are committed to it, so this variation in the perception of the training programme is something which would need to be addressed if training is to be adopted in laboratories.

3.6.2 The Effects of Age, Sex and Housing on Success Rates and Time Investment

The literature shows a variable picture on the influence of age and sex on learning in primates. Neither of these factors affected learning in this study, but no old individuals were included in this study, and as the most consistent trend seems to be that aged animals perform less well than younger animals, this is unlikely to have been seen.

In this study the sex of the individual did not affect how quickly they learnt the task. Other training studies have found the same result in both common marmosets (McKinley *et al*, 2003) and chimpanzees (*P. troglodytes*, Videan *et al*, 2005) suggesting that this is a general trend across species. Gender differences seen in foraging tasks have been attributed to the males deferring to females (Box, 1997), and this may be relevant in training programmes where mixed sex pairs or groups are studied. However no differences were seen when opposite sex pairs were trained (McKinley *et al*, 2003), supporting results from this study that there were no actual differences between males and females in their trainability.

That neither age nor sex impacts on the trainability of an individual common marmoset is important and useful for the implementation of training programmes in the laboratory. Some research requires a particular animal demographic, for example reproductive research may require females only, and if training is to be used success and time investment will not be affected by this requirement. This means that it is possible to successfully use training across all ages and both sexes of common marmosets in the laboratory.

It has been suggested that animals housed in the lower tier of racked caging are subjected to a number of disadvantages in terms of their quality of life, notably reduced illumination leading to behavioural changes and reduced human interaction (Reinhardt & Reinhardt, 2000). If these lower tier individuals are more stressed or are more fearful of humans this may affect their trainability as they may be less willing to interact with humans. In this study no differences were seen between the animals housed in upper and lower tier cages in the time investment required for

them to learn the task suggesting that this is not the case, in this laboratory at least. Training was carried out carefully however to ensure that monkeys were able to retreat above the height of the trainer, which may reduce the immediate impact of fearfulness, and it should be noted that care needs to be taken with this when implementing training programmes. There is now UK legislation which prohibits two tier housing and therefore removes this variable, but these data suggest that for training at least there is no disadvantage to being housed in the lower tier, and this should not affect time investment needed.

There was a correlation between the time investment required to train the first trained individual in the pair and the time investment required for the second trained. This suggests that either the first trained was monopolising training, particularly in the early stages of training or that the second trained marmoset was the subordinate and was intimidated by the presence of the dominant individual so did not interact until that marmoset could be reliably moved away from the training location. There is also a possibility of some social learning taking place, either of the task itself or of increased socialisation with humans. Whether any or all of these possibilities explain this correlation is impossible to determine from the available data. Other studies have approached this problem by combining individual time investments so that time investment is recorded per pair (McKinley *et al*, 2003) or by only measuring the performance of the dominant individual (Fernström *et al*, 2009), both of which approaches also have flaws, in that they eliminate either the performance of the best or worst performing individuals. Whilst this might be relevant for the purposes of those studies, where individual differences are being studied it is necessary to measure the performance of all individuals. Training the

marmosets away from their cagemate may have overcome this, but possibly would have slowed training due to the marmosets being more stressed by the separation, and also not be practical for a laboratory setting. Results when only the first trained marmoset of the pair was analysed are similar enough to those when all marmosets are analysed it is reasonable to conclude that the social influences above are not significantly altering the findings of this study.

3.6.3 The Effect of Age, Sex and Housing on Response to the Temperament Tests

The novel object test in this study was a simplified interpretation of foraging tests used in other studies, where a device containing food has been introduced to animals who are required to manipulate the object in order to receive the food (Cameron & Rogers, 1999, Yamamoto *et al*, 2004). It is also very similar to novel objects used in other studies (Majolo *et al*, 2003a). Whereas some of these studies found that females performed better than males (Box, 1997, Yamamoto *et al*, 2004), females were no more or less likely to touch the novel object than males in this study, in accordance with Cameron and Rogers (1999). Males however were much faster to access the food and four times faster to hand feed in the human interaction test in this study in direct contrast to published data where differences have been found (Box, 1997, Yamamoto *et al*, 2004). In both this study and that of Cameron and Rogers (1999) the monkeys were housed in single sex groups whereas studies which have seen females outperform males have housed their monkeys in mixed sex groups. This may be significant, as it has been suggested that the males defer to the females in foraging tasks (Box, 1997, Yamamoto *et al*, 2004). Perhaps the results of these tests are more representative of an individual's true temperament when housed

in same sex pairs/groups than in mixed sex pairs/groups. This however would not explain the performance of the males in this study. Care therefore would need to be taken if administering and interpreting this test when mixed sex pairs are being tested, and this is further borne out by the fact that when all data were analysed no differences were seen between males and females in any of the tests. The apparent difference may therefore be an artefact of the different success rates in the task between males and females.

Marmosets housed in lower tier cages took twice as long as those housed in upper tier cages to touch the novel object, but no differences were seen in latency to access the food or to hand feed. Buchanan-Smith *et al* (2002) showed that marmosets housed in the lower tier of caging are less likely to feed from the floor of the cage than those housed in the upper tier, suggesting an unwillingness to go to the cage floor in lower tier housed marmosets which is reproduced here. This relative reluctance of lower tier housed marmosets to go to the cage floor to touch the novel object may reflect the vulnerability felt by those individuals, possibly due to their increased risk of predation (Prescott & Buchanan-Smith, 2002). Obviously the floor of the lower tier cage is much lower than the floor of the upper tier cage, and for an arboreal species being that low to the ground represents a risk which may not be as pronounced for upper tier housed marmosets. However, analysis of the full data set did not show a difference between the upper and lower tier in the latency to touch the novel object, although it is a very borderline result. It is therefore prudent to treat this result with caution, though it may become more marked in full height cages where the difference between the cage floor and the top of the cage is obviously much greater. It has been suggested that monkeys housed in the lower

tier are less likely to receive human interaction (Reinhardt & Reinhardt, 1999, 2000), but the results presented here suggest that this is not the case, or that the amount of prior interaction does not affect willingness to hand feed from a human as no differences were seen between the two housing conditions and the latency to take food from a person.

3.6.4 Relationships Between the Temperament Tests and Time Investment

Novel Object Test

Of the marmosets tested in this study, 80% were willing to touch the novel object, and 47% accessed the food. In other studies 100% success rates for interacting with food retrieval type task apparatus have been reported in common marmosets, with 80% successfully accessing the food (Cameron & Rogers, 1999). It is interesting that the success rate in this task was lower in this study. This may be due to the position in which the task was presented, in this study the novel object was placed on the cage floor whilst in the study of Cameron and Rogers (1999) it was presented on a platform 28cm above floor level. This still required the marmosets to go close to the bottom of the cage however, and it is questionable whether this small difference in the height of the object would have accounted for differences in success rates. The marmosets in this study were tested with their cagemates, but Cameron and Rogers (1999) were able to separate their marmosets from the rest of the group, whilst keeping them in their home cage, so individuals could be tested alone. This may have meant that the marmosets were able to interact with the object

without interference from cagemates. However being alone may have caused them to be more fearful and therefore less likely to interact with something novel.

As all marmosets were successfully trained, whether they touched the novel object or accessed the food inside was not a predictor of success, but how individuals responded to the test did predict the speed at which they learnt the training task. Those marmosets who did not touch the novel object were significantly slower to learn the training task than those who accessed the food, taking over 4.5 sessions longer to reach criterion. Although those who touched the novel object took nearly 3.5 sessions less than those who did not touch the novel object, this was not significant due to high variability. There was no correlation between the latency to touch the novel object or access the food and the number of training sessions required to reach criterion, which suggests that this test is not as sensitive at predicting training time investment as the human interaction test. Nevertheless this test proved to be a useful predictor of the time investment required to train marmosets. Response to a novel object has been found to be a predictor of training success in rhesus macaques (Coleman *et al*, 2005), but was not seen in cynomolgus macaques in an earlier study (Chapter 2), and as discussed in Chapter 2 this may be due to the selection of the novel object and how it is presented rather than actual differences in the usefulness of this type of test. However this study showed a novel object type test was a practical and useful indicator of trainability in common marmosets.

Human Interaction Test

Just under two thirds of the marmosets tested were willing to take food from a person prior to any training, and those who took food reached criterion approximately 4.5 sessions faster than those who did not take the food. As discussed in Chapter 2, interacting with, and taking food from, a person is vital in training programmes, so this is not surprising. It is however particularly impressive that even those individuals who were not willing to hand feed prior to training still completed the training programme within the allocated time limit, something which did not prove possible with the cynomolgus macaques (Chapter 2). Further to this there was a good correlation between the latency to hand feed and the number of sessions required to reach criterion, suggesting that this is a sensitive predictor of trainability. As discussed previously a marmoset who will hand feed quickly wastes less time in a training session, and therefore is likely to make faster progress than a more hesitant individual. Furthermore, marmosets willing to approach a person quickly are likely to be bolder in general than those who take more time to interact. Once again this emphasises the importance of hand feeding in relation to training and also the general welfare of laboratory-housed primates. Hand feeding is a simple task which all staff can participate in, requiring no specific training and a small time investment, but has huge potential to improve laboratory primate welfare by reducing fear.

Response to a Stressor

Of the recorded behaviours only self scratching at PRE TRAIN/BASE was found to be related to the trainability of the marmosets. The frequency of self scratching was

found to be positively correlated with the number of sessions an individual required to reach criterion, and as self scratching has been identified as a marker of anxiety in primates (Baker & Aureli, 1997) this may give weight to the argument that high trait anxiety and poorer cognitive ability, at least in relation to training, are positively related (Eysenck, 1985). However, in common with the cynomolgus macaques discussed in Chapter 2, this correlation was only seen in PRE TRAIN/BASE observations and not in PRE TRAIN/STRESS observations as predicted. It is possible that self scratching is not actually measuring trait anxiety. However that the frequency of self scratching predicts trainability this finding is still of value. Those marmosets exhibiting higher levels of self scratching are likely to be more anxious individuals, and therefore more likely to at least be hesitant about interacting with humans and therefore take longer to train. Marmosets who exhibit higher levels of self scratching may be those individuals who are more stressed in general, so this may suggest that more relaxed individuals are better able to learn, or at least cope with the demands of the training programme. This further supports the premise that fear is closely related to the trainability of a monkey.

3.6.5 Relationships Between the Temperament Tests

That the latency to touch the novel object was positively correlated to the latency to access the food within it is not particularly surprising given that in order to access the food the marmosets must have first touched the novel object. No correlation was found however between these latencies and the latency to hand feed, indicating that individual marmosets perceived the tests differently. That is to say marmosets who were quick to hand feed were not necessarily quick to touch the novel object, suggesting that these tests were measuring different aspects of temperament and that

boldness in relation to a novel object may not be related to boldness with humans. Overall marmosets were significantly faster to take food from a person than to either touch the novel object or access the food, a finding which indicates they found the novel object test more challenging, or unfamiliar, than interacting with a person. This is positive from a training point of view, in that the marmosets did not appear to be particularly fearful of people. However when all marmosets were included in the analysis rather than just those who were successful in the temperament tests, there was no longer a significant difference between the latencies to touch the novel object and to hand feed, which may suggest that there a general fear or neophobia in some animals.

3.6.6 Behavioural Observations

Observations showed that the marmosets' behaviour changed both in response to the stressor and training. Following the stressor untrained marmosets exhibited increased levels of agitated locomotion and decreased levels of sitting, when compared to PRE TRAIN/BASE, but no other behaviours show this response. This suggests that the stressor is stressful for the marmosets but significant differences in other behaviours, in particular self scratching, would have provided stronger evidence for this. The marmosets' response to the stressor appears to be changed quite substantially by training however. They exhibit lower levels of agitated locomotion and watchful behaviour following the stressor once trained (POST TRAIN/STRESS) than they did prior to training (PRE TRAIN/STRESS), and increased levels of sitting and foraging. Overall behaviour was no different following the stressor once the marmosets were trained than at baseline prior to training. No significant differences were seen across any of the observations in the

frequencies of the behaviours recorded as events (ano-genital presentations, scent mark, tsik vocalisations, phee vocalisations and self scratching) suggesting that these may not be as sensitive as duration behaviours at identifying stress in marmosets, in contrast with Bassett *et al* (2003) who suggest self-scratching is a sensitive measure of stress in marmosets. A summary of behavioural changes seen across the three observations are shown in Tables 3.14 and 3.15.

Behaviour	**PRE TRAIN/STRESS**	**POST TRAIN/STRESS**
locomotion	=	=
agitated locomotion	↑	=
sit	↓	=
contact sit	=	=
watch	=	=
nest box	=	=
forage	=	=

Table 3.14 Summary of behavioural changes seen in response to the stressor and to training as compared to PRE TRAIN/BASE. ↑ indicates an increase in response to the treatment, ↓ indicates a decrease and = indicates no significant difference.

Behaviour	POST TRAIN/STRESS
locomotion	=
agitated locomotion	↓
sit	↑
contact sit	=
watch	↓
nest box	=
forage	↑

Table 3.15 Summary of behavioural changes seen in response to the stressor and to training as compared to PRE TRAIN/STRESS. ↑ indicates an increase in response to the treatment, ↓ indicates a decrease and = indicates no significant difference.

The overall pattern of behaviours indicate that the stressor lead to a reduction in the welfare of the marmosets prior to training, but that following training no such reduction was seen, suggesting that training helps marmosets to cope with routine stressors. This finding is further supported by work carried out by Bassett *et al* (2003) who found that trained marmosets exhibited lower levels of self scratching than untrained marmosets following a stressor, and made the same conclusion. The relevance of specific changes in behaviour is discussed below.

Locomotion

Normal locomotion was not significantly different across any of the three observations, but agitated locomotion was seen to increase in response to the stressor in untrained animals, but a similar increase following the stressor was not seen once the marmosets were trained. As discussed in Chapter 2 the interpretation

of changes in locomotion can be difficult, however increases in locomotion are most often seen in response to stress (e.g. Laudenslager *et al*, 1990, Levine, 1993, Hennessy *et al*, 1995) so it is generally regarded as an indicator of reduced welfare unless increased locomotion is a specifically desired outcome (e.g. Tripp 1985). Normal locomotion did not change in this study but did increase following the stressor and decrease following training in cynomolgus macaques (Chapter 2), so it may be that it is not such a sensitive measure in marmosets. Bassett *et al* (2003) however found that in marmosets locomotion increased in response to a stressor, but training had no effect on levels of locomotion, so this would suggest that this behaviour is a sensitive enough measure to identify stress and reduced welfare in this species. Agitated locomotion however increased following the stressor prior to training, but no similar increase was seen in response to the stressor once training had occurred; indeed post stressor levels of agitated locomotion were significantly lower once the marmosets were trained as compared to pre-training.

Agitated locomotion therefore seems to be a more sensitive measure of reduced welfare, with changes in this behaviour being seen even when changes in normal locomotion are absent. As agitated locomotion comprises a small proportion of total locomotion, changes are unlikely to be identified if all locomotion is measured together. This continues to support the suggestion proposed earlier that the qualitative nature of a behaviour is important as well as the total amount of that behaviour. That agitated locomotion showed the pattern it does in this study provides evidence that the stressor was stressful and that training reduced the effect of the stressor on the marmosets' welfare.

Sitting

Sitting alone was seen to decrease in the marmosets following the stressor prior to training, but the stressor following training elicited levels of sitting which were no different to PRE TRAIN/BASE and significantly higher than PRE TRAIN/STRESS. A similar result has been previously reported in marmosets (Bassett *et al*, 2003), with inactivity being lower in untrained marmosets following a stressor than prior to a stressor. However this pattern was repeated in trained marmosets, suggesting that training had no impact on the levels of inactivity marmosets exhibit in response to a stressor, and when pre- and post-stressor data were pooled trained and untrained marmosets spent the same amount of time inactive. It is therefore likely that inactive sitting is a sensitive measure of stress in marmosets. That the marmosets show no changes in sitting behaviour following the stressor once trained gives further evidence for the positive welfare benefits of positive reinforcement training, especially in relation to their ability to cope with stressful situations. Cynomolgus macaques showed no change in sitting in response to a stressor, but in this species training lead to a decrease in inactive sitting (Chapter 2). It may be therefore that changes in sitting are a better measure of stress in marmosets than macaques.

Contact sitting showed no significant changes across the observations. Contact sitting is an affiliative behaviour (Stevenson & Poole, 1976), which in macaques at least decreases following a stressor (Chapter 2). That this was not seen in marmosets suggest this behaviour is not such a sensitive measure in this species. It is interesting that in the two species studied different types of inactivity responded differently to the stressor and to the training programme, once again highlighting

the importance of recording subtle qualitative differences within a particular behaviour.

Vigilance behaviour

Watchful behaviour was significantly lower at POST TRAIN/STRESS than PRE TRAIN/STRESS observations, but no significant statistical differences were seen between this behaviour at PRE TRAIN/BASE and PRE TRAIN/STRESS. Watchful behaviour can be an indicator of levels of fear or interest in something, and that training reduces the levels of watchful behaviour in a stressful situation suggests that the majority of vigilance behaviour exhibited by the marmosets in this study is as a result of fear. Marmosets however have been shown to exhibit increased levels of vigilance behaviour following training (Bassett, 2003), possibly due to an increased interest in the trainer and the food they deliver.

Cynomolgus macaques did not exhibit different levels of vigilance behaviour following training but did show more vigilance following a stressor (Chapter 2), and in another study marmosets showed increased levels of vigilance behaviour following training (Bassett *et al*, 2003), so alongside the decrease in vigilance behaviour seen in this study it is clear that this is a difficult behaviour to interpret. However it is likely that given the other behavioural changes seen in this study, the changes in vigilance behaviour exhibited by the marmosets are related to fear rather than a positive interest.

Foraging

Recorded levels of foraging behaviour were low in all three observations, however significantly more foraging behaviour was seen at POST TRAIN/STRESS than PRE TRAIN/STRESS. As foraging is a behaviour which is often encouraged in enrichment programmes, and seen as an indicator of positive welfare, this suggests that training improved the welfare of the marmosets. In cynomolgus macaques training was seen to increase foraging behaviour, and as this is also seen in the marmosets it is likely that this behaviour is a good indicator of primate welfare. As discussed previously for macaques, the willingness of the marmosets to go to the cage floor to forage is likely to be related to their perception of threat and fear. Further, an increase in the relaxed activity of foraging may be indicative of reduced boredom.

Nest box use

The marmosets did not show different levels of nest box use across the three observations, and overall nest box use was low. It might have been expected that they would spend more time in their nest boxes in response to the stressor if they felt threatened and viewed the nest box as a safe place (Buchanan-Smith, 2010). The nest boxes in this laboratory however were used to capture the marmosets, thus being closely related to the stressor, so this may have influenced the marmosets perception of them as a safe haven. Similarly Bassett (2003) found no effect of either stressor or training on nest box use, but as this research was carried out in the same laboratory, where the same capture practices were in use, the same confounds apply.

Use of nest boxes in other species has been shown to reduce stress (silver foxes, *Vulpes vulpes*, Pedersen & Jeppeson 1993) and provide a retreat from disturbing stimuli (blue foxes, *Alopex lagopus*, Pedersen & Jeppeson 1993), so it is not unreasonable to suggest that they may have the same effect in primates, providing they are not also linked with the stress of being trapped for capture. That there is no significant change in nest box use across the observations may be due to high individual variability in nest box use, possibly due to conflict between wanting to hide in the nest box and being fearful of entering or remaining inside it. If alternative methods of capture were used, avoiding associating the nest box with aversive events, the time spent in the nest box might be a useful measure of stress and anxiety in marmosets. However in the current study time spent in the nest box did not prove to be a useful indicator of stress and anxiety in marmosets.

Social behaviours

Neither ano-genital presentations nor scent marking showed any differences in the frequency at which they were performed by the marmosets across the three observations. Ano-genital presentations are commonly seen when marmosets are anxious, particularly during aggressive interactions (Epple, 1975, Cillia & Piper, 1997). Marmosets may direct these displays at a threat, so if a human observer is perceived as a threat there may be an increase in this behaviour. A subsequent decrease may then be seen if the marmosets become less anxious in the presence of a human observer. However very low frequencies of this behaviour were recorded and no differences were identified, and therefore ano-genital presentations are not likely to be a useful measure of stress and welfare in marmosets.

Scent marking behaviour is seen at higher levels when marmosets are in new environments and so is considered to be related to territorial behaviour, and is also implicated in social dominance and sexual communication (Epple, 1970, Smith & French, 1997). It would therefore be expected that scent marking would increase following the stressor as not only were the marmosets in a new environment but also in a state of arousal. Indeed it has been found that that this is the case following a similar stressor, but only when sample size was increased through the pooling of data (Bassett, 2003). Further to this, scent marking was not found to be statistically significantly lower in response to a stressor (a taxidermised predator) following the administration of anxiolytic drugs, although it actually ceased to occur (Barros *et al*, 2000), which the authors suggest was due to a small sample size and low levels of the behaviour in the control group. This suggests that this behaviour is not a particularly sensitive measure of stress, but nevertheless may reflect emotionality in marmosets.

Self directed behaviours

Self scratching in primates has been identified as a displacement activity and has been widely reported to increase in response to stressors (Schino *et al*, 1988, Maestripieri *et al*, 1992, Baker & Aureli, 1997). However in this study self scratching was not seen to differ across the observations. Bassett *et al* (2003) reported an increase in self scratching in response to a stressor in untrained marmosets, but no concurrent increase was seen in trained animals, which may suggest that the trained marmosets perceived the stressor as being less stressful than the untrained marmosets did. The authors suggest that self scratching is a sensitive

measure of welfare in primates, and this is borne out by other studies (e.g. Maestripieri *et al*, 1992). Self scratching also proved to be a useful measure of welfare in cynomolgus macaques (Chapter 2). As no differences in self scratching were seen in this study, this may suggest that the stressor was not stressful; however other behaviours recorded indicate that this was not the case. Self scratching showed high levels of variability between individuals, and although it did not reach significance, the median frequency at POST TRAIN/STRESS was less than one-third of that at either behavioural observations prior to training, so it may be that with a larger sample statistical significance may be achieved.

Vocalisations

The frequencies of the two vocalisations recorded did not differ across the observations. Phee vocalisations are considered to be a contact and territorial call (Norcross & Newman, 1993), and tsik calls are a mobbing call emitted in response to a threat (Epple, 1970). Phee calls may have increased following the stressor, and the marmosets' return to their new cage as pairs re-establish their territorial claims in the room, however this was not seen.

Similarly tsik mobbing calls are frequently associated stressful situations, but usually in response to a specific perceived threat (Epple, 1970, e.g. a snake model, Cross & Rogers, 2006). It has been found than tsik vocalisations help to calm marmosets in stressful situations, and cortisol levels are lowered when marmosets are exposed to familiar tsik vocalisations when experiencing stressful situations

(Cross & Rogers, 2006). It would be reasonable to predict an increase in this type of call following the stressor, however this was not seen.

Coo vocalisations were seen to be a good indicator of stress in cynomolgus macaques (Chapter 2), further providing evidence that vocalisations can be indicators of stress and welfare in primates. It was however much easier to identify the individual who made the call in macaques than marmosets due to their larger size, slower movement and also the relative emptiness of their home cage. Collecting reliable data on vocalisations in marmosets has proved to be difficult, and it is likely that they were underreported in this study. Calls were only recorded if a positive identification of the caller was possible, so calls emitted when the marmoset was facing away from the observer were probably missed. There was also relatively high individual variation, which reduces the likelihood of statistical significance.

3.7 SUMMARY

Whilst the focus of many studies on training primates has been chimpanzees and macaques, there is now a small body of literature on the training of New World primates, most notably marmosets. There, however, remains a paucity of data regarding the factors which influence the success of training in these species. Twenty-four laboratory-housed common marmosets (*C. jacchus*) underwent a training programme whereby they were target trained then trained to remain calm in a transport box away from their home cage. Behaviour was recorded to assess the impact of training on the welfare of the marmosets.

The training of laboratory-housed marmosets proved to be very successful, with all marmosets reaching criterion in the training task within 17 sessions, with a mean time investment of just under eight sessions, or 40 minutes. Neither age nor sex influenced the time investment required to reach criterion. The temperament tests provide a useful way of predicting trainability, with both willingness to interact with a novel object and latency to hand feed predicting the time investment required to train an individual. Further to this, the behaviour of the marmosets prior to any training also predicted trainability, with those individuals who exhibited more self scratching (an anxiety-related behaviour) taking longer to train than those who exhibited less self scratching. The behaviour of the marmosets indicated that they experienced anxiety as a result of capture and return to a new cage, but experience of a training programme reduced this, thus helping the marmosets to cope with this stressful procedure.

CHAPTER 4

THE EFFECTS OF TRAINER TRANSFER ON SUCCESS RATES, TIME INVESTMENT AND BEHAVIOUR IN COMMON MARMOSETS (*Callithrix jacchus*)

In laboratories a number of staff is usually involved in the care of the animals, but training requires the animal being trained to develop a relationship of predictability and consistency with the trainer, suggesting that having a single trainer is desirable. However, limited resources may prevent each animal having just one person training them, and staff absences would leave individual animals without a trainer. It may therefore be beneficial if more than one person is involved with training of individual primates. This study was designed to determine whether multiple trainers affected training progress of marmosets, and to determine whether marmoset behaviour was affected when a new trainer started.

Twenty-four common marmosets (*C. jacchus*) were trained to enter a transport box, 12 by a single trainer and the remaining 12 by two trainers. Behaviour was recorded to assess if the transfer of training impacted upon the monkeys' welfare. No differences were seen in the time investment required for a single trainer to train the behaviour, and when two trainers were involved. Behaviour was affected by a change in trainer, with the marmosets showing increased levels of locomotion following a training session with a new trainer. Training can therefore be successfully transferred between trainers. However, it is suggested that as few trainers as possible are responsible for training to maintain consistency.

4.1 INTRODUCTION

In laboratories it is common for a number of staff to be involved in the care of the animals. In some institutions specific members of staff are involved in the routine care of the animals whilst others work specifically on experimental procedures, but in other laboratories some or all staff may undertake both roles. The use of training requires the animal being trained to develop a relationship of predictability and consistency with the trainer, which may be undermined by the trainer also undertaking aversive experimental or husbandry procedures. This may suggest that it is preferable to have a single person responsible for all training; however it is unlikely that sufficient resources are available to dedicate one member of staff purely to training, and this would also mean that periods of holiday or illness would leave the laboratory without a trainer. Further to this, it is likely that the tasks trained for are those which most if not all staff would make use of, for example transport box training, meaning that they would need to have a training relationship with the animals and an understanding of the principles involved in positive reinforcement. It is therefore desirable that most, if not all, staff have some training knowledge and experience (Scott, 1990, Laule, 2010).

If most members of staff are to be involved in the training programme, this raises the issue of how training should be allocated between staff. Primates are capable of distinguishing between people as proficiently as humans (Sands & Wright, 1982), so they will be perfectly aware that different members of staff are interacting with them. The style in which carestaff interact with animals in their care will influence the behaviour of those animals, with animals responding more calmly to interactions with a

gentle person than a more forceful person (Heymann & Holighaus, 1998), and show preferences for people with whom they have had positive experiences (Bloomsmith *et al*, 1997), and this extends to the training interaction where the skill and proficiency of the trainer will also affect how the monkey responds to the trainer (McKinley, 2004).

Any training relationship built with one trainer may not automatically extend to other members of staff, although there is evidence that positive interactions with one member of carestaff creates a reduced level of fear of all humans (Baker & Springer, 2006). It may be that changes in the trainer lead to confusion and uncertainty for the individual being trained, potentially disrupting their progress and affecting the success of the training programme and increasing the time investment required. The quality and consistency of training can be improved though the use of ongoing staff training in this field, and good lines of communication and documented progress but there will still be differences between trainers which the individuals themselves are unaware of (McKinley, 2004). However, where high levels of consistency between trainers is reported to be maintained, there do not appear to be any differences in the speed at which baboons (*Papio hamadryas*) learn a task dependent on which trainer is training them, when training is split between two trainers (O'Brien *et al*, 2008). No comparisons however were made with the rate of learning when only a single trainer works with an individual animal, so it may be that leaning rates would be different in this case.

Where training is discussed in the literature, the number of trainers and how training is allocated has not generally been well reported, despite this having the potential to affect the success of the training. This is particularly true in earlier studies (e.g. Reinhardt *et al*, 1990). However more recently the importance of the relationship with the trainer has

been recognised, with training recommendations and protocols suggesting that only one trainer is involved with establishing a new behaviour, and that if transfer to other trainers is required, this is done only once the desired behaviour is established (Colahan & Breder, 2003, Laule, 2010). In a study with chimpanzees Bloomsmith *et al* (1998) only transferred the maintenance of the trained behaviour of moving into the indoor part of their cage to a member of carestaff once the chimpanzees (*Pan troglodytes*) were performing this behaviour reliably. Similarly Videan *et al* (2005) only transferred the behaviour of presenting a limb for injection in chimpanzees once it was established and available on cue by a single trainer. In other studies, a single trainer has had responsibility for all the training of a particular animal or group, so that the monkeys only interact with one trainer, and the transfer of training is not discussed (Bassett *et al*, 2003, Coleman *et al*, 2005, Schapiro *et al*, 2005, Fernström *et al*, 2009). Interestingly whilst McKinley (2004) reports that three trainers were involved in the training of stumptail macaques (*Macaca arctoides*) to present a limb for venipuncture, and that training sessions were split between these trainers with no overall pattern, a discussion on the differences between the trainers is provided, but how this affects the speed or success of training and the behaviour of the macaques following training is not discussed.

