Training in a Laboratory Environment: Methods, Effectiveness and Welfare Implications for Two Species of Primate

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Abstract

The use of Positive Reinforcement Training (PRT) for co-operation during routine husbandry and laboratory procedures is widely advocated as a means of promoting the welfare of nonhuman primates. However, while research originating in US zoos provide qualitative descriptions of how PRT may be used in the training of a wide variety of species, quantitative data and evidence to support the view that PRT reduces stress predominately comes from laboratory studies of primates whose training may have used other methods. Despite official guidelines, training is rarely carried out in the UK and the educational and wider organisational structures concerning training, present in the US are largely absent.

The techniques used in the UK were assessed through detailed observations recorded when four stump-tailed macaques were trained to co-operate during venipuncture. Data recorded during training sessions showed that although food rewards were given, their delivery was slow and inconsistent. A certain amount of coercion was used which violates a principle of PRT which states that co-operation should be voluntary. The macaques showed increasing resistance to the process and a mild but detrimental effect on the subsequent behaviour of the study animals. When training resumed 18 months later there were considerable improvements in the techniques used. The macaques showed a greater willingness to participate and there were no significant changes in their behaviour when training days were compared to those when training did not take place. The behaviour of the macaques during venipuncture was judged to be arising from engineered compliance rather than voluntary co-operation. However, it was concluded that the technique observed, if carried out correctly, was a reasonable compromise between forced restraint and voluntary co-operation given the paucity of evidence showing the effectiveness of

PRT for invasive procedures. However, it was also concluded that the use of coercion should be recognised and provide a focus for future refinement.

The effectiveness and welfare implications of PRT was assessed through the training of common marmosets to target and allow in-homecage weighing and to provide urine samples. It was found that the trained animals performed reliably and that time invested in training could be recouped through faster data collection. Following a period of training or increased positive contact with humans, observations of marmoset behaviour showed a decrease in stress related behaviours and an increase in allogrooming supporting the view that improved relations with humans had a beneficial effect. Following exposure to a mild stressor, trained marmosets showed no elevation in levels of urinary cortisol or stress related behaviours. Untrained animals showed increased levels of locomoting and self-scratching following exposure to the same stressor. It was concluded that PRT successfully reduced the stress associated with the presence of, and manipulation by, humans.

Final recommendations were that training can promote the welfare of nonhuman primates and should be used in UK laboratories to a greater extent than is currently the case. However, the lack of educational opportunities for animal trainers in the UK needs to be addressed. It was also recommended that in light of the growing evidence showing the benefits that can arise from training and good relations with humans, the zero-handling policy practiced in many UK zoos should be re-assessed.

Chapter 1

Training and the Welfare of Captive Nonhuman Primates

"The least distressing method of handling is to train the animal to co-operate in routine procedures."

Current Home Office Code of Practice for the housing and care of animals used in scientific procedures (1986, Para. 3.50).

"Training sessions, where positive rewards are offered to the animals, can contribute to creating trustful relationships between the animals and their caregivers."

Report of the Scientific Committee on Animal Health and Animal Welfare (European Commission; 2002, p41)

"Programmes of animal training using positive reinforcement techniques have been successful in reducing the stress that normally accompanies manipulation by humans."

(U.S. Dept of Agriculture; 1999, p25).

1.1 Training nonhuman primates in laboratories and zoos

The above extracts from a variety of official guidelines concerning the welfare of laboratory-housed animals reflects a growing consensus that training represents a valuable tool in the promotion of the psychological well-being of captive non-human primates. This view is reiterated by the Institute for Laboratory Animal Research and National Research Council (ILAR/NRC, 1998) and the International Primatological Society (1989).

Such a strong consensus between a variety of welfare organisations would suggest that the relationship between animal training and animal welfare is unproblematic yet this impression is misleading. Training techniques have frequently undermined animal welfare and some of the earliest welfare laws were introduced specifically to outlaw cruel training practices (Baratay & Hardouin-Fugier, 2002). Poor training is responsible for many of the behavioural problems seen in domestic

animals such as dogs *(Canis lupus familiaris)* (Mugford, 1995; Pryor, 1999) and horses *(Equus caballas)* (Kiley-Worthington, 1997; McGreevy, 1996). The opinions expressed in the opening quotations actually represent a profound shift in attitude which is by no means universal. This change has occurred as the result of a growing body of evidence that suggests that training can indeed promote the psychological well-being of non-human primates in a variety of captive environments.

This evidence comes from two main sources: reports concerning animals that have been trained in laboratories, and those trained in zoos. It is important to distinguish between these two settings as, although some similarities do exist, both the procedures where training could be useful and the settings in which training is carried out are likely to be different in many respects. One obvious difference is that while zoos tend to house small groups from a wide variety of species, laboratories usually contain large numbers from a restricted range, predominantly macaques (Boyd Group, 2000). Laboratory-housed animals generally participate in a variety of procedures on a regular basis, venipuncture being one of the most common (Reinhardt, 1997a). For the vast majority of zoo animals, the closest equivalent to this would be veterinary procedures and routine health checks, carried out much less frequently. In addition, laboratories are designed to allow easy access to individual animals and contain devices such as cages equipped with squeeze-back mechanisms which also make restraint of animals easy. Many zoo enclosures either pre-date such considerations or were designed for architectural merit rather than practical considerations (Baratay & Hardouin-Fugier, 2002). Access to the animals, along with the numbers of animals trained and the requirements of various husbandry and handling routines, will affect the types of training methods employed and this is reflected in the literature. The following review is intended to provide an overview of the training literature and

highlight some important issues. In order to avoid undue repetition, a more detailed examination of specific areas is provided in the relevant chapters.

Two of the most common procedures where training has been employed in a laboratory setting is moving animals between locations and venipuncture. For caged animals, one simple trained behaviour is entering transport cages on request. Reinhardt (1992b) found that some rhesus macaques *(Macaca mulatta)* would spontaneously enter a transport cage placed in front of their homecage. While other had to be "encouraged" by prodding with a stick, the practice of rewarding them on return to the homecage appeared to encourage future cooperation. When a similar technique was employed with cynomolgus monkeys *(M. fascicularis)* the animals initially had to be captured and placed in a transport box. However, after repeated trials where the monkeys received food during the process, they began to enter the box spontaneously (Heath, 1989).

The capture and transportation of group-housed animals provides a greater challenge, largely solved by enclosure design and the circular "chute" system where the animals are trained to enter a narrow tunnel that connects to a transport box or cage where individuals can be isolated for treatment. Following treatment, the animals follow the tunnel which reconnects to their main enclosure. This technique has been employed successfully in a number of institutions (Bunyak, Harvey, Rhine & Wilson, 1982; Knowles, Fourrier & Eisele, 1995; Luttrell, Acker, Urben & Reinhardt, 1994; Phillippi-Falkenstein & Clarke, 1992; Reinhardt, 1990; Walker, Gordon & Wilson, 1982). Training to travel along tunnels towards a sampling cage has also been used with caged animals in order to reduce the risk of back injury in laboratory personnel (Cowley, Vertain, Pape & Reinhardt, 1993).

When the chute system is employed, venipuncture can be carried out in a cage inserted into the tunnel system. Here, the technique employed is similar to that used when blood samples are collected from caged animals. Typically, these monkeys are taught to extend a limb through an opening in the cage door and are rewarded for co-operation. Initially a squeeze mechanism is employed to restrict the animal to the front portion of the cage. A limb is then grasped and drawn through the cage door. Over time, the monkeys begin to present a leg voluntarily. This behaviour has been most commonly trained in rhesus macaques (Reinhardt, 1991, 1992a; Vertain & Reinhardt, 1989) but also in stump-tailed macaques *(M. arctoides)* (Reinhardt & Cowley, 1992), vervet monkeys *(Chlorocebus aethiops)* (Wall, Worthman & Else, 1995) and capuchin monkeys *(Cebus apella)* (Dettmer, Phillips, Rager, Bernstein & Fragaszy, 1996).

While articles concerning training for co-operation during venipuncture dominate the literature, there are a number of additional procedures reported. A similar technique to that described above (rewarding for presentation of a limb) has been used to teach stump-tailed macaques to present their heads through a hole in the cage front and allow application of various substances to the forehead (Reinhardt & Cowley, 1990).

Turkkan (1990) trained single-housed olive baboons *(Papio hamadryas anubis)* to reach from their homecages and grasp a post placed at the end of a narrow shelf. Initially rewarded for touching the shelf itself, the monkeys were subsequently required to reach for the post itself then hold it for progressively long periods, a process known as shaping (definitions of training terms are provided later). Next, an inflatable cuff used to measure blood pressure was introduced with time allowed for habituation to this device. The baboons were then rewarded for allowing a

stethoscope to touch their arm. At this point, the quality of the food reward was increased (fresh fruit or apple sauce from a pump rather than dry pellets) and the cuff was inflated. Once the animals would sit quietly and tolerate the entire procedure, auscultatory blood pressure measurements were taken. It took a mean of 12 weeks to train these animals (range 2-36 weeks). However, this period was shortened following the introduction of apple sauce as a reward. This had the advantage of allowing continuous reinforcement during co-operation yet allowed instant termination of the reward as soon as any animal withdrew his arm. The animals trained following this modification co-operated fully within three weeks. The technique of rewarding for grasping an object outside the homecage has been used to teach baboons to tolerate injections (Levison, Fester, Nieman & Findley, 1964).

A shaping procedure was used to teach hamadryas baboons *(Papio hamadryas hamadryas)* to exercise on a moving treadmill. Initially rewarded for mounting the stationary treadmill, the monkeys were reinforced for touching, then standing on the treadmill as it moved at a slow speed. Once they were walking they would receive a food reward every two minutes. They were then required to walk at a constant rate of 1.6km/hr which, if maintained, would result in a reward every minute. The procedure was subsequently modified when the baboons were transferred to social housing which allowed movement to the treadmill without removal from the homecage. All 18 study animals were trained within six weeks (Rogers, Coelho, Carey, Ivy, Shade & Easley, 1992).

The procedures reported above all require that the study animals be taught to perform the required behaviour and that the provision of rewards is an essential part of this process, not only for learning but as part of the process of building up a trusting relationship between the animals and their trainers (European Commission,

2002). However, this is not always the case and in some cases it is questionable just how much training was required. For example, Marks, Kelly, Rice, Ames, Marr, Westfall, Lloyd and Torres (2000) utilised "restraint chair training" as a means of preparing baboons housed in single cages for transfer to housing in a social group. This initial stage of this process used the pole and collar system as a means of placing baboons in a restraint chair. The monkeys were fitted with a collar while anaesthetised and the pole placed within their cage to allow familiarisation. The pole was then attached to their collars and they were manoeuvred around the room. The restraint chair was introduced and left in the room for a two week period before the monkeys were placed in it. Once an individual baboon would quietly tolerate being manoeuvred by the pole and placed in his/her chair, chairs containing another animal were placed within reach to allow familiarisation and limited physical contact before the monkeys were released together to create a social group. The main advantage of this procedure was the gradual introduction of each stage. However, as no rewards were used (except contact with another animal in the latter stages) their behaviour can be most likely attributed to habituation rather than active learning (see below). Moreover, both the pole and collar system, and the use of restraint chairs can cause considerable distress (Reinhardt *et al*, 1995).

Lutz, Tiefenbacher, Jorgensen, Meyer and Novak (2000) attempted to teach rhesus macaques to provide a sample of saliva. Their first technique required that the macaques lick a piece of gauze, flavoured with "Kool-Aid", placed behind a small mesh screen (to prevent the gauze being eaten) at the end of a pole. An alternative apparatus attached a piece of flavoured rope to the pole-end, which the macaques then chewed. Habituation to the apparatus took approximately two weeks although the extent to which the macaques had to be actively taught to chew the flavoured gauze or

rope is debatable. It could be assumed that once they realised that these items had a pleasant taste they would lick or chew anyway. However, for many tests, saliva is a useful substitute for blood and this study demonstrates that it can be collected using simple, non-invasive methods.

The above example suggests that some techniques can require habituation to certain apparatus, but that the required behaviour may occur spontaneously. There are other examples where behaviour is attributed to training when it may simply represent a species-typical response to a particular situation. For example, Reichard *et al.* (1993) claim that female primates are taught to present their perineal regions for inspection whereas others attribute this behaviour to "a natural submissive gesture of presenting the hindquarters which can be used by the "dominant" technician"(Burt & Plant, 1990, p179). However, while initial presentation may be spontaneous, remaining stationary during vaginal swabbing still requires some degree of training (Bunyak *et al.*, 1982).

Laboratory-based reports tend to include fairly detailed qualitative descriptions of the training procedures involved along with qualitative data regarding success and time investment required for training (e.g. Dettmer *et al*, 1996; Luttrell *et al.*, 1994; Phillippi-Falkenstein & Clarke, 1992; Reinhardt, 1991, 1992a, 1992b; Reinhardt & Cowley, 1992; Vertain & Reinhardt, 1989). In addition, much, if not all of the evidence to support the view that training actually reduces the stress associated with manipulation by humans comes from laboratory studies and in particular, the use of physiological measures such as the 'stress' hormone cortisol.

In such studies, levels of plasma cortisol in blood samples collected from trained animals are compared to samples obtained using traditional methods such as forced restraint. Such studies consistently find significantly elevated plasma cortisol

in samples obtained through traditional methods, but not in those obtained from trained animals (Elvidge, Challis, Robinson, Roper & Thorburn, 1976; Reinhardt *et al.*, 1990, 1991; Reinhardt & Cowley, 1992). Dettmer *et al.* (1986) reported elevated plasma cortisol levels in blood collected from capuchin monkeys during the training process. However, an important difference here was that the capuchin monkeys studied were removed from their homecage during sampling, which in itself causes distress and was something that the training process aims to avoid (Reinhardt, Liss & Stevens, 1995). Samples taken towards the end of the study showed no elevation in plasma cortisol in animals who appeared behaviourally habituated to the procedure.

The above studies have served a dual purpose in that, in addition to demonstrating the benefits of training, examination of plasma cortisol has identified the physiological consequences of stressful handling techniques (Reinhardt *et al,* 1995) and the resulting implications concerning the validity of research using such methods (Reinhardt, 1999). This has contributed greatly to the drive towards finding better handling techniques with positive implications for the psychological well-being of laboratory-housed animals. However, such studies predominately address the training of macaques and similar publications concerning the training of callitrichid species are rare, despite the fact that these primates are commonly found in laboratories (Boyd Group, 2000). The literature concerning the training of callitrichids is not extensive and is examined in Chapter 6).

Many reports of training in zoo settings tend to give general descriptions of the procedures employed and the wider principles underlying the establishment of a training regime at a particular institution (e.g. Baker, 1991; Bloomsmith, 1992; Desmond & Laule, 1994; Katka *et al.*, 2001; Laule, 1993, 1994; Laule & Desmond, 1991, 1998; Petinot, 1995; Sevenich, 1995; Shellabarger, 1992). The literature is also

less extensive than at first appears as many articles appear to be revised versions of previously published reports (e.g. Bloomsmith, Laule, Thurston & Alford, 1992; Bloomsmith, Laule, Alford & Thurston, 1994). While these reports are interesting in that they demonstrate the range of species that can be trained, along with the variety of procedures that can be carried out using trained animals, they often lack sufficient detail to allow a close examination of the training processes employed. Indeed, many provide an impressive list of successfully trained behaviours but with no details of how these behaviours were trained, and no quantifiable data by which success can be measured. However, much of the zoo-based literature specifically concerns the training of a variety of primate species and some articles do describe the techniques employed to solve particular management problems including veterinary procedures, promoting positive social behaviour and solving behavioural problems.

Reichard, Shellabarger and Laule (1993) report that a variety of primates at the Toledo Zoo including gorillas (*G. gorilla gorilla),* chimpanzees *(Pan troglodytes)* and orangutans *(Pongo pygmeaus)* were trained to present limbs for injections, provide sperm samples and present for artificial insemination. They were also trained to present a variety of body parts for inspection and accept a variety of veterinary procedures such as venipuncture, injections and blood pressure measurements (see also Shellabarger, 1992).

Priest (1990, 1991) taught a diabetic drill *(Mandrillus leucophaeus)* to cooperate with the administration of daily insulin injections. Training has been used to encourage such co-operation from diabetic animals in a number of other species including chimpanzees (Laule, Thurston, Alford & Bloomsmith, 1996); hamadryas baboons (Ferreri, 1996); De Brazza *(Cercopithecus neglectus)* and red-tailed moustached guenons *(C. cephus cephus)* (Stringfield & McNary, 1998). The

techniques used, along with those employed to similar end in laboratories are discussed in Chapter 3.

Zoo-housed animals have been trained to move between locations through techniques known as "targeting" and "stationing" (see below). Positive social behaviours have been increased and aggressive behaviours diminished through rewarding animals for activities such as allogrooming (Desmond & Laule, 1991; Laule, 1993) and allowing subordinate animals to feed in peace (Bloomsmith *et al.*, 1992, 1994; Laule & Desmond, 1991). The technique of rewarding desirable behaviours and thus increasing their frequency has been used to produce a corresponding decrease in unwanted behaviours such as stereotypies (Laule, 1993).

What is clear from the zoo-based literature is that training in US zoos is a well organised activity. Considerable effort is made when planning training interventions and there is a well-developed education programme to both train the trainers through classroom education in operant theory and supervised practice (Laule, 1992; Petiniot, 1995; Sevenich, 1995). It is also clear that US zoos are promoting one specific type of training technique, which is positive reinforcement training (PRT). These trainers stress that only PRT techniques should be employed in both a zoo (Mellen & Ellis, 1996) and laboratory environment (Laule, 1999) and it is PRT that is specifically recommended in the US Department of Agriculture guidelines. The importance of this distinction is clarified through examination of the theories underlying the majority of training techniques.

1.2 Training techniques and operant theory

It is generally believed that animals learn through four processes; habituation, classical and operant (instrumental) conditioning and complex (cognitive) learning

(Mellen & Ellis, 1996). Most training techniques rely on either habituation, or classical and operant conditioning, also known as stimulus-response (S-R) learning and associative learning.

Habituation

Habituation can be defined as "the waning of a response due to repeated presentation of the eliciting stimulus" (Mellen & Ellis, 1997, p88). The most commonly seen example of this is the fear response elicited by a particular situation, object or person. If an animal is repeatedly exposed to the feared stimulus without harm, then the fear response will eventually be extinguished and disappear. However, habituation is of limited value in training due to the phenomenon of "spontaneous recovery". If some time passes between habituation and re-exposure to the feared stimulus, the fear response may return when that stimulus is again presented (Lieberman, 1993). For example, horses are generally habituated to entering a lorry or trailer for transport. In the UK, many events requiring transportation only take place in the summer months. Young animals in particular may enter a horsebox and travel without fuss all summer but then, following a period of no travel over the winter, will refuse to load the following spring. Their natural fear of dark, enclosed places has spontaneously returned.

Another problem with habituation is that it is difficult to be sure that the fear response has truly gone. 'Pseudo-habituation' occurs when the animal still experiences fear but the outward signs have become too subtle to be easily detected (Caine, 1987, 1990).

Classical conditioning

Classical conditioning involves the association between two stimuli, one which already produces a particular response (the unconditioned stimulus or US) and

one initially neutral stimulus (the conditioned stimulus or CS) (Lieberman, 1993). Sometimes called Pavlovian conditioning in honour of Ivan Pavlov who first demonstrated this type of learning under laboratory conditions, classical conditioning is responsible for a considerable amount of animal training both deliberate and inadvertent. For example, if the delivery of food is always preceded by particular sounds created during preparation, then the animals will learn to associate these sounds with the arrival of food. Equally, if a painful procedure is always carried out by technicians using a particular piece of apparatus, then this will become associated with the pain and subsequently feared. An additional use of classical conditioning in training occurs when teaching an association between a particular sound (e.g. a verbal request) and a desired behaviour.

Classical conditioning provides a better means of removing a fearful response than simple habituation (Wolpe, 1997). Counter-conditioning refers to the process where a feared object or situation is repeatedly paired with a pleasant stimulus such as food. Here a new response (anticipation of food) is conditioned in place of the pre-existing one (fear). The practice of feeding treats during potentially aversive procedures such as handling allows counter-conditioning to occur. In the treatment of human phobias, counter-conditioning is regarded as much more efficient than habituation as the risk of spontaneous recovery is considerably reduced (Davidson, 1968).

There are a number of important factors which determine the effectiveness of conditioning including contiguity, contingency and frequency. With regards to temporal contiguity, optimal conditioning occurs when the CS precedes the US by about half a second with a general rule that the shorter the time interval, the more effective the conditioning (Lieberman, 1993). Spatial contiguity also has an effect.

Testa (1975) found strongest conditioning occurred when rats were exposed to a light and blast of air occurring in the same area of their box. If training is a pleasant experience, then the close proximity of the trainer will allow an association to be formed, fostering good animal/human relationships. However, training such as conditioning in an operant chamber where no human is present is unlikely to produce any such effect.

In addition to contiguity, the CS must be a reliable indicator that the US will occur (Rescorla, 1968). For example, if a person carrying out an aversive procedure only approached a particular animal for that purpose, then that person would become a reliable indicator that the procedure was likely to occur and a conditioned fear response to that person would develop. In this example, the procedure would occur when the person was present, but not when the person was absent which is a positive contingency. However, if that person approached the animal on other occasions such as during feeding then their presence is not a reliable indicator so conditioning is unlikely. The more accurately the CS predicts the occurrence of the US, the stronger the conditioning (Rescorla, 1968).

Additional important factors include the nature of the stimulus and the frequency with which it is presented. The strength of the conditioned response increases most during the early stages of conditioning and intensity, and the more vivid or intense the stimuli involved, the stronger the conditioned response. Painful or extremely frightening stimuli could produce conditioning in a single trial (Lieberman, 1993). In practical terms, the above factors suggest that when attempting to classically condition an animal, a trainer should be fast, consistent and allow sufficient trials to allow conditioning to occur. The number of trials required will depend on the nature of the stimuli involved.

Operant (instrumental) conditioning

During operant conditioning, an association is formed between a particular action and its consequences. Thorndike's "Law of Effect" states that;

"Of several responses made to the same situation, those which are accompanied or closely followed by satisfaction to the animal will, all other things being equal, be more firmly connected with the situation, so that, when it recurs, they will be most likely to recur."

"...those which are accompanied, or closely followed by discomfort to the animal will, all other things being equal, have their connections with the situation weakened, so that, when it recurs, they will be less likely to occur."

Thorndike, 1911, p24, p224

The basic principles of operant conditioning are that, if an action is followed by a pleasant outcome, then that act is more likely to be repeated in the future, however, if an action is followed by unpleasant consequences, then that action is less likely to be repeated in the future. The possible outcomes can be categorised into three types, positive reinforcement, negative reinforcement and punishment.

Positive reinforcement occurs when behaviour is rewarded through gaining something pleasant for example. Negative reinforcement is still a reward, but the reward is gained through the removal of an unpleasant stimulus. Learning through negative reinforcement is sometimes called escape/avoidance learning because the animals performs an action in order to escape an unpleasant stimulus or to avoid its occurrence altogether. The final possible outcome is punishment where the performance of an action is followed by unpleasant consequences.

Reinforcers can fall into two categories, primary reinforcers and secondary reinforcers. A primary reinforcer is something that is intrinsically rewarding, for example, food. Care must be taken to ensure that a reward truly is pleasurable to the animal. For example, patting and stroking may be rewarding to some animals such as

domestic dogs, but such contact with humans may be merely tolerated, or experienced as aversive by many primates.

A secondary reinforcer is something that was initially neutral but has become rewarding through classical conditioning as a result of repeated pairing with a primary reinforcer. Once conditioning has occurred, the secondary reinforcer itself becomes rewarding although periodic presentation with the original primary reinforcer is required to prevent extinction of the conditioned response (Lieberman, 1993). An obvious example would be food paired with verbal praise although other stimuli such as whistles and clickers can be conditioned in exactly the same way. Secondary reinforcers are particularly useful in a situation where a primary reinforcer cannot be delivered quickly (see below). Here, a sound such as verbal praise or a click can act as a "bridging stimulus", effectively signals to an animal that the correct response has been made and that a food reward will follow (Laule, 1999; Mellen & Ellis, 1997)

Important factors in operant conditioning are the speed and frequency with which reinforcement is delivered. As with classical conditioning, the shorter the interval between performance of the behaviour and delivery of the reward, the more rapidly the association between these events is formed (Grice, 1948). With regards to frequency, in general, initial learning occurs fastest when every occurrence of the desired behaviour is rewarded (a continuous reinforcement schedule). The disadvantage of rewarding all occurrences is that when the rewards cease, extinction of that behaviour occurs and the animal stops responding. However, once a behaviour is established, reinforcement can be switched to a ratio schedule where reinforcement depends on the number of responses that have occurred. One of the most effective schedules is a variable ratio schedule where reinforcement is delivered at unpredictable intervals. This schedule tends to produce high levels of responding and

a behaviour that is resistant to extinction (Lieberman, 1993; Mellen & Ellis, 1997). An important point to note is that the occasional reinforcement of an unwanted behaviour (e.g. using food to distract an animal that is being aggressive towards the trainer) will make that unwanted behaviour difficult to extinguish. As with classical conditioning, effective training requires that the trainer reward the desired behaviour quickly and, at least during initial training, consistently. This is why bridging stimuli such as clickers are so useful as they allows the rapid delivery of reinforcement at the right moment, even when the animal is some distance from the trainer. The correct delivery of reinforcement during training is an important issue that is addressed further in Chapter 3.

1.3 Limitations of operant theory

The theories of both classical and operant conditioning are limited in that they make no allowance for either species or individual differences. Indeed, the theories suggest that any animal can be trained to perform any behaviour in exactly the same way. This assumption tends to be reflected, at least implicitly, in the literature where species-specific differences are rarely considered. However, the assumption that all species learn in exactly the same way is clearly false (Domjan, 1998).

The classic article by Breland and Breland (1961) demonstrated that operant techniques could not train a behaviour that conflicted with a naturally occurring one. In one example, racoons *(Procyon lotor)* were trained to deposit coins in a piggy bank as part of an advertising campaign. However, the animals initially performed well, they gradually stopped performing the required behaviour and began to pick up the coins and rub them together in the way they would normally handle food items. Breland and Breland coined the term "instinctive drift" for the phenomenon that

occurs when an animal has a strong natural instinct that interferes with a conditioned response and stated that when this occurs "learned behavior drifts towards instinctive behaviour" (1961, p684). This suggests that behaviour that runs counter to genetic pre-dispositions will be difficult to train. However, training is most successful when it works with an animal's natural pre-dispositions (Kiley-Worthington, 1990).

"Preparedness" is a term that refers to a genetic pre-disposition that can make it particularly easy or difficult for an animal to learn a particular task. This was demonstrated through examination of taste-aversion learning in rats. Rats rapidly learn to avoid food that subsequently makes them ill, which is probably an adaptive behaviour in opportunistic feeders. However, while they readily learn to associate the taste of food with illness, they find it difficult to associate taste with electric shocks (Garcia & Koelling, 1966).

The fact that a genetic pre-disposition to perform or learn certain behaviours can both enhance or inhibit the training process can be illustrated in the behaviour of domestic dogs. Not only are these animals extensively trained, the relationship between genetics and their behaviour has been extensively studied and strong breed differences reported (Scott & Fuller, 1965). For generations, dogs have been selectively bred to perform specific tasks and breeds are categorised according to their original purpose, for example, 'hounds' were used for hunting, 'working dogs' for herding and guarding livestock and 'gundogs' for locating and retrieving game animals (Glover, 1977). It is well recognised that success of training depends on initially choosing the appropriate breed (Willis, 1995). For example, while it is relatively easy to train a border collie to herd sheep it would be extremely difficult to teach any terrier the same behaviour. The most successful guide dogs in recent years

have been labrador/golden retriever crossbreeds and most guide dog associations now breed their own animals (Willis, 1995).

Even within breeds, individual differences in trainability are apparent. Experienced shepherds select potential sheepdogs by watching the behaviour of puppies as young as eight week. Suitable puppies spontaneously display the required stalking and herding behaviours towards other animals such as chickens. Dogs who fail to display these behaviours rarely work well and are generally sold as pets (B.Duncan, pers. comm.). The success of training is influenced by traits such as fearfulness, distractibility and excitability, all of which appear highly heritable (Willis, 1995).

A final limiting factor concerning operant theory is that associative learning is that this is not the only way in which animals learn. This limitation became apparent when operant theory could not account for a behaviour as simple as maze learning by rats (reviewed by Goldstein, Krantz & Rains, 1965). Complex, or cognitive learning is a general term that can apply to a range of abilities from forming a spatial representation or "cognitive map" of a specific area to social learning from observations of other animals. However, unlike associative learning, complex learning by animals is poorly understood (King, 1999; Lea & Kiley-Worthington, 1996; Vauclair, 1996; Whiten, 1993; Whiten & Ham, 1992). As training techniques are based on operant theories, the principles of classical and operant conditioning can be used to explain the results of the training process. However, the influence of species-specific behaviours and the possibility that the animals are learning in ways in addition to simple conditioning cannot be entirely ignored.

1.4 Principles and techniques of PRT

One of the fundamental principles of PRT is that only positive reinforcement techniques are used. Training proceeds by rewarding the desired behaviour while incorrect responses are ignored. When this principle is followed, the animals are voluntarily co-operating with the training process as no coercion is being employed. Negative reinforcement should only be used once all alternatives are exhausted. When negative reinforcement is used, it should always be balanced by positive reinforcement (Desmond & Laule, 1998; Laule, 1999). Negative reinforcement requires the use of some aversive stimulus, which violates the principle of voluntary co-operation. In addition, the use of an aversive stimulus can elicit fear or anxiety in the animal which both inhibits learning, undermines the psychological well-being of the animal and increases the risk to personnel (Laule, 1999).

Punishment is undesirable as it can produce the same unwanted effects as negative reinforcement. In a study of domestic dogs, there was a significant positive correlation between the number of behavioural problems reported and the number of tasks for which the dogs were trained using punishment, but not using rewards (Hiby, Rooney & Bradshaw, 2004). In addition, punishment is not particularly effective. Skinner (1938) found that rats punished for pressing a lever did initially stop but gradually began to respond again until they returned to performing at the same frequency as control animals who had never been punished. Later studies found that punishment could work, but only is the intensity of the punishment was relatively severe (Boe & Church, 1967). However, the more intense the punishment, the greater the risk of unwanted effects such as an increase in fear or aggression. During PRT, physical punishment should only be used in extreme, life-threatening situations. On

the rare occasions when punishment is used, the preferred method is "time out" where a training session is terminated following, for example, aggression towards the trainer or a conspecific (Laule, 1999).

The principle of only rewarding desired behaviours has led to the development of a number of training techniques;

- 'Shaping' is a technique where the training of the required behaviour is broken down into a series of stages that build upon each other until the required behaviour is achieved. The animal is rewarded for performing each stage, or successive approximation of the required behaviour. The technique used to train baboons to co-operate during blood pressure measurement (Turkkan, 1990, described above) is an example of a shaping procedure.
- 'Target' is a versatile behaviour used to station an animal in a particular location, move individuals around an enclosure or extend a body part for inspection or treatment. The animal is initially rewarded simply for touching the target then a shaping procedure is used to train the animal to hold the target for progressively long periods. When training in groups, individuals can be trained to their own specific target, which can provide a useful way of separating them from the other animals.

Although commercially manufactured targets are available, a variety of commonly available objects could be used provided they are appropriate, distinctive, safe and easily replaced if broken or lost. The actual choice of object will depend on the situation. For example, a long stick or pole can be useful when training nervous (or dangerous) animals as it allows the maintenance of some distance between the trainer and the animal.

When training group living animals to specific targets, it is important to ensure that the objects used are truly distinctive to the animals. For example, although Old World monkeys and apes are trichromatic with colour vision similar to humans, colour vision in most New World species is polymorphic and while some females are trichromatic, other females, and all males are dichromatic with limited colour vision (Caine, 2002). Coloured targets are therefore suitable for some primate species, but not others. "Target training" is discussed further in Chapter 6.

- 'Stationing' is similar to targeting except no specific object is used and the animal is simply required to move to and then remain in the desired location. Occasional rewards are given as long as the animal remains in position.

As stated, there is little difference between stationing and targeting other than the absence of a specific object. This may have some advantages in certain situations. For example, the behaviour can be elicited quickly (for example, if the animals fight or escape) without the need for fetching or constantly carrying the required targets. The need to have the required targets readily available could be a disadvantage in large facilities or with larger species as generally, the stronger the animals, the more robust (and therefore more cumbersome) the target has to be. In addition, some primate species may grasp the target readily enough, but then show considerable reluctance to let go. This poses the risk of confrontation with the trainer which is something to be avoided whenever possible.

- 'Familiarisation' or 'desensitisation' is used to teach an animal to tolerate an aversive object or situation. Throughout exposure, the animal is offered

frequent food rewards. When an animal is instinctively fearful, by pairing rewards with the aversive situation, the animal is conditioned to associate that situation with a pleasant stimulus, which in turn reduces or eliminates the fear. If the fear is a result of a previously conditioned response, then this pairing of food with the aversive stimuli if a form of counter-conditioning which, as stated above, replaces an existing conditioned response with a new one.

The distinction between different training methods can be illustrated by comparison between the techniques used to solve a particular husbandry problem that occurs in both laboratories and zoos. In laboratories, the recent move towards the practice of housing primates in social groups has presented laboratory personnel with the challenge of obtaining regular samples from animals no longer confined in small cages. This has added an additional element to training for co-operation during venipuncture described above, and a closer examination of the techniques used suggests that co-operation by the animals may not be entirely voluntary. Although some variation exists, the basic procedure employed is very similar across a number of studies. Large enclosures are designed to include a narrow area or chute that connects to a smaller holding pen, which in turn connects back to the enclosure, effectively forming a circular loop. The monkeys are trained to enter the chute where they can be isolated in turn in a transport cage or a sampling cage built into the chute itself. The main purpose of training is to get the monkeys to enter the chute.

What appears to be clear is that the initial stages of training involve chasing the animals into the chute (Reinhardt, 1990). In various institutions they are "prompted by shouting and arm-waving" (Luttrell, Acker, Urben & Reinhardt, 1994,

p137), or directed by an "auditory cue" created by "hitting the (PVC) pipes against the metal support poles" (Phillippi-Falkenstein & Clarke, 1992, p 83). Phoenix (1975) reports that technicians need to be "fleet of foot" to manage macaques in an outdoor corral. In the report by Phillippi-Falkenstein and Clarke (1992) the fact that all of the animals would defecate while in the chute and refused to accept food rewards while in the sampling cage suggests that they found the procedure stressful. Many institutions do offer food once the animals are released (Knowles, Fourrier & Eisele, 1995; Luttrell *et al.*, 1994; Reinhardt, 1990) yet strictly speaking, this rewards the animals for returning to the main enclosure rather than for entering the chute system. Boccia, Broussard, Scanlan & Laudenslager (1992) describe how an identical procedure to those described above was developed for the handling of pigtail *(M. nemestrina)* and bonnet macaques *(M. radiata).* However, it this example, the procedure used was not described as a training technique, but as a way of minimising the confounding effects of human contact. In addition, analysis of plasma cortisol showed that the monkeys who had experienced the procedure several times became habituated to it, even though food treats were never provided.

Most of the above studies concern the training of rhesus macaques but there is some evidence to suggest that the technique is less successful when employed with stump-tailed macaques. While co-operative on many occasions, males will sometimes stand their ground and when this occurs the best solution appears to be to try again later (de Waal, 1989). Bunyak, Harvey, Rhine and Wilson (1982) found it was necessary to remove all male stump-tailed macaques and keep them occupied with a favoured food before attempting to capture the females. Even so, females with young infants were found to be too difficult to manipulate using the chute method.

While it is claimed that co-operation with this procedure is the result of training, it is worth noting that the practice of herding animals into a progressively restricted area is not unique to laboratories and can be found on most farms. An example would be handling sheep for dipping or shearing where the animals are herded from the fields into a small holding pen then individually down a narrow passage for capture. It is clear that farm animals become accustomed to the procedure and farmers acknowledge that the dogs that carry out the herding require training. However, it seems unlikely that farmers ever claim to have trained the sheep!

While the chute system requires that the animals move away from humans, PRT teaches the animals to move towards the trainer using either the 'target' or 'station' method. As stated previously, once an animal has been trained to come to his/her target, he/she can be moved to a particular location simply by placing the target there (Laule, 1999; Mellen & Ellis, 1996). Stationing is essentially the same as the 'sit, stay' behaviour commonly taught to domestic dogs (Prior, 1999) and the animal is rewarded for approaching the trainer when his/her name is called (Laule, 1999; Mellen & Ellis, 1996).

Targeting and stationing techniques have been used in a number of zoos and with a variety of species (Laule, 1992; Laule & Desmond, 1998; Reichard, Shellabarger & Laule, 1993; Sevenich, 1995; Shellabarger, 1992). The advantage of these techniques is that only positive reinforcement is used and participation by the animal is truly voluntary as the animal is not forced to approach the trainer and may retreat at any time. However, there is a paucity of detailed information regarding effectiveness and time investment required for training. In addition, as is usually the case with zoo-based studies, evidence that such training actually reduces stress comes from the voluntary participation by the animals with the assumption that if the

procedure was stressful the animals would not choose to co-operate. There is a lack of more detailed examinations of behaviour and physiological responses found in laboratory-based studies. However, this point illustrates something of an anomaly in the literature in that the little quantitative evidence available to support the view that PRT reduces stress may come from animals that have not been trained exclusively by PRT techniques.

The factors described above raised the question of exactly which techniques were being used while training primates in a laboratory setting. As stated, the recommended method is PRT but the description of many training regimes suggests that the fundamental principles are not always followed. However, there is another feature of the existing literature that is potentially cause for concern.

With a few laboratory-based exceptions (e.g. Heath, 1989), almost all reported instances of primate training originate in the US. At the start of this research it was not possible to find a single article originating in a UK zoo and what little training appeared to be conducted in British zoos was confined to a limited number of species. Indeed, the cultural norm in Britain appeared to be that a 'hands off' policy, keeping contact between caregivers and animals to a minimum, was the most desirable option (Kiley-Worthington, 1990).

One of the main objections to close relationships between caregivers and zoo animals is that this is unnatural. A related belief is that close contact with humans renders animals unsuitable for reintroduction and may even result in the domestication of wild species. While carrying all the undesirable elements of close contact, training, where an animal is deliberately taught to perform specific behaviours on request, arouses the additional fear that this will disrupt normal social behaviour as animals become 'fixated' on gaining food treats from humans (Kiley-

Worthington, 1990). The most obvious reason for the absence of UK-based training articles was that practically no training was intentionally being carried out. Of course, it is highly probable that a great deal of training is carried out inadvertently. For example, a common technique used to encourage the animals to come into their indoor quarters at night involves placing food in those quarters and then calling to let the animals know that food has been provided. When the animals enter and find food they have been effectively rewarded for coming to call.

There is a paucity of studies examining the effects of inadvertent training or indeed the effects of deliberate training beyond the actual training sessions themselves. To address this issue data were collected on the behaviour of all the study animals out with training sessions. The purpose of this was twofold. First, to assess the psychological well-being of the animals through the measurement of stress-related behaviours and secondly, as studies conducted with laboratory housed animals can have implication for those in zoos (Crockett, 1998) behaviour was examined to look for any significant changes as a result of both training and increased positive contact with humans (Chapters 4 & 7).

As reported above, training is a well-organised activity in the US with educational programmes in place to ensure that those training animals themselves receive adequate training. This is clearly not the case in the UK at the present time. The Animals (Scientific Procedures) Act (1986) requires that laboratory personnel complete a mandatory course of training. While the second module contains information on general handling and restraint techniques, there is no specific reference to training techniques or operant theory. Even then, veterinary surgeons, experienced animal technicians and holders of overseas qualifications may only be required to complete Module 1 which only covers ethical and legal issues with no

reference actual animal husbandry. In addition, the description of the types of learning used in training contained in one handbook, *Guidelines on the handling and training of laboratory animals* (The Biological Council, 1992) clearly confuses negative reinforcement with punishment.

These factors raised the question of how well the training that was being conducted in the UK was actually being carried out. This has significant welfare implications as while good training practice can improve animal/human relationships, bad training has the potential to increase fear of humans thus undermining psychological well-being (Jones, 1997; Rushen, Taylor & de Passillé, 1999; Waran, 1995). The first study recorded the training of macaques as carried out in a UK laboratory in greater detail than currently recorded in the literature and assessed the techniques used within the framework of operant theory (Chapter 3)

As reported above, a close examination of the existing literature suggests that there are considerable differences between the techniques used to train primates in zoos and those housed in laboratories. Zoo-based studies report the use of PRT but provide little quantitative data regarding required time investment and effectiveness while the data contained in laboratory-based studies comes from techniques that employ a considerable amount of negative reinforcement. As such, there is little evidence to support the view that PRT is a practical approach to the management of primates in a laboratory situation. This research aimed to explore the practicality of training that only used positive reinforcement techniques in a laboratory setting. This was an important consideration as initiatives intended to improve welfare have little chance of success if they are not practical, as they will simply not be carried out. As there is a paucity of information regarding non-macaque species, this was explored through the training of common marmosets *(Callithrix jacchus)* (Chapter 6).

1.5 Training and animal welfare

1.5.1 The importance of animal welfare

Animal welfare is an emotive issue with much debate as to what constitutes good welfare (Broom & Johnson, 1993; Fraser, Weary, Pajor & Milligan, 1997; Mason, 1990) how it can be assessed (Barnett & Hemsworth, 1990; Mason, 1991; Mason & Mendl, 1993; Mench; 1993; Newman & Farley, 1995; Rosenblum, 1919; Widowski, 1990; Woolverton, Ator, Beardsley & Carroll, 1989) and even why it matters (Blum, 1994; Hunt, 1991; Singer, 1990).

In the latter category, one view is that animal welfare is important because we have an ethical obligation towards animals (Singer, 1990; Sandøe, Crisp & Holtug, 1997). Without such an ethical obligation there would be no duty of care and consequently no welfare issues. However, ethics are moral judgements and as such, are contestable and this is reflected in the various philosophical approaches to the question of animal welfare.

The view of the 'animal rights' approach is that as living creatures, animals have rights that should not be violated regardless of the potential benefits to humans. In this approach there is no justification for the use of animals, be it in farming, laboratories or zoos (Regan, 1995). The utilitarian view tries to balance the interests of animals with those of humans, which results in an ethical evaluation of the costs and benefits to each. In this view, research involving animals must consider these factors and the potential benefit to humans must outweigh the suffering experienced by the experimental animals (Rollin, 1985). This view is reflected in legislation such as the Animals (Scientific Procedures) Act (1986) that provides the basis of all guidelines regarding the care and use of laboratory housed animals in the UK.

Additional considerations arise from practical considerations. Poor welfare in farming leads to low productivity as a result of poor growth and high mortality (Grandin, 1997). In laboratories, the growing recognition of the physiological consequences of psychological distress has led to doubts concerning the validity of research where the confounding effects of stress are ignored (Greek & Greek, 2002; Reinhardt, 1997a, 1999). In addition, different responses to stressful conditions may increase variability within experimental results thus requiring more animals to increase sample sizes with obvious financial implications (Chance, 1957).

It is also worth noting that to many people, practical issues and the question of ethics and moral responsibility are irrelevant and the importance of animal welfare is an emotional rather than an intellectual issue. Animal welfare matters because animals themselves matter (Serpell, 1996). While some writers have been criticised for advocating an approach to welfare based on compassionate feelings (Dawkins, 1990), it has also been argued that empathy can play an important role in the understanding of animal suffering (Arluke, 1992).

1.5.2 Theories of animal welfare

The stress concept

Of the many theories and models proposed to examine animal welfare, there are three that are particularly appropriate, the first of which is the stress concept initially proposed by Hans Selye (1973). Following observations of physiological changes such as gastro-intestinal ulcers, immune system degeneration and increased secretion of glucocorticoids from the adrenal cortex in rats exposed to a series of aversive stimuli, Selye proposed that the physiological responses to such stimuli (or stressors) were essentially the same regardless of whether physiological or

psychological in nature. When an animal confronts stressors in the environment, the sympathetic branch of the autonomic nervous system becomes active, stimulating the adrenal medulla to secrete epinephrine, norepinephrine and steroid stress hormones including the glucocorticoid hormones that effect glucose metabolism, thus preparing the animal for action, the so-called 'fight or flight' response. When the threat posed by environmental stressors is past, the parasympathetic branch of the autonomic nervous system returns the body to homeostasis (Broom & Johnson, 1993). Selye termed this triphasic reaction of arousal, response and return to homeostasis the General Adaptation Syndrome and added that should exposure to the stressor continue, adaptation can fail leading to exhaustion and death (Selye, 1973).

Although hugely influential, Selye's theory has been criticised on a number of issues. The physiological response to stressors is not as coherent as Selye believed and similar physiological responses can occur following both stressful (in the sense of being aversive) and non-stressful stimuli or events such as mating (Broom & Johnson, 1993). Engel (1967) proposed two distinctly different modes of response to stress. The short-term reaction leads to the 'fight or flight' response mediated by the sympathetic adrenal-medullary system and the animal takes action to resolve the situation. The behavioural response taken by an animal will depend on a number of factors such as genetics, rearing conditions and other prior experiences. If the response shown is futile, the long-term effect is depression of behaviour, which serves to conserve energy. This stage is mediated by different physiological changes originating in the pituitary adrenal cortical system. In Engel's model, the effects of genetics and prior experience play a critical role in how a stressor is assessed and which behaviour responses will occur.

In a continuation of Engel's (1967) theory, Moberg (1986) proposed a three stage model of stress where perception of a challenge is followed by a stress response involving behavioural, autonomic and physiological changes. The exact nature of such changes will depend on the initial perception of the stressor and the genotype, experience and physiological condition of the animal itself. Severe or prolonged challenges pose a risk to the animal's psychological and physiological well-being which may manifest itself in a number of ways including abnormal behaviour, increased susceptibility to disease and impaired reproduction.

Both Engel's and Moberg's models of the stress response emphasis the importance of psychological factors in both the perception of, and reaction to environmental stressors. Such factors can both increase and decrease arousal in response to aversive stimuli (Weinberg & Levine, 1980). Examination of four psychological variables can provide a framework for understanding how training could contribute to the welfare of captive animals. Important factors are:

- The predictability of stressors. The training process may promote welfare by helping to make a variety of laboratory and husbandry routines predictable.
- The ability of the animal to exert control over, or make coping responses during stressful situations. The principle of voluntary co-operation is closely linked to the principle of control. If an animal is free to retreat from the training situation then it has control of the process. In addition, training can actively teach an animal an appropriate response to a number of essentially alien situations.
- The effects of information available to the animal immediately following its response to aversive stimuli which indicates that the threat has passed

and the correct response has been made. In this context, the provision of rewards can provide a clear indicator that the correct response has been made.

- The previous history of the animal with regard to the above factors. This factor points to the importance of good training techniques as poor or inconsistent training can undermine rather than promote the benefits of training with regard to the above factors. It is also likely that an important element within the animal's history concerns previous experience with humans as animal/human relationships are an essential part of the training process (Reinhardt, 1997a, 1997b).

The concept of stress has done much to stimulate research demonstrating both the psychological and physiological consequences of poor animal welfare. In addition, measurements of activity in the sympathetic-adrenal-medullary system and in the hypothalamic-pituitary-adrenal cortex system can provide useful indicators of how well animals cope with environmental stressors (Broom & Johnson, 1993) (Chapter 7).

The 3 Rs

The concept of the 3 Rs is not as much a model of animal welfare but a framework in which improvements to animal welfare can progress. The concept was first proposed in Russell and Burch's (1959) *The principles of humane experimental technique* and has since been adopted by a number of welfare agencies such as the Home Office Animals (Scientific Procedures) Inspectorate (Richmond, 2000). The "Rs" refer to the concepts of Replacement, Reduction and Refinement. These are defined as follows:

- *Replacement* as "the substitution for conscious living material" for example, tissue culture, computer modelling and the use of invertebrate species.
- *Reduction* as using the minimum number of animals necessary "to obtain information of given amount and precision" with an emphasis on using the right number rather than too few or too many.
- *Refinement* as any decrease in the nature, severity or incidence of inhumane procedures to those animals which still have to be used.

(Richmond, 2000, p 84)

With regards to reduction, parametric statistical tests calculate the relationship between means, variance and sample size (Howell, 1995). When the variability between study animals is large, a larger sample size thus more animals are required. However, much variation can be the result of different responses to stressful situations (Reinhardt, 1997a). If training can reduce the stress associated with laboratory procedures then the variance between study animals is also reduced. Measures such as minimising contact and handling all animals in exactly the same way in order to reduce variability are ineffective as differences in prior experience cause different behavioural and physiological responses anyway (Boccia, Broussard, Scanlan & Laudenslager, 1992). As such, training all study animals to a common standard may reduce variability more successfully.

With regards to refinement, the detrimental psychological and physiological effects of a wide range of common laboratory techniques and procedures have been documented (Reinhardt *et al.*, 1995). Techniques using trained animals represent a refinement of existing practice. In addition, as more information regarding the effectiveness of various techniques is gathered, these in turn can be improved, in

effect becoming a refinement of a refinement. In addition, refinement also aims to reduce the quantity of invasive techniques. Training could be used to collect samples using non-invasive methods, for example urine in place of blood.

However, the rather narrow definition of refinement outlined above has been criticised in recent years as it suggests that the concept takes the rather negative approach of addressing issues of poor welfare and aiming to bring animals to an effectively neutral state. Rennie & Buchanan-Smith (in prep.) have argued that the concept of refinement be widened to include the promotion of positive welfare experiences. In addition, refinements should be made to every practice that a laboratory-housed animal experiences throughout his/her life.

These suggestions have significant implications for training. One factor that is common to all laboratory-housed animals throughout their lives is the presence of humans therefore improvements in animal/human relationships represent a refinement that could appreciably promote the welfare of these animals. By helping to establish such positive animal/human relationships (Heath, 1989; Laule, 1999; Laule & Desmond, 1998; Reinhardt, 1997b; Reinhardt, 1997c) training could be useful, not only in minimising the stress associated with specific laboratory procedures, but it actively promoting the psychological well-being of laboratory-housed animals. Given the paucity of studies concerning the training of callitrichid species, this issue was addressed by examining the response of common marmosets to changes in their relationships with humans following PRT or a period of increased positive contact, and the reactions of trained and untrained common marmosets following administration of a mild environmental stressor (Chapter 7).

To summarise, the training of laboratory-housed primates has the potential to promote their physiological and psychological well-being in a number of ways. The

stress associated with a laboratory environment could be reduced by making routine procedures predictable, giving the animals control over their environment, teaching appropriate coping responses and improving relationships with human caregivers. In addition, a reduction in variability between study animals could reduce the number of animals required. Standard laboratory techniques can be refined and non-invasive alternatives developed. However, such benefits depend on the quality of the training techniques used. At best, poor training will result in no improvement to the psychological well-being of captive primates. At worst, poor training has the potential to make the situation significantly worse.

1.6 Aims of Thesis

To summarise, the aims of the thesis were as follows:

- To examine the training techniques currently used in a UK laboratory by recording the training of macaques in greater detail than currently reported in the literature and assess the techniques used within the framework of operant theory and the effects on the behaviour of the trained animals (Chapter 3).
- To explore the practicality and welfare implications of training that only used positive reinforcement techniques in a laboratory setting through the training of common marmosets (Chapter 6).
- To examine the behaviour of the study animals out-with training sessions in order to assess the psychological well-being of the monkeys through the measurement of stress-related behaviours following training sessions (Chapter 4) and look for any significant changes in

behaviour as a result of both training and increased positive contact with humans (Chapter 7).

- To test the hypothesis that trained animals would experience less stress when exposed to a standard laboratory procedure than untrained ones (Chapter 7).

Chapter 2

Stump-tailed Macaques *(Macaca arctoides)*: Introduction to the Species and Methods

2.1 INTRODUCTION

2.1 The stump-tailed macaque

"They are extremely noisy and appear to fear nothing, at times not even man... They are apparently very pugnacious when disturbed. The Nagas are somewhat timid of them at times, on account of their vicious habits."

McCann (1933) cited by Bertrand, 1969, p147.

"...at first sight stumptails do look, let us say, somewhat unusual. This is the reason few zoos display the species. Anyone who knows stumptail monkeys better, though, is smitten with their charming personality."

de Waal, 1996, p145.

Statistical data recorded between 1965 and 1975 show that of all macaque species, the cynomolgus macaque *(Macaca fascicularis)* and the rhesus macaque *(M. mulatta)* were the most commonly imported with 53,432 and 39,263 animals respectively arriving in the UK (Burton, 1978). The same period saw the importation of 1415 stump-tailed macaques (Burton, 1976), then classified as *M. speciosa*, but subsequently changed to *M. arctoides* in 1976 (Rowe, 1996).

Although intensively studied in captivity, little is known of the behaviour of this species in the wild, partly due to their elusive nature and relatively inaccessible habitat of southern China and Southeast Asia (Bertrand, 1969; Fooden, 1989). The stump-tailed macaque has adapted to a wide range of environmental conditions and has been found at altitudes ranging from sea level to 2,400m (Bertrand, 1969). Stump-tailed macaques are relatively large, powerful monkeys with wild males

weighing around 12.2 kg and females weighing around 8.4kg (Fooden, 1989). Diurnal and largely terrestrial, these animals live in multimale-multifemale groups of up to 60 individuals with a median group size of 25 animals (Fooden, 1989). They feed on leaves, fruit, seeds and animal prey such as birds, eggs and insects. Feeding primarily occurs between dawn and 1000h-1100h with a second bout from around 1700h till dusk. Between feeding bouts, these monkeys rest but also spend a considerable amount of time grooming (Bertrand, 1969).

In the United States, interest in the stump-tailed macaque arose partly due to the apparently aggressive nature of rhesus macaques and the hope that a more tractable substitute could be found for use in biomedical research (Orbach & Kling, 1964). In 1963 and 1964, Orbach and Kling published a series of articles recommending the stump-tailed macaque as a laboratory animal due to the placid nature of this species which appeared to be docile when handled and thus easily managed. The authors went so far as to find the behaviour of stump-tails comparable to that of lobotomised rhesus macaques (Orbach & Kling, 1963). However, the animals studied by Orbach & Kling were all relatively young (the eldest believed to be under four years old). As data concerning the behaviour of these animals accumulated, it became clear that the temperament of this species was considerably more complex than originally supposed. Indeed, such studies reveal some contradictory findings that could explain the very different views of stump-tailed macaque nature expressed by McCann and de Waal in the passages quoted above.