The two studies where the transfer of training is discussed involve training for quite different behaviours: a neutral behaviour of entering another part of the cage (Bloomsmith *et al*, 1998) and an aversive event of having an injection (Videan *et al*, 2005). Interestingly the two studies find quite different results in response to the transfer. The behaviour of entering the indoor enclosure becomes slightly less reliable when training is transferred to a new trainer, whilst remaining above the authors'

threshold for reliability, although this soon returns to pre-transfer levels of reliability. With chimpanzees trained for injection it seems that transferring the training actually improves the reliability of the behaviour, with significantly more trainer-transferred animals continuing to perform the behaviour long-term than those where training was not transferred (Videan *et al*, 2005). Both of these studies however looked only at one species, chimpanzees, in relation to the transfer of training, and details of the trainer competency and the familiarity and relationship with the chimpanzees is not discussed. Other primates may show different responses to a change in trainer, especially those species considered to be more flighty and nervous, such as common marmosets (*Callithrix jacchus*).

Whilst information relating trainer transfer to the success of training is scarce, there is nothing looking at the effect of this on the behaviour of primates, out with training. How changes in the predictability of the trainer, as a new person with a slightly different training style takes over, affects the monkeys has not been researched. It may be that the animals remain unconcerned by the new trainer providing it is a person with whom they are familiar such as a member of carestaff involved with their day-to-day care, or it may take time to build a relationship with a new trainer. Potentially, a new trainer may cause the animal to experience stress, and whilst this may be reflected in changes in success rates and time investment of training, more subtle changes may be picked up by recording their behaviour following training sessions.

4.2 AIMS OF THE STUDY

The aim of this study was to identify any differences between the time investment required to teach a group of animals who were trained by a single trainer to that required by a group trained by two trainers, thus evaluating if the common practice of having multiple trainers was detrimental or beneficial to the success of a training programme. Further to this the behaviour of the marmosets was also recorded to assess if changing trainers affected the way they responded to the training sessions.

4.3 METHODS

4.3.1 Study Animals

This experiment was carried out at in the Laboratory Animal Science Department of GlaxoSmithKline, Stevenage, UK. All animals had been purpose bred in captivity in the UK and had been housed in the department for at least 1 year prior to the start of the experiment. The marmosets used in this study were aged between 2 years 2 months and 6 years 8 months at the start of the study. They were housed in 4 female-female pairs, 3 male-male pairs and 5 male-female pairs, meaning that 13 female and 11 male marmosets were used. Although an earlier study (Chapter 3) indicated that there was no difference between males and females in the speed or ability of learning a training task between males and females, an approximately even split between the sexes was chosen in order to prevent any potential bias due to gender.

Housing and Husbandry

Marmosets were kept in established single sex and mixed sex pairs. Each pair was housed in a metal cage measuring 1.10m by 0.97m by 2.00m. Cages were furnished with branches, perches, ropes and a metal nest box. The cage floor was covered with a layer of sawdust. Some cages had a mesh veranda projecting from the front which the marmosets could enter allowing them greater visual contact with neighbouring marmosets. Rooms were swept and mopped daily. Once a week, on a regular timetable, marmosets were removed from their home cage and placed in a new, clean cage which was placed in the same position in the room. At this time the room was given a thorough clean. Marmosets were given a general health check whilst being moved to their new cage to look for signs of injury and illness, and everyday given a visual health and welfare assessment. Each room housed eight cages, four along each side, with a gap of 2m between. Animals in this study were kept in two separate rooms. Each room backed onto another marmoset room allowing olfactory and auditory contact between the rooms. Visual contact between some individuals was possible when people moved between the two rooms but this was generally discouraged and was therefore infrequent. Marmosets were able to see people as they passed the room via a window in the door. Lights went on at 0700h and were switched off at 1900h, with a 30 minute dusk/dawn period of increasing/decreasing light at each end of the day. Humidity was maintained at 55% and temperature at 24-25ºC.

Food was delivered to the marmosets twice daily. The first feed of the day occurred at around 0900h and on four days a week consisted of dry Mini Marex primate diet pellets, whilst on the remaining three weekdays was a Mini Marex primate diet soaked in banana milkshake until a ‘mash’ consistency. Once a week this was supplemented

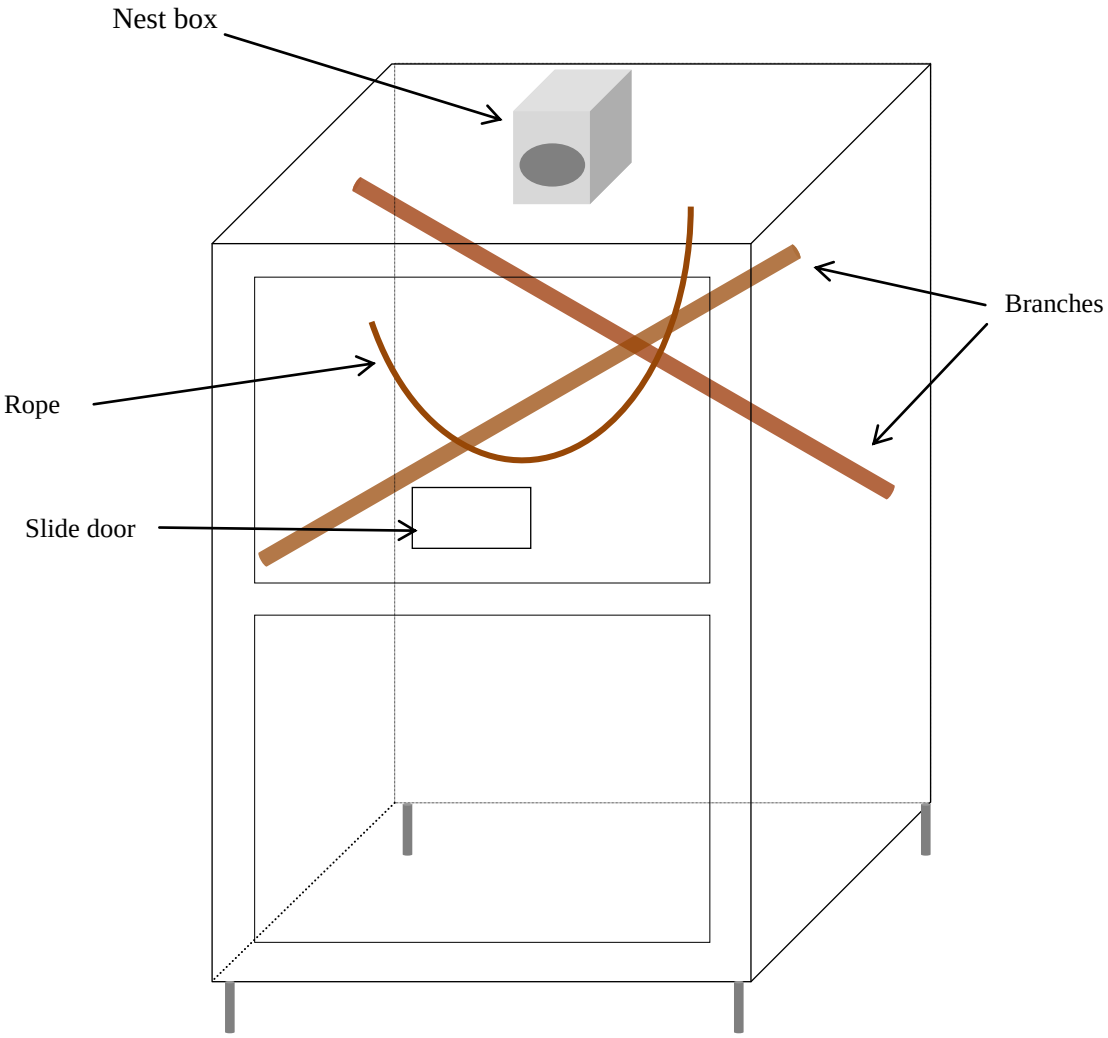

Figure 4.1 Schematic diagram of layout of marmoset cage. Not to scale.

with bread. In the afternoon, at approximately 1500h, the marmosets were offered either baby rusks, a forage mix (Lillico), baby rice or fruit (apple, orange or banana). Both of these were fed in a dish which attached to the front of the cage approximately 1.5m from the floor.

4.3.2 Experimental Design

Twenty-four pair-housed common marmosets (*C. jacchus*) were used in this study. Six pairs were allocated at random to act as a control group and be trained by a single trainer ("control") whilst the remaining six pairs were allocated to the "transfer" group where training would be transferred to a second trainer part way through the training programme. Prior to any training the marmosets were habituated to the unfamiliar Trainer A (myself) for approximately two hours per day for one week. During this time Trainer A sat and walked around the room and talked gently to the marmosets. No specific habituation was carried out for Trainer B as he was a familiar member of the carestaff. Trainer B was an inexperienced trainer who had received practical training from Trainer A in how to use positive reinforcement to box train marmosets. He was also a member of the carestaff in the laboratory and was involved in both routine animal husbandry and some experimental procedures.

At the end of the habituation week Trainer A offered all 24 marmosets a piece of marshmallow to ensure that all were hand feeding prior to the start of training as this has been shown to be an important factor in the time investment required to learn a task (Chapter 3). As this study was looking for differences between training methodologies

rather than between marmosets it was desirable that all marmosets were willing to hand feed prior to the start of training. As it was laboratory practice to regularly hand feed, after habituation all 24 marmosets took the marshmallow within five seconds of being offered it.

Training protocol

Training was carried out in two phases. Phase I was entirely carried out by Trainer A and Phase II which was split between Trainer A and Trainer B. Phase I consisted of training the marmosets to hold a target and Phase II was training them to enter and remain calm in a transport box. All training was positive reinforcement using small pieces of marshmallow (approximately $^{1}/_{8}$ of a small marshmallow), which was paired with a secondary reinforcer (bridge), in this case the click of a retractable pen. All training was carried out between 1100h and 1500h, when the marmosets had had plenty of opportunity to eat and when the laboratory routine meant that disturbances were minimised. A maximum of two marshmallows (16 rewards) were available to each marmoset in each training session, which was also limited to a total of five minutes per marmoset. In all cases the marmoset was considered to be reliable in performing a desired behaviour if it performed it on nine out of ten occasions it was requested. If at any point a marmoset failed to perform at a particular level the trainer went back and repeated the last level.

Training was carried out in two phases as described below.

Phase I – Initially all marmosets were trained to associate a secondary reinforcer (a click from a retracting pen) with a food reward. This was done by pairing the click with the food reward until the marmosets anticipated the food on hearing the click. Once this was established the targets were introduced, two different coloured plastic spoons (white and blue). Each individual marmoset in the pair had a specific colour of spoon which they were to be trained to touch, and this was kept consistent across all training sessions. The marmosets were then rewarded for touching and, through a process of successive approximations, holding their target. Initially the target was always presented at approximately 1.5 m from the floor, on the right for those with a white target, and on the left for those with a blue target. This helped to reduce aggression caused by the presence of a desired food item. If any aggression occurred however a time out was taken where the trainer stood away to the cage with his or her back to it for 30 seconds, before resuming training. Once the marmoset was reliably holding the target, even briefly, the cue word of "hold" was introduced when they were holding the target. Subsequently this was used to prompt the behaviour of holding the target. The marmosets were then requested to hold their target when it was presented in various locations around the front of the cage, and for increasing lengths of time up to 30 seconds.

Phase II – At the start of Phase II a perspex transport box measuring 25cm x 25 cm x 25cm was attached over the small slide door in the cage front. The transport box had a number of small holes drilled into the front (furthest away from the cage) which allowed the marmosets to reach out and hold their target as well as to receive food rewards. The slide doors in the cage and transport box were opened and the marmosets

were allowed to enter the box in order to explore it. After two minutes the marmosets were requested to enter the transport box by placing their target at the front of it and giving them the verbal cue of "hold". Once the marmosets were reliably holding the target in the box for a period of up to 20 seconds, they were requested to hold their target whilst the door between the cage and transport box was closed and opened, then whilst the box was moved and finally totally removed from the cage. Marmosets were considered to have reached criterion when they would return to the transport box within 30 seconds of return to the homecage, and this could be repeated three times. Trainer B was not present whilst the control group was being trained, but Trainer A remained present in the room whilst the transfer group were trained. Trainer A however did not interact with either the marmosets or Trainer B during training sessions.

4.3.3 Data Collection

Data were collected using a handheld Workabout computer using THE OBSERVER V5.0. Data were recorded for five minutes per individual, with cagemates being recorded consecutively. All data were collected between 1100h and 1500h when routine cleaning had finished and at least two hours after feeding, and was the period during which the marmosets experienced the least disturbance. Five behavioural observations were made per marmosets, the first prior to any training as a baseline (B). Behavioural data were then collected following the first training session of Phase I (T1), following the last training session of Phase I (T2), following the first training session of Phase II (T3) and following the last training session of Phase II (T4) (Table 4.1). Behaviour was recorded as for previous studies, using the behavioural categories below (Table 4.2).

Abbreviation	Phase	Training session
B	Pre	N/A
T1	I	First
T2	I	Last
T3	II	First
T4	II	Last

Table 4.1 Summary of abbreviations used for baseline, training phase and training session

Behaviour Class	**Behaviour**	**Recorded as behavioural state (S) or event(E)**	**Description**
locomotion	locomotion	S	Normal relaxed movement; walking running, climbing
	agitated locomotion	S	Quick movement, sudden movements, usually upwards in direction
sitting	sit	S	Still, relaxed, in one location, not actively watching anything
	contact sit	S	Still, relaxed, in contact or within 10cm of cagemate
vigilance	watch	S	Actively either observer or other person/event outside cage
foraging	forage	S	Engaging in searching for or ingesting food
	nest box	S	Out of sight in nest box
social	fight	E	Initiates aggressive physical encounter with cagemate
	threat	E	Physical lunge towards cagemate or observer, or aggressive display
	retreat	E	Move away from fight or threat from cagemate
	ano-genital present	E	Present rear region with tail raised exposing genitals to cagemate or observer
	play	E	Friendly, boisterous interaction between cagemates
	scent mark	E	Rub ano-genital region on part of cage or fittings to leave olfactory deposit
	allogroom	E	Manipulation of fur or body parts of cagemate
vocalisation	tsik	E	Short, sharp, repeated mobbing calls
	phee	E	Long, tonal, whistle, contact call
self directed	drink	E	Intake water from water bottle
	self scratch	E	Rapid, agitated touching or manipulation of single body area
	groom	E	Calm manipulation of own body and fur
	urinate	E	Elimination of urine
	defecate	E	Elimination of faeces
	object manipulate	E	Physical interaction with item in cage

Table 4.2 Description of behaviours collected during recording sessions, adapted from Stevenson & Poole (1976)

4.3.4 Statistical Analysis

Training data

Data were tested for normality using Kolmogorov-Smirnov tests before the decision was made to use parametric statistics. Training data were analysed per pair, as it is likely that the speed of learning by one individual affected that of his or her cagemate. Analysis was also carried out on the fastest marmoset in the pair to learn the task. Time to reach criterion at Phase I, Phase II and in total was compared between the two treatment groups (Control/Transfer) using between subjects t-tests.

Behavioural data

As the behaviour of one marmoset is likely to influence that of his or her cagemate, behavioural data from each pair was pooled and a mean value for each cage for each behaviour was calculated, reducing sample size from 24 to 12. As the data were normally distributed, mixed model ANOVAs were used, with pair (between subject), observation (within subject), group (between subject, control or transfer), as factors in the analysis of the state behaviours. Planned post-hoc Tukey tests were carried out on significant behaviours for pair and observation, and on the comparisons between the control group and transfer group at each observation for the interaction. The behaviours of 'foraging' and 'nest box' were excluded from the analysis as only three occurrences of the former and eight of the latter were recorded, meaning insufficient data were available. Similarly only 'self scratch' and 'scent mark' were analysed for the event behaviours as the frequencies of other behaviours were too low to allow valid analysis. These two behaviours were not normally distributed so were analysed using Wilcoxon

signed rank tests over three observations, comparing the control group and transfer group at T1, T2, T3 and T4, which allowed a good comparison between the two groups but avoided large numbers of comparisons which would have been likely to increase the chances of Type I errors. Significance was set at 0.05, and no corrections were initially applied, however results of subsequent Bonferroni corrections are used to highlight particularly robust results. Results are presented ± the standard error of the mean.

Variable	Between/Within Subjects	Levels
Observation	Within	B T1 T2 T3 T4
Group	Between	Control Transfer

Table 4.3 Summary of analysis

4.4 RESULTS

4.4.1 Success Rates and Time Investment

All 24 marmosets were successfully trained to both hold a target and enter and remain inside a transport box. The time investment required to reach criterion in Phase I ranged from 2 - 5 sessions (mean = 2.6 ± 0.19 sessions), and for Phase II from 2 - 7 sessions (mean = 4.1 ± 0.43 sessions), whilst total time investment ranged from 4 - 11 sessions (mean = 6.7 ± 0.53 sessions). No significant differences were seen between the control

group and transfer group in the time investment required to reach criterion at either Phase I or Phase II or in total for the two phases of training (Figure 4.2, Table 4.4).

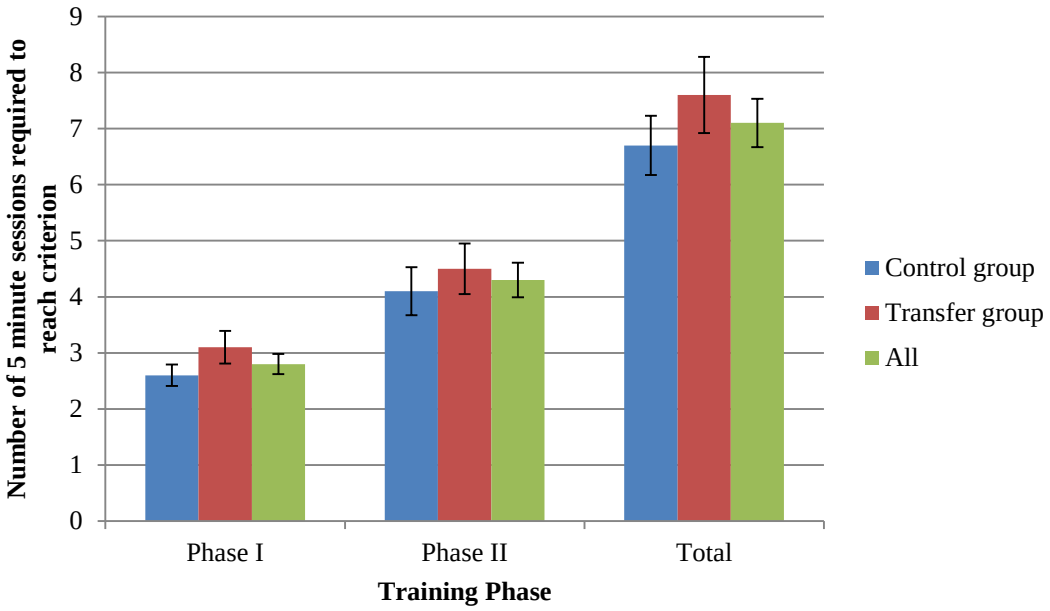

Figure 4.2 Time investment required by the control group, transfer group and all marmosets to reach criterion in Phase I, Phase II and in total. Error bars represent standard error of the mean.

	df	t	p
Phase I	10	-1.44	NS (0.17)
Phase II	10	-0.66	NS (0.51)
Total	10	-1.07	NS (0.30)

Table 4.4 Results of t-tests comparing time investment required by the control group and transfer group to reach criterion at Phase I, Phase II and in total

4.4.2 Behavioural Data

Analysis of the data for all marmosets shows that the behaviour of the marmosets changes across the five observations. The amount of time the marmosets spent engaged in locomotion, sitting and contact sitting varied significantly, however the time they spent in agitated locomotion and watchful behaviour did not differ with observation (Table 4.5).

Behaviour	analysis	df	F	p
locomotion	observation	4,50	18.47	<0.001
	group	1,50	4.51	< 0.05 (0.039)
	observation*group	4,50	3.52	< 0.05 (0.013)
agitated	observation	4,50	0.21	NS (0.093)
locomotion	group	1,50	1.26	NS (0.27)
	observation*group	4,50	0.65	NS (0.63)
sit	observation	4,50	26.26	<0.001
	group	1,50	1.94	NS (0.17)
	observation*group	4,50	1.20	NS (0.32)
contact sit	observation	4,50	22.86	<0.001
	group	1,50	0.17	NS (0.68)
	observation*group	4,50	1.60	NS (0.19)
watch	observation	4,50	0.05	NS (0.99)
	group	1,50	4.86	< 0.05 (0.032)
	observation*group	4,50	1.14	NS (0.35)

Table 4.5 Results of ANOVA on durations of recorded behaviours across the five observations, two treatment groups and interaction between observation and group

The marmosets spent significantly more time engaged in locomotion following the first training session of Phase I (T1) and the first training session of Phase II (T3) than

baseline (B), significantly more time at T1 than at the last training session of Phase I (T2) and the last training session of Phase II (T4), and more time at T3 than at T4 (Figure 4.3, Table 4.6). Following Bonferroni correction for multiple comparisons whereby significance was set at 0.01, these results all reached significance.

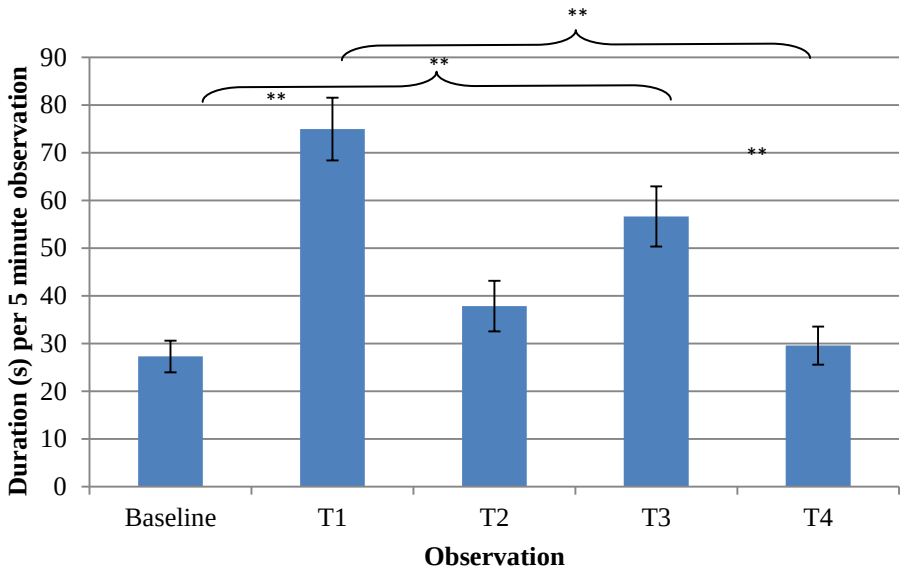

Figure 4.3 Mean duration (in seconds) of locomotion across observations (* $p < 0.05$, ** $p < 0.01$) **Error bars represent standard error of the mean.**

Comparison	T	P
B v T1	7.17	< 0.001
B v T2	1.59	NS (0.51)
B v T3	4.41	< 0.001
B v T4	0.34	NS (0.99)
T1 v T2	-5.57	< 0.001
T1 v T3	-2.75	NS (0.06)
T1 v T4	-6.82	< 0.001
T2 v T3	2.81	NS (0.05)
T2 v T4	-1.25	NS (0.72)
T3 v T4	-4.07	< 0.01 (0.002)

Table 4.6 Results of post-hoc Tukey tests for locomotion across the five observations

There was also an effect of group on the amount of locomotion the marmosets exhibited, with marmosets in the transfer group, trained by both Trainer A and Trainer B, displaying more of this behaviour than those in the control group, trainer entirely by Trainer A (Figure 4.4), although this did not stand up to the more stringent p value of 0.01 following a Bonferroni correction.

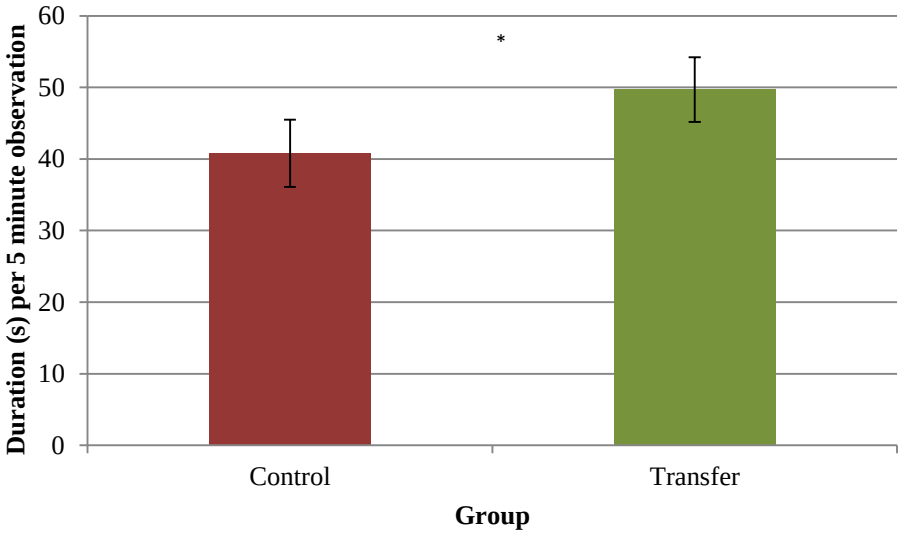

Figure 4.4 Mean duration (in seconds) of locomotion by treatment group (Control/Transfer) across all observations (* p < 0.05, ** p < 0.01). **Error bars represent standard error of the mean.**

Marmosets in the two treatment groups exhibited significantly different amounts of locomotion at T3, but not at any other of the other observations (Figure 4.5, Table 4.7), though following a Bonferroni correction this did not reach significance.

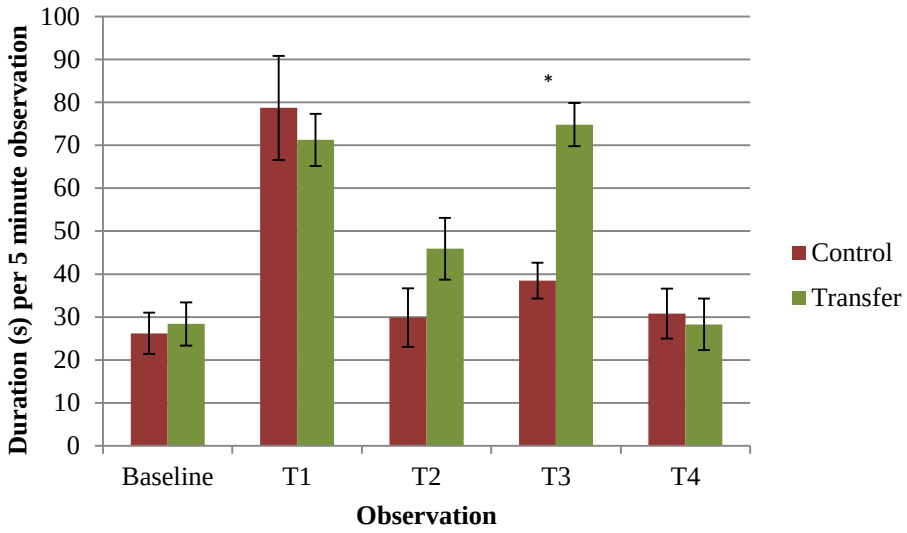

Figure 4.5 Mean durations (in seconds) of locomotion by treatment group (Control/Transfer) and observation (* $p < 0.05$, ** $p < 0.01$). **Error bars represent standard error of the mean.**

Observation	T	p
Baseline	0.24	NS (1.0)
T1	-0.79	NS (1.0)
T2	1.70	NS (0.79)
T3	3.86	0.01
T4	-0.27	NS (1.0)

Table 4.7 Results of post-hoc Tukey tests, comparisons between Control and Transfer group at each observation

The amount of time the marmosets spent sitting alone was significantly greater at Baseline than following any of the training interactions, but time spent sitting did not differ between training sessions (Figure 4.6, Table 4.8).

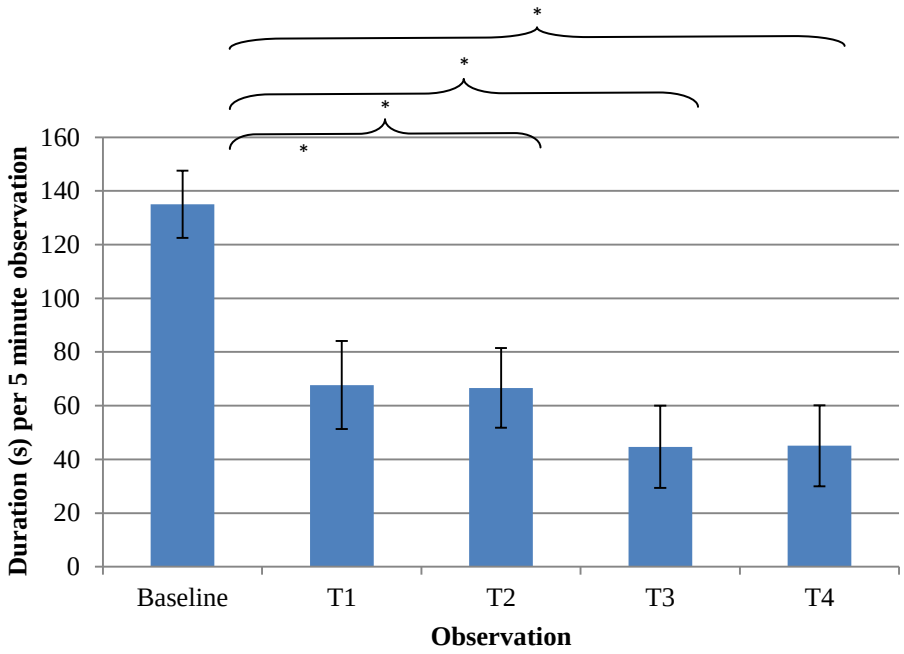

Figure 4.6 Mean duration (in seconds) of sitting across observations (* $p < 0.05$, ** $p < 0.01$**). Error bars represent standard error of the mean.**

Comparison	T	p
B v T1	-6.59	< 0.001
B v T2	-6.69	< 0.001
B v T3	-8.84	< 0.001
B v T4	-8.80	< 0.001
T1 v T2	-0.11	NS (1.00)
T1 v T3	-2.25	NS (0.18)
T1 v T4	-2.21	NS (0.19)
T2 v T3	-2.15	NS (0.22)
T2 v T4	-2.11	NS (0.23)
T3 v T4	0.04	NS (1.00)

Table 4.8 Results of post-hoc Tukey tests for sitting across the five observations

The marmosets spent significantly more time contact sitting at T2, T3 and T4 than at Baseline, more time at T2, T3 and T4 than at T1, and more time at T4 than at T2 (Figure 4.7, Table 4.9).

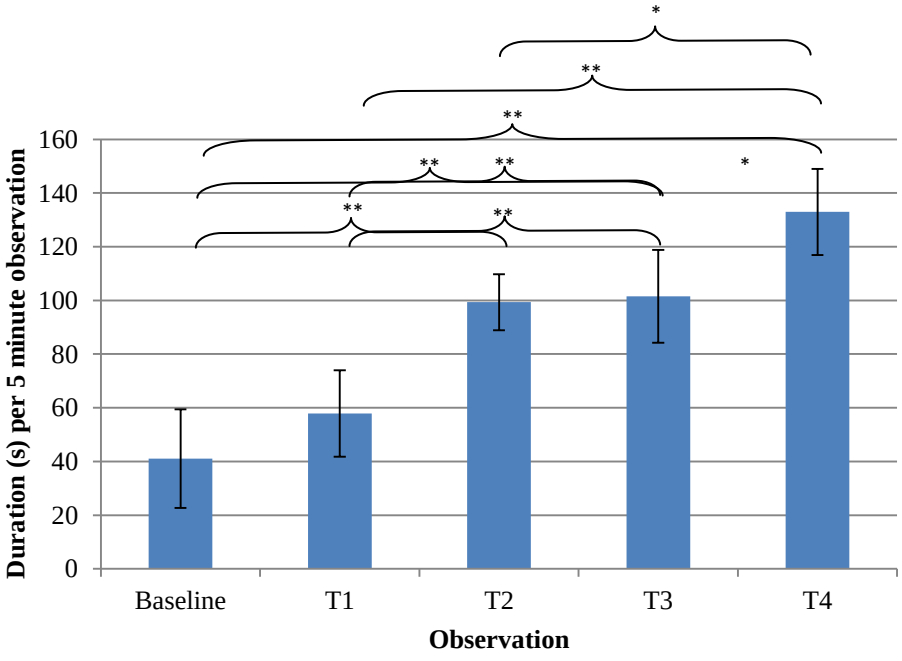

Figure 4.7 Mean duration (in seconds) of contact sitting across observations (* p < 0.05, ** p < 0.01). **Error bars represent standard error of the mean.**

Comparison	T	p
B v T1	1.54	NS (0.54)
B v T2	5.35	< 0.001
B v T3	5.55	< 0.001
B v T4	8.43	< 0.001
T1 v T2	3.80	< 0.01 (0.003)
T1 v T3	4.01	< 0.01 (0.002)
T1 v T4	6.89	< 0.001
T2 v T3	0.20	NS (1.00)
T2 v T4	3.09	< 0.05 (0.026)
T3 v T4	2.89	< 0.05 (0.044)

Table 4.9 Results of post-hoc Tukey tests for contact sitting across the five observations

Whilst watchful behaviour did not vary with observation, there was a significant difference between the two treatment groups in the amount of time they spent engaged in this behaviour, with the marmosets in the control group exhibiting more of this behaviour than those in the transfer group (Figure 4.8).

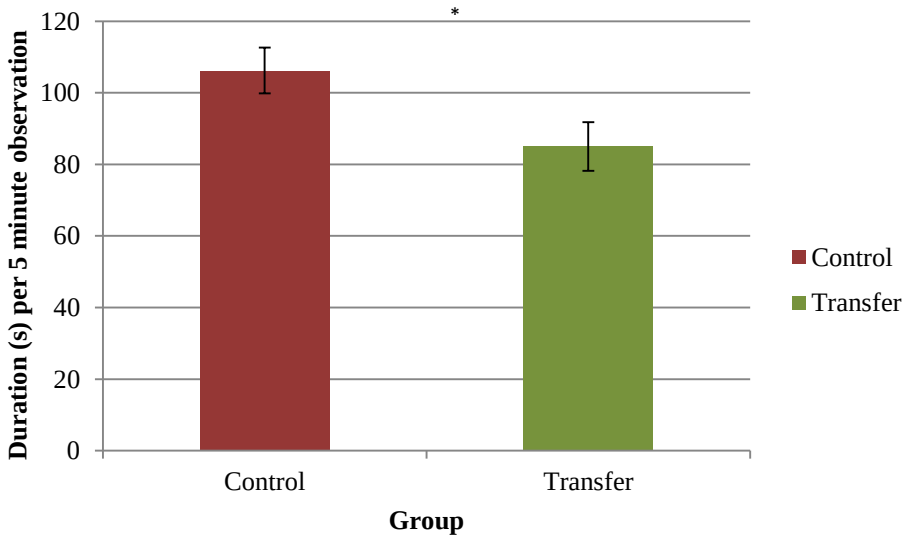

Figure 4.8 Mean duration (in seconds) of watchful behaviour by treatment group (Control/Transfer) across all observations (* p < 0.05, ** p < 0.01**). Error bars represent standard error of the mean.**

Comparisons of the amount of self scratching and scent marking exhibited by the marmosets in the control and transfer group at each of the observations reveal no significant differences (Table 4.10).

Behaviour	Comparison	n	W	p
self scratch	B	8	1.96	NS (0.11)
	T1	8	1.77	NS (0.14)
	T2	9	-1.58	NS (0.18)
	T3	5	0.09	NS (0.93)
	T4	8	-0.17	NS (0.87)
scent mark	B	6	0.92	NS (0.40)
	T1	7	0.96	NS (0.38)
	T2	6	0.40	NS (0.70)
	T3	6	0.90	NS (0.41)
	T4	8	0.03	NS (1.00)

Table 4.10 Results of Wilcoxon signed rank test for self scratch and scent mark, comparison between control and transfer group at each observation

4.5 DISCUSSION

4.5.1 Success Rates and Time Investment

All of the marmosets were successfully trained both to hold a target and to enter and remain in a transport box. The number of sessions required (6.7 ± 0.53) is broadly in agreement with those found in another colony, where the mean number of sessions required to accomplish the same task was 7.9 sessions (Chapter 3). There were no differences in the speed in which the marmosets learnt the task and their treatment group; the marmosets who had their training transferred to a second trainer did not take any longer than those who were trained by a single trainer throughout. Where trainer

transfer is reported in the literature it appears that it does not have a long-term effect on the learning of the task; chimpanzees respond less initially to a new trainer but this was quickly overcome (Bloomsmith *et al*, 1998). Further to this, chimpanzees who have been trained for injection and then were transferred to a second trainer actually perform better in the long-term than those who do not have their training transferred, a finding which leads the authors suggest that training can only really be considered successful once it is transferred (Videan *et al*, 2005). That no differences were found in this study suggests that the marmosets had little difficulty in building a new training relationship with a second trainer, and one with whom they had an ambiguous relationship in that he was involved in both positive interactions (such as feeding) and more aversive events such as capture and experimental work. Certainly in other laboratory-housed primates, the willingness to take food from one person increases the likelihood of taking food from a new person (Baker & Springer, 2006). This suggests that all staff can be involved in the training of marmosets, especially in the early socialisation phases, without compromising the training programme. It may be that a more disrupted training programme, where, for example Trainers A and B alternated training sessions may have lead to differences in time investment. However it seems that transferring training once between trainers does not affect the success or time investment of a training programme. Once again, it is emphasised that good lines of communication are critical and that Trainer A taught trainer B may have assisted with consistency and hence the success of transfer.

4.5.2 Behavioural Data

Two recorded behaviours, locomotion and watchful behaviour, changed significantly across the five observations, dependent upon which treatment group they belonged to. Locomotion proved to have the most complex relationship with the training programme, with levels increasing in response to the first training session (T1) before returning to baseline levels by T2, and then increasing again following T3 before returning again to lower levels at T4. Locomotion is commonly used as an indicator of anxiety in primates, and has been shown to increase in response to a number of stressors (Laudenslager *et al*, 1990, Levine, 1993, Hennessy *et al*, 1995, Bassett *et al*, 2003), which suggests that the marmosets found the first training sessions of both Phase I and Phase II arousing. However agitated locomotion, which has proved to be a more sensitive indicator of stress in both macaques (Chapter 2) and marmosets (Chapter 3), was not affected by the training interactions. If the marmosets found the training interaction stressful it would be expected that significant increases in agitated locomotion would have been recorded, which did not occur. That all the marmosets showed an increase in locomotion following the first training session therefore indicates that training interactions may be more arousing, and indicative of uncertainty rather than stress.

Treatment group also proved significant for locomotion, as did the interaction between observation and group which showed that at all observations except T3, the first observation of Phase II, both groups exhibited similar amounts of locomotion. That the transfer group showed increased levels of locomotion following this first session with a new trainer when the control group, with their familiar trainer, did not, suggests that the

marmosets found the interaction with a new trainer arousing, perhaps as noted above due to the uncertainty of events. It is likely that the reduction in performance of a trained behaviour when a new trainer is working with the animals, as reported by Bloomsmith *et al* (1998) may be due to this uncertainty. That no effects are seen in time investment and that levels of locomotion are not different by the end of Phase II (one to six sessions later), suggests that this uncertainty soon dissipates.

When data from both control an transfer groups was pooled, sitting alone was higher at baseline than following any of the training sessions, which may suggest that the training sessions are stressful for the marmosets, with reduced levels of sitting having been observed following a stressor (Bassett *et al*, 2003). It is however necessary to combine the two sitting behaviours to understand the full picture. Contact sitting increases across the observations which may be an indicator of training improving the social behaviour of the marmosets, or may be a further indicator of the training interaction leading causing stress to the marmosets resulting in them seeking comfort with their cage-mate. However, as affiliative behaviour is generally considered to be positive in terms of welfare, it is likely that the increase in this behaviour indicates an improvement in welfare.

Watchful behaviour has been shown to increase in primates in response to stressors (Chapter 2) but also in response to training, possibly as the animals show an increased interest in their trainer in the expectation of food (Bassett, 2003). The marmosets in the control group did however exhibit more vigilance behaviour than those in the transfer group, possibly due to them having a more predictable indicator of a training interaction in Trainer A than those in the transfer group. As Trainer A also carried out the

behavioural observations it may be that the marmosets in the transfer group spent less time watching during observations as, following the transfer of training, Trainer A was considered a less reliable source of food by this group.

Self scratching has been shown to increase in response to stress in primates (Schino *et al*, 1988, Maestripieri *et al*, 1992, Baker & Aureli, 1997, Bassett *et al*, 2003), so that this behaviour did not increase following any of the training sessions suggests that the marmosets did not find the training interaction stressful. However self scratching also did not change in response to capture by any method in this thesis, which may suggest that either capture is not stressful, a finding not in accordance with the other behavioural results reported, or more likely that rates of this behaviour were low and inter-animal variation high, leading to a lack of statistical significance.

Marmosets appear to show an arousal or uncertainty response to the first training session. It may be something about learning which elicits this response, but more likely it is the close interaction with humans which is responsible. Even if marmosets are willing to hand feed, they may retreat to the back of the cage to eat their reward, and interactions rarely last as long as a training session. The prolonged and more intense interaction required by training seems to elicit this response, but it is quickly reduced and the marmosets' behaviour returns to more normal levels. However when a new trainer takes over the marmosets once again exhibit this uncertainty response. This again quickly diminishes, but nevertheless suggests that care needs to be taken when introducing a new trainer, and possibly that the number of trainers working with any individual or group should be minimised. There is little however to suggest that the current recommendations of only transferring training once a behaviour is established

should be adhered to, as transferring training part way through box training did not increase the time investment required. The behaviour being trained in this case however was a neutral one, and the findings here may not extrapolate to more complex or aversive behaviours.

4.6 SUMMARY

In laboratories it is common for a number of staff to be involved in the care of the animals. Successful training requires the animal being trained to develop a relationship of predictability and consistency with the trainer, which may suggest that it is preferable to have a single person responsible for all training. However, it is unlikely that sufficient resources are available to dedicate one member of staff purely to training, and this would also mean that periods of holiday or illness would leave the laboratory without a trainer. Further to this, it is likely that the tasks trained for are those which most if not all staff would make use of, for example transport box training, meaning that they would need to have a training relationship with the animals and an understanding of the principles involved in positive reinforcement.

Marmosets trained by two trainers do not show a reduced success rate or an increase time investment when compared to those trained by a single trainer. However the first training session with a new trainer was arousing to the marmosets as shown by increased levels of locomotion. It is therefore recommended that training is limited to as few trainers as possible, whose consistency and lines of communication are good, but that training can be transferred at any stage of training.

CHAPTER 5

TRAINING COMMON MARMOSETS (*Callithrix jacchus*) TO COOPERATE WITH CAPTURE: EFFECTS ON CORTISOL RESPONSE AND BEHAVIOUR

Whilst there is an increasing amount of research on training New World primates, in particular marmosets, most of this has been for tasks which are likely to be perceived as enjoyable or neutral by the animals. There is no information regarding the success of training marmosets to cooperate with aversive procedures. Training was carried out with seven common marmosets to attempt to train them to come to a specially designed panel and remain there whilst they were captured by hand. The cage mates of these marmosets had no specific training but did receive intensive socialisation, primarily in the form of hand feeding. The behaviour and cortisol response to training for standard hand-capture was measured, as well as responses to trained hand-capture to assess if the different methods of capture, and training or socialisation affected the welfare of the monkeys.

The time investment to train marmosets for this task was relatively high in comparison to that for non-aversive procedures, and the success rate was also lower at only 57%. However, that it was possible to train for this task is an interesting result. Saliva collection and analysis for cortisol assay was successful, with over 77% of samples resulting in a recordable cortisol concentration. Although salivary cortisol provided useful data on the responses of the marmosets to capture, the behavioural data collected seemed to provide a fuller picture of the animals' responses, and welfare interpretation should incorporate both. As expected, any method of capture was stressful for the marmosets (as indicated in particular by the behavioural data), but trained hand-capture did not seem to be less stressful than standard capture as indicated by both behaviour

and cortisol response. Sample size however was very low. Both training and positive interactions significantly reduced the stress responses of the marmosets following capture, seen in both the behavioural and cortisol responses, and it is therefore recommended that these types of interactions with animals should be routine for laboratory staff.

5.1 INTRODUCTION

Changes in physiology have been used across a range of species to assess the stress they experience in variety of situations. The most commonly used measure of stress physiology is the glucocorticoids (GCs); cortisol in humans, other primates, and ungulates, corticosterone in rodents and a mixture of both, for example, in pigs (Lane, 2006). Other measures such as heart rate and blood pressure (e.g. Shively & Kaplan, 1984, Line *et al*, 1989a,b), hair loss (Honess *et al*, 2005a) and leukocyte activity (e.g. Honess *et al*, 2005b) have been used to measure stress in primates, but GCs are widely considered as "the stress hormone" and most commonly used owing to this, and to its relatively simple and well elucidated release mechanism and well validated assay techniques. GCs can be found and measured in blood, faeces, urine, hair, milk and saliva, with each having its advantages and disadvantages. However the use of cortisol is not without its difficulties and limitations, of which the primary ones are discussed below. Variations occur across day and season, can be affected by how the sample is collected, the history of and the day to day life of the individual it is collected from as well as the stressor the experimenter is trying to quantify (Reinhardt, 1990, 2003, Cross *et al*, 2004, de Kloet *et al*, 2005). It is therefore important to understand the effects of these factors to interpret the data fully and properly.

5.1.1 Cortisol Physiology

Cortisol (or its analogous hormone corticosterone) is released as a result of stimulation of the hypothalamic-pituitary-adrenal (HPA) axis, whereby the hypothalamus in the brain releases corticotrophin releasing hormone (CRH) which acts on the pituitary gland leading to the release of adrenocorticotropic hormone (AHCH). This then acts on the adrenal cortex causing the production of the glucocorticoid cortisol/corticosterone, which then travels around the body. GCs are important for normal function in animals, being involved in the production of glucose, immune responses and anti-inflammatory reactions (Munck *et al*, 1984). However when levels are elevated, particularly over extended periods, cortisol can lead to pathological conditions (Weiss, 1970, Sapolsky, 1990). Most of the cortisol produced (about 90%) is bound to proteins which prevent it crossing membranes, whilst the remaining free fraction is biologically active and can cross biological membranes (Breuner & Orchinik, 2002).

Cortisol has been used to assess chronic stress in a range of species; and whilst it has proved useful in some situations, in others cortisol appears to return to baseline levels on exposure to long term stressful environments, a phenomenon known as allostasis, where the HPA axis appears to be reset (Selye, 1950). In allostatis the HPA axis continues to be activated but levels of circulating GCs are reduced. Social stress has been used as a model for chronic stress in primates (Levine, 1993), and squirrel monkeys (*Saimiri sciureus*) who were stressed by having to increase their foraging effort had elevated blood plasma cortisol levels for the full 10 weeks of the study (Champoux *et al,* 1993), suggesting that this was not long enough to elicit allostasis.

In humans cortisol levels are found to be elevated in individuals suffering from depression (Tse & Bond, 2004, van Praag, 2004), post-traumatic stress disorder (Sher *et al*, 2004) and in people who are suicidal (Westrin *et al*, 1999) suggesting that cortisol and the perception of stress may be linked. Similarly in animals who are in a state of learned helplessness, considered to correlate to depression in humans, increased GC levels are found (pigs, *Sus scrofa*, Gregory, 2004). Depression has been showed to lead to a decreased sensitivity to reward (anhedonia, Pryce *et al*, 2005), and this could be relevant to training paradigms, as those animals in severely "depressed" states may not learn well due to their inability to appreciate the positive feedback of the reward.

More pertinently to this study increased cortisol levels are linked to acute stressful events. Studies with rhesus macaques (*M. mulatta*) saw raised blood plasma cortisol levels in individuals following an electric shock stressor (Feldmann & Brown, 1976), and capture and venipuncture (Herndon *et al*, 1984) whilst short-term separation from its mother was linked with infant rhesus macaques having elevated blood plasma cortisol (Norcross & Newman, 1999). Furthermore research has suggested that elevated levels of cortisol were particularly linked to psychological stressors, for example a noise previously associated with electric shock lead to elevated plasma cortisol levels in rhesus macaques (Mason & Brady, 1956). Common marmosets (*C. jacchus*), when subjected to social isolation and disturbance, showed elevated salivary cortisol levels (Cross *et al*, 2004) and urinary cortisol is raised in response to both social isolation and restraint (Jones *et al*, 2004). Stressors such as loud music and the removal of a cage or room-mate also lead to increases in salivary cortisol in common marmosets (Pines *et al*, 2004). Similarly Wied's black tufted-ear marmosets (*C. kuhli*) exhibited raised urinary cortisol levels following isolation in a small cage and handling (Smith & French, 1997).

The evidence therefore indicates that cortisol can be a useful tool for assessing whether an individual finds an experience stressful.

5.1.2 Measuring Cortisol

Blood serum has historically been the most commonly used method of measuring cortisol, being relatively easily obtained from restrained, and more recently unrestrained, individuals. Early studies which looked at the relationship between cortisol and stress measured the levels in blood but the method of collecting the blood sample in itself can be stressful involving restraint and removal from the group, and this in turn leads to the release of cortisol, possibly masking the effect of the stressor which is being assessed (Reinhardt, 1991, 1999). Catheterisation with the use of tethers has been used in some species allowing blood samples to be collected without restraint and close human contact (e.g. Norman, 1994) and whilst this may reduce the stress experienced by the animal in the course of taking the blood sample, it still requires the animal to be housed individually and probably in a smaller than normal cage or pen with the associated stress this may cause. More recently blood has been collected from trained individuals who freely cooperate with this procedure, therefore reducing the stress of collection and leading to a more accurate measurement of cortisol in relation to a hypothesised stressor (Reinhardt, 1991, 1999). Blood however allows only the measurement of the total circulating cortisol rather than the free fraction, which is the level of cortisol which is relevant to the stress response. The free fraction of cortisol is that which is not bound to corticoid binding globulin (CBG) and it is therefore the biologically active fraction (Vinning *et al*, 1983). This free fraction can cross the

membranes to enter the saliva, whereas the bound portion cannot, meaning that only the free fraction is measured when measuring cortisol in saliva (Riad-Fahmy *et al*, 1983).

As a result of these welfare and measurement problems with blood collection other non-invasive ways of collecting samples which can be assayed for cortisol have been investigated. Faecal cortisol has been measured in some species and has value in that it causes no direct stress to the animal as a result of the collection, although animals may have to be removed from their home cage in order to collect the faeces which may be stressful. It offers a good measure for the evaluation of chronic stress but responses to acute stressors are not easily identified due to the length of time the faeces spends in the body and therefore represents a relatively long period of the day (Lane, 2006). If animals are group housed identifying which faecal sample belongs to which individual can be problematic, and although feeding different coloured non-harmful dyes can help in this, there is also the risk of cross contamination of samples. Cortisol measured from urine suffers from some of the same drawbacks as faeces in that it provides a measure of cortisol which will show some evidence of acute stressors, but may miss some of the more subtle changes, and can prove difficult to collect. Urine is however the predominant excretory route for cortisol in primates (Bahr *et al*, 2000) so may therefore prove preferable to faecal collection where possible. Urine has traditionally been collected by placing the individual in isolation in a metabolism cage (e.g. Hearn & Lunn, 1975, Lunn, 1989), which in itself is likely to be a stressful experience leading to increased cortisol values. More recently methods of collecting urine have been developed which allow the individual to remain in his or her home cage. Training animals to enter a specific compartment immediately upon waking has been used, and has provided reliable and useful individual results (Anzenberger & Gossweiler, 1993).

This method of urine collection however only allows collection of samples first thing in the morning, making the assessment of acute stressors difficult due to the time lag from stressor to the elimination of urine. It is possible to train some animals to provide a urine sample (Smith *et al*, 2004), and even to do so at a specific location on request (Bassett *et al*, 2003), which may begin to overcome some of these issues. However the capture of the behaviour of urination was through rewarding the marmosets for scent marking when they deposit a few drops of urine. The increase in scent marking may confound results or potentially cause irritation to the individual. Increases in scent marking are associated with the stress response (Bassett *et al*, 2003) and therefore it may not be desirable to encourage this behaviour, however no increase in scent marking was seen outside of training sessions (McKinley, 2004). The measurement of urinary cortisol may be affected by the dilution of the urine, so measurements are usually made per µg/mg Creatinine (Cr), a waste product of protein metabolism secreted by the kidneys. This controls for the dilution of the urine sample. Despite this, there is a correlation between urinary cortisol and that of blood plasma (humans, Lindholm & Schultzm, 1973)

Milk in species such as cows and goats can be easily collected during the normal milking process (e.g. Gygax *et al*, 2006) but will only allow cortisol assay of female animals who are lactating, and only those who are tolerant of being milked, so is of limited use. Hair can be collected easily and with minimal stress to the individual by shaving a small patch, either when awake or during routine anaesthesia for health checks. Any restraint, whilst not desirable from a welfare perspective, will not impact upon the cortisol levels as these are not immediately deposited in hair. Rather, hair

provides a useful measure of chronic stress over very long periods of time, and does not show the effect of an acute stressor (Davenport *et al*, 2006).

Saliva sampling has recently been providing a useful method of providing a fluid which can be sampled for cortisol. Samples can be collected non-invasively from an individual and this is relatively stress free providing that the animal is used to close human interaction as it requires no restraint or manipulation of the individual other than chewing a cotton bud. Unlike the collection of blood or urine, collection of saliva requires minimal training making it easier to collect from larger numbers of animals and reducing the time investment of doing so. Saliva samples have been collected from a range of species including pigs (Hillmann *et al*, 2004), dogs (*Canis familiaris*, Rooney *et al*, 2007b), dolphins (*Tursiops truncates*, Pedernera-Romano *et al*, 2006) and a number of primates (rhesus macaques, Lutz *et al*, 2000, squirrel monkeys, Tiefenbacher *et al*, 2003, common marmosets, Cross & Rogers, 2004, Cross *et al*, 2004). Salivary samples are sensitive enough to establish diurnal rhythms (Cross & Rogers, 2004) and collection has proved reliable (Cross & Rogers, 2004, Cross *et al*, 2004, Pines *et al*, 2004). As only the free fraction of the cortisol can cross the membranes, the cortisol measurement from saliva is measuring the biologically relevant levels (Hubert & de Jong-Meyer, 1989). These factors suggest that saliva is the best medium for the collection of a sample for cortisol assay, particularly when looking at acute stressors.

5.1.3 Cortisol as a Measure of Welfare

Although cortisol has been used as a measure of stress in mammals, there are factors which can confound the results of such assessments, and therefore care has to be taken

to avoid or reduce the impact of these factors. Firstly cortisol secretion follows a diurnal pattern, with levels in most mammals decreasing from a peak in the early morning to a low around midnight (e.g. Fulkerson & Tang, 1979). This means that samples taken on different days should be taken at similar times so as to ensure that they are comparable.

There is considerable variation in sex differences between males and females across species and study. In deer (*Cervus elaphus*) (Huber *et al*, 2003) and bonobos (*Pan paniscus*) (Dittami *et al*, 2008) studies have shown no difference in basal levels between males and females. In rhesus macaques (Lado-Abeal *et al*, 2005), Wied's marmoset and common marmosets baseline plasma cortisol levels for females have been shown to be significantly higher than in males (Johnson *et al*, 1996, Cunha *et al*, 2007), although there were no reported differences between males and females when salivary cortisol was measured in common marmosets (Cross & Rogers, 2004), and no differences were seen between males and females in plasma cortisol in black tufted-ear marmosets (*C. penicillata*) (Boere *et al*, 2005).

Further to this there is evidence to suggest that cortisol response differs depending on sex. Most work has been carried out with humans (for review see Kudielka *et al*, 2009) although similar trends have been shown in other primates (Lado-Abeal *et al*, 2005). In humans the response differences between males and females depend on how the person perceives the stressor (Kudielka *et al*, 2009) and it is suggested that this may be extended to non-human animals. Male and female Wied's marmosets respond with differing magnitudes to competitive social stress, with males showing a greater cortisol response than females. In humans males show an increased stress response to tasks such as mental arithmetic, those considered as 'achievement tasks' (Kudielka *et al*, 2009)

and although there has not been any comparable study in non-human primates it is perhaps worth considering this might be a factor in learning and training paradigms.

A number of studies have looked at the relationship between social status and cortisol levels and within one group of species, in this case primates, there is no overall pattern in how cortisol and dominance interact. Differences are seen between species and even between sexes within the same species, and this is likely to be related to social structure. Dominant and subordinate female squirrel monkeys show similar levels of cortisol (Saltzman *et al*, 1991), as do male rhesus monkeys (Bercovitch & Clarke, 1995). Subordinate female cynomologous macaques (*M. fascicularis*) (Shively *et al*, 1997) and male squirrel monkeys (Coe *et al*, 1979) have higher cortisol levels that dominant individuals, whilst in cotton top tamarins (*Saguinus oedipus*) (Ziegler *et al*, 1995, Ginther *et al*, 2001) and common marmosets (Abbott *et al*, 1997, Cross & Rogers, 2004, Saltzman *et al*, 2004) cortisol levels are lower in subordinates than dominant animals.

There is also evidence to suggest that early life stress can lead to alterations in the physiology of the HPA axis which leads to a decreased response to a stressor in later life. Mother-raised rhesus macaques had higher cortisol responses to stressors such as a new cage than peer-raised individuals (Clarke, 1993). Common marmosets subjected to early repeated removal from the family group exhibited a reduced cortisol stress response when placed in social isolation in a novel environment compared to normally raised animals (Dettling *et al*, 2002). This emphasises the important of knowing the rearing history of subjects; in marmosets hand rearing is often performed for individuals

from large litters (triplets or quads) and macaques may be peer-raised and weaned early. This may have long-term consequences on the magnitude of the stress response.

Eating however can lead to changes in cortisol secretion, with levels decreasing more sharply after the provision of food in animals as varied as sheep (*Ovis aries*, Simonetta *et al*, 1991) and common marmosets (Cross & Rogers, 2004) when they were fed once daily. In sheep however this was not seen when they were fed on several occasions during the day, suggesting that the cortisol drop could be as a result glucose production, since glucose can affect cortisol levels (Simonetta *et al*, 1991).