Trollope (1968) found that stump-tailed macaques were indeed steadier, easier to handle, less prone to flight and panic, and less destructive than rhesus macaques. However, under certain conditions they would threaten and even attack personnel. While testing stump-tailed macaque' responses to closely positioned human

observers, Blurton Jones and Trollope (1968) recorded open-mouth threats on 30 per cent and attacks on 12.2 per cent of trials. The same authors reported that although females appeared unconcerned by attempts to observe their infants, an infant scream or an attempt to groom infants through cage bars easily provoked an attack. Stump-tailed macaque males are particularly tolerant and protective of infants and readily attack humans if an infant is threatened (Weisbard & Goy, 1976).

A common observation is that this species is prone to contagious aggression, which follows a predictable pattern. Screams produced by a frightened or wounded animal trigger aggressive vocalisations in others which then increase in intensity, arousing the group in preparation for attack (Bertrand, 1969; de Waal, 1989). There have been a number of reports of human hunters being killed following attack by stump-tailed macaque troops (Young, 1967, cited by Bertrand, 1969). Contagious aggression could well be adaptive in the wild in that defence of the group is more successful when several individuals attack a predator simultaneously (Bertrand, 1969). However, this tendency poses problems in captivity where an attacked conspecific has little possibility of escape. It also presents a potential danger to humans, particularly those working with group-housed stump-tailed macaques if a negative reaction by one animal leads to an aggressive response by other animals in the vicinity.

Some studies have reported that these monkeys can also be extremely aggressive towards each other with fights resulting in serious injury or even death (Bertrand, 1969). Brüggemann and Grauwiler (1972) recommended that breeding males be removed as soon as mating was concluded due to their tendency to fight. However, fighting may have been the result of the practice of confining individually caged animals in a narrow space solely for the purpose of mating. While some studies

have found group-living stump-tailed macaques to be slow to settle and as aggressive as rhesus macaques (Chamove, 1981), other have found that these animals are relatively placid and adapt quickly to changes in group composition (Rhine, 1973).

Although serious fights do occur among stump-tailed macaque groups, such events are uncommon. Low intensity disputes occur frequently yet rarely escalate into actual physical harm and are reconciled on at least 50 per cent of occasions (de Waal, 1989). One striking characteristic of these animals is the relative tolerance shown with regard to the dominance hierarchy which is less strictly enforced than in some other macaques species (Chaffin, Friedlen & de Waal, 1995). For example, de Waal (1989) found that subordinate animals will often stand their ground when approached by dominants and may even respond to threats with counter-threats. Threatening dominants are often ignored and are avoided on only 50 per cent of occasions. Subordinate stump-tailed macaques are more confident than their rhesus counterparts and initiate contact with dominants on around 50 per cent of occasions, as opposed to 30 per cent for rhesus. While male rhesus rarely become involved in female disputes, male stump-tailed macaques frequently intervene, protecting infants, juveniles and subordinate group members (de Waal, 1989). Disputes can also be resolved through ritual biting, a behaviour peculiar to this species. Here, a part of the body, usually a limb, is grasped and held between the teeth for a few seconds although this never results in injury. A striking feature is the relaxed posture of the animal being bitten, who may even have initiated the behaviour by offering a limb (Demaria & Thierry, 1990).

What emerges is a picture of an animal that is generally placid but extremely dangerous when roused. In addition, stump-tailed macaques have a complex and egalitarian social system characterised by frequent, but easily resolved squabbles and

a tolerant dominance hierarchy. Indeed, these animals invest a considerable amount of time to maintaining social relationships. The key to understanding the complex nature of this species may lie in their natural response to danger. Stump-tailed macaques are relatively slow moving and are not particularly agile which suggests that the apparent docility shown by these monkeys may be the result of a species-typical "freezing" response to danger (Blurton Jones & Trollope, 1968). When animals are poorly adapted for flight, their remaining options are to hide or fight (Bertrand, 1969). As such, the stump-tailed macaques' first response to danger is to become still and inconspicuous. If pushed they will attack as a group, a response made more effective by their tendency towards contagious aggression. High levels of social behaviours such as grooming and a tolerant social hierarchy may be the results of a need for close relationships if co-operative group behaviour is an adaptive response to danger (de Waal, 1989). Whatever the reasons, the complex nature of these animals presents some interesting challenges to humans working with them.

Early studies on the establishment of captive stump-tailed macaque colonies do not report the high mortality rates found in callitrichid species (see Chapter 5). Although some deaths are reported (Chamove, 1981), these animals appeared to survive and breed in captivity relatively easily (Brüggeman & Grauwiler, 1972; Chamove, 1981; Rhine, 1973) although abnormal behaviours often developed in response to poor housing conditions (Burt & Plant, 1990). However, despite early promise, the stump-tailed macaque is not widely found in biomedical research in the UK with the cynomolgus macaque accounting for between 80 to 90 per cent of macaques used between 1990 and 1995 (Boyd Group, 2000). This low use of stump-tailed macaques may be in part due to their large size and tendency towards obesity (K. Morris, per. comm.) and their relatively low reproductive rate (Fooden, 1985).

They have never been a popular species in zoos due to their unattractive appearance and apparent inactivity (de Waal, 1989). Another contributing factor to their decline in captivity may have been the introduction of testing for the Herpes simiae virus. In one colony alone, all wild-caught animals and 80 per cent of captive-bred animals housed with them tested positive and were subsequently sold or destroyed (Chamove, 1981). At present, the MRC Human Reproductive Science Unit in Edinburgh is the only UK laboratory housing this species (K. Morris, pers. comm.). However, stump-tailed macaques are still used in other countries in particular in studies of sexual behaviour and reconciliatory behaviour (Call *et al.,* 1999; Nieuwenhuijsen, Slob & van der Werff ten Bosch, 1988; de Waal, 1989).

2.1.2 Choice of stump-tailed macaques as study animals

Given the considerable differences in attitudes and practice concerning training in the UK and the US (Chapter 1), one of the aims of this thesis was to investigate existing training as carried out in a UK laboratory. The MRC Human Reproductive Science Unit in Edinburgh was identified as an institution that both carried out training and was willing to have its methods closely observed. As stump-tailed macaques were one of two species housed at this unit, the decision to study this species was largely opportunistic.

That said, the availability of these monkeys was fortuitous for a number of reasons. Firstly, they provided an interesting contrast to the second species studied, common marmosets, due to considerable difference in species-typical characteristics and pre-existing relationships with humans (see Chapter 5). Secondly, as most of the studies reporting the training of laboratory-housed primates concern the training of rhesus macaques (Reinhardt, 1997a), there was the possibility that observations of a

different macaque species could help contribute to an understanding of how primates in general respond to the training process. Finally, the specific character of the stump-tailed macaque provided an additional possibility. The placid nature of these animals suggested that they should respond well to training if handled appropriately. However, as shown above, stump-tailed macaques respond aggressively when provoked and show little fear of humans under such circumstances. This suggested that any aversive elements in the training process were unlikely to be tolerated. This aspect of the nature of stump-tailed macaques potentially provided the opportunity to detect differences between good and poor training practice.

2.2 MRC Stump-tailed Macaque Colony

2.2.1 Housing

At the start of the study, 34 stump-tailed macaques were housed at the MRC Human Reproductive Sciences Unit. The monkeys were maintained in six social groups, with the actual study animals forming one of these groups. Housing for each group consisted of indoor enclosures or "gang rooms" measuring 2.7 x 2.8 x 4.8m. These were furnished with wooden logs and additional metal shelves and ladders to allow climbing and provide a variety of perches. Enclosure floors were covered with a thick layer of wood-shavings among which meals were scattered to allow foraging. Throughout the study period, additional enrichment devices such as hanging tyres, puzzle boxes and television were occasionally present as such objects were rotated between the various groups (see Plate 1). The front wall of each gang room contained a large viewing window (1 x 0.88m). This window allowed unobstructed visual access to the whole room and also allowed the monkeys to view activity in the technicians' access corridor (see Plates 2 & 3). Gang rooms were constructed in

pairs, separated by a central "cage room" containing two rows of eight standard laboratory cages arranged in two tiers (see Plate 4). Each bank of connected cages was placed along opposing walls and could be accessed from one gang room via slide doors. This allowed neighbouring groups visual and auditory, but not tactile contact. The use of two doors placed at opposite ends of the cage row prevented subordinate animals becoming trapped in the cages by more dominant individuals. Although the macaques normally had free access to both gang room and cages, slide doors could be used to confine the animals to the gang room, cage room or within individual cages. See Figure 2.1 for a schematic diagram of macaque housing at the MRC unit.

Figure 2.1 Plan of macaque housing at the MRC Human Reproductive Sciences Unit (not to scale)

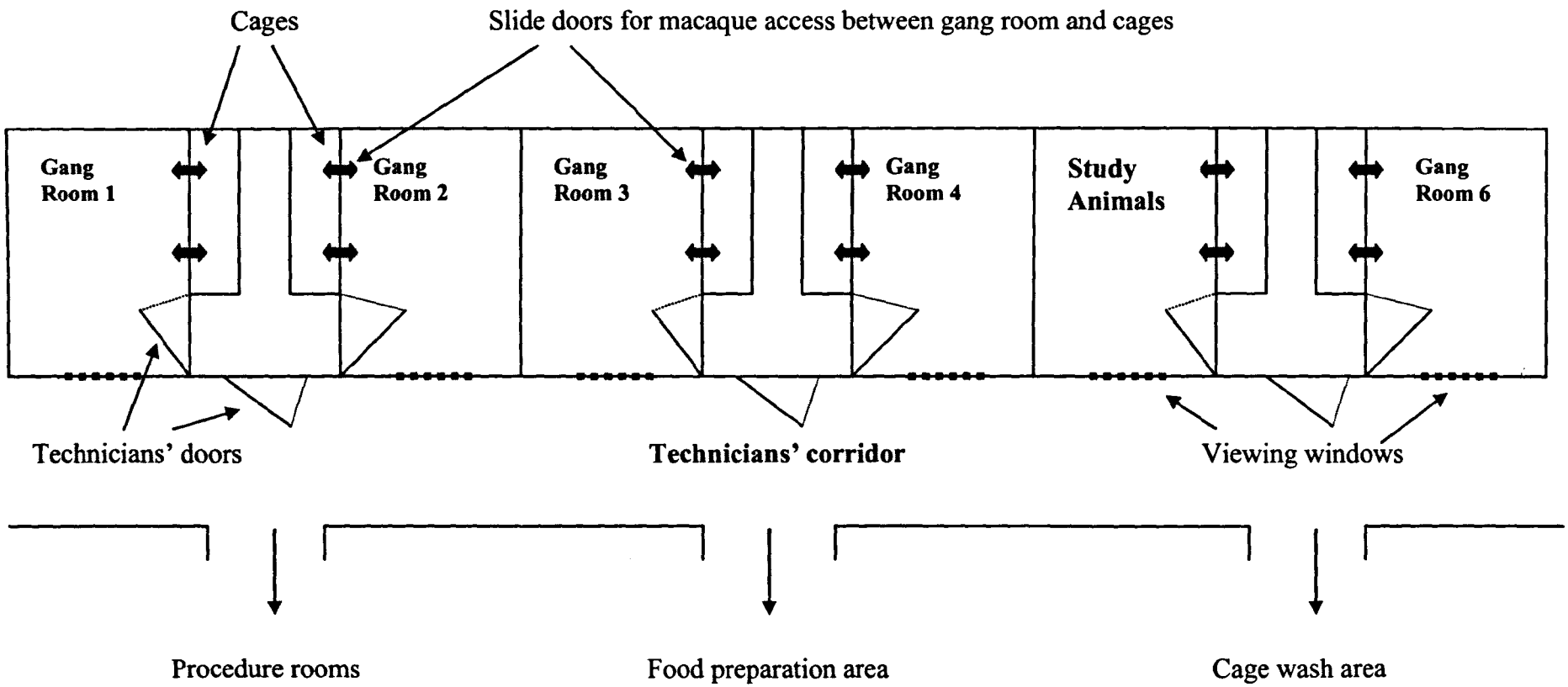


Plate 1 Technicians access corridor (macaques)
(viewing windows are on the left)

Plate 2 Macaque watching human activity through the viewing window

Plate 3 The study animals as seen through the viewing window

Plate 4 Macaque cage room

2.2.2 Husbandry

Most routine cleaning procedures took place in the morning, usually between 0830 and 1030h, with a final check on the animals conducted between 1600 and 1700h. When enrichment activities such as modifications to the enclosures and provision of puzzle feeders or novel objects occurred, they were generally carried out in the afternoon. Gang rooms were cleaned out twice a week when all used wood-shavings were removed and replaced. This was accompanied by power hosing every fortnight. Cage rooms were dry cleaned daily and power hosed twice a week. No cleaning was conducted at weekends. Experimental procedures such as blood sampling and vaginal swabbing were generally carried out once cleaning was completed.

2.2.3 Feeding

At the start of the study, food was provided twice per day on weekdays and once at weekends. A single piece of fruit was given in the morning, usually before 1030h. The main feed was provided at around 1400h – 1430h when laboratory staff returned after their own lunch. This practice was vulnerable to changes in staff routine as the one hour staff lunch break did not begin until all morning tasks were completed. A delayed lunch break meant that staff returned to work late and as a result, there was considerable variation in the times that the macaques were actually fed. When the results of a study showing that disruption of feeding routines had a detrimental effect on the welfare of the animals became available (Waitt *el al.*, 2001), this procedure was changed (see Chapter 4). Subsequently, the macaques were fed once per day between 1230h and 1330h with the feeding schedule becoming much more consistent.

The macaques were provided with a mixture of Old World primate pellets, fresh fruit and vegetables (banana, apple, pear, orange, tomato, grapes, cucumber, cabbage and carrot). On entry to the gang rooms during feeding, staff would hand each monkey a piece of their favourite food then scatter remaining items around the room to encourage foraging. Further encouragement was provided by the use of a "forage mix" of grains, nuts, seeds and dried fruit scattered throughout new wood-shavings whenever the gang room was cleaned. In addition, treats such as bread and honey or yoghurt were provided once or twice per week on an unpredictable schedule.

2.2.4 Staff/macaque relations

Good relations with human caretakers have been shown to promote the welfare of non-human primates (Heath, 1989). The policy at the unit was to encourage the development of positive relationships between laboratory staff and the monkeys and this was particularly noticeable with the macaques. The welfare of the animals was a high priority and staff time was allocated to environmental enrichment. All six members of staff were involved in the care of the animals but only three carried out procedures such as venipuncture. All adult female macaques in the unit had been trained to present for vaginal swabbing, a procedure carried out in order to monitor reproductive cycling. A number of females had also been trained to present a limb for blood collection. Male macaques were never used in experimental procedures and were kept primarily due to their role in regulating disputes among the females (Chamove, 1981; de Waal, 1989).

Although the macaques at the unit had both names and identification numbers, they were always referred to by name. Reinhardt (1997c) has argued that names are important in that they lead animals to be viewed as distinct individuals. The

combination of a relatively small number of animals, each with a name and distinct appearance, made it easy for the macaques to be viewed in this way, as opposed to large numbers of rather similar, unnamed marmosets. The overall impression was that the staff at the unit were genuinely interested in, and cared about, the stump-tailed macaques in their care. Staff frequently discussed the monkeys, relating anecdotes about their behaviour and personalities and there was remarkable consistency in the way that individual humans viewed individual monkeys.

In addition to individual differences, staff were also aware of differences between groups and this affected the amount and quality of primate- human interactions (Waitt *et al.*, 2002). For example, staff would often spend time in the cage rooms simply talking to the monkeys and hand-feeding treats through the cage bars. With some groups, staff would enter the gang rooms or let the monkeys loose in the cage room to interact with them. Groups that contained monkeys who would readily interact with humans received the most attention. Groups that contained aggressive animals were rarely treated in this way. Staff also avoided giving treats to groups where this would lead to aggression by one of the dominant animals towards the others. Two staff members in particular were sensitive to the relationships between the monkeys and would adapt the way in which they worked with the animals accordingly.

2.3 STUDY ANIMALS

The study animals were five adult stump-tailed macaques housed in the same social group. These animals had originally been part of a large breeding group acquired from another laboratory. Approximately eight months prior to the start of this study, serious fighting had occurred within this group when the dominant male

(Blackie) was challenged and deposed by a younger animal. Blackie was subsequently removed with four of the females who had supported him to form the group studied here. The group was regarded by laboratory staff as an easy one to work with as none of the animals were considered aggressive. The study followed the training of the four female members. Although not included in training sessions, Blackie was included during observations of the social behaviour of these animals conducted before and after each session. Details of the study animals are given in Table 2.1 (see Plate 5).

Table 2.1 Details of the study animals including name, sex, date of birth and relationship to other group members.

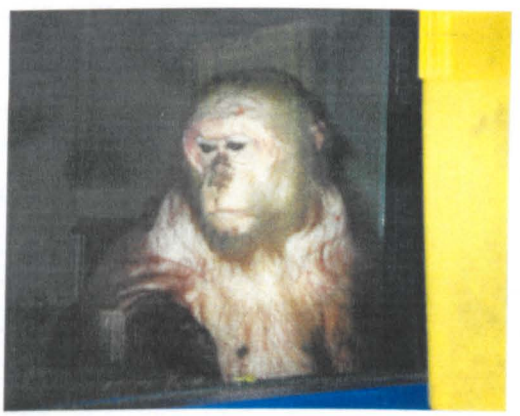
Name	**Sex**	**Date of Birth**	**Relationship to other group members**
Blackie	M	Unknown	Sire of Kelly & Noreen
Jane	F	02/01/83	Dam of Kelly & Noreen, paternal half-sister to Mirrium
Kelly	F	07/12/94	Daughter of Blackie & Jane
Noreen	F	25/01/92	Daughter of Blackie & Jane
Mirrium	F	13/08/86	Paternal half-sister to Jane

The training process inevitably involves some type of human-animal relationship, the nature of which is influenced by many factors including the personalities of the animals involved and their pre-existing relationships with humans. Although subjective reports should always be treated with some caution, individuals' perceptions of the animals in their care do affect how they react towards them (Serpell, 1986; Waitt & Buchanan-Smith, 2003). Any understanding of the training process would be incomplete if this was ignored. Given the importance of these factors, there is some justification for including a description of the study animals beyond the usual reporting of age, sex and living accommodation.

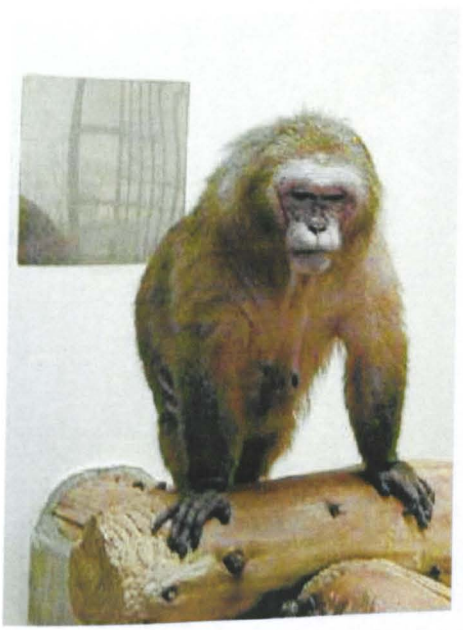
Plate 5 The Study Animals

Blackie

Kelly



Noreen



Jane

Mirrium

As reported above, Blackie's age was unknown although both his appearance and stiff "arthritic" way of moving suggested that he was an elderly animal. This assumption was supported by the fact that his arrival in captivity pre-dated consistent record keeping. He was regarded by laboratory staff as a placid animal but not one who could be forced to do anything he did not wish to. Observations suggested that he took little interest in humans, even when they entered the gang room. Approaches for food were always made by the females with Blackie only showing interest when they appeared to receive something. On occasions he would be groomed by the females and would intervene in disputes between them. However, in many ways he appeared to be a solitary animal who chose to spend a considerably amount of time sitting apart from the others. As the study progressed, he began to spend increasing amounts of time with Mirrium and would support her in disputes with the other females.

Jane, the dominant female, was considerably larger and more powerful than the other females in the unit. Staff regarded her as a domineering animal, but not an aggressive one (i.e. it was generally believed that Jane would be quick to retaliate if provoked but was safe to work with if treated carefully). She was the only one of the group who would threaten humans looking through the viewing window but only if they made eye contact with her. Her threats were never more than stares and she would quickly lose interest.

de Waal describes how stump-tailed macaque relations "continuously alternate between friendliness and minor hostility, like an animated human family at the dinner table." (1989, p166). This quote describes the relationship between Kelly and Noreen perfectly. There was considerable competition between the sisters,

especially over access to Jane. Rhine (1973) found that access to high-ranking animals can be viewed as a resource as they are the most desirable associates, particularly in relation to grooming. Grooming sessions involving all three animals were common with Jane lying, apparently asleep, on the uppermost shelf. During these sessions, Noreen and Kelly would often groom their mother while simultaneously threatening each other. This pattern of frequent agonistic interactions between related individuals has been previously observed in this species (Nieuwenhuijsen *et al.*, 1988). Although younger than her sister, Kelly appeared the more dominant animal as it was Noreen who was usually displaced following competition over access to Jane. If either Blackie or Jane became involved in the sisters' disputes, it was usually Kelly who was supported. In addition, ritual biting has been found to be most common between individuals who are close in rank, have a close bond and frequent agonistic interactions (Demaria & Thierry, 1990). This behaviour was easily observed between this pair and it was always Kelly who bit Noreen, again suggesting that she was the more dominant animal. However, these bouts of 'sibling rivalry' were intermittent and the sisters generally got on well and spent a considerable amount of time together. Kelly was usually the first animal in the colony to be introduced to new activities or puzzle feeders as she was regarded as a particularly clever macaque while Noreen was regarded as a quiet, harmless animal. During interactions with humans, neither sister showed any sign of aggression although Kelly was both bolder, and more interested than her sister. Kelly was usually the first animal to approach humans entering the gang room and the most likely to make physical contact, for example, searching pockets for food.

Mirrium was the most subordinate animal and a somewhat peripheral member of the group at the start of the study. Her position depended largely on the prevailing

relationship between Noreen and Kelly. Following agonistic interactions, each sister in turn would spend time sitting beside or grooming Mirrium. If they were getting on, both would exclude or even bully her. During the course of the study, Mirrium began to spend increasing amounts of time with Blackie. Towards the end of observations, he began to intervene in disputes, supporting Mirrium against Noreen and Kelly. Shortly after this behaviour began, Mirrium would run to Blackie whenever threatened and became noticeably bolder in her interactions with the other females.

Of all the macaques in this group, Mirrium was the most interested in humans. While the others generally ignored anyone watching through the viewing window, Mirrium would frequently glance at them. If Kelly did not prevent her, she would often climb onto the shelf beside the window to interact with humans through the glass. She was also the most vocal animal, especially when food was not delivered when expected. When this occurred, she would glance repeatedly at any observer and vocalise, sometimes as frequently as every few seconds.

2.4 OBSERVATIONS OF GROUP BEHAVIOUR

2.4.1 Observational protocol

Observations of the group's behaviour before and after training sessions were conducted through the viewing window at the front of the gang room (see Plate 2). This allowed a clear view of the entire gang room (see Plate 1). However, it was not possible to observe animals in the cage room from this position. To ensure consistency across conditions, recording of behaviour began as soon as I took up position at the window, rather than standing for a short period to allow the animals to become accustomed to my presence. This was due to the study design which required that recording of post-training data began as soon as the macaques were released from

the cage room, thus preventing any 'settling' period. The macaques appeared well habituated and generally paid little attention to human observers beyond the occasional glance or stare, all occurrences of which were recorded. Data were recorded onto checksheets with an electronic 'beeper' marking each sample interval. The beeper was set at a volume that, although audible in the corridor where I was standing, was unlikely to be heard in the gang room itself.

2.4.2 Selection, categories and definitions of observed behaviours

Data concerning the behaviour of the study animals were collected before and after training sessions primarily to identify any changes in the social behaviour of the macaques occurring as a consequence of these sessions. In this regard, the situation with the macaques was different to that with the marmosets in that good relations with humans existed before training for venipuncture commenced. As stated previously, the macaques had already been taught to present for vaginal swabbing, were very tame and showed little or no fear of humans. While their relationships with humans may already have affected their overall behaviour, it seemed unlikely that further training would produce much in the way of additional long-term change. Of greater interest was the possibility of short-term changes brought about by the training sessions themselves, particularly if the macaques found such sessions stressful (Chapter 4). The second purpose of group observations was to record behaviours believed to be associated with stress in these animals. Behaviours were chosen and categorised following a period of preliminary observations and with reference to existing literature. Preliminary observations identified behaviours that were commonly observed in the particular group studied and helped eliminate behaviours that may have provided useful indicators of welfare in other circumstances but were

inappropriate in this particular situation. For example, although foraging occurred throughout the day, this activity was not recorded as a distinct behaviour as it was clearly influenced by non-training factors such feeding (Waitt *et al.*, 2001) and cleaning schedules (when forage mix was added to newly laid wood-shavings). Equally, although the performance of stereotypies can indicate poor welfare (Mason, 1991), none of the macaques observed displayed such behaviours. Jane was reported to occasionally self-bite although this behaviour occurred too infrequently to be useful as a distinct behavioural category. However, as self-biting is a form of self-abusive behaviour (Reinhardt & Russell, 2001), it was included in the 'aggression' category.

Although vocalisations can provide a useful welfare indicator (Mench & Mason, 1997) this behaviour was not included for a number of reasons. Primate vocalisations serve a variety of functions and in order to provide an accurate indicator of stress, it is necessary to identify specific calls rather than recording vocalisations *per se*. With only a short interval in which to conduct preliminary observations, I was not confident in my ability to correctly identify specific calls, particularly as observations were conducted from the corridor where subtle utterances could not be heard clearly. Unambiguous vocalisations such as screams and barks were extremely rare. In addition, the most commonly heard vocalisations occurred around feeding times and fitted Blurton Jones and Trollope's (1968) description of food calls. Mirrium in particular produced these calls frequently. A final factor was that stump-tailed macaques may respond to a stressful or threatening situation by falling silent. Bertrand (1969) found that these animals could suspend all vocalisations for long periods, especially when aware of human observers. Interpretation of any changes detected in vocalisations becomes difficult in a species that may respond to stress or threat by increasing or decreasing frequencies of this behaviour.

A similar difficulty occurs with changes in locomotor activity. Locomotion is commonly recorded in studies concerning animal welfare and environmental enrichment (Woolverton, Ator, Beardsley & Carrol, 1989) and an increase in locomotion is generally viewed as a positive change, particularly in studies of zoo animals where many interventions are designed to increase activity (Crocket, 1989; Mench, 1998). However, changes could arise for a number of different reasons, not all of them positive. For example, activity may increase as a result of practices such as scatter-feeding or increasing the complexity of the environment as the animals have been provided with the opportunity to perform additional behaviours (Anderson & Chamove, 1984; Reinhardt, 1997). However, locomotor activity could also increase due to agitation caused by aversive situations (Broom & Johnson, 1993). While the marmosets showed distinct patterns of locomotion, one of which appeared related to arousal (Chapter 5), this was not the case with the macaques. The study animals were relatively inactive and locomotion consisted almost exclusively of quadrupedal walking and climbing (Bertrand, 1969). Although they appeared to walk slightly faster following events that may have caused agitation such as being confined in the cages, this was not distinct enough to categorise independently and was indistinguishable from activity preceding positive events such as being provided with food treats such as yoghurt. However, locomotor activity was important as obesity can be a problem in this species (Chamove, 1981) and in the study animals in particular (K. Morris, pers. comm). Locomotion was recorded in the hope that any changes occurring could be interpreted in conjunction with other behaviours.

Preliminary observations showed that the study animals spent a considerable amount of time huddling, nestling or sitting in close contact with each other. These behaviours are frequently observed in this species (Bertrand, 1969) and are believed

to play an important role in maintaining social relations (Call, Aureli & de Waal, 1999). Rhine (1973) suggests that close physical contact provides a useful measure of social acceptance and is most commonly observed among animals of similar social rank. During preliminary observations, subordinate individuals such as Noreen and Mirrium would often sit close to, but not in physical contact with other group members for a short period before attempting to join them. Sitting in close proximity was usually accompanied by frequent glances towards the other monkey followed by an attempt initiate some other social behaviour such as sitting in close contact or grooming. Preliminary observations suggested that such attempts were more successful when the group was relaxed. As sitting in physical contact and sitting in close proximity seemed to provide an indicator of group mood and cohesiveness they were included among recorded behaviours.

Grooming is an activity that has been shown to occupy a high percentage of activity budgets in this species (Goosen, 1974a; Rhine, 1973) with this activity accounting for between 19 per cent (de Waal, 1989) to 40 per cent of recorded behaviours (Bertrand, 1969). Allogrooming is an important social activity across the primate order (Goosen, 1974a, 1974b) and is also used during reconciliation (de Waal, 1989) therefore any decrease in its performance could indicate that the welfare of the animals has been compromised. However, while allogrooming is regarded as a positive social behaviour, auto-, or self-grooming can be interpreted as a displacement activity employed as a coping response to a stressful situation (Maestripieri, Schino, Aureli & Troisi, 1992). Autogrooming can increase following conflict (Call *et al.*, 2001) while Goosen (1974b) found that this activity increased when single-housed stump-tailed macaques were denied physical contact with a potential grooming partner. Given the different welfare implications of these different patterns of

grooming, allogrooming and autogrooming were recorded separately. In addition to autogrooming, self-scratching is another displacement activity frequently used as an indicator of stress (Maestripieri *et al.*, 1992). Instances of this behaviour were also recorded.

Time spent in the cage room was recorded for two reasons. First, all training was conducted in the cage room. If the training process was experienced as aversive, this might result in a reluctance to return there with a corresponding decrease time recorded for this category. Secondly, an individual's presence in the cage room was assumed whenever that animal was out of view. Brief behaviours such as aggressive or affiliative gestures were calculated as mean occurrences per 30 minute recording session. However, a decrease in such behaviours could simply occur because an individual was out of view for a significant proportion of the observation periods. Recording the proportion of time spent in the cage room allowed this to be taken into account when analysing any 'all occurrences' data.

Affiliative and aggressive behaviours provide much information about the social behaviour of primate species. However, interpretation of such behaviours must allow for species-specific behaviour patterns. For example, de Waal (1989) reports an average of 38 aggressive acts per ten hours of observation per individual for stump-tail macaques, compared to 18 aggressive acts for rhesus macaques. This could lead to the conclusion that stump-tailed macaques are the more aggressive species. However, when the type of aggression is taken into account, a different picture emerges. Stump-tailed macaque aggression is generally low intensity and rarely escalates into actual physical harm, while the escalation rate is eighteen times higher in rhesus macaques (de Waal, 1989). The literature suggests that aggressive threats should be relatively common, but actual physical aggression should be rare. In light

of this, aggressive threats were recorded in a different category from physical aggression.

Another feature of stump-tailed macaque behaviour is that affiliative behaviours also occur at relatively high frequencies, particularly after conflict (Call *et al.*, 1999; de Waal, 1989). Brief affiliative gestures include presenting, oral contact, genital inspection and 'hold bottom' where one animal grasps the hips of another (de Waal, 1989). While some behaviours are unambiguous, others can differ in meaning depending on the context in which the behaviour occurs. For example, de Waal (1989) describes how reconciliation can occur when one protagonist presents to another who then performs the 'hold-bottom' gesture. However, if an individual responds to presentation by turning away, the affiliative gesture offered by one animal has been rejected by another. Furthermore, on occasions, one individual may try to impose the "hold-bottom" gesture on another, a behaviour that can be interpreted as aggressive. This complexity creates problems when attempting to categorise this behaviour. For the purpose of this study, the behaviour of each individual was recorded separately. Presenting was classed as an affiliative behaviour, as was the 'hold bottom' gesture if performed following voluntary presentation. Attempts to perform this behaviour forcibly were classed as a threat because although physical contact was made, intent to cause harm cannot be assumed as with biting or scratching. While ignoring the presentation of another is clearly not an affiliative behaviour, it cannot truly be classed as threat or aggression either and was therefore not recorded.

Another example of an ambiguous behaviour is the 'ritual bite'. Performance of this behaviour can be interpreted as an expression of dominance (Demaria & Thierry, 1990) or as a form of chastisement or warning directed at subordinate

animals (Niemeyer, 1980). In this sense, ritual biting can be categorised as a threat alongside other aggressive gestures. However, ritual biting can occur outwith agonistic interactions or immediately prior to or during reconciliation, suggesting that this behaviour can also be used in affiliative interactions. In these circumstances, the subordinate animal usually offers a limb for biting (de Waal & Ren, 1988). In this study, inflicting a ritual bite was classed as an aggressive gesture while offering a limb to be bitten was classed as affiliative.

The final behavioural category recorded was observer-directed behaviours. As reported previously, the macaques generally took little notice of humans watching through the viewing window, beyond the occasional glance. However, if training sessions were regarded as stressful or threatening it was possible that this behaviour might increase due to heightened vigilance. Equally, if training was experienced as aversive or threatening, this might lead to an increase in threats or submissive behaviours directed towards the observer, depending on the nature of the animal. Preliminary observations of the macaques suggested that dominant animals such as Jane tended to respond to unwanted human activity (e.g. chasing into the cage room during cleaning) by becoming more aggressive whereas subordinate animals tended to respond to the same situation by showing submissive or appeasement behaviours (such as presenting).

Behaviours of relatively long duration (behavioural states) were recorded using instantaneous scan sampling while those of relatively short duration (behavioural events) were recorded as 'all occurrences' (Martin & Bateson, 1993). A 30 second sample interval was chosen following preliminary observations. Martin and Bateson (1993) suggest that in order to maximise the accuracy of the instantaneous sampling method, the sample interval should be as short as possible.

This was particularly important in this study as the opportunity to observe the behaviour of the macaques immediately following a training session was very limited. Although a 30 second interval may seem short given that five animals were being observed, their relative inactivity and the ease with which individuals could be identified meant that it was possible to record their behaviour within this time frame.

Table 2.2 Behavioural categories and definitions used for stump-tailed macaques.

Behavioural category	Definition
• **Locomotion**	Travelling around the enclosure, walking, climbing on furnishings or running.
• **Proximity (touch)**	Sitting or lying on branch, shelf or floor in physical contact with another animal. Includes huddling, nestling and sleeping while in contact with another.
• **Proximity (< 1 metre)**	Sitting or lying on branch, shelf or floor in a position less than 1 meter from another animal.
• **Allogroom**	Includes grooming or being groomed by another. Moving fingers through the hair or picking at the skin of another animal.
• **Autogroom**	Passing hand through hair or picking at skin while watching movement of hand.
• **Cage Room**	Not in view in gang room therefor must be located in the cage room.
• **Other**	All other behavioural states not otherwise listed.
* **Threat**	Stare, open-mouth and bared teeth threat. Includes infliction of "hold bottom" or ritual bite when behaviour is not initiated by the recipient.
* **Aggression**	Act of physical aggression including pushing, slapping and biting.
* **Affiliate**	All brief affiliative behaviours including touching and presenting. Also includes presentation of limb for ritual biting.
* **Scratch**	Scratching any part of the body. Distinguished from self-grooming by brief duration and the animal not watching movement of hand.
* **Observer directed behaviours**	Glancing at observer through the viewing window, threats (stare and open-mouthed stare) and submissive behaviours (presenting, grin, teeth chatter) directed towards the observer.

• denotes behaviours recorded by instantaneous scan sampling.
* denotes all occurrences of behaviour recorded.

2.3.3 Training Sessions

In addition to observational data collected before and after training sessions, data were collected throughout the sessions themselves. The methods used to record these sessions are reported in Chapter 3.

Chapter 3

Training stump-tailed macaques to co-operate during venipuncture.

3.1 INTRODUCTION

3.1.1 Training techniques

"The training recommended as the approach of choice is positive reinforcement training. Animals are reinforced with pleasurable rewards for the desired behavioural response. ...on the rare occasions when an escape-avoidance technique (negative reinforcement) is necessary, it is kept to a minimum and balanced by positive reinforcement at all other times."

(Laule & Desmond, 1998, p302)

As reported in Chapter 1, the use of positive reinforcement training (PRT) is increasingly recommended as a means of promoting the welfare of captive nonhuman primates. The above quote emphasises the basic principles underlying this type of training that is, that the trained animal is rewarded for performing a desired behaviour through the provision of a pleasurable reward and punishment is used only used in a situation that presents considerable danger to the trainer or the animal (Laule & Desmond, 1998).

While the difference between reward and punishment is unambiguous, differentiation between positive and negative reinforcement appears to be more problematic yet this distinction is important for a number of reasons. Firstly, an important principle of PRT is that the animals are voluntary participants in the training process (Laule *et al.*, 1992). However, negative reinforcement techniques usually involve the use of some aversive stimulus that leads the animal to perform the

desired behaviour. The reward is thus avoiding something unpleasant (negative reinforcement) rather than gaining something pleasant (positive reinforcement). However, a pleasurable reward can be provided in addition to negative reinforcement. This can reduce the impact of any aversive element in the training process through counter-conditioning as the negative element becomes paired with a positive outcome (see Chapter 1). Nonetheless, whenever negative reinforcement is employed, the resulting behaviour could still be viewed as resulting from "engineered compliance" rather than truly voluntary co-operation.

Secondly, the use of aversive stimuli in the training process can lead the animal to experience fear or anxiety, which in turn inhibits learning (Lieberman, 1993) and increases the threat to personnel. This is especially probable if the trained behaviour includes some aversive element thus the negative stimulus must be even more aversive in order to be effective. In such instances, training means that the animals effectively choose between two threatening situations, a situation that is unlikely to enhance their well-being (Laule, 1999).

The different effects of positive and negative techniques suggests that the effectiveness of training as a means of promoting welfare is likely to depend on the training methods used. However, in much of the literature the descriptions of training techniques are vague yet such details are important as many traditional training protocols rely on escape/avoidance learning (Kiley-Worthington, 1990). When sufficient information is provided, a closer examination of the techniques employed in zoo and laboratory-based training reveals some striking differences in the ways in which training is employed to teach similar behaviours.

Venipuncture is one of the most commonly carried out procedures in a laboratory environment and traditionally, this procedure involved removal from the

home-cage and the forced restraint of the animals (Reinhardt, Liss & Stevens, 1995). A growing awareness of the considerable distress caused by this procedure has led to the development of alternative techniques (reviewed by Reinhardt, 1997a). Vertein and Reinhardt (1989) developed a procedure used to train pair-housed, female rhesus macaques *(Macaca mulatta)* to co-operate during in-homecage venipuncture. The study animals were initially moved into new homecages equipped with squeeze-back mechanisms. Following a weeklong habituation period, training took place over 24 consecutive working days. During training, the following shaping procedure was used:

- On days 1-5, the back wall of the squeeze cage was brought forward, reducing the space available to the monkeys by about 75 per cent. The trainer then offered food for two minutes then left the animals alone for five minutes. This was repeated then the trainer moved the back of the cage to its normal position then offered food for a further minute.
- On days 6-7, this procedure was repeated and the trainer gently touched the animal's leg during the times when food was offered.
- On days 8-23 the trainer gently pulled the animal's leg through the cage door and stroked the animal for approximately twenty seconds before releasing the leg and rewarding the animal.
- Venipuncture was carried out for the first time on day 24.

The authors report that by day four, all eight animals took food offered by the trainer and subsequently allowed their legs to be held by day nine. When venipuncture was carried out on day 24, three monkeys actively offered a leg and while the remaining five were less co-operative, they showed no fear or resistance

when their legs were pulled through the cage door. The authors also report that training took a total of 250 minutes in total, 31 minutes for each individual animal.

This study describes a gradual technique, which allows the study animals time to habituate to both human contact and the squeeze-back mechanism before venipuncture is introduced. The technique does include an aversive element as monkeys do respond negatively to squeeze-mechanisms (Fuller, Hobson, Reyes, Winter & Faiman, 1984; Pun, Puri & Anand-Kumar, 1981; Sainsbury, Eaton & Cooper, 1989). However, this is balanced by positive reinforcement through the frequent provision of food. While training appears to have been successful, the study ends with the first occasion on which venipuncture actually took place. While none of the animals showed any fear or resistance, and three offered their legs, this occurred before the most aversive element of the procedure (i.e. venipuncture) had been introduced.

In a subsequent paper, Reinhardt (1991) describes a modified version of the above procedure used while training male rhesus macaques for the same purpose, co-operation during in-homecage venipuncture. Of the 15 study animals, ten were pair-housed with the remaining five singly housed. In the double cages used by the pairs, one half was equipped with a squeeze back mechanism, which was also present in all the singly housed animals' cages. A 'privacy panel' divided the cages used by the pair-housed animals and when the back wall of one half of the cage was pulled forward, the passage hole was cut off, confining one animal in the front portion of the cage. None of the animals had been restrained by this mechanism prior to training.

The main difference between the method described in this paper and that described by Vertain and Reinhardt (1989) is that the period of habituation is considerably shorter. The squeeze back was brought forward to a position that

restricted the monkeys' movement (14 – 16 cm from the front of the cages), and their legs were grasped from the first session. Venipuncture was carried out after "several training sessions" (p13). When an animal stopped resisting it was restrained without venipuncture during subsequent sessions while the squeeze back was placed farther away from the cage front, allowing the monkey greater mobility. After "a few training sessions" (p13), venipuncture was again carried out.

Reinhardt reports, "under no circumstances were training sessions terminated before the subject's leg was successfully pulled out of the cage for one minute" (1991, p13). Once the animal did co-operate he was released, the squeeze mechanism pushed back and favoured food offered. Here, a mixture of both negative (release of the squeeze mechanism) and positive reinforcement (food) was given with the rewards delivered after the animal had been released.

The results showed that all animals initially resisted either by changing position or attempting to slap or bite the trainer. However, all animals accepted food once the session was over. It took an average of 24.1 ± 18.3 minutes to obtain the first blood sample (while the monkey was squeezed by the cage mechanism), with this time spread over 5.8 ± 4.8 sessions. The total time spent with each animal before he would "present" a leg varied from 16 – 74 minutes with a mean of 40.4 ± 18.8 minutes. Training sessions were carried out over 2 to 16 days. Although this is fewer days than was required for the females (24 days) (Vertain & Reinhardt, 1989), the mean time required to get the macaques to present a leg is actually greater (31 minutes for females versus 40.4 minutes for the males). While this may reflect differences between male and female rhesus macaques, it could also mean that the more gradual shaping procedure may actually be more effective. However, with no

information on the behaviour of the females after venipuncture had been performed this is difficult to determine.

Subsequent reports showed that the modified technique described above is not always successful. Reinhardt (1992) described the training of juvenile female rhesus macaques. Unlike both the adult male and female macaques described above, who were familiar with the trainer and would accept food from his hand prior to training, these animals had no such prior relationship with the trainer. During initial selection, fourteen animals were screened by bringing the squeeze back of their home-cages forward then attempting to catch them by hand. The six monkeys that did not attempt to bite Reinhardt were selected for training. Only two of these animals were successfully trained. An additional pair allowed their legs to be pulled from the cage after 24 and 34 sessions respectively but would co-operate no further. The remaining two showed continued signs of fear and resisted all attempts to hold their legs even after 75 sessions at which point training was abandoned.

Reinhardt (1992) attributes these problems to the greater difficulty that juveniles have overcoming their fear of humans although it is worth noting that the modified procedure provided less opportunity to do so than the original method described by Vertain and Reinhardt (1989). In addition, although food was offered at the end of each session, Reinhardt (1992) does not report if was actually accepted. When negative reinforcement is balanced by positive reinforcement, this will only work if the animals actually accept the food rewards. Fearful and distressed individuals are unlikely to do so. However, it should be noted that the procedures described above were developed as part of a pioneering programme aimed at improving the welfare of laboratory-housed macaques (Reinhardt, 1997b) and the comparisons between outcomes of various modifications play a major role in

balancing welfare innovations with practicality. In a recent article, Reinhardt recommends a technique similar to the original, gradual one with emphasis placed on the importance of establishing a good relationship with the animals prior to the commencement of training itself (Reinhardt, 2003). In addition, whatever variation was used, there is evidence to show that these techniques were a considerable improvement on forced restraint.

Although all of the above studies concern the training of rhesus macaques, the technique has been used with stump-tailed macaques (Reinhardt & Cowley (1992). The study animals were six adult females, pair-housed in cages identical in design to those described by Reinhardt (1991). It is reported that the squeeze back of the cage was made of mesh thus allowing each monkey visual contact with her cage-mate throughout the procedure. All of the study animals were familiar with the trainers and the following procedure was used:

- Food was used to entice one monkey into the restraint compartment
- The squeeze back was pulled forward to a position where the macaque was restricted, but not squeezed. She was then scratched through the mesh and offered food.
- The macaque was then "enticed with food or coaxed with the help of a rod" (p252) until she turned to face one side of the cage. She was again rewarded.
- A leg was pulled through a gap in the cage front and blood taken. Another reward was then given.

Once the macaques would tolerate the procedure without resistance, the squeeze back was pulled to a position that still restricted the movement of the macaques, but to a lesser extent than during initial training sessions. Total training

time ranged from 15-45 minutes, with a mean of 33.5 minutes. This is comparable to the time required for male and female rhesus macaques (40.4 and 31 minutes respectively) (Reinhardt, 1991; Vertain & Reinhardt, 1981). Active co-operation was achieved within 9-23 training sessions (with each session lasting between 49 and 351 seconds) with individuals receiving up to three sessions per day.

It is unclear whether venipuncture was carried out after a number of preparatory sessions or if so, how many. Although not explicitly stated, the report that "no training session was terminated before the goal of that training *step* was achieved" (p253) suggests that venipuncture was not carried out during the first session. This assumption is supported by the very short duration of these sessions (49-351 seconds) and the report that the fastest macaque tolerated venipuncture after eight minutes of training, with the slowest animal requiring 22 minutes. However, it is possible that prior to reaching the criteria of "passive tolerance" the macaques did experience venipuncture but continued to resist.

Evidence that training reduces the stress associated with handling procedures comes largely from studies that compare the levels of cortisol in blood collected from trained animals with that of blood collected using mechanical restraint devices. In one such study, blood was collected from ten, singly housed female rhesus macaques which had been taken from their homecages and placed in restraint apparatus, a procedure they had undergone for several years. The levels of cortisol in these blood samples was compared to that taken from fifteen trained animals, ten pair-housed and five single-housed. Cortisol levels in blood taken 60-90 seconds after the technicians entered the room did not differ between the three groups. When samples taken fifteen minutes later were examined, levels for animals in the restraint apparatus were on average 50 per cent higher than at baseline, a significant difference. Cortisol levels

from the trained monkeys had increased by 18 per cent for the single-housed animals and 14 per cent for the pair-housed animals and neither of these differences was significant. However, the mean level of serum cortisol concentration in the restrained group was 29.8 μg/dl, significantly higher than the mean of 22.2 μg/dl for the trained pairs, but not significantly different from the trained single-housed animals (24.2 μg/dl) (Reinhardt, Cowley, Scheffler, Vertein & Wegner, 1990).

As there is considerable variation between individuals, even at baseline, mean cortisol levels are not the only measure worth examining. At baseline, cortisol levels in blood taken from the restrained group ranged from 14 – 28.1 μg/dl (S.D. = 4.2). Cortisol levels for the trained single-housed animals ranged from 17.3 -23.8 μg/dl (S.D. = 2.1) while for trained pairs the range was 15.3 – 23.7 μg/dl (S.D. = 2.9). At baseline, the restrained animals appear to show the greatest variance.

For the second samples, the range for the restrained group was 23.6 – 39.0 μg/dl (S.D. = 5.2), while the range for the trained, single-housed animals was 19.9 – 35.4 μg/dl (S.D. = 5.7) and that for the trained pairs 13.5 – 31.7 (S.D. = 6.4). This suggests that some of the trained animals were more stressed than the restrained ones and that the restrained animals no longer show the greatest variance. This could simply have occurred because the restrained animals had experienced this procedure for several years and may have become habituated to it whereas the other animals were tested 2-4 weeks after training. The overall results do suggest that training was beneficial and were replicated in a subsequent study of male rhesus macaques (Reinhardt, Cowley, Eisele & Scheffler, 1991) and female stump-tailed macaques (Reinhardt & Cowley, 1992). However, in common with other studies, these studies examined the behaviour of animals that have completed training and did not explore the possibility that the training procedure itself may be stressful.

One study that does address this issue is that conducted on brown capuchin monkeys *(Cebus apella)* by Dettmer, Phillips, Rager, Bernstein and Fragasy (1996). In this study, cortisol levels were measured in blood collected during the training process. In this procedure, the monkeys were required to enter a transport box with the squeeze mechanism on their homecages being used if necessary. They were then placed in a modified squeeze cage and, if an animal did not offer a leg the squeeze mechanism was employed to allow that monkey's leg to be grasped. When co-operation occurred, the squeeze back was released (negative reinforcement) and food offered (positive reinforcement). The leg was then shaved and blood drawn. The amount of resistance shown was recorded, as were other behaviours such as vocalisations. This procedure was carried out three days per week for six weeks. Analysis of blood cortisol levels showed a significant increase over the first five weeks of training followed by a return to baseline levels on weeks six and seven.

In the second phase of the study, four of the eight study animals were classed as habituated as they showed little resistance and few vocalisations during sessions. The remaining four subjects continued to show active resistance and were classed as non-habituated. Blood was again collected from these animals, the first sample taken on average, 6.59 minutes after capture and the second 60 minutes later. In addition matched samples were taken from eight naïve animals using the same procedure.

Analysis showed no difference between the three groups for the first sample and the trained habituated animals showed no increase in cortisol levels between the immediate and later blood draws. Significant increases were shown both by the naïve animals and the trained, unhabituated ones.

This study suggests that training itself produced a stress response during the initial stages. A subsequent reduction in stress only occurred in animals that

displayed behavioural indications that they had habituated to the process. The use of a different species makes comparisons between this and other studies difficult, although Dettmer *et al.* (1996) suggest that capuchin monkeys, who generally respond positively towards humans should be easier to train than the more aggressive and commonly studied macaques. In addition, an important difference between this and the procedure described by Reinhardt and his colleagues is that the monkeys were removed from their homecages and in some cases, their companions. Reinhardt (1997a, 1997b, 1999) has suggested that one of the main benefits of training is that it removes the necessity of doing this. Although monkeys can be trained to enter transport cages, this in itself seems to involves some degree of coercion, for example, being prodded with a stick, at least in the initial stages (Reinhardt, 1992).

Zoo-housed animals rarely experience procedures such as venipuncture or injections as frequently as those living in laboratories. However, an exception to this is insulin-dependent diabetic primates and the techniques used to train for co-operation with daily injections provide an interesting comparison to those used in laboratories. Stringfield and McNary (1998) describe the training of David, a 24 year old, wild caught, moustached guenon *(Cercopithecus cephus cephus).* In the first stage of training, a clicker and coloured target (see Chapters 1 & 6) was paired with food rewards readily accepted by this animal. After two months David would put his arms through the cage bars and touch the target. Although unwilling to have his arm held, he would present by lying on his back and allow parts of his body to be scratched. Within the next two months, training progressed from scratching David's back to pinching the skin, prodding with a needle to finally administering insulin. In 1998, David would still permit insulin injections, but not blood collection.

Laule, Thurston, Alford and Bloomsmith (1996), provide a more detailed account of the training procedure used to teach a three year old, diabetic chimpanzee to accept venipuncture. This animal, Allie, had been hand reared and already co-operated when blood was collected by heel puncture during which she was carried from her cage and places on a fleece-covered tabletop. For venipuncture, she was initially taught to sit upright on this table and allow her arm to be held by the trainer. She was then desensitised by having her arm touched by the trainer's finger, followed by a cotton swab, a needleless syringe, a blunt needle and then a sharp needle. Throughout this process she was rewarded for remaining calm and tolerating the procedure. Blood was drawn without any outward signs of distress or discomfort on the eighteenth training session, after 275 minutes of training. The authors report that in the four years following training, this animal has never resisted this procedure. Although this procedure appears to have been highly successful, the authors acknowledge that it may become unsuitable as the animal matures when further training and the use of a sleeve device may become necessary.

Sleeve devices consist of a metal tube with a space to allow access to the animal's arm and a metal bar fixed across one end. Primates are trained to insert their arms into the tube and grasp the bar. This allows blood collection while preventing the animals grabbing hold of the trainer. Priest (1990, 1991) trained Loon, a diabetic male drill *(Mandrillus leucophaeus)* to co-operate with this procedure when it became apparent that the method initially employed to administer insulin – restraint in a squeeze-backed cage, could not be continued long-term due to Loon's increased exhibition of aggressive and abnormal behaviours.

Priest initially taught Loon, to grasp the bar at the end of the tube, using a clicker as a signal that the rod could be released and a food reward collected. This

was achieved within a few days although Loon remained aggressive towards the trainer for several weeks. Loon was then required to hold the rod for increasingly long periods and was desensitised by having various objects drawn across his skin. After six weeks blood was successfully drawn while Loon continued to hold the rod and he was still co-operating one year after training was completed.

This technique can be seen as requiring voluntary co-operation as Loon was free to release the bar at any time. However, the number of animals successfully trained in this way is small and the procedure appears to be considerably more time-consuming than that employed in laboratories. In some cases where apparently similar techniques have been used, there are not enough details provided to allow a detailed examination of the methods used (e.g. Ferreri, 1996; Stringfield, 1998).

3.1.2 Study Outline

This investigation aimed to document the training of a group of stump-tailed macaques housed in a UK laboratory. The aim was to examine how training was actually carried out in a "real life" situation and record events in as much detail as possible in order to identify the methods used, the strengths and weaknesses of the training programme and the resulting effect on the trained macaques. All training to co-operate during venipuncture was carried out by laboratory staff over two distinct phases separated by an 18 month interval. The data presented in this chapter are those collected during training sessions while additional data collected before and after the training sessions are presented in Chapter 4.

3.2 METHODS

3.2.1 Study animals

The study animals were four adult female stump-tailed macaques housed in the same social group at the MRC Human Reproductive Sciences Unit in Edinburgh. The male housed in the same group was not included in the training process. Brief details of the study animals are given in Table 3.1. Full details of the study animals, housing and husbandry routines are provided in Chapter 2.

Table 3.1 Details of the study animals including name, sex, dominance rank and age at start of study.

Name	Sex	Age
Jane * ♦	Female	16yr 11mth
Kelly *	Female	5yr 4mth
Noreen * ♦	Female	7yr 11mth
Mirrium *	Female	13yr 3mth

* denotes participated in training phase 1
♦ denotes participated in training phase 2

3.2.2 General Procedure

Training for co-operation during venipuncture was carried out over two distinct phases (henceforth referred to as Phase 1 and Phase 2) separated by an interval of 18 months.