Other factors can affect cortisol secretion, for example painful stimuli have been shown to lead to an extended period of increased cortisol (e.g. in sheep, Mears & Brown, 1997). Large parasite burdens can also cause increased cortisol levels (chimpanzees, *P. troglodytes*, Muehlenbein, 2006). Exercise can affect cortisol levels, and this is unsurprising given that it is involved with the synthesis of glucose required for energy. However it seems that only high levels of exercise affect cortisol levels and that gentle or moderate exercise to not have this effect in humans (McCarthy *et al*, 1992, Stupnicki & Obminski, 1992, Duclos *et al*, 1997) so whilst it is important to measure activity, unless this is great it is unlikely to unduly influence cortisol levels or mask changes caused by stressful experiences. These studies however have been carried out in humans and there is a paucity of information pertaining to this in other animals. It is however reasonable to suggest that a stressor may result in increased activity, which may contribute to increased cortisol levels, so behaviour needs to be considered alongside physiological recordings.

5.1.4 Cortisol in Primates

Cortisol levels have been assayed in many primates, in apes, Old World and New World monkeys and levels have been found to vary greatly amongst these groups and also between studies. Chimpanzees in the wild have had urinary cortisol measured in the range of approximately ten times higher than laboratory-housed individuals (wild, Anestis & Bribiescas, 2004, laboratory, Muller & Lipson, 2003). Faecal cortisol in chimpanzees has been reported in the range of 1.82 - 17.77 ng/g (Muehlenbein, 2006) and 1.7 ± 0.3 ng/g (Whitten *et al*, 1998) whilst gorillas (*Gorilla gorilla*) had faecal cortisol concentrations in the range 0.15–21.66 mg/g (Peel *et al*, 2005). In rhesus macaques blood plasma cortisol has been reported in the region of 33 – 46 µg/dl (Bercovitch & Clarke, 1995). Faecal concentrations of cortisol metabolites have been reported to be 420.1 ± 189.6 ng/g dry matter with a range of 84.5–902.4, and salivary cortisol ranging from 0.27 to 1.66mg/dl with a mean of 0.73 mg/dl ± 0.15 (Lutz *et al*, 2000). Hair cortisol in the same species has been shown to be in the range of 32.1 to 254.3 pg/mg, with a mean of 110.3 ± 10.2 pg/mg (Davenport *et al*, 2006)

In callitrichids much of the data reports blood plasma cortisol values, but values for salivary, urinary and faecal cortisol levels are available, and recently values for hair cortisol have been published (Table 5.1). Measurement descriptions vary depending upon medium but efforts have been made to harmonise data given below to allow comparison.

Reference	**Species**	**Sample method**	**Mean Concentration ± sem unless otherwise specified**
(Torii *et al*, 1998)	*C. jacchus*	Blood plasma	Males 200-300 ng/ml (200-300 µg/dl) Females Late pregnancy 300-500 ng/ml (300-500 µg/dl) Parturition < 100 ng/ml (< 100 µg/dl) 50 days postpartum 200-300 ng/ml (200-300µg/dl)
(Johnson *et al*, 1996)	*C. jacchus*	Blood plasma	Morning Males Pair - dominant 43.5 ± 6.5 µg/dl subordinate 90.6 ± 12.3 µg/dl Isolate - dominant 203.3 ± 57.8 µg/dl subordinate 126.1 ± 18.6 µg/dl Unstable peer - dominant 255.4 ± 41.1 µg/dl subordinate 317.5 ± 82.2 µg/dl Stable peer - dominant 112.3 ± 12.9 µg/dl subordinate 113.2 ± 20.6 µg/dl Females Pair – dominant 116.1 ± 14.5 µg/dl subordinate 141.3 ± 21.3 µg/dl Isolate – dominant 154.3 ± 9.0 µg/dl subordinate 126.1 ± 18.6 µg/dl Unstable peer – dominant 317.0 ± 26.5 µg/dl subordinate 231.0 ± 31.3 µg/dl Stable peer – dominant 177.1 ± 42.5 µg/dl subordinate 100.6 ± 29.3 µg/dl Evening Males Pair - dominant 29.6 [1] µg/dl subordinate 91.0 ± 25.8 µg/dl Isolate – dominant 31.2 ± 2.8 µg/dl subordinate 89.0 ± 45.8 µg/dl Unstable peer – dominant 132.0 ±18.0 µg/dl subordinate 195.0[1] µg/dl Stable peer – dominant 98.8 ± 55.6 µg/dl subordinate 97.6 ± 30.3 µg/dl Females Pair – dominant 119.0 ± 65.0 µg/dl subordinate 179.5 ± 44.8 µg/dl Isolate – dominant 145.4 ± 80.6 µg/dl subordinate 228.0 ± 45.9 µg/dl Unstable peer – dominant 215.5 ± 55.5 µg/dl subordinate 109.8 ±31.8 µg/dl Stable peer – dominant 226.7 ± 2.0 µg/dl subordinate 74.7 ± 21.5 µg/dl Calculated mean – males 126.6 µg/dl females 166.3 µg/dl dominant 146.7 µg/dl subordinate 144.4 µg/dl morning 164.1 µg/dl evening 128.9 µg/dl

(Norcross & Newman, 1999)	*C. jacchus*	Blood plasma	Females 102.8 ± 9.9 µl/dl
(Saltzman *et al*, 1994)	*C. jacchus*	Blood plasma	Females 0900h 214.1 ± 21.5 µg/dl 1145h 171.7 ± 17.0 µg/dl
(Schultz-Darken *et al*, 2004)	*C. jacchus*	Blood plasma	Females 5.14 µmol/l (95% confidence 5.05 – 5.23 µmol/l) (182.07µg/dl 95% confidence 182.81 – 189.33µg/dl)
(Whitehouse & Abayasekara, 2000)	*C. jacchus*	Blood plasma	Females 3858 ± 429 ng/ml (3858 ± 429µg/dl)
(Boere *et al*, 2005)	*C. pencillata*	Blood plasma	Males 1089.85 ± 278.65 ng/ml Females 1251.95 ± 159.83 ng/ml Juvenile 922.68 ± 181.15 ng/ml Adult 1170.90 ± 155.94 ng/ml
(Sousa & Ziegler, 1998)	*C. jacchus*	Faeces	Non-breeding females Min 30.10 Max 897.27 ng/g
(Sousa *et al*, 2005)	*C. jacchus*	Faeces	Females Dominant 129.12 ± 22.09 ng/g Subordinate 116.97 ± 20.02 ng/g Cycling subordinate 193.11 ± 476.91 ng/g Non-cycling subordinate 47.69 ± 90.78 ng/g
(Clara *et al*, 2008)	*C. jacchus*	Hair	< 40 µmol/g
(Cross *et al*, 2004)	*C. jacchus*	Saliva	Quiet period 561 ± 85 nmol/l (20.2 ±3.1 µg/dl) Disturbed period 2004 ± 334 nmol/l (72.1±12.0 µg/dl)
(Dettling *et al*, 2002)	*C. jacchus*	Urine	Normally reared juveniles 113.9 ± 83 µg/mg Cr Early deprived juveniles 75.9 ± 9.0 µg/mg Cr
(Torii *et al*, 1998)	*C. jacchus*	Urine	Males 15-30 µg/mg Cr Females Late pregnancy 30-65 µg/mg Cr Parturition < 20 µg/mg Cr 50 days postpartum 20-35 µg/mg Cr
(Smith *et al*, 1998)	*C. kuhli*	Urine	Breeding males and females 15.15 ± 2.29 µg/mg Cr
(Smith & French, 1997)	*C. kuhli*	Urine	Males 14.24 ± 1.9 µg/mg Cr Females 21.42 ± 1.7 µg/mg Cr Small cage Males 49.5 ± 9.0 µg/mg Cr Females 26.4 ± 2.1 µg/mg Cr Small cage and Handling Males 43.7 ± 6.2 µg/mg Cr Females 39.1 ± 3.5 µg/mg Cr

Table 5.1 – Reported cortisol values from different collection mediums in callitrichids

Reported cortisol measurements vary greatly between collection methods and even within the same collection method between studies, as well as variation due to experimental manipulation. Johnson *et al* (1996) give one of the most comprehensive studies of blood cortisol levels, with sex, social status, housing and time of day taken into account. Levels in this study range from 31.2 ± 2.8µg/dl (excluding 'means' from only one individual) in dominant males in isolation in the evening to 317.5 ± 82.2µl/dl in subordinate males in unstable peer groups for morning samples. This is obviously a huge range and indicates how difficult cortisol is to use as a measure of welfare. Males and females seem to differ in their response to different housing conditions but patterns in the data are hard to identify. Whilst, for example, dominance in the group does not affect cortisol in stable peer groups, in unstable peer groups dominant males have higher baseline levels than females but in subordinates the reverse is true. This again highlights the care needed in the interpretation of cortisol data.

The cortisol levels reported in saliva in Table 5.1 are in the range expected if 10% of the total circulating cortisol is the free fraction for low stress recordings and 20-30% following stressors; 20.2 ± 3.1 µg/dl salivary cortisol concentration would equate to somewhere in the region of 200 µg/dl for blood plasma, which is well within in the range reported, and 72.1± 12.0 µg/dl salivary cortisol concentration would be equate to somewhere in the region of 240 – 360 µg/dl, again in good agreement with reported data for plasma cortisol following a stressor. This suggests that measuring salivary cortisol concentration is a valid method of measuring circulating cortisol.

5.2 CAPTURE OF MARMOSETS

Capture of marmosets can be stressful for them (see Chapter 3). Methods vary across facilities and include

a. net capture
b. negative reinforcement to encourage the marmoset into a nest box or transport box from which the marmoset is then hand-captured, or positive reinforcement to enter the transport box
c. hand capture within home cage using latex or leather gloved hand.

Net capture is never recommended (Rennie & Buchanan-Smith, 2006b) as the monkeys often rush frantically around the cage, and the potential for injury is high. Nets can also create high anxiety in the whole room and have an adverse impact on the health and welfare of the colony. Encouraging the marmoset into the nest box or transport box is a preferable method, but unless they are trained using PRT it uses negative reinforcement (usually avoiding the threat of a gloved hand). There is often a time when the marmosets are left in the box, and the marmosets still have to be removed from the box. Whilst calm experienced staff can do this in a manner that does not appear to distress the marmosets, they will often attempt to avoid capture or cling to the wire mesh of the box, potentially damaging their nails and claws. Hand capture within the home cage should only be attempted with calm marmosets and if the technician is experienced enough to do it swiftly and calmly. A latex gloved hand is far preferable to a leather glove, so the correct pressure is applied (Buchanan-Smith, 2010). Nevertheless, sometimes the marmosets will still try to grip to the cage, with the concomitant potential damage to their claws and nails. Given that none of the above capture methods

is without problems, an alternative method of capture was sought, and behaviour and cortisol measured to determine whether it was an improvement on the laboratory's current method of hand capture, which is done with a latex-gloved hand. This is referred to as STANDARD capture (see below).

5.3 AIMS OF THE STUDY

The aim of this study was firstly to establish is if was possible to train marmosets to cooperate with capture, a potentially aversive and stressful experience, and if so the time investment this would require. The second aim was to assess the feasibility of using salivary cortisol as a measure of stress in laboratory-housed marmosets, and determine whether salivary cortisol concentrations alongside behavioural observations can quantify the stress response of marmosets. The overall objective is to use both the physiological and behavioural responses of the marmosets to capture and training to determine the stress experienced following training and capture, and to establish whether different methods of capture lead to reduced stress. The combined data are be used to compare the stress response of trained and untrained marmosets to capture and to allow recommendations to be made on the best way of catching laboratory-housed marmosets.

5.4 METHODOLOGY

5.4.1 Study Animals

This experiment was carried out at in the Laboratory Animal Science Department of GlaxoSmithKline, Stevenage, UK. All animals had been purpose bred in captivity and

had been housed in the department for at least 1 year prior to the start of the experiment. The marmosets used in this study were aged between 2 years 6 months and 8 years 3 months at the start of the study. They were housed in 2 female-female pairs, 3 male-male pairs and 2 male-female pairs, meaning that 6 female and 8 male marmosets were used. Although an earlier study (Chapter 3) indicated that there was no difference between males and females in the speed or ability of learning a training task between males and females, an approximately even split between the sexes was chosen in order to prevent any potential bias due to gender.

Housing and Husbandry

Full details of the housing and husbandry of the marmosets are provided in Chapter 4.

5.4.2 Experimental Design

Fourteen pair-housed common marmosets (*C. jacchus*) were used in this study. One marmoset in each of the seven pairs was randomly allocated to the training group ("PANEL") and the other marmoset in the pair to the positive human interaction group ("INTERACT"). "PANEL" marmosets were trained according to the protocol below, whilst "INTERACT" marmosets received a matched reward every time their "PANEL" cagemate did, therefore acting as a control. Habituation to my presence followed procedures described in Chapter 4.

5.4.3 Training Protocol

The aim of the training programme was to establish in the "PANEL" group of marmosets the behaviour of coming to a detachable panel and remaining there whilst the cage door was opened and the trainer touched and subsequently held them. The "INTERACT" group received no specific training but received a food reward matched with their "PANEL" group cagemate. All training was positive reinforcement using small pieces of marshmallow (approximately $^1/_8$ of a small marshmallow), which was paired with a secondary reinforcer (bridge), in this case the click of a retractable pen. All training was carried out between 1100h and 1500h, when the marmosets had had plenty of opportunity to eat and when the laboratory routine meant that disturbances were minimised. A maximum of two marshmallows (16 rewards) were available to each marmoset in each training session, which was also limited to a total of five minutes per marmoset. In all cases the marmoset was considered to be reliable in performing a desired behaviour if he or she performed it on nine out of ten occasions it was requested. If at any point a marmoset failed to perform at a particular level the trainer went back and repeated the last level. Panel design and positioning on the cage are shown in Plate 2 and Figure 5.1.

As only the "PANEL" group of marmosets was actively trained, the training protocol only applies to these marmosets. Before any training was carried out the detachable panel was introduced to the cage for 10 minutes to allow the marmosets to investigate it freely. The panel was then placed into the cage approximately two minutes prior to each training session, and removed at the end of each session. It was always positioned in the same place, approximately 1.2m from the floor, on the inside of the upper half of the

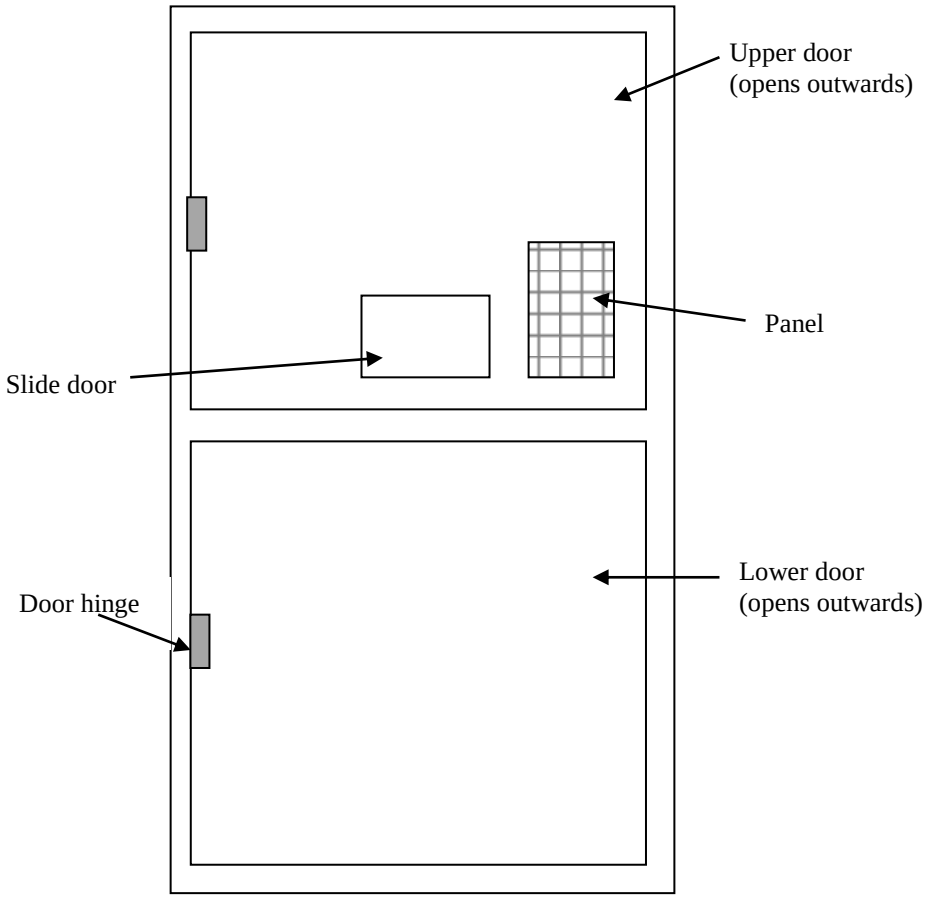

Figure 5.1 Schematic diagram of placement of training panel in marmoset cage (not to scale)

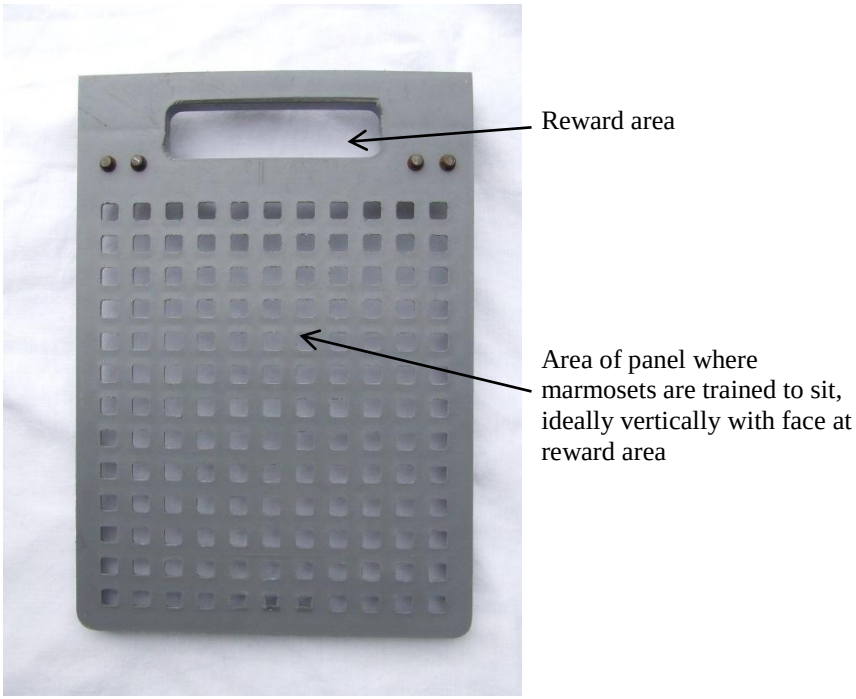

Plate 2 Photograph of capture panel showing 'marmoset eye' view

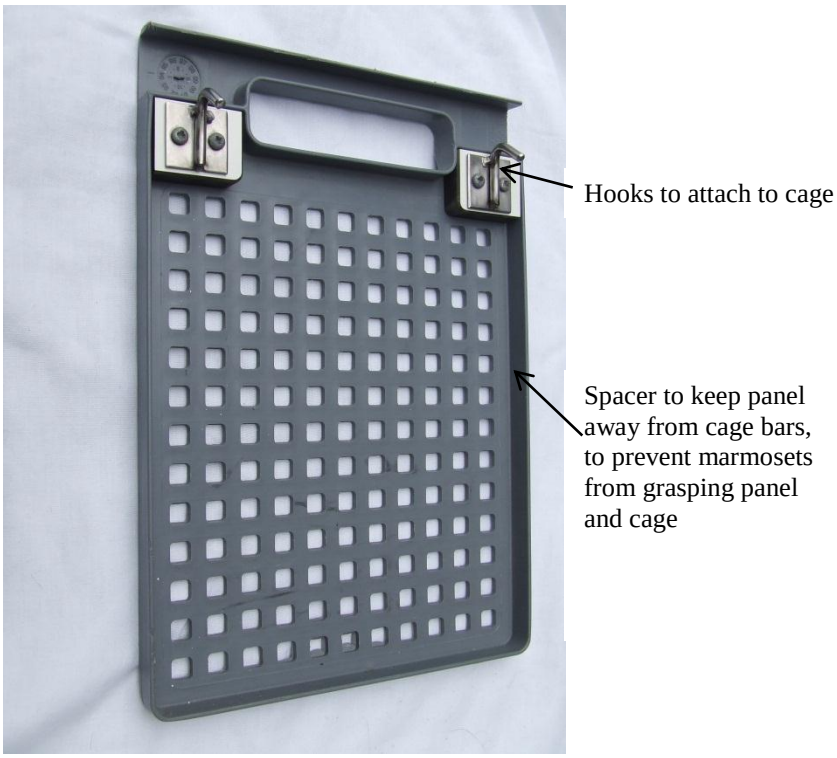

Plate 3 Photograph of capture panel showing back of panel

cage and on the side furthest from the door hinge. This meant that it could be accessed by opening the lower cage door only and reducing the risk of escapes. Training was broken into nine stages outlined below.

- Stage 1 - The first stage of the training programme required the marmoset to come to the front of the cage and take the reward from the trainer, at which point the secondary reinforcer (click) was added. Food was delivered directly into the mouth of the marmoset and the secondary reinforcer paired with the food by clicking on the delivery of food.
- Stage 2 - Once the association between the click and food was established the marmosets were shaped to sit on the detachable panel. All food rewards from this point onwards were delivered through the gap at the top of the panel ('reward area') and this was paired with the 'panel' command. Once the marmoset was reliably coming to the panel the length of time they had to remain there before receiving their reward was extended, up to a maximum of 90 seconds.
- Stage 3 - Once the panel behaviour was established the cage was then rattled slightly to simulate the noise of the cage door opening, and the marmoset rewarded for staying on the panel. The lower cage door was then gradually opened and closed whilst the marmoset remained on the panel.
- Stage 4 - When the marmoset was confident to remain on the panel during the door opening, the trainer then started to put their hand inside the cage and move it towards the marmoset, making sure where possible that this was visible to the marmoset to prevent him/her being startled. The trainer then gently touched the tail of the marmoset, increasing contact strength and duration.

- Stage 5 – The trainer was able to firmly grasp the marmoset's tail, briefly at first building up to several seconds. At no point was this used to restrain the marmoset if he or she wished to escape, any resistance from the marmoset lead to the tail being released, but no reward was provided.
- Stage 6 - Once the marmoset was comfortable with the tail hold, his or her body was gently touched until they were confident to remain on the panel in this position for several seconds.
- Stage 7 – The marmoset was held firmly round his or her body whilst remaining on the panel, briefly at first building up to remaining in this position for several seconds.
- Stage 8 - The panel was then gently moved, whilst remaining attached to the cage, then removed from the cage front, but still being inside the cage, whilst the marmoset was held round his or her body.
- Stage 9 - The marmoset and panel were then removed entirely from the cage, at which point he or she was allowed to drop the panel by gradually and carefully increasing the weight of the panel supported by the marmoset (the marmoset was never left to support the full weight of the panel him or herself, as it may have caused damage to hands and feet). Once out of the cage and being held by the trainer the marmoset was rewarded and returned to the cage. The panel was replaced and the marmoset was always asked to come back to the panel for a simple stay following any capture.

Those marmosets in the "INTERACT" group were provided with a food reward every time their "PANEL" cagemate received one. This reward was presented at random

locations around the front of the cage, but was always hand fed and delivered directly to the marmoset's mouth. This was done by a single trainer (myself) and was done as soon as possible after presenting the 'PANEL' marmoset with their reward.

5.4.4 Data Collection

Salivary cortisol collection

All saliva samples were collected between 1200h and 1500h. It has been shown that as well as the gradual decline in cortisol levels over the day the provision of food causes cortisol levels to decrease in marmosets (Cross & Rogers, 2004), so this time frame was chosen to attempt to avoid the sharpest decreases in cortisol due to either of these factors. For each pair, each stage sample was collected within 30 minutes of samples collected on previous days in order to reduce the effect of circadian cortisol rhythms. Two samples were collected for each stage on consecutive days (excluding weekends) to allow a mean value to be established for each individual at each stage and to reduce the effect of missing or short samples, whereby there was insufficient saliva collected to be analysed. The schedule of saliva collection is provided in Table 5.2.

Stage	Code	Description	Groups collected for
Baseline	B	Sample collected prior to any intervention	"PANEL" & "INTERACT"
Standard Capture Baseline	CB	Sample collected within 5 mins of return to cage following a standard capture, prior to any intervention	"PANEL" & "INTERACT"
Standard Capture Baseline +30	CB+30	Sample collected 30-35 mins after return to cage following a standard capture, prior to any intervention	"PANEL" & "INTERACT"
Training session 1	S1	Sample collected within 5 mins of end of 1st training/interaction session	"PANEL" & "INTERACT"
Training session 10	S10	Sample collected within 5 mins of end of 10th training/interaction session	"PANEL" & "INTERACT"
Trained Capture	TC	Sample collected within 5 mins of return to cage following a trained capture	Trained "PANEL" only
Trained Capture +30	TC+30	Sample collected 30-35 mins of return to cage following a trained capture	Trained "PANEL" only
Standard Capture	SC	Sample collected within 5 mins of return to cage following a standard capture, after training/intervention programme	"PANEL" & "INTERACT"
Standard Capture +30	SC+30	Sample collected 30-35 mins after return to cage following a standard capture, after training/intervention programme	"PANEL" & "INTERACT"

Table 5.2 – Saliva sampling stages and the groups that saliva was collected from at each stage

Saliva was collected by firstly rubbing a cotton bud (Johnson and Johnson) into a banana until it was coated with a thin layer of banana. Banana has been shown to be a preferred food of marmosets, and this technique has been shown to be effective for the collection of saliva in marmosets (Cross *et al*, 2004, Cross & Rogers, 2004, Pines *et al*, 2004). The cotton bud was then presented to an individual marmoset who was

encouraged to lick and chew the end of the cotton bud in order that they deposited saliva onto the bud. If possible the cotton bud was gently moved into the cheek of the marmoset, outside the teeth, where saliva could be easily collected in greater quantities. Once the cotton bud was well soaked with saliva, after approximately 3-4 minutes, it was removed from the marmoset, who was then given a small piece of banana. The bud was then checked for traces of blood which may have rendered the cortisol assay inaccurate, and taken for processing.

The saliva soaked cotton bud was then cut so that approximately 2cm of the stick was protruding from the cotton wool end. The bud was then placed stick down into an Eppindorfer tube which was sealed and placed in a centrifuge. The saliva samples were spun for 20 minutes at 3200rpm. On the completion of the centrifugation buds were removed from the tube. The tubes and their contents were then frozen at -19°C for less than one week until they were moved to a -70°C freezer where they were stored until assayed (less than 3 months).

Cortisol Assay

Saliva samples were thawed and brought to room temperature for analysis, which was carried out by Sue Heggarty of GlaxoSmithKline. Coat-a-count kits by Diagnostic Product Corporation (DPC) were used to assay the saliva samples for cortisol. In this assay the cortisol in the saliva sample competes with radiolabelled cortisol in a pre-prepared polypropylene tube. The level of radioactivity can then be measured using a gamma counter and the amount of cortisol in the sample converted from this figure on a calibration curve.

Behavioural data collection

Behavioural data were collected using a handheld workabout computer using THE OBSERVER V5.0. Data were recorded for 5 minutes per individual, with cagemates being recorded consecutively. All data were collected between 1100h and 1500h, and each recording was taken after training for that particular pair, and after saliva collection, so behavioural data were collected at approximately 5-10 minutes post-training/capture for monkey 1 and 10-15 minutes post-training/capture for monkey 2. The order in which data were collected was randomly allocated to the "PANEL" and "INTERACT" individual, but always kept consistent between cagemates. Behavioural data for each sample point was only collected on one day, the first day at that sample point.

Behaviour was collected as for previous studies, the categories recorded are defined in Chapter 4, Table 4.2. Behavioural data were collected on the same timetable as saliva collection described above (Table 5.2).

5.4.5 Statistical Analysis

For all analysis the level of significance was set at 0.05. This is despite multiple analyses being carried out, where it is recommended that corrections are used. This was done as despite a risk of Type I errors (false positives), it reduces Type II errors (false negatives). The corrections needed would have led to significance being set at such a high level that the risk of Type II errors increased. As it is desirable to look for biologically relevant patterns in the data, a significance level of 0.05 was used, but caution taken in interpreting the results. Data were then subjected to a Bonferroni

correction for multiple comparisons, and where statistical significance was retained, results are highlighted. Data were tested for normality using Kolmogorov-Smirnov tests and if normal parametric tests were used. Where means are reported, standard errors are also provided. Non-normally distributed data were analysed using non-parametic tests, and presented with interquartile ranges.

Cortisol data

Analysis of the cortisol data was carried out in Minitab v12. Comparisons were carried out using mixed measures ANOVA followed by post-hoc planned comparisons allowing the identification of significant results. The factors were observation (within subject), treatment (between subject - PANEL and INTERACT as levels) and whether successfully trained (between subject) (Table 5.3). Paired t-tests were also used where comparisons between two sample points was required.

Variable	**Between/Within Subjects**	**Levels**
Observation	Within	Baseline Standard Capture Baseline Standard Capture Baseline + 30 Training 1 Training 10 Trained Capture Trained Capture + 30 Standard Capture Standard Capture + 30
Treatment	Between	PANEL INTERACT
Trained?	Between	Successfully trained for capture Not Successfully trained for capture

Table 5.3 Summary of statistical analysis of cortisol and behavioural data

Behavioural data

Although the behaviour of one marmoset is likely to influence the behaviour of his or her cagemate, due to the different training or interaction programmes experienced by cagemates, data were analysed for individuals rather than being combined for each cage. Repeated measures ANOVAs were used to analyse the data, with planned post-

hoc comparisons. Factors are described above and paired t-tests were also used where comparisons between two sample points was required.

5.5 RESULTS

5.5.1 Success Rates and Time Investment

Of the seven marmosets in group "PANEL", four were successfully trained. The number of five minute sessions required to achieve this ranged from 21 to 35, meaning a time investment of between 1 hour and 45 minutes and 2 hours and 55 minutes. The mean time investment to reach criterion was 29.0 ± 2.94 sessions or 145 ± 14.7 minutes. The three remaining marmosets in the "PANEL" group were trained to allow their tails to be touched but were not able to be trained any further within the 42 session limit (3 hours 30 minutes time investment).

5.5.2 Cortisol Data

Of the 184 samples attempted (28 x B, 28 x CB, 28 CB +30, 14 x T1, 14 x T10, 8 x TC, 8 x TC +30, 28 x SC, 28 x SC +30), 41 were either not successfully collected or analysed, meaning a total of 143 samples were successfully analysed, which equates to 77.7% of samples. Where two saliva samples were successfully collected for one sampling point, means were calculated and these were used in the analysis to try to reduce the effect of large variations. The data were tested for reliability using a Pearson correlation, and the collected cortisol values per marmoset for each sample point were found to be correlated ($df = 62$, $r = 0.37$, $p = 0.014$), validating the use of mean values.

The mean cortisol baseline level (B) for all the marmosets was 1222.0 ± 122.0 nm/l. Whilst the mean value for female marmosets was higher than that of males (1288 ± 221 nm/l versus 997 ± 98 nm/l) baseline cortisol levels were not found to differ significantly between males and females in this study (t-test, df = 6, t = -1.2, p = 0.27, NS). As dominance was not assessed no analysis based on this was carried out. The baseline cortisol levels for "PANEL" (1212 ± 179 nm/l) and "INTERACT" (1032 ± 140nm/l) groups were not significantly different (t-test, df = 11, t = 0.79, p = 0.45, NS). The variation across cortisol measurements was high, with baseline cortisol ranging from 598.5nm/l to 2173.5 nm/l. Figure 5.2 shows the variation amongst the "PANEL" group across the sampling points, and Figure 5.3 the variation amongst the "INTERACT" group.

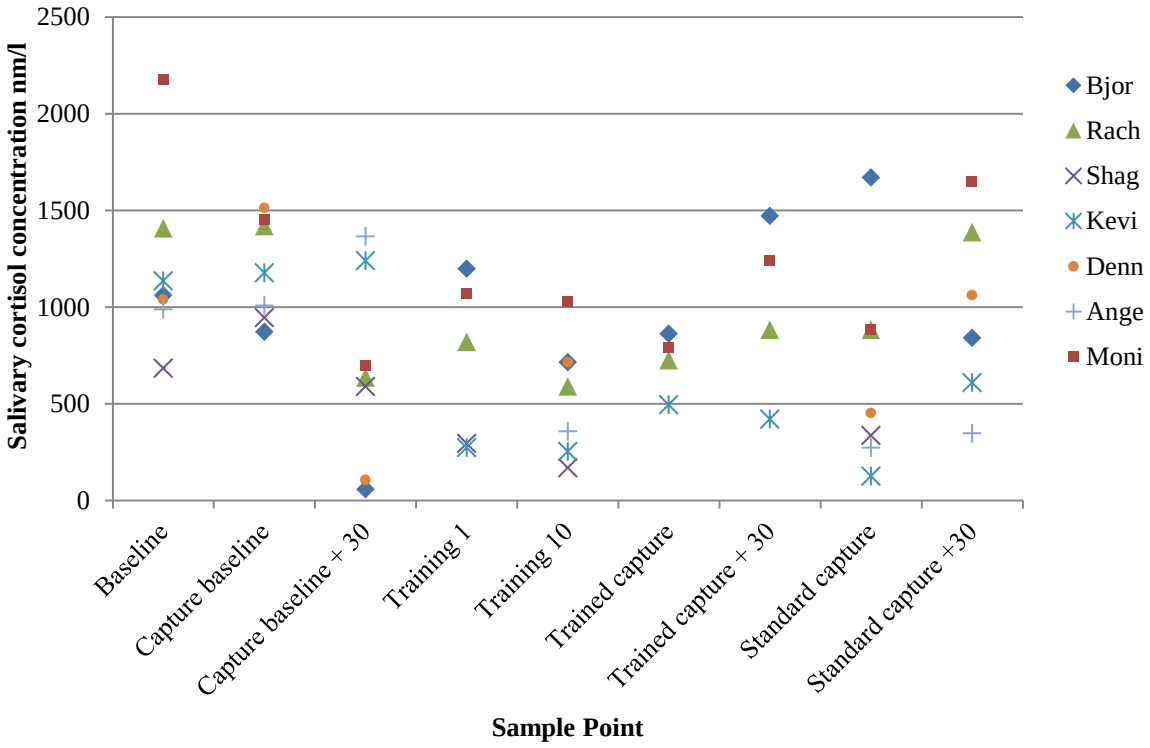

Figure 5.2 Individual variation in salivary cortisol concentrations (nm/l) for "PANEL" group across sample points

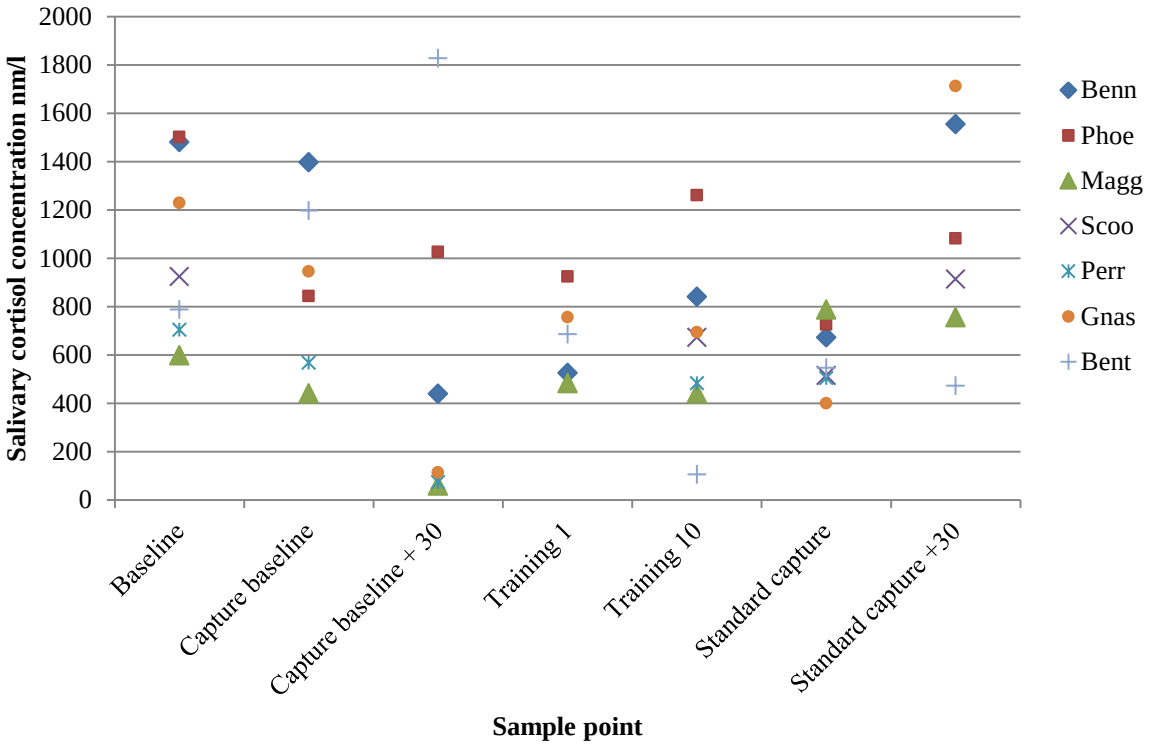

Figure 5.3 Individual variation in salivary cortisol concentrations (nm/l) for "INTERACT" group across sample points

Baseline data

Analysis of the baseline data (B, CB, CB+30) showed that whilst cortisol concentrations were unaffected by the treatment group ("PANEL", n = 7 or "INTERACT", n = 6), whether the marmosets were successfully trained (trained, n = 4, untrained, n = 9) or the interaction between training success and observation, there were significant differences between observations. The salivary cortisol concentrations of the marmosets was significantly higher at baseline (B) than at capture baseline + 30 (CB + 30), whilst no differences were seen between B and capture baseline (CB) and CB and CB + 30. (Figure 5.4, Table 5.4, Table 5.4). This significance is primarily due to one outlier (Moni in the PANEL group), as when her data are removed from the analysis,

there is no longer a significant difference between observations ($F_{2,29}$ = 2.94, p = 0.07, NS).

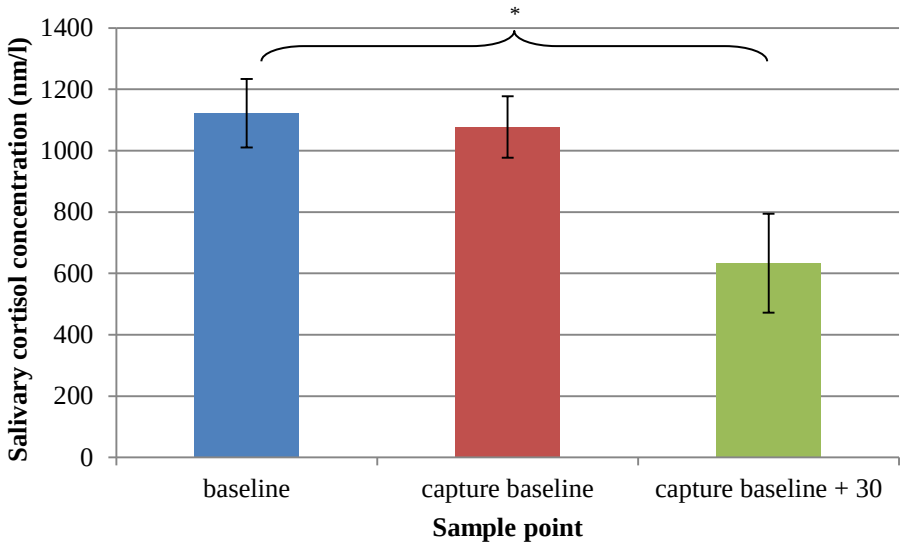

Figure 5.4 Mean salivary cortisol concentrations (nm/l) of all marmosets at baseline, capture baseline and capture baseline + 30 sample points (* p < 0.05, ** p < 0.01) **Error bars show standard error of the mean.**

	df	F	P
observation	$_{2,32}$	4.92	<0.05 (0.014)
trained?	$_{1,32}$	0.91	NS (0.35)
group	$_{1,32}$	0.11	NS (0.74)
observation*trained?	$_{2,32}$	0.56	NS (0.58)

Table 5.4 Results of repeated measures ANOVA for recorded salivary cortisol concentrations at baseline, capture baseline and capture baseline + 30 for all marmosets

	T	p
B v CB	-0.54	NS (0.85)
B v CB +30	-2.96	< 0.05 (0.016)
CB v CB +30	-2.38	NS (0.060)

Table 5.5 Results of post-hoc Tukey tests, comparisons between baseline, capture baseline and capture baseline + 30 for salivary cortisol concentrations for all marmosets

This result is robust enough to remain significant when a Bonferroni correction for multiple comparisons changes the p value to 0.0167.

Effect of training and positive human interactions

Comparisons of the salivary cortisol data for baseline with the first training session (T1) and tenth training session (T10) showed that concentrations varied across observations and also with whether the marmosets were successfully trained (trained, n = 4, untrained, n = 6), but not with their treatment group ("PANEL", n = 5, "INTERACT", n = 5) (or with the interaction between observation and their training success (Figures 5.5, 5.6, Table 5.6). Post-hoc analysis revealed that baseline concentrations were greater than those at either T1 or T10, but that concentrations at T1 and T10 did not differ significantly (Table 5.7).

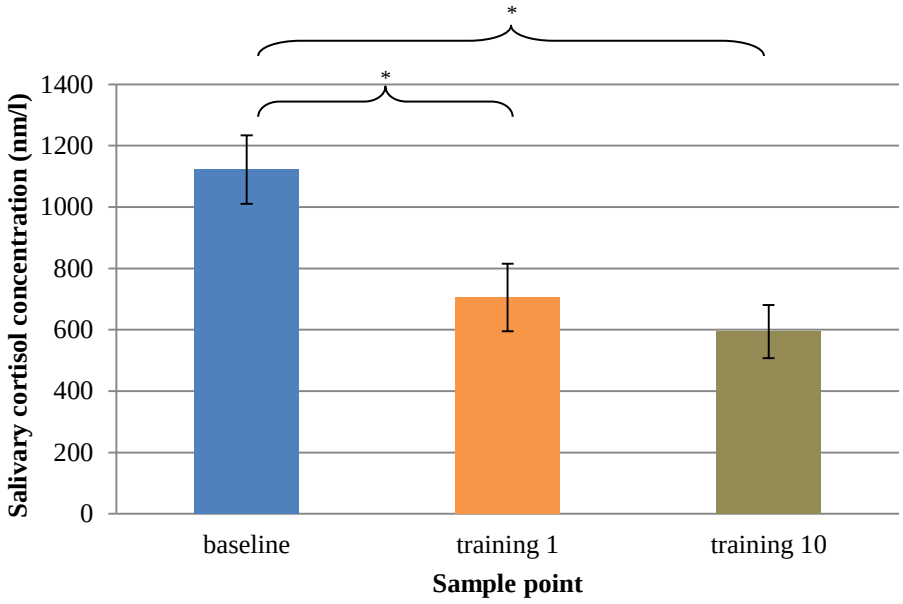

Figure 5.5 Mean salivary cortisol concentrations (nm/l) of all marmosets at baseline, training session 1 and training session 10 (* $p < 0.05$, ** $p < 0.01$) **Error bars show standard error of the mean.**

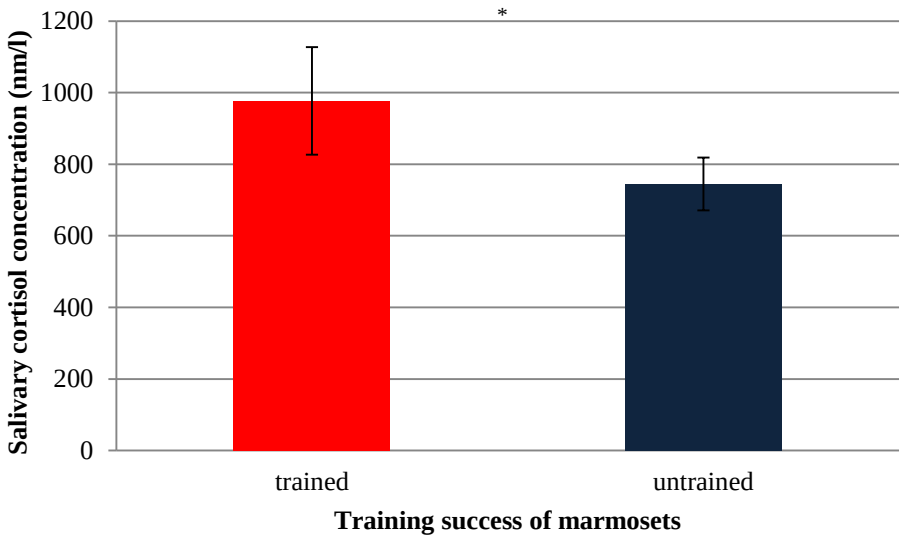

Figure 5.6 Mean salivary cortisol concentrations (nm/l) of all marmosets pooled across baseline, capture baseline and capture baseline + 30 by whether they were successfully trained (* $p < 0.05$, ** $p < 0.01$) **Error bars show standard error of the mean.**

	df	F	p
observation	$_{2,30}$	9.99	<0.001
trained?	$_{1,30}$	5.97	< 0.05 (0.021)
group	$_{1,30}$	0.11	NS (0.18)
observation*trained?	$_{2,30}$	0.56	NS (0.44)

Table 5.6 Results of repeated measures ANOVA for recorded salivary cortisol concentrations at baseline, training session 1 and training session 10 for all marmosets

	T	p
B v T1	-3.30	<0.01 (0.007)
B v T10	-2.96	< 0.01 (0.001)
T1 v T10	-2.38	NS (0.16)

Table 5.7 Results of post-hoc Tukey tests, comparisons between baseline, training session 1 and training session 10 for salivary cortisol concentrations for all marmosets

When a Bonferroni correction is aaplied to the results of the post-hoc Tukey tests, and significance is set at 0.0167, these results are robust enough to rtain their significance.

Trained animals

When the baseline and trained capture data (B, CB, CB + 30, TC and TC + 30) from the four successfully trained marmosets were analysed separately there is an indication that salivary cortisol concentration varies with observation (ANOVA, n = 4, $F_{4,15} = 2.8$, p = 0.04), but due to the very small sample sizefurther post-hoc analysis was not carried out. However, when the data for just capture baseline and trained capture were analysed separately, the comparison of most interest, there is a trend for salivary cortisol

concentrations being greater at CB than at TC, with concentrations at CB approaching double those found at TC, (CB n = 4 mean = 1229 ± 134 nm/l, TC n = 4 mean = 717 ± 79 nm/l; t-test df = 3, t = 3.06, p = 0.055, NS), but this again fails to reach statistical significance, likely due to the small sample size (n = 4) (Figure 5.7).

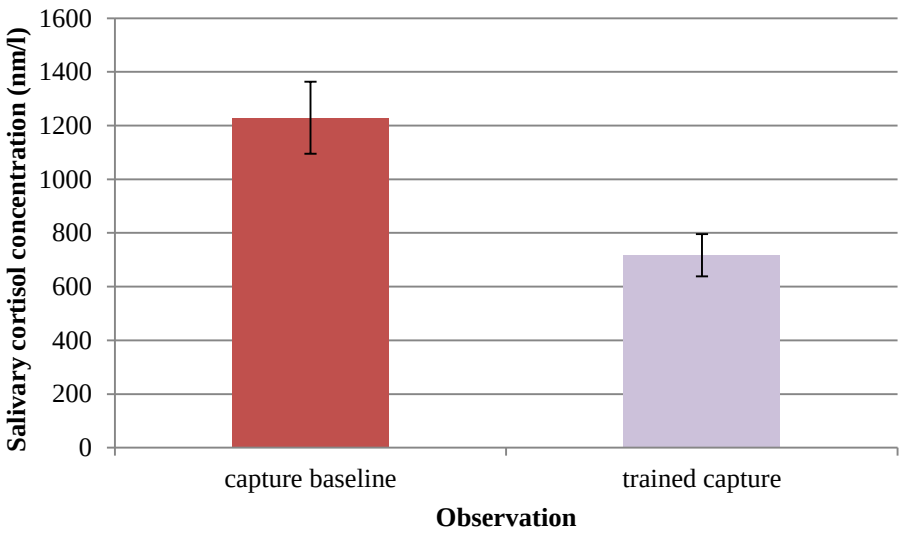

Figure 5.7 Mean salivary cortisol concentrations (nm/l) of trained marmosets at capture baseline and trained capture (* $p < 0.05$, ** $p < 0.01$) **Error bars show standard error of the mean.**

Response to capture following training/positive interaction

When all collected data were analysed together salivary cortisol concentrations did not vary across the capture baseline (CB) and standard capture (SC) dependent upon treatment group, training success or the interaction between observation and whether they were successfully trained. However cortisol concentration did vary across the observations, being significantly higher following SC than TC (Figure 5.8, Table 5.8).

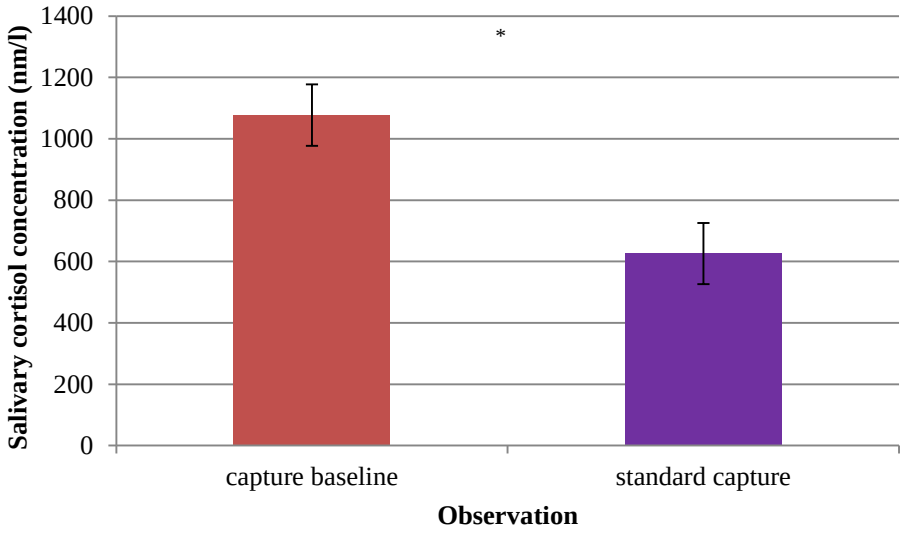

Figure 5.8 Mean salivary cortisol concentrations (nm/l) of all marmosets at capture baseline and trained capture (* $p < 0.05$, ** $p < 0.01$) **Error bars show standard error of the mean.**

	df	F	p
observation	$_{1,21}$	7.42	< 0.05 (0.013)
trained?	$_{1,21}$	0.00	NS (0.95)
group	$_{1,21}$	2.55	NS (0.13)
observation*trained?	$_{1,21}$	0.22	NS (0.64)

Table 5.8 Results of repeated measures ANOVA for recorded salivary cortisol concentrations at capture baseline and standard capture for all marmosets

5.5.3 Behavioural Data

Analysis of the data showed that the behaviour did not differ between the "PANEL" and "INTERACT" groups of marmosets, and also that no interactions were seen between the data by observation or treatment group. Therefore the "PANEL" group and the "INTERACT" group data presented below are for all 14 marmosets. Due to the

relatively short amount of time the marmosets spent engaged in agitated locomotion as compared to the other behaviours, the results from these data are plotted separately for clarity. Data for time spent in the nest box or out of sight were not analysed as this behaviour was only recorded on two occasions, each for less than two seconds, meaning that there were insufficient data.

Baseline data

An initial analysis was carried out on the baseline data to identify any effects of capture on the behaviour of the marmosets. The analysis of baseline (B), capture baseline (CB) and capture baseline + 30 (CB30) showed that whilst the duration of locomotion, contact sitting and watchful behaviour did not change significantly across the three observations, the durations of agitated locomotion and sitting alone were both significantly affected by the baseline capture (Figures 5.9, 5.10, Table 5.9). The duration of agitated locomotion increased significantly following CB, rising to over eight times the baseline levels following capture. Thirty minutes after capture (CB + 30) levels of agitated locomotion had fallen back to levels close to those at baseline, and were significantly lower than immediately after capture. The marmosets spent significantly less time sitting alone following capture (CB) than at baseline (B), but 30 minutes following capture (CB + 30), the amount of sitting alone was not significantly different from either B or CB (Table 5.10).

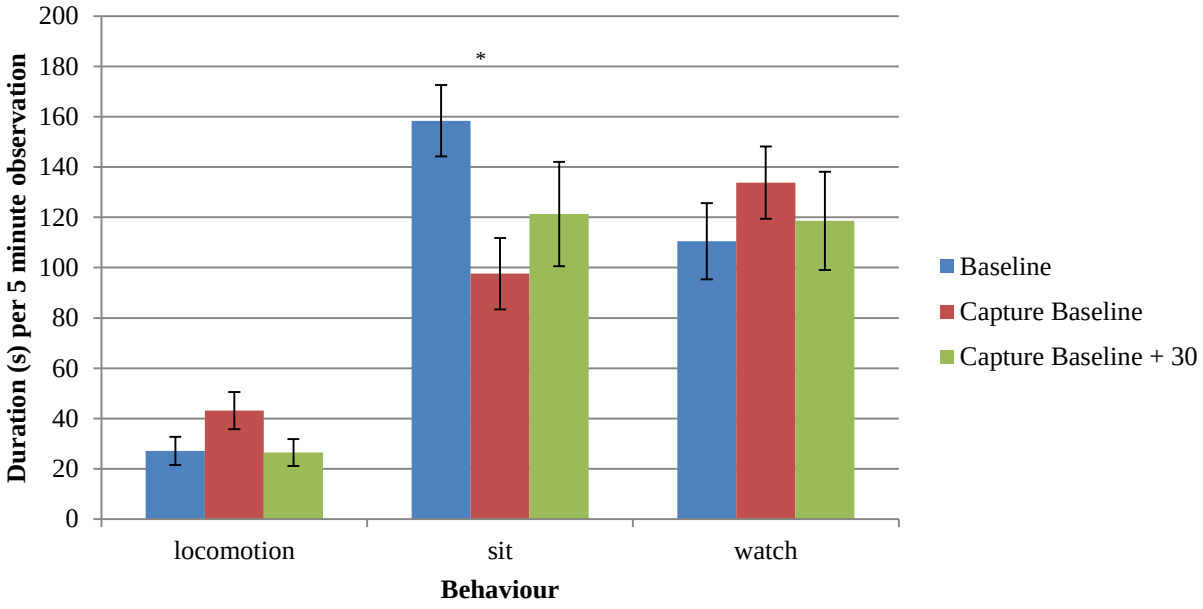

Figure 5.9 Mean durations (in seconds) of longer duration behaviours (> 20 s) per 5 minute observation across baseline, capture baseline and capture baseline + 30 (* $p < 0.05$, ** $p < 0.01$) **Error bars show standard error of the mean.**

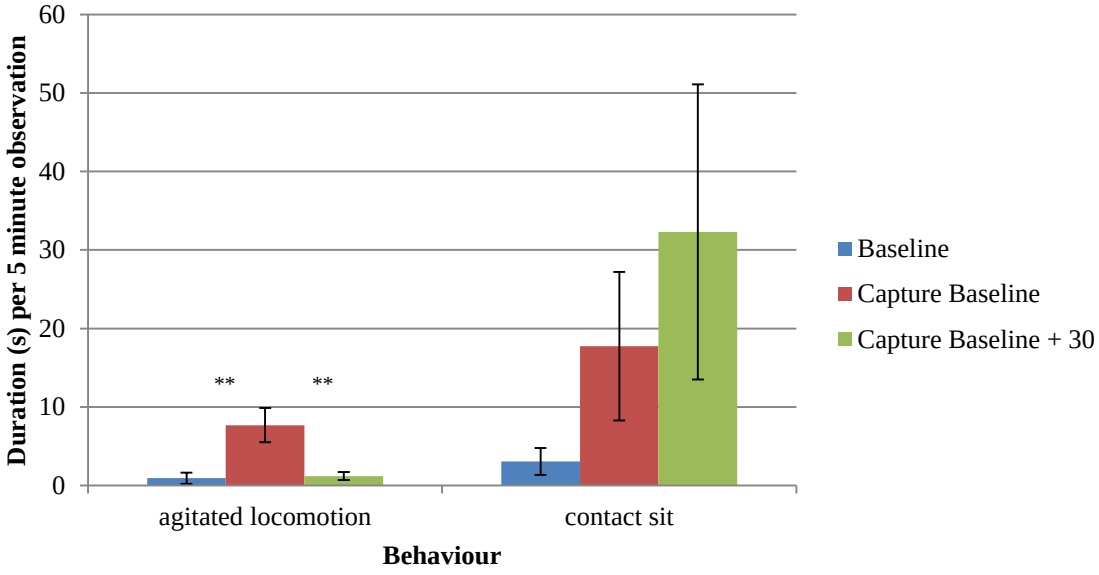

Figure 510 Mean durations (in seconds) of shorter duration behaviours (< 20 s) per 5 minute observation across baseline, capture baseline and capture baseline + 30 (* $p < 0.05$, ** $p < 0.01$) **Error bars show standard error of the mean.**

Behaviour	$F_{2,36}$	p
locomotion	2.38	NS (0.11)
agitated locomotion	7.31	<0.01 (0.002)
sit	3.61	<0.05 (0.037)
contact sit	1.47	NS (0.24)
watch	0.27	NS (0.77)

Table 5.9 Results of repeated measures ANOVA on durations of recorded behaviours across observations at baseline, capture baseline and capture baseline + 30 for all marmosets

Behaviour	Comparison	T	p
agitated locomotion	**B v CB**	3.41	<0.01 (0.005)
	B v CB +30	0.18	NS (0.98)
	CB v CB +30	-3.20	<0.01 (0.008)
sit	**B v CB**	-2.63	< 0.05 (0.033)
	B v CB +30	-1.78	NS (0.19)
	CB v CB +30	0.84	NS (0.68)

Table 5.10 Results of post-hoc Tukey tests, comparisons between baseline, capture baseline and capture baseline + 30 for agitated locomotion and sitting for all marmosets

With a Bonferroni correction placing significance at 0.008 the Tukey test results for agitated locomotion retain their significance (although the result for CB v CB +30 is borderline), but the result for sitting fails to meet this criterion.