Phase 1: Before training began, I spent several weeks conducting preliminary observations and allowing the study animals time to habituate to my presence. Throughout the study, I participated in routine husbandry procedures and conducted observations on days when no training occurred (Chapter 4) so it is unlikely that the monkeys learned to associate my presence solely with the training process.

All four study animals were trained over eight sessions. These began on 11/11/99 and were conducted on an irregular schedule, once or twice per week with the final session conducted on 16/12/99. Training was always conducted in the latter

part of the morning with sessions beginning between 1105h and 1121h and ending between 1148h and 1218h with a mean duration of 30.5 minutes (± S.D. = 7.31 mins). A male staff member (henceforth referred to as 'Trainer A') conducted the first six sessions, with the remaining two sessions conducted by a different male technician (henceforth referred to as 'Trainer B').

Phase 2: Two of the original study animals (Jane and Noreen) participated in Phase 2. Training resumed on 30/7/01 with venipuncture conducted daily for the first four weeks. During the first two weeks, sessions were observed every day bar Sundays then on all weekdays for the next two weeks. Training was then conducted three times weekly for a subsequent three weeks. All but the final two of these sessions were observed making a total of 29 training day observations. Trainer A conducted 12 of these sessions while a female technician, henceforth referred to as 'Trainer C', conducted an additional 17 sessions. Trainer B carried out the remaining session.

Although training was generally conducted in the latter part of the morning, there was more variation than during Phase 1. Observed sessions started between 1050h and 1146h, ending between 1108h and 1224h. The mean duration was 18 minutes 49 seconds (±S.D = 4.49 mins). However, it should be noted that this is not comparable with Phase 1 as only two of the macaques were trained and sessions included vaginal swabbing of all four animals, something that had not occurred previously.

3.2.3 Data recording

Throughout the study, a diary was kept noting the methods used to get the monkeys into the cage room, reactions to the squeeze mechanism, the type of reward used and any additional information. The general procedure was to confine each

monkey in an individual cage where they remained until training was completed and all the monkeys released simultaneously. Detailed observations for each individual began as soon as the squeeze mechanism was pulled forward and the guillotine section of the cage door raised.

Data were recorded on a palm top computer using THE OBSERVER 3.0 software (Noldus, 1993). The use of the computer was advantageous in that it enabled data to be entered quickly and recorded the time interval between events in a way that would not have been possible using traditional check sheets. Before observation of the macaques began, the planned observation technique was piloted using observations of the training of domestic dogs. This allowed practice in entering data into the computer quickly and examination of the type of data that the procedure was likely to produce.

The technique used to record training sessions was loosely based on a research technique used in social psychological research, Bales' (1950) Interaction Process Analysis (IPA) (described by Brown, 1988). IPA is a coding scheme used in the observation and analysis of interactions within human social groups. The idea is that observations can be broken down into a series of acts with each "act" being the smallest piece of meaningful behaviour that the observer can detect. These acts are then classified according to their purpose and examined to explore group interactions.

Training sessions are essentially a series of animal-human interactions, which can be separated into four categories as follows:

- Initiation (by trainer)
- Response (by monkey)
- Reaction (by trainer)
- Outcome (monkey)

The trainer must perform some act in order to initiate the desired behaviour, to which the animal responds. In turn, the trainer reacts and the animal counter-responds producing the final outcome. The success or otherwise of different training techniques depends on how these human-animal interactions proceed (see Chapter 1). The aim of training observations was to record sessions in a way that would allow the identification of good or poor training practice.

For example, the trainer must have some way of indicating what behaviour is required. Verbal requests will only work once the animal has had the opportunity to learn what a particular word or phrase refers to. In the early stages of training a verbal request should be paired with some other means of initiating the desired behaviour. The trainer may simply wait until the behaviour occurs spontaneously or gently guide the animal in the desired direction. However, if strong physical force or threats are used as initiators then participation by the animal cannot be said to be voluntary.

The response of the animal could be to perform the desired behaviour or an approximation of that behaviour. Equally, she could ignore the trainer or respond fearfully or aggressively. Depending on that response, the trainer could react using positive reinforcement, negative reinforcement, ignoring the response or punishment. Following the principles of PRT, negative reinforcement should only be used once positive alternatives have been exhausted and then negative reinforcement is used it should always be accompanied by positive reinforcement (Laule, 1999). In the early stage of training, secondary reinforcers such as clickers or verbal praise will only be effective is previously paired with a primary reinforcer such as food. If this has not occurred then reinforcement is meaningless. Equally, rewards such as patting or stroking will only be effective if the animal actually enjoys such contact with humans.

Punishment should only be used in extreme situations with the preferred method being "time out" when sessions are terminated following aggressive behaviour. Ideally, incorrect responses should be ignored. Of course, this technique relies on the assumption that training enjoyable. If training is experienced as aversive then "time out" becomes a form of negative reinforcement that rewards the undesirable behaviour.

Clues as to the effectiveness of reinforcement can come from the final outcome or counter-response shown by the animal. If food is refused, or taken and discarded, then the offered reward is unlikely to be effective. Refusal to accept the reward could indicate that the food is not sufficiently attractive or that the animal is distressed. If an animal takes food and remains in proximity to the trainer during consumption then it is likely that that animal is fairly relaxed while attempts to retreat before consumption could indicate otherwise. In some cases, aggressive or fearful responses might be expected at the beginning of the training process but these should diminish over time. An increase in such responses would indicate that training is not being successful.

When behaviours occurred concurrently (e.g. verbal request and physical manipulation, verbal praise and food) they were recorded as combinations as simultaneous presentation would allow the animal to form associations between them. When verbal requests or verbal punishments were used, the tone of voice (quiet or loud) was also recorded. The behaviours recorded in each category are shown in Table 3.2.

Table 3.2 Behaviours recorded during training observations grouped by behavioural category

Category	Behaviour	Definition	Modifiers
Initiation (trainer)	Request (verbal)	Trainer uses a word or phrase to initiate behaviour	Tone (quiet or loud)
	Request (gesture)	Trainer uses some hand or other non-verbal gesture without physical contact with animal	
	Request (physical)	Trainer grasps limb or pushes animal into a desired position.	Force (gentle or strong)
	Negative stimuli	Unpleasant stimulus such as poking or tightening the squeeze mechanism further	
	Passive	Trainer waits for the desired behaviour to occur spontaneously	
Response (monkey)	Desired	Presents limb	
	Approximation	Allows limb to be grasped and held but does not present	
	Aggression	Threats, cage rattling, attempts to grab, slap, nip or bite trainer	
	Fear/avoidance	Fear grin, chatter, urination or defecation, attempt to escape	
	No response	Animal ignores trainer	
Reaction (trainer)	Positive (food)	Food reward is offered as positive reinforcement	
	Positive (pat/stroke)	Patting or stroking as positive reinforcement	
	Positive (verbal)	Verbal praise given as positive reinforcement	Tone (quiet or loud)
	Negative reinforcement	Cessation of any negative stimuli, release of squeeze mechanism	
	Punish (verbal)	Verbal punishment, e.g. "no"	Tone (quiet or loud)
	Punish (physical)	Hitting, shaking or any unpleasant physical contact	
	Ignores	Trainer makes no response	
Outcome (monkey)	Takes food (proximity)	Animal accepts and eats reward without attempting to move position	
	Takes food (moves)	Animal accepts reward but attempts to move or withdraw limb before eating	
	Rejects food (proximity)	Animal refuses or discards reward without attempting to move position	
	Rejects food (moves)	Animal refuses or discards reward and attempts to move or withdraw limb	
	Aggression	As above	
	Fear/avoidance	As above	

3.2.4 Analysis

As only four animals were studied in Phase 1 and two in Phase 2, only descriptive statistics were used. Where appropriate, means for the group were calculated but as there were considerable individual differences, the results from individual macaques are also presented. In addition, trainers may interact differently with individual animals depending on how that individual is perceived (Kiley-Worthington, 1990). Data from individual monkeys allowed examination of this possibility. The OBSERVER 3.0 software computer programme (Noldus, 1993) produced a detailed record of the order and time at which each recorded behaviour occurred and this allowed a number of factors to be examined. Where appropriate, this was supplemented with additional information recorded in the diary kept during the training periods.

One indicator that the animals were learning to co-operate during venipuncture could be the time taken to collect blood samples. If the monkeys are uncooperative then the process is likely to take longer than when they comply. However, the total time taken as measured from beginning to end of each session is a poor indicator as laboratory practice was to apply pressure to the vein for a period sufficient to prevent bruising. This period could vary for a number of reasons (e.g. a punctured artery must be held considerably longer than a vein) and would affect the results. In addition, the time taken to draw a sample varied according to the vein or artery punctured and the quantity of blood required. For these reasons, the time between raising the cage door to begin the training process and blood actually appearing in the syringe was recorded and used as a measure of co-operation.

As outlined above, an important factor in training is the way in which behaviours are reinforced. The data were examined to determine what type of reinforcement (positive or negative) was used and when. Reinforcers tend to be associated with the action taken immediately prior to their delivery. If reinforcement follows an unwanted response then undesirable behaviours are effectively being rewarded (Lieberman, 1995). All instances of reinforcement were therefore identified in the data sheets and the behaviour that preceded them identified.

Finally, in addition, the speed with which reinforcement is delivered is an important factor as the shorter the interval between response and reward the more readily the animal learns (Grice, 1948; Laule, 1999; Perkins, 1947). In all cases where a desired behaviour was followed by positive reinforcement, the time interval between these events was calculated.

Another indicator of the monkeys' response to the training process is the number of aggressive or fearful responses shown. Aggressive responses were rare and as both aggressive and fearful behaviours indicate distress (de Waal, 1989) these categories were combined for analysis. In addition, aversion to the training process could be indicated by a reluctance to enter the cages where training was conducted. The methods used to bring the monkeys into the cage room were recorded throughout.

3.3 RESULTS

3.3.1 Phase 1

Each individual session followed the same basic procedure which was similar to that described by Reinhardt (1991). All the monkeys were confined in individual cages. In turn, the squeeze mechanism was brought forward and the front door of the cage lifted to allow a leg to be drawn through. Sessions began either with a verbal

request given as the trainer grasped the monkey's leg or by simply grasping the leg. There was no discernable pattern and no consistent word or phrase used. It is most likely that the macaques simply learned to associate the touch on their leg with the request for the desired behaviour.

A leg was drawn through the gap created by raising the guillotine section of the cage door. The actual leg used varied across days to allow as much healing as possible before the next draw. As the monkeys always sat side-on and the leg nearest the cage front taken, this was usually achieved by pulling the squeeze back forward at the moment the monkey was facing in the desired direction. Once this was in place it was difficult for the macaques to turn round. Venipuncture was then attempted and this occurred from the first session. The trainer did not hold the leg tightly and at times the monkeys managed to draw back into the cage. Following completion of the blood draw, pressure was then applied until the trainer was sure that any bleeding had stopped. The monkey's leg was then released and the door closed and the squeeze mechanism pushed back, ending that individual's training session.

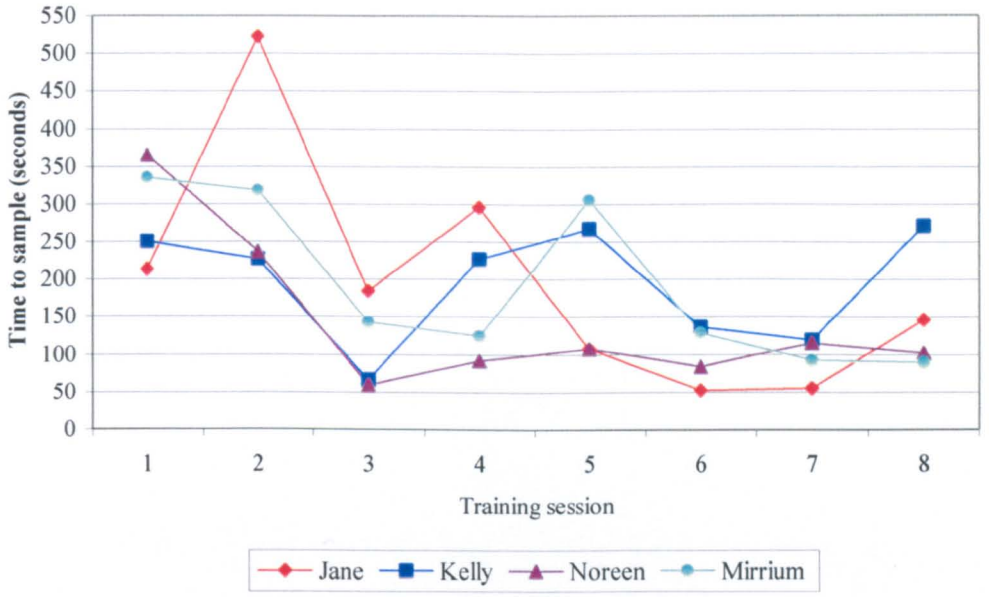
Food rewards consisted of a variety of sweets such as fruit gums, jelly babies or chocolate covered peanuts. Attempts to use fruit such as grapes were unsuccessful as the monkeys simply discarded them although they would accept a sweet reward given immediately afterwards.

Time required to begin blood collection

When the group data were examined, the mean time between the start of each individual's session and the start of actual blood collection (and indicated by blood appearing in the syringe) was 182.44 seconds although there was a great deal of variation (S.D.= 110.75 secs). The time required did decline from a mean of 291.13 seconds (S.D. = 71.16 secs) during the first session to 152.48 seconds (S.D. = 82.62

secs) by the eighth session. However, there was no smooth pattern of increasing cooperation over time and one of the macaques (Kelly) showed the greatest resistance during the final session. The actual times required by each macaque over the eight training sessions are shown in Figure 3.1.

Figure 3.1 Time delay between start of procedure to actual blood collection for each study animal over the eight training sessions.



Reinforcement

Negative reinforcement (i.e. release of the squeeze mechanism) was used on seven occasions, but only twice following a desired response (allowing a leg to be drawn through the cage door) during Sessions 7 and 8. On one of the remaining five occasions the mechanism was pushed back (though not completely) when the monkeys objected strongly to being squeezed. Although this was in response to fearful behaviour rather than used as a reward, on each occasion the monkeys subsequently settled down.

Verbal punishment was used on five occasions and always consisted of the word "no" spoken in a quiet voice. This always followed aggressive behaviour and occurred during Sessions 5 and 7 (Mirrium) and Session 6 (Kelly). A loud voice was never used, nor was physical punishment. Most aggressive acts were simply ignored. Positive reinforcement was used on 94 occasions and a summary of the frequencies of the types of reward is given in Table 3.3.

Table 3.3 Total instances of positive reinforcement by type of reinforcer used over the eight training days.

	Food only	Food + verbal praise	Food + pat/stroke	Verbal praise only	Pat/stroke only	Verbal praise + pat/stroke
n =	**21**	**40**	**11**	**3**	**4**	**15**

Positive reinforcement most commonly consisted of food paired with verbal praise. Stroking sometimes occurred when the leg was being held once venipuncture was completed. As the monkeys showed no indication that either verbal praise or stroking were actually reinforcing, only instances where food was given (77 per cent of all positive reinforcement) were examined further.

Between them, the macaques earned 72 food rewards over the eight training days. Trainer A tended to deliver rewards more frequently than Trainer B. The total number of rewards earned by each study animal along with the mean rewards per session are shown in Table 3.4. Food was generally accepted and only refused on four occasions. Two of these occurred when grapes were used instead of sweets. Once the macaques accepted a reward they generally sat quietly during consumption. On only one occasion was any attempt made to move away or withdraw a leg once food had been accepted (Noreen).

Table 3.4 Mean number of food rewards given to each study animal in total and by trainer.

	Mean rewards per session (all)	Mean rewards per session (Trainer A)	Mean rewards per session (Trainer B)
Jane	2.5	2.7	2
Kelly	2.25	2.7	1
Noreen	2	2	2
Mirrium	2.25	2.5	1.5

Ideally, food rewards should have consistently followed performance of either the desired behaviour (presenting a leg) or its approximation (allowing the leg to be taken and held) and this did occur most of the time. The monkeys were occasionally rewarded for continuing to sit and have their legs held while post-venipuncture pressure was applied. However, there was a tendency to use food to distract or calm the monkeys at times when they were resisting and this was particularly noticeable with Jane who received 25 per cent of her rewards following avoidant or aggressive behaviour. Table 3.5 shows the number and percentage of the total rewards given according to whether they were delivered following a desirable behaviour, for continued co-operation or following an unwanted behaviour.

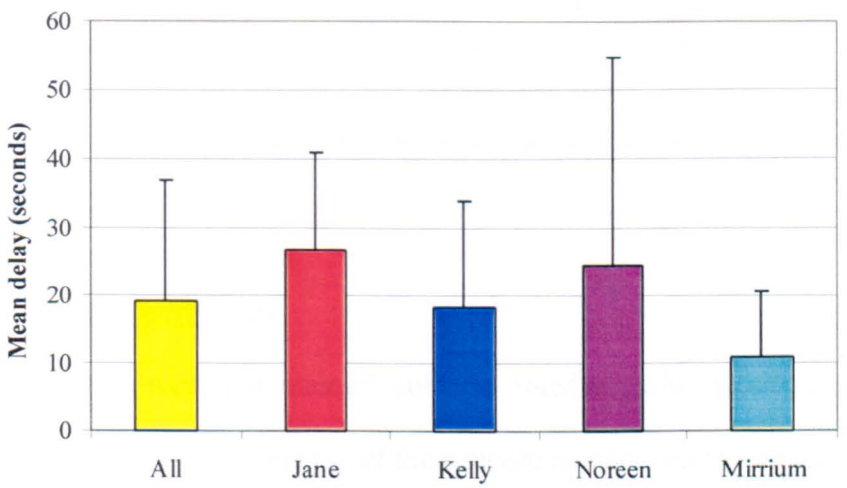
Table 3.5 Summary of food rewards delivered grouped according to the preceeding behaviour shown by the study animals

	Total	Reward following desired response		Reward for continued co-operation		Reward following unwanted response	
	N =	n =	%	n =	%	n =	%
Jane	20	9	**45**	6	**30**	5	**25**
Kelly	18	13	**72.2**	3	**16.7**	2	**11.1**
Noreen	16	13	**81.3**	0	**0**	3	**18.7**
Mirrium	18	15	**83.3**	1	**5.6**	2	**11.1**

Speed of Reinforcement

There was considerable variation in the time between performance of a desired behaviour and delivery of reinforcement. Even when verbal praise was used, it tended to be delivered at the same time as the food rather than immediately as a bridging stimulus (Chapter 1). Overall, the mean delay was 19.13 seconds (S.D. = 17.67), with a range of 2.7-107.9 seconds. There was also variations between individual monkeys with Mirrium on average receiving her rewards faster than the other macaques. The mean delay between performance of a desired behaviour and reward overall and for each individual monkey is shown in Figure 3.2.

Figure 3.2 Mean delay between performance of a desired behaviour and reward overall and for each study animal (bars represent Standard Deviations)

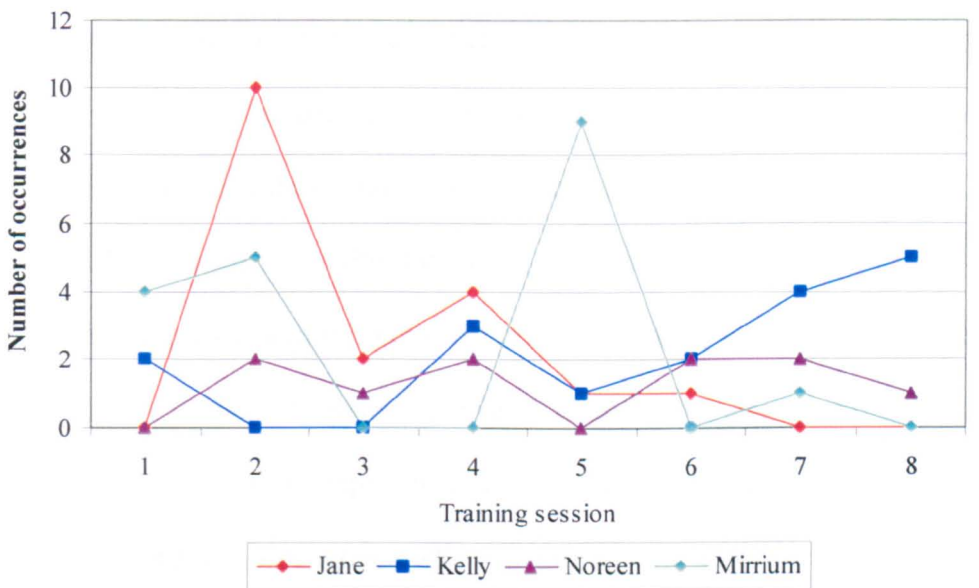


Aggressive and fear/ avoidance responses

The only aggressive action observed was attempting to nip the trainer's hand and ripping his latex gloves. This occurred 19 times in total with Noreen the only animal that never displayed aggressive behaviour. The most common fear/avoidance responses were pulling the leg back into the cage and/or attempting to move upwards and these were noted on 45 occasions. Figure 3.3 shows that while Kelly and Noreen

remained fairy consistent over the eight training days, Jane and Mirrium each had one particularly "bad" session. It was noted that Mirrium had a fresh cut on her left hip on Session 5 and it is likely that this was a contributing factor. The total instances of aggressive and fearful/avoidant acts shown by each macaque are shown in Figure 3.3.

Figure 3.3 Instance of aggressive and fearful/avoidant behaviours shown by each study animal over the eight training sessions.



Additional observations

There were a number of problems noted out-with the observations reported above. One was the tendency of the squeeze mechanism to jam when pulled forward. This was often caused by the study animals, who clearly found this aversive and quickly developed their own methods to avoid being squeezed. For example, Jane would attempt to hit the squeeze back as it moved forward. As the mechanism moved along a central bar, this sometimes twisted the back sideways, which although at only a slight angle, was enough to jam the mechanism. Kelly and Noreen would perch on the central bar, grasp the cage bars with their feet and brace themselves against the squeeze-back. If they leaned to one side, this again caused the mechanism to jam.

There was a consistent difference in the way in which the two trainers dealt with this problem. Trainer A simply moved the monkey to the adjoining cage and started again. Trainer B would use a broom handle to knock the squeeze-back straight and free it. This resulted in a loud bang that clearly startled the monkeys, particularly the one who was confined in the cage at the time.

Another problem was that it became increasingly difficult to persuade the monkeys to enter the cage room. They entered when called on Session 1 and were already confined on Session 2 as they had not been released after the gang room had been cleaned. On day three they initially refused to enter but did come eventually. However, the lock on one of the slide doors was broken and they managed to escape back to the gang room and subsequently had to be chased back through. On Sessions 4, 5 and 6 they would only enter if Trainer A chased them through from the gang room. When Trainer B attempted to chase them into the cage room on Session 7, Jane stood her ground and only moved when threatened with a net. On Session 8, both Jane and Kelly resisted and the net was used again. This time Jane actually attacked the net although she eventually followed Kelly into the cage room.

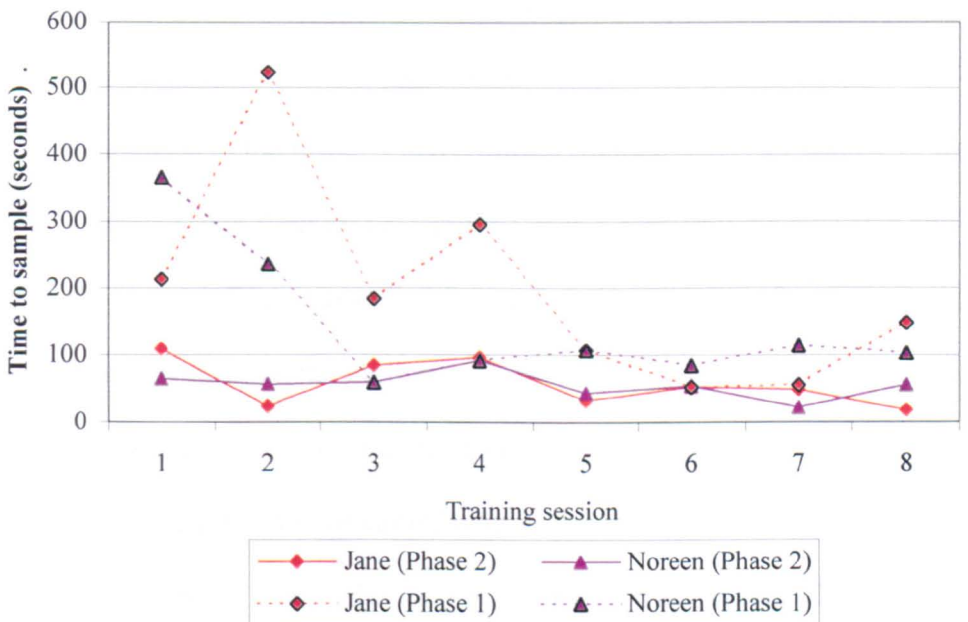
3.3.2 Phase 2

Time required to begin blood collection

When the data were examined, the mean time between the start of each individual's session and the start of actual blood collection was 50.35 seconds (S.D.= 25.64 secs) which was substantially less than during Phase 1 (182.44 secs). This faster mean time than previous sessions was not due to the greater number of training sessions as the mean time required for the first eight sessions was similar (mean =

59.27, S.D. = 25.25). To allow comparison with Phase 1, the actual times required by each macaque over the first eight training sessions are shown in Figure 3.4.

Figure 3.4 Time delay between start of procedure to actual blood collection for each study animal over the first eight training sessions (Phase 2) (Phase 1 data included for comparison).



Reinforcement

Negative reinforcement was used on six occasions with both macaques during the final three sessions conducted by Trainer A. This occurred after collection of blood was completed, during the time when pressure was being applied to the leg. On all other occasions, the squeeze mechanism was released at the end of each individual's session. However, although the squeeze-back was pulled forward, it was never used to actually squeeze the monkeys as had occurred previously. No punishment of any description was recorded.

During Phase 2, the data concerning rewards given through verbal praise alone, pat/stroke and verbal praise combined with pat/stroke were not collected. This

was due to Trainer C who praised the monkeys constantly as soon as their legs were held. In addition, she stroked their legs throughout the time that pressure was being applied. As a result the number of rewards delivered in this way was difficult to record accurately. Verbal praise was recorded only when immediately followed by a food reward. Trainer B offered no food rewards to either study animal. As only one session by Trainer B was observed, all subsequent data in this chapter are those from the sessions conducted by Trainers A and C.

In addition to the rewards given during training itself, the monkeys were always rewarded with food for entering the cage where training occurred and again as soon as the squeeze-back was pulled forward. As the training observations recorded by computer began when the guillotine section of the cage door was lifted (as during Phase 1) these initial food rewards were not included in analysis.

The two study animals earned a total of 181 food rewards over the 29 observed sessions. Of these, 131 (72.4 per cent) were accompanied by verbal praise. The total number of rewards earned by each study animal along with the mean rewards per session are shown in Table 3.6. Food was always accepted.

Table 3.6 Mean number of food rewards earned by each study animal in total and by trainer.

	Mean rewards per session (all)	Mean rewards per session (Trainer A)	Mean rewards per session (Trainer C)
Jane	3.28	2.42	3.88
Noreen	2.97	3.88	3.41

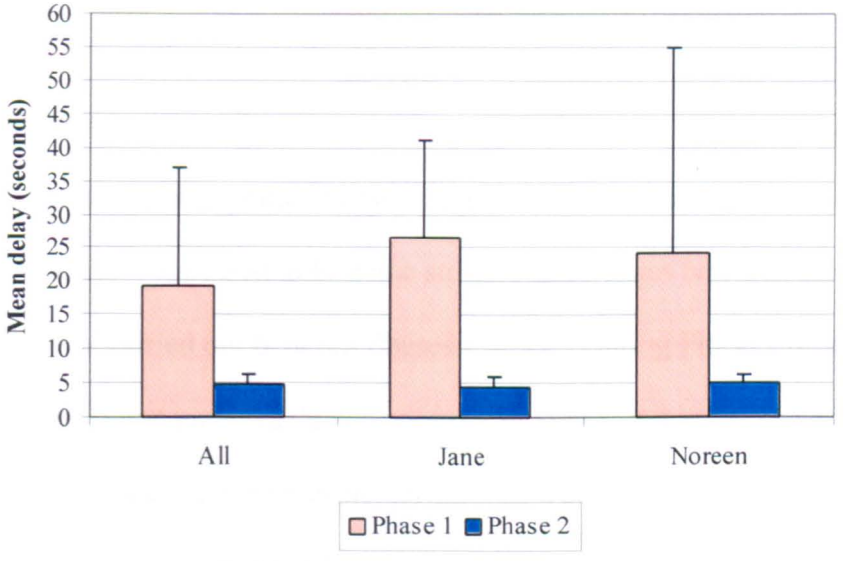
Delivery of food rewards was much more consistent than during Phase 1. The monkeys never received food following an unwanted behaviour. Throughout, both

study animals were rewarded as soon as they allowed their leg to be held and again when the needle was withdrawn following venipuncture. On seven occasions, Jane presented her leg as soon as the cage door was raised (twice with Trainer A, five times with Trainer C). Any additional rewards were delivered for continued co-operation during the period when pressure was being applied to the leg.

Speed of Reinforcement

Overall, the mean delay between allowing the leg to be drawn from the cage (or voluntarily presenting) and delivery of the food reward was 4.67 seconds (S.D. = 1.41 secs). Reinforcement was both faster, and more consistent than during Phase 1 when the mean delay was 19.13 seconds (S.D. = 17.67 secs). This result was consistent between the two study animals. The mean delay between behaviour and reward for Jane was 4.64 seconds (S.D. = 1.54 secs) and for Noreen 4.89 seconds (S.D. = 1.26 secs) (see Figure 3.5). Examination of the data also showed consistency between the two trainers with a mean delay for Trainer A of 4.4 seconds (S.D. = 1.51 secs) and a mean delay of 4.82 seconds for Trainer C (S.D. = 1.34 secs).

Figure 3.5 Mean delay between performance of a desired behaviour and reward overall and for each study animal (Phase 1 data included for comparison) (bars represent Standard Deviations)



Aggressive and Fear/ avoidance responses

Both aggressive and fearful/avoidant responses were rare with only three fear/avoidant responses shown when training was conducted by Trainer A (Noreen during day 18). When Trainer C carried out the procedure, fearful or aggressive behaviours were never shown. The only other negative behaviours occurred during the session conducted by Trainer B. These consisted on three aggressive acts by Jane, and three avoidant responses by Noreen.

Additional observations

The problems persuading the monkeys to enter the cages where training took place that were observed during Phase 1 were not generally repeated. On fourteen observation days the monkeys were waiting in the cages before the trainer entered the cage room. The only time resistance was shown was on day 18 and on the final day. On both occasions the trainer (Trainer A) persuaded them to enter by rattling the jar that contained the food rewards. On both occasions, Trainer B had conducted the

previous day's training. On the final day, Noreen was found to have a bruise on the venipuncture site, the first that had been noted. For this reason, no further sessions were conducted.

3.4 DISCUSSION

Although the training methods used were broadly similar to those described by Reinhardt (1990) there appeared to be some striking differences between the way in which training was carried out between Phases 1 and 2. During Phase 1, rewards were inconsistent in that undesirable behaviours were rewarded as well as desirable ones and this was particularly noticeable during Jane's sessions. Twenty-five per cent of her food rewards were obtained when she attempted to pull her leg free, nip the trainer's hand or hit the squeeze back. Equally, 30 per cent of Jane's rewards were earned for continued co-operation as pressure was applied to her leg following blood collection. However, this can have the effect of rewarding aggressive behaviour and should be avoided (Bloomsmith, Laule, Thurston & Alford, 1992) as it was during Phase 2.

Kiley-Worthington (1990) observed that humans respond to individual animals in different ways, with greater care taken with those who are either particular favourites or perceived as threatening. Jane was undoubtedly the most powerful and domineering of the study animals and the tendency to use food to bribe her may have resulted from this. By contrast, Noreen was the least aggressive and most co-operative of the study animals yet she alone was never rewarded for continued co-operation, suggesting that her 'good behaviour' tended to be taken for granted. While this did not appear to affect her behaviour during the actual training sessions, Noreen did become one of the monkeys most reluctant to enter the cages. When questioned,

neither trainer was aware that there was any difference in the way they treated individual study animals. Without detailed observations, such subtle differences could pass unnoticed.

During Phase 1 there was a considerable delay between co-operation and delivery of a food reward, sometimes over a minute. Curiously, the most subordinate monkey, Mirrium, tended to receive her rewards faster than the other macaques and again, this may have been influenced by the way she was regarded by staff. Mirrium was the only macaque who would actively solicit food on a regular basis by glancing towards humans and vocalising. However, during Phase 2, rewards were consistently delivered within four to five seconds, which would have made it much easier to associate food with a particular action (Grice, 1948). During Phase 2, the laboratory staff had begun to place a supply of sweets within easy reach throughout each animal's session and this helped to ensure that rewards were on hand for rapid delivery. The fruit gums that were given as rewards could take as long as a minute to eat, and therefore the monkeys were often still eating the reward they had earned for entering the cages as the squeeze back was pulled forward and eating the reward gained for tolerating this as their leg was grasped and drawn forward. This in itself may have distracted them or helped desensitise them to the more aversive elements of the training process. Turkkan (1990) used an infusion pump to deliver apple sauce to baboons during training to co-operate with blood pressure monitoring. This allowed continuous reinforcement for as long as the monkeys continued to co-operate. Chewy sweets have a similar effect with the added advantage that they are handed to the monkey by the trainer, which allows the recipient to associate the trainer with the reward.

An additional difference was that the macaques were rewarded more frequently during Phase 2 and were also rewarded for entering the cages and tolerating the squeeze mechanism. However, the resistance shown during Phase 1 was never repeated, even before the macaques were rewarded. One explanation for this is that although the squeeze back was pulled forward, the monkeys were never actually squeezed as occurred during Phase 1. Negative reinforcement through releasing the squeeze mechanism was rarely used and, as reported previously, sometimes occurred to loosen the squeeze back when the monkeys showed a particularly aversive reaction. This did have the effect of calming them and it did appear throughout that the squeeze mechanism was the most aversive element of the procedure. Learning theory would predict that reinforcing resistance to the squeeze back should have increased the frequency of this behaviour (Lieberman, 1993). However, this occurred so infrequently that any effect was unlikely and the resistance shown during phase one was never recorded again. However, whenever Trainer A described the procedure to visitors, he would report that the squeeze back was always released as soon as the macaques co-operated. As with the differences in treatment with regards to individual monkeys, what people think they are doing can differ from what they are actually doing.

Curiously, during both phases, most resistance was shown prior to actual venipuncture. It might have been expected that the macaques would have attempted to withdraw their leg when they felt the needle pierce their skin. This was never observed, even during sessions where a great deal of resistance was shown when attempts were made to grasp a leg. When their leg was drawn from the cage, there appeared to be a distinct change in their behaviour in that they suddenly relaxed and showed no more resistance. Their attitude appeared remarkable similar to that

observed during performance of the 'ritual bite', a behaviour unique to this species (Demaria & Thierry, 1990; see Chapter 2). This behaviour involves passive resistance to the clasping and mock bite of a limb and it would be interesting to know if the macaques were interpreting the actions of the trainer in a similar way.

The more frequent, consistent and rapid rewards shown during Phase 2 should have predicted an increase in the amount of co-operation shown by the monkeys and indeed, that is what occurred. Aggressive and fearful or avoidant responses effectively disappeared and Jane occasionally presented a leg voluntarily. Throughout Phase 2, both Jane and Noreen appeared relaxed and this was reflected in their consistent willingness to enter the cage room.

As stated above, the only days when any reluctance was shown followed days when training was conducted by Trainer B. Although this trainer was only observed on one occasion, during that session, Jane was rewarded once and Noreen not at all. Although he was reluctant to have his methods observed, it was noted that he often went to collect blood from other macaques in the unit without taking the rewards jar with him. A second reason why few sessions conducted by Trainer B were recorded was that both Trainers A and C were aware of his resistance to the use of rewards and tried to conduct the procedure themselves in order to keep his involvement to a minimum. As reported previously, this trainer's methods such as his reluctance to reward the monkeys and his habit of freeing a jammed squeeze back by hitting it with a broom cannot be advocated. Shortly after completion of this study, Trainer B left the unit.

Differences between individual personnel are likely to have a significant impact on the well-being of animals during the training process. However, the rather

broad descriptions of training techniques provided in the existing literature do not allow identification of such differences.

The fact that the macaques had to be chased into the cages during Phase 1 does illustrate that, although the training process did predominantly use positive reinforcement, it cannot truly be called positive reinforcement training as participation by the macaques was not entirely voluntary. In addition, the monkeys were confined in relatively small area and a squeeze mechanism was used throughout. However, this technique has been shown to be a considerable improvement on the practice of forcibly removing monkeys from their homecage and forcibly restraining them (Reinhardt & Cowley, 1992; Reinhardt *et al.*, 1990, 1991). The aversive reactions shown during Phase 1 did not continue once the methods used had improved and the monkeys entered the training area voluntarily throughout Phase 2. This difference in response between the two phases was also detected during behavioural observations (Chapter 4).

The differences between Phases 1 and 2 may have been due to experience rather than training technique as the fastest stump-tailed macaque trained by Reinhardt and Cowley (1992) did not begin to tolerate the procedure until after nine sessions. However, with an 18 month interval between Sessions 8 and 9 this would seem unlikely and it is difficult to compare the technique used in the training of Reinhardt and Cowley's (1992) macaques with that described here as they were cage-housed and it is unclear when actual venipuncture was introduced.

Although there is a paucity of information to draw any firm conclusions, it is possible that only using positive reinforcement techniques to train for venipuncture may be too time consuming to be practical in a laboratory environment. If that is the case, then the technique observed in the second phase of this study could be seen as a

reasonable compromise. However, a likely important additional factor was the pre-existing good relationship between the macaques and their caregivers as these animals had been shown to be less affected by human activity than those without such a relationship (Waitt *et al.*, 2002). Even during Phase 1 the monkeys were willing to approach the trainers to accept food and would readily enter the cage room outside the training period (Chapter 4). It would appear that training that is not entirely voluntary can still have a beneficial effect and there is an intermediate area between forced restraint and voluntary co-operation although techniques that allow true co-operation have an advantage in that they allow the animal control, which is an important factor in reducing stress (Weinberg & Levine, 1980). However, as there is no evidence as yet to support the view that PRT represents a practical means of training for invasive procedures in a laboratory environment, techniques such as the one described here may be the best alternative until methods that allow true co-operation are developed. In order to categorise such training methods more accurately, it might be useful to use the term 'engineered compliance' to refer to techniques where the behaviour of the animal is not entirely voluntary but where co-operation is rewarded and aversive elements are balanced by the provision of food.

As reported above, the techniques used by Trainer A changed considerably over the two phases and it is hard to say for certain why this occurred. During the eighteen-month interval there had been considerable discussion about training methods both within the MRC laboratory and in the wider research community. The laboratory staff had also been told of the broad results of the Phase 1 observations. The second phase occurred after a considerable amount of training had been carried out with the marmosets and it is possible that any or all of these factors had an effect. What was clear was that at the beginning of Phase 1, none of the staff had been

specifically trained in operant theory and none were aware of basic training terminology. Each had learned to perform venipuncture following instruction from whatever staff member had been carrying out the procedure when they themselves had started working with macaques.

An additional observation was the importance of the design and maintenance of laboratory equipment. One reason that negative reinforcement was rarely used was that the squeeze mechanism was awkward to operate. This was particularly true for Trainer C who was physically small and needed both hands to operate to move the squeeze back in either direction. The poor design of this apparatus also caused additional disruption when the actions of the monkeys caused it to jam. Trainer B's technique of striking it free with a broom handle caused unnecessary distress. Additional disruption was caused by the monkeys' occasional escape through the faulty slide door. This was eventually jammed in the closed position, which left only one entrance between the gang and cage rooms. This in turn made it difficult for subordinate animals to escape from the more dominant animals. This in itself may have contributed to a reluctance to enter the cages.

The conclusions of this chapter are limited by the use of descriptive, rather than inferential statistics throughout although this could be remedied in future research using the same technique with a larger sample size. However, the observations do appear to illustrate the following points:

- Training techniques need to be examined in greater detail as not all training is truly following the principles of PRT. While techniques that encourage 'engineered compliance' are clearly an improvement on forced restraint, failure to acknowledge that some coercion is being used can obscure the need for future refinement.

- These more detailed observations than are currently reported in the literature reveal a number of important factors that are largely ignored. For example, when training to co-operate during venipuncture, rewards should be frequent, consistent and delivered quickly. Published reports do not give sufficient details as to precisely when this occurs. Equally, there was considerable difference between events as recorded at the time and descriptions given later by the trainers. Post-hoc, subjective reports regarding the training process are clearly unreliable which is unsurprising given the re-constructive nature of human memory (Loftus & Hoffman, 1989).

- Educational opportunities for animal trainers need to be improved as trainers should be aware of the operant principles underlying training techniques and understand why factors such as timing of the delivery of rewards are important. In addition, it was difficult for the laboratory technicians to gain access to the literature containing training information. Without access to a university library, the information available was limited to that contained in journals freely available on the Internet. The staff at the MRC unit were extremely keen to learn about training but it was difficult for them to find the information or indeed the time that was required for them to do so.

Chapter 4

Effects of Training on the behaviour of stump-tailed macaques

4.1 INTRODUCTION

4.1.1 Training and primate social behaviour

As reported in Chapters 1 and 3, in US laboratories, the uses and benefits of training are widely recognised, largely due to the pioneering work of researchers such as Viktor Reinhardt. However, much of the research concerning training to co-operate during venipuncture has concerned reactions to the procedure itself (Reinhardt, 1997) and little attention has been paid to effects on primate behaviour beyond training or blood draw sessions. In addition, many of the animals trained using the methods described in Chapter 3 were either singly or pair housed with the emphasis placed on the reactions of the animals as individuals rather than as members of a complex social system. Even when the training of animals living in social groups is reported, any post-training reactions reported tend to be those directed towards humans rather than conspecifics (e.g. Phillippi-Falkenstein & Clarke, 1992; Smith, 1981). There is a paucity of studies examining any effects of training on the social behaviour of laboratory-housed primates maintained in groups, an important omission as while the benefits of group housing are increasingly recognised (Reinhardt *et al.*, 1987; de Waal, 1991), disruption to social relationships can have a detrimental effect on welfare (Castles, Whiten & Aureli, 1999; Shively, Laber-Laird & Anton, 1997).

Heath (1989) introduced a programme of training and increased positive contact with humans with a group of female cynomolgus monkeys *(M. fascicularis).* Qualitative reports showed that the animals became more docile and easier to handle while "undesirable" behaviour traits decreased with a corresponding rise in "natural"

behaviours (behavioural definitions were not provided). However, it should be noted that the group was not an established one but was formed at the start of the study with the study animals specifically selected for good temperament. The introduction of training was accompanied by a move to an environment considerably enriched in comparison to the single cages that the animals had been accustomed to. In addition, not all of the animals benefited with the most subordinate animal removed from the group following the onset of stress-related chronic diarrhoea.

When Dettmer *et al.* (1996) attempted to train capuchin monkeys *(C. apella)*, to co-operate during venipuncture success was mixed with only four of the eight study animals responding positively to the training process (see Chapter 3). The outcome appeared independent of whether the animals were housed singly or with companions although the reactions of pair- or group-housed animals upon return to the homecage are not reported.

As reported previously (see Chapter 3), group-housed animals are often trained to enter a narrow chute or tunnel connected to a sampling cage where individuals can be temporarily isolated for experimental procedures. Phillippi-Falkenstein and Clarke (1992) did not report any behavioural changes when employing this technique with rhesus macaques *(M. mulatta)* while Walker, Gordon and Wilson (1982) found that reproductive performance in this species was unaffected following the introduction of identical procedures. Knowles, Fourrier and Eiselle (1995) found that a beneficial effect of this procedure was that staff became more sensitive to group dynamics and would alter their handling of the monkeys accordingly. Another major advantage of this technique is that individuals can choose the order in which they enter the chute. When such choice is allowed, the order of entry appears to become relatively predictable and it is not necessarily dominance

ranking that affects the order. Boccia *et al.* (1992) found that animals with extensive experience of such procedures tended to be the first to present for sampling and had lower blood cortisol levels than those sampled later, a result found in both bonnet *(M. radiata)* and pigtail macaques *(M. nemestrina).*

Additional information regarding the effects of training on social behaviour can be found in zoo-based studies. As outlined in Chapter 1, the wider effects of training have been of considerable concern in zoos where maintaining normal social behaviour is of high priority (Laule & Desmond, 1991). Indeed, this issue is one of the reasons that training has not been widely used in British zoos, along with concerns that animals will become 'fixated' on gaining food treats. By contrast, North American zoos have used positive reinforcement techniques to both reduce abnormal behaviour and promote desirable social behaviour in primates (Laule, 1992, 1993; Laule & Desmond, 1994).

Bloomsmith, Baker, Ross and Lambeth (1999) found an increase in agonistic behaviour in chimpanzees *(Pan troglodytes)* during training sessions although this was accompanied by an increase in general activity and playful interactions. Chimpanzee aggression during feeding times has been reduced by rewarding aggressive individuals for remaining seated and allowing others to feed in peace. The changes recorded appeared specific to feeding times with no reported changes outwith this specific situation (Bloomsmith, Laule, Thurston & Alford, 1992; Bloomsmith, Laule, Alford & Thurston, 1994; Laule, 1992). Desmond, Laule and McNary (1987) successfully trained a group of drills *(Mandrillus leucophaeus)* by reinforcing positive social behaviours such as grooming. In addition, dominants were rewarded for allowing subordinates access to the trainers and a previously excluded animal was integrated into the group by pairing him with a female who was rewarded for staying

close during feeding sessions. Such programmes have led to an increase in a range of affiliative behaviours (Cox, 1987). Similar techniques have been used to promote social behaviour in gorillas *(G. g. gorilla)* (Laule & Desmond, 1991; Petiniot, 1995; Shellabarger, 1992).

While the above studies suggest that training actually promotes positive social relationships, it should be noted that the programmes reported represented deliberate attempts to alter positive social behaviour. In addition, the programmes reported were carried out by skilled and experienced trainers, something that may not always be the case, especially in the UK when comprehensive training programmes for personnel do not exist (Kiley-Worthington, 1990). While the potential for disruption to social relationships is recognised (Laule & Desmond, 1991), there is still a paucity of studies examining accidental effects on social behaviour. Moreover, most evidence presented is anecdotal and systematically collected empirical data are scarce.

When the benefits of training are examined, consideration of the effects on group behaviour makes evaluation considerably more complex yet such considerations are important for a number of reasons. Firstly, during actual training sessions, there is the question of whether it is better to separate group members or train the group as a whole. Each method has potential problems. Separation may be stressful and there is the potential for disruption when the animals are reunited. However, when training in groups there is the possibility of increased aggression or the exclusion of subordinate animals. When position within the dominance hierarchy is considered, positive reinforcement training may benefit some individuals but not others. Access to the rewards offered represent a resource that could be subject to competition like any other. In this scenario, training could be stressful to subordinate

animals if they are subjected to aggression as dominants seek to monopolise access to rewards.

Secondly, there are the possible effects on behaviour beyond actual training sessions. Early behaviourist studies found that discontinuing positive reinforcement can lead to a temporary increase in aggressive behaviour (e.g. Azrin, Hutchinson & Hake, 1966; Thompson & Bloom, 1966). Although these studies concerned the conditioning of animals in operant chambers, the end of any training session involves the discontinuing of positive reinforcement. However, an important difference is that in an operant chamber, the cues to perform a specific behaviour (e.g. the presence of a lever) remain after reinforcement ceases whereas in training involving animal-human interactions both cues and rewards are removed when the trainer leaves. It is possible that the reported increase in aggression arises from frustration when a behaviour is performed but not rewarded.

The above factors suggest that even if positive reinforcement training itself is beneficial, there may be some inadvertent after-effects thus it is important to study behaviour beyond the actual training sessions, especially when animals are housed in social groups. Conversely, if training techniques are themselves stressful, any negative effects could be heightened through displaced aggression or other disruption to group relationships.

4.1.2 Study Outline

This investigation was conducted concurrently with the observations of the training techniques employed at the MRC Human Reproductive Sciences Unit reported in Chapter 3. As training occurred in two distinct stages, this presented the opportunity for two studies concerning the wider effects of training on the behaviour of the study animals. These were:

Study 1 – Effects of training on behaviour during the initial training period.

Study 2 – Effects of training on behaviour during the second training period.

4.2 STUDY 1 – Effect of training on behaviour during initial training period

4.2.1 Aims and Methods

The study reported in this chapter was conducted for two reasons. These were:

1. To examine the wider effects of training on the social behaviour of an established stump-tailed macaque group.
2. To provide additional information regarding the training procedure itself. As reported previously (Chapter 3), the techniques used to train for co-operation during venipuncture are not always successful (Reinhardt, 1992) and it has been suggested that the training procedure itself may be stressful (Dettmer *et al.*, 1996). While observations of the training sessions themselves provided one opportunity to record the reactions of the monkeys, observations of their behaviour following each session provided the opportunity to collect additional data.

In this regard I was fortunate in that the study animals had previously participated in a number of studies examining the effects of human activity on their behaviour. When examining the reactions of macaques classed as 'friendly' (which included the present study animals) as opposed to 'unfriendly' animals, Waitt, Buchanan-Smith and Morris (2002) found that the former group were less disturbed by husbandry routines, more likely to approach and less likely to threaten caretakers. The same authors found that disruption to routine husbandry routines, and in particular delays in feeding had a detrimental effect on behaviour (Waitt *et al.*, 2001). These studies were particularly useful, both in identifying behaviours that could prove

useful in examining the effects of training but also potential confounding variables that should be recorded.

As during training observations, no attempt was made to influence events as the aim was to assess training as currently carried out in a UK laboratory. While this resulted in a study high in ecological validity, there was a corresponding lack of control. Although attempts were made to standardise the methods used as much as possible, some variation was unavoidable due to the nature of the study.

4.2.2 Study animals

The study animals were five adult stump-tailed macaques housed in the same social group at the MRC Human Reproductive Sciences Unit in Edinburgh. The group consisted of the four females whose training is described in Chapter 3 and the adult male housed with them. Brief details of the study animals are given in Table 4.1. Full details of the study animals, housing and husbandry routines are described in Chapter 2.

Table 4.1 Details of the study animals including name, sex, and age at start of study.

Name	Sex	Age
Blackie	Male	Unknown
Jane	Female	16yr 11mth
Kelly	Female	5yr 4mth
Noreen	Female	7yr 11mth
Mirrium	Female	13yr 3mth

4.2.3 Procedure

Data were collected when initial training to co-operate during venipuncture (Chapter 3) was conducted. As this occurred over eight sessions, data were collected on 16 days (the eight training days and eight corresponding control days). On each training day, data were collected before training began, immediately following each

session and again two hours later. Pre-training data were collected following completion of the morning cleaning routine, beginning between 1015h and 1025h. Post-training observations began as soon as the slide doors were raised following training, releasing the monkeys into the gang room. Timing varied depending on the start and duration of each training session but all post-training observations began between 1138h and 1220h. For each individual day, the starting time was recorded with the second post-training observation session beginning two hours later.

As stated above, for each training day, data were collected on a corresponding day when no training took place with the start of each control observation period timed to match the corresponding training day. As training did not occur either on a daily basis or on consistent days of the week (Chapter 3), it was possible to collect control data during the training period. This had an additional advantage it that it was unlikely that the monkeys learned to associate my presence with the occurrence of a training session.

No training was conducted on days when the gang room was power hosed. As this was an activity likely to have some effect on behaviour, either through frustration at being confined in the cages for a considerable period or through the addition of foraging material to the fresh substrate (Chapter 2), control data were never recorded on days when this occurred.

All observations were conducted through the viewing window at the front of the gang room. Observation periods lasting 30 minutes were chosen as this was the maximum period that seemed likely to fit around normal staff routines. As THE OBSERVER 3.0 software (Noldus, 1993) used to record training sessions (Chapter 3) did not allow simultaneous collection of scan and all occurrences data, behaviours were recorded onto checksheets. Behavioural states, behaviours of relatively long

duration, were recorded using instantaneous scan sampling with a 30 second sample interval. Behaviours recorded this way included: locomotion; sitting or lying in physical contact with another monkey, henceforth referred to as 'proximity (touch)'; sitting or lying in a position less than 1m from another monkey, henceforth referred to as 'proximity (<1m)'; allogroom; autogroom; and out of view therefore located in the cage room, referred to as 'cage room'. All other behavioural states were recorded in the category henceforth referred to as 'other'.

All occurrences of behavioural events, behaviours of relatively short duration, were also recorded. Behavioural categories included: 'threat'; 'aggression'; 'affiliate'; 'scratch' and 'observer directed behaviours'. Full definitions of behavioural categories are given in Chapter 2. In addition, events likely to influence the results such as cleaning routines and disruption to feeding schedules were also recorded.

4.2.4 Statistical Analysis

The data were found to be normally distributed therefore parametric tests were used throughout. For each animal, the mean percentage of the total activity budget spent engaged in each behaviour was calculated. For behavioural categories where all occurrences were recorded, the mean frequency per 30 minute observation period was calculated using the scan sampling data. As the amount of time spent out of sight in the cage room clearly effected the frequency with which these behaviours were observed, means were calculated using the time that each animal was visible rather than the total number of observation periods *per se*.

A repeated-measures ANOVA was performed with two factors: Day (training and control) and Time (pre-training, post-training and 2 hours post-training). In

instances where equal variances could not be assumed, as indicated by a significant result of Mauchly's Test of Sphericity, any significant outcome was determined using the more conservative Greenhouse-Geisser test (Howell, 1995). When significant interactions occurred, post-hoc *t*-tests were used to determine where significant differences lay. However, as the risk of Type 1 errors increases when multiple *t*-tests are employed, the Fisher's least significant difference (LSD) procedure was used with the requirement that the result for the overall ANOVA was significant before further comparisons were made (Howell, 1995). Although the LSD test is one of the most liberal of the multiple comparison procedures, this test was chosen due to the small sample size (N = 5). Significance was set at $p < 0.05$ throughout the analyses.

Caveat When any conclusions concerning the results presented below are drawn, there are a number of limitations that must be considered. The most obvious was the small sample size. In addition, during pre and post-training observations, behaviour was recorded using scan sampling and, as the behaviour of each animal was likely to have been affected by other group members, observations cannot be considered to be independent (Martin & Batson, 1993). Ideally, analysis should have used means for the group rather than individuals, producing a mean activity budget for the group as a whole, the procedure used when analysing the marmoset data (Chapter 7). However, this would have resulted in one figure per condition, making further analysis impossible. Another factor that must be considered is the use of a repeated-measures design throughout. While this is the only method possible with a limited number of study animals, the repeated-measures design is more powerful than the between-subjects design, thus increasing the probability of rejecting a false null hypothesis (Howell, 1995). Although this factor is balanced to some degree by the small sample size, it should also be borne in mind when interpreting the results.