Effect of training and positive human interaction

Analysis of the data from baseline and the two training sessions recorded was undertaken to assess the impact on the behaviour of the marmosets of training or interaction with a person. When behaviour at baseline (B) was compared with behaviour following both the first (T1) and tenth (T10) training session durations of locomotion, sitting and contact sitting were all significantly different across the three observations. Neither agitated locomotion nor watchful behaviour was similarly changed (Figures 5.11, 5.12, Table 5.11). Post-hoc analysis showed that locomotion following the first training session (T1) was significantly higher than that exhibited by the marmosets either at baseline or following the tenth training session. The marmosets spent significantly longer sitting alone at baseline than following either T1 or T10 whilst the amount of contact sitting increased significantly across the three observations, with more recorded at both T1 and T10 than baseline, and more at T10 than at T1 (Table 5.12).

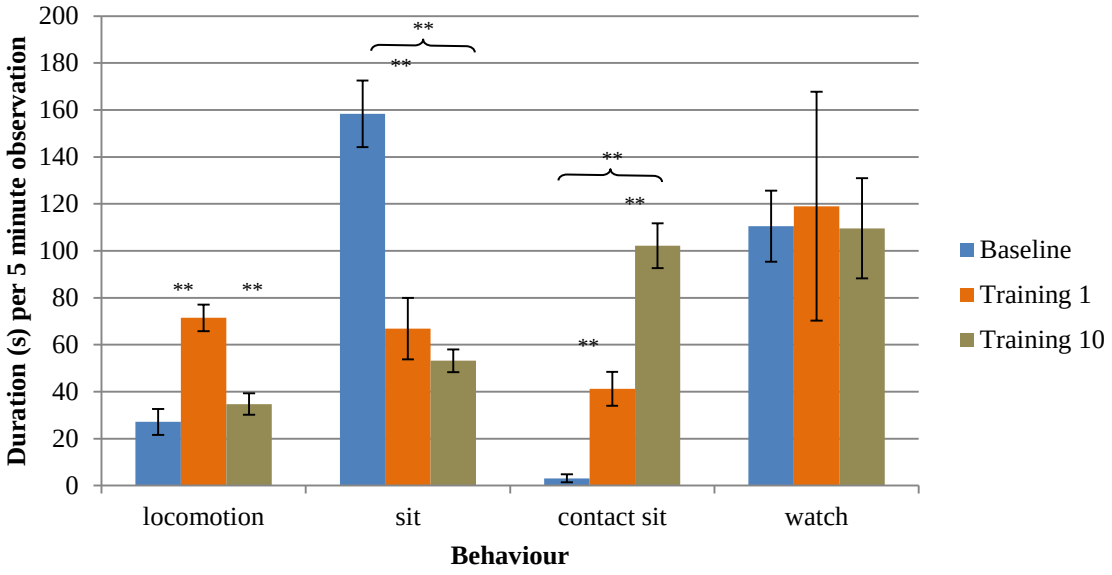

Figure 5.11 Mean durations (in seconds) of longer duration behaviours (> 20 s) per 5 minute observation across baseline, training session 1 and capture training session 10 (* $p < 0.05$, ** $p < 0.01$) **Error bars show standard error of the mean.**

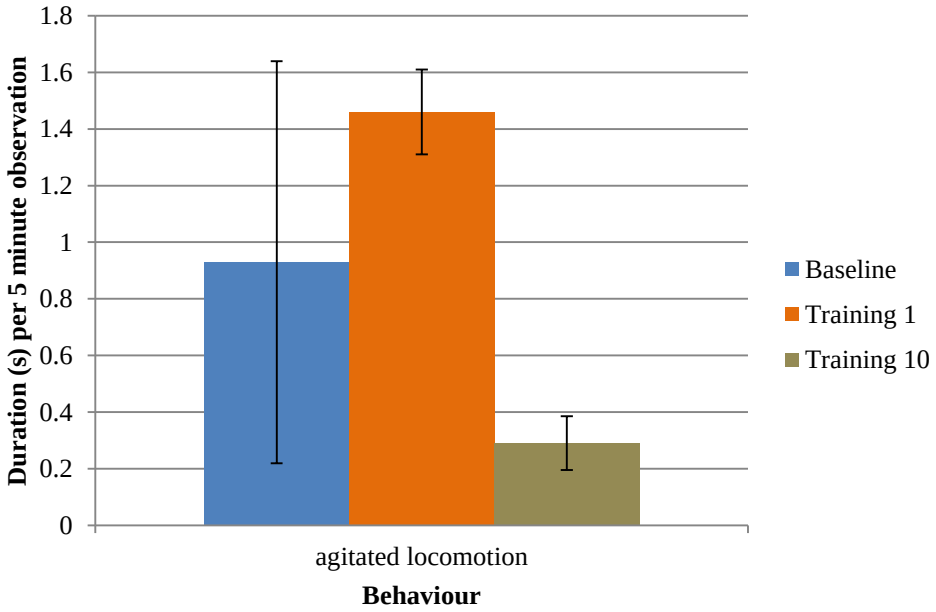

Figure 5.12 Mean durations (in seconds) of shorter duration behaviours (< 20 s) per 5 minute observation across baseline, training session 1 and training session 10 (* $p < 0.05$, ** $p < 0.01$) **Error bars show standard error of the mean.**

Behaviour	$F_{2,36}$	p
locomotion	20.56	<0.001
agitated locomotion	1.86	NS (0.17)
sit	23.1	<0.001
contact sit	50.8	<0.001
watch	0.15	NS (0.86)

Table 5.11 Results of repeated measures ANOVA on durations of recorded behaviours across observations at baseline, training session 1 and training session 10 for all marmosets

Behaviour	Comparison	T	p
locomotion	**B v T1**	5.99	<0.001
	B v T10	1.02	NS (0.57)
	T1 v T10	-4.97	<0.001
sit	**B v T1**	-5.43	<0.001
	B v T10	-6.25	<0.001
	T1 v T10	-0.81	NS (0.70)
contact sit	**B v T1**	3.84	0.001
	B v T10	9.99	<0.001
	T1 v T10	6.15	<0.001

Table 5.12 Results of post-hoc Tukey tests, comparisons between baseline, training session 1 and training session 10 for locomotion, sitting and contact sitting for all marmosets

All of the results for these post-hoc Tukey tests are still significant when a Bonferroni correction sets the level for significance at 0.0056.

Trained animals

Analysis of the data from the four successfully trained marmosets was carried out in order to identify any patterns of behaviour which may be related to the training programme and also to assess if the marmosets behavioural response to a capture using the detachable panel, trained capture (TC), differed from that seen when the marmosets were captured in their usual way prior to any training (CB). Durations of locomotion, agitated locomotion, sitting and contact sitting were all significantly different across the five observations (B, CB, CB + 30, TC and TC + 30) included in the analysis, whilst only watchful behaviour remained unaffected (Table 5.13).

Behaviour	**$F_{4,15}$**	**p**
locomotion	6.53	< 0.01 (0.003)
agitated locomotion	3.90	< 0.01 (0.023)
sit	6.36	< 0.01 (0.003)
contact sit	14.28	<0.001
watch	0.47	NS (0.76)

Table 5.13 Results of repeated measures ANOVA on durations of recorded behaviours across observations at baseline, capture baseline, capture baseline + 30, trained capture and trained capture + 30 for trained marmosets

Post-hoc analysis of the locomotion data revealed that the marmosets were engaged in this behaviour significantly more following the baseline capture (CB) than 30 minutes later at baseline capture + 30 (CB + 30). The marmosets also spent significantly more

time in locomotion at CB than at trained capture + 30 (TC + 30). Interestingly there were no differences in the duration of locomotion between the two types of capture (CB and TC) (Figure 5.13, Table 5.14).

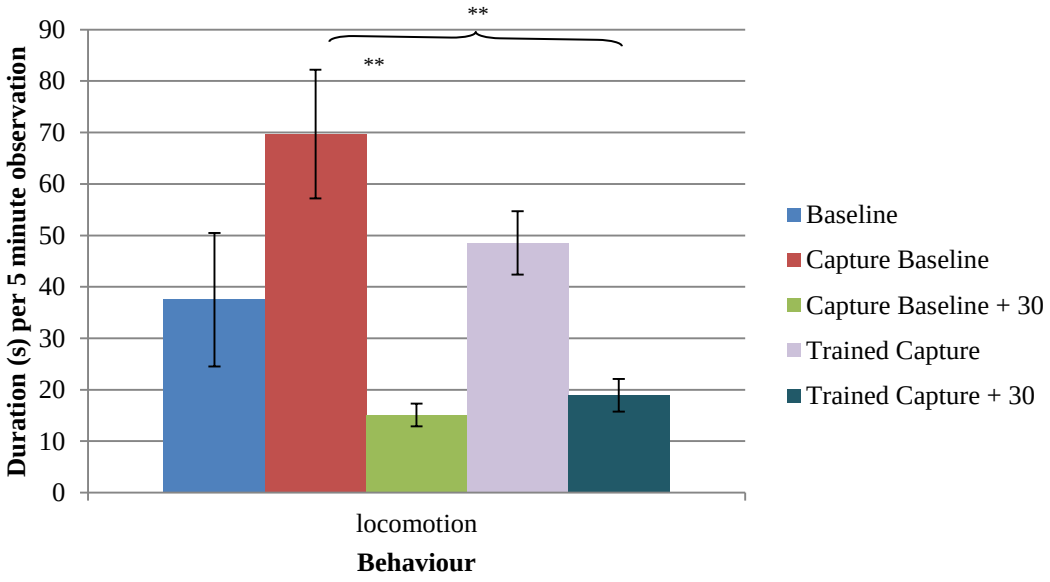

Figure 5.13 Mean duration (in seconds) of locomotion per 5 minute observation across baseline, capture baseline, capture baseline + 30, trained capture and trained capture + 30 for trained marmosets (* $p < 0.05$, ** $p < 0.01$) **Error bars show standard error of the mean**

Behaviour	Comparison	T	p
locomotion	**B v CB**	2.60	NS (0.12)
	B v CB + 30	-1.81	NS (0.40)
	B v TC	0.89	NS (0.90)
	B v TC + 30	-0.50	NS (0.58)
	CB v CB + 30	-4.41	< 0.01 (0.004)
	CB v TC	-1.70	NS (0.46)
	CB v TC + 30	4.10	< 0.01 (0.007)
	CB + 30 v TC	2.70	NS (0.10)
	CB + 30 v TC + 30	0.31	NS (1.00)
	TC v TC + 30	-2.39	NS (0.17)

Table 5.14 Results of post-hoc Tukey tests, comparisons between baseline, capture baseline, capture baseline + 30, trained capture and trained capture + 30 for locomotion for trained marmosets

The amount of time the marmosets spent sitting was significantly greater at B than at TC and TC + 30 and was also significantly greater at CB + 30 than at TC and TC + 30 (Figure 5.14, Table 5.15).

The result for CB v CB +30 is still significant when a p value corrected for multiple comparisons by a Bonferroni correction is set at 0.005, however the result for CB v TC +30 fails to reach significance at this new level.

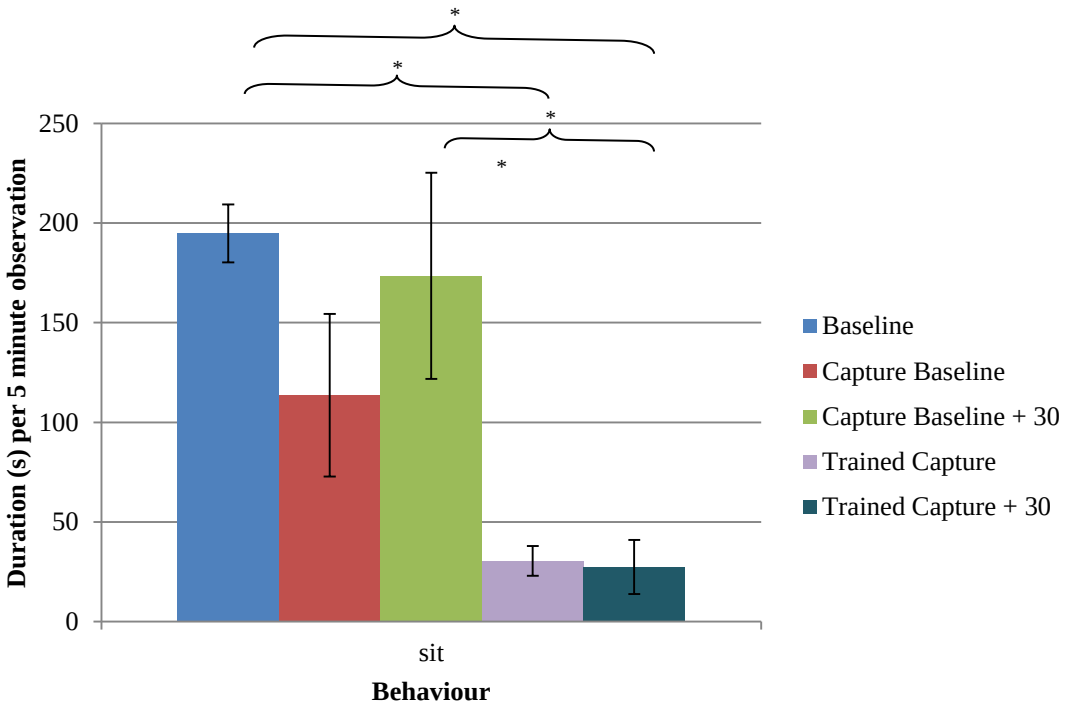

Figure 5.14 Mean duration (in seconds) of sitting per 5 minute observation across baseline, capture baseline, capture baseline + 30, trained capture and trained capture + 30 for trained marmosets (* $p < 0.05$, ** $p < 0.01$) **Error bars show standard error of the mean.**

Behaviour	Comparison	T	p
sit	**B v CB**	-1.85	NS (0.38)
	B v CB + 30	-0.49	NS (0.99)
	B v TC	-3.75	< 0.05 (0.014)
	B v TC + 30	-3.82	< 0.05 (0.012)
	CB v CB + 30	1.37	NS (0.66)
	CB v TC	-1.90	NS (0.36)
	CB v TC + 30	-1.97	NS (0.33)
	CB + 30 v TC	-3.27	< 0.05 (0.036)
	CB + 30 v TC + 30	-3.34	<0.05 (0.031)
	TC v TC + 30	-0.07	NS (1.00)

Table 5.15 Results of post-hoc Tukey tests, comparisons between baseline, capture baseline, capture baseline + 30, trained capture and trained capture + 30 for sitting for trained marmosets

The amount of time the marmosets spent contact sitting hugely increased following training. This behaviour was seen infrequently in the observations prior to training, but accounted for over one-third of the time budget following training. Contact sitting at TC was significantly greater than at B, CB and CB + 30. Similarly the marmosets spent significantly more time engaged in this behaviour at TC + 30 than at B, CB and CB + 30 (Figure 5.15, Table 5.16). None of these results are significant when a Bonferroni correction is applied and a p value of 0.005 is used.

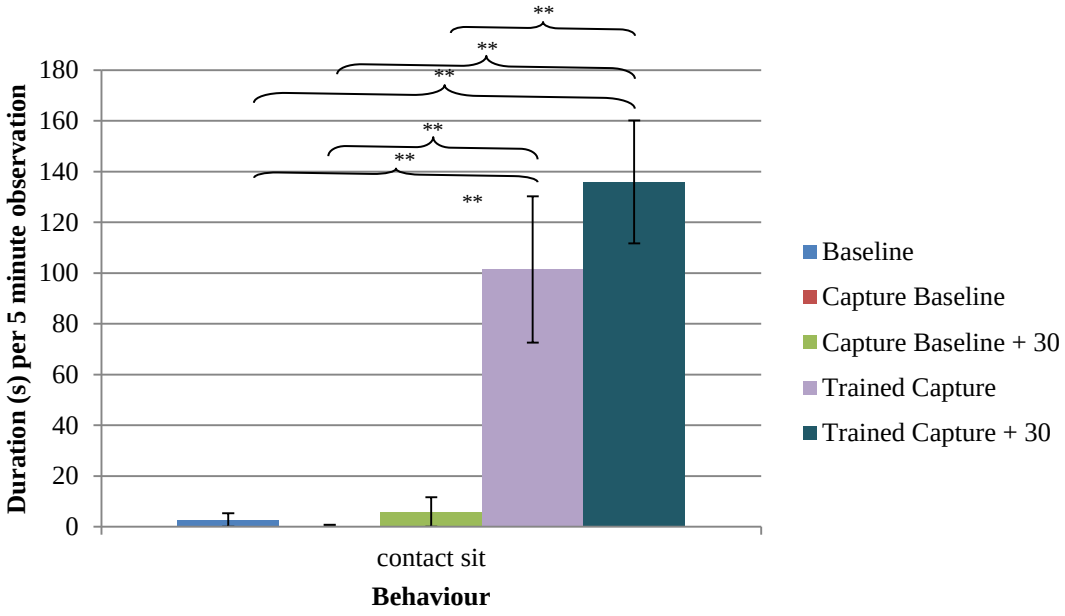

Figure 5.15 Mean duration (in seconds) of contact sitting per 5 minute observation across baseline, capture baseline, capture baseline + 30, trained capture and trained capture + 30 for trained marmosets (* $p < 0.05$, ** $p < 0.01$) **Error bars show standard error of the mean.**

Behaviour	Comparison	T	p
contact sit	**B v CB**	-0.09	NS (1.00)
	B v CB + 30	0.13	NS (1.00)
	B v TC	4.08	< 0.01 (0.007)
	B v TC + 30	5.52	< 0.01 (0.005)
	CB v CB + 30	0.22	NS (1.00)
	CB v TC	4.18	<0.01 (0.006)
	CB v TC + 30	5.61	<0.001
	CB + 30 v TC	3.96	0.01
	CB + 30 v TC + 30	5.39	<0.001
	TC v TC + 30	1.43	NS (0.62)

Table 5.16 Results of post-hoc Tukey tests, comparisons between baseline, capture baseline, capture baseline + 30, trained capture and trained capture + 30 for contact sitting for trained marmosets

The results of this Tukey test for B v TC + 30, CB v TC + 30 and CB + 30 v TC + 30 are particularly robust, and remain significant when a Bonferroni correction increases the criteria for significance to 0.005.

Watchful behaviour did not differ significantly across the five observations included in this analysis (Figure 5.16). The duration of agitated locomotion exhibited by the marmosets was significantly greater at CB than at CB + 30 and TC + 30. There was also a non-significant trend for less agitated locomotion at TC than at CB (Figure 5.17, Table 5.17). None of these results were significant when a Bonferroni correction was applied and a p value set at 0.005.

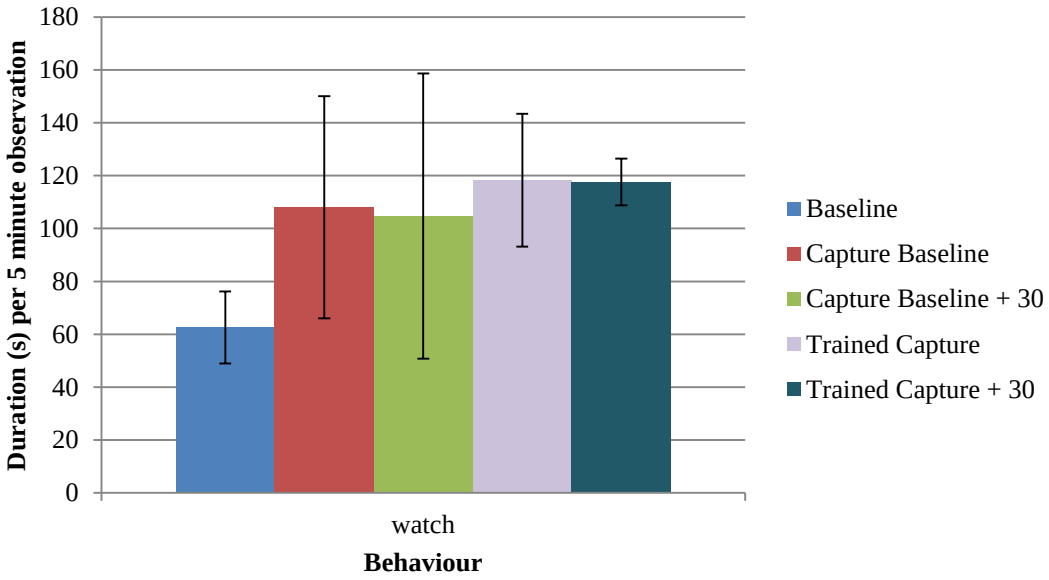

Figure 5.16 Mean duration (in seconds) of watchful behaviour per 5 minute observation across baseline, capture baseline, capture baseline + 30, trained capture and trained capture + 30 for trained marmosets (* $p < 0.05$, ** $p < 0.01$) **Error bars show standard error of the mean.**

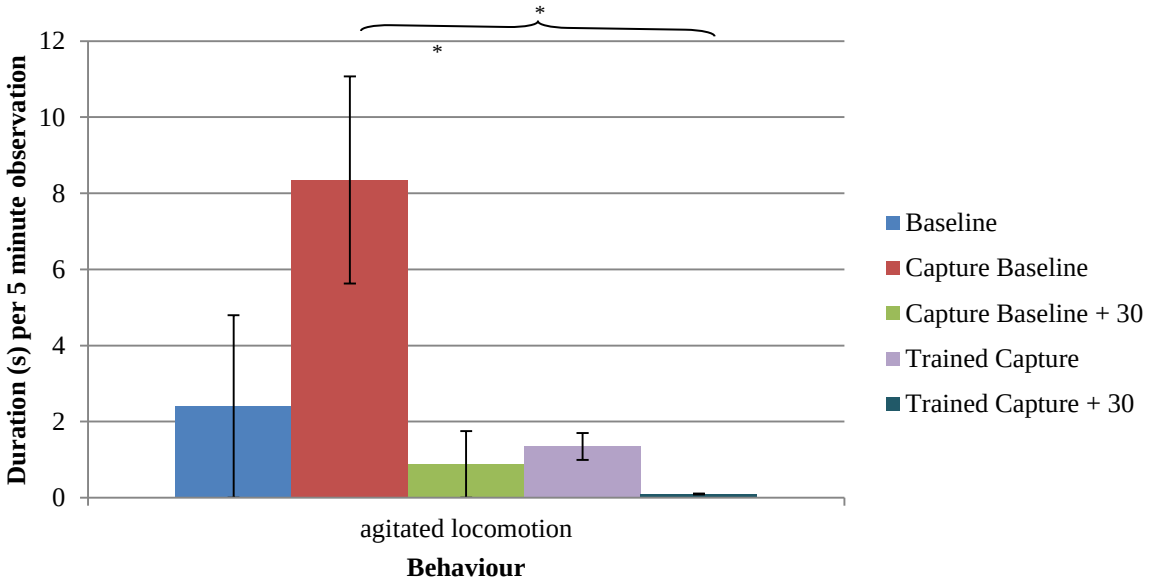

Figure 5.17 Mean duration (in seconds) of locomotion per 5 minute observation across baseline, capture baseline, capture baseline + 30, trained capture and trained capture + 30 for trained marmosets (* $p < 0.05$, ** $p < 0.01$) **Error bars show standard error of the mean.**

Behaviour	Comparison	T	p
agitated locomotion	**B v CB**	2.51	NS (0.14)
	B v CB + 30	-0.64	NS (0.97)
	B v TC	-0.44	NS (0.99)
	B v TC + 30	-0.97	NS (0.86)
	CB v CB + 30	-3.15	< 0.05 (0.04)
	CB v TC	-2.95	NS (0.06)
	CB v TC + 30	-3.48	< 0.05 (0.02)
	CB + 30 v TC	0.20	NS (1.00)
	CB + 30 v TC + 30	-0.33	NS (1.00)
	TC v TC + 30	-0.53	NS (0.98)

Table 5.17 Results of post-hoc Tukey tests, comparisons between baseline, capture baseline, capture baseline + 30, trained capture and trained capture + 30 for agitated locomotion for trained marmosets

Further analysis of the data from the successfully trained marmosets comparing their behaviour following baseline capture (CB) and standard capture (SC) showed that whilst locomotion, agitated locomotion, sitting and watchful behaviour were not significantly different between these two observations, the duration of contact sitting did change significantly. The amount of contact sitting was significantly higher following SC than following CB (Figure 5.18, 5.19, Table 5.18). This result did not reach significance however following a Bonferroni correction with significance set at 0.01.

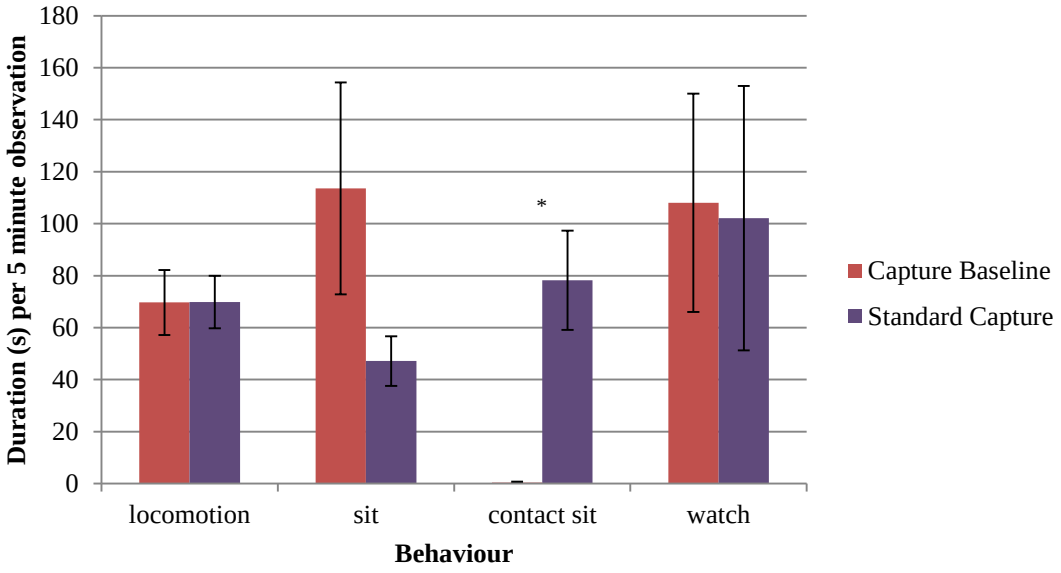

Figure 5.18 Mean durations (in seconds) of longer duration behaviours (> 20 s) per 5 minute observation across capture baseline and standard capture for trained marmosets (* $p < 0.05$, ** $p < 0.01$) **Error bars show standard error of the mean.**

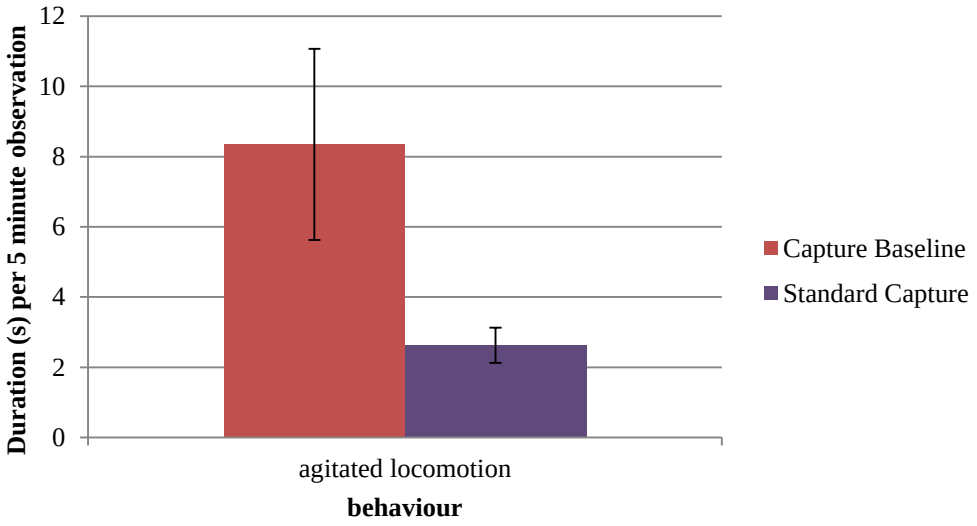

Figure 5.19 Mean durations (in seconds) of shorter duration behaviours (< 20 s) per 5 minute observation across capture baseline and standard capture for trained marmosets (* $p < 0.05$, ** $p < 0.01$) **Error bars show standard error of the mean.**

Behaviour	$F_{1,5}$	p
locomotion	0.00	NS (0.99)
agitated locomotion	4.10	NS (0.09)
sit	2.36	NS (0.18)
contact sit	7.10	< 0.05 (0.04)
watch	0.01	NS (0.91)

Table 5.18 Results of repeated measures ANOVA on durations of recorded behaviours across observations at capture baseline and trained capture for trained marmosets

Response to capture following training/interaction

When data from all 14 marmosets were analysed together, recorded durations of locomotion, agitated locomotion, sitting, contact sitting and watchful behaviour were all significantly different at standard capture (SC) than at baseline capture (CB). Durations of both locomotion and contact sitting were significantly greater at SC than at CB, whilst the amount of time the marmosets spent engaged in sitting, watchful behaviour and agitated locomotion was significantly lower at SC than CB (Figure 5.20, 5.21 Table 5.19). Locomotion and contact sitting were still found to be significantly higher following standard capture, and sitting alone significantly lower, when a Bonferroni correction was applied and the level of significance set at 0.01.

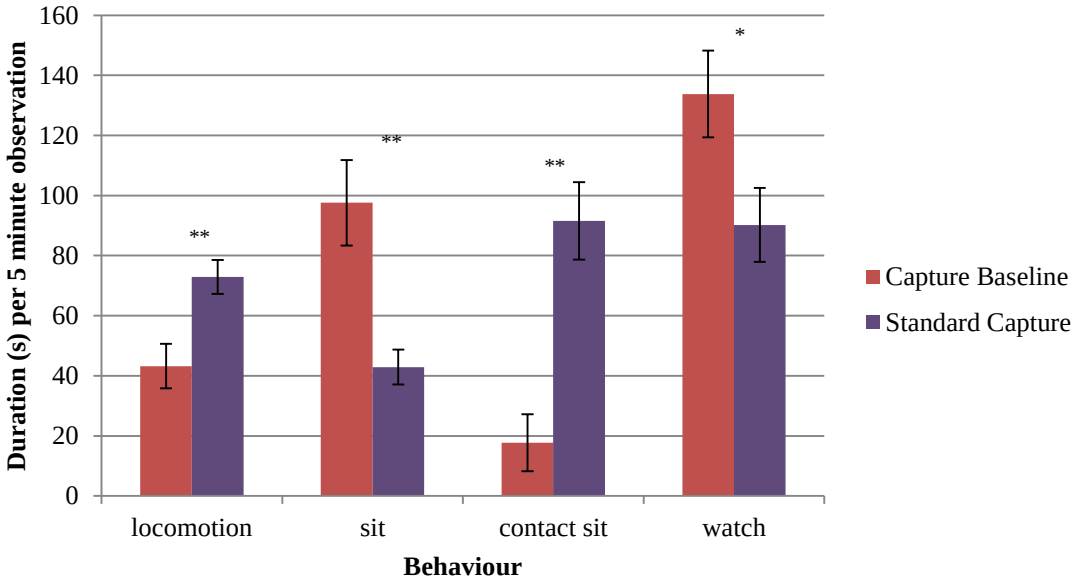

Figure 5.20 Mean durations (in seconds) of longer duration behaviours (> 20 s) per 5 minute observation across capture baseline and standard capture for all marmosets (* $p < 0.05$, ** $p < 0.01$) **Error bars show standard error of the mean.**

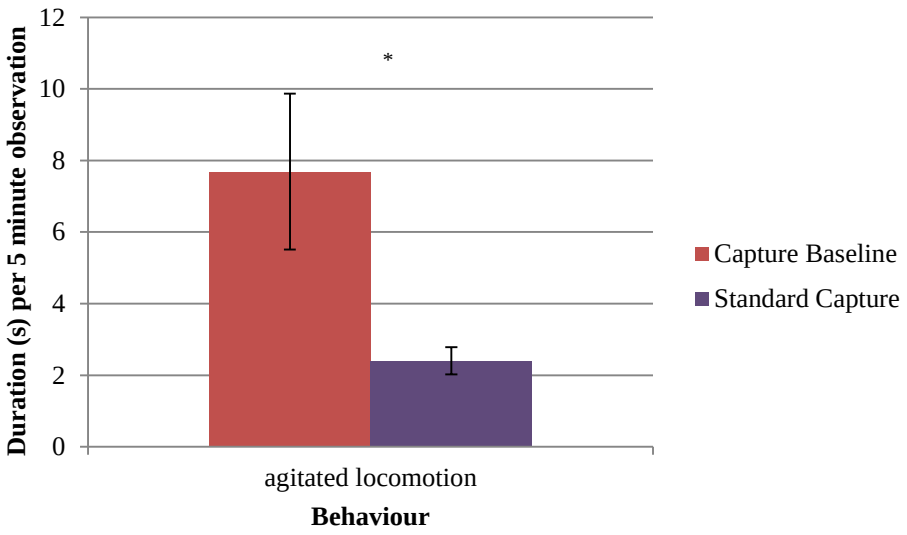

Figure 5.21 Mean durations (in seconds) of shorter duration behaviours (< 20 s) per 5 minute observation across capture baseline and standard capture for all marmosets (* $p < 0.05$, ** $p < 0.01$ **Error bars show standard error of the mean.**)

Behaviour	$F_{1,26}$	p
locomotion	10.14	< 0.01 (0.004)
agitated locomotion	5.78	< 0.05 (0.024)
sit	12.72	0.001
contact sit	21.22	<0.001
watch	5.29	< 0.05 (0.030)

Table 5.19 Results of repeated measures ANOVA on durations of recorded behaviours across observations at capture baseline and trained capture for all marmosets

5.6 DISCUSSION

5.6.1 Success Rates and Time Investment

Training marmosets to come to a panel and cooperate with capture proved to be possible with a time investment of less than three hours for some individuals but not for all. Due to the small sample size it is not possible to determine any factors which may explain these differences, however this was not the aim of the study. Showing that this behaviour can be achieved in a laboratory setting is an important finding in itself. There has been little work with marmosets which has considered training for more aversive tasks such as this, as it has been often considered to be beyond the capabilities or temperament of the species. Whereas apes and Old World monkeys have been trained for complex and often aversive tasks (e.g. Schapiro *et al*, 2005, Videan *et al*, 2005, Coleman *et al*, 2008) the training of New World monkeys in the laboratory has been limited neutral or less challenging tasks such as weighing and target training (e.g. Bassett *et al*, 2003, McKinley *et al*, 2003). Establishing that it is possible to train individuals who have experienced normal laboratory life, with the challenges this presents, is encouraging for the further use of this type of training. Whilst it may not be feasible to train all marmosets in a laboratory, there may be individuals and studies which may benefit from training this behaviour, for example individuals who will be long term residents in the laboratory, and studies which require frequent capture, although the cooperation of individuals may deteriorate if capture is followed by invasive and aversive procedures such as blood collection.

Although no temperament tests were carried out in this study, temperament has been shown to affect training success and time investment in marmosets (Chapter 3) and other primate species (cynomolgus macaques, Chapter 2, rhesus macaques, Coleman *et al*, 2005), so it may be that those individuals who learnt the task were temperamentally suited to cooperating with this type of task, perhaps by being bolder than other marmosets, or perhaps less fearful of humans. It is also interesting that it appeared that some individuals would not learn the task irrespective of time investment, perhaps again due to temperament or possibly due to previous experience, or a combination of both. Training this behaviour at a young age, prior to any experimental work, may help remove the effect of previous experience, but temperamental differences will remain. A larger scale study would be required to identify these differences.

The time investment required to learn the task ranged quite considerably, with the marmoset who took the longest to learn requiring over 1.5 times the time than the fastest marmoset. Again this suggests individual differences play an important role in the learning of this task. Whilst individual differences are seen in the speed of learning less aversive tasks (Chapter 3), these are likely to be accentuated by the more aversive nature of this task. There was, however, less variability in this task than in the box training where the slowest marmoset took over four times longer than the fastest marmoset, albeit with a much larger sample size. The time investment required was not inconsiderable, and this might prove to be problematic in a laboratory with large numbers of animals. As discussed above one of the main aims of this study was to assess if training for an aversive task was possible with marmosets, and it is feasible that refinements in training protocol, such as training at an early age prior to other potentially aversive experiences, may help reduce time investment. The results are

encouraging in that it has proved possible to train the marmosets, and the time investment may be feasible for smaller groups of animals specifically selected for particular long term studies.

5.6.2 Cortisol Data

Measuring cortisol from saliva samples proved to be successful, with over three quarters of samples taken resulting in a cortisol measurement. It is likely that with further experience and refinement of technique this could be increased, showing that this is a promising non-invasive method for cortisol measurement in marmosets as previously determined by Cross and colleagues (Cross & Rogers, 2004, Cross *et al*, 2004, Pines *et al*, 2004) .

Baseline data

There was considerable variation between individuals in their recorded salivary cortisol measurements, as show in Figures 6.1 and 6.2, however it is possible to identify individual patterns, for example that levels recorded for the marmoset named Monica (Moni, Figure 6.1) remained at the upper end of the range at each sample point.

Although mean baseline levels for females were approximately one-third higher than those for males, this did not prove to be significant, most probably due to the high levels of variation. That this pattern was seen (even if not significant) shows good agreement with Johnson *et al* (1996) who measured blood cortisol in larger numbers of marmosets, and when I calculated means for all of their data the females had around one-third higher cortisol levels than males. The recorded salivary cortisol levels are

however considerably greater than those measured previously for common marmosets (Cross & Rogers, 2004, Cross *et al*, 2004). Previously reported salivary cortisol levels have been found to be around 500nm/l at 1300h, the midpoint of data collection in this study (Cross & Rogers, 2004). That the mean salivary cortisol levels are over twice this level in this study gives cause for concern. They are however more comparable to those measured when marmosets are disturbed due to high levels of noise and activity in the laboratory (Cross *et al*, 2004), although these data were recorded later in the day when cortisol levels have been shown to be lower than in the mornings (Cross & Rogers, 2004). This may suggest that the data collected at baseline were not a true representation of baseline levels but that perhaps the marmosets were stressed either by the saliva collection or by external factors. No unusual events took place on any of the days on which these data were collected, so it seems unlikely that this was the cause of such high cortisol levels, leading to the conclusion that the collection of salivary cortisol leads to increased stress levels in the marmosets. This stress may be due to the actual physical process of taking the sample or by the necessity of closely interacting with a person, or a combination of the two. The marmosets however were free to choose whether to come and chew the cotton bud to give a sample, so even if they were stressed by the process the availability of a favoured food outweighed this stress. Interestingly a similar result has been found in baboons (*Papio hamadryas*), whereby salivary cortisol was higher at baseline than either pre- or post-training collections (O'Brien *et al*, 2008). The authors attributed this to increased stress from the animals associating the training area with previous medical interventions, which had dissipated by the time training took place.

Habituation to the saliva collection data was considered but not carried out as it was felt that this may undermine the results of the baseline capture data due to the marmosets receiving positive interactions with people prior to the start of the study. As one of the key aspects being recorded was how this type of interaction differed from PRT interactions this was not desirable. Further to this, in other studies, marmosets who were hand feeding did not show increased cortisol levels as a result of the sample collection (Cross & Rogers, 2004).

If baseline cortisol levels were increased due to the stress of the sample collection, this may also explain why no differences were seen between baseline levels and those recorded following capture baseline, as under both circumstances the marmosets were experiencing some stress. Thirty minutes after capture baseline (CB + 30) the marmosets' mean salivary cortisol levels were much more closely comparable with those seen in other studies at 632.5 nm/l, as compared to a mean concentration of approximately 500mn/l at 1300h reported by Cross and Rogers (2004), and significantly lower than concentrations recorded for baseline, but not capture baseline. This suggests that by the time these post-capture data were collected (the fourth and sixth time saliva collection was attempted) the marmosets were beginning to become habituated to the process. It may be therefore that the data collected at capture baseline + 30 represents a more accurate representation of baseline salivary cortisol concentrations than those data collected at the baseline sampling point.

Effects of training and positive human interaction

In both the "PANEL" and "INTERACT" groups salivary cortisol concentrations were significantly lower following training sessions than when recorded at baseline. This is true at both training session 1 (T1) and session 10 (T10). Training may lead to lower cortisol concentrations due to some form of calming effect or possibly due to a decrease in activity, and this may explain this result. However given the results of the analysis of the baseline data it seems more likely that this provides further evidence for the baseline concentrations recorded representing a stressed level rather than an unstressed cortisol concentration. O'Brien *et al* (2008) found that baboon salivary cortisol concentrations were lower pre- and post-training than at baseline, a finding which they too attributed to the baseline measurements being raised due to previous experience, rather than the training-related measurements being lowered. Salivary cortisol concentrations following the training sessions seem more comparable to those seen at capture baseline + 30, which may be a better indicator of true unstressed baseline levels. If this is the case then it seems that training may not cause the marmosets to experience physiological stress, or at least one represented by an increase in cortisol levels. There is however no evidence from this result that laboratory-housed marmosets are stressed by the training process, a finding which is important given the recent increase in the use of training with these animals, and is in agreement with data from baboons (O'Brien *et al*, 2008). However if baseline levels are artificially high due to the increased stress caused by interacting with humans, it may be that early training interactions may cause marmosets to become stressed. This seems to decrease relatively quickly with positive interactions. Given that marmosets in this institution are handled at least once per week,

it seems that any negative associations they have formed with people can be quickly and easily overcome through the use of relatively few positive interactions.

Interestingly, in this analysis, whether the marmosets went on to be successfully trained or not proved to be significant, with those who were successfully trained having significantly higher cortisol concentrations than those untrained in both treatment groups. Training records show that by training session 10 all four of the successfully trained marmosets had completed Stage 3 of the training protocol (remaining on panel when door opened), and were working on Stage 4 (remaining on panel when tail touched), so were having very close interactions with the trainer. The remaining three marmosets in the "PANEL" group had successfully reached Stage 2 (coming to the panel), and were working on Stage 3, but had not reached the point of the trainer being able to open the door. This may have meant that the trained marmosets were more stressed by T10 due to the closer interaction they were having with the trainer than the other marmosets, and possibly due to the conflict between wanting to remain on the panel for reward but also wanting to flee to the back of the cage as they normally would have done when the cage was opened. There was however no evidence of an interaction between observation and whether the marmosets were successfully trained, so nothing suggests that by T10 they were more stressed than the untrained marmosets, but small sample sizes and high variation mean interactions of this kind are difficult to identify.

Trained animals

Analysis of the data for the four trained marmosets show that there was a weak trend for cortisol concentrations to differ significantly across the baseline, capture baseline,

capture baseline + 30, trained capture and trained capture + 30 observations. However it was not valid to carry out further post-hoc analysis. When the results from capture baseline and trained capture were compared directly, although again a weak trend was seen for cortisol concentrations to be lower at TC than at CB, this was not significant. It is therefore not possible to conclude that the marmosets find the trained capture less stressful than the capture baseline, at least from the cortisol data. Mean cortisol concentration at CB was around 1.7 times greater than that at TC, so it may be that with a greater sample size some significant differences would be seen in these data. By providing the marmosets with an opportunity to cooperate with capture, it likely improves their welfare by providing them with predictability and control, both of which are known to positively impact on welfare (Bassett & Buchanan-Smith, 2007), so this method of capture may have further benefits which are not evident in this analysis.

Response to capture following training/positive interaction

Recorded salivary cortisol concentrations were significantly lower following standard capture than following capture baseline, but no other factors were significant in this analysis. So whilst experiencing training and positive interactions reduces the stress of capture, there is no evidence to suggest that training in itself has any additional benefits in comparison to positive human interactions in terms of reducing the physiological stress of capture. It may be the case that the reduction of fear of humans is the key factor in reducing the stress of capture, and this would explain why both the "PANEL" and "INTERACT" groups respond in the same way to the stressor even after their training or interaction. It is also possible however that the trained marmosets find the standard capture more stressful than a trained capture as the standard capture is unusual

to them after becoming accustomed to the trained capture protocol, but that it is still less stressful than capture baseline. Due to the small sample size and lack of significant differences for other analyses, this analysis was not carried out on this data set.

That training and positive interactions reduce the physiological stress of capture, as measured by salivary cortisol, is however promising. Whilst training may have additional benefits in terms of environmental enrichment (e.g. Bourgeois & Brent, 2005, Coleman & Maier, 2010), and improving laboratory husbandry and procedures, that simple positive interactions prove to have such a benefit to the welfare of laboratory-housed marmosets is extremely encouraging. Positive interactions take very little time and require minimal staff training, so are cheap and easy to implement. This means they can be introduced quickly and easily into laboratory routines, and therefore have the potential to improve the welfare of a large number of laboratory-housed primates, irrespective of the institution they are in, the study they are taking part in or their housing conditions. Indeed, the majority of technical staff follow this career as they like to interact with animals, and given the clear evidence for the benefit, socialisation with primates should become routine. It benefits the animals, the staff, and most likely the scientific output also.

5.6.3 Behavioural Data

Baseline data

Analysis of the baseline data (baseline, capture baseline and capture baseline + 30) showed that the marmosets' behaviour changes significantly across these three

observations. They exhibited much more agitated locomotion following the capture baseline and less sitting alone, whilst levels of normal locomotion, contact sitting and watchful behaviour remained the same following this event. Behaviour at capture baseline + 30 did not differ significantly from that observed at baseline. Contact sitting was found to be ten times greater at this observation than baseline, but this did not reach significance.

Normal locomotion has been seen to increase in response to a comparable stressor in both macaques (Chapter 2) and in marmosets (Bassett *et al*, 2003), but remained unaffected in another study of marmosets (Chapter 3). Increased levels of locomotion are typically associated with reduced welfare (Chamove, 1989, Chamove & Anderson, 1989) and increased stress (Bassett *et al*, 2003), so it seems slightly incongruous that in this study locomotion does not increase following capture which is potentially stressful. It may be that, in this colony at GSK at least, the marmosets do not find capture stressful, however this seems counter-intuitive and is not supported by the other behaviours the marmosets exhibit. In both of these cases agitated locomotion was recorded separately and this behaviour increased significantly following the stressor. As this is a behaviour which was not recorded in the majority of the studies which saw an overall increase in locomotion, perhaps this accounts for the change in locomotion, although in macaques increases in both types of locomotion are seem (Chapter 2).

That agitated locomotion increases so strikingly following capture provides good evidence that the marmosets find being caught stressful, and supports earlier studies which find this also occurs in other colonies of marmosets (Chapter 3) and in macaques (Chapter 2). It is also seen less in marmosets housed in larger cages than in smaller

cages, and less in animals with access to outdoor cages than those housed entirely indoors (Badihi, 2006). Agitated locomotion seems to be a particularly strong indicator of stress across a range of primates, and one which perhaps deserves to be more widely used. The amount of agitated locomotion exhibited by the marmosets 30 minutes after capture is not significantly different from the level seen at baseline observations, and is significantly lower than that seen at capture baseline, suggesting that they have recovered from any stress which capture causes relatively quickly.

Whilst sitting alone decreased significantly following the stressor, levels of this behaviour were not significantly different at capture baseline + 30 than at either baseline or capture baseline. Sitting alone, or inactivity, seems to be another good indicator of stress in marmosets with exposure to a stressor leading to decreases in the performance of this behaviour (Bassett, 2003, Chapter 3 this thesis), whilst in macaques sitting behaviour is seen to be unaffected by stress (Chapter 2), emphasising the importance of identifying species-specific behaviour stress related patterns. Contact sitting is unaffected by exposure to the stressor in marmosets, in agreement with the previous study (Chapter 3), however in cynomolgus macaques this behaviour decreases under the same circumstances (Chapter 2). Similarly that watchful behaviour remains at the same level following the stressor supports earlier findings that in marmosets at least this behaviour does not seem to be an indicator of stress, whereas in macaques watchful behaviour increases post-stressor (Chapter 2).

The overall pattern of behaviour for these baseline observations suggest that whilst the marmosets find being captured stressful, they recover relatively quickly, within 30 minutes, from this stress. If however this stress could be reduced by finding alternative

methods of capture, such as training them to cooperate with it, it could go some way to improving the welfare of laboratory-housed marmosets.

Effects of training and positive human interaction

Whilst there is an increasing amount of literature considering the effect of a training programme (e.g. Bourgeois & Brent, 2005, Coleman & Maier, 2010), few studies have looked at how primates respond to the actual training interaction (e.g. O'Brien *et al*, 2008). There was no effect of whether the marmosets were in the "PANEL" or "INTERACT" treatment group on how they responded to the training or positive human interaction. By the tenth training session all marmosets in the "PANEL" group were coming to the detachable panel and being rewarded there, so were having a different experience to the "INTERACT" marmosets who were just being hand fed, any differences therefore in how they perceived the interaction should have been apparent by this stage. That there was no difference between the two groups provides evidence that the process of learning is perceived no differently by the marmosets than simply interacting in a positive manner with a person. There is nothing to indicate that the marmosets find training more stressful than interacting, or *vice versa*.

Recorded durations of locomotion, sitting and contact sitting all change significantly across observations at baseline (B), first training session (T1) and tenth training session (T10), whilst levels of agitated locomotion and watchful behaviour remain unchanged. Locomotion is significantly higher following the first training session than the baseline or tenth training session which may suggest that on their first introduction to training or interacting the marmosets experience a degree of anxiety or uncertainty, a finding also

reported in Chapter 4. However there was no corresponding change in agitated locomotion which has been shown to be the most reliable and strongest behavioural indicator of a stress response (Chapter 2, Chapter 3). Increases in locomotion have been shown to occur in response to a stressor in primates (Coe *et al*, 1982, Chamove, 1989, Bassett *et al*, 2003), but this behaviour does not seem to be as reliable an indicator of stress in marmosets when agitated locomotion is also recorded (Chapter 3). Locomotion however may be an indicator of a slightly different but related emotional state. Perhaps increases in locomotion in response to novelty and mild stress indicate a more uncertain, 'disturbed' state rather than a fearful 'stressed' one, as suggested in Chapter 4. It would certainly be interesting to explore this further to establish if this was the case with other events. However the marmosets seem to find their initial encounter with the trainer somewhat 'uncomfortable', but by the tenth session this has diminished and there is no evidence of behavioural disturbance.

The marmosets spent more time sitting alone at baseline than at either T1 or T10, but contact sitting increased across the three observations. Sitting alone is generally considered to be an indicator of reduced welfare, whilst contact sitting is normally regarded as a more positive behaviour (Kitchen & Martin, 1996). It seems that in response to the first training session the marmosets spend more time engaged in locomotion at the expense of sitting, whilst by T10 they spend the time contact sitting rather than sitting alone. That the training programme leads to an increase in contact sitting provides further evidence that training and positive human interactions have benefits for the welfare of laboratory-housed marmosets.

Watchful behaviour remains stable across the three observations suggesting that the marmosets are no more or less interested in humans with an increased level of interaction. Whilst training does not appear to make them pay less attention to people, it does not lead to them focussing more on humans in the hope of receiving food, in contrast with the findings of Bassett (2003) who found that trained marmosets spent more time watching people than untrained animals.

Behavioural responses of the trained marmosets

Although the sample size for those marmosets that were successfully trained was small, some interesting results have nevertheless been shown. Unlike when data from all 14 marmosets were analysed for the trained group only, locomotion at capture baseline + 30 (CB + 30) is significantly lower than at capture baseline (CB), and this behaviour is also significantly lower at trained capture + 30 (TC + 30) than at CB. This may indicate that with this group of animals locomotion increased in response to capture baseline (CB), however with such a small sample size caution needs to be exercised in such interpretations. No differences were seen in levels of locomotion between capture baseline and trained capture (TC), suggesting that the trained capture was no less stressful than normal capture, however as discussed above normal locomotion may not be the best indicator of stress in marmosets. Agitated locomotion followed a similar pattern with the amount the marmosets exhibited at CB being greater than that seen at either CB + 30 or TC + 30. There was however a weak trend for the marmosets to engage in more agitated locomotion following CB than TC. If as discussed above agitated locomotion is a more sensitive measure of stress in marmosets this may indicate that the trained capture is less stressful than the capture baseline, which would

be a very positive and interesting finding. However with the small sample size and lack of a strong statistical significance this is difficult to validate.

Both time spent sitting alone and contact sitting change across the observations for these animals. The marmosets spend more time sitting alone at baseline than following the trained capture or at TC + 30, more time at CB + 30 than TC or TC + 30, more time contact sitting at both TC and TC + 30 than at B, CB and CB + 30. The pattern of these data indicates that irrespective of the whether the marmosets had been captured, training leads to decrease in sitting alone and an increase in contact sitting. This is, to some extent, in agreement with Bassett (2003) who found that training lead to a decrease in inactivity, and the findings for macaques (Chapter 2) where sitting alone was found to decrease following training. Marmosets however have previously shown no change in sitting behaviour following a stressor once trained as compared to a pre-training baseline (Chapter 3). Where contact sitting has been recorded, the time primates spend engaged in this behaviour does not seem to be affected by training (Chapter 2, Chapter 3), although there is some evidence from this study that training leads to increased performance of this behaviour. As discussed above this difference may be due to the very low levels of contact sitting observed prior to training, however it is still encouraging that a positive behaviour can be increased in this manner.

Response to capture following training/interaction

Perhaps the most interesting finding is that when the data for all marmosets are analysed, the behavioural response to standard capture does not differ for the "PANEL" and "INTERACT" groups. The marmosets spend less time engaged in agitated

locomotion, sitting alone and watchful behaviour, but more time in locomotion and contact sitting following SC than they did following CB. The decrease in the performance of agitated locomotion, sitting alone and watchful behaviour alongside the increase in contact sitting provide a convincing argument that both training and positive human interactions go some way to improve the welfare of laboratory-housed marmosets. The increase in locomotion following training could be problematic with the previously considered interpretation of locomotion as an indicator of stress. However given the premise discussed above that increased locomotion is more of an indicator of disturbed behaviour rather than stress, it would seem to fit well with these data. Nevertheless, as has previously been suggested, even brief positive interactions with humans improve the welfare of laboratory-housed primates (Scott, 1990, Baker & Springer, 2006, Rennie & Buchanan-Smith, 2006a), highlighting the importance of socialisation.

Given that the two groups ("PANEL" and "INTERACT") respond in the same way to being captured following training or positive human interaction, there seems little evidence to suggest that the four trained marmosets had become habituated to handling in the process of training, and were therefore less stressed by it. It is therefore likely that the primary benefit of training is the reduction of fear of humans, which can be achieved by positive human interactions, rather than as a result of the choice and control over their capture.

5.6.4 Comparisons Between Cortisol and Behavioural Data

It is not possible to directly map the cortisol data onto the behavioural data for individuals as the cortisol data provides mean values across two days. The patterns in the data are nevertheless interesting. The overall picture provided by the salivary cortisol and behavioural data is a little unclear, but there does seem to be a general pattern for the training or positive human interaction to reduce the signs of stress exhibited by the marmosets. Table 5.20 provides an overview of the results of the comparisons analysed.

Comparison	Indicator					
	cortisol	locomotion	agitated locomotion	sit	contact sit	watch
B v CB	=	=	↑	↓	=	=
B v CB +30	↓	=	=	=	=	=
CB v CB +30	=	=	↓	=	=	=
B v T1	↓	↑	=	↓	↑	=
B v T10	↓	=	=	↓	↑	=
T1 v T10	=	↓	=	=	↑	=
CB v SC	↓	↑	↓	↓	↑	↓

Table 5.20 Summary of physiological and behavioural changes seen in response to comparisons between data collection points. Arrows represent change from first observation indicated in comparison to the second.

There seem to be a number of comparisons where behavioural differences are seen, but the cortisol concentrations either remain the same or contradict the behavioural findings, for example when baseline is compared to the first training session (B v T1),

salivary cortisol concentrations are found to be reduced, but two behavioural indicators of stress, locomotion and sitting alone, both change in a way which indicates a reduction in welfare.

There do not seem to be any further relationships between the behavioural and physiological responses measured. The behavioural data seem to provide a more detailed representation of the marmosets response to the interactions carried out, so whilst the collection of physiological data in the form of cortisol proves to be a useful adjunct in terms of validating the behavioural measurements, these data does not seem to provide further insight into the marmosets stress response. This further emphasises the difficulties in relying upon a single measure of welfare.

5.7 SUMMARY

The published data regarding the training of common marmosets (*C. jacchus*) and other New World primates (NWPs) has primarily been directed at tasks which are likely to be perceived as, at worst, neutral by the animals. There is no information regarding the success of training NWPs to cooperate with more aversive procedures. Training was carried out with seven common marmosets to attempt to train them to come to a specially designed panel and remain there whilst they were captured by hand. The cagemates of these marmosets had no specific training but did receive intensive socialisation, primarily in the form of hand feeding. The behaviour and cortisol response to training and capture was measured to assess if the different methods of capture and training or socialisation affected the welfare of the monkeys.

The success of the training programme shows that it is possible to train laboratory-housed marmosets to cooperate with a potentially aversive procedure such as capture. The time investment however was relatively large, in comparison to that for less aversive procedures, and success rates were only 57%. The collection of saliva proved to be a good way of measuring cortisol concentrations with over 77% of all attempted samples resulting in a recorded cortisol concentration. Both behaviour and salivary cortisol concentration were shown to be useful measures of stress in marmosets, but behaviour seemed to provide a fuller picture of the animals' responses. Both behavioural and physiological data showed that capture is a stressful procedure for marmosets, and that training seemed to initially cause some slight uncertainty, but that this soon dissipated. There was little evidence to suggest that the marmosets found trained capture less stressful than capture baseline, although the sample size was very low. However strong evidence was found that following both training and positive human interactions the marmosets coped better with capture and stress was reduced, These types of interactions can therefore improve the welfare of laboratory-housed marmosets, and it is recommended that such interaction should become routine for laboratory staff.

CHAPTER 6

GENERAL DISCUSSION

6.1 SIMILARITIES AND DIFFFERENCES IN THE TRAINING OF MACAQUES AND MARMOSETS IN THE LABORATORY

The results of studies presented in earlier chapters show that both cynomolgus macaques (*Macaca fascicularis*) and common marmosets (*Callithrix jacchus*) housed in research laboratories can be successfully trained to cooperate with husbandry procedures. The temperament tests carried out proved to be good predictors of training success and time investment in both species, whilst no differences were seen between training success in males and females, or between ages, in either species. The success of the training programmes for common marmosets was shown to be greater than that for cynomolgus macaques, with all marmosets being successfully trained in neutral tasks (target training, transport box training), whilst training cynomolgus macaques for a comparable neutral task (target) was less successful, with only 62% reaching criterion. These results are comparable with those seen in other studies where success rates for marmosets has been shown to be 100% (e.g. Bassett *et al*, 2003, McKinley, 2004), whereas success rates for rhesus macaques (*M. mulatta*) have been more variable, ranging from approximately 60% success rates (Coleman *et al*, 2005) to over 96% (Fernström *et al*, 2009). When training for more complex or aversive tasks is attempted, success rates for both marmosets and macaques drop (Chapter 5, Fernström *et al*, 2009), although Coleman *et al* (2008) report 100% success for training rhesus macaques to present a limb for venipuncture. Coleman *et al*'s (2008, p 38) study however, employs

"predominantly positive reinforcement with some selective reinforcement techniques" so cannot be classed as truly cooperative training where the animal is allowed choice over whether it engages in the activity.