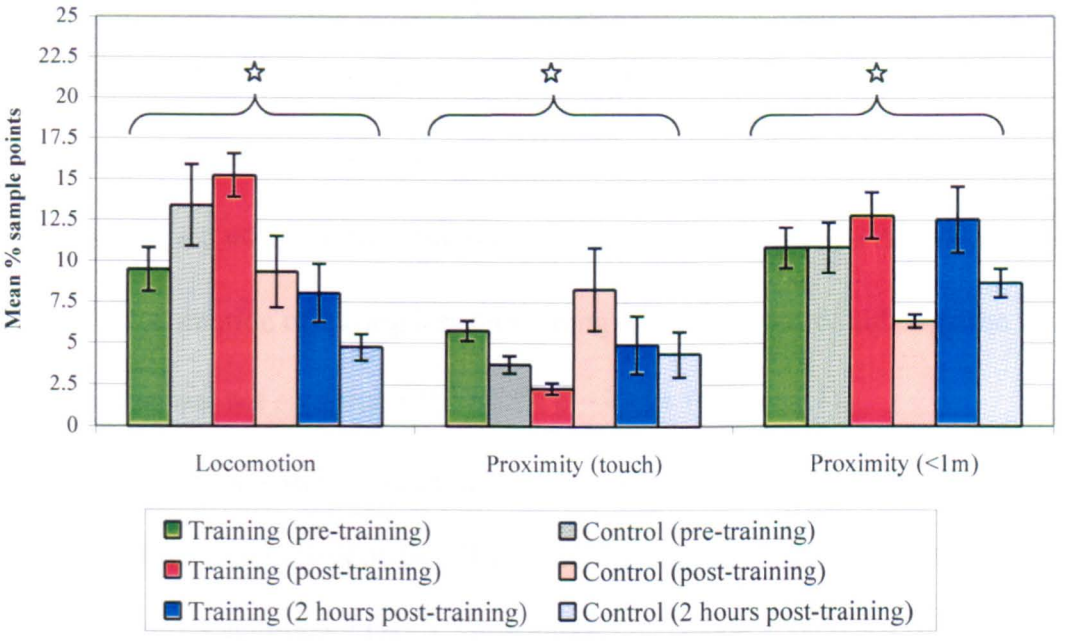
Further discussion regarding these limitations and the rationale for the data collection methods can be found in the appendix.

4.3 RESULTS

4.3.1 Activity budgets

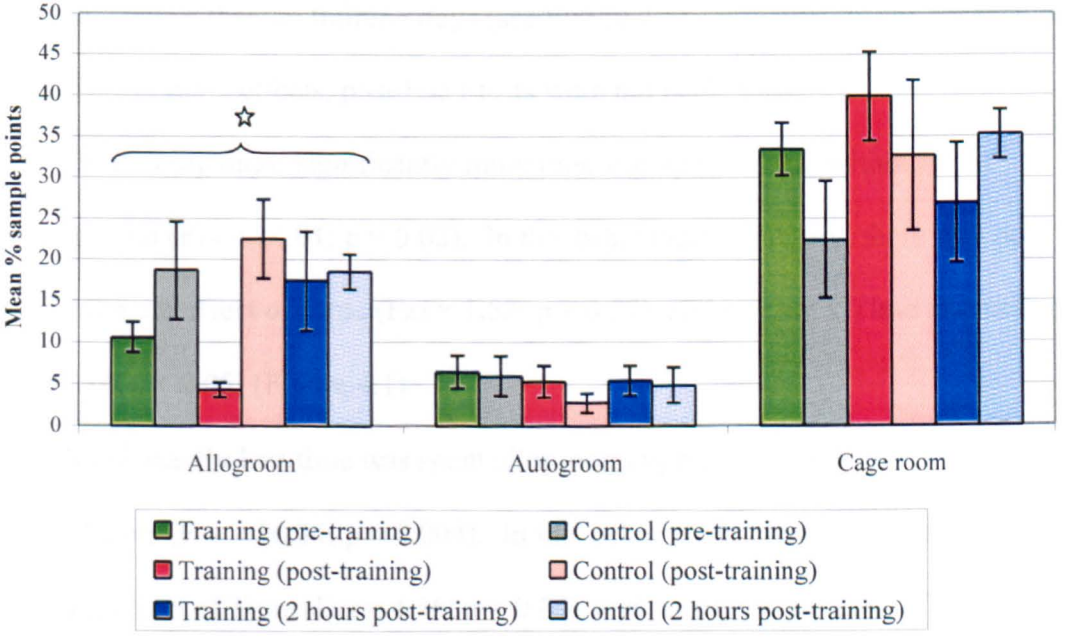
The results for the behavioural categories recorded using instantaneous scan sampling are initially presented, followed by those recorded as 'all occurrences'. In each case, the means and standard errors for all categories are presented first, followed by the ANOVA results from all categories where significant differences were found with those categories producing no significant results presented last. For clarity, the results from the six behavioural categories are presented over two graphs. The mean percentage sample points spent engaged in the first three categories, (Locomotion, Proximity (touch) and Proximity (<1m)) recorded using scan sampling on training and control days are shown in Figure 4.1. The results from the remaining three categories (Allogroom, Autogroom and Cage room) are presented in Figure 4.2.

Figure 4.1 Percentage sample points spent engaged in behavioural categories Locomotion, Proximity (touch) and Proximity (<1m) on training and control days across the three observation periods. (bars represent standard errors)



☆ denotes behavioural categories where significant differences were found

Figure 4.2 Percentage sample points spent engaged in behavioural categories Allogroom, Autogroom and Cage room on training and control days across the three observation periods. (bars represent standard errors)



☆ denotes behavioural categories where significant differences were found

The macaques spent more time locomoting on training days than on control days ($F_{1,8}$=16.88; p = 0.01). In the 'locomotion' category there was also a significant effect of Time ($F_{2,8}$ = 25.71; p = 0.01), and a significant Day x Time interaction. Post-hoc *t*-tests showed that the macaques spent more time locomoting on training days during post-training observations (t_4 = 3.97; p = 0.02) and 2 hours post-training observations (t_4 = 2.99; p = 0.04). Pre-training observations showed a different pattern with locomotion occurring less on training days than control although the difference here was not significant (t_4 = 2.39; p = 0.07) (Figure 4.1).

When the time spent in the Proximity (touch) category was examined, there was no significant main effect of Day ($F_{1,4}$ = 1.05; p = 0.3), or Time ($F_{2,8}$ = 0.21; p = 0.81). There was a significant Day x Time interaction ($F_{2,8}$ = 3.38; p = 0.01. It appeared that on training days, more time was spent in Proximity (touch) than on control days during pre-and 2 hour post-training observations. This pattern was reversed during post-training observations when more time was spent in Proximity (touch) on control than on training days (see Figure 4.1). However, in the absence of any significant main effects, post-hoc *t*-tests were not performed.

On training days, significantly more time was spent sitting within 1m of another animal ($F_{1,4}$ = 14.01; p = 0.02). In this behavioural category, there was no significant main effect of Time ($F_{2,8}$ = 1.52; p = 0.27), and no Day x Time interaction ($F_{2,8}$ = 4.58; p = 0.05) (Figure 4.1).

Significantly less time was spent allogrooming on training days as compared to control days ($F_{1,4}$ = 36.84; p = 0.004). In this behavioural category there was no significant effect of Time ($F_{2,8}$ = 4.16; p = 0.06), and no significant Day x Time interaction ($F_{2,8}$ = 4.16; p = 0.18) (see Figure 4.2).

There were no further significant differences found in behavioural categories recorded using instantaneous scan sampling. Table 4.2 contains a summary of the results were where no effects of Day or Time were found.

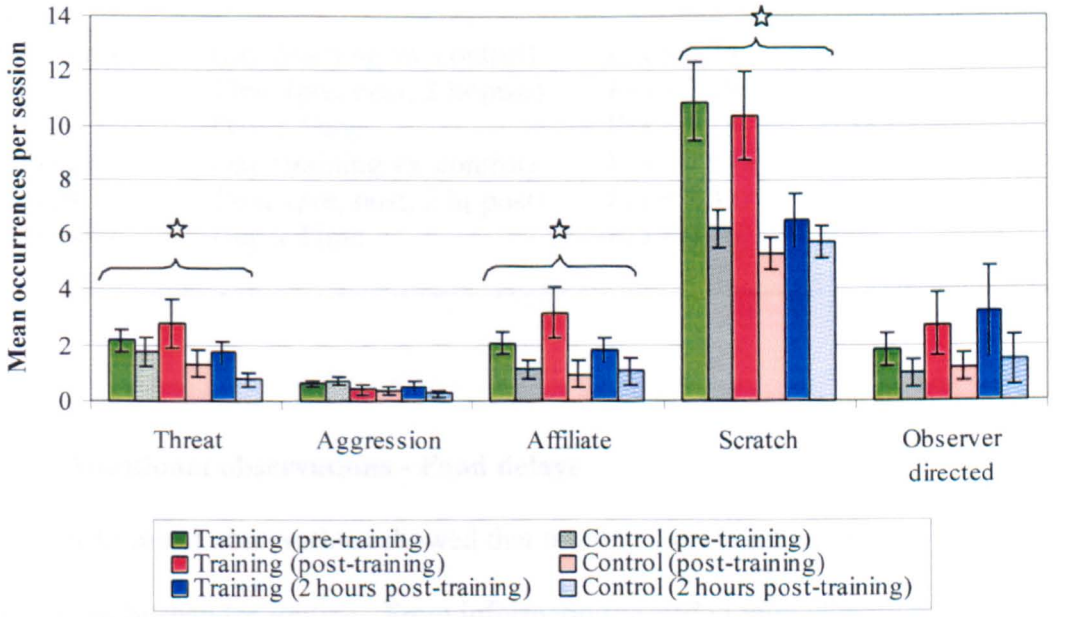
Table 4.2 Results of ANOVAs for behavioural categories where no significant main effects of Day (training or control) or Time (pre-, post- and 2 hours post-training were found.

Behavioural category	Factor	F =	P =
Autogroom	Day (training vs. control)	$F_{1,4} = 1.19$	0.34
	Time (pre, post, 2 hr post)	$F_{2,8} = 2.29$	0.16
	Day x Time	$F_{2,8} = 1.2$	0.35
Cage room	Day (training vs. control)	$F_{1,4} = 0.89$	0.4
	Time (pre, post, 2 hr post)	$F_{2,8} = 4.38$	0.05
	Day x Time	$F_{2,8} = 2.07$	0.18

4.3.2 Behaviours recorded as 'all occurrences'

The mean frequency per 30 minute observation session for each behavioural category is presented in Figure 4.3.

Figure 4.3 Mean frequency per 30 minute observation session (pre-, post- and 2 hour post-training) for training and control days across the three observation periods (bars represent standard errors)



☆ denotes behavioural categories where significant differences were found

Threats occurred more frequently on training than on control days ($F_{1,4} = 8.66$; $p = 0.04$). In this category there was no significant main effect of Time ($F_{2,8} = 2.6$; $p = 0.13$), and no significant Day x Time interaction ($F_{2,8} = 1.53$; $p = 0.27$). However, affiliative behaviours were also more frequent on training days ($F_{1,4} = 18.09$; $p = 0.01$). There was no significant effect of Time ($F_{2,8} = 1.37$; $p = 0.3$) and no significant Day x Time interaction ($F_{2,8} = 5.55$; $p = 0.05$).

The mean frequency of scratching was significantly higher on training than on control days ($F_{1,4} = 24.82$; $p = 0.008$). In this category there was no significant effect of Time ($F_{2,8} = 2.72$; $p = 0.13$), and no significant Day x Time interaction ($F_{2,8} = 2.89$; $p = 0.11$).

There were no significant differences found in frequencies of aggressive or observer-directed behaviours. The results from these two behavioural categories are presented in Table 4.3.

Table 4.3 Results of ANOVAs for behavioural categories where no significant main effects of Day (training or control) or Time (pre, post and 2 hours post-training were found.

Behavioural category	Factor	F =	P =
Aggression	Day (training vs. control)	$F_{1,4} = 0.74$	0.43
	Time (pre, post, 2 hr post)	$F_{2,8} = 3.19$	0.09
	Day x Time	$F_{2,8} = 1.25$	0.34
Observer-directed behaviours	Day (training vs. control)	$F_{1,4} = 7.51$	0.05
	Time (pre, post, 2 hr post)	$F_{2,8} = 1.31$	0.32
	Day x Time	$F_{2,8} = 0.76$	0.49

4.3.3 Additional observations - Food delays

Additional observations showed that training days resulted in some disruption to the usual husbandry routine. From information recorded immediately prior to the start of pre-training observations, it was noted that delivery of the morning fruit was

delayed on six of the eight training days but on only one control day. On four of the training days, food had not yet been delivered by the time the post-training observations were completed. The macaques had always been fed by this time on control days. During 2 hour post-training observations, the main feed had not been delivered on four of the training days and one of the control days (see Table 4.4).

Table 4.4 Summary of disruption to feeding routines observed during the first training period. Figures indicate the number of sessions on which feeding was delayed.

Number of observation sessions when food was delayed	Pre-training	Post-training	2 hours Post-training
Training Days	6	4	4
Control Days	1	0	1

Delays to feeding schedules had previously been shown to have an adverse effect on the behaviour of these animals (Waitt *et al.*, 2002). To examine the importance of food delays as a possible confounding variable, analysis of the observational data was repeated with sessions grouped according to the presence of food (delayed or delivered) rather than training or control days. The results proved very similar to those reported above. The results for behavioural categories recorded using instantaneous scan sampling are presented in Figure 4.4 while those for behavioural categories recorded as 'all occurrences' are shown in Figure 4.5. The results of the ANOVAs performed during re-analysis are presented in Table 4.5.

Figure 4.5 Percentage sample points spent engaged in each behavioural category during sessions when food was present and those when food was delayed during the three observation periods.
(bars represent standard errors)

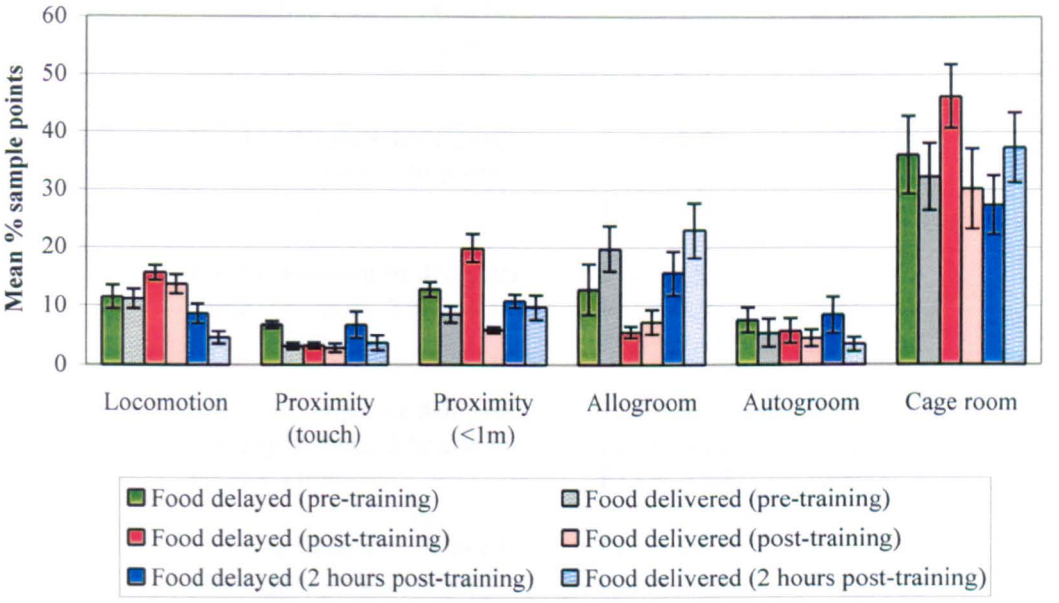


Figure 4.6 Mean frequency per observation session (pre-, post- and 2 hour post-training) when food was present and those when food was delayed (bars represent standard errors)

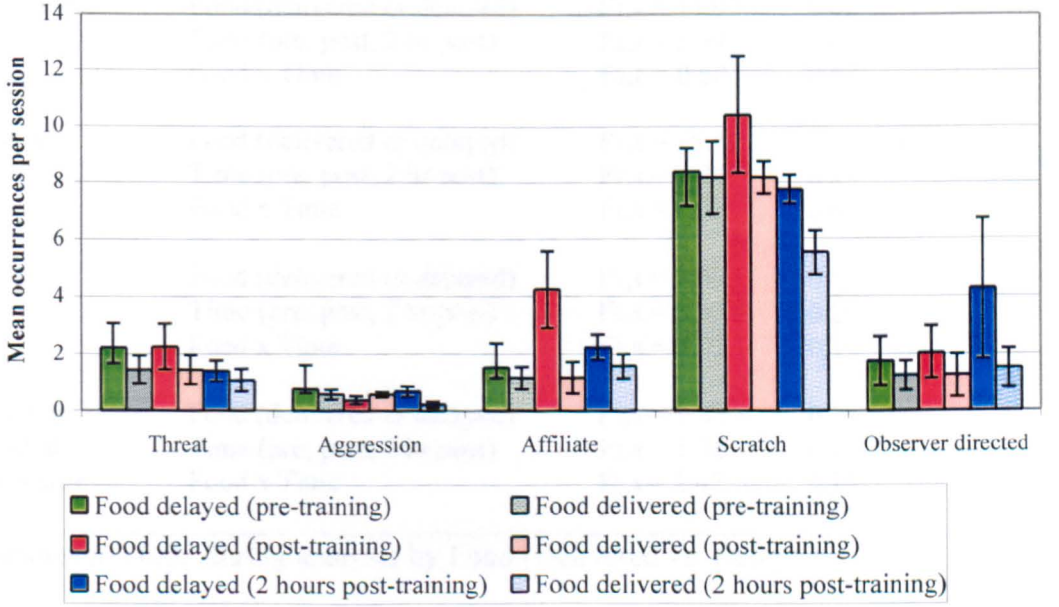


Table 4.5 Results of ANOVAs performed on observational data re-analysed by Food (delayed vs. delivered)

Category	Factor	F =	P =
Locomotion	Food (delivered or delayed)	$F_{1,4} = 41.48$	0.003 * ♦
	Time (pre, post, 2 hr post)	$F_{2,8} = 33.62$	0.001 * ♦
	Food x Time	$F_{2,8} = 1.65$	0.25
Proximity (touch)	Food (delivered or delayed)	$F_{1,4} = 8.28$	0.05
	Time (pre, post, 2 hr post)	$F_{2,8} = 2.37$	0.16
	Food x Time	$F_{2,8} = 2.33$	0.16 ♦
Proximity (<1m)	Food (delivered or delayed)	$F_{1,4} = 15.43$	< 0.01* ♦
	Time (pre, post, 2 hr post)	$F_{2,8} = 3.93$	0.07
	Food x Time	$F_{2,8} = 9.46$	0.08 * ♦
Allogroom	Food (delivered or delayed)	$F_{1,4} = 10.42$	0.03 * ♦
	Time (pre, post, 2 hr post)	$F_{2,8} = 10.21$	0.06
	Food x Time	$F_{2,8} = 1.03$	0.39
Autogroom	Food (delivered or delayed)	$F_{1,4} = 10.39$	0.03 *
	Time (pre, post, 2 hr post)	$F_{2,8} = 0.50$	0.63
	Food x Time	$F_{2,8} = 0.49$	0.63
Cage room	Food (delivered or delayed)	$F_{1,4} = 0.59$	0.48
	Time (pre, post, 2 hr post)	$F_{2,8} = 1.47$	0.39
	Food x Time	$F_{2,8} = 3.83$	0.07
Threat	Food (delivered or delayed)	$F_{1,4} = 1.01$	0.37 ♦
	Time (pre, post, 2 hr post)	$F_{2,8} = 2.09$	0.19
	Food x Time	$F_{2,8} = 1.03$	0.40
Aggression	Food (delivered or delayed)	$F_{1,4} = 13.03$	0.02 *
	Time (pre, post, 2 hr post)	$F_{2,8} = 5.44$	0.03 *
	Food x Time	$F_{2,8} = 0.60$	0.57
Affiliate	Food (delivered or delayed)	$F_{1,4} = 15.69$	0.02 * ♦
	Time (pre, post, 2 hr post)	$F_{2,8} = 3.04$	0.15
	Food x Time	$F_{2,8} = 6.67$	0.06
Scratch	Food (delivered or delayed)	$F_{1,4} = 3.69$	0.13 ♦
	Time (pre, post, 2 hr post)	$F_{2,8} = 1.43$	0.29
	Food x Time	$F_{2,8} = 2.02$	0.19
Observer-directed behaviour	Food (delivered or delayed)	$F_{1,4} = 1.82$	0.24
	Time (pre, post, 2 hr post)	$F_{2,8} = 1.76$	0.23
	Food x Time	$F_{2,8} = 2.47$	0.15

* denotes $p < 0.05$ during analysis by Food (Delivered vs. Delayed)

♦denotes $p < 0.05$ during analysis by Day (Training vs. Control)

As shown above, re-analysis produced similar significant differences in six behavioural categories. Locomotion, sitting in proximity (<1m) to another animal, and affiliative behaviours all occurred more frequently on training days or when food was delayed. Equally, allogrooming decreased on these days. No significant differences were found in time spent in the cage room nor in the frequency of observer-directed behaviours during either analysis.

There were some differences. The analysis by Day (training vs. control), showed that the frequency of threats increased on training days while aggression remained unchanged. Analysis by Food (delayed vs. delivered) produced the opposite result with an increase in aggression but not threats. A significant increase in autogrooming was only found in the analysis by Food while analysis by Day showed an increase in scratching. Overall though, the trends shown in the two data sets were very similar suggesting that disruption to the feeding routine did present a major confounding variable during this study.

4.4 Conclusions

The results indicated that the training sessions were having a detrimental effect on the behaviour of the macaques. The significant increase in locomotion observed on training days could be seen as a positive change (Crocket, 1989; Mench, 1998) especially given that the macaques were very overweight. This rise in activity was accompanied by an increase in the frequency of self-scratching, threats and a decrease in social grooming, suggesting that the change was due to agitation. However, the increase in the frequency of threats was accompanied by an increase in affiliative behaviours and time spent in physical contact with another group member. Equally, when training and control days were compared, threats may have increased

but actual aggression remained unchanged. Overall, instances of aggression were low with the most commonly observed acts being pushes and slaps. Aggression serious enough to cause flight by the recipient was only recorded on two occasions. No actual injuries were observed.

What changes were observed did suggest a detrimental, but relatively mild effect and this was in accord with the results of observations made during the training sessions themselves. However, the finding that time spent in the cage room was unchanged following training sessions suggests that the monkeys did not develop any aversion to entering the area where training occurred. Equally, there were no changes in behaviours directed towards myself, something that might have occurred if the monkeys had associated humans with a strongly aversive experience or if they were becoming fixated on humans to receive food rewards.

However, it is not possible to conclude that any of the changes observed were actually due to the training process due to the concurrent disruption to the usual feeding routines. It was primarily due to this problem that the observations were repeated when training resumed eighteen months later. However, it is reasonable to conclude that it is important that the introduction of a training programme should not lead to the disruption of normal husbandry routines, given the detrimental effect that this is known to have (Waitt *et al.*, 2001).

4.5 STUDY 2 - Effects of training on behaviour during the second training period

4.5.1 Aims and Methods

As reported above, disruption to normal feeding schedules proved a major confounding variable during the initial training period. By the time the second training period commenced, this problem had been recognised and changes to staff routines implemented to ensure that feeding occurred on a consistent schedule (Chapter 2). The second training period provided the opportunity the replicate behavioural observations with the same study animals when disruption to feeding routines was no longer a confounding factor.

Although training was conducted daily for four weeks then three times weekly for another three weeks, observational data were collected over eight training and eight control days in order to match that collected during Study 1. The procedures used to determine the timing of observation periods and conduct observations were identical to that described above (Study 1). However, there was a difference in the distribution of training and control days. As training was conducted on a daily basis for the first four weeks, it was not possible to begin control observations during this period. Four control days were observed when sessions were conducted three times per week with the remaining control observations conducted when training ceased.

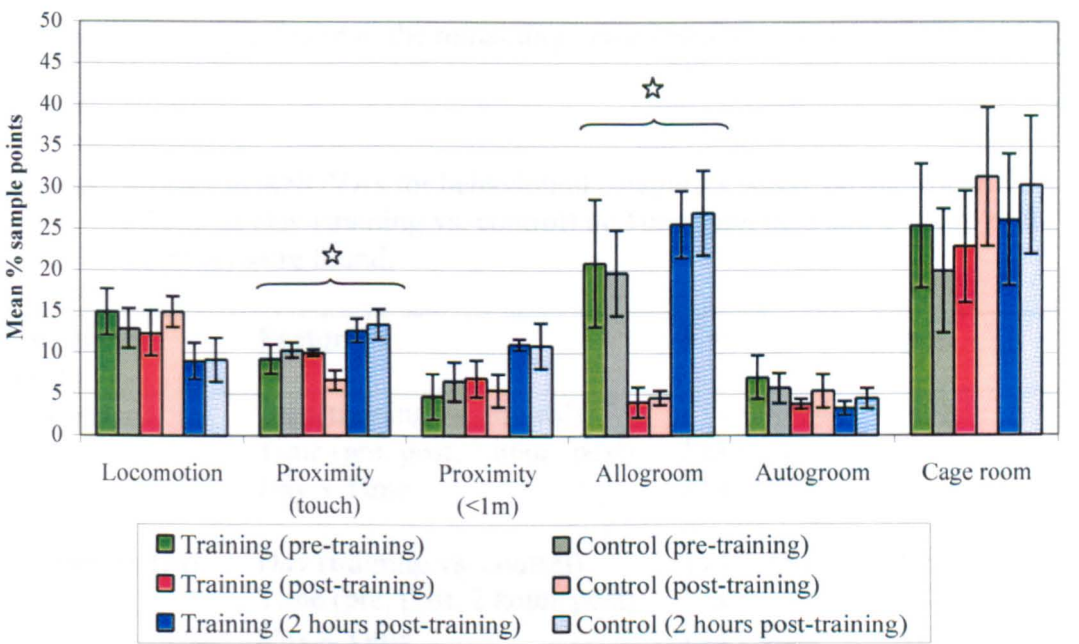
In addition, training sessions continued over weekends, something that had not occurred during Study 1. As the husbandry routine was considerably different at weekends, data were not recorded. Data were collected on weekdays, avoiding Thursdays when the gang room was power-hosed. The factors reported above meant that neither training nor control observations exactly matched those conducted during Study 1 but were as close as circumstances allowed.

4.6 RESULTS

4.6.1 Activity Budgets

The mean percentage sample points spent engaged in each behavioural category recorded using instantaneous scan sampling are shown in Figure 4.6.

Figure 4.6 Mean percentage sample points spent in each behavioural category on training and control days across the three observation periods. (bars represent standard errors)



☆ denotes categories where a significant effect of Time was found

Changes were observed in two behavioural categories. In the Proximity (touch) category, there was no significant main effect of Day (training vs. control) ($F_{1,4} = 1.05$; $p = 0.36$). There was a significant effect of Time ($F_{2,8} = 8.14$; $p = 0.01$), but no significant Day x Time interaction ($F_{2,8} = 1.04$; $p = 0.39$). Post-hoc t-tests showed that time spent in this category was significantly higher during 2 hour post-training observations on control days ($t_4 = 2.89$, $p = 0.04$).

There was no significant main effect of Day (training vs. control) in the allogroom category ($F_{1,4} = 0.016$; $p = 0.91$) but there was a significant effect of Time ($F_{2,8} = 14.84$; $p = 0.009$). Post-hoc t-tests showed that more time was spent allogrooming during 2 hour post-training observations on both training days ($t_4 = 8.9$, $p < 0.001$) and control days ($t_4 = 4.45$, $p < 0.001$). There was no significant Day x Time interaction ($F_{2,8} = 0.21$; $p = 0.76$).

There were no further significant differences in the activity budgets. A summary of the results found in the remaining behavioural categories is presented in Table 4.6.

Table 4.6 Results of ANOVAs for behavioural categories where no significant effects of Day (training vs. control) or Time (pre, post and 2 hours post-training) were found.

Behavioural category	Factor	F =	p =
Locomotion	Day (training vs. control)	$F_{1,4} = 0.154$	0.72
	Time (pre, post, 2 hours post)	$F_{2,8} = 2.60$	0.14
	Day x Time	$F_{2,8} = 0.38$	0.59
Proximity (<1m)	Day (training vs. control)	$F_{1,4} = 0.01$	0.98
	Time (pre, post, 2 hours post)	$F_{2,8} = 4.42$	0.08
	Day x Time	$F_{2,8} = 0.50$	0.62
Autogroom	Day (training vs. control)	$F_{1,4} = 0.71$	0.45
	Time (pre, post, 2 hours post)	$F_{2,8} = 1.73$	0.25
	Day x Time	$F_{2,8} = 1.29$	0.33
Cage room	Day (training vs. control)	$F_{1,4} = 0.29$	0.62
	Time (pre, post, 2 hours post)	$F_{2,8} = 1.33$	0.32
	Day x Time	$F_{2,8} = 1.24$	0.32

4.6.2 Behaviours recorded as 'all occurrences'

The results of the ANOVAs performed on data collected in categories recorded as "all occurrences" showed no significant differences. The mean frequency of each behaviour per 30 minute observation period is shown in Figure 4.7 and the results of the ANOVAs are given in Table 4.7.

Figure 4.7 Mean frequency per observation period for each behaviour on training and control days across the three observation periods. (bars represent standard errors)

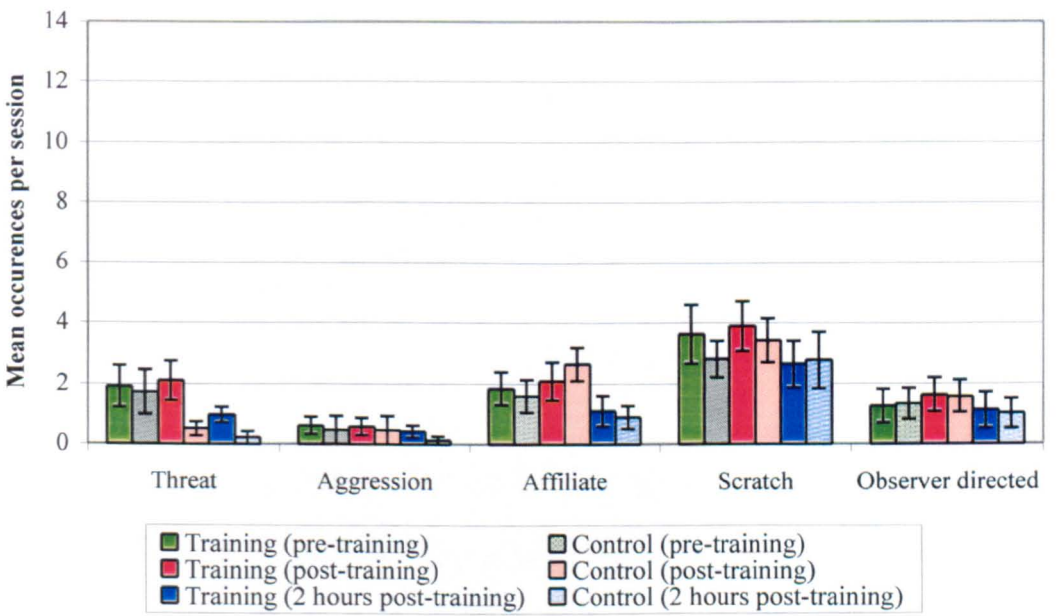


Table 4.7 Results of ANOVAs for behavioural categories where no significant effects of Day (training vs. control) or Time (pre-, post- and 2 hours post-training) were found.

Behavioural category	Factor	F =	p =
Threat	Day (training vs. control)	$F_{1,4} = 5.97$	0.07
	Time (pre, post, 2 hours post)	$F_{2,8} = 4.34$	0.08
	Day x Time	$F_{2,8} = 1.17$	0.34
Aggression	Day (training vs. control)	$F_{1,4} = 0.96$	0.34
	Time (pre, post, 2 hours post)	$F_{2,8} = 1.76$	0.24
	Day x Time	$F_{2,8} = 0.09$	0.08
Affiliate	Day (training vs. control)	$F_{1,4} = 0.01$	0.92
	Time (pre, post, 2 hours post)	$F_{2,8} = 5.99$	0.05
	Day x Time	$F_{2,8} = 2.19$	0.20
Scratch	Day (training vs. control)	$F_{1,4} = 2.05$	0.19
	Time (pre, post, 2 hours post)	$F_{2,8} = 2.99$	0.11
	Day x Time	$F_{2,8} = 0.32$	0.64
Observer directed behaviour	Day (training vs. control)	$F_{1,4} = 0.01$	0.92
	Time (pre, post, 2 hours post)	$F_{2,8} = 2.72$	0.14
	Day x Time	$F_{2,8} = 1.00$	0.84

4.7 Conclusions

There were few changes observed in the behaviour of the macaques with no significant differences found between training and control days. The decrease in social grooming noted during post-training observations and subsequent increase during 2 hour post-training observations was found on both training and control days. The decrease in time spent in proximity to another animal (Proximity, touch) during post-training observations appeared more pronounced on control days but, as there was no significant main effect of Day (training vs. control) it cannot be concluded that this was due to training. Overall, the results of Study 2 provided no evidence to suggest that the training sessions were having any effect on the behaviour of the study animals.

4.8 DISCUSSION

The two studies reported in this chapter produced very different results. The results of Study 1 suggested that the training sessions were having a detrimental effect on the social behaviour of the study animals. While an increase in time spent locomoting could be viewed as a positive change in a group of relatively inactive animals, the accompanying decrease in time spent in proximity and social grooming and the increase in self-scratching and threats suggests otherwise. However, the changes observed were relatively mild and the increase in threats was accompanied by an increase in affiliative behaviours. High levels of low-level agonistic interactions coupled with frequent reconciliatory behaviours are commonly observed in this species (de Waal, 1989).

The differences observed during Study 1 were not replicated when observations were repeated during Study 2. Here, the only two changes in behaviour recorded, were due to the time of day when the observations were conducted rather than the occurrence of training sessions. This could suggest that the detrimental effects of training observed during the first training phase had been overcome. This explanation is supported by the behaviour of the monkeys observed during the actual sessions. As reported in Chapter 3, during Study 1, the monkeys became increasingly uncooperative until training was abandoned after eight sessions. Although training data on group behaviour was only collected during the first two weeks of the second phase of training, it should be noted that the monkeys continued to co-operate for a subsequent six weeks, four of which required that blood be collected on a daily basis. As reported in Chapter 3, the training techniques used during Study 2 had improved considerably when compared to those employed previously. It is possible that the disruption to the macaques' social behaviour was due to agitation caused by the poor

methods used during the first training period. When training techniques improved the monkeys were relatively unconcerned and this was reflected in their behaviour as recorded during Study 2.

However, there is the possibility that the results reported had little to do with training. As shown above, disruption to the laboratory's regular feeding schedules proved a major confounding variable. This was the main reason that the initial observations were repeated when training resumed and the improvement in training technique was unforeseen. As reported previously (Chapter 2), the results of a study showing the negative consequences of feeding delays (Waitt *et al.*, 2002) had led to changes in the husbandry routines. The practice of providing a piece of fruit in the morning ceased with the main feed delivered around 1300h rather than when the staff returned to work after 1400h. As a result, the feeding schedule became much more regular, with no subsequent differences between training and control days. It was curious that delays or failure to deliver the morning fruit had such a noticeable effect as the monkeys never appeared particularly hungry. There was always food available and the morning fruit was often left untouched or only partially eaten.

Another change that occurred in the interval between studies was the arrival of a new staff member and the installation of a vacuum system to clean the gang rooms. The result was that cleaning routines were completed faster than had occurred previously. The effects of the changes in husbandry routines were noticeable throughout the colony. Following a period of activity while routine husbandry routines were being carried out, the monkeys tended to settle down to a period of grooming or resting while huddled together once morning cleaning had been completed. A period of increased activity was noticeable around the time when feeding occurred with the monkeys again resting and grooming in the afternoon.

These distinct patterns of behaviour had not been as noticeable during Study 1 and the whole colony appeared more settled.

Although data from the two studies was not compared statistically due to the numerous confounding variables, comparisons between graphs suggest some positive changes in social behaviour. In Study 2, the amount of time spent engaged in social grooming and huddling appears to have increased. As grooming is an important social activity reported to occupy a high percentage of activity budgets in this species (Bertrand, 1969; de Waal, 1989; Goosen, 1974a; Rhine, 1973), this can be viewed as a positive change. A final category that appears noticeably different between studies is the reduced frequency of self-scratching during Study 2. This again suggests that the monkeys were more relaxed given that this behaviour is a commonly used indicator of stress (Maestripieri *et al.*, 1992).

While these changes in the behaviour of the study animals are likely to be the results of changes in husbandry routines, the absence of any significant differences between training and control days suggests that the training process was doing nothing to undermine such improvements. Factors such as the disruption to feeding routines observed during Study 1 and the improvement to training techniques during Study 2 makes it difficult to draw any firm conclusions. This is always a risk when attempting to observe 'real life' events rather than conduct carefully controlled experiments. However, the results do highlight the need to consider the wider impact of introducing a training regime. If staff time is re-allocated to training then there is a risk of disruption elsewhere. The results presented in this chapter suggest that this factor should be taken into consideration when planning training programmes and that that care should be taken to ensure that disruption to normal husbandry routines is minimised.

Chapter 5

Common Marmosets *(Callithrix jacchus)*: Introduction to the Species and Methods

5.1 INTRODUCTION

5.1.1 The common marmoset as a laboratory animal

"The marmosets are all gentle and playful in disposition...but they are very delicate, and rarely survive long in confinement after the advent of the Northern winter."

Forbes, 1896, p132.

Throughout the 1960's and 70's, a number of research institutions started to look for a new non-rodent species to aid the testing and development of new drugs. The preferred species would be cost-effective in terms of procurement and husbandry and be safe to handle, both in terms of injury to personnel and transmission of disease (Hiddleston, 1978). New World monkeys of the family Callitrichidae (marmosets and tamarins) appeared to be ideal candidates to become "a laboratory animal and not a zoological specimen used in the laboratory" (Hiddleton, 1978, p173), due to their small size, early sexual maturity and ability to produce large numbers of offspring (Arrunda, Yamamoto & Bueno, 1986; Epple, 1970; Hearn, 1983). However, the enormity of the transformation to the 'laboratory animal' desired by Hiddleton (1978) is highlighted by a brief account of the natural history of this species.

Common marmosets are small neotropical primates, naturally distributed throughout northeast Brazil where their natural habitat includes evergreen lowland rainforest, humid semi-deciduous forest, scrub and mangroves. Within high rainforest, they tend to occupy the lower strata between 5 -19m (Stevenson & Rylands, 1988). Diurnal and arboreal (Emmons & Feer, 1990), marmosets are

frugivore-insectivores and feed largely on fruit, gum and animal prey including insects, lizards, snails and frogs (Stevenson & Rylands, 1988). Plant exudates are an important component of the diet and wild common marmosets have been observed spending as much as 30 per cent of their activity time feeding on gums (Stevenson & Rylands, 1988). All marmoset species have specialised dentition, enabling tree-gouging (Hershkowitz, 1977) and an additional adaptation consists of specialised claw-like nails which allow marmosets to cling to vertical trunks while feeding (Garber, 1992). It has been estimated that the home range of a group of marmosets should include at least 50 gum trees in order to provide sufficient quantities of this food source (Scanlon, Chalmers & Monteiro da Cruz, 1989), and these animals have been observed feeding from at least ten different tree species (Stevenson & Rylands, 1988). Home range sizes have been estimated as between 2.5 and 6.5ha. (Scanlon *et al.*, 1989), and 0.72 to 1.63ha. (Hubrecht, 1985). Marmoset groups defend their territories against neighbouring groups and although these confrontations tend to be largely vocal, fights do occur. These instances tend to occur where territories overlapped and groups appear to avoid incursions into the core area of their neighbours' home ranges (Hubrecht, 1885).

The daily activity patterns reported by Stevenson and Rylands (1988) showed a 12-hour activity cycle, beginning around 0500h and ending when the marmosets returned to their sleeping site around 30 minutes before sunset (1700h). It was estimated that these animals spent around 35 per cent of their time moving and foraging, 12 per cent feeding, 10 per cent in social activities such as grooming and 53 per cent resting. Feeding on fruits and gum tended to occur in bouts, particularly during early mornings, whereas animal prey foraging occurred intermittently throughout the day.

The social structure of wild marmosets groups is variable and although groups of up to twenty have been reported (Koening, 1995), the mean group size has been estimated at 8.56 (Hubrecht, 1984). The most common group structure consists of multiple males and females with only the dominant female breeding (Rylands, 1996). Although relatedness in free-living populations is difficult to determine, the results of a number of studies suggest that these are co-operative family groups where elder offspring assist with the rearing of their younger siblings (Ferrari, 1992; Koenig & Rothe, 1991) with the dominant female suppressing ovulation in her daughters (Abbott, 1984). This system of co-operative breeding has been related to the high costs of raising multiple litters with twin births and short inter-birth intervals of between five to eight months being the norm (Ferrari, 1992). Callitrichid species are vulnerable to a wide range of predators (Prescott & Buchanan-Smith, 2002; Terborgh, 1990) and this has been related to the high fecundity of these animals (Ferrari & Digby, 1996). However, although this trait is considered desirable in a laboratory animal, the selection pressure imposed by high levels of predation also select for animals that are adapted to both detect and avoid predators (Prescott & Buchanan-Smith, 2002). In the laboratory environment, this could result in animals that are nervous, highly vigilant and easily stressed by the presence of large, potentially dangerous animals, that is, humans.

Given the complexity of the natural and social environment of these animals, it is perhaps not surprising that early attempts to manage various callitrichid species appeared to confirm Forbes' (1896) belief that these animals did not survive well in captivity, especially when compared to Old World monkeys (Epple, 1970). Some early attempts to establish laboratory-housed colonies were abandoned (Hiddleston, 1978) and attempts to breed these animals in zoos were equally unsuccessful (Hearn,

1983). Hiddleston (1978) reports mortality rates of between 10 and 70 per cent, depending on the condition of imported animals on arrival in the UK. Only 74 of the 156 wild-caught common marmosets sent to the MRC Unit of Reproductive Biology in Edinburgh survived - a mortality rate of 52.6 per cent. Of these, 35.9 per cent died within six weeks of arrival (Lunn & Hearn, 1978).

Despite this high mortality rate, large numbers of these animals continued to be imported. In the UK between 1965 and 1975, licences were granted allowing the importation of 8,560 animals of 11 different callitrichid species, of which 5,877 (69 per cent) were listed as *Callithrix jacchus*. It should be noted that until 1988, this name referred to a number of species now classified separately for example, the buffy tufted-eared marmoset *(Callithrix aurita)*, Geoffroy's tufted-eared marmoset *(C. geoffroyi)* and the black tufted-eared marmoset *(C. penincillata)* (Groves, 1993). As a result, the number of common marmosets (now *Callithrix jacchus)* imported during this period may be over-estimated. In addition, the number of licences granted does not accurately reflect the actual numbers of animals. During this period, demand for wild-caught animals was already outstripping supply and in reality, actual imports were around 50 – 70 per cent of the number of animals for which licences were granted (Burton, 1978). Figures from 1965 – 1975 reflect a steady decline from 1507 licences in 1965 to 163 in 1975. These years also show changes in the declared purpose of importation. In 1965, scientific research accounted for 40 per cent of granted licences, with breeding and exhibition (including zoos) accounting for 0.4 per cent and the pet trade 0.6 per cent. The largest number of licences was granted to dealers (59 per cent). By 1975, scientific research accounted for 94 per cent of licences with zoos granted the remaining 6 per cent. No licences were requested by dealers nor the pet trade. Difficulties with supply, high mortality and the introduction

of a compulsory six month quarantine period appeared to have made dealing in marmosets uneconomical (Burton, 1978).

Although the importation of wild-caught animals declined during the above period, demand for marmosets continued to grow (Bleby, 1978). In 1976, a Laboratory Animals Centre report stated that although 90 per cent of laboratory-housed marmosets at that time had been obtained from wild populations, problems with conservation, disease and high mortality meant that the establishment of captive breeding colonies was essential (Bleby, 1978). The decline of wild populations finally led to an embargo on exportation from source countries (Ogden, Wolfe & Deinhardt, 1978) and all laboratory-housed common marmosets in the UK are now captive bred (Boyd Group, 2002).

The challenge then presented to institutions housing these animals was not simply keeping them alive, but to establish successful breeding colonies. Throughout this period, a number of papers were published outlining a variety of attempts to solve the problems of managing a species about which little was known. A review of such literature reflects not only how knowledge about the physiology and behaviour of these animals has grown, but also how attitudes towards what constitutes good welfare and management have changed.

When examining early work in this area, it should be noted that the changes in the classification system reported previously means that some studies apparently referring to common marmosets may in fact refer to another species. In the early sixties, the term "marmoset" was used, not only for species of the genus *Callithrix* but also for some tamarin species (genus *Saguinus*). Stellar (1960) provides no Latin names so it cannot be concluded that the animals described below really are common marmosets. The animals studied in Hampton, Hampton and Landwehr (1966)

"Observations on a successful breeding colony of the marmoset," referred to as "*Oedipomidas oedipus*", would now be identified as cotton-top tamarins *(Saguinus oedipus).* However, both marmosets and tamarins belong to the family Callitrichidae and are similar in size, social behaviour and husbandry requirements (European Commission, 2002; NRC/ILAR, 1998; Poole, Hubrecht & Kirkwood, 1991). As the purpose of the review was to examine how management practices have changed it would seem reasonable to assume that data from any callitrichid species would have some relevance.

In 1953, the Institute of Neurological Sciences at the University of Pennsylvania began an attempt to establish a colony of marmosets housed under laboratory conditions. Although early results showed them to be "delicate animals", the author believed that successful maintenance could be achieved (Stellar, 1960). The most critical factor appeared to be adaptation from the natural diet (fruit, gum and animal prey, Rylands, 1993) to a laboratory diet. This consisted of:

"40 per cent Beechnut mixed cereal, 40 per cent whole powdered milk and 20 per cent wheat germ by weight to which is added an equal weight of water containing 2.0cc of Poly-Vi-Sol for each 100cc. Made up as a dough, this prepared diet stays fresh for at least 24 hours." (Stellar, 1960, p2)

Evidence to support the success of this diet is the observation that a marmoset will consume its daily ration of between 30 and 50gm within 15 minutes to one hour. This was considered preferable to providing a variety of natural foods as this resulted in the marmosets feeding throughout the day. An additional "benefit" was the rapid weight gain shown by the marmosets. A number of later studies also found that captive common marmosets became heavier than their wild counterparts and this was

in turn related to breeding problems as litter size and infant weight also increased (Epple, 1970).

Much of the literature reports various recipes for a standardised laboratory diet and by 1966 one paper alone was able to refer to 23 different suggestions for marmoset diets (Hampton *et al.*, 1966). These were largely concerned with supplying the correct balance of protein, carbohydrate, vitamins and minerals. While a balanced diet is important, and valuable insights such as need for relatively high levels of vitamin D3 were made (Hampton *et al.*, 1966; Hiddleston, 1976; Stellar, 1960), the importance of feeding behaviour and food as a source of enrichment was largely ignored. A single food source does little to meet what is believed to be an innate drive to forage and process food (Reinhardt & Roberts, 1997). While some laboratories persist in the belief that a prepared diet is sufficient, primates are adapted to a varied diet and a single food may not be eaten in sufficient quantities (NRC/ILAR, 1998). The benefits of a varied and interesting diet were recognised by Epple (1970) and current guidelines recommend that marmosets be fed on a variety of fruits and sources of protein, supplemented with additional vitamins as necessary (Home Office, 1999; NRC/ILAR, 1998; Poole *et al.*, 1999).

In addition to recommendations regarding feeding, Stellar (1960) also reports that marmosets can be single-housed in cages measuring 18 by 18 by 36 inches high. Although no problems are reported and the single-housed marmoset is described as "an active, alert animal" (p3), the additional observations of how "the marmoset... in occasional bursts of activity, leaps from the floor of its cage to the shelf to the perch and back" (p4) could possibly be interpreted as a stereotyped behaviour. Broom defines a stereotypy as "a repeated, relatively invariate sequence of movements which has no obvious function" (1993, p178). These repetitive behaviours have been

widely used as an indicator of welfare problems (Broom & Johnson, 1993) and are frequently observed in single-housed social animals (Broom & Johnson, 1993; Mason, 1991). The detrimental effects of an impoverished environment were documented by Mitchell as early as 1970 and were vividly illustrated by Schoenfeld (1989) who recorded the behaviour of a family group of common marmosets as they were moved from an enriched enclosure to progressively smaller and more barren cages. Over this period, behaviours such as play, proximity, follow, groom and locomote all declined. Kerl and Rothe (1996) reported reduced inactivity and increased locomotion, exploratory behaviour and grooming, along with lowered mean night heart rate with increasing cage size. However, improving the complexity of the environment proved as important as providing a larger cage.

Despite its optimistic tone, Stellar's (1960) article contains little factual information that allows an accurate assessment of the welfare of these animals to be made. Although it is reported that ten marmosets had been kept for two years with "nothing more than minor illnesses" (p3), the summary refers to the author's experience with at least 30 marmosets yet the fate of the remaining 20 animals is never mentioned. Subsequent studies did recognise the importance of social housing (Epple, 1970; Rothe & Koenig, 1991; Stevenson & Poole, 1982) and it is now recommended that marmosets should be maintained in stable male/female pairs or family groups (European Commission, 2002; Evans & Poole, 1984; Home Office, 1999; Hubrecht, 1984; NRC/ILAR, 1998; Poole *et al.*, 1999; Woodcock, 1982).

Early data regarding breeding success also suggest that a great deal needed to be learned. Epple (1970) reports that of 32 infants studied, 10 were stillborn while of the 22 live birth, only 13 (40.6 per cent) survived beyond twelve weeks of age. Other colonies did have more success. Lunn and Hearn (1978) report a survival rate of 60.4

per cent of 275 births, while Hiddleston (1978) found that of 2,351 births, 1,640 (69.7 per cent) survived at least until weaned between 14 and 16 weeks. Huntington Life Sciences have reported survival rates of up to 92 per cent when supplementary feeding was provided for litters containing more than two infants (Hazlewood, 2002).

Improved diet through the addition of extra vitamins and fresh fruit has played a part in increasing breeding success (Epple, 1970; Hampton *et al.*, 1966; Hiddleston, 1978). As noted above, improved nutrition also led to an increase in litter size. The percentage of triplet births in one laboratory rose from 11.5 per cent in 1974 to 43.3 per cent three years later although the weakest triplet normally died within days (Hearn, 1983). Hiddleston (1978) reported an increase in the percentage of triplet births from 17.1 to 41.1 per cent over a five year period. Although once again the weakest infant usually died and hand-rearing did not prove cost-effective, the possibility of selective breeding for successful rearing of triplets was considered. This does not appear to have been successful as, although the percentage of triplet births remains high (for example, triplets account for 52 per cent of litters born at Huntington Life Sciences), survival of all three infants largely depends on human intervention (Hazlewood, 2002; Poole *et al.*, 1999).

Despite the contribution made by improved nutrition, a greater understanding of the social behaviour of callitrichid species has also proved important (Hearn, 1983). At the ICI facility at Alderley Park, the reproductive success of 60 animals housed in two groups, each comprising of 15 males and 15 females was recorded. After 12 months no females had bred, yet when the groups were split into individual pairs due to fighting, 80 per cent of females became pregnant within six weeks (Hiddleston, 1978). The same laboratory weaned all infants between 14 and 16 weeks and subsequently housed them in groups of up to 60 individuals. Although the author

reports that this policy presented no subsequent problems when these animals in turn bred, the same paper lists poor parental care as the second most common cause of infant death after surplus triplets. In a comparative study of monogamous, polygynous and polyandrous groups, fewer than 50 per cent of infant born to non-monogamous groups were successfully reared (Rothe & Koenig, 1991).

Another possible source of difficulties that was identified was human activity. Hampton *et al.* (1966) found a correlational relationship between stressful events such as moving to a new cage, recapture or handling and abortion. During a unspecified period of time when building work required the use of power tools, Hiddleston (1978) reported 87 stillbirths in a colony of 320 pairs. Similar results were also reported by Johnson, Kamilaris, Carter, Gold and Chrousos (1991) when the breeding records of 24 common marmoset females revealed that 64 per cent of pregnancies occurring during a ten month period of construction work ended with spontaneous abortion.

The observation that marmosets are highly excitable and dislike handling has been made on numerous occasions (Epple, 1970; Hampton *et al.*, 1966; NRC/ILAR, 1998; Poole *et al.*, 1999; Stellar, 1960). The solution to this problem has largely consisted of recommendations that handling should be avoided as much as possible and that the nestbox should be used as the principle means of capture. In 1966, Hampton *et al.* stated that, "The use of the nestbox ... eliminates many occasions for restraint. The gloved hand or a net suffices at other times." (p269). Although it is now advised that nets should be used with caution due to the risk of injury, the practice of confinement in the nestbox is still recommended (NRC/ILAR, 1998; Poole *et al.*, 1999). However, Poole (1998) reports that a secure place to hide or rest is one of the fundamental psychological needs of mammals. As marmosets both sleep in the

nestbox and retreat there when threatened, there is a potential welfare problem in using nestboxes as a means of capture.

It seems strange that while considerable improvements in feeding, housing and other husbandry areas have been made, the way in which marmosets are handled has remained unchanged in nearly forty years. While there have been numerous investigations to improve caregiver-animal relationships and develop improved handling techniques with macaque species (Heath, 1989; Reinhardt, 1997), the marmosets have largely been ignored. One of the main aims of this study was to address this issue.

5.1.2 Choice of common marmosets as study animals

Common marmosets were chosen as a study species in addition to stump-tailed macaques for a number of reasons. Firstly, both macaques and marmosets are commonly found in UK laboratories (Boyd Group, 2000). While stump-tailed macaques could be regarded as representative of the Old World (Catarrhine) monkeys, and common marmosets representative of the New World (Platyrrhine) primates (Groves, 2001), this factor was less important than the contrast provided, not only in terms of size and temperament, but also in terms of the way in which they are regarded and handled in a laboratory environment.

With regards to size and behavioural characteristics, stump-tailed macaques are relatively large (6 - 16kg; Bertrand, 1969), whereas marmosets, weighing between 300 and 600g are among the smallest of the simian primates (Poole *et al.*, 1999). Stump-tailed macaques are powerful, prone to contagious aggression towards humans when provoked, and potentially very dangerous (Bertrand, 1969; de Waal, 1990).

Marmosets are nervous animals and easily frightened by the presence of humans, to whom they pose no threat (NRC/ILAR, 1998; Poole *et al.*, 1999).

With regards to training, the pioneering work of researchers such as Viktor Reinhardt had raised awareness of the possibilities for improved handling techniques for macaques that training provided (Chapters 1 & 3). As shown above, very few attempts to train marmosets had been made. This paucity of information was another reason for investigating the application of PRT training techniques in this species, given that they are housed in laboratories in very large numbers (European Commission, 2002).

This difference between the training of marmosets and macaque species throughout the literature was reflected at the MRC Unit. As training was already carried out with macaques, this provided the opportunity to assess the application of training techniques in a 'real life' situation with a group of animals where a close relationship with humans already existed (Chapter 3). By contrast none of the marmosets had ever been trained and contact with humans was limited to that necessitated by routine husbandry procedures. With regards to a wider application of the research findings, the situation with the marmosets with regards to animal/human relationships was similar to that in zoos promoting a 'hands off' management policy. The marmosets provided the opportunity to introduce a programme of PRT with a group of animals with no pre-existing close relations with humans and monitor the results. The difference in research questions and methods used with the two species reflects the differences in terms of species, husbandry practice and animal/human relationships that existed before the study began. On a practical note, the presence of both species within the same laboratory, one that was willing to have the behaviour of both the animals and the staff observed, was also a consideration.

5.2 MRC Common Marmoset Colony

5.2.1 Housing

The MRC Reproductive Sciences Unit houses a large colony of approximately 300 common marmosets. These are housed in six identical colony rooms measuring 2.7m x 3m x 5m, maintained at a temperature of 22 – 24°C and a relative humidity of 50% (Plate 6). Rooms are maintained on a twelve-hour light/dark cycle with some natural daylight via large windows in the top half of each room door. These windows also allowed some of the marmosets visual access to the activity of laboratory staff (Plate 7). Each room contained eight large housing units, four along each of two opposing walls. These units could be used entire or be subdivided into two or four separate cages, depending on the size of group being housed (Plate 8). All marmosets that served as study animals were housed in pairs, each occupying a quarter of a housing unit forming a cage measuring 55cm wide x 95cm high x 110cm deep (Plate 9). Cages had wood shavings as a floor substrate and were furnished with a nestbox, shelves and two wooden logs. Some cages contained additional enrichment devices.

5.2.2 Husbandry

Most routine husbandry procedures took place in the morning with a final check on the animals conducted between 1600 and 1700h. Cleaning routines consisted of the daily removal of uneaten food and used paper dishes. On weekdays floors were also cleaned. Wood shaving were replaced weekly and these procedures were carried out between 0800 and 1030h. Once a month, marmosets were transferred to new cages as whole units were removed and washed in a commercial cage washing machine. This procedure allowed various enrichment devices to be

Plate 6 Marmoset colony room

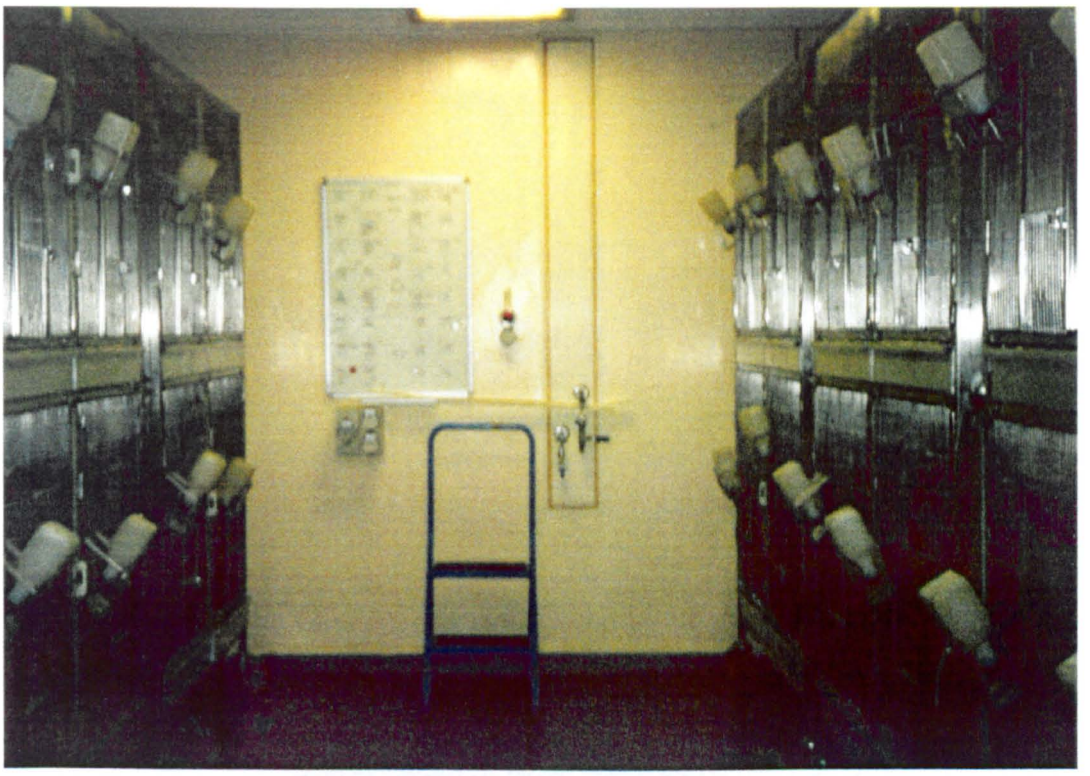


Plate 7 Marmoset colony room viewed from access corridor

Plate 8 Common marmoset cage unit

Plate 9 Cage section used for housing common marmoset pairs

rotated around the colony. However, it did create additional disruption and the prolonging of husbandry routines therefore no data were collected on the same day as cage washing.

5.2.3 Feeding

Food was provided once a day at around 1230h. The marmosets were fed with commercially manufactured primate pellets (Mazuri Primate Diet, E; Witham, Essex, England) and a variety of fresh fruit daily (banana, apple, pear, orange, tomato and grapes). Three times weekly this was supplemented by "porridge" containing yoghurt, baby rice, protein powder, Abidec multivitamin drops and vitamin D3. On remaining days marmosets were given a mixture of dried fruit and nuts. Of the food items available, the primate pellets were the least preferred and the most likely to be removed uneaten during morning cage cleaning. To encourage their consumption, the proportion of fresh fruit in the diet was reduced over weekends. Water was available *ad libitum* from a bottle mounted on the front mesh of the cage.

5.2.4 Staff / marmoset relations

As stated previously, macaques were all named and regarded as individual "personalities" by the staff. In general, staff attitudes towards the marmosets were rather different. While talk about individual macaques was common, staff rarely discussed the marmosets in this fashion. Marmosets were numbered rather than named and were not seen as distinct individuals. When new animals arrived that had been named, the names were not used and quickly forgotten.

In common with many institutions housing large numbers of animals (Serpell, 1999) there were few animals with names, usually those who had some physical or

behavioural characteristic that made them distinctive. This could be seen in the chosen names that tended to reflect why a particular animal had been distinctive, for example "Supermum" was the colony's most prolific breeder while "Stormin' Normin" was particularly aggressive. By contrast, macaques born and named within the colony were always given human names, for example, Hamish, Charlie, Sylvie and Joe.

The Unit's programme of environmental enrichment included the marmosets but again, there were differences between species. Much macaque enrichment involved primate/human interactions for example, encouraging tool use. When puzzle feeders or new toys were provided, these were handed to the animals and staff would stay to watch their reactions. Most marmoset enrichment involved alterations to the animals' cages and this was usually carried out on units that were currently out of use, preventing staff interactions with the animals.

Possible reasons for this difference in attitude include the relatively large number of marmosets kept and their lack of distinguishing features. While stump-tailed macaques are among the easiest of primates to tell apart (de Waal, 1989), marmosets are very similar in appearance, necessitating the use of identity tags. Another important difference was that unlike macaques, marmosets were used in terminal studies so avoidance of naming and personification may reflect attempts to maintain a psychological distance from these animals (Serpell, 1986).

5.2.5 Handling

None of the study animals were being concurrently used in any MRC research and therefore were only handled when required by routine husbandry procedures. When being moved between cages, marmosets were first captured by confining them

in the nestbox which was then removed, placed in the new cage and the animals released. This method avoided handling and allowed an item marked with the animals' own odour to be retained. It was also fairly easy in that the marmosets tended to enter the nestbox as soon as the front of the cage was opened. However, as already mentioned, Poole (1998) reports that a secure place to hide or rest is one of the fundamental psychological needs of mammals and there is a potential welfare problem in using nestboxes as a means of capture.

Routine procedures that required actual handling included weighing, identification tag cleaning and for females, uterine palpation to detect pregnancy. For these procedures, the marmosets were first removed from the homecage in the nestbox and then captured by hand, the technician wearing thick protective gloves. There were two techniques. One technician would insert his hand into the nestbox, detecting and capturing an animal by touch. All other staff slid the nestbox door open with their gloved hand cupped around the entrance. As the marmoset emerged they would close their hand around his/her waist. Differences in the level of struggling and vocalising observed suggested that this second method was the less stressful method of capture. Although the use of nets can result in injury (NRC/ILAR, 1998), nets were used to recapture any escapees.

Despite training macaques to co-operate with routine procedures (Chapter 3) the possibility of training the marmosets in this laboratory had never been considered. The techniques used for capture and handling marmosets were no different to that described in some of the earliest studies of this species as laboratory animals. In addition, the basic "tool kit" for managing these animals (i.e. a nestbox, a net and a stout pair of gloves), was identical to that described by Hampton *et al.* in 1966 (Plate 10).

Plate 10 Standard marmoset handling kit

5.3 STUDY ANIMALS

All the marmosets used in the study were pair-housed, with a total of 66 individuals. Thirty-six marmosets were trained; either target trained to allow in-homecage weighing, trained to provide urine samples or both. Eighteen animals received increased levels of positive contact with humans and the remaining 12 marmosets served as control animals when stress responses to standard weighing procedures were examined. Details of the trained animals are given in Table 5.1 and details of contact and control animals are given in Table 5.2.

Table 5.1 Details of trained common marmosets including name, sex, date of birth and behaviours taught.

Name	I.D. number	Sex	Date of Birth	Target trained	Urine trained	Urine trained (revised method)
Adam	784BK	M	10/10/97	*	-	-
Allie	850R	F	10/10/97	*	-	-
Billy	432BK	M	10/11/86	*	-	-
Bella	890R	F	20/06/98	*	-	-
Cecil	843BK	M	10/09/98	*	*	-
Coco	952R	F	15/02/99	*	*	-
Derby	702BK	M	12/10/94	*	*	-
Doris	327R	F	01/08/84	*	x	-
Eddie	657BK	M	03/07/93	*	-	-
Eva	676R	F	25/03/93	*	-	-
Freddie	832BK	M	18/07/98	*	*	-
Foxy	895R	F	18/07/98	*	*	-
Georgie	734BK	M	03/03/96	*	*	-
Gracie	795R	F	21/10/96	*	x	-
Harry	871BK	M	18/05/99	*	-	-
Helen	965R	F	18/05/99	*	-	-
Iggy	833BK	M	19/07/98	*	*	-
Iris	829R	F	22/06/97	*	*	-
Jambo	683BK	M	10/05/94	*	*	-
Jilly	975R	F	29/07/99	*	*	-
Kipper	878BK	M	09/07/99	*	*	-
Keltie	946R	F	01/02/99	*	*	-
Leo	813BK	M	11/05/98	*	*	-
Lala	902R	F	22/07/98	*	*	-

Table 5.1 (Cont.)

Alba	898BK	M	28/02/00	-	-	*
Abby	1Y	F	28/02/00	-	-	*
Brian	905BK	M	04/04/00	-	-	*
Bonnie	998R	F	25/02/00	-	-	*
Charlie	935BK	M	06/11/00	-	-	*
CC	24Y	F	28/06/00	-	-	*
Dopey	904BK	M	04/04/00	-	-	*
Daisy	13Y	F	15/04/00	-	-	*
Eric	803BK	M	15/03/98	-	-	*
Elsie	9W	F	16/08/99	-	-	*
Frankie	846BK	M	09/11/98	-	-	*
Fifi	26Y	F	29/06/00	-	-	*

* denotes behaviours trained
x denotes failed attempt to train this behaviour
- denotes no attempt made to train this behaviour

Table 5.2 Details of non-trained common marmosets including sex, date of birth and group (increased positive contact with humans or control).

I.D. number	Sex	Date of Birth	Group
813BK	M	20/05/98	Contact
929R	F	07/11/98	Contact
729BK	M	25/02/96	Contact
352R	F	05/06/85	Contact
785BK	M	10/10/97	Contact
823BK	M	18/06/98	Contact
658BK	M	03/07/93	Contact
814BK	M	11/05/98	Contact
809BK	M	19/04/98	Contact
825BK	M	26/06/98	Contact
887R	F	18/06/98	Contact
886R	F	18/06/98	Contact
897R	F	20/07/98	Contact
898R	F	20/07/98	Contact
955R	F	06/03/99	Contact
983R	F	26/09/99	Contact
733R	F	13/02/95	Contact
764R	F	01/07/96	Contact

Table 5.2 (Cont.)

864BK	M	22/04/99	Control
678R	F	29/03/93	Control
878BK	M	24/06/99	Control
971R	F	24/06/99	Control
788BK	M	24/11/97	Control
902R	F	22/07/98	Control
870BK	M	04/05/99	Control
685R	F	15/06/93	Control
804BK	M	15/03/98	Control
909R	F	11/08/98	Control
802BK	M	04/03/98	Control
940R	F	23/12/98	Control

5.4 OBSERVATIONAL DATA

5.4.1 Observational protocol

The presence of a new caregiver has been shown to adversely affect the behaviour of macaques (Heath, 1989) and it would seem reasonable to assume that this holds true for marmosets. However, I had regularly participated in routine procedures such as cleaning, feeding and identity tag cleaning for a period of eight months prior to the start of the study. In addition, I always wore clothing identical to that worn by laboratory staff so it seems reasonable to assume that at the start of the study my relationship with the marmosets was comparable to that of regular technicians.

All observational data collection took place when the marmosets were in their homecages. During collection, I sat on a set of steps commonly left within the colony room and with which the marmosets were familiar. The steps were placed directly in front of the homecage but as far back as possible, sitting just in front of the opposite cage. This position allowed a good view of all four pairs in a single housing unit and the marmosets were only out of view when in the nestbox. When matched trained and contact pairs were observed consecutively (Chapter 7), this placement allowed both

sets of observation without changing position. Data were recorded onto checksheets with an electronic 'beeper' marking each sample interval. As marmosets are easily startled an earpiece was used. Although the marmosets never habituated to the presence of humans (see below), I always sat quietly for five minutes before data collection began to allow them to settle. Although attempts to use a hide had been made in the past, the marmosets appeared to find this alarming and always retreated to their nestbox when it was in use.

5.4.2 Selection, categories and definitions of observed behaviours

Selection

The difficulties involved in the selection of appropriate behaviours to serve as welfare indicators has already been discussed (Chapter 2). As with the macaques, the challenge was choosing behaviours appropriate to the species, the situation and the research questions. The main aim behind the collection of observational data was to assess the effects of both training and increased levels of positive contact with humans of the behaviour of the marmosets out-with training sessions (Chapter 7). Behaviours were chosen in two ways. Primarily, behaviours were selected through reference to existing literature specifically relating to callitrichid species. This was the main source of behaviours previously shown to relate to arousal in these animals. Secondly, some behaviours were chosen following preliminary *ad libitum* observations of the colony. This identified behaviours that may have proved important in the specific setting where observations were conducted. For example, the observer effect (Martin & Bateson, 1993) was studied rather than avoided. Interpretation of these behaviours was reached through reference to existing literature.

Behaviours

Although it has been reported that in some situations, the presence of a familiar observer will not influence the behaviour of common marmosets (Stevenson & Poole, 1976), preliminary observations showed that this was clearly not the case in this laboratory. The marmosets showed intense interest whenever a human entered their room. The monkeys would commonly cling to a position at the highest point of their cage door and remain watching until the human left (see Plate 11). This behaviour was so pronounced that even fighting pairs could be difficult to identify as when staff, alerted by an sharp increase in vocalisations entered to investigate, hostilities would cease as the fighting pair took up this position. When an individual pair was approached, the more nervous monkeys (as indicated by willingness to take food from the observer's hand) would retreat inside the cage but continue to watch.

Watching humans can be interpreted in a number of ways. It could be that the marmosets simply find human activity interesting (Ely, Freer, Windle & Ridley, 1998) or they may feel the need for vigilance as they feel threatened when humans are present (Hampton *et al.*, 1966). While it can be difficult to determine what motivates this behaviour, the fact that the more nervous animals retreat to within the cage when approached suggests that vigilance play a greater part than interest when watching from this position. As the present study was interested in the behaviour of the marmosets when a human was present, these two behaviours, watch observer from cage door (wire watch), and watch observer from inside the cage (cage watch) were recorded.

Stevenson and Poole (1976) identified two distinct patterns of locomotory behaviour seen in marmosets. 'Normal' locomotion includes walking, running, climbing and jumping and is characterised by a relaxed gait and extended tail.

Plate 11 Common marmoset watching the observer

The second pattern, labelled 'excited locomotion' in the present study includes running with an exaggerated gait and rapid 'to and fro' movements. The tail is extended or arched. In the MRC laboratory, this behaviour was clearly distinguishable from normal locomotion and the two forms of locomotion were recorded separately as the latter was a possible measure of arousal.

Affiliative behaviours such as close association and grooming have been shown to decrease in a number of species following brief threatening events (Chamove & Moodie, 1990) although other studies have shown an increase in affiliative behaviours (Moodie & Chamove, 1990). Proximity (both in contact with and less than 10cm from cage mate) was recorded. However, when watching the observer, close proximity may be a result of a shared desire for the best viewing position rather than affiliative behaviour so proximity was only recorded if the marmoset was not simultaneously watching the observer. Other affiliative behaviours included social grooming, touching, and nuzzling.

The final affiliative behaviour was food sharing. This behaviour, where one animal acquires food from another, can be interpreted in a number of ways. Sutcliffe and Poole (1984) interpret what they regard as food stealing as an agonistic behaviour. However, these authors report that food is stolen by infants as young as 2-5 weeks and in 43 per cent of cases, young juveniles stole food from adults. An agonistic interpretation would seem more plausible if dominant adults were stealing from juveniles. Some authors insist that true 'sharing' should involve the active giving of food, a behaviour rarely seen in non-human primates, the more commonly observed behaviour being interpreted as "tolerated scrounging" (de Waal, 1996). Arguably, the key feature is that such behaviour is tolerated therefore a number of authors have interpreted such incidents as affiliative (de Waal, 1996).

During preliminary observations, two distinct forms of behaviour were noted. In the first, the 'scrounger' would quietly approach the 'donor' until their bodies were touching. He/she would then reach towards the food with either a hand or the mouth. The striking feature was the absence of threatening behaviours and it was not uncommon for two animals to eat from the same piece of fruit simultaneously. This behaviour was regarded as food sharing and was classed as affiliative.

The second form, labelled food stealing, involved the rapid snatching of a food item, always by the dominant animal, and was usually accompanied by some form of threat or actual physical aggression. Food stealing was classed as an aggressive behaviour. Other behaviours that were recorded as 'aggressive' included cuffing, biting, and scratching cage-mates. Angry 'chatter' calls were also recorded as aggressive behaviour (Epple, 1968).

Time spent in the nestbox was recorded, as it is possible that the marmosets would hide in there if frightened. The marmosets did tend to retreat to the nestbox as soon as the technicians opened their cage door. Conversely, a desire to maintain close observation of humans may result in a reluctance to enter the nestbox.

Allogrooming was rarely observed and this may indicate that the marmosets were not sufficiently relaxed to perform this behaviour when humans were present. However, excessive self-grooming (Maestripieri *et al.*, 1992) may indicate tension therefore these behaviours (groom other and groom self) were recorded separately.

As mentioned above, many behaviours were suspended as soon as a human entered the room where the marmosets were housed. Although behaviours such as foraging, feeding and interacting with enrichment devices were occasionally observed inside the room, they were observed more frequently through the glass panels on the room door (Plate 7) and were often terminated as soon as the door was opened. As

these behaviours were clearly affected by human activity they were included in the 'other' category.

Additional brief behaviours shown to indicate arousal in callitrichid species were also recorded. Scratching has been shown to increase as a response to stress (Moodie & Chamove, 1990; Maestripieri *et al.*, 1992), as has scent marking (Epple, 1970; Sutcliffe & Poole, 1978). Epple (1970) found genital presenting increased when both male and female marmosets were aroused. During preliminary observations, the marmosets frequently presented to the observer and it was this behaviour, genital present to observer that was recorded. Another behaviour noted during preliminary observations and included in the present study was a sudden startled movement to the upper section of the cage or nestbox. This behaviour, labelled "vertical flight" usually occurred in response to the sudden appearance of a human or an unexpected noise.

Vocalisations are also widely used as a measure of arousal. However, marmosets use a wide variety of calls for different reasons and not all are easy for a human observer to locate (Epple, 1968). Open-mouth 'phee' calls are both easy to distinguish and have been shown to increase with distress (Epple, 1968). 'Tsk' calls are again distinctive and used when marmosets are alarmed (Epple, 1968). Instances of these calls were recorded. The categories of behaviour used during observations and their definitions are given in Table 5.3.

Behaviour states, behaviour of relatively long duration, were recorded using instantaneous scan sampling (Martin & Bateson, 1993). Preliminary observations showed that the marmosets alternated between the different behavioural categories relatively quickly. For example, time spent watching the observer was interrupted by brief periods of activity. The faster movement of the marmosets and the fact that

fewer animals were to be observed simultaneously mean that a shorter sample interval than that used for the macaques (30 seconds) was appropriate. A period of 15 seconds was chosen. For analysis, the percentage of the total activity budget spent engaged in each behaviour was calculated. All occurrences of behavioural events, i.e. behaviours of relatively short duration, were also recorded and the mean frequency per 10 minute observation period calculated.

Table 6.3 Behavioural categories and definitions used for common marmosets. Descriptions of scent marking adapted from Stevenson and Poole (1976); vocalisations from Epple (1968).

Behavioural category	Definition
♦**Watch observer (wire)**	Located in a position either clinging to the wire cage-front or sitting on the shelf attached to the upper section of the cage door. Eyes looking towards the observer.
♦**Watch observer (cage)**	Positioned at any point in the cage other than the door. Eyes looking towards the observer.
♦**Locomotion (normal)**	Walking, running or climbing. Relaxed gait and tail extended.
♦**Locomotion (excited)**	Rapid movement returning to starting position without pause. Tail stiffly extended or arched.
♦**Proximity (touch)**	Sitting or lying while torso in contact with cage-mate. Not watching the observer.
♦**Proximity < 10cm**	Sitting or lying with torso within 10cm of cage-mate. Not watching the observer.
♦**Nestbox**	Not visible in cage therefore must be in the nestbox.
♦**Groom self**	Moving fingers through hair or picking at skin. Watching movement of hand.
♦**Groom other**	Grooming or being groomed by cage-mate.
♦**Other**	All other behaviours including foraging, interacting with objects in cage and located away from cage-mate but not watching the observer.

Table 5.3 (cont.)

* **Affiliate**	Brief, affiliative behaviours such as touching, nuzzling and food-sharing.
* **Aggression**	Cuffing, biting, scratching or food stealing. Includes angry 'twitter' vocalisations when directed at cage-mate.
* **Scratch**	Rapid scratching of any part of the body. Distinguished from self-grooming by brief duration and the marmoset not watching movement of hand.
* **Scent mark**	Animal sits and rubs anogenital or sternal area on branch or other area of the cage. May be preceded by gnawing marked area.
* **Genital present**	Orienting body away from observer while raising tail and showing genitals. Turns head to watch observer over the shoulder.
* **Vertical Flight**	Sudden, startled movement to upper section of cage or nestbox.
* **Vocalise**	Open mouthed 'phee' calls and 'tsk' calls.

♦ denotes behaviours recorded by instantaneous scan sampling, 15 second sample interval.
* denotes all occurrences of behaviour recorded

5.4.3 Training Sessions

In addition to observational data collected out-with training sessions, data were collected throughout the training process. The methods used both to conduct and record the training process are reported in Chapter 6.

Chapter 6

Positive Reinforcement Training – Common marmosets

7.1. INTRODUCTION

7.1.1 Training callitrichid species

One of the most striking features about the literature concerning the training of callitrichids is how little exists. While both the UFAW handbook (Poole, 1999) and NCR/ILAR (1998) guidelines report that marmosets can be trained, the sole reference supplied, Hearn (1983) actually gives very little information as to how this might be accomplished. In this paper, Hearn describes how the marmosets are given a reward after each procedure and the importance of talking to the animals in a calm voice and moving quietly and slowly is emphasised. Marmosets handled regularly in this way were maintained and bred more successfully than those who were handled only when absolutely necessary (Hearn, 1983). However, the claim that the marmosets were actually trained to enter the nestbox requires a little more scrutiny. From the photographs supplied, the caging used is very similar to that currently in used at the MRC unit. Here, the marmosets always enter the nestbox as soon as the cage door is opened. It could be argued that to claim that this behaviour is the result of training is analogous to claiming that sheep are trained to move together and form a tight group whenever a sheepdog comes near. They are not actually trained to do this and the behaviour is simply a species typical response to a perceived threat, the 'selfish herd' effect (Dawkins, 1989). Caged marmosets retreating to the nestbox as a response to the close proximity to a human could be interpreted in the same way. The provision of a food treat whenever the marmosets were handled is valuable due to counter-conditioning as the practice pairs a pleasant stimulus with a potentially aversive event

(Laule & Desmond, 1998). Hearn's recognition of this and the importance of careful, sensitive handling should not be undervalued but the techniques described in his paper cannot be interpreted as PRT.

Pearce, Crofts, Muggleton, Ridout and Scott (1999) trained marmosets to participate in a number of cognitive tests using an apparatus attached to the front of the homecage. An important refinement was that the marmosets were free to enter and leave the testing area at will which removed the distress associated with forced removal and isolation in a testing chamber (Snowdon, 1979). Previous attempts to conduct cognitive testing with marmosets met with limited success as the animals showed little persistence with the task (Snowdon, 1979). Pearce *et al.* (1999) overcame this problem by rewarding responses with a food treat (banana milkshake) rather than a food item from the regular diet. This represents a refinement of the practice of food deprivation reported by Stellar (1960). However, the training technique described by Pearce *et al.* is effectively an adaptation of the operant chamber in that training was accomplished with no active human involvement in training. Moreover, the technique is applicable to a specific research requirement (cognitive testing) and does not really apply to routine husbandry procedures.

Training to provide urine samples has been undertaken. Anzenberger and Gossweiler (1993) developed a technique for training marmosets to provide urine samples without the need for capture or confinement in a metabolism cage which is a commonly used method (Hearn, 1983). This technique exploited a natural tendency to urinate shortly after leaving the nestbox when first awakening. Each member of a family group was trained to enter a different area of a specially designed 'urine collection apparatus' placed between the nestbox and the main cage area. The marmosets remained in the apparatus until urination occurred, after which they were

released. This is a useful method where housing arrangements permit the permanent installation of a device measuring approximately 50cm x 38cm x 60 cm. In this laboratory, marmosets were kept in large rooms measuring between 35^3 and $45m^3$. The application for use with standard laboratory cages (at the MRC unit pair accommodation measures 55cm x 95cm x 110cm) is limited. In addition, the technique only allows collection of first void samples rather than repeated collection throughout the day.

Wied's black tufted-ear marmosets *(Callithrix kuhli)* have also been trained to provide urine although no details of how this was accomplished are given other than that the marmosets were trained to "urinate in return for a desired food item" and that "urine was collected in hand-held aluminium pans" (Smith & French, 1997, p227). The same study also avoided capture by "enticing the animal into a small transport cage ...attached to the home cage" (1996, p226). This appears to be positive reinforcement training but no detailed methodology is provided.

The above brief review covered the literature on the training of laboratory-housed marmosets that was available when the present study began. This paucity of information was also reflected in the zoo-based literature. Although PRT techniques have been employed successfully with a wide variety of animals from marine mammals to ungulates, the absence of reference to callitrichids is particularly striking when the range of trained primate species is considered. These include golden monkeys *(Pygathrix (Rhinopithecus) roxella*: Mellen & Ellis, 1996), drills *(Mandrillus leucophaeus*: Desmond *et al.*, 1987; Mellen & Ellis, 1996; Priest, 1991), red-tailed moustached guenon *(Cercopithecus c. cephus)* and de Brazza guenon *(C. neglectus*: Stringfield & McNary, 1998), colobus monkeys *(Colobus guereza*: Reichard *et al.*, 1993), chimpanzees *(Pan troglodytes*: Bloomsmith, 1992;

Bloomsmith *et al.,* 1992; Desmond & Laule, 1994; Laule *et al.*, 1996; Mellen & Ellis, 1996; Petiniot, 1995), gorillas *(Gorilla g. gorilla*: Baker, 1991; Mellen & Ellis, 1996; Petiniot, 1995; Shellabarger, 1992) and orangutans *(Pongo pygmaeus*: Baker, 1991; Mellen & Ellis, 1996; Petiniot, 1995; Sevenich, 1995; Shellabarger, 1992). The training of golden lion tamarins *(Leontopithecus rosalia)* reported by Box (1991) describes their introduction to a semi-natural enclosure in preparation for release into the wild rather than the application of operant techniques to train a specific behaviour. At the start of training for this study it was not possible to find any zoo-based studies that specifically referred to any marmoset or indeed any New World species.

A report prepared by the Boyd Group examining welfare considerations for both marmosets and macaques asks why macaques are more easily trained (Boyd Group, 2000). Perhaps an equally pertinent question would be, why are institutions more prepared to train macaques? Scant information on how to train marmosets may be one reason. Equally, individuals may be unwilling to train as there is little evidence that this can be done successfully yet the reason there is so little evidence could be because so few people have tried.

Broom (1999) suggests that the first component in any welfare investigation is to recognise that there is a problem. One reason that marmosets have not been widely trained may be that the need for improved methods has not yet been perceived. From a human viewpoint, marmosets are easily handled due to their small size, and a stout pair of gloves is all that is required to protect the handler from bites and scratches. While handling these monkeys poses few problems in terms of human safety, it is widely noted that this can cause considerable distress, not only for the animal itself, but for others housed in the same area (NRC/ILAR, 1998).

Motivation to encourage co-operation is greatly increased when handling animals that are potentially dangerous when handled in any other way (Kiley-Worthington, 1990). This point was demonstrated when the macaques refused to enter the cage room (Chapter 3) and there was nothing that the technicians could do about it. Pryor (1981) suggests that one of the reasons that PRT originated with the training of dolphins was that methods to force compliance could not be applied to an animal that could simply swim away. Paradoxically, one of the potential dangers of training is that loss of inhibition around humans may result in the animals becoming dangerous (European Commission, 2002). However, marmosets are unlikely to pose any serious threat due to their small size. Poole, Hubrecht and Kirkwood (1999) even recommend that the use of heavy gloves should be avoided as marmoset teeth are delicate and easily damaged. It would be ironic if the relative helplessness of these small primates made them particularly suitable candidates for training while simultaneously reducing any incentive to do so.

There is no real reason to assume that marmosets cannot be trained. Presumably the ladies of the French Court described by Hearn (1983) used some form of training, however inadvertent, to persuade their marmosets to remain in the décolletage. Marmosets do respond readily to operant techniques (Stellar, 1960), without the need for food deprivation (Pearce *et al.*, 1999). However, when alternative methods of handling are sought, there are a number of practical reasons why PRT may not be considered. Many laboratory animals are destined for terminal studies thus their time in the laboratory may be limited, decreasing the return on initial time investment. Equally, as fewer blood samples can be taken (Hearn, 1983), the requirement for repeated capture may be less than for macaques. While appropriate in a zoo setting with relatively few callitricids, training is not widely

regarded as practical in a laboratory housing hundreds of animals. This is an important issue as changes intended to promote welfare stand little chance of being widely implemented unless they can be shown to be practical.

6.1.2 Aims

The aim of this area of research was to conduct a broad investigation of the use of PRT as a tool in the management of the common marmosets. The third element in the concept of the "Three Rs" states that experiments should be refined so that any animal suffering is reduced (Russell & Burch, 1959). As stated previously, the use of PRT could represent such a refinement, but only if the techniques are effective. The first question was whether it was actually possible to train these animals using only positive reinforcement techniques. This was addressed by an attempt to teach two different behaviours:

1) Target (to allow in-homecage weighing)

2) Provide urine samples.

The second aim addressed concerned practicality in a laboratory situation. As stated above, a pragmatic approach cannot help but conclude that no management technique is likely to be implemented unless it can be shown to be a practical alternative to current practice. The two most commonly expressed concerns are the time investment required to train and the reliability of trained animals. One of the fundamental principles of PRT is that the animals must choose to co-operate (Laule, 1999). Conversations with various scientists and technicians suggested that the argument that animals will co-operate voluntarily without food or water deprivation is met with considerable scepticism. Kiley-Worthington (1990) suggests that such beliefs are a legacy of the era of "Skinner box" experiments on reinforcement and a

glance through any behaviourist textbook does reveal numerous studies on the behaviour of hungry and thirsty rats (e.g. Lieberman, 1993; Pearce, 1997).

There is no doubt that PRT does require that time be invested in training and my third aim was to provide quantitative data on how much. However, the possibility that such investment can be recouped if data collection from trained animals proves faster than that using conventional methods is often overlooked. To investigate this possibility, the time taken to collect data from trained animals was compared to that taken with the current methods used in the MRC unit.

Reliability of target-trained animals was assessed by recording the number of trained animals who co-operated during the above procedure and reliability of urine training was assessed during a study conducted in collaboration with Lois Bassett and Dr. Tessa Smith, then of Belfast University (Chapter 7). This study required that a large number of urine samples be collected and reliability was assessed by comparing the actual number of samples collected with those required by the experimental protocol.

The procedure used to train the marmosets to provide urine samples was developed to meet the specific conditions that existed at the MRC unit. As the procedure had not been described elsewhere, the final aim of this investigation was to refine the training technique in light of the results of the first attempt. In addition, as all laboratories experience staff turnover, illness etc., co-operating with different people is an important consideration when assessing practicality. The last group of marmosets were tested to see if they would co-operate with someone other than the original trainer.

6.1.3 Study Outline

As this investigation into the use of PRT with common marmosets hoped to address a number of different issues, it was conducted as three studies. These were:

Study 1 – Initial training of a single behaviours (target training to allow in-homecage weighing) to examine time investment and reliability.

Study 2 – Training two behaviours (target and provide urine samples) to examine time investment and reliability in comparison with current laboratory procedure.

Study 3 – Refined method for training to provide urine samples to compare time investment with the earlier method and transfer from the original trainer.

6.2 STUDY 1 – Initial training of a single behaviour (target)

6.2.1 Aims and Methods

As stated above, the aim of the first study was to investigate the possibility of training common marmosets using only PRT techniques and to quantify the time investment required to do so. Although the basic principles of PRT are the same regardless of the species being trained (Chapter 1), it is important to consider the specific characteristics of each species and the environment in which training is carried out (Laule, 1994; Mellon & Ellis, 1996; Sevenich, 1995).

Firstly, marmosets are small nervous animals. Preliminary observations suggested that some appear to find close contact with human more aversive than others. When the front of the cage was approached, some animals retreated to the back of the cage while some remained and tried to reach through the bars to grasp anyone who came close. All marmosets would enter the nestbox if the front of the cage was opened. If animals do not have a pre-existing close relationship with

humans the training process itself can be stressful (Dettmer, Phillips, Rager, Bernstein & Fragaszy, 1996).

Secondly, the marmosets were trained while confined in relatively small, laboratory cages (see Plate 9). This affected the training process in a number of ways. The small space available did not allow the animals to remain at a comfortable distance from the trainer and they certainly had very limited space in which to flee upward, especially when housed in the lower tier. In a large, walk-in enclosure the animals can choose to remain out-with their "flight distance", the distance at which they flee from danger (Kreger, Hutchins & Fascione, 1998). In addition, trainers can hold both targets and rewards at arms length, maximising the distance between their bodies and the animals (see Plates 12 & 13). This is not possible when training cage-housed animals.

Although it was not possible to exclude all nervous animals, a selection procedure was used to ensure that at least one pair member was reasonably bold. It was hoped that the nervous animals would follow their cage-mates' example once they had observed that they came to no harm. Throughout training, I behaved in the least threatening way possible, avoiding eye contact and sitting on the floor when training animals housed in the lower tier. The marmosets may have been more comfortable accepting rewards from the uppermost part of the cage, above eye level (Boyd Group, 2000). However, the design of the cage, with the small access hatch at the foot of the door, meant that the scales for weighing would have to be placed at the bottom of the cage. Attaching the scales to the top section would have meant opening the cage front, which the marmosets find alarming anyway (as shown by their tendency to enter the nestbox whenever this occurred). As the marmosets were going to have to learn to come to the bottom of the cage, rewards were delivered there from

the start. Lower tier branches all sloped downwards towards the front corners of the cage and these provided a convenient 'runway' that allowed the marmosets to move back and forth easily. Initial nervousness also meant that the rewards would have to be sufficiently enticing to overcome any reluctance to approach closely.

The small size of the cages also made it difficult to maintain any reasonable distance between the pairs. The danger here was that it would be very easy for the more dominant animal to steal the reward from the subordinate, especially as marmosets (unlike the macaques) move very quickly. Initial measures to address this problem included excluding any particularly aggressive animal during the selection process. Rewards were chosen that could be delivered quickly and in very tiny pieces that could be eaten rapidly, thus reducing the opportunity for theft. Each animal was only ever rewarded in one particular location, the bottom left hand corner of the cage for males and the right corner for females.

The behaviour 'target' was chosen as it is both simple and versatile (Laule & Desmond, 1998) (see Chapter 1). At the start of the study, no training had been attempted with the marmosets nor were any of the study animals accustomed to being hand-fed and contact with humans was limited to that required by routine husbandry procedures. In many ways the situation with the marmosets at the MRC Unit was analogous to any institution introducing a programme of training for the first time. Study 1 was the first stage in developing, monitoring and refining such a programme.

Plate 12 Common marmosets working above head height in a walk-in enclosure

Plate 13 Common marmosets working below head height – a behaviour that requires considerable confidence

6.2.2 Study animals

Study animals were nine male / female pairs housed in the same colony room (room 6) at the MRC Human Reproductive Sciences Unit, Edinburgh (Plate 6). When data collection began, room 6 contained 30 different groups of marmosets, 26 of which were pairs. Details of housing and husbandry routines are given in Chapter 5. Criteria for selection were that at least one member of a pair would accept food from my hand and that no aggression was shown between cage-mates during hand-feeding. All study animal were then given names, chosen so that the first letter would identify which pair an individual belonged to and sounded phonetically distinct from the cage-mate's name. The ability to discriminate between human speech sounds has been demonstrated in pygmy marmosets *(Callithrix pygmaea*: Snowdon, 1979) and it was assumed that each animal would eventually learn to recognise its own name although this was not essential. The ages of the study animals ranged from 395 to 5715 days (approx. 13 months to 15 years 10 months) and the mean age was 1586.11 days (approx. 4 years, 4 months; ± S.E. 360.48). Not one of the females was past the first trimester of pregnancy, as determined by transabdominal uterine palpation and contraceptive measures were employed with seven pairs. Details of the study animals are given in Table 6.1. Once the study animals were selected, observational data were collected to record their behaviour before any intervention (Chapter 7). As soon as data collection was complete training began.

Table 6.1 Details of study animals including name, sex, age at start of training and relationship to cage-mate.

Pair	Name	Sex	Age	Relationship
A	Adam	M	2yr 8mth	Siblings (twins)
	Allie	F	2yr 8mth	
B	Billy	M	13yr 7mth	None
	Bella	F	2yr	
C	Cecil	M	1yr 9mth	Siblings
	Coco	F	1yr 4mth	
D	Derby	M	5yr 8mth	None
	Doris	F	15yr 10mth	
E	Eddie	M	6yr 11mth	None
	Eva	F	7yr 3mth	
F	Freddie	M	1yr 11mth	Siblings (twins)
	Foxy	F	1yr 11mth	
G	Georgie	M	4yr 3mth	None
	Gracie	F	3yr 8mth	
H	Harry	M	1yr 1mth	Siblings (twins)
	Helen	F	1yr 1mth	
I	Iggy	M	1yr 11mth	None
	Iris	F	3yr	

6.2.3 Selection of food rewards

Food deprivation was never used. A number of different food items were tested by being offered to members of the family groups housed in a different colony room. The study animals were not used at this stage as feeding treats before training began may well have influenced the training process, particularly with regards to the time investment required.

As stated previously, the rewards had to be sufficiently motivating and easy to deliver and consume quickly. A liquid reward such as the banana milkshake used by Pearce *et al.* (1999) was not considered as this would have removed the marmosets' option of retreating to the back of the cage before consumption. Although some macaque studies have shown that these animals will work for food items normally present in the diet (Reinhardt, 1990; Reinhardt *et al.*, 1991), the only food item the

marmosets would accept with any consistency was banana. However, this was impractical as it was difficult to handle. Pieces of grape or dried fruits were accepted once or twice and then discarded. Whole dates, chopped into small pieces were accepted more readily, possibly because they were a new addition to the diet and still relatively novel. However, dates were only eaten in the morning (before the marmosets were fed) and discarded in the afternoon. Rice crispies were accepted readily but were easily crushed when taken. Cornflakes proved popular with some animals and were easy to handle. The most successful food item was marshmallow in that it was highly desirable and easy to deliver, even when cut into tiny pieces. The three food rewards used in this study (in decreasing order of desirability) were:

- Marshmallow
- Cornflakes
- Chopped dates

6.2.4 Targets

Plastic teaspoons were used as targets, black spoons for males and white for females. Colour vision in marmosets is polymorphic and while some females may be trichromats with colour vision similar to humans, other females and all males are dichromats with limited colour perception (Caine, 2002). As a result, colour of the targets is an important consideration. Black and white targets were chosen to provide maximum visual contrast. Plastic spoons had the advantage of being cheap and easy to clean or replace.

6.2.5 Training procedure

Training was conducted either in the morning, once all regular husbandry procedures had been completed, or in the afternoon. The marmosets were easily distracted so I only trained when alone in the colony room. If anyone else entered training was suspended until they left. No training was conducted on mornings when cage rotation took place although afternoon sessions continued as normal.

Each training session lasted a maximum of ten minutes, ending sooner if each animal had earned 12 rewards. Once an animal would hold the target for 20 seconds the scales were introduced. Animals were considered trained when they would remain on the scales long enough to allow their weight to be noted. A shaping procedure was used with training progressing in stages. These were:

1) The target was held at the front of the cage with the food reward held behind it. Simultaneously, the verbal request "hold" was given, preceded by the marmoset's name. As already stated, males were offered a black target placed on the left hand side and females a white target placed on the right. Initially the target was touched accidentally as the marmoset reached for the food. A reward was given when the correct target was touched, paired with verbal praise "good". Incorrect responses were ignored.

2) The target was presented without the reward held behind it. Marmosets were rewarded when target touched.

3) The time the target had to be held for before reward was given was gradually increased (Plate 14).

4) Scales for weighing were placed in the cage and the target held in front of them. The marmoset was rewarded for climbing onto the scales and holding the target (Plate 15).

Plate 14 Target training common marmoset pairs
Female common marmoset holds her target as male is given his.

Plate 15 Female marmoset sits on scales during in-homecage weighing as male waits until his target is presented

When pair members learned at different rates, the more advanced animal was simply required to hold the target for longer periods of time thus spreading the maximum number of rewards allowed over the full ten minute period.

6.2.6 Countering aggression

For ethical reasons, the training procedure was flexible to allow measures to be taken to minimise aggression between pairs. As stated above, the most aggressive animals were rejected during the selection procedure but it was still possible that agonistic behaviours such as stealing food rewards would occur once training commenced. 'Time out', terminating a session the instant aggression occurred, was used and pairs with an aggressive member were switched to a lower value reward at the next attempt. Whenever a session was terminated, that pair was given one further trial approximately 15 minutes later to allow the opportunity to end that day's training on a good note. A full session was not conducted until the next day. All instances of aggression were recorded to allow identification of any other counter-measures that could be taken such as altering the times at which training was carried out.

6.2.7 Data collection and analysis

Use of the palm-top computer as with the macaques (Chapter 3) was not a practical option when simultaneously training and recording data. Responses were recorded onto audiotape using a radio microphone worn during sessions. The use of the verbal request "hold" preceded by an individual marmoset's name allowed identification of each trial. "No?" with a rising intonation indicated that an animal had not attempted to touch the offered target, while verbal praise "good" indicated a correct response and "clever" that the food reward had been given. As reported

above, the marmosets were gradually shaped to hold the target for increasingly long periods. When the audiotape from each session was replayed, the use of two different words allowed the length of time that each individual had held the target to be determined.

When the marmosets were holding the target for approximately twenty seconds the scales were introduced at the next session. The total number of sessions required for each individual animal to remain on the scales long enough to allow their weigh to be recorded was used to estimate the time investment required. Differences between males and females were calculated using an independent sample t-test (Howell, 1995). Once all the marmosets were trained, an attempt was made to record the weight of each animal within one session, with a maximum of ten minutes allowed per pair. As 18 weights were required from the nine pairs, reliability was assessed by comparing this to the number of weights successfully recorded at this time.

6.2.8 Results

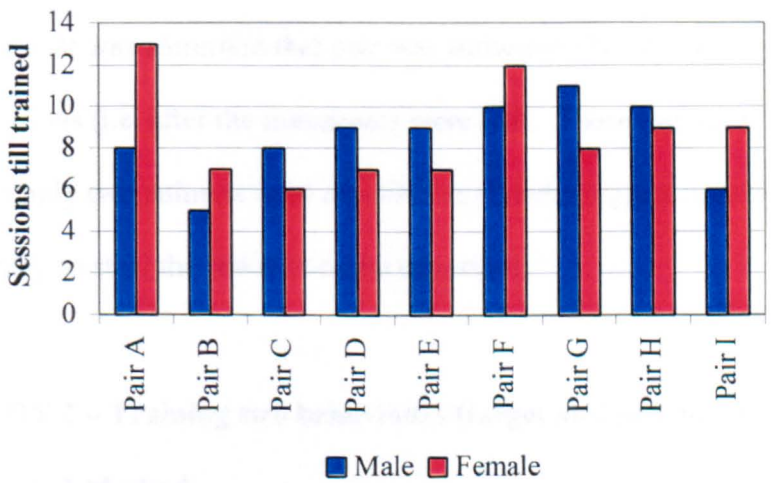
Time investment: All the study animals learned the task although there was considerable variation in that number of sessions required ranged from 5 -13. The mean number of sessions required was 8.56. Males learned in a mean of 8.33 sessions, while females required a mean of 8.73 sessions (Table 6.2). The difference between males and female was not significant ($t_{(16)} = 0.43$, $p = 0.43$). Figure 6.1 shows the number of sessions required by individual animals. However, as the marmosets were trained in pairs, i.e. two individual sessions were conducted simultaneously, the time required to train each pair is equivalent to the number of sessions required for the slowest animal. By this calculation, the mean number of

sessions required to train a pair was 9.78, a time investment of around 1 hour, 40 minutes. However, it should be noted that this time investment was calculated by examining the total number of 10 minute sessions required. As sessions sometimes ended sooner as the pair had each earned the maximum number of rewards, required time as calculated may over-estimate the actual time required.

Table 6.2 Summary of number of 10 minute sessions required to target train for in-homecage weighing.

	Mean sessions	S.E.
All marmosets	8.56	0.5
Males	8.33	0.62
Females	8.73	0.81
Per pair	9.78	0.64

Figure 6.1 Number of training sessions required to achieve in-homecage weighing by individual study animals.



Aggression was low with only seven instances recorded. In each case the dominant animal tried to steal the food reward. All of these instances occurred during morning sessions before the monkeys were fed. Three occurred on a Monday after two weekend days when the proportion of fresh fruit in the diet was reduced.

Reliability: All study animals proved reliable with 100 per cent of weights successfully recorded (n = 18).

6.2.9 Conclusions

The results show that marmosets could be trained using solely positive reinforcement techniques and that trained animals performed reliably once trained. Although there was considerable variation, all the animals learned the task fairly quickly, on average within 8.5 sessions, a time investment of around one hour twenty-five minutes. However, as two animals were trained simultaneously, the time investment required per pair was around one hour forty minutes. Aggression did occur, particularly when the animals were hungry. Whenever aggression occurred the training session was terminated and later resumed with a lower value food reward. The difference in behaviour between morning and afternoon sessions was quite noticeable with the marmosets appearing more excitable in the morning. Once an aggressive animal was identified that pair was subsequently trained only during afternoon sessions (i.e. after the marmosets were fed). These measures appeared to be successful as only two animals were recorded as showing aggression more than once, each attempting to steal the reward on two occasions.

6.3 STUDY 2 – Training two behaviours (target and provide urine samples)

6.3.1 Aims and Methods

When the above phase was completed, observational data on the behaviour of the marmosets were again recorded (Chapter 7). When that was completed, the next phase of training, Study 2 began. As stated above, one of the aims of this study was to train animals to provide urine samples that would be used as part of a collaborative

investigation (Chapter 7). Data were collected on the training process for a new behaviour as well as additional data on target training. This study also explored the possibility of recouping time investment in training by comparing the speed of data collection using trained animals versus that using current laboratory techniques.

6.3.2 Study animals

The experimental protocol included analysis of cortisol present in the urine and pregnant females were not suitable for this purpose (Chapter 7). As a result, pairs A and E had to be replaced as Allie and Eva were both past the first trimester of pregnancy, as determined by transabdominal uterine palpation. In addition, it was clear that Billy was becoming increasingly frail so pair B was also excluded. Pair H had been separated when Helen was paired with a new male in a different room and so were not used in this study. Pairs D and G began training but were not included in the overall analysis as one of each pair failed to learn to provide urine samples. Positive contact with these animals continued and they were still fed treats although no further data were collected. Three new pairs were selected, none of which had been housed in room 6 during Study 1. The final study group (n=12 animals) had a mean age of 867.92 days (± S.E. 142.81). Details of the study animals are given in Table 6.3. All housing and husbandry procedures are described in Chapter 5.

Table 6.3 Details of study animals including name, sex, age at start of urine training and relatedness to cage-mate.

Pair	Name	Sex	Age	Relationship
◆C	Cecil	M	2yr 1mth	Siblings
	Coco	F	1yr 8mth	
◆D	Derby	M	6yr	None
	Doris *	F	16yr 2mth	
◆F	Freddie	M	2yr 3mth	Siblings (twins)
	Foxy	F	2yr 3mth	
◆G	Georgie	M	4yr 7mth	None
	Gracie *	F	4yr 2mth	
◆I	Iggy	M	2yr 3mth	None
	Iris	F	3yr 4mth	
■J	Jambo	M	6yr 5mth	Father/daughter
	Jilly	F	1yr 3mth	
■K	Kipper	M	1yr 3mth	Siblings
	Keltie	F	1yr 8mth	
■L	Leo	M	2yr 5mth	None
	Lala	F	2yr 3mth	

◆ denotes pairs who were target trained first
■ denotes pairs who were trained to provide urine samples first
* denotes animals who failed to learn to provide urine samples

6.3.3 Training procedure – Target

The three new pairs, J, K and L were target trained to allow in-homecage weighing after they had been trained to provide urine samples. Apart from training order, the procedure used was identical to that described for Study 1 above.

6.3.4 Training procedure – Training to provide urine samples (Urine training)

As before, sessions were conducted either in the morning once all regular husbandry procedures had been completed or in the afternoon, sessions being suspended if anyone entered the room. As the animals were most aggressive on Monday mornings during Study 1, no training was conducted at that time. Pairs with an aggressive member, as identified during Study 1, were only trained during afternoon sessions. Each training session lasted a maximum of 10 minutes, ending sooner if each animal had earned 12 rewards. Food rewards were the same as before.

Marmosets were rarely observed urinating but scent marked frequently, depositing a few drops of urine each time. Scent marking is a behaviour that occurs fairly frequently in common marmosets (Epple, 1970; Stevenson & Poole,1976) and in this population in particular (HBS, personal obs: see also Chapter 7). It proved more practical to reinforce this behaviour, rather than waiting for urination. The criterion for success of urine training was that each animal scent marked on request 12 times per 10 minute session.

As with target training, a shaping procedure was used with training progressing in stages. To allow immediate reinforcement of desired behaviours, a clicking sound was used as a bridging stimulus. Without a bridge, there was the possibility that the marmosets would learn to associate the reward with coming to the front of the cage, rather than scent marking. Commercially available "clickers" proved too loud and startled the marmosets therefore I created the sound by clicking my tongue. This had the added advantage of leaving both hands free to deliver rewards. The stages employed during training were:

1) The marmosets were taught to associate tongue-clicking with a food reward (i.e. I clicked my tongue and then rewarded both pair members). The association was considered formed when the marmosets moved rapidly to the front of the cages and reached for food as soon as the clicking sound was made. The marmosets learned this within one session. One of the new animals appeared to have learned to associate the clicking sound with the offer of food, as she would move towards the front of the cage whenever the sound was made but was initially too nervous to actually take the reward.

2) Each pair was observed in turn until scent marking occurred spontaneously. Whenever a marmoset scent marked a branch, I made a clicking noise and rewarded that animal.

3) When the rate of scent marking had increased, the verbal request "go on then" was given as the animal moved towards the sites where scent marking occurred. If the animal then scent marked, I clicked and he/she was rewarded.

4) Once the marmoset would scent mark on verbal request, rewards were given only for marking one or two specific sites.

5) Holes were drilled at sites used by the marmosets to allow insertion of collecting vials (Plates 16 & 17).

If members of a pair learned at different rates, reinforcement for the more advanced member was switched from an all occurrences to a variable ratio schedule (Chapter 1). Once a behaviour is established, this schedule can maintain high levels of responding (Mellen & Ellis, 1997) and its use meant that rewards were spaced throughout the ten minute session. Individuals who had been target trained and failed to earn rewards during urine training were presented with their targets at the end of each session in order to finish on a positive note.

6.3.5 Data collection (training sessions)

Throughout, the number of rewards earned by each animal in each session was recorded onto audiotape, along with instances of aggression and stage of training reached by the end of each session. The total number of sessions required by each animal in order to reach criterion was calculated as in Study 1.

Plate 16 Branch with hole drilled to allow insertion of collecting vial

Plate 17 Collecting vial inserted into branch

6.3.6 Reliability

Once training was complete, all marmosets who had successfully learned both behaviours were weighed as described for Study 1 and the number of weights successfully recorded calculated as a percentage of the number of weights required. Reliability of animals who had been urine trained was assessed when samples were collected for urinary cortisol analysis (Chapter 7). Again, the number of samples collected was calculated as a percentage of the number of samples required by the experimental design.

6.3.7 Time investment – Comparison between data collection using trained animals versus standard laboratory procedures.

Weighing: The time taken to record the weights of the trained animals was compared to that taken using the current standard procedure. Data were collected when the study animals were weighed during the cortisol study (Chapter 7). Timing of the standard weighing procedure began when the cage door was opened. The marmoset pairs were confined in their nestbox and then taken to the procedure room. Each was in turn removed and placed in a weighing cage, weighed and then returned to the nestbox. Timing ended when cage door was closed after the monkeys were returned to their home cage. For the trained procedure, timing began when the cage door was opened to allow insertion of the scales and ended when the door was closed after removal.

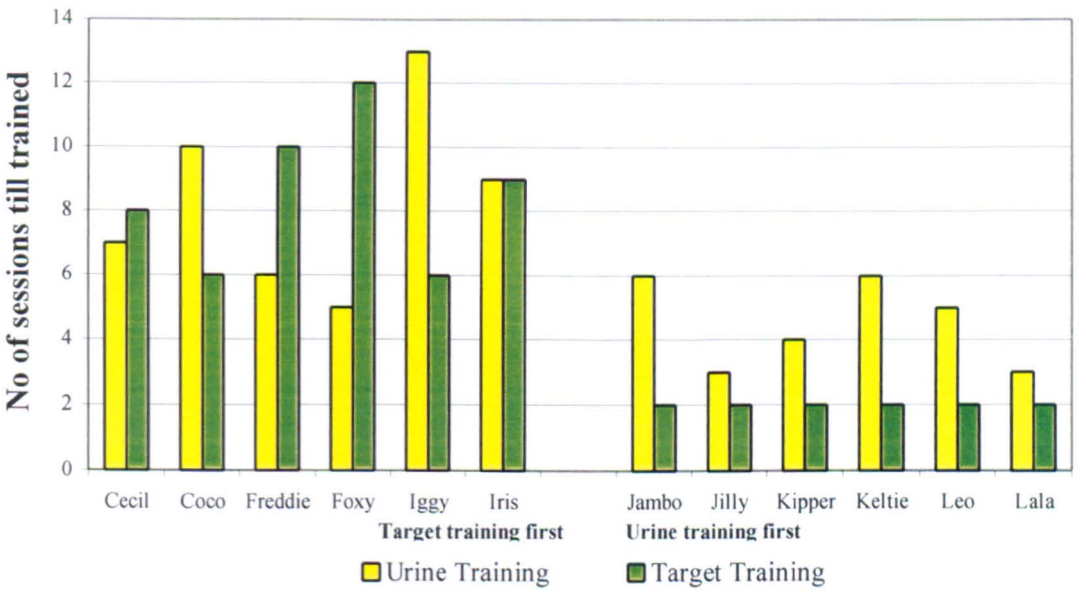
Urine collection vs. Blood sampling: Urine was not routinely collected in this laboratory therefore time taken to collect urine samples was compared to that taken to collect blood samples as many tests conducted on blood can also be carried out using urine. Data were collected during observations of routine blood draws conducted with experimental animals housed in room 7 (n = 14). The standard practice was to collect

all animals due to have samples taken simultaneously. This was done by confining them in their nestboxes and transporting them to the procedures room. Once all samples are collected, the animals are returned to their home cage. Timing began when the first cage door was opened and ended when the last animal was returned to the home cage. For urine sampling, timing began when first cage door was opened to allow insertion of the first collecting vial and ended when the last sample was removed and the door closed. In all cases, time recorded was divided by the number of samples obtained to give an estimate of time taken per sample.

6.3.8 RESULTS

Time investment: As before, there was considerable variation in the speed with which each animal learned to perform the tasks. The time required to complete target training ranged from 2 - 12 sessions and the mean number of sessions required was 5.25, S.E. = 1.08 (20min - 2h, mean = 1h 4min), while urine training was accomplished in 3 - 13 sessions with the mean number of sessions required being 6.42, S.E. = 0.86 (30min - 2h 10min, mean = 52min). Figure 6.2 shows the number of sessions required for individual animals. As the marmosets were trained in pairs, the actual time investment for target training (as calculated from the number of sessions required for the slowest pair member) was 5.83 sessions or approximately 1 hour per pair, while urine training required 7.67 sessions or approximately 1 hour 20 minutes per pair.

Figure 6.2 Number of sessions required to train for in-homecage weighing and provision of urine samples by individual study animals.



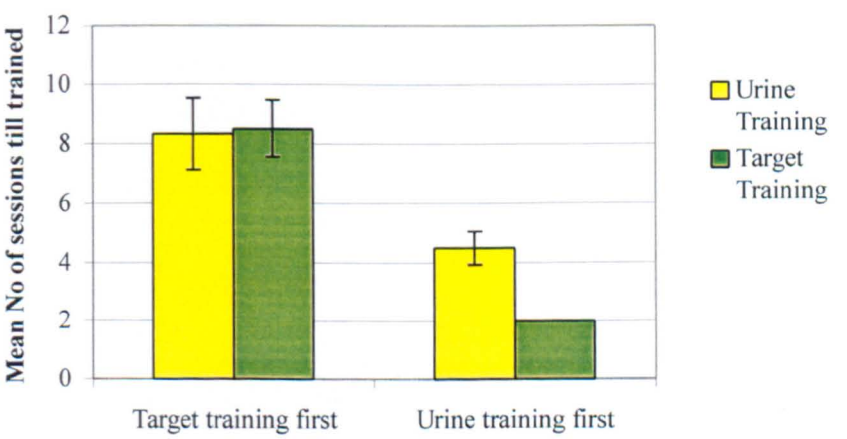
A mixed-design ANOVA, with one within factor, Behaviour (target vs.urine), and one between factor, Sex (males vs. females) revealed no significant difference between the number of sessions required for either task ($F_{1,10} = 8.17$, $p = 0.33$). There was no effect of Sex ($F_{1,10} = 0.01$, $p = 0.92$), and no Task x Sex interaction ($F_{1,10} = 0.34$, $p = 0.57$). A summary of the mean number of sessions required is presented in Table 6.4.

Table 6.4 Summary of the number of sessions 10 minute required to reach criterion for target and urine training.

	Target training		**Urine training**	
	Mean	S.E.	Mean	S.E.
All	5.25	1.08	6.42	0.86
Males	5	1.44	6.83	1.3
Females	5.5	1.75	6	1.21
Per pair	5.83	1.80	7.67	1.28

As indicated in Figure 6.2, when the marmosets were grouped according to which behaviour was learned first, animals who were urine trained first learned significantly faster than those who were target trained first ($F_{1,10} = 157$, $p < 0.001$). When urine training was conducted first, this behaviour was established within a mean of 4.5 sessions (around 45 minutes). Target training was accomplished within two sessions (20 minutes). Figure 6.3 shows the mean number of sessions required for each behaviour depending on training order.

Figure 6.3 Mean number 10 minute of sessions required per individual for each behaviour by training order (bars represent standard errors).



Aggression: No instances of aggression were observed with the original study animals. The sole agonistic act occurred during a morning session when one of the new animals (Jambo) tried to steal a reward earned by his cage-mate. All subsequent training with this pair was conducted in the afternoon and no further aggressive acts were recorded.

Reliability: After training, during formal data collection, the trained animals proved extremely reliable with 100% of weights (n = 12), and 95% of required urine samples, being successfully collected (n = 312).

Comparison between trained animals and routine laboratory procedures

When the time taken to record the weights of the trained animals was compared to weights recorded by the current standard procedure, data collection from trained animals was considerably faster (Table 6.5). Time taken per urine sample was less than that typically taken to collect blood samples.

Table 6.5 Mean time required per sample collected using trained animals as compared to standard laboratory procedures.

Procedure	**Mean Time per Sample**
Weighing (Standard procedure)	174.25 seconds (2 min 54 sec)
Weighing (Trained animals)	14.75 seconds
Blood Sample Collection	542.8 seconds (9 min 3 sec)
Urine sample Collection	184.6 seconds (3 min 5 sec)

During the standard weighing procedure, pair members were captured and removed to the procedure room simultaneously. This meant that these animals were removed from the homecage for approximately six minutes. The standard practice for blood collection required that all animals be collected simultaneously. As seven marmosets were observed over two days, this meant that each animal was removed from the homecage for approximately one hour.

6.3.9 Conclusions

Time required for target and urine training was similar, requiring an average of 5.25 and 6.42 ten-minute sessions per animal (1 hour and 1 hour 20 minutes per pair) respectively. As in Study 1 there were considerable individual differences although no significant difference between males and females was found. The most important factor was training order with animals trained to provide urine samples first learning significantly faster. Measures taken to minimise aggression were successful with only one instance recorded during this study. The trained animals co-operated reliably during recording of weights and collection of urine samples. These data were collected faster when compared to the time taken using standard laboratory procedures suggesting that time invested in training can be recouped. For example, if weights from a trained pair can be recorded five minutes 30 seconds faster than those using standard methods (3 minutes vs.15 seconds per animal), the 1 hour 20 minute training time is recovered after weighing has been carried out 15 times. For pairs who learned the target behaviour after being trained to provide urine samples, the twenty minute training investment could be recovered after the animals had been weighed four times.

6.4 STUDY 3 – refined method for training to provide urine samples

6.4 1 Aims and Methods

Data collected during Study 2 showed that, although sessions were scheduled to last for ten minutes, the full time was rarely required. As time is limited in a busy laboratory, the first refinement examined the feasibility of cutting the length of training sessions to only five minutes. The second refinement concerned the selection process as previous study animals had been selected by temperament. However, in

many laboratories or zoos this may not be possible and a good procedure should be applicable to all animals. Selection had initially been carried out to avoid undue stress through nervousness or aggression and the results of the first two studies suggested that these problems could be overcome through the training process. To ensure that this was the case, the animals in this study were selected at random from any male/female pairs not used previously.

As stated previously (Chapter 3), another important consideration is that animals will co-operate with laboratory staff other than the original trainer. In this study a test of reliability was carried out by one of the regular laboratory technicians.

6.4.2 Study animals

Study animals were 12 common marmosets housed as male/female pairs in four different colony rooms (rooms 2-5). With one exception, these marmosets had not experienced the period of habituation that previous study animals had during pre-training observations. However, one female (Elsie) had been one of a group of marmosets that had arrived from another laboratory. On arrival, these animals were noticeably more fearful (as indicated by long periods of time spent in the nestbox) than marmosets born into the colony. As the benefits of improved relations with humans had been demonstrated before the arrival of these animals (Chapter 9), both myself and unit staff had spent time talking to these recently arrived animals and feeding them treats. The mean age of the study animals was 952.08 days (± S.E. 79.94). This was not significantly different from the mean age of the marmosets who were urine trained in Study 2 ($t_{(22)} = 0.51$, $p = 0.61$). Details of the study animals are shown in Table 6.6. Details of housing and husbandry routines are reported in Chapter 5.

Table 6.6 Detail of study animals including name, sex, age at start of training and relatedness to cage-mate.

Pair	Name	Sex	Age	Relationship
A_2	Alba Abby	M F	2yr 5mth 2yr 5mth	Siblings (twins)
B_2	Brian Bonnie	M F	2yr 4mth 2yr 5mth	None
C_2	Charlie CC	M F	1yr 9mth 2yr 1mth	None
D_2	Dopey Daisy	M F	2yr 4mth 2yr 4mth	Siblings (twins)
E_2	Eric Elsie	M F	4yr 5mth 3yr	None
F_2	Frankie Fifi	M F	3yr 9mth 2yr 1mth	None

6.4.3 Training procedure

The study animals were trained to provide urine samples rather than target trained as the results of Study 2 suggested that this was a good initial behaviour to teach. Training followed the same basic procedure as described in Study 2 with the following modifications:

- Training was only carried out after feeding as this had been shown to minimise aggression.
- The duration of sessions was cut from ten to five minutes as during training for Study 2, the full ten minutes was rarely necessary.
- Only one location per animal was ever rewarded. In Study 2, most of the study animals tended to favour one or two sites. Scent marking occurred with sufficient frequency that rewarding all occurrences, regardless of location, was unnecessary and tended to prolong the training process.

- Criteria for training was cut from 12 to 8 scent marks per session. When urine was collected for cortisol analysis, it was found that 8 scent marks was usually sufficient to obtain a sample. It was also found that any marmoset that will scent-mark eight times on request would continue to do so if asked.

  The mean number of sessions required till criterion was calculated and possible male/female differences calculated using an independent sample t-test (Howell, 1995).

6.4.4 Transfer from the original trainer

Once all the study animals had reached criterion, collection of urine samples was attempted by one of the female laboratory technicians (Trainer C, Chapter 3). Before the attempt was made, I demonstrated how the procedure was carried out using a seventh pair that had been trained specifically for that purpose. Once each marmoset had scent marked three times, Trainer C took over until the criterion of eight marks per animal was reached. This took approximately ten minutes. She then spent another ten minutes practising, spending five minutes with Leo and another five minutes with Iggy (two of the original animals from Study 2).

Once she was comfortable carrying out the procedure, urine collection from the study animals began. Collection was carried out in a single session with a maximum of ten minutes permitted with each experimental pair. During each attempt I sat or stood alongside, recorded each time an animal co-operated and timed each session. Timing began when the cage door was opened to allow insertion of the collecting vials and ended when the door was closed following removal. As before (Study 2), the total time required was divided by the number of samples obtained to give an estimated mean time per sample. Only one attempt per pair was allowed.

6.4.5 Results

Time Investment

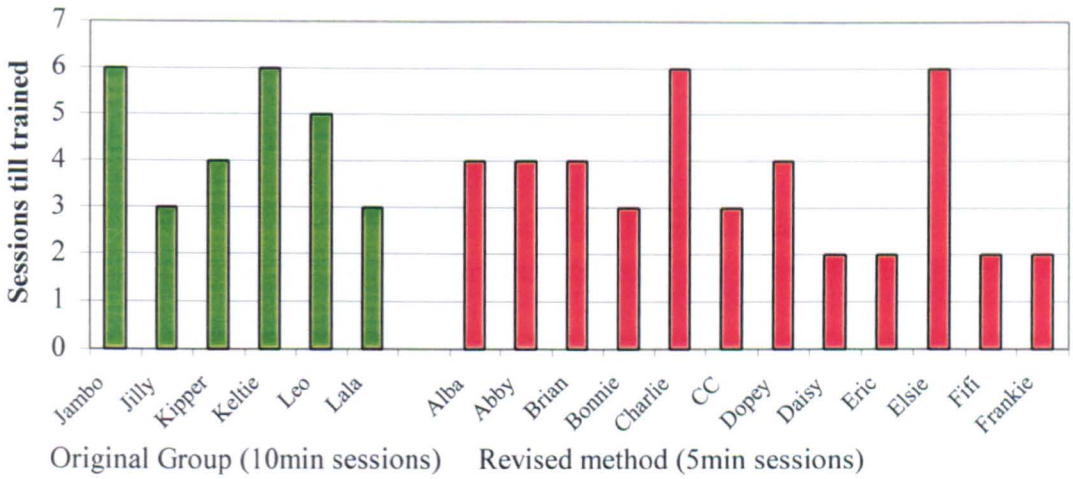
The mean number of sessions required until criterion was reached varied from two to six sessions with a mean of 3.5 sessions, (S.E. = 0.42) with no significant difference between males and females ($t_{(10)} = 0.38$, $p = 0.71$). As the marmosets were trained in pairs, the actual time investment per pair was that required by the slowest member. This was a mean of 4.33 sessions or, as sessions lasted for five minutes, a time investment of approximately 22 minutes per pair (Table 6.7).

Table 6.7 Summary of sessions required to reach criterion for and urine training using the refined method.

	Mean Sessions	S.E.
All marmosets	3.5	0.42
Males	3.67	0.61
Females	3.33	0.61
Per pair	4.33	0.61

There was no significant difference in the number of sessions required for training when these animals were compared to those who were urine trained first in Study 2 ($t_{(16)} = 1.40$, $p = 0.18$). However, as the sessions were half as long, training was achieved in less time using the refined method. The number of sessions required for each individual animal are shown in Figure 6.4. Sessions required for the marmosets trained in Study 2 (study animals who were urine trained first) are included for comparison. No instances of aggression were recorded.

Figure 6.4 Number of sessions required by individual study animals to reach criterion. (Results from the original group in Study 2 included for comparison).



Transfer to another trainer

Ten of the 12 study animals reached criterion within ten minutes giving a success rate of 83.2 per cent. The mean time required to reach criterion was 224.6 seconds (3 minutes 45 seconds). Of the two who failed to reach criterion, Charlie only scent marked once while Elsie scent marked three times.

6.4.6 Conclusions

Although the animals in this study had been randomly selected, the measures taken to counter aggression were successful in that no instances of aggression were observed during training. Cutting the duration of training sessions to five minutes did not result in a greater number of sessions being required, as there was no significant difference between these animals and those who were initially trained to provide urine samples in Study 2. Trained behaviour transferred successfully from the original trainer to one of the laboratory staff with only two of the twelve animals failing to co-

operate. It is interesting that these two animals were those who took longest to train initially, and that one of them (Elsie) was already habituated to humans prior to the study beginning.

6.5 GENERAL DISCUSSION

The performance of the marmosets over three studies demonstrated that training to co-operate with routine laboratory procedures could be accomplished using only positive reinforcement techniques. Problems with aggression were less than might have been anticipated and the measures taken to overcome them appeared to be successful as shown by the diminishing number of aggressive acts over the three studies. All agonistic acts were similar and occurred when the dominant animal tried to steal the reward. All of these incidences occurred when the monkeys were hungry (e.g. before they were fed), with five occurring on a Monday (after two days when the proportion of fresh fruit in the diet was reduced). This observation, and the solution to the problem, training pairs with an aggressive member only after they had been fed and rewarding responses with cornflakes (a less preferred food) rather than marshmallow at the start of each session, have far-reaching implications. They counter the widely held belief that food deprivation is necessary for successful training. Indeed they even suggest that food deprivation may be counter-productive to the training process. Of course, food-deprived animals may more willing to work for items normally included in the diet rather than treats. However, when training in pairs or groups, the finding that hunger increases aggression must add to the ethical considerations when evaluating the welfare costs of this approach.

While the marmosets learned both behaviours fairly quickly, there was considerable variation between individual marmosets with the results showing that the

order in which behaviours were taught influenced learning. This suggests that overcoming fear of humans was an important factor in their performance. Initially the marmosets were nervous and tended to take the food reward then retreat to the back of the cage before eating. They would only remain holding their targets once this nervousness had been overcome during the training process. Some primates scent mark more frequently when nervous (Sutcliffe & Poole, 1978; Watson, Ward, Davis & Stavinsky, 1999) and this did appear to be the case with these animals. Initial nervousness, while a hindrance during target training, was actually helpful during urine training as frequent scent marking allowed frequent reinforcement of this behaviour. By the time urine training was complete, the marmosets had become quite tame and this made target training easier.

Two of the marmosets target trained during Study 1 failed to learn to provide urine samples during Study 2. This was due to the fact that they rarely scent marked during training sessions and tended to remain at the front of the cage. The practice of presenting the targets at the end of each session to allow unsuccessful animals to earn rewards and finish on a good note probably did not help as this encouraged them to wait for the targets to appear. However, the practice was intended to minimise frustration, especially when the cage-mate had learned the task and was being rewarded. At times a compromise between the need to teach the task and the welfare of the animals is required and this appeared to be one of these occasions. The two marmosets involved, Doris and Gracie, were both very bold, dominant animals and there was the possibility that allowing them to become frustrated would have led to displaced aggression towards their cage-mates.

When the reliability of the trained animals was assessed they showed high levels of co-operation and data collection during Study 2 was considerably faster than

that using standard laboratory techniques. When the training time per pair is examined, the 1 hour 40 minutes required to train for in-homecage weighing as the first behaviour could be recovered within 15 weighing sessions. The twenty minutes required to target train a pair already trained to provide urine samples could be recovered after the animals have been weighed four times.

As the important factor appeared to be overcoming fear of humans, it is likely that this short training time could be achieved if targeting was used as the first trained behaviour, provided a good relationship with humans already existed. The basic behaviour is extremely versatile and, once established, could be used for other procedures such as entering transport cages or handling (Laule & Desmond, 1998). In addition, when used for in-homecage weighing, the need for capture in the nestbox and separation from companions is eliminated. However, although target was originally selected as a suitable initial behaviour to train, the findings of Study 2 suggest that training to provide urine samples may be a better choice. In addition, the results of Study 3 showed that the procedure used to train this behaviour could be modified to reduce the time investment required with no subsequent loss in performance.

In both Study 1 and 2, weights were collected with 100% reliability. Around five percent of urine samples were lost, largely due to the same animal, not because he failed to provide a sample but because he became adept at removing the collecting vial before the trainer. In a comparison of urine vs. blood collection, it should be noted that it could be difficult to collect daily blood samples over a long period of time without damage to the femoral vein (Ferrell, 2003). In addition, there is a limit to how much blood can be taken from such a small animal before its health is compromised with a recommended maximum of 3ml per week or 0.3 ml per day if

sampling is carried out over a number of weeks (Hearn, 1983). Hearn (1983) estimates the mean daily production of urine as around 17 ± 0.34ml. Training to provide urine samples could be particularly useful for studies of relatively long duration.

However, as scent marking is linked with stress in marmosets (Epple, 1979; Sutcliffe & Poole, 1978, Watson *et al.*, 1999) there is the issue of rewarding a stress-related behaviour. However, the study animals did not continue to scent mark at high rates outside training sessions despite the fact that this behaviour had been rewarded (Chapter 7). Many substances such as cortisol can now be measured in saliva (Lutz, Tiefenbacher, Jorgensen, Meyer & Novak, 2000) and this may prove a more satisfactory replacement for blood and urine. Saliva can be collected at very regular intervals (i.e. 5 minutes) and training is minimal (Chapter 1).

Training to provide urine samples did transfer successfully from the original trainer to one of the regular laboratory staff. A likely contributing factor to her success was the fact that she already handled the marmosets in the quiet, gentle way recommended by Hearn (1983). As reported above, this was the same technician who had successfully gained the co-operation of the macaques (Chapter 3). It should be noted that it was not possible to examine how the marmosets would have responded to a novel male handler although shortly after these studies were completed, further training using the methods described above was successfully conducted by a male trainer (McDermott & Smith, 2003). However, the macaques demonstrated that the way in which individual technicians carried out procedures had a profound effect on their willingness to co-operate (Chapter 3) and the same is likely to be true of marmosets. Staff attitude and staff training is important and training skills do take

practice (Laule & Desmond, 1998), but this is the case for any laboratory procedure (European Commission, 2002; Home Office, 1999).

That marmosets are prepared to co-operate with more than one person lends weight to the belief that the use of PRT is a practical approach to the management of these animals. In laboratories, procedures are carried out by different people, staff change and the animals themselves sometimes move between institutions. The results also suggests that if existing staff lack either the time or knowledge to train themselves, the actual training process could be conducted out by outside personnel. When laboratory animals are not bred in-house, basic training could be conducted at breeding establishments.

Overall, the results showed that positive reinforcement training was both an effective and practical alternative to current laboratory techniques. One unexpected result was the change in staff attitude towards the marmosets. As reported, all the trained animals were named at the start of the study. Staff began to use these names rather than identification numbers and the behaviour and personalities of the study animals became a topic of conversation in a similar way previously shown with macaques. The study animals had effectively become individualised. The technician who participated in Study 3 reported that she found working with the trained animals enormously satisfying. By examining largely practical issues, this chapter examined the potential benefits the PRT could contribute to the management of common marmosets from the perspective of their human caregivers. The potential benefits to the marmosets themselves are examined in Chapter 7.

Chapter 7

Effects of Training on the Behaviour of Common Marmosets

7.1 INTRODUCTION

7.1.1 Animal / human relationships

"The behaviour of an animal during a procedure depends on the confidence it has in its handler. This confidence is developed through regular human contact and, once established, should be preserved."

Home Office, 1999, 3.39

The above statement, taken from the Home Office Code of practice for the housing and care of animals used in scientific procedures, contradicts much common practice and beliefs about the desirability of close contact between captive animals and their caregivers. This is an important issue with regards to positive reinforcement training as such contact is an essential element in the training process. While close relations can be established without training, PRT cannot be carried out without establishing a positive relationship between the trainer and animals (Mellen & Ellis, 1996). In institutions where resistance to the suggestion that positive contact between humans and animals is beneficial exists, PRT is unlikely to be considered as a means of refining animal husbandry. Misgivings about the value of regular human contact as recommended above exist in both laboratories and zoos but arise for different reasons including issues of safety, animal welfare and scientific rigour. However, a closer examination of these issues suggest that much current practice is based, not on scientific evidence showing benefits to the animals, but on ideas arising from 'common sense' assumptions based on cultural beliefs regarding animal-human relationships.

Concerns about the safety of personnel working with primates are a recurring theme in laboratory handbooks containing warnings that "primates can injure personnel severely if adequate restraint is not used" (Whitney, Johnson & Cole, 1973, p50). In this context, primates can be portrayed as "viciously aggressive creatures who pose a life threatening risk..." (Reinhardt, 1997, p93). Equally, "the potential of primates to carry fatal zoonotic diseases should not be under-estimated" (Wolfensohn & Lloyd, 1994, p84). The consequence of such fears is the belief that contact should be minimised in order to protect personnel.

While it is true that there is a risk of disease transmission between human and non-human primates, as with physical injury, some primates pose a greater risk than others. Many handbooks make no distinction between Old and New World species despite differences in their susceptibility to transmissible diseases. For example, macaque, but not callitrichid species are host to Mycobacterium tuberculosis (NRC/ILAR, 1998). In addition, captive-bred animals pose a lesser risk than those that have been wild-caught and imported (Wolfensohn & Lloyd, 1994) with the risk further reduced in closed colonies (European Commission, 2002). Since primates first began to be used as laboratory animals, there have been tremendous advances in the ability to identify individual carriers of specific diseases. Current European Commission (2002) guidelines recommend that regular haematology, bacteriological, virological and parasitological tests be carried out on all laboratory-housed primates with the results recorded in each individual's file. With such information available, it should be possible to identify potentially infectious individuals rather than preclude contact with all primates. Equally, staff training and pre-employment health screening can further minimise the danger of cross-infection (European Commission, 2002).

When considering the danger of physical injury, many sources make no distinction between large species such as macaques that are potentially dangerous and the much smaller callitrichid species. In the UK, early perceptions of the dangers posed by primates may have been influenced by the fact that they were relatively unknown, exotic animals in a country with no native primate species. Risk perception is influenced by familiarity. For example, many people fear flying but not car journeys despite the fact that flying is statistically a much safer way to travel. Strictly speaking, there is another common laboratory-housed animal that, having large incisors and powerful hind legs, could inflict greater injury than a marmoset. However, rabbits are not generally regarded as dangerous animals. While it is unlikely than anyone would dispute that evaluations of appropriate handling techniques must consider the potential risk to the handler, these should be based on the actual risk involved. In this context, generalisations about 'primates' are not particularly helpful given the diversity of the primate order.

For species that could potentially inflict serious injury, the technique of 'protected contact' can be employed where animal-human interactions are conducted across a safety barrier such as the cage bars (Mellon & Ellis, 1996). Even individual differences within a particular species can lead to differences in appropriate techniques. However, such flexibility in handling techniques can only be employed with considerable knowledge of both the species and individuals involved. It could be argued that many accidents and even deaths occur, not as a result of contact *per se*, but as a result of inappropriate contact.

If risk of injury to personnel is an insurmountable obstacle to establishing positive animal-human relationships, it is strange that most primate studies showing the benefits of such relationships concern potentially dangerous species such as

macaques and chimpanzees rather than marmosets (e.g. Baker, 1997; Bayne *et al.*, 1993; Heath, 1989; Reinhardt, 1997). It is possible that the situation regarding animal-human relationships is similar to that regarding training described in Chapter 6. The fact that these animals are potentially dangerous produces incentives to develop safer means of handling them, especially as rigorous precautions do not actually prevent injuries occurring (Reinhardt, 1997). With little or no threat present, there is little incentive to establish good relationships therefore little evidence is produced to show that this would be of benefit to the animals. This is a serious omission as it is possible that it may be both easier and more desirable to establish good relationships with potentially dangerous species that perhaps have less reason to fear humans. The same approach may be detrimental to the welfare of relatively harmless but nervous animals.

However, a common observation in studies of common marmosets is that they do not like being handled (Hampton *et al.*, 1966; Hiddleston, 1978; Stellar, 1960). The logical response to this is to avoid handling whenever possible (Boyd Group, 2000; NRC/ILAR, 1998; Poole *et al.*, 1999). However, Hearn's (1983) description of the establishment of a laboratory-house colony suggests that such a policy may not be the best solution in all circumstances. When managing his initial nine marmoset pairs, Hearn followed the advice of more experienced colleagues and avoided contact with the five pairs designated as 'breeders'. However, the four 'experimental' pairs were captured and handled daily using the techniques described in Chapter 6. Hearn reports that while the experimental group thrived, with all the females becoming pregnant, the breeding animals remained nervous and no pregnancies ensued. As a result, the policy of regular, careful handling was adopted throughout the colony thus

Hearn's (1983; Hearn & Dixon, 1984) recommendations with regards to the handling of common marmosets conflict with those in the sources cited above.

Concerns about welfare underpin much reluctance to handle animals in zoos. Once again, there is some intuitive sense in the idea that if animals do not respond well to contact with humans, the answer is to keep contact to an absolute minimum. This 'hands off' policy appears to have become the cultural norm in the UK. However, a possible outcome of this policy is that when handing does occur, it is inevitably aversive. If close contact with humans is always accompanied by stressful events such as restraint, transportation or the discomfort associated with many veterinary treatments, the resulting conditioned fear response may well intensify any existing aversion to humans. This has the potential to make an already stressful situation significantly worse which may exacerbate any illness or even result in the death of the animal (Hinshaw, Amand & Tinkelman, 1996).

In zoos, a further objection to close relationships between caregivers and zoo animals is that this is unnatural. A related belief is that close contact with humans renders animals unsuitable for reintroduction. In addition, while carrying all the undesirable elements of close contact, training, where an animal is deliberately taught to perform specific behaviours on request, arouses the additional fear that this will disrupt normal social behaviour as animals become "fixated" on gaining food treats from humans (Kiley-Worthington, 1990).

Both Arluke (1992) and Serpell (1999) found that laboratory technicians are often strongly discouraged from forming close relationships with the animals in their care. A similar effect has been found among scientists where close contact comprises "exactly the kind of stuff we were trained to avoid" (Davis & Balfour, 1992, p1). Such attitudes arise from a number of concerns. Firstly, close relations will

compromise strict objectivity and make it difficult for personnel to carry out any studies that involve discomfort, injury or the death of the animal (Davis & Balfour, 1992). Secondly, contact with humans is a confounding variable as it changes the behaviour and physiology of the animal, therefore, "It taints our research" (Davis & Balfour, 1992, p1). Minimising contact reduces such contamination and when contact during experimental procedures is unavoidable, it should be carefully controlled with all animals handled in the same way (Boccia, Broussard, Scanlan & Laudenslager, 1992). The ideal situation is one where the animals have become completely habituated so that humans become an insignificant part of the environment and are ignored (Estep & Hetts, 1992).

Such beliefs have come under attack for a number of reasons, some concerning animal welfare and some concerning the benefits to science. There is a growing recognition that some relationship between captive animals and caregivers is inevitable. Good or bad, close or minimal, such relationships will affect research and cannot be ignored (Davis & Balfour, 1992; Estep & Hetts, 1992; Reinhardt, 1997). The stress associated with the presence of, and manipulation by humans will always represent a confounding variable (Novak & Suomi, 1988). If such stress can be reduced by improving animal-human relations, then animals who experience frequent positive interactions will be better research subjects (Markowitz & Line, 1992). The practice of treating all primates in an identical manner will only produce a consistent outcome if the animals were initially all the same. This is not the case as individual differences in temperament and responses are clearly apparent and reported in a wide variety of sources (e.g. Boccia *et al.*, 1992; de Waal,1989; Markowitz, 1989; Suomi & Novak, 1991; Thorndike, 1911). Any innate differences can be further increased by past experience of human behaviour and laboratory procedures (Boccia *et al.*, 1992;

Estep & Hetts, 1992). The variation in the time investment required for training reported in Chapter 6 suggests considerable individual differences among marmosets. Consistency may be better achieved by taking steps to reduce fear of humans in all study animals while recognising that this will require more effort for some than others.

As stated above, one desirable human-animal relationship occurs when the animals are believed to have habituated to the presence of humans. If the animals appear to ignore human activity, then the effect of the researcher can be discounted as minimal. However, there is some question as to the degree to which habituation actually occurs. Caine (1987, 1990) found that apparently habituated red-bellied tamarins *(S.labiatus)* actually adopted subtle anti-predator strategies when observers were present. Studies of macaques have found physiological responses to the presence of humans even when no behavioural change is apparent (Bowers, Crockett & Bowden, 1998; Line, Morgan, Markowitz & Strong, 1989; Tatoyan & Cherkovich, 1972). Such studies suggest that the belief that habituated animals are not being influenced by human activity is mistaken.

Minimal contact, standardised treatment and attempts to habituate in the sense of learning to ignore humans all arise from the assumptions underlying Western science. Such assumptions produce a demand for strict objectivity, a denial of personality, feeling or emotions on the part of the animals, a strong fear of anthropomorphism and a desire for research animals 'uncontaminated' by contact with humans (Lehman, 1992). However, such assumptions are the product of Western philosophies, not universal 'truths' about how science should be conducted. This can be illustrated by contrast with Eastern science. A strict separation between humans and animals is not part of the Japanese cultural heritage. In primatology,

pioneering researchers such as Kinji Imanishi based their methods on identification with the animals and on a "disciplined subjectivity" (Haraway, 2001, p256). A related approach proposed by Kawai is the concept of *kyokan* (feel-one), which proposes

"...the particular method and attitude resulting from feelings of mutual relations, personal attachment and shared life with the animals as the foundation of reliable scientific knowledge." (Haraway, 2001, p259)

Another feature of Japanese primatology is the emphasis on recognising animals as individuals and documenting behaviour in terms of individual kinship relationships, friendships, rivalries and interactions within the social order (de Waal, 2001). The value of such an approach can be seen in the work of researchers such as Jane Goodall and Frans de Waal. However, for this chapter, the main purpose of the comparison between Western and Japanese science is to illustrate that close relationships between animals and researchers may promote, rather than undermine the pursuit of scientific knowledge. Avoidance or denial of the animal-human relationship can promote insensitivity to the complexity of animal behaviour which in turn leads to distorted understanding (Davis & Balfour, 1992).

The main purpose of this introduction was to demonstrate that much current practice arises, not from scientific evidence demonstrating benefits to the animals, but from a complex mix of assumptions and social and cultural traditions. The literature regarding animal-human relationships also contains many poorly defined concepts and unhelpful generalisations. The distinction between different forms of contact is rarely explicitly made. 'Handling' in the sense that the animal is actually touched may well be aversive for some species or individuals. Bennett and Davis (1989) acknowledge the importance of this as, although they recommend training and the

avoidance of indiscriminate restraint, they also suggest that humane treatment is further promoted by prohibiting all touching and petting unless initiated by the monkeys. Aversion to handling is particularly likely for small primates that can be picked up and held in the hands, which is effectively a form of capture. From the animals' perspective, being picked up and held is possibly very different from voluntarily climbing onto a human hand or arm. For the purpose of this chapter, all non-training interactions described as 'positive contact' refer to interactions where the marmosets are talked to and offered food treats but where physical contact is restricted to that initiated by the animal.

Demonstrating the weaknesses in the argument that contact between animals and their caregivers should be minimised is no substitute for evidence that increased contact is of benefit to the animals. Such evidence has been increasing hence the growing recognition that positive relationships can do much to promote welfare (Home Office, 1999; NRC/ILAR, 1998). Much of the evidence available comes from studies of farm animals (reviewed by Grandin, 1997; Rushen, Taylor & de Passillé, 1999). However, it is not difficult to foresee that proponents of a "hands off" policy could argue that studies of domesticated species have little relevance to the husbandry of wild animals although the argument that there are strong distinctions between the two is itself open to question (Kiley-Worthington, 1990).

A further source of evidence comes from the beneficial effects of early handling in laboratory animals, mainly rodents. Once again there is the question of how far these results can be generalised across species, especially as recent studies of mice suggest that the consequences of early handling are more complex than originally thought. While some laboratory strains do become tame, for others, early

handling has no effect and one strain, C37BL6, actually becomes more aggressive (Ridley, 2003).

As stated previously, evidence showing the benefits of good animal-human relationships does exist. However, much of this evidence comes from a relatively narrow range of primate species, mainly macaques (Reinhardt, 1997c). Norcross and Newton (1999) found that well-handled marmosets showed no elevation in plasma cortisol when moved to a novel environment yet beyond this, and Hearn's (1983) observations, there is a paucity of studies specifically relating to common marmosets. The small size, defencelessness and nervousness of these animals mean that studies based on larger, bolder species may be misleading. The main aim of this chapter was to examine the effects of changing existing animal-human relationships on the behaviour of common marmosets, both in the mere presence of humans and in response to a potentially stressful laboratory procedure.

7.1.2 Study Outline

The study reported in this chapter addressed a number of issues through two separate but related experiments. These were:

Experiment 1 – The effects of positive reinforcement training and increased positive contact with humans on the behaviour of common marmosets.

Experiment 2 – The behavioural and physiological responses of trained marmosets to a potentially stressful laboratory procedure.

7.2 EXPERIMENT 1 – The effects of PRT and increased positive contact with humans on the behaviour of common marmosets.

7.2.1 Aims and Methods

As stated previously (Chapters 2 & 5), relations between staff and the macaques at the MRC unit was very different to that existing with the marmosets. This provided the opportunity, not only to examine the effects of PRT, but also the consequences of increasing the amount of positive interactions with humans experienced by these animals. Data were collected to see if PRT did reduce the stress associated with the presence of humans. In addition, given the concerns that training may disrupt normal social behaviour as animals become 'fixated' on gaining food treats from humans (Kiley-Worthington, 1990), the behaviour of the study animals was recorded before and after training to examine any changes that did occur. Matched data were collected from animals that were not trained but experienced increased positive contact to examine if such contact with humans actually differed in effect to that of training the animals to perform a specific task.

7.2.2 Study animals

Eighteen pairs of common marmosets *(Callithrix jacchus)* were selected from the 52 animals pair-housed in the same colony room (room 6) at the MRC Human Reproductive Sciences Unit, Edinburgh. Details of housing and husbandry routines are given in Chapter 5. The study animals were divided into two groups. The 'trained' group, comprising nine male/female pairs, were the animals described in Chapter 6, Study 1. The remaining animals, 2 male/female, 3 male/male and 4 female/female pairs, formed the 'contact' group.

As reported in Chapter 6, the study animals were selected by temperament with the criteria that at least one pair member would take food from my hand and that neither animal showed aggression towards his/her cage-mate. For the trained group, male/female pairs were required in order to examine sex differences during training. As reported above, not all the 'contact' group were housed in male/female pairs and the sex distribution was not equal as the contact group contained eight males and ten females overall. While it would have been better if the sex distribution between groups had been identical, the experimental design and availability of study animals meant that this was not possible. However, sex differences in the behaviour of common marmosets are neither strong (Woodcock, 1982; Harrison & Tardif, 1989) nor consistent across studies (Kerl & Rothe, 1996). For example, while common marmosets consistently show higher levels of aggression towards same-sex individuals (Stevenson & Rylands, 1988), some studies have shown a greater tendency for aggression in females (Michels, 1998) while others have found that males direct more agnostic behaviours outside the homecage (Evans & Poole, 1984). As stated previously, any pair (mixed or same-sex) with an aggressive member was excluded during the selection procedure. Any pre-existing differences between the groups were examined during statistical analysis of the pre training / contact data.

The ages of the trained group ranged from 395 – 5715 days (approx. 13 months to 15 years 10 months) and the mean age was 1586.11 days (approx. 4 years, 4 months; ± S.E. 360.48). The ages of the contact group ranged from 275 – 5475 days (approx. 9 months to 15 years) with a mean age of 1213.61 days (approx. 3 years, 4 months; ± S.E. 283.43). There was no significant difference in age between the trained and contact groups ($t_{(34)} = 0.81$; $p = 0.42$). None of the females were past the first trimester of pregnancy as determined by transabdominal uterine palpation.

Figure 7.1 Details of study animals including name or I.D. number, sex, age at start of study and relationship to cage-mate.

Pair	Name	Sex	Age	Relationship
*A	Adam	M	2yr 8mth	Siblings (twins)
	Allie	F	2yr 8mth	
*B	Billy	M	13yr 7mth	None
	Bella	F	2yr	
*C	Cecil	M	1yr 9mth	Siblings
	Coco	F	1yr 4mth	
*D	Derby	M	5yr 8mth	None
	Doris	F	15yr 10mth	
*E	Eddie	M	6yr 11mth	None
	Eva	F	7yr 3mth	
*F	Freddie	M	1yr 11mth	Siblings (twins)
	Foxy	F	1yr 11mth	
*G	Georgie	M	4yr 3mth	None
	Gracie	F	3yr 8mth	
*H	Harry	M	1yr 1mth	Siblings (twins)
	Helen	F	1yr 1mth	
*I	Iggy	M	1yr 11mth	None
	Iris	F	3yr	
♦Ac	813BK	M	2yr 1mth	None
	929R	F	1yr 7mth	
♦Bc	729BK	M	4yr 4mth	None
	352R	F	15yr	
♦Cc	897R	F	1yr 11mth	Siblings (twins)
	898R	F	1yr 11mth	
♦Dc	887R	F	2yr	Siblings (twins)
	886R	F	2yr	
♦Ec	733R	F	5yr 4mth	None
	764R	F	3yr 11mth	
♦Fc	955R	F	1yr 3mth	Siblings
	983R	F	9mth	
♦Gc	285BK	M	2yr 8mth	None
	823BK	M	2yr	
♦Hc	809BK	M	2yr 2mth	None
	825BK	M	2yr	
♦Ic	658BK	M	6yr 11mth	None
	814BK	M	2yr 1mth	

* Denotes trained group
♦ Denoted contact group

7.2.3 Procedure

Pre training / contact observations

Once the study animals were selected, each contact pair was matched to one of the trained pairs. When necessary, contact pairs were moved between housing units so that they were housed next to, and on the same tier as the matched trained pair. The locations of the study animals within room 6 are shown in Figure 7.1.

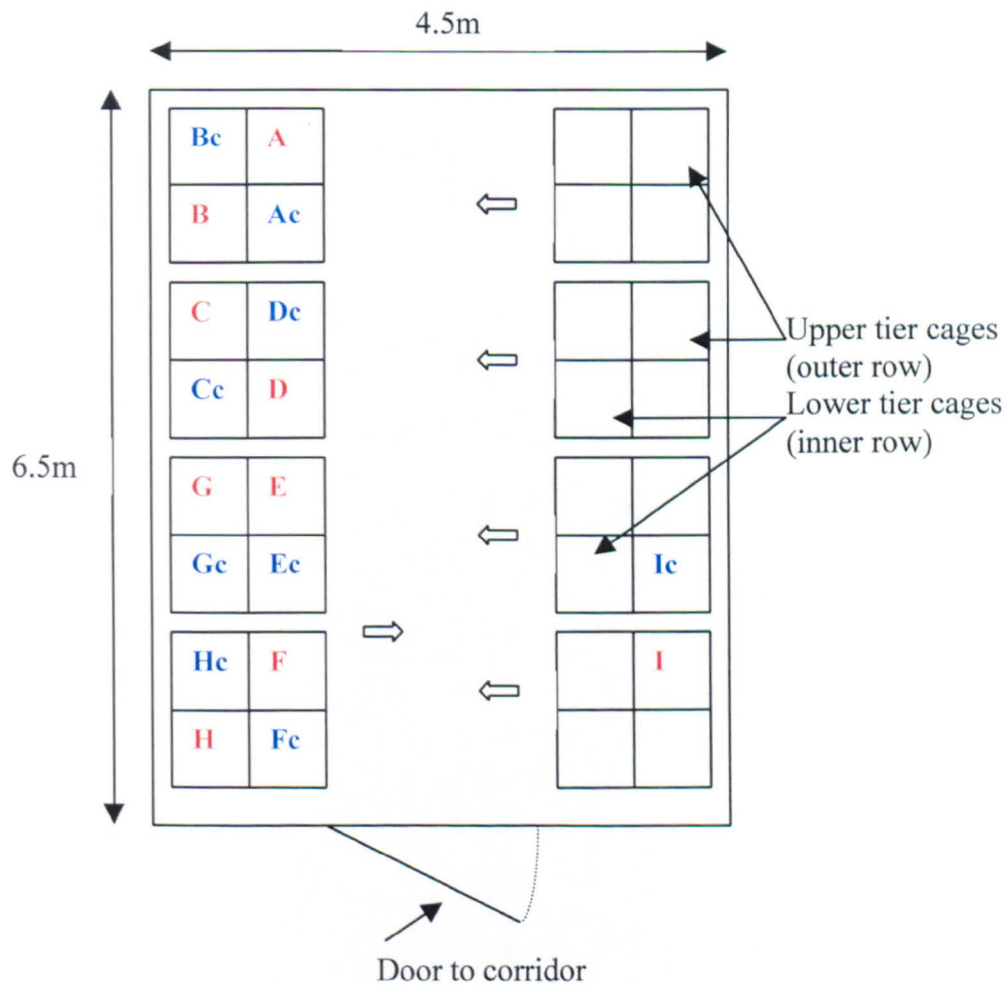
Each pair was observed for eight, ten-minute sessions. As reported previously, routine husbandry procedures were normally carried out in the morning and the laboratory was noticeably quieter in the afternoon. As there was a clear difference in the amount of human activity at these times, half of the observation sessions were conducted in the morning and half in the afternoon. Morning sessions were conducted after all husbandry procedures were completed and ended at least 30 minutes before the animals were due to be fed. Each experimental and matched contact pair was observed consecutively with the order counterbalanced across observations. The protocol for collection of observational data and definitions of all behavioural categories are reported in Chapter 5.

Behavioural states, behaviours of relatively long duration, were recorded using instantaneous scan sampling with a 15 second sample interval. Behaviours recorded in this way included: watching the observer from the wire front of the cage, henceforth referred to as 'watch (wire)"; watching the observer from a position inside the cage, referred to as 'watch (cage)'; 'locomotion (normal)'; 'locomotion (excited)'; 'proximity (touch)'; 'proximity (< 10cm); 'autogroom'; 'allogroom'; 'nestbox' and 'other'.

All occurrences of behavioural events, behaviours of relatively short duration, were also recorded. These included: 'affiliate'; 'aggression'; 'scratch'; 'scent mark';

'genital present', 'vertical flight' and 'vocalise'. When all observations were complete, the training / contact phase began.

Figure 7.1 Schematic diagram of marmoset colony room 6 showing the location of the trained and contact pairs (not to scale).



Red font denotes trained pairs.

Blue font denotes contact pairs.

⇨ indicates positions from which observations were conducted.

Training / Contact Phase

The trained group were target-trained to allow in home-cage weighing as described in Chapter 6. As reported, training sessions lasted a maximum of ten minutes, ending sooner if each animal had earned the maximum of twelve rewards. Following each pair's training session, I then spent the same amount of time talking to the matched contact pair who were fed the same number of treats that the trained pair had earned. However, as the contact group did not have to perform any specific behaviour in order to earn their rewards, treats were fed to both animals simultaneously whenever possible to minimise aggression. If morning sessions for any trained pair were discontinued due to aggression (Chapter 6), morning sessions for the contact pair also ceased. If any trained pair finished a session before the allotted ten minutes had expired, time spent with the matched contact pair was adjusted accordingly. This process continued until all of the animals in the trained group would remain holding their targets long enough for their weights to be recorded (Chapter 6).

Post-training Observations

Once training was complete, observations were repeated exactly as conducted during the pre-training phase. At this stage, additional measurements of vocalisations were taken. Vocalisations recorded during observations were 'tsk' and 'open mouth phee' calls as these had previously been shown to relate to arousal (Epple, 1968) and were easy to distinguish. However, other calls such as the 'closed mouth phee' call were not recorded as, although they could be heard, it was not always possible to determine which individual marmoset was calling.

As reported previously, the laboratory was noticeably quieter in the afternoon when husbandry activities were rarely carried out, suggesting that vocalising was at least partly influenced by human activity. In addition, during the training / contact phase, the marmosets in room 6 appeared to be getting calmer and quieter, a change distinct enough to be noticed and commented on by laboratory staff. To gain an objective measure of this change, sound levels in room 6 were measured and compared to those in room 5 where the composition of marmoset groups was similar. Details of the population of each room are given in Table 7.2.

Table 7.2 Details of marmosets housed in colony rooms 5 and 6 by group composition.

Number of marmosets by group size	**Room 5**	**Room 6**
Single housed	3	2
Pair housed	44	52
Group of three	6	6
Group of four	4	0
Total	**57**	**60**

Data from both rooms were collected on the same day, chosen to ensure the same basic cleaning procedure was carried out in both rooms (see Chapter 5) and that no additional cleaning procedures such as changing the woodshaving substrate or cage rotation were scheduled. Sound levels were measured using a Brüel and Kjaer electronic sound-meter set on fast. Measurements were taken every 30 minutes, beginning at 1000h and finishing at 1700h, with the room order counterbalanced across sample points. On each occasion, I entered the room and stood in the same central position, immediately taking the first reading. I then stood quietly for five minutes then recorded the sound level for a second time.

7.2.4 Statistical Analysis

Observational data: For each pair, the percentage of the total activity budget spent engaged in each behaviour was calculated. For behavioural categories where all occurrences were recorded, the mean frequency per pair per 10 minute observation period was calculated using the scan sampling data. Data were found to be normally distributed throughout and hence parametric tests were used. A mixed-design ANOVA was performed with two within factors: Stage (pre-training or post-training) and Time (morning or afternoon); and one between factor: Group (trained or contact). In instances where equal variances could not be assumed, as indicated by a significant result of Mauchly's Test of Sphericity, any significant outcome was determined using the more conservative Greenhouse-Geisser test (Howell, 1995). Significance was set at $p < 0.05$ throughout the analyses.

Sound recording: Two matched-sample t-tests were performed, the first comparing sound levels recorded immediately upon entry into each room and the second comparing sound levels recorded five minutes later. As above, significance was set at $p < 0.05$.

7.3 RESULTS

7.3.1 Activity budgets

The results for the behavioural categories recorded using instantaneous scan sampling are presented first, followed by those recorded as 'all occurrences'. In each case, the means and standard errors for all categories are presented first, followed by the ANOVA results from all categories where significant results were found with those categories producing no significant results presented last. For clarity, the initial results are presented over two graphs. The mean percentage of sample points spent

engaged in each behavioural category recorded using instantaneous scan sampling for both the trained and contact groups are shown in Figures 7.2 (morning observations) and 7.3 (afternoon observations).

Figure 7.2 Percentage sample points spent in each behavioural category for the trained and control groups during morning observations, before and after treatment. (bars represent standard errors)

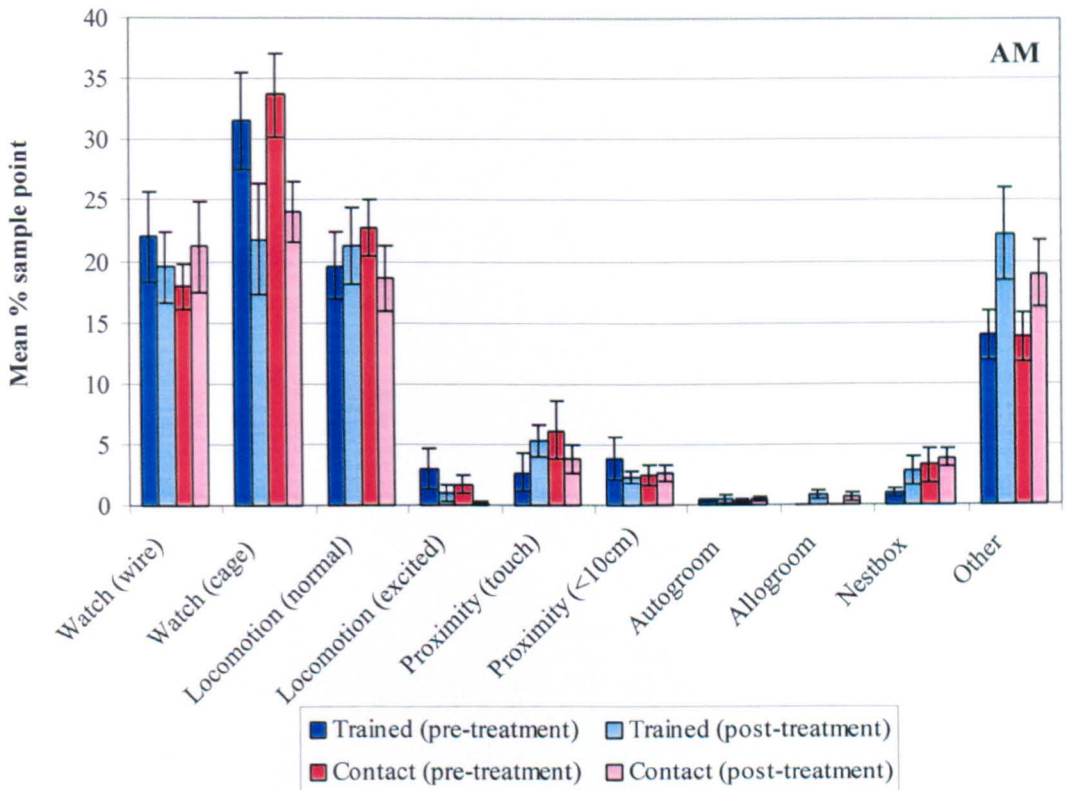
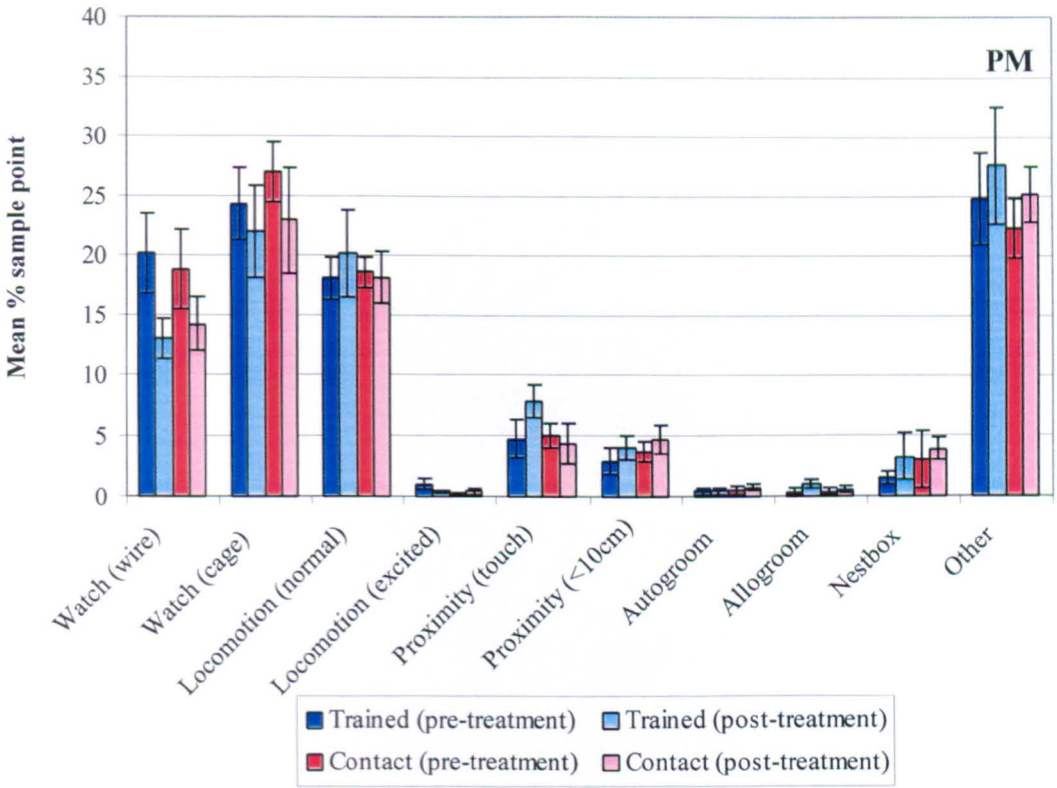


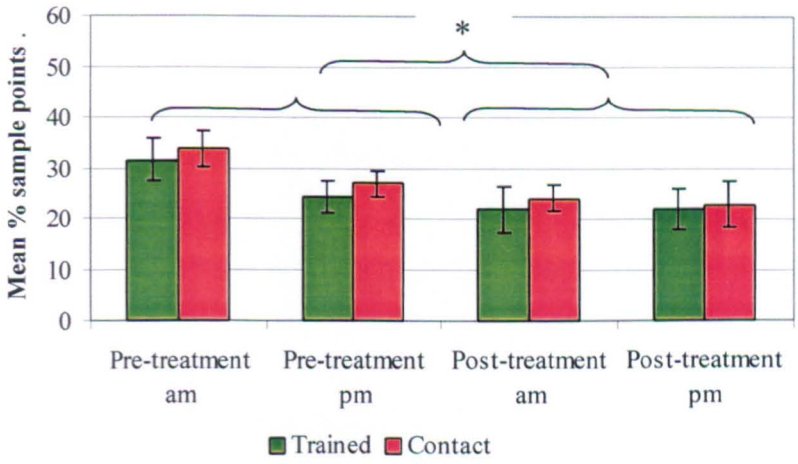
Figure 7.3 Percentage sample points spent in each behavioural category for the trained and control groups during afternoon observations, before and after treatment. (bars represent standard errors)



Effect of training / increased positive contact.

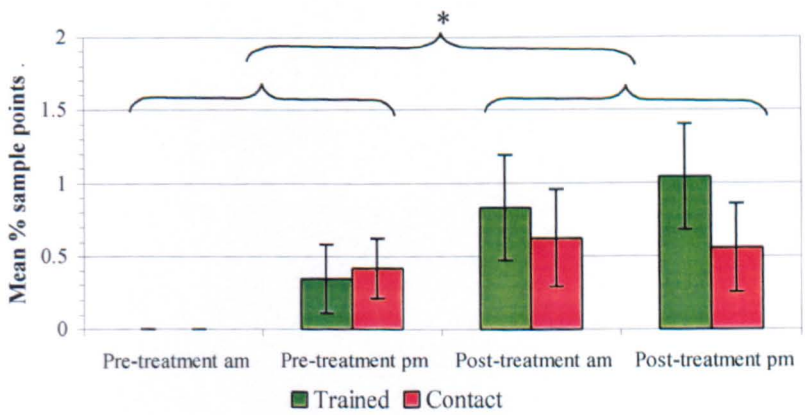
The marmosets spent significantly less time watching the observer from inside the cage after treatment ($F_{1,16} = 8.39$; $p = 0.01$), with no difference between the trained and contact groups ($F_{1,16} = 0.3$; $p = 0.59$) (see Figure 7.4). More time was spent allogrooming post-treatment ($F_{1,16} = 6.05$; $p = 0.03$) with no difference between groups ($F_{1,16} = 0.65$; $p = 0.43$) (see Figure 7.5). A similar pattern was found in the "other" category ($F_{1,16} = 8.12$; $p = 0.01$), again with no difference between groups ($F_{1,16} = 0.43$; $p = 0.52$) (see Figures 7.6).

Figure 7.4 Mean percentage sample points spent watching the observer from inside the cage (Watch; cage) pre- and post-treatment (training or contact). (bars represent standard errors).



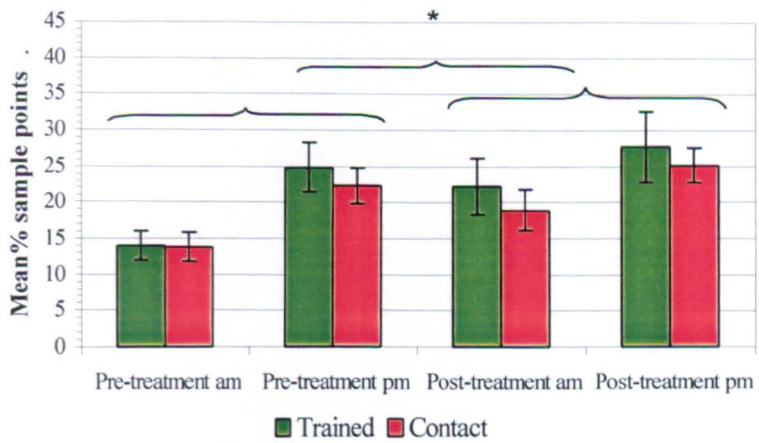
* denotes significant difference between categories

Figure 7.5 Mean percentage sample points spent "allogroom" pre- and post-treatment (training or contact). (bars represent standard errors)



* denotes significant difference between categories

Figure 7.6 Mean percentage sample points engaged in 'other' behaviours pre- and post-treatment (training or contact). (bars represent standard errors)



* denotes significant difference between categories

Results of other behavioural categories

There were no significant differences between the trained and contact groups in any category. There were two significant effects of time. The marmosets spent more time watching the observer when clinging to the wire cage front during morning observations ($F_{1,16} = 7.90$; $p = 0.01$). More time was spent engaged in 'other' behaviours during afternoon observations ($F_{1,16} = 19.89$; $p < 0.01$). Table 7.3 contains a summary of the results where no significant effects of training or increased contact were found.

Table 7.3 Results of ANOVAs for behavioural categories where no significant main effect of treatment (training or contact) was found.

Behavioural category	Factor	$F_{1,16}$ =	p =
Watch (wire)	Stage (pre vs. post treatment)	1.83	0.19
	Time (am vs. pm)	7.90	0.01*
	Group (trained vs. contact)	0.06	0.82
Locomotion (normal)	Stage (pre vs. post treatment)	0.01	0.91
	Time (am vs. pm)	2.46	0.14
	Group (trained vs. contact)	0.01	0.91
Locomotion (excited)	Stage (pre vs. post treatment)	0.93	0.35
	Time (am vs. pm)	0.64	0.43
	Group (trained vs. contact)	2.18	0.16
Proximity (touch)	Stage (pre vs. post treatment)	0.19	0.67
	Time (am vs. pm)	0.73	0.41
	Group (trained vs. contact)	0.04	0.84
Proximity (< 10cm)	Stage (pre vs. post treatment)	0.10	0.76
	Time (am vs. pm)	3.74	0.07
	Group (trained vs. contact)	0.01	0.92
Autogroom	Stage (pre vs. post treatment)	1.19	0.18
	Time (am vs. pm)	1.01	0.33
	Group (trained vs. contact)	0.10	0.76
Nestbox	Stage (pre vs. post treatment)	1.28	0.27
	Time (am vs. pm)	0.03	0.86
	Group (trained vs. contact)	2.52	0.13

* $p<0.05$

9.3.2 Behaviours recorded as 'all occurrences'

The mean frequency per observation period and standard error for each behavioural category is presented in Figure 7.7 (morning observations) and Figure 7.8 (afternoon observations).

Figure 7.7 Mean frequency per observation session for each behaviour for trained and contact animals in each experimental condition (morning observations) (bars represent standard errors)

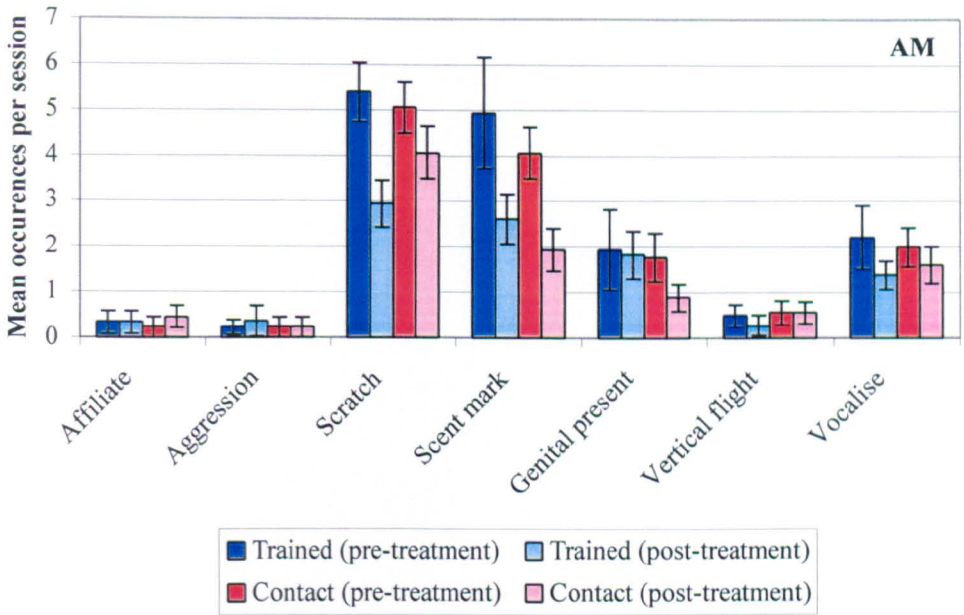
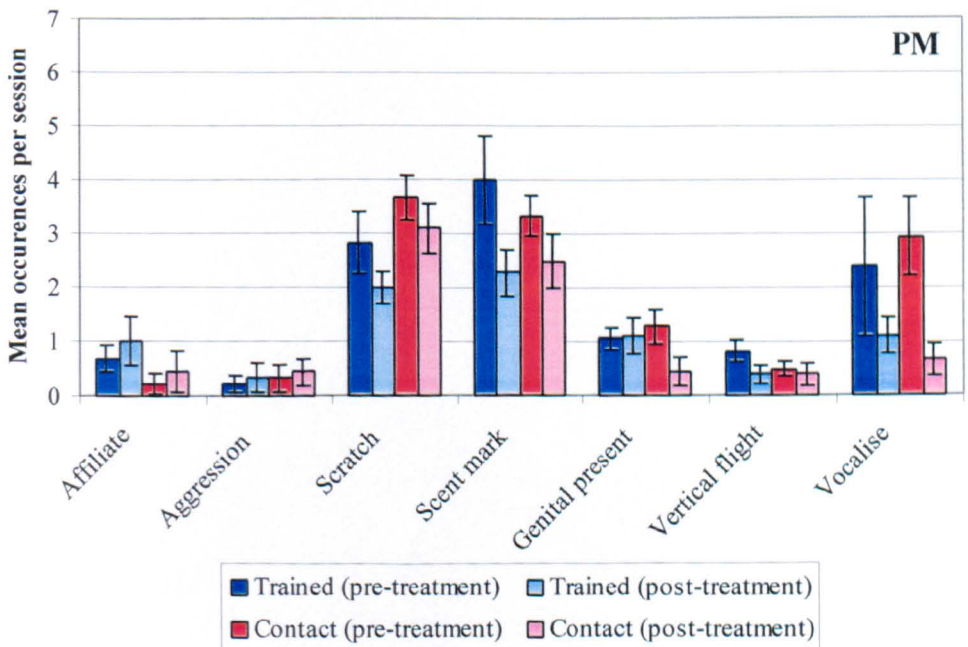


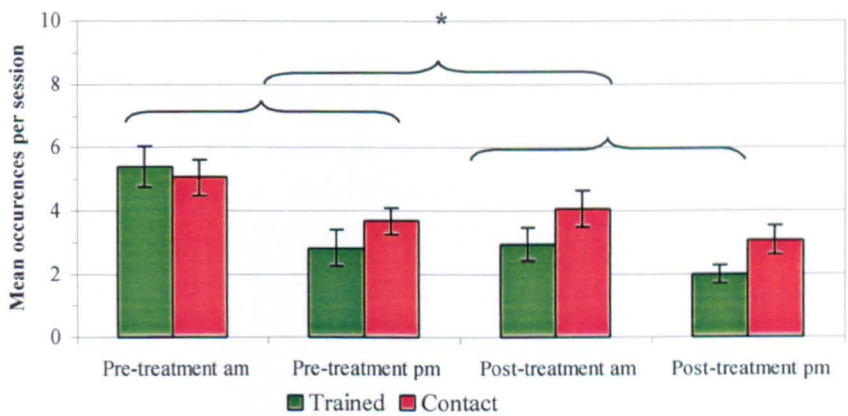
Figure 7.8 Mean frequency per observation session for each behaviour for trained and contact animals in each experimental condition (afternoon observations) (bars represent standard errors)



Effect of training / increased positive contact

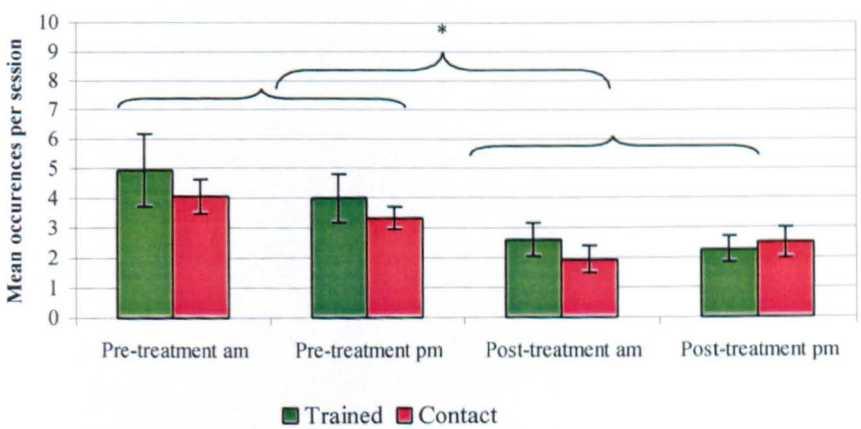
Instances of scratching were significantly lower after treatment ($F_{1,16} = 8.12$; $p = 0.01$) with no significant difference between the trained and contact groups ($F_{1,16} = 3.23$; $p = 0.09$) (Figure 7.9). Scent marking also decreased ($F_{1,16} = 19.55$; $p < 0.01$) with no differences between groups ($F_{1,16} = 0.44$; $p = 0.52$) (Figure 7.10).

Figure 7.9 Mean occurrences per observation session of 'scratch' pre- and post-treatment (training or contact). (bars represent standard errors).



* denotes significant difference between categories

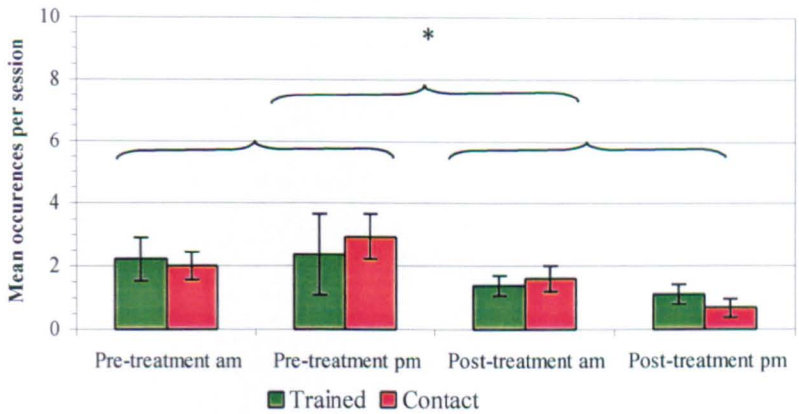
Figure 7.10 Mean occurrences per observation session of 'scent mark' pre- and post-treatment (training or contact). (bars represent standard errors)



* denotes significant difference between categories

Vocalisation also decreased ($F_{1,16} = 6.03$; $p = 0.03$) with no significant differences between the trained and contact groups ($F_{1,16} = 0.003$; $p = 0.96$) (Figure 7.11).

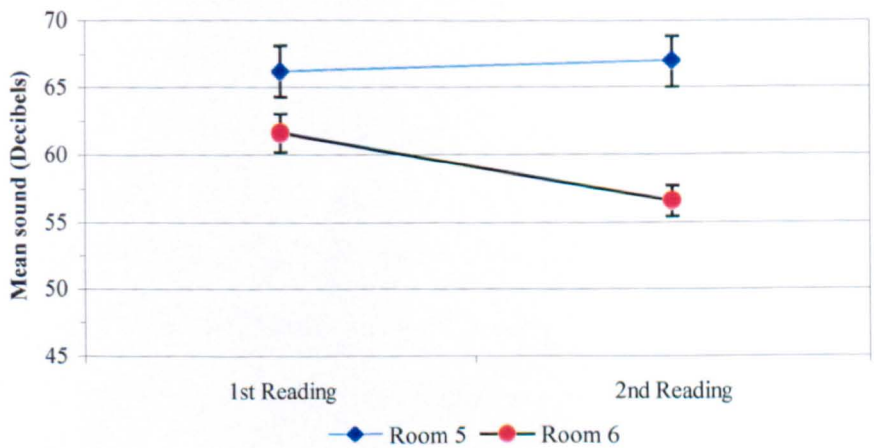
Figure 7.11 Mean occurrences per observation session of 'vocalise' pre- and post- treatment (training or contact). (bars represent standard errors)



* denotes significant difference between categories

When sound levels recorded in room 5 and room 6 were compared, there was no difference in the sound levels recorded immediately on entry ($t_{(15)} = 2.19$; $p = 0.05$). However, sound levels in room 6, where the experimental animals were housed, were significantly lower when recordings were taken five minutes after entry ($t_{(15)} = 5.82$; $p < 0.001$) (see Figure 7.12).

Figure 7.12 Mean sound levels (decibels) recorded in rooms 5 and 6 upon entry and after 5 minutes elapsed. (bars represent standard errors)



Behavioural categories where no effect of training / increased contact was found.

Two categories showed a significant effect of time. Instances of scratch were lower during afternoon observations ($F_{1,16} = 24.87$; $p < 0.001$). Affiliative behaviours were more frequent during afternoon observations ($F_{1,16} = 5.55$; $p = 0.03$). There was no effect of time in any other category and no differences between the trained and contact groups. The results of the ANOVAs for categories with no effect of treatment are given in Table 7.4.

Table 7.4 Results of ANOVAs for behavioural categories where no significant main effect of treatment (training or contact) was found.

Behavioural category	Factor	$F_{1,16}$ =	p =
Affiliate	Stage (pre vs. post treatment)	1.03	0.33
	Time (am vs. pm)	5.55	0.03*
	Group (trained vs. contact)	0.09	0.76
Aggression	Stage (pre vs. post treatment)	0.28	0.60
	Time (am vs. pm)	0.29	0.60
	Group (trained vs. contact)	0.02	0.88
Genital present	Stage (pre vs. post treatment)	1.55	0.23
	Time (am vs. pm)	3.84	0.07
	Group (trained vs. contact)	1.70	0.21
Vertical flight	Stage (pre vs. post treatment)	1.90	0.19
	Time (am vs. pm)	0.26	0.62
	Group (trained vs. contact)	0.001	1.0

* $p < 0.05$

7.4 CONCLUSIONS

During observation periods, the marmosets spent a considerable amount of time watching the observer. Overall, when the means for the two 'watch observer' categories were combined, these behaviours accounted for nearly 44 per cent of the activity budgets of both the trained groups and contact groups. Although the amount

of time spent watching from the front of the cage remained unchanged, the marmosets did spent less time watching from within the cage after treatment. As stated previously, watching human activity could be interpreted as either interest (Ely *et al.*, 1997) or vigilance in the presence of a perceived threat (Caine, 1992; Hampton *et al.*, 1966). The fact that it was watching from within the cage that decreased, a behaviour shown more frequently by nervous animals or when humans approached closely, suggests that the marmosets were more relaxed and therefore less vigilant. This interpretation is supported by the finding that the reduction in time spent watching was matched by an increase in time spent in other behaviours such as foraging, exploring and resting, rather than behaviours that could indicate arousal or fear such as excited locomotion or hiding in the nestbox. The fact that time spent engaged in 'other' behaviours was greater during afternoon sessions is probably explained by the inclusion of foraging in this category. As morning sessions were conducted before the marmosets were fed, no fresh food was present at this time.

Allogrooming also increased although overall grooming was rarely observed with combined scores for both self and social grooming accounting for less than 1 per cent of all observations. Social grooming bouts were also very brief as they were never recorded over two consecutive sample points, indicating that no bout lasted longer than 15 seconds.

Affiliative and aggressive behaviours were unaffected. Affiliative behaviours occurred more frequently during afternoon observations and, as with "other" behaviours, this may be due to the fact that food was present at this time. The 'affiliative' category included food sharing and this was one of the most commonly observed behaviours. Instances of 'genital present' and 'vertical flight' were low and no significant differences were found between pre- and post-training / contact

observations. However, instances of 'scratch', 'scent mark' and 'vocalise', all behaviours previously shown to indicate arousal (Epple, 1968, 1970; Maestripieri *et al.*, 1992; Moodie & Chamove, 1990; Sutcliffe & Poole, 1978) all decreased, suggesting that the marmosets were more relaxed following treatment.

Overall, the changes observed in the activity budgets were not pronounced and no evidence was found to support the belief that either training or increased contact with humans has a major negative impact on the social behaviour of these animals. Nor was there any evidence that the study animals had become 'fixated' on receiving treats. Indeed, the changes that did occur suggested that the impact of human activity was decreased rather than increased. At no point was any difference found between the trained and contact groups suggesting that training does not differ in effect from increasing the amount of positive contact with humans experienced by these animals.

7.5 EXPERIMENT 2 – Effects of training on response to a stressor

7.5.1 Aims

As stated previously, the data reported in this section were collected as part of a collaborative project, the overall aim of which was to validate the use of both behaviour and urinary cortisol as reliable and sensitive measures of stress in the common marmoset (Bassett, Buchanan-Smith, McKinley & Smith, 2003). The value of the study from the perspective of evaluating the effectiveness of PRT was twofold. Firstly, as reported in Chapter 6, if PRT is to be adopted as a refinement to current practice, it must be shown to be a practical alternative. As the success of the collaborative project required that sufficient urine samples be collected, this experiment allowed the trained animals to be tested under 'real life' conditions (see Chapter 6). Secondly, while the first experiment examined the effects of training on the general behaviour of trained marmosets in the presence of humans, this experiment provided both behavioural and physiological data examining the response of trained marmosets to a stressful event, that is, capture for weighing.

Broom and Johnson (1993) identify a number of challenges faced by captive animals as short-term problems in the sense that they consist of events lasting a few hours or less. Examples provided include human interventions such as close approach, handling, certain training methods, transport, operations, accidents, attacks and threats. In this sense, the routine laboratory and husbandry procedures that PRT techniques are employed to facilitate can be viewed as short-term stressful events. Measurements of activity in the sympathetic-adrenal-medullary system and in the hypothalamic-pituitary-adrenal cortex system can provide useful indicators of how well animals cope with such events (Broom & Johnson, 1993). These systems produce an increase in andrenocortical activity in response to the perception of

aversive or threatening situations – the so-called 'stress' or 'fight or flight' response (Barnett & Hemsworth, 1990; Carlson, 1998). During such a response, the sympathetic branch of the autonomic nervous system becomes active, stimulating the adrenal medulla to secrete epinephrine, norepinephrine and steroid stress hormones including the glucocorticoid hormones that effect glucose metabolism. When a threat is perceived by the animal, glucocorticoid hormones perform of functions including breaking down protein for conversion to glucose, making fats available for energy, increasing blood flow and stimulating behavioural responsiveness (Carlson, 1998). In rodents and domestic poultry, the predominant glucocorticiod produced in response to a stressor is corticosterone while in most ungulates, dogs, cats and primates it is cortisol (Broom & Johnson, 1993).

The techniques used to analyse cortisol levels are relatively straightforward (Bahr, Palme, Möhle, Hodges & Heistermann, 2000) and as a result, measurements of this hormone have become a valuable indicator of welfare, particularly in response to environmental stressors. Moreover, cortisol has been shown to produce a graded response with levels increasing with the severity of the stressor (Smith & French, 1997). Although measurements of cortisol can be taken from blood plasma, as discussed previously, procedures used to collect blood can themselves lead to elevations in cortisol levels (Crockett *et al.*, 1993; Dettmer *et al.*, 1996; Reinhardt *et al.*, 1995). However, analysis can also be carried out using saliva, urine and faeces, all of which can be collected using non-invasive techniques (Broom & Johnson, 1993; Lutz *et al.*, 2000). Such measurements have been used to assess the welfare of a wide variety of domestic species including pigs (Baldwin & Stevens, 1973), cattle (Johnson & Buckand, 1976; Wohlt, Allyn, Zajac & Katz, 1994) and sheep (Kent, Molony &

Robertson, 1993; Parrott, Thornton & Robertson, 1988) and dogs (Beerda, Schilder, Janssen & Mol, 2000).

Cortisol analysis has also been used to assess the welfare and responses to a variety of stressors in a wide range of primate species including small-eared bushbabies *(Otolemur garettii)* (Watson, Ward, Davis & Stavisky, 1999); capuchin monkeys *(Cebus apella)* (Dettmer *et al.,* 1996); squirrel monkeys *(Saimiri sciureus)* and titi monkeys *(Callicebus dubius)* (Hennessy, Mendoza, Mason & Moberg, 1995); hamadryas baboons *(Papio hamadryas)* (Goncharov, Tanarov, Antonichev, Gorlushkin & Aso; 1979); cynomolgus monkeys *(Macaca fascicularis)* (Clarke, Czekala & Lindburg, 1995; Laudenslager *et al.,* 1999; Stavisky, Adams, Watson & Kaplan, 2001); pigtailed macaques *(M. nemestrina)* (Crockett, Shimoji & Bowden, 2000); lion-tailed macaques *(M. silenus)* (Clarke *et al.,* 1995), rhesus macaques *(M. mulatta)* (Capitanio *et al.,* 1996; Pun *et al.,* 1981; Reinhardt *et al.,* 1995) and stumptailed macaques *(M. arctoides)* (Kling & Orbach, 1963b).

In callitrichid species, corsisol levels have been shown to rise following capture in male cotton-top tamarins (*Saguinus oedipus)* (Ziegler, Wegner & Snowdon, 1996) although Ziegler, Scheffler and Snowdon (1995) found that in females, cortisol levels were not significantly increased on the day following capture although levels were elevated on 54 per cent of occasions. However, this study only analysed first void urine samples and so, depending on when capture occurred, there may have been a considerable temporal dissociation between the stressor, the physiological response and collection of urine for analysis. Elevated cortisol levels have been found in Weid's black tufted-ear marmosets *(Callithrix kuhli)* following transfer to a new laboratory (Schaffner & Smith, 1999), separation and transfer to novel housing (Smith, McGreer-Whitworth & French, 1998), isolation and restraint (Smith &

French, 1997). In common marmosets, similar elevated levels of cortisol have been found in blood plasma samples following isolation in a novel environment (Norcross & Newman, 1999).

Although physiological measurements can provide useful indicators of welfare, it should be noted that indices such as cortisol levels are measures of arousal and can occur in response to a number of factors in addition to environmental stressors. They can fluctuate due to diurnal variation (Sousa & Ziegler, 1998), the reproductive cycle (Ziegler *et al.,* 1995) and events such as mating (Szechtman, Lambrou, Caggiula & Redgate, 1974). However, taken in conjunction with behavioural measures to allow interpretation of any changes found, methods such as cortisol analysis can in turn add validity to such behavioural measures (Broom & Johnson, 1993).

7.5.2 Study animals

The study animals were 24 common marmosets, 12 males and 12 females, housed at the MRC Human Reproductive Sciences Unit, Edinburgh. Details of housing and husbandry routines are given in Chapter 6. The study animals were divided into two groups. The trained group were the six male/female pairs described in Chapter 6, Study 2, all housed in colony room 6. The control group in this experiment were six naïve male/female pairs selected from animals housed in colony room 5. None had been housed in room 6 during the period of observations and none had experienced increased human contact as described previously. Contact with myself was limited to that experienced during routine feeding and occasional cleaning. When data collection began, the mean age of the trained animals was 1188 days (approx. 3 years 3 months, ± S.E. 232.37 days). The mean age of the control

group was 989 days (approx. 2 years 10 months, ± 145.55 days). There was no significant difference in age between the two groups ($t_{(12)} = 0.72$; $p = 0.13$). None of the females was past the first trimester of pregnancy as determined by transabdominal uterine palpation. Details of the study animals are given in Table 7.5.

Figure 7.5 Details of study animals including name or I.D. number, sex, age at start of study and relationship to cage-mate.

Pair	Name	Sex	Age	Relationship
*C	Cecil	M	2yr 4mth	Siblings
	Coco	F	1yr 11mth	
*F	Freddie	M	2yr 6mth	Siblings (twins)
	Foxy	F	2yr 6mth	
*I	Iggy	M	2yr 6mth	None
	Iris	F	3yr 7mth	
*J	Jambo	M	6yr 8mth	Father/daughter
	Jilly	F	1yr 6mth	
*K	Kipper	M	1yr 6mth	Siblings
	Keltie	F	1yr 11mth	
*L	Leo	M	2yr 8mth	None
	Lala	F	2yr 6mth	
♦A5	864BK	M	1yr 9mth	None
	678R	F	7yr 9mth	
♦B5	878BK	M	1yr 7mth	Siblings (twins)
	971R	F	1yr 7mth	
♦C5	788BK	M	3yr 2mth	None
	902R	F	2yr 6mth	
♦D5	870BK	M	1yr 9mth	None
	685R	F	7yr 6mth	
♦E5	804BK	M	2yr 10mth	None
	909R	F	2yr 5mth	
♦F5	802BK	M	2yr 10mth	None
	940R	F	2yr 1mth	

* Denotes trained group, housed in room 6
♦ Denoted control group, housed in room 5

Training to target for in-homecage weighing and provide urine samples as described in Chapter 6 was completed by 3rd November 2000 and collection of urine for this experiment began on 1st February 2001. When the animals were fully trained, morning sessions that had been discontinued for pairs with an aggressive member were resumed. By this time, aggressive animals had learned that they would only receive a reward in their own corner (left side of the cage for males, right for females) and no further instances of aggression occurred. Once a behaviour is established, it is important that it is routinely repeated so that it does not become extinguished (Mellen & Ellis, 1996). During the intervening period, a practice session was performed with each of the trained pairs once each week. When possible, practice sessions were conducted when one of the technicians was in the room in order to accustom the marmosets to working in the presence of another person. There was a three week period without practice during December with no deterioration in performance.

7.5.3 Procedure

Observational data

Throughout the data collection period, all marmoset handling and urine collection was carried out by myself while behavioural data were recorded by Lois Bassett using her own protocol. During data collection, she stood (when observing animals housed in upper-tier cages) or sat on the floor (lower-tier cages) around 1.5 metres from the front of the cage. As with myself, habituation never completely occurred and the marmosets spent a considerably amount of time watching her. Data collection sessions lasted for 5 minutes. Data were recorded on a palm top computer using THE OBSERVER 3.0 software. As this software does not allow simultaneous scan and all occurrences sampling, all behaviour was recorded using instantaneous

scan sampling with an interval of 15 seconds between sample points. In order to establish that it was valid to use instantaneous sampling to record behavioural events, behaviours of relatively short duration, correlations were carried out on data sets generated during observations of the trained. These examined the relationship between frequencies of behaviours (self-scratch, scent mark and vocalise) recorded by instantaneous sampling with those simultaneously recorded using all occurrences recording. There were significant correlations between the scores obtained by the two sampling methods for all three behavioural categories (self-scratch, Pearsons $r = 0.77$; $n = 6$; $p < 0.05$; scent mark, $r = 0.99$; $n = 6$; $p < 0.001$; vocalise, $r = 0.99$; $n = 6$; $p < 0.001$, all tests one tailed). It was therefore considered valid to use instantaneous sampling throughout.

Behaviours recorded included: inactive, alert and watching the observer (henceforth referred to as 'watch observer'); inactive, alert and not watching the observer (referred to as 'inactive, alert'); 'locomote'; 'self-scratch'; 'scent mark'; 'vocalise' and 'forage'. An 'other' category was used and included behaviours such as inactive, inalert behaviour and allogrooming. In addition, for each scan, it was recorded whether the animal was in or out of the nestbox. Behavioural definitions are given in Table 7.6.

Table 7.6 Behavioural categories and definitions used by Lois Bassett during observations of common marmosets. (description of scent marking based on Stevenson & Poole, 1976).

Behavioural category	Definition
Inactive (watching observer)	Animal remains in one location, without engaging in any other activity, whilst watching observer.
Inactive (not watching observer)	Animal remains in one location, without engaging in any other activity. Does not watch observer, but looks at the surroundings or another individual.
Locomote	Animal moves between locations by walking, climbing, running or jumping.
Forage	Animal is engaged in any activity directly related to acquiring or ingesting food.
Nestbox	Animal is in the nestbox
Self-scratch	Animal scratches itself with a hand or foot.
Scent mark	Animal sits and rubs anogenital area on branch or other area of enclosure (anal scent mark), or rubs sternal area along substrate (sternal scent mark).
Vocalise	Animal emits any kind of vocalisation audible to observer. Animal must also be seen to vocalise for this behaviour to be scored.
Other	Any behaviour not otherwise listed (e.g. allogrooming).

Data collection protocol

The first period of data collection was carried out to examine whether circadian rhythms were associated with fluctuations in behaviour and levels of urinary cortisol throughout the day. Behavioural data and urine samples were collected from the trained animals every hour from 0900h – 1600h. A total of at least eight urine samples per animal and eight behavioural samples per pair were obtained. Data from different pairs were collected on different days over a two week period. Urine samples were collected over a range of 3-7 days and behavioural data over a range of 2-8 days. When more than one urine sample was obtained per animal for any single hour (i.e. a sample obtained at 1100h on six different days), a mean was used for the purpose of the analysis. Following data collection on the day that the stressor was administered (see below) behavioural data and urine samples were collected at 1000h

for a further three days (1, 2, and 7 days post-stressor). Some of these data were used part of an investigation of stress indicators in the common marmoset (Bassett *et al.*, 2003). The results reported here concern data collected at baseline and on the day that the stressor was administered. For the control animals, baseline observational data were collected at 1000h, 1200h, 1400h and 1600h one day prior to administration of the stressor using the same protocol as that for the trained animals.

Response to a stressor

A mild stressor, capture, removal from the homecage and weighing was administered to the trained and control animals on two consecutive Wednesdays with the trained animals tested first and the control animals the following week. Wednesdays were chosen as this allowed data collection on two subsequent weekdays while avoiding Mondays when the animals were likely to be hungry following fruit reduction over the weekend. The procedure was carried out between 0930h and 1030h.

Throughout, the standard laboratory procedure for capture described in Chapter 6 was followed. Both members of a pair were chased into the nestbox, which was then removed from the cage. The nestbox was then taken to the laboratory procedure room and the marmosets removed one at a time and transferred by hand to a small cage and weighed. To ensure that both groups received identical treatment gloves were used although this was not really necessary when handling the trained animals. Following weighing, the animals were returned to the nestbox which was then replaced in the homecage. As soon as one pair was returned, the procedure was repeated with the next pair. For the trained animals, the procedure took between 4 min and 4 min 30 seconds (mean time 4 min 9 sec per pair; ± S.E. 4.73 sec). For the control animals, the procedure took between 3 min 45 seconds and 4 min 30 seconds

(mean time 4 mins 14 sec; ± S.E. 7.24 sec). There was no significant difference in the amount of time taken to perform the procedure for trained or control pairs ($t_{(10)} = -0.578$; $p = 0.58$).

For both trained and control animals, once the stressor was administered, matching behavioural data were collected at 1200h, 1400h and 1600h. For each trained pair, urine was collected immediately after their behavioural data were recorded. As the stressors were administered at around 1000h and it is known that cortisol takes some time to show in the urine, baseline samples collected at 1000h and samples collected at 1000h the morning after the stressor were included.

7.5.4 Statistical Analysis

Observational data

To ensure statistical independence, a single mean was calculated from both animals in each pair. Data used consisted of mean sample points per session with a total of 20 sample points obtained per pair per five minute observation period.

To test for the effects of stress, a two-factor repeated-measures ANOVA was performed with the first factor: Stress (pre- vs. post-stressor) and the second factor: Time (1200, 1400 and 1600). Separate analyses were carried out for the trained and control groups. In addition, a three-factor mixed ANOVA was performed using behavioural data from both groups to further examine the effect of training and increase the sample size by combining both sets of data. The within factors were: Stress (pre- vs. post-stressor) and Time (1200, 1400 and 1600) with the between factor: Group (trained vs. control). Where significant main effects were found, where appropriate, post-hoc pairwise t-tests with Bonferroni correction were used to identify

where differences lay while controlling against Type II errors (Howell, 1995). Significance was set at $p < 0.05$ throughout.

Cortisol Enzyme Immunoassay (EIA)

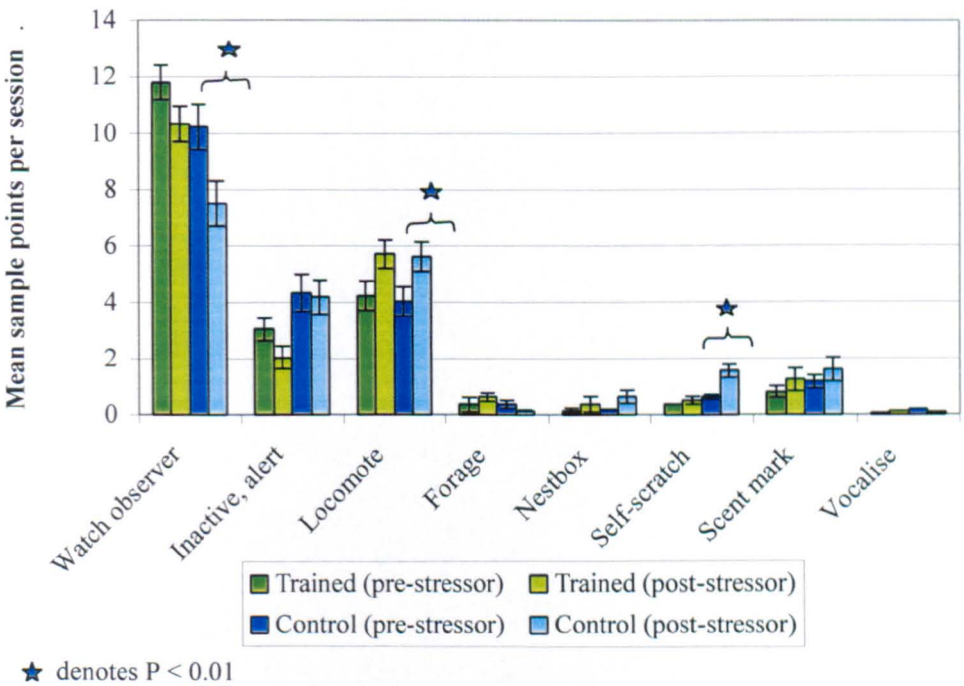
Dr. T. E. Smith measured cortisol concentrations in all urine samples and provided the following details of the procedure used. The enzyme immunoassay was immunologically validated as described by Reimers, Salerno and Lamb (1996). Serial dilutions of four urine pools gave parallel displacement curves with a standard solution. This confirmed that the cortisol in the urine samples was immunologically identical with standard cortisol preparations (from Sigma Chemical company). Recovery of known amounts of cortisol standard (n = 5 stds: 500, 250, 125, 62.5, 31.25 pg/50ul) from high to low concentrations of a urine pool had a mean of 80.83 ± S.E. 1.9 (n = 3 repeats for high pool and 3 repeats for low pool). Intra-assay coefficients of variation for high and low concentration pools were 4.68% and 1.91% respectively (n = 11). Inter-assay coefficients of variation for high and low concentration pools were 9.30% and 14.89% respectively (n = 11). Sensitivity was 1.95 pg/50ul, equivalent to 39 pg/1ml. To correct for urine dilution, creatinine concentrations were quantified for each sample (Tietz, 1976) and cortisol expressed as μg cortisol/mg Cr/ml. To test for the effect of stress on urinary cortisol concentrations, a two-factor within-subjects ANOVA was performed with the first factor: Stress (pre- vs. post-stressor) and the second factor: Time (1200, 1400 and 1600). Significance was set at $p < 0.05$.

7.6 Results

7.6.1 Behavioural data.

The activity budgets of both the trained and control animals, pre- and post-stressor are presented in Figure 7.13.

Figure 7.13 Mean sample points spent performing each behaviour by the trained and control groups, before and after the stressor (collapsed across 1200, 1400 and 1600h) (bars represent standard errors).



Trained and control groups analysed separately

The trained animals showed no significant difference in the amount of time spent watching the observer following administration of the stressor ($F_{1,5} = 5.42$; $p = 0.07$), while the control animals spent significantly less time in this activity ($F_{1,5} = 29.16$, $p < 0.01$). There were no significant differences in the 'inactive, alert' category for either group (trained, $F_{1,5} = 0.96$; $p = 0.37$; control, $F_{1,5} = 0.38$; $p = 0.57$).

There was no significant differences for 'locomote' for the trained animals ($F_{1,5} = 4.00$; $p = 0.10$) while the control animals showed a significant increase in this

activity ($F_{1,5} = 60.06$, $p < 0.01$). The trained animals showed a significant effect of time on observation of 'locomote' ($F_{2,10} = 4.37$; $p < 0.05$). However, following the Bonferroni correction, no significant differences between the individual observation times were found (Table 9.7).

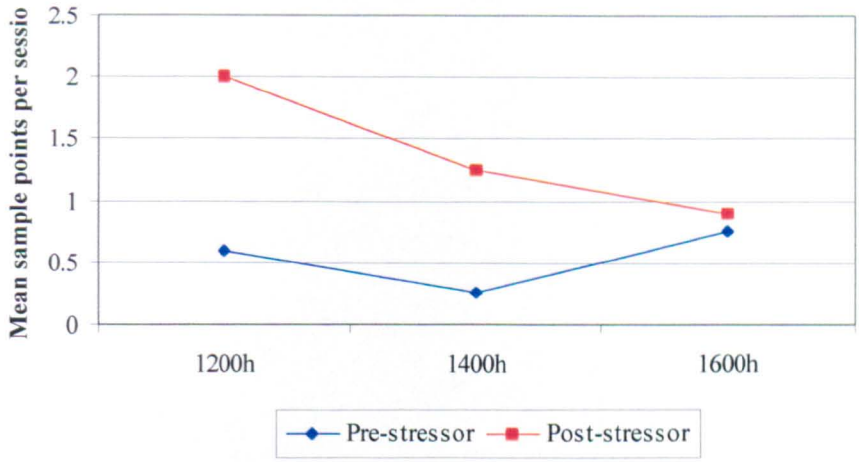
Table 7.7 Post-hoc t-test t and p values for effects of time on 'locomote' (trained animals, pre and post-stressor combined).

Time	**t_5 =**	**p** (uncorrected)	**p** (following Bonferroni correction)
1200 vs.1400h	0.34	0.75	1.00
1200 vs.1600h	3.29	< 0.05*	0.07
1400 vs. 1600h	2.47	0.06	0.17

The amount of time spent foraging was unchanged for both groups (trained, $F_{1,5} = 4.00$; $p = 0.10$; control, $F_{1,5} = 2.07$, $p = 0.21$). Neither trained or control animals showed any significant difference in the amount of time spent in the nestbox (trained, $F_{1,5} = 0.68$; $p = 0.45$; control, $F_{1,5} = 4.05$; $p = 0.10$).

There was no significant difference in self-scratching for the trained animals ($F_{1,5} = 0.63$; $p = 0.47$) while the control animals showed a significant increase in self-scratching following the stressor ($F_{1,5} = 50.37$; $p < 0.01$). The control animals also showed a significant interaction between time and stress ($F_{2,10} = 9.83$; $p < 0.01$) with levels much higher at 1200h than recorded during the pre-stressor observations (see Figure 7.14).

Figure 7.14 Interaction between 'time' and 'stress' for 'self-scratch' (control animals)



There were no significant differences in scent marking for either group (trained, $F_{1,5} = 4.22$; $p = 0.10$; control, $F_{1,5} = 2.42$, $p = 0.18$) and vocalising also remained unchanged (trained, $F_{1,5} = 0.65$; $p = 0.46$; control, $F_{1,5} = 2.50$; $p = 0.18$). Beyond the effect of time on 'locomote' shown by the trained animals and the time by stress interaction shown by the control animals for 'self-scratch', there were no further significant effects of time or time by stress interactions by either group in any behavioural category. A summary of the main effects of stress for all behavioural categories is given in Table 7.8.

Table 7.8 Results of within-subjects ANOVAs of effects of stress on all behaviours for the trained and control groups (collapsed across 1200, 1400 and 1600h).

	Trained group		**Control group**	
Behaviour	**$F_{1,5}$ =**	**P =**	**$F_{1,5}$ =**	**P =**
Watch observer	5.43	0.07	29.16	< 0.01**
Inactive, alert	0.96	0.37	0.38	0.57
Locomote	4.00	0.10	60.06	<0.01**
Forage	4.00	0.10	2.07	0.21
Nestbox	0.68	0.45	4.05	0.10
Self-scratch	0.63	0.47	50.37	< 0.01**
Scent mark	4.22	0.10	2.42	0.18
Vocalise	0.65	0.46	2.50	0.18

Trained and control groups combined; Effects of stress and time

When the main effects of 'stress' were calculated, the marmosets spent significantly less time watching the observer after the stressor than before ($F_{1,22}$ = 9.48; $p < 0.05$). 'Locomote', 'self-scratch' and 'scent mark' all increased (see Table 7.9). There was also a significant main effect of time on the 'watch observer' ($F_{2,22}$ = 5.32; $p < 0.05$) and 'locomote' ($F_{2,22} = 4.12$; $p < 0.05$) categories. The effects of 'stress' and 'time' for all behavioural categories are shown in Table 7.9.

Table 7.9 Results of within-subjects ANOVAs of effects of stress (collapsed across 1200, 1400 and 1600h) and time (1200h, 1400h and 1600h, pre- and post-stressor combined) on all behaviours for the trained and control groups combined.

	Effect of stress		Effect of time	
Behaviour	**$F_{1,22}$ =**	**P =**	**$F_{2,22}$ =**	**P =**
Watch observer	9.48	<0.05*	5.32	<0.05*
Inactive, alert	1.90	0.20	0.96	0.40
Locomote	7.08	<0.05*	4.12	<0.05*
Forage	0.20	0.66	0.08	0.93
Nestbox	1.74	0.22	0.28	0.76
Self-scratch	14.47	<0.01**	2.79	0.09
Scent mark	6.24	< 0.05*	0.44	0.65
Vocalise	0.14	0.72	0.89	0.43

Sig; ** $p < 0.01$; * $p < 0.05$

Interactions between 'stress' and 'time'

There was a significant stress / time interaction ($F_{2,20} = 0.54$; $p < 0.05$) in the 'inactive alert' category (see Figure 7.15). More time was spent engaged in this behaviour pre-stressor for the times of 1200h and 1400h than there was post-stressor. However, by 1600h this pattern was reversed with more time spent in this behaviour post-stressor. The second significant stress / time interaction occurred in the 'self-scratch' category ($F_{2,20} = 5.03$; $p < 0.05$). Pre-stressor rates remained similar over the three time periods while after the stressor rates were higher at 1200h. This difference decreased over time and virtually disappeared by 1600h (see Figure 7.16). There were no significant interactions in any other behavioural category (Table 7.10).

Figure 7.15 Interaction between 'stress' and 'time' for 'inactive, alert' (trained and control groups combined)

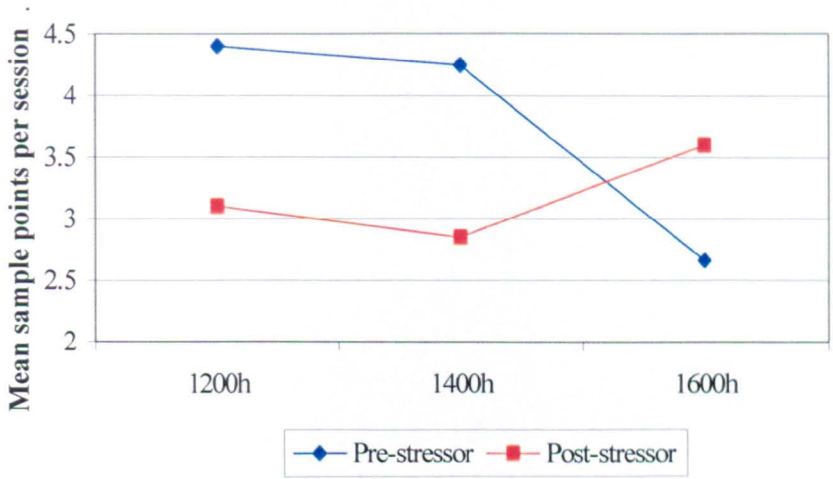


Figure 7.16 Interaction between 'stress' and 'time' for 'self-scratch' (trained and control groups combined)

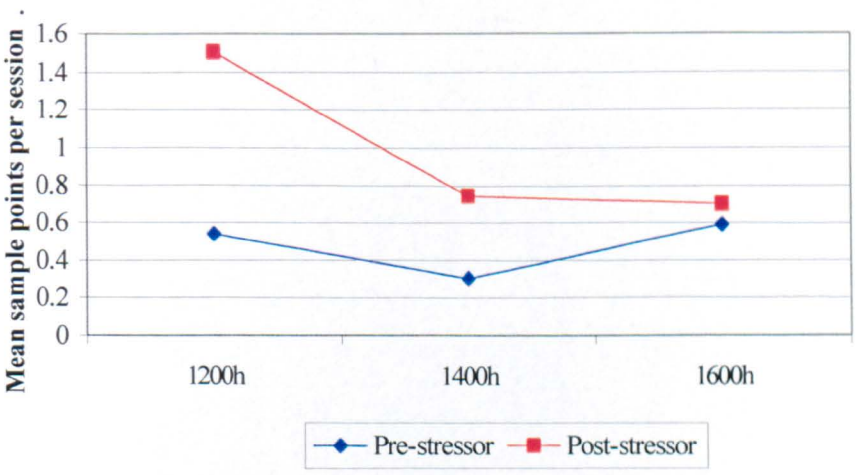


Table 7.10 Results of ANOVAs for interaction between effects of 'stress' and 'time' (1200h, 1400h and 1600h) on all behavioural categories (trained and control groups combined)

Behaviour	**$F_{2,20}$ =**	**p**
Watch observer	0.54	0.59
Inactive, alert	3.62	< 0.05*
Locomote	1.57	0.23
Forage	0.13	0.88
Nestbox	0.30	0.75
Self-scratch	5.03	< 0.05*
Scent mark	0.16	0.86
Vocalise	1.46	0.26

Sig; * $p < 0.05$

Effects of training

When frequencies of all behaviours (pre- and post-stressor combined) were examined, frequencies of 'inactive, alert' and 'self-scratch' were significantly lower in the trained than control animals ($F_{1,10} = 8.33$; $p < 0.05$, $F_{1,10} = 5.17$; $p < 0.05$ respectively). The mean sample points spent engaged in each behaviour are presented in Figure 7.17 and full results of between-subjects ANOVAs of effects of training are given in Table 7.11.

Figure 7.17 Mean sample points spent performing each behaviour for trained and control groups (collapsed across 1200 1400 and 1600h) (bars represent standard errors).

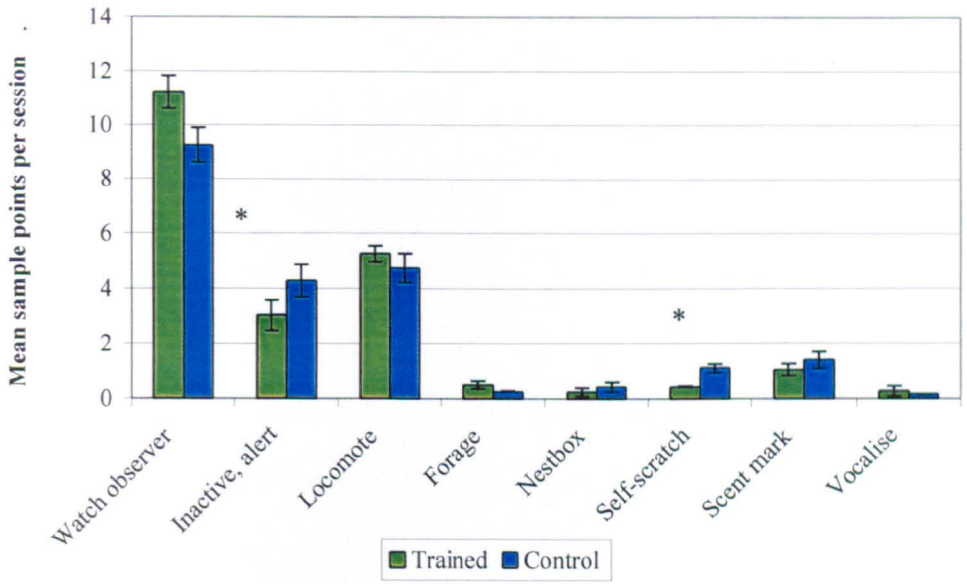


Table 7.11 Results of between-subjects ANOVAs of effects of 'training' on all behaviours (pre- and post-stressor combined, collapsed across 1200h 1400h and 1600h.

Behaviour	**$F_{1,10}$ =**	**P =**
Watch observer	4.23	0.07
Inactive, alert	8.33	< 0.05*
Locomote	0.14	0.72
Forage	0.01	0.91
Nestbox	0.01	0.94
Self-scratch	5.17	< 0.05*
Scent mark	1.46	0.25
Vocalise	0.20	0.66

* $p < 0.05$

There was a significant interaction between 'training' and 'stress' for 'forage' ($F_{1,10} = 5.95$; $p < 0.05$). The trained animals spent more time foraging after the stressor while the control animals spent less time foraging post-stressor (see Figure 7.18). There was also a significant training/stress interaction for 'self-scratch'. Following the stressor, the trained animals showed a slight increase in the amount of 'self-scratch' while the control animals showed a large increase. Although both groups showed very similar levels of this behaviour before the stressor, the control animals scratched considerably more afterwards (see Figure 7.19). Results of all ANOVAs for interactions between 'training' and 'stress' for all behaviours are presented in Table 7.12. There were no significant interactions between 'training'and 'time' for any behaviour and no significant three-way interactions between 'training' 'stress' and 'time' (see Table 7.13).

Figure 7.18 Interaction between 'training' and 'stress' for 'forage' (collapsed Across 1200 1400 and 1600h)

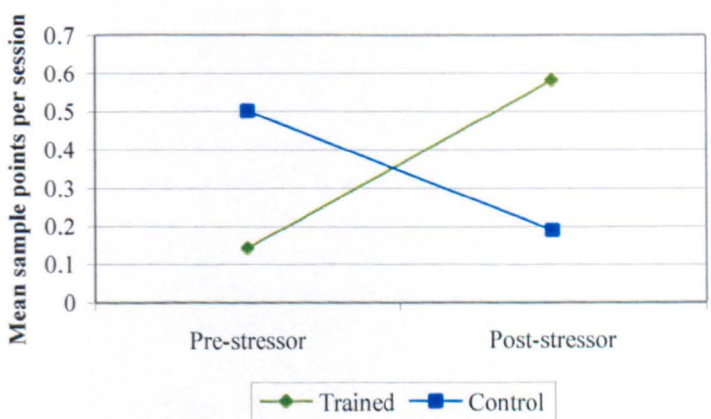


Figure 7.19 Interaction between 'training' and 'stress' for 'self-scratch' (collapsed across 1200 1400 and 1600h)

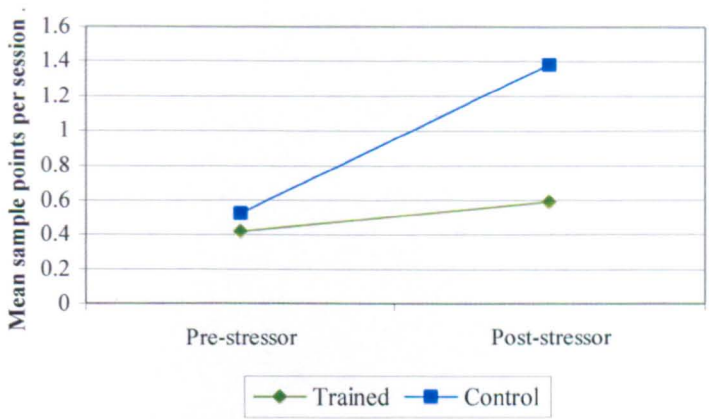


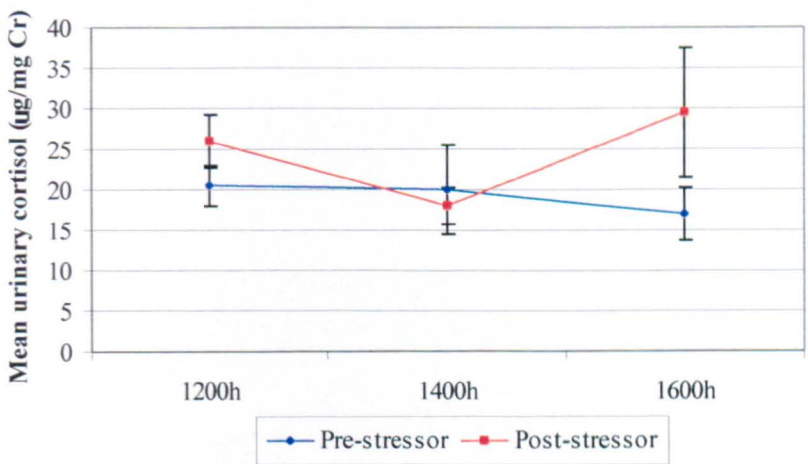
Table 7.13 Results of ANOVAs for interaction between effects of 'training' and 'time' (1200 1400 and 1600h) and three-way interaction between effects of 'training', 'stress' and 'time'(1200 1400 and 1600h) on all behaviours.

	Interaction 'training' and 'time'		**Interaction 'training', 'stress' and 'time'**	
Behaviour	**$F_{2,20}$ =**	**P =**	**$F_{2,20}$ =**	**P =**
Watch observer	1.63	0.22	<0.01	1.00
Inactive, alert	0.85	0.44	0.62	0.55
Locomote	3.44	0.06	0.68	0.52
Forage	1.10	0.35	<0.01	1.00
Nestbox	2.14	0.14	1.06	0.37
Self-scratch	0.07	0.93	2.05	0.16
Scent mark	1.56	0.23	1.09	0.35
Vocalise	2.14	0.14	1.46	0.26

7.6.2 Results of cortisol analysis

When pre-stressor data were compared with data collected on the day the stressor was administered (at 1200 1400 and 1600h), there were no significant effects of time or stress on urinary cortisol ($F_{2,18} = 0.92$; $p = 0.42$ and $F_{1,9} = 4.45$; $p = 0.06$ respectively) (see Figure 7.20).

Figure 7.20 Mean concentrations of urinary cortisol pre-stressor and following administration of the stressor at 1000h (bars represent standard errors)



When pre-stressor cortisol levels at 1000h were matched with values from samples collected at 1000h the day after the stressor and included in the analysis, there was still no significant effect of stress on urinary cortisol ($F_{1,8} = 3.59$; $p = 0.10$). There was a significant effect of time on cortisol concentration ($F_{3,24} = 3.03$; $p < 0.05$) (Figure 7.21). However, post-hoc t- tests using the Bonferroni correction revealed that mean cortisol concentrations were not significantly different at any of the individual time periods (see Table 7.14).

Figure 7.21 Mean concentrations of urinary cortisol pre-stressor and following administration of the stressor at 1000h. Data include 1000h samples collected pre-stressor and the day after administration of the stressor (bars represent standard errors).

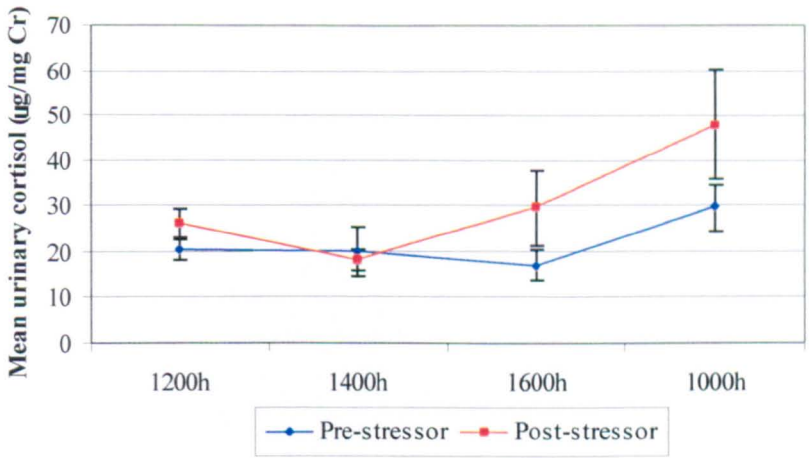


Table 7.14 Results of post-hoc t-tests for concentrations of urinary cortisol at the four different time periods (pre- and post-stressor values combined).

Time periods	**t =**	**p** (uncorrected)	**p** (following Bonferroni correction)
1000h vs. 1200h	2.33	< 0.05*	0.24
1000h vs. 1400h	2.40	< 0.05*	0.21
1000h vs. 1600h	2.05	0.07	0.39
1200h vs. 1400h	1.59	0.14	0.83
1200h vs. 1600h	0.74	0.48	1.00
1400h vs. 1600h	0.45	0.66	1.00

7.7 Conclusions

There were a number of differences between the trained and control animals. Control animals spent less time watching the observer following administration of the stressor and more time locomoting and scratching. Scratching was highest during 1200h observations, the period closest to the time that the stressor was administered. When pre- and post-stressor data were combined, there was still a significant difference in this behaviour with lower levels of scratching observed in the trained animals. The combined data also produced two trained/stress interactions with trained animals again showing less scratching than controls and also spending more time foraging post-stressor while this activity decreased in the control animals.

The trained animals did show some effect after the stressor. When the data from both groups were combined, increasing the sample size, the marmosets spent less time watching the observer and more time locomoting, scratching and scent marking. However, the effect of the stressor on the trained animals was clearly less than that on controls.

Analysis of cortisol levels produced no significant differences between pre- and post-stressor samples. This would appear to support the results of the behavioural observations that suggest that the trained animals were not greatly affected by the weighing procedure.

7.8 DISCUSSION

The results of both experiments support the view that training can help reduce the stress associated with human activity. In the first experiment, stress-related behaviours such as scratching, scent marking and vocalising were lower during post-training observations. The absence of any differences between the trained animals

and those who received increased positive contact with humans suggests that it was this contact that produced the effect rather than additional properties of the training process itself (in the sense of teaching the marmosets to perform a specific behaviour). In both conditions, the animals received food treats, allowing an association between my presence and positive rewards to be formed. This alone can teach animals to tolerate initially frightening stimuli (Laule & Desmond, 1998) and is in effect a very simple form of training.

Throughout both training and positive contact, the marmosets were also talked to. This has been recognised as useful technique by many people who work with animals. Hediger (1992) suggests that while the actual words are unimportant, talking provides a medium of non-verbal communication by activating facial expressions and providing cues through intonation, sound intensity, posture and movement. Hediger also suggests that talking may provide animals with additional cues that are undetectable through human senses.

Of course, both feeding treats and talking to the marmosets meant spending time with them and it is possible that the animals simply habituated to my presence. To explore this possibility, there should have been a third group who experienced the same increase in time spent with them but without any form of interaction. However, given that the other marmosets in the room could observe treats being given to the experimental animals, it could have been unethical to simply stand in front of their cages and given them nothing. Such a practice could have led to frustration and aggression between pair members and compromised the welfare of the animals. The actual practice was to offer a food treat to every marmoset in the room when each day's sessions were completed. As a results, the only suggestion that habituation did not explain the changes in the marmosets' behaviour came from comments by

laboratory staff who had not observed similar changes following any prior observational studies that had been carried out at the MRC unit.

Not all changes in the marmosets' behaviour were regarded as positive by all laboratory staff. One remarked that some of the experimental animals had become difficult to catch in that they no longer entered the nestbox as soon as the front of the cage was opened. This supports the possibility that this behaviour is motivated, at least in part, by fear. Reduced fear of humans has led to difficulties with other animals. For example, tamed pigs have been shown to take longer to move from their pen than those with minimal exposure to humans (Day, Spoolder, Burfoot, Chamberlain & Edwards, 2001). This is not an unfamiliar phenomenon on farms where the majority of animals are herded (effectively a form of chasing) but where well-handled show animals are led or simply come to call. It is possible that training animals for one laboratory procedure may necessitate changes in others. It is also possible that comparing the behaviour of tamed and untamed animals could help identify techniques that rely on fear to be effective and should be refined.

The fact that the control animals did show negative behavioural changes following the standard laboratory procedure used for weighing provides evidence to negate the view that this procedure is quick, easy and does not cause stress. The results show that this is clearly not the case and that this procedure should be replaced. The data presented in Chapter 6 show that target training to allow in-homecage weighing is a relatively easy and practical alternative. In addition, once marmosets have learned to come to their target, this technique could be used to train them to enter a transport box thus removing the need to capture in the nestbox for any reason.

Although the two experiments are not directly comparable due to the differences in methodology, it was interesting that the control, but not the trained animals spent less time watching the observer following administration of the stressor. This result is hard to explain. If watching human activity indicates vigilance, why did these marmosets become less vigilant while performing other behaviours associated with stress? If this behaviour is motivated by interest, why did the control animals find human activity less interesting while interest in other activity in the room remain unchanged? One possibility is that they were simply spending their time engaged in other activities as locomoting and scratching increased. When activity budgets are calculated, an increase in one behavioural category necessitates a decrease somewhere else. During the first experiment, scratching was recorded independently of behaviours such as watching the observer as, although the behaviours can be defined as mutually exclusive, the marmosets are capable of performing both simultaneously. The pre-observation data sheets showed that this was a common occurrence. However, when both behaviour were recorded using point sampling, scratching occurring on the sample point would be noted with no record of what the marmosets were doing while they were scratching.

Throughout the second experiment (pre- and post-stressor), the trained animals scratched less than controls. They also spent less time engaged in 'inactive, alert' behaviours. Beyond that, there were no further differences between the two groups prior to administration of the stressor. It was interesting that there was no difference in the amount of time spent watching the observer, given that this was one of the categories that decreased in the first experiment. Again, the results are not directly comparable. Only six of the original animals participated in the second experiment and there was a different observer and a different definition of the 'watch observer'

category. The position of the observer during data collection was closer to that used during training than that during the original observations. However, the results do suggest that the change in time spent watching the observer recorded during the first experiment was either not strong or failed to generalise to a different situation or observer. The pooled results for the trained and control animals did show a stronger effect for increases in locomoting, scratching and scent marking which suggest that the stressor did affect the trained marmosets to some extent although they were clearly less affected than the untrained animals. In addition, administration of a stressor is an accepted method of validating stress indicators therefore the changes observed in the above categories validates their inclusion among the behaviours recorded throughout this study.

Another encouraging result was that there was no difference in scent marking between the trained and control animals during the second experiment. One concern was that training to provide urine sample by reinforcing a stress-related behaviour would lead to an increase in its performance beyond the training sessions. The data suggest that this did not occur.

Beyond those listed above, there was no additional change in the general behaviour of the trained marmosets. With regard to the views that training or increasing positive contact with humans disrupts the social behaviour of non-human primates and leads to a 'fixation' with gaining food treats from humans, the results do not support these views and found that neither intervention had any significant negative impact on the social behaviour of the study animals. In the first experiment, the reduction in the time spent watching the observer from inside the cage with a subsequent increase in other activities such as foraging and grooming suggests that the marmosets became slightly less interested in human activity.

Of course, pre-training observations suggest that they were strongly interested in human activity before the study began and thus may not reflect the behaviour of marmosets housed under different conditions. However, there was no evidence to suggest that the experimental interventions made the situation worse so fears that training or even increasing contact with humans will lead to animals 'fixated' on humans may not be justified.

The results of urinary cortisol analysis supported the results of behavioural analysis suggesting that the trained animals were relatively unaffected by administration of the stressor. While this was a positive result from the perspective of training, it was unfortunate given that analysis was carried out as part of a collaborative project seeking to validate urinary cortisol as a reliable indicator of stress in the common marmoset. From this perspective, the results could be interpreted as providing no evidence to show that it is. However, given that measures of cortisol have been successfully used in a wide variety of species, it is unlikely that this is the case. A more plausible explanation was that the stressor was too mild to produce a measurable effect in the trained animals. A closely related species, Weid's black tufted-ear marmoset, showed significantly elevated levels in urinary cortisol following administration of a stressor (Smith & French, 1997). However, the stressor in this study was 11 hour isolation in a small cage, whereas the animals in the experiment described here were only removed from their homecage for a matter of minutes. Apart from the few seconds required for transfer to the weighing cage, the marmosets had continuous visual contact with their cage-mate (although this was also true for the control animals). This may also have minimised the effect of the procedure. Smith *et al.* (1998) found that while Weid's black tufted-ear marmosets

showed elevated urinary cortisol levels when housed alone in a novel cage but not when housed in a novel cage in the presence of a heterosexual pair mate.

A further possibility was that the weighing procedure was carried out by the same person who trained the animals and was therefore the person the marmosets were most accustomed to have working with them. With hindsight, it might have been better if the procedure had been carried out by someone else. The behaviour of the control pairs suggests that the procedure was stressful to some extent but not stressful enough for the trained animals to show elevated cortisol levels.

For the vast majority of research, training to reduce the stress associated with experimental procedures promotes the welfare of the study animals and will result in better science (Lehman, 1992; Reinhardt, 1997b, 1999). The results of the experiments reported in this chapter suggest that the benefits of training or increasing positive contact with humans reported in other primate species apply equally to common marmosets.

Chapter 8

General Discussion

"The most striking testimony to the mental advance of the monkeys...is given by the difficulty of making clear emphatic statements about them."

(Thorndike, 1911)

8.1 Animal welfare and aims of thesis

As stated in Chapter 1, the overall aim of this research was to examine the use of training as a means of promoting the psychological well-being of nonhuman primates housed in a laboratory environment. More specifically, the aims were:

1) To examine the training techniques currently used in a UK laboratory by recording the training of macaques in greater detail than currently reported in the literature and assess the techniques used within the framework of operant theory and the effects on the behaviour of the trained animals (Chapter 3).

2) To explore the practicality and welfare implications of training that only used positive reinforcement techniques in a laboratory setting through the training of common marmosets (Chapter 6).

3) To examine the behaviour of the study animals out-with training sessions in order to assess the psychological well-being of the monkeys following the training sessions themselves (Chapter 4) changes in animal/human relationships and manipulation by humans during a standard laboratory procedure (Chapter 7).

These original aims have been met and as a consequence, clear recommendations regarding the use of training can be made.

8.2 Training techniques

8.2.1 Techniques as observed during the training of macaques

Laboratory staff carried out the training of stump-tailed macaques to co-operate during venipuncture during two distinct phases separated by an eighteen-month interval. As reported in Chapter 3, there were considerable differences in the techniques used during Phase 1 and Phase 2 of training. During Phase 1, reinforcement was both slow and erratic with unwanted behaviours rewarded on occasion. Training was not particularly successful in that no consistent decline in aggressive of fearful behaviours was detected and the monkeys became increasing reluctant to enter the cages where training occurred. This conclusion was supported by observations of the macaques' behaviour following training sessions (see below). During Phase 2, reinforcement was applied more often (including rewards for entering the cages and tolerating the squeeze mechanism) and was delivered faster, consistently and appropriately. Although the use of descriptive rather than inferential statistics limits the confidence that can be placed in the results, the differences were considerable. The mean delay between performance of a desired behaviour and delivery of a food reward was 19.13 seconds during phase 1 and 4.67 seconds during phase 2. During Phase 1, 16.5 per cent of the food rewards were delivered following performance of an unwanted behaviour. This never occurred during Phase 2.

Overall, the techniques could not be said to elicit truly voluntary co-operation by the study animals. They were confined in a relatively small cage with the available area further reduced by the use of the squeeze back which itself appeared to be the most aversive element in the entire procedure. The macaques' legs were drawn through the cage door and, although some movement was allowed, they were not

released until venipuncture had been completed. However, the technique observed was similar to that pioneered by Viktor Reinhardt and the welfare benefits of this practice over forced restraint following removal from the homecage has been well documented (reviewed by Reinhardt, 1997a; Reinhardt *et al.*, 1995). In addition, the negative reactions shown by the macaques disappeared once additional rewards were introduced, suggesting that they had been successfully desensitised (Laule, 1999). The technique observed was neither forced restraint (as the term is applied to traditional techniques) nor was it truly PRT (in the sense that a certain amount of coercion was used and the monkeys were not free to withdraw from the training process. I coined the term 'engineered compliance' to refer to techniques that are not entirely voluntary but where co-operation is rewarded and aversive elements balanced by the provision of food. When this was done consistently, there was a considerable improvement in the reactions shown by the study animals. Moreover, they continued to voluntarily enter the cages for the seven-week duration of the study.

One additional important factor was the pre-existing good relationships between the macaques and their caregivers (Waitt *et al.*, 2002). Reinhardt (1997c) suggests that macaques are quick to forgive when such a relationship exists and this certainly appeared to be the case with the present study animals. Following release back into the gang room, they would approach the trainer without hesitation if, for example he/she entered to provide their normal food or distribute treats. This behaviour was observed during both phases.

Venipuncture is an invasive procedure and it is possible that training for co-operation during this procedure may require a certain degree of coercion. Successful training carried out solely using PRT has only been reported in two cases (Laule *et al.*, 1996; Priest, 1990). Both required a substantial time investment. The

chimpanzee trained by Laule *el al* (1996) was hand-reared and the drill trained by Priest (1990) was confined in a relatively small area during the initial stages of training. At present, PRT alone has not been demonstrated to be a practical means of training for co-operation during venipuncture and the technique pioneered by Reinhardt may represent a reasonable compromise. However, both the existing literature and the observations reported in Chapter 3 suggest that subtle differences in the way in which training is carried has a considerable effect on both effectiveness and welfare. In his most recent article, Reinhardt (2003) recommends that time be taken to establish a close and trusting relationship before any training begins. He then recommends that additional time be taken to introduce the squeeze mechanism while providing food treats, thus desensitising the animals to this part of the procedure before any attempt to take a limb is made. The observations reported in Chapter 3 support such recommendations.

Following Reinhardt's work in training for co-operation during venipuncture from early reports (Vertain & Reinhardt, 1989) to the present day (Reinhardt, 2003) reveals a constant process of refinement and evaluation. In order to understand the refinement process, there are a number of factors that should be considered. As detailed in Chapter 1, Weinberg and Levine (1980) report that four psychological variables mediate the response to environmental stressors shown by animals. To recap, these include:

- The predictability of stressors
- The ability of animals to exert control over, or make coping response during stressful situations

- The effects of information available to the animal immediately following its response to aversive stimuli which indicate that the correct response has been made, and
- The previous history of the animal with regard to the above factors.

The use of a consistent procedure during training for co-operation during venipuncture will help to make the process predictable. However, it should be noted that any procedure, no matter how aversive, would become predictable if repeated in a consistent manner (Boccia *et al.*, 1992). Training can also help the animal learn what responses are appropriate with immediate information provided through the provision of rewards (Pryor, 1981). This is one of the reasons why the prompt and consistent delivery of such rewards is so important. However, the use of coercion and especially the squeeze mechanism does limit the degree of control that the animal actually has. This in turn suggests that further refinements to this technique would be desirable.

The reduction of stress (or indeed the promotion of good welfare) most likely depends on numerous different factors. If a technique is demonstrated to be successful in reducing stress, then a closer examination of that technique could help to explain why this occurs and also what factors are not being addressed which in turn could provide a focus for future refinement. As stated above, training for co-operation during venipuncture may be promoting some important factors (for example, making the process predictable, teaching appropriate responses and allowing desensitisation through pairing aversive elements with food treats which in turn promotes a better relationship with the person providing the treats) but is weak in other areas (the animal has little control). Equally, connecting an operant chamber to the homecage to allow free access rather than physically placing an animal in it

(Scott, Pearce, Fairhall, Muggleton & Smith, 2003) gives the animal control in that he/she can choose whether or not to enter, but does nothing to promote animal/human relationships. This is an important omission as the observations reported in Chapter 7 support the view that good animal/human relationships play a vital role promoting the psychological well-being of captive animals.

At present, the term 'training' seems to apply to a multitude of procedures and descriptions are often accompanied by a 'shopping list' of desirable, stress-reducing effects. In order to develop the best techniques possible for a specific behaviour in a specific situation, more attention needs to be paid to the actual techniques used and why they succeed (or fail) in promoting the psychological well-being of the trained animals.

8.2.2 Personnel

Comparisons between the trainers showed that it is important to record training sessions in sufficient detail to observe subtle differences in technique. One thing that became clear both during the observations reported here and those conducted during pilot sessions with domestic dogs was that there is often a considerable difference between what people think they are doing and what they are actually doing.

The observations reported in Chapter 3 revealed differences in the ways in which individual trainers worked both within and between the two training periods. The technique employed by Trainer A showed considerable improvement during Phase 2 in that he rewarded the monkeys more often, more consistently and faster. Although Trainer B was only observed on one occasion during Phase 2, what was recorded did not suggest any comparable improvement. Trainer C worked in an almost identical manner to that observed with Trainer A during Phase 2. In addition,

during Phase 1, the individual characteristics of the study animals appeared to lead to subtle differences in the techniques used. However, when questioned, the trainers were unaware that there was any difference in the way they treated individual macaques.

At the beginning of the study, none of the staff had been introduced to operant theory nor were they familiar with basic training terminology and it does appear that there is a lack of educational opportunities for individuals who are going to train animals. By the time Phase 2 of the macaques' training was conducted, training had been a topic of conversation for some time, the basic results from Phase 1 were known and much of the marmoset training had been observed. All of these factors may have had an influence but, even if it did, this is no substitute for a properly planned education programme. In addition, the training that was observed was the first attempt to train any macaques following the move from cage to gang room accommodation that occurred when the MRC unit relocated to its present location. The fact that potential problems getting the macaques into the cage room were not foreseen and that the training programme resulted in disruption to other husbandry routines shows a lack of planning. This is in stark contrast to the measures taken in US zoos where there is a well-developed education programme and training regimes are carefully planned (Colahan & Breder, 2003; Laule, 1992; Petinot, 1995; Savastino *et al.*, 2003; Sevenich, 1995).

As reported in Chapter 1, the mandatory basic training course undertaken by laboratory personnel (The Animals (Scientific Procedures) Act, 1986) does not contain specific instruction in PRT or operant theory. If training is to be introduced as a means of promoting the welfare of any laboratory housed animal then this issue needs to be addressed. This is by no means the first time that the need for a proper

education programme for animal trainers has been identified. Kiley-Worthington (1990) came to the same conclusion following her investigation of training practices in UK circuses.

Trainer B differed from the other personnel at the unit in that he failed to accept that rewarding the animals was important. The attitudes and other characteristics of personnel play a vital role in the success of any training programme (Laule, 1999). Reinhardt (1997c) reiterates this view and states that the 'macho' individual is out of place in the laboratory and that anyone lacking a basic understanding of the behavioural rules and needs of macaques should not be permitted to work with them.

However, while these are perfectly valid points, the situation is likely to be considerably more complex and finding appropriate people to carry out training may involve more than simply selecting individuals with an appropriate 'personality'. The 'Fundamental Attribution Error' (Ross, 1977) refers to a tendency to assume internal or dispositional causes for the behaviour of individuals and underestimate the effects of the situation. A number of authors have suggested that apparent insensitivity and even callousness with regards to laboratory animals arises from the psychological mechanisms employed by laboratory personnel to make it easier to deal with the deaths of the animals in their care (Arluke, 1992; Serpell, 1999; Walshaw, 1994). Arluke (1992, 1994) also points out that laboratory personnel become socialised to accept the rules and norms of their working environment and this can include the prohibition of close relationships and encouragement of a tendency to regard laboratory animals as objects (Chapter 6).

To dismiss Trainer B as 'macho' or conclude that he did not care about animals would be both inaccurate and unfair. He was never deliberately unkind and

was never observed to shout at, or even show anger towards any monkey. Even caring people may become seemingly uncaring when exposed to a laboratory environment. If close animal/human relationships are to be encouraged, this is an issue that needs to be addressed. In addition, if maintaining a psychological distance between themselves and the animals in their care is employed by laboratory staff as a coping mechanism, then removing that option could have unforeseen consequences. Arluke (1992) reports that mental health problems are already common among laboratory staff and failure to address this issue could make it difficult to retain appropriate personnel. Recommendations that laboratory staff develop close relationships with animals that are subsequently hurt or even killed in the name of science are demanding a great deal. These issues raise ethical concerns regarding the psychological well-being of both nonhuman and human primates in a laboratory environment. In addition, staff shortages caused by absenteeism due to stress-related illness are detrimental to both laboratory personnel and the animals in their care.

8.3 Training common marmosets using PRT techniques

As reported in Chapter 6, PRT proved to be a practical means of training marmosets to co-operate during in-homecage weighing and during the collection of urine samples. The performance of the trained animals was reliable, they learned the required behaviours relatively quickly and what time was invested could be recouped through faster data collection. A major factor in their performance appeared to be overcoming their initial nervousness of humans, a conclusion supported elsewhere (Savastano, Hanson & McCann, 2003). Even when trained using a faster, refined technique, the marmosets performed reliably both for myself and one of the regular laboratory staff. In addition, the technique was subsequently used by another

individual to successfully train marmosets housed in family groups (McDermott & Smith, 2003). Overall, the results presented in Chapter 6 suggested that PRT techniques were both effective and practical when used to train common marmosets to perform non-invasive procedures.

While training was accomplished fairly quickly, it should be noted that although the marmosets did not experience the close relationships with laboratory staff observed with the macaques, they had not experienced strongly negative experiences either. The marmosets were handled only when necessary and staff at the MRC unit (where the animals had spent their entire lives) aimed to handle all animals carefully. A history of strongly negative experiences with humans is likely to affect the time taken to train these animals by making their initial fear stronger and harder to overcome and increase the likelihood that the animal will experience stress during the training process itself (Weinberg & Levine, 1980).

As reported in Chapter 6, the marmosets at the MRC unit were only identified by number, and the trained animals were given names at the start of the study. An unexpected observation was that laboratory staff began to refer to the trained animals by name and began to discuss their individual personalities in a way that had previously occurred with the macaques. Reinhardt (1997c) states that giving laboratory animals names is important in that this encourages caregivers to regard each animal as an individual thus promoting close animal/human relationships. Serpell (1999) suggests that this is precisely why laboratory animals are not named (see Chapter 6). Naming animals would seem to be a relatively simple way of promoting the welfare of laboratory housed animals with the advantage that it would cost nothing to implement.

The prospect of finding and remembering names for 200+ animals that are all very similar in appearance does present some practical difficulties. This can be aided by choosing names according to some organised system. The trained pairs in the present study all had names beginning with a different letter of the alphabet thus pair A were called Adam and Allie, pair B, Billy and Bella and so on.

The clearest identifying feature that laboratory housed marmosets have is the tag carrying their identity number. A system could be used that created names based on the last two digits of that number which would both organise a naming system and provide a visual cue (the tag) to help staff remember the names of each animal.

In this system, the penultimate digit corresponds to a particular consonant with the final digit corresponding to a vowel as shown below.

Penultimate digit	0	1	2	3	4	5	6	7	8	9
Corresponding letter	**B**	**C**	**D**	**F**	**G**	**H**	**J**	**L**	**M**	**N**

Final digit	0	1	2	3	4	5	6	7	8	9
Corresponding letter	**A**	**A**	**E**	**E**	**I**	**I**	**O**	**O**	**U**	**U**

If a marmoset had the identity number 864, then the last two digits correspond to the letters J (6) and I (4) which then become the first two letter of his/her name (for example, Jimmy or Jilly). Marmoset number 897 would have a name with the first two letters N (9) and O (7) for example, Norman or Norma.

Of course, this system requires that all marmosets do wear identity tags, which is not the case in all laboratories (HB-S, pers. obs). However, if laboratory animals are to be regarded as individuals, there must be some way of ensuring that they are perceived as such. In species that lack sufficient individual characteristics easily detected by humans, some artificial means of identification should be used. In addition, animals should not be given names that may encourage staff to view them in a negative way, for example Nasty or Biter. While this (or any) system for naming

laboratory animals would require some practice, if names help to personalise individual animals and thus promote good animal/human relationships then this effort would be well worth while.

8.4 The effects of training on behaviour

8.4.1 Stump-tailed macaques

The conclusion that the poor techniques observed during Phase 1 were not promoting the psychological well-being of the study animals was supported by the results from the observations recorded on training and control days. Although the increase in locomotion observed during training days could be interpreted as a positive change given that the macaques were overweight, the corresponding increase in threats and scratching and decrease in allogrooming suggests otherwise. During Phase 2, there were no significant differences in any behavioural category.

However, it should be noted that the changes observed were neither severe nor long-lasting. Although threats increased, actual aggression did not. In addition, the increase in threats was matched by a corresponding increase in affiliative behaviours, a pattern of behaviour that would have been expected in these animals (de Waal, 1990).

The confounding variable presented by disruption to the feeding schedule made it difficult to be certain that the observed changes were really due to training. However, this observation served to highlight the importance of planning a training regime and considering the wider impact on normal husbandry routines. The obvious conclusion is that training should be carried out using appropriate methods and that additional disruption to normal routines should be avoided.

The observations reported in Chapter 3 also illustrate the need for planning with regards to the actual training process. Problems caused by broken slides and poorly designed squeeze mechanisms could have been foreseen. Small but important details such as having a plentiful supply of food rewards within easy reach were not considered until Phase 2. The need for a practical and safe (from both an animal and human perspective) environment in which to conduct training should be considered both during modifications to existing buildings and in the design of new facilities. Problem solving is an inevitable part of any training regime (Laule, 1994) yet whenever possible, the approach taken should be pro-active rather than reactive. However, the ability to foresee problems requires considerable knowledge of issues that must be considered which again illustrates importance of training for laboratory personnel themselves.

8.4.2 Common marmosets

The results presented in Chapter 7 showed a mild but positive change in the behaviour of common marmosets that had either been trained using PRT or simply experienced increased positive contact with humans. Following either intervention, the amount of time spent watching the observer from inside the cage decreased suggesting decreased vigilance, while allogrooming increased. Stress related behaviours such as self-scratching, scent marking and open-mouthed 'phee' and 'tsk' vocalisations all decreased. The reduction in stress-related behaviour was a positive improvement and there was no evidence that the marmosets had become 'fixated' on receiving treats from humans and the reduction in time spent watching the observer suggests the reverse. The marmosets appeared perfectly able to distinguish whether I was in their room to train or simply observe them and there were certainly a number of cues that would have allowed them to do this. These included my position in

relation to their cage and the presence of the steps, clipboard and electronic 'beeper' used during observation periods but absent during training. The implications of these results with regards to zoo-housed animals are discussed later.

The lack of difference between the trained animals and those who received increased positive contact with humans suggested that the two interventions did not differ in effect. As overcoming fear of humans appeared to be important in the training process (Chapter 6), it seems likely that this factor was at least partly responsible for the observed changes in behaviour. Following exposure to a mild stressor, the trained animals showed no subsequent elevation in levels of urinary cortisol nor any significant behavioural changes. These results showed that training had successfully reduced the stress associated with the standard laboratory procedure used for weighing these animals. However, the changes observed in the behaviour of the control animals demonstrate that this procedure is aversive and should be replaced. The results presented in Chapter 6 suggest that training to allow in-homecage weighing is a practical alternative.

The question remains as to why the trained animals were less affected by the weighing procedure than the control animals. It cannot be the case that training made the procedure more predictable or allowed the animals to learn appropriate responses to the situation as the stressor used was not a procedure they were trained to co-operate with. Nor can control be an issue, as the trained animals had no more control during the procedure than the untrained animals. In this case the most plausible explanation is the improved relationship with humans, which supports the view that this factor has a wide impact on behaviour. With hindsight, it would have been interesting to have included the marmosets who had experienced increased levels of positive contact during the first experiment as, if the reduction in stress was due to the

animal/human relationship then there would have been no difference between them and the trained animals.

What was interesting was that outwith the actual sessions, training resulted in a positive change in the behaviour of the marmosets, had a detrimental effect on the behaviour of the macaques during Phase 1 and no significant effects on behaviour during Phase 2. This meant that for the macaques, the best result was that training had no noticeable effect on behaviour once the issues of poor training technique and feeding schedules were resolved. While it is possible that only PRT can actively promote positive behaviour change and that other techniques at best do no harm, this was not an issue addressed here although it is certainly one worth examining. An equally likely explanation lies with the pre-existing differences between the two species in relation to animal/human relationships. The marmosets did not have much close positive contact with humans before training began. The improvement in their behaviour may have come about as a result forming closer relationships. However, with the macaques, such a relationship already existed (Waitt *et al.*, 2002) so perhaps there was less to be gained.

8.5 Implications for zoo housed animals

While the broader implications with regards to welfare discussed above apply equally to laboratory and zoo-housed primates, an additional aim was to address the concerns regarding the wider effects on behaviour found in the zoo community, especially with regards to the widely-practiced 'hands off' policy. The main objections to training given by zoo personnel can be summarised as follows:

- Training has an adverse effect out-with the actual training sessions in that the natural behaviour of the animals will be altered. Preserving natural behaviour is important both in terms of conservation and public education.
- Training creates close relationships with humans, which are unnatural. Such relationships may be suitable for domestic animals but not for wild species.
- Training will produce animals that are unsuitable for re-introduction.

The results reported in Chapters 4 and 7 produced no evidence to suggest that training, if carried out well, would have any additional behavioural effect beyond that brought about by close contact with humans. The initial detrimental effect observed on the behaviour of the macaques disappeared following changes in training practice the introduction of measures to minimise disruption to normal husbandry routines. Following these modifications, there were no observed differences between training and control days. When measures were taken to improve animal/human relationships with the marmosets, there were no differences between animals that were trained, and those who experienced increased positive contact with humans. As reported above, the changes reported were mild but positive. The decrease in time spent watching the observer suggested that, out-with training sessions, these animals became less, not more fixated on humans. As intended, scent marking increased during training sessions and at subsequent urine collection times but this change did not persist outwith these specific situations. Of course, improved relationships with humans did have an effect on the marmosets but with growing evidence that this is of benefit to the animals (Bayne *et al.*, 1993; Heath, 1989; Savastano *et al.*, 2003; Waitt *et al.*, 2002a) it is time that the policy of keeping contact between zoo housed animals and humans to a minimum was re-examined.

Not all British zoos follow a 'hands off' policy, for example, Chester zoo handles some animals although the rational behind why some species are chosen and not others is unclear (Kiley-Worthington, 1990). Close contact is encouraged at both Twycross Zoo and Howletts Wild Animal Park, where management policies regarding contact between keepers and animals seem to arise from the personal philosophies of the zoos' founders (Kiley-Worthington, 1990). As is often the case in Britain, disapproval of such policies is easily detected during post-conference discussions but is rarely, if ever, written about. Both the above zoos first opened during a period that saw a number of institutions founded, not by zoological societies but by individuals keen to establish relationships with their animals and challenge perceptions of ferocity (Baratay & Hardouin-Fugier, 2002). However, conditions at such establishments were often poor and contributed to the escalation of protests about zoos and circuses that occurred during the 1970s. In order to protect their collective image, many established zoos quickly distanced themselves from all such institutions along with their tendency towards personification, anthropomorphism and close contact with the animals (Baratay & Hardouin-Fugier, 2002). Current criticisms tend to focus on one or two distinct issues such as the high number of hand-reared apes at Twycross and serious keeper injuries at Howletts. While these are valid concerns, they also reveal a certain amount of dichotomous thinking regarding animal-human relationships; that is, contact is avoided completely or the animals must be tamed and treated as pets. The idea that there may be an optimum level of contact that is both species and context specific is rarely considered. Some species pose little threat or a lesser threat than is currently perceived. For example, in zoos, zebra are chemically immobilised to allow foot trimming, a procedure performed regularly on circus animals without the need for drugs (Kiley-Worthington, 1990).

With regards to other objections to training, or indeed any form of close relationship between captive wild species and caregivers, it is perfectly true that such relationships are unnatural. However, it could also be argued that the captive environment, with its absence of predators, hunger and other challenges is unavoidably unnatural anyway (Poole, 1998). Veterinary treatment of disease and injury is another feature of the captive environment absent in the wild. Training has been shown to facilitate such treatment and, by reducing the stress associated with such interventions (Desmond & Laule, 1994). The results presented in Chapter 7 are in accord with numerous studies demonstrating the welfare benefits of both close relationships with human caregivers and positive reinforcement training. Where is the evidence to support the view that a 'hands off' policy is in the best interests of captive animals? In addition, there is the question of how much such a policy does prevent 'contamination' of animal behaviour through contact with humans. As reported in Chapter 7, laboratory studies have shown that humans inevitably affect the behaviour of captive animals even when measures are taken to prevent this occurring (Boccia *et al.*, 1992; Caine, 1987, 1990; Davis & Balfour, 1992; Estep & Hetts, 1992; Novak & Suomi, 1988). Even when no deliberate training is carried out, the question remains as to how much training is conducted inadvertently.

There may well be validity in the belief that close relations with humans creates problems with animals destined for reintroduction and this issue does need to be addressed. Losing all fear of people while in captivity would clearly be problematic in species that may hunt, or be hunted by humans in the wild. There is also the danger of disease transmission from humans to wild populations via released animals (Warren & Swan, 2002). However, the existence of such problems does not justify the belief that close relations with humans preclude return to the wild. While

my research does not address this issue, there are a number of points worth making in order to challenge the view that this is always and unavoidably the case.

Firstly, contact with humans often continues after release either through provisioning until the animals are able to feed themselves (Kierulff, 2002) or post-release monitoring. Such monitoring is now considered "one of the most important components of a re-introduction or translocation project..." (IUCN/SSC, 2002; p42). The presence of a familiar caregiver has proved useful in the re-introduction of chimpanzees *(Pan troglodytes)* (Farmer, 2002), and drills *(Mandrillus leucophaeus)* (Gadsby, 2002). Primates have been successfully returned to the wild despite close contact with humans and this includes animals released from rehabilitation centres dealing with orphans of the bushmeat trade, reared by humans and kept as pets (Farmer, 2002; Russon & Galdikas, 1993).

While discussion of re-introduction focuses exclusively on the deliberate re-introduction of wild species, some important clues as to the effect of contact with humans can be overlooked. For example, some of the most successful journeys back to the wild have been undertaken by domestic animals. For example, Australian dingos *(Canis dingo)* are descended from the domestic dog *(Canis lupus familiaris).* The free living herds of mustangs in the US and brumbys in Australia are all descendants of escaped domestic horses *(Equus caballus).*

With regards to animals destined for re-introduction, at present there is simply not enough evidence to support any judgement regarding the desirability of increasing positive contact with caregivers or introducing PRT and many questions still need to be addressed. Does a close relationship with familiar caregivers generalise to all humans? Does affinity with humans continue once animals are no longer dependent on them for food? How does the behaviour of tamed animals influence the behaviour

of their young? Until such questions are answered, there are legitimate reasons for caution. However, many zoos contain animals that stand no realistic chance of a return to the wild and withholding techniques that could promote the welfare of these individuals is harder to justify.

There is one final point in relation to zoos. In addition, to conservation and research, zoos include education among their stated aims (Baratay & Hardouin-Fugier, 2002). Although this is in generally with regard to wild species and conservation issues, there seems to be no good reason why this could not be broadened. There is a great deal of training conducted with domestic animals, much of it using inappropriate methods and no real understanding of animal learning (Hiby *et al.*, 2004; Kiley-Worthington, 1997; McGreevy, 1996; Mugford, 1995; Pryor, 1999). Unlike laboratories, zoos are open to the public and some US zoos allow visitors to observe training sessions and provide information about the techniques used (Savastano, Hanson & McCann, 2003). Such measures could do much to educate the public about animals training and thus promote the welfare of many domestic animals.

8.6 Limitations and future research

There were some clear methodological issues, some of which have already been discussed in the appropriate chapters with additional information provided in the appendix. As such, the limitations discussed here predominantly consider some important issues that were not addressed during this research.

The reasons behind the decision to choose stump-tailed macaques as study animals are outlined in Chapter 2. However, it is true that this species is rarely found in UK laboratories and that most commonly used species is the cynomolgus macaque

(Boyd Group, 2000). As species-specific information is important, there is a need for research with regards to the training of these primates. In addition, the majority of cynomolgus macaques used in UK laboratories are imported from breeding stations in countries such as Mauritius, the Philippines and China (Boyd Group, 2000). Past experiences of human contact are likely to have a significant effect on responses to training and this was another factor that was not addressed here but should be in future research. More information is needed, not only on the ways in which primates are handled overseas but also in the UK. The records of the study animals were examined but, although the identity of the facilities where they had been housed previously were provided, along with data such a prior veterinary treatment, no information was provided as to what experimental or training procedures the macaques had been exposed to in the past.

All of the animals trained during this research were adults and issues concerning the effects of training on juveniles were not addressed. The terms 'critical periods' and 'sensitive periods' refer to stages during development when animals are particularly sensitive to particular environmental influences (Mellen & Ellis, 1996). Studies of domestic animals have shown that socialisation to humans occurring within such a sensitive period has a profound lifelong effect on subsequent animal/human relationships (Scott & Fuller, 1965). The possibility that close contact with humans early in life leads to a different relationship that than created by close contact during adulthood raises a number of issues. Would the beneficial effects of training be increased is contact with humans began at an early age, and if so, at what age? Scott and Fuller (1965) found that in domestic dogs, the sensitive period for maximum socialisation to humans *ends* at seven weeks old. Of course, primates must be

socialised to their own species and there is a possibility that early close contact with humans, especially during infancy, could interfere with this process.

The question of sensitive periods is only one of a number of issues that should be addressed in future research. More issues are illustrated by the fact that the training procedures discussed here were analysed in terms of operant theory, the limitations of which were discussed in Chapter 1. At present, additional cognitive or social learning processes are poorly understood but it is highly unlikely that these are not being used during the training process. For example, when training in pairs or groups, do animals learn from watching the behaviour of conspecifics? Observations of the marmosets certainly suggested that the behaviour of cage-mates helped to overcome any initial fear of humans. Initial practice sessions with single-housed animals and subsequent demonstrations of training techniques conducted with some of the large breeding groups suggested that marmoset boldness increases with group size. In addition, the behaviour of some of the more timid members of the trained pairs did not show a gradual reduction in fear (as would have been indicated by a tendency to take the reward but retreat to the back of the cage before consumption). Several of these animals suddenly switched from refusing to participate at all to holding their target for as long as required within one session. While observing their bolder cage-mate may have taught timid marmosets that I was safe to approach, a phenomenon that has been reported elsewhere (Savastano *et al.*, 2003), any learning beyond this is hard to determine. Certainly, the marmosets that failed to learn to provide urine samples did not appear to benefit from frequently observing their cage-mate being rewarded for performing the required behaviour. However, if observations of unconcerned conspecifics help to reduce fear, then the question of

how animals are affected by watching training with aversive elements or for invasive procedures must be addressed.

The training process is also likely to be effected by species-typical behaviours (Breland & Breland, 1961). As reported in Chapter 1, training is more likely to be successful when the techniques used work with, rather than against natural behavioural tendencies and any understanding of how animals interpret the training process could be enhanced by considering how these factors interact.

For example, one intriguing possibility concerns how the different species may interpret the offering of food treats or rewards. Food sharing does not form part of the macaque behavioural repertoire (de Waal, 1989) therefore a human voluntarily offering food may be a difficult situation for macaques to interpret. By contrast, food sharing is common in marmosets, particularly between caregivers and infants (Sutcliffe & Poole, 1984). During observations of the marmosets, of all possible affiliative behaviours, food sharing was the one most frequently recorded. Here, a natural behaviour exists that the taming or training process may be exploiting.

Hediger (1965, cited by Estep & Hetts, 1992) provided a conceptual framework for understanding animal-human relationships by suggesting that animals may regard humans in a number of different ways: as predators, as prey, as a socially insignificant part of the environment, as symbiont and as conspecific. These categories form a continuum with animals regarding individual humans as belonging to one or a combination of categories, a perception that can change with experience. The prevalence of escape behaviour and other fearful responses among animals with little contact with humans suggests a pre-disposition towards regarding human as predators. A shift towards a symbiotic, and therefore more positive relationship can only occur if the animal learns that there is some benefit to association with humans.

This can be aided by communication through signals that are not species typical but have been learned through repeated interactions. However, such communication is easier, and attachments formed more readily, if conspecific signals or behaviours can be modified and then utilised. This can be seen in the methods used by Fulani tribesmen in their interactions with cattle (Estep & Hetts, 1992) and in those developed by Monty Roberts for training horses (Roberts, 1992). In this context, marmosets may interpret the offering of food treats as an affiliative behaviour, which could facilitate the taming process. With no concept of food sharing, macaques have no reason to interpret to provision of treats in this way.

Throughout both training and positive contact, the marmosets were also talked to. This has been recognised as useful technique by many people who work with animals. Hediger (1992) suggests that while the actual words are unimportant, talking provides a medium of non-verbal communication by activating facial expressions and providing cues through intonation, sound intensity, posture and movement. Future research could examine how such factors could be used to make improvements to training techniques beyond those suggested by operant theory.

Although the training of the common marmosets was completed successfully, it should be noted that the behaviour were very simple ones and that participation did not require any exposure to painful or even uncomfortable stimuli. PRT may be less effective when used to train for procedures where this does occur. Training to provide urine samples could replace the need for venipuncture in some, but not all situations. Improving the techniques used to collect blood samples from marmosets should be addressed in future research. Equally, PRT successfully reduced the stress associated with a mild stressor but may not have been successful had the procedure used been more severe.

The focus of this research was very much on the animal side of the animal/human relationships. However, it became increasingly clear that the human element was vitally important as the success of training depends as much on the behaviour of the trainer as that of the animal. The UK is not a particularly reward-oriented society and a tendency to rely on negative reinforcement and punishment can be found, not only in animal training (Kiley-Worthington, 1997; McGreevy, 1996; Mugford, 1995; Pryor, 1999) but also in the education of human children (Cowley, 2001). The need for discipline and a drive for obedience rather than co-operation can easily be detected in a culture where proposals to ban the physical punishment of human infants is greeted by outrage in many quarters. Laboratory personnel do not emerge from a cultural vacuum and a tendency to revert to social norms or 'common sense' ideas with regards to coercion and punishment could undermine attempts to introduce PRT principles and techniques. Breland and Breland (1961) called a tendency for instinctive behaviours to override learned behaviours 'instinctive drift'. Although 'cultural drift' may be a better term when applied to humans, pre-existing attitudes are likely to have considerable effect on the successful application of training as a means of promoting welfare and this is another area where more research is required. Research with regards to animal welfare has always required input from a range of different disciplines. Perhaps it is time to encourage greater participation from those researchers who specialise in the study of human behaviour.

Finally, in terms of welfare, the focus of this research was a somewhat negative one in that training was examined as a mean of alleviating a negative state of well-being rather than promoting a positive one. The behaviours recorded during observations of the monkeys were predominantly those used to indicate poor, rather than good welfare. While there are a number of well-validated behavioural indicators

of negative psychological states, there are few that can be used to indicate a state of positive rather than neutral well-being. Training may do more than alleviate stress and could be a source of enrichment. This issue was not addressed here but should be in future research.

8.7 Conclusions and recommendations

The results of the studies reported in this thesis have been used as the basis of the following conclusions and recommendations:

- PRT techniques can be used to promote the psychological well-being of laboratory-housed nonhuman primates and should be used to a greater extent than is currently the case.

However, it will take time to develop and implement the necessary changes although preparatory measures such as naming laboratory-housed primates and encouraging close animal/human relationships could be implemented relatively quickly.

- All training and laboratory procedures should be recorded in greater detail and subjected to greater scrutiny that is currently the case.

The information gained should then be used to identify weaknesses and areas where refinements could be made. Even standard procedures could be improved by the provision of rewards at appropriate moments and this could represent the first step to refining these procedures.

- The absence of any regulated educational programme for animal trainers needs to be remedied.

Individuals who train or handle trained animals should be aware of theories concerning animal learning and how these relate to the training process and this

should be included in the compulsory training course undertaken by all laboratory personnel. Individuals responsible for the implementation and supervision of training programmes should receive additional training with regards to the wider organisation of training regimes including issues of staff training, consistency and supervision and the impact of training schedules on existing laboratory routines. In addition, steps should be taken to ensure that information regarding training innovations and the results of training-related studies are available to the people who care for laboratory animals on a day-to-day basis. At present this is not the case and much relevant information is contained in academic journals that laboratory staff cannot easily obtain.

- In light of the growing evidence that training and positive animal/human relationships can do much to promote the psychological well-being of captive animals, UK zoos need to re-examine the factual and logical arguments underlying their 'hands off' policy with regards to the management of the animals in their care.

8.8 Recent developments

The final recommendation listed above may already be superfluous as recently there has been a noticeable change in attitude and interest in training has grown considerably in both laboratories and zoos. Following discussion of training-related issues at a numbers of meetings (see conference presentations), in 2003, the Laboratory Animal Veterinary Association and the Laboratory Animal Science Association jointly organised a meeting specifically entitled "Animal Training as a Refinement: Basis and Benefits". At this meeting, papers were presented regarding the training of a number of species in addition to primates and illustrated that the

research community in general was beginning to recognise the potential benefits both in terms of animal welfare and the quality of research data obtained. However, it was clear that the term 'training' was used rather loosely, covering everything from PRT to conditioning in an operant chamber. Furthermore, there were some indicators that the training process was not always understood (confusion regarding negative reinforcement being the most common) and in some cases 'innovative' training techniques, for example, training dogs to stand quietly on tables during examination, have been used in other situations for years (for example, every small dog exhibited at shows is taught to 'table'). The above points further illustrate the need for improved education and cross-discipline communication with regards to training.

The presentation of a number of training-related papers at the 5th Annual Zoo Research Symposium held at Marwell Zoo in 2003 elicited a considerable amount of interest. This was a considerable change from the response shown to presentations given by US trainers during the second International Conference on Environmental Enrichment held in 1999, where the reactions of UK delegates ranged from disinterest to open hostility. Subsequently, an additional meeting was organised at Paignton Zoo specifically to discuss the potential applications of PRT, and to provide practical guidance.

Finally, in November 2003, the *Journal of Applied Animal Welfare Science* released a special issue containing articles relating specifically to PRT. Originating from a symposium entitled "Training primates" at the XIXth Congress of the International Primatological Society, a number of the articles addressed some of the issues reported in Chapter 1. The principles of PRT were again explained with specific reference to the potential detrimental effects of training regimes employing negative reinforcement. In addition, helpful suggestions regarding a change to a PRT

regime were provided (Laule, 2003). This issues also contained two US zoo-based articles containing quantitative data concerning the behaviour of primates during training and time investment required for a number of different behaviours (Savastano *et al.*, 2003; Schapiro, Bloomsmith & Laule, 2003) and an extensive account of the organisation behind the training programme used at Disney's Animal Kingdom (Colahan & Breder, 2003).

Of particular interest was Savastano *et al. 's* report on the training of 86 monkeys from 17 New World species including a variety of callitrichids. Observations, including quantitative data on required time investment, are reported regarding six marmoset and nine