Similarly, the time investment required to train marmosets reported here is considerably less than that of macaques, with target training for macaques taking approximately twice as long as transport box training did for marmosets (Chapter 2, 3, & 4), and in other studies target training of macaques takes a similar amount of time to the transport box training of marmosets reported here (Fernstrom *et al*, 2009, Chapter 3, 4). Whilst differences in training protocols and trainer competencies may account for some of these differences, the emerging pattern of relative difficulty in the training of macaques as compared to marmosets is an interesting one. There could be a number of reasons as to why marmosets appear to be more trainable in the laboratory than macaques, and differences in their natural history and the factors associated with trainability identified previously (Chapter 2) may be significant.

6.1.1 Natural History and Ecology

Marmosets and macaques are physically quite different primates, however, with regards to their natural histories and ecology, there are a number of similarities. Marmosets have adapted to, and indeed thrive in, living in areas of disturbed habitat (Kinzey, 1997) which may be quite different from the habitat in which they evolved (Rylands, 1996). Marmosets often live in city parks, in close proximity with humans to whom they may become habituated and reliant for food (Buchanan-Smith, personal communication). Similarly cynomolgus macaques have adapted to living in close proximity with humans,

and will interact with people in order to access food (Lucas & Corlett, 1991, Fuentes *et al*, 2008, Sha *et al*, 2009). This suggests that neither species has an inherent shyness of humans, as wild populations demonstrate an ability to overcome any fear of humans in return for a food reward. It also provides evidence to suggest that both species are adaptable to their environment, and this adaptability has meant that they will live and breed in laboratories even when their living conditions have been poor, as we have seen in the past before welfare became a prerogative. Another similarity is that both marmosets and macaques are subject to predation from a range of other species; cynomolgus macaques to snakes, monitor lizards, raptors, felids and domestic dogs (Palombit, 1992, van Noordwijk & van Schaik, 1999) and marmosets to snakes, raptors, mustelids and felids (Kinzey, 1997), so as prey animals both species exhibit high levels of vigilance, although the small size of marmosets may make them even more vulnerable (Koenig, 1998). From this there do not seem to be any factors within the ecology of the two species which might predict the greater trainability of marmosets as opposed to macaques.

There may be differences in cognitive abilities between macaques and marmosets, certainly macaques have bigger brains in relation to their body weight than marmosets do (e.g. Armstrong, 1985, Dunbar, 1993). In terms of performance on a T-maze spatial learning task marmosets outperform rhesus macaques (Easton *et al*, 2003, Murray *et al*, 1989), however both are outperformed by rats (*Rattus norvegicus*, Markowsa *et al*, 1989) so perhaps this not the best indicator of ability to learn. It is more likely that these differences are due to differences in the strategy the animals use to establish the correct response; marmosets and rats use spontaneous alternation, which the macaques did not (Easton *et al*, 2003). Cognitive ability may not be particularly relevant to training

however as tasks being trained for are relatively simple, and therefore are well within the abilities of both species.

In terms of their species temperament, and how they are perceived, especially in the laboratory, marmosets are commonly thought of as nervous and easily frightened (NRC, 1998, Poole *et al*, 1989), whilst cynomolgus macaques have been described as fearful (Kling & Orbach, 1963) and are considered to be reserved and more passive than liontailed macaques (*M. silenus*) (Clarke & Lindburg, 1988, 1993). Cynomolgus macaques also exhibited greater levels of fear behaviour when confronted by an observer than rhesus or liontailed macaques (Clarke & Mason, 1988), and showed greater increases in heart rate and plasma cortisol levels following the stress of physical restraint, novel environments and negative reinforcement training (Clarke *et al*, 1988a, b, 1994) than rhesus or liontailed macaques (Clarke *et al*, 1988a, b), so could be described as one of the more nervous macaques. Given the nervous and fearful disposition of both species, alongside the fact that being used in a laboratory is in itself stressful, it is likely that fear is a major factor in the lives of laboratory-housed common marmosets and cynomolgus macaques, and this impacts upon their trainability and also on their welfare.

6.1.2 Aspects of Trainability

Motivation for food

Very little research has been carried out to assess the motivation of either macaques or marmosets to obtain food when they have not been food deprived. Motivation to obtain

a food reward is important in training, especially when the task being trained for is in some way aversive and therefore causes the individual some discomfort or anxiety, as the positive reward must outweigh the aversive experience; the 'consequence' must be worth the 'gain'. In an experiment with bonnet macaques (*M. radiata*) Andrews and Rosenblum (1993) found individuals had a preference for either food or video rewards, although Washburn *et al* (1997) found a much more consistent pattern in rhesus macaques of preference for food alone *versus* food and a 10 second video. Although differences in experimental protocols may account for these disparities, it is also possible that there are species differences in motivation for reward types, and there are certainly individual differences in reward motivation. Non food-deprived macaques and marmosets have been shown to continue to perform neutral activities such as computerised discrimination tasks for food rewards over long periods of time (e.g. Andrews *et al*, 1995, Williams *et al*, 2006) suggesting that the motivation to obtain these rewards is high in both species.

Macaques have been shown to have strong preferences for sweet tastes (Sato *et al*, 1977), even when they are novel (Johnson, 2007), and Caldwell *et al* (2009) verified the widely held view of those who work with marmosets, that marshmallow (followed by banana) is a favourite taste, so it is likely that the foods offered to the monkeys were amongst the best food rewards available. It would seem therefore that the value of the reward could not be increased, and therefore the 'consequence' of the task was not worth the 'gain' of the reward, however good that reward was, in both macaques (Chapter 2) and marmosets (Chapter 5). In the temperament tests I carried out, in both marmosets and macaques, individuals were more likely to touch a novel object than take food from a person, but whilst more macaques accessed food from the novel object

than took food from a person, the reverse was true for marmosets. This suggests that for the marmosets the 'consequence' of going to the cage floor was perceived as less worth the 'gain' of the reward than the 'consequence' of interacting with a person, and macaques had the opposite perception. It may be that cynomolgus macaques are more comfortable at ground level than marmosets, who are rarely seen to descend to ground level (Hubrecht, 1985, Prescott & Buchanan-Smith, 2004). However cynomolgus macaques are reported to spend just 2% of their time at ground level, though this may rise to 10% in the absence of an observer (Wheatley, 1980, reported in Rodman 1991). The proportion of individuals who were willing to hand feed prior to training was approximately the same for both species, which suggests that the two species perceived the 'consequence' of interacting with a person and the 'gain' of the reward in approximately the same way, that is to say neither species appeared to be more fearful of humans. However it appears that the marmosets overcame their fear of humans more easily than the macaques did, as all marmosets were trained for neutral tasks, but not all macaques were, with the macaques' fear potentially being deeper or more ingrained than that of the marmosets.

Motivation or drive to work

Primates have been shown to work for food when free food is available (contrafreeloading, Inglis *et al*, 1997). For example Anderson and Chamove (1984) found that stumptailed macaques (*M. arctoides*) would forage in the cage substrate for food which was freely available. To my knowledge, no study has looked at "contrafreeloading" in common marmosets or cynomolgus macaques, but there is nothing to suggest that they would differ substantially in this respect as both have

complex diets in the wild and are adapted to spend considerable periods of time searching for and acquiring food (van Schaik *et al*, 1983, Ahlborn & Rothe, 1997). Therefore there is no evidence that the two species differ in this aspect of trainability, and thus that motivation to work does not explain the differences between them.

Distractibility/concentration

Both macaques and marmosets have been shown to successfully undertake computerised cognitive tasks when they are undertaken in, or attached to, the homecage rather than in the less interesting (but possible more anxiety-inducing) environment of a separate test room (e.g. common marmosets, Crofts *et al*, 1999, rhesus macaques, Washburn *et al*, 1994). Further to this, marmosets show no loss of performance in tests when attached to homecage as opposed to when taken away to be tested (Crofts *et al*, 1999), suggesting that they are capable of concentrating even when distractions are present. Alternatively one might argue that marmosets are less stressed when tested in the home environment and therefore one might see better performance, given that stress adversely impacts upon learning. None-the-less in relation to this thesis, although individual differences in distractibility are likely, overall both species are able to concentrate sufficiently to learn the relatively simple training tasks used in these studies, and therefore this is not an important factor in explaining the differences I found. The different social situations in which the marmosets and macaques live however may have influenced their ability to concentrate on the task, as the marmosets were all pair-housed, whilst the macaques were housed in groups of three. Previous training literature does not suggest group size impacts on training success, although much of the research with rhesus macaques has been with single-housed animals (e.g.

Coleman *et al*, 2005, 2008) and where group-housed animals are used, multiple group sizes were included meaning it was not possible to identify the influence of this factor on training success (Fernström *et al*, 2009). There is evidence that low ranking rhesus macaques will perform less well than higher ranking individuals in groups (Drea & Wallen, 1999), and therefore the influence of group size on training success deserves further investigation.

Sociability with/attention to humans

Sociability to humans is likely determined both by the natural history of the species and by the experience of the individual. Whilst both common marmosets and cynomolgus macaques have adapted to live in close proximity with humans (Buchanan-Smith, personal communication, Lucas & Corlett, 1991, Fuentes *et al*, 2008, Sha *et al*, 2009) differences in their behaviour, in particular in food-sharing behaviour may play a greater role in the success of training. Marmosets are seen to food-share with other individuals in their social group, but this has not been observed in macaques beyond mother-infant interactions (reviewed in Brown *et al*, 2004). It may be that the concept of food-sharing is more familiar to marmosets, and mean they are more amenable to taking food from humans than macaques. However sociability with humans is also very likely to be related to previous experience, and differences in rearing and early experience discussed below are probably more important in the case of laboratory-housed primates.

In both species, just over 60% of individuals were willing to take food from a person prior to any training, and this proved to be a significant factor in how trainable they

were. However, whilst in both marmosets and macaques it predicted the time investment, in macaques it also predicted training success, with only one individual who did not hand feed reaching criterion. This suggests that the macaques found it harder to overcome their fear of humans than the marmosets, or that the marmosets fear was less than that of the macaques. Again this may be due to differences between the behaviour of the species but also their early experience.

Shyness/boldness and inhibition/exploration

The macaques displayed a greater level of exploration or boldness than the marmosets in the temperament test carried out here. All but one of the macaques touched the novel object, whilst just four-fifths of the marmosets did so in their tests. Over 70% of the macaques, but less than half of the marmosets accessed the food, suggesting that the macaques displayed a greater level of boldness/exploration. The marmosets were more successfully trained however, suggesting that at a species level this test did not predict trainability. The level of inhibition or boldness which rhesus macaques display has been related to their training success (Coleman *et al*, 2005), and this is the only temperament factor on which data have been published in relation to training in primates. However whilst response to a novel object, the means used to determine this temperament factor both here and by Coleman *et al* (2005), proved a useful predictor of trainability in marmosets, it did not do so in macaques in this study (Chapter 2). It may be that an element of social facilitation affected the macaque results. In my studies, the macaques were group-housed whilst Coleman and colleagues singly-housed their animals. Alternatively, or additionally there may be differences between rhesus and cynomolgus macaques in this respect, as seen in other studies comparing the two species (Clarke &

Mason, 1988, Clarke *et al*, 1988a, b, 1994). Therefore, whilst inhibition/exploration may be an important factor, the fear of humans is probably more important; indeed results of novel object tests are likely to be influenced by the individual's perception of humans. When a human observer is present those animals more fearful of people are probably less likely to place themselves in a vulnerable position by going to the cage floor than those more confident around humans.

6.1.3 Trainability and Fear

Based on the natural history and ecology of the two species studied there do not seem to be any reasons why training success would differ so strikingly. Further, no differences are apparent in the factors identified as important in trainability, which leads to the question of why the training of marmosets was more successful than that for macaques. The only factors which appeared to be significant in trainability was how the individuals interacted with a human, and in particular how easily any fear of humans was overcome, and to a lesser extent, and in marmosets only, the response to a novel object. The marmosets who did not hand feed prior to training seemed to learn this behaviour relatively quickly, whilst the macaques who were similarly timid with humans never truly overcame this and remained reticent throughout. This suggests that fear, and more specifically fear of humans may be the most important factor in the trainability of primates in the laboratory

6.2 FEAR AND ANXIETY IN THE LABORATORY

Laboratory-housed primates are likely to experience fear and anxiety for a number of reasons, but perhaps the biggest source of fear for these animals is the humans who

work with them, as it is likely that they identify humans as predators or intruders (O'Neil, 1989). A person simply entering the room can cause physiological changes; in rhesus macaques this lead to an increase in leukocyte levels (Capitanio *et al*, 1996). Further, stumptailed macaques show behavioural signs of fear and aggression towards people with whom they associate unpleasant or negative experiences (McKinley, 2004). Conversely, when humans are associated with positive interactions, primates will choose to interact with them (chimpanzees, *Pan troglodytes*, Bloomsmith *et al*, 1997).

Whilst unpleasant interactions, such as those sometimes associated with experimental work, will cause animals to become fearful, there is now strong evidence that positive interactions with people can improve welfare (Bassett *et al*, 2003, Rennie & Buchanan-Smith, 2006a). This means that whilst reducing unpleasant interactions as far as possible will improve the welfare of laboratory-housed primates, further benefits can be gained through positive interactions such as socialisation and training. One can ask whether there are benefits to different people being responsible for unpleasant and pleasant interactions. It is well established that animals prefer predictable negative events with reliable signals (reviewed in Bassett & Buchanan-Smith, 2007) and if animals were always given a reliable signal that something negative is to occur, the rest of the time they can enjoy safe periods. However, the response to signalled negative events may be exacerbated: it is well known that those veterinarians who only visit to perform task perceived as the animals as being aversive, are often met with extreme alarm amongst laboratory animals. Although guidelines suggest carestaff signal their intent (NRC, 1999), to my knowledge, there are no published data on this, by giving a signal (e.g. different clothes) may improve the positive interactions with the same

humans at time when only positive husbandry is on-going. The benefits of positive socialisation with humans are described below.

6.2.1 Early Experience

Fear is greatly influenced by early experience, and this early experience is often quite different for marmosets and macaques used in research. The common marmoset is more frequently bred in-house; as a species it breeds well in captivity (Poole *et al*, 1989). Rennie and Buchanan-Smith (2005) report that in 2001 48% of marmosets used (in four European countries) were bred in-house. The same report found that 87% of Old World primates were imported, although again data are from a limited number of countries. In the UK, whilst the source of primates is reported in the official statistics, data on the source of primates are not provided separately for Old World and New World primates, and data are reported by number of procedures rather than numbers of animals. However it is possible to see a pattern emerge in the data, whereby prior to 2007 (when my research was carried out), the majority of primates were imported, and from 2007 onwards most were bred in the UK (Table 6.1). This change is likely due to the establishment of the UK Centre for Macaques, a rhesus macaque breeding facility, and the requirement that rhesus macaques used in research funded by the MRC, BBSRC, Royal Society, Wellcome Trust and other member charities of the Association of Medical Research Charities (AMRC) are sourced from here (National Centre for the Replacement, Refinement and Reduction of Animals in Research, 2006). However it is still valid that marmosets generally are bred in the UK, whist macaques, especially the cynomolgus macaques used in contract research are imported (The Boyd Group, 2002, Honess *et al*, 2010).

Year	Acquired from within own designated establishment	Acquired from within other breeding or supplying establishment in UK	Acquired from EU countries	Acquired from other sources[a]
2004	19.4%	17.0%	0	63.5%
2005	13.3%	13.7%	3.3%	69.8%
2006	17.3%	15%	0.9%	66.7%
2007	9.7%	53.5%	2.2%	34.6%
2008	6.5%	51.1%	2.2%	40.2%
2009	13.1%	43.3%	2.2%	41.5%

Table 6.1 Percentage of scientific procedures performed on primates by source of animals, 2004-2009 (Home Office, 2005, 2006, 2007, 2008, 2009, 2010). [a] In the case of primates, this is likely imported from breeding colonies in habitat countries.

In the three laboratories in which my research was carried out, marmosets were bred in-house or were sourced from UK breeders, whilst the macaques were imported. Prescott and Buchanan-Smith (2007) report that all three contract research organisations questioned imported macaques, whilst all universities and government or pharmaceutical research organisations either bred in-house or sourced from UK, thus the laboratories in which I conducted my research were representative of the general situation in the UK.

Those animals bred in-house have the advantage of being familiar with laboratory routines, food, and likely carestaff prior to any experimental work starting, which will help reduce the fear and anxiety they experience (Rennie & Buchanan-Smith, 2006a, JWGR, 2009). Those bred in the UK may have to adapt to a new environment, but do not face long and stressful transportation. It is also possible for socialisation with new carestaff prior to moving to the new facility which may reduce the stress of the move.

Those imported from abroad however have long and stressful journeys alongside major changes in housing, temperature, possibly food and routines, and this leads to extended periods of reduced welfare (cynomolgus macaques, Honess *et al*, 2004). This may go some way to explaining the differences between macaques and marmosets in terms of their trainability; imported macaques are more likely to be stressed, especially early on in their time in the laboratory, and are also likely to be more fearful of humans during this time.

The importance of early experience on the welfare of primates has been well documented, especially in relation to maternal deprivation (Mineka & Suomi, 1978, Wallen *et al*, 1981, Pryce *et al*, 2005, Latham & Mason, 2008). Whilst it is recommended that weaning occurs at around 12 - 18 months for macaques (IPS, 2007), it is not uncommon for this to take place at around 6 months (Honess *et al*, 2010), and this may affect the fear response of the animals. Marmosets however usually remain with their birth group until they are 18 months old (The Boyd Group, 2002), although earlier weaning for research purposes is not uncommon (Majolo *et al*, 2003b) and has been standard practice in UK breeding establishments (Buchanan-Smith, personal communication). Further, marmosets are more likely to have more similar rearing histories than do macaques. Marmosets are usually one of a twin, and are reared within their family group, being attended to by both parents as well as older siblings (e.g. Mills *et al*, 2004). Cynomolgus macaques are usually reared in large groups in gang cages once weaned, although breeding systems vary from timed breeding in solitary housing to expansive corrals (Honess *et al*, 2010). Further, factors such as the matrilineal dominance hierarchies of the mothers (and later the young animals themselves) may lead to some more dominant or confident individual receiving more attention than those

more submissive animals (Laule, 2010). This is likely to lead to disparities being exaggerated, and the confident and reticent animals becoming more so over time. In turn this may lead to bigger differences in temperament in macaques than marmosets.

Although both marmosets and macaques will likely have had negative experiences of humans prior to the start of any experimental procedures, for example capture and physical examinations, these may be more pronounced for macaques due to their experience of importation and the handling this entails. Further stressful events are particularly well remembered (Joëls *et al*, 2006), so this may further increase macaque fear and anxiety of humans. Gaining the voluntary cooperation of an animal whilst it is overwhelmed by fear will be virtually impossible (Laule, 2010), therefore not only is it important to reduce fear for welfare reasons, in terms of practicalities, it is necessary in order for training to be successful.

6.3 THE ROLE OF SOCIALISATION

A number of studies have shown the benefits to primates of socialisation with humans. In laboratory-housed chimpanzees, human socialisation in leads to reduced levels of abnormal and anxiety-related behaviour, but also decreased levels of sociality in their group (Bloomsmith *et al*, 1997, 1999). Manciocco *et al* (2009) found that marmosets exposed to a four week programme of positive interaction (without food) with a caregiver exhibited less locomotion and self-scratching and more grooming and play, indicating a raised level of welfare. Interestingly, the effects of socialisation did not seem to extend to the observer, as no differences were seen in the amount of agonistic behaviours directed towards the observer. In comparison Baker and Springer (2006)

showed that a feeding enrichment programme, whereby primates (primarily rhesus macaques) were hand fed by carestaff, increased the likelihood of individuals taking food from an unfamiliar person. Following the human feeding enrichment programme 53% of primates would hand-feed, but 47% of individuals were still unwilling to interact with a stranger. No details of the proportion of animals which were willing to hand- feed from a familiar person are provided, but the authors seem to imply that all animals would do so. It seems therefore that food-based socialisation (i.e. being a form of positive reinforcement training) has a greater level of generalisation between humans. Whilst an increased number of primates were willing to feed from an unfamiliar person in the Baker and Springer study, it may be that the primates still experienced some anxiety in doing so. Results of the study on the transfer of training from one trainer to another (Chapter 4) show that, even when the second trainer was a familiar member of carestaff, the marmosets still experience some anxiety on the new interaction. This, alongside the data provided by Baker and Springer (2006), shows that whilst some generalisation occurs, it is no substitute for careful socialisation with all members of carestaff. Interestingly, it is proposed that as animals voluntarily cooperate with training, it must be stress-free (Hemsworth & Barnett, 2000), however this does not appear to be entirely true as shown in Chapter 4, with animals experiencing some uncertainty or anxiety on a training interaction with a new person.

In a survey of laboratories using primates in the UK, the lack of early socialisation, and the subsequent increased levels of fear seen in young macaques, was identified as a major difficulty in the habituation and desensitisation training of these animals (Prescott & Buchanan-Smith, 2007). This was highlighted in Chapter 2, where the aim of box training of young macaques was changed to the interim step of just target training them

due to low levels of reliable hand feeding and the knock on effect of slow training progress. Starting socialisation programmes early, as is recommended (Laule, 2010) may be one of the most positive changes to be made to reduce fear and anxiety in laboratory-housed primates. Not only will it improve welfare outwith study protocols, but if it facilitates training, it will be another important refinement, as training itself can have benefits, as discussed below.

6.4 THE ROLE OF TRAINING

There are a number of benefits to both animals and staff in the training of laboratory-housed primates, as discussed in Chapter 1, notably giving animals' choice, predictability and control over aspects of their lives, reducing the fear of interactions with people and acting as an enrichment to reduce boredom. Indeed it may also be rewarding for carestaff as the training process if dynamic, and most technicians choose this career to spend more time with animals, and improve the human-animal bond (Laule, 2010). For both carestaff and primates, training gives a sense of achievement, one of the four basic mammalian needs (together with security, novelty and complexity, Poole, 1992).

Not all training may be classified as an enriching experience however. If training is for a possibly painful or frightening experience such as venipuncture or capture, it may be the case that the training itself is not rewarding for the individual. Whilst it is less stressful for both caregiver and monkey to train an animal to cooperate in these situations, it may be problematic to imply that this training is enriching for the animal, as has been suggested (Bayne, 2003). It seems that whilst training macaques and

chimpanzees to cooperate with aversive tasks is possible, the training of marmosets in comparable tasks is more difficult (Chapter 5, Bowell *et al*, 2005). It may however be that the training of these behaviours in itself is enriching, but the performance of them is not so; for example the training of marmosets for hand capture as described in Chapter 5 might be considered as enriching up to the point where physical contact between the trainer and monkey occurs. It is interesting that cortisol responses to a trained capture do not differ from those of a standard capture. Certainly the psychological impact of the trained response to aversive procedures, as opposed to the impact of a training programme, is one worthy of further investigation, having been only studied in a neutral behaviour in baboons previously (O'Brien *et al*, 2008). None-the-less, the benefits of trained capture might be seen in other areas, such as a reduction in injuries to claws, or improved behaviour outwith capture as the marmosets have higher predictability and are familiar with procedures. Overall there is an increasing body of work showing that training is beneficial to laboratory-housed primates, with results shown in Chapters 2, 3 and 5 in agreement with this.

Both the macaques and marmosets in this study involved in training and socialisation programmes exhibited more relaxed activity and less inactivity, suggestive of a reduced level of boredom, and were better able to cope with stressful experiences (Chapters 2, 3, Bassett *et al*, 2003), and exhibited increased positive sociality (Bloomsmith *et al*, 1997, 1999). Further, training has been seen to decrease the performance of stereotypic behaviour (Bourgeois & Brent, 2005, Coleman & Maier, 2010) another indicator of compromised welfare (Mason, 1991). Pigs (*Sus scrofa*) who experienced a cognitive enrichment programme were shown to be less fearful in an open field test (Puppe *et al*, 2007), providing further evidence for the benefits of learning and the contingency

between behaviour and outcome in improving animal welfare. These findings suggest that the benefits of training extend beyond the immediate reduction in stress related to the task being trained, and that training, for neutral tasks at least, may fulfil the criteria for environmental enrichment, reducing boredom and enhancing welfare both within and outwith training sessions. Most of the time primates are not directly involved in research and testing; they spend most of their time in their home cage, often getting bored. Human socialisation and training for pleasant or even neutral tasks in the homecage provides the primates with something to do, the lack of 'something to do being' identified as key to boredom (Wemelsfelder 1990, 2005). This also gives them a sense of achievement and control, which may be vital in promoting positive welfare (Puppe *et al*, 2007). Training also allows the primates to lose fear of humans and therefore allows them to be more resilient, and relax and become more explorative within their home environment.

6.5 PRACTICALITIES OF IMPLEMENTING PRT

One of the main aims of this study was to identify practical ways in which training can be used in large research laboratories in the UK. The results of the experimental work I carried out show that the practicalities for marmosets and macaques may be quite different, resulting, most probably, from potential differences in socialisation, rearing and prior experience as discussed above.

Macaques

Whilst the training of cynomolgus macaques proved to be reasonably successful, with over 60% becoming target trained, the time investment may prove to be a barrier to the

widespread uptake of PRT in large laboratories, unless early socialisation programmes are put in place. Spending on average over 1.5 hours training a macaque to do the relatively simple task of holding a target may be feasible when only a few animals are held, and when large numbers are used, as in contract research, this level of time investment may not be realistic. When the staffing costs of this are considered alongside the extra expense of housing the monkeys for longer to allow training, the cost of this training could be prohibitive. Further training for aversive tasks such as venipuncture would incur further costs and therefore be even less likely. However, this first step, 'learning to learn', is critical in the training process and paves the way for faster training later (Schapiro *et al*, 2005). Many laboratories are however committed to trying to improve primate welfare, and given the benefits training can provide to animals in their care, resources may be available to implement training programmes. Given the impact which good socialisation with humans has on the speed and success of training, early positive socialisation is likely a cost-effective way of reducing time investment and thus cost, whilst being relatively easy to implement.

Macaques are commonly moved from larger gang housing to smaller group cages around four weeks prior to the start of any testing, which would allow enough time for target training. As this is a useful and versatile behaviour which can be built upon once experimental work has commenced, it provides a good foundation for later training (Prescott *et al*, 2005a). Temperament has also proved to be a reliable predictor of training success and time investment (Chapters 2 & 3, Coleman *et al*, 2005), so selection of trainable macaques for long-term studies where training will be required is one way of maximising benefits whilst reducing time investment. This should, however, not be at the expense of the socialisation of animals not in training programmes.

Further, formal socialisation on arrival at the facility, but prior to movement to the smaller group cages could be of great benefit, as long as all animals received appropriate attention, and not just those bold enough to interact already.

Marmosets

In comparison to macaques, the training of marmosets was more successful and faster. This, alongside the way marmosets are used in the laboratory, and that marmosets tend to be held in smaller numbers than macaques, means that overall time investment would be less. This suggests that training programmes could be relatively easily implemented for them in many laboratories. Temperament tests were good predictors of trainability in marmosets, so further reductions in time investment may be possible where only selected animals are trained. Time investment can also be reduced by ensuring that all animals are well socialised and willing to hand feed before any formal training takes place. Further, training can be successfully shared by two trainers without affecting success rates and time investment, with the added flexibility this gives to training programmes, though taking into consideration the uncertainty that interacting with a new trainer causes the marmosets.

Training marmosets to cooperate with an aversive task was less successful than training for neutral tasks. However, four out of seven marmosets with whom this training was carried out were trained to cooperate with capture, a promising finding given that so little has been done to train this species for aversive tasks. Time investment was, unsurprisingly, much greater than for transport box training, but still reasonable at around 2.5 hours over six weeks. It may be possible to use temperament tests to identify

individuals who would be more likely to succeed in capture training, and to select these animals for long-term studies.

6.6 FINAL CONCLUSION

There is still much to be learnt about PRT; for example, understanding why there is not greater uptake, how best to implement it, and how to overcome more of the practical difficulties. In some cases, management and individuals within the carestaff team appear to be the reason why there is not greater uptake, and future efforts should be focussed on changing the perception of training, how to create and manage a team to implement it successfully, as well as looking at the welfare benefits of training. The findings obtained from research reported within this thesis together with other research confirm the benefits of PRT.

Given the positive impact that sociability with humans has not only on primate welfare but also on training success, it is imperative that wide ranging human socialisation programmes are established in both breeding and research facilities. The importance of this has been recently highlighted (JWGR, 2009), and results presented here provide a sound evidence base to support this. The use of PRT is practicable in laboratories, and should remain the ultimate goal. None-the-less to facilitate wider uptake initial programmes may initially wish to pre-select animals, and identify specific longer-term studies where training may have the greatest impact.

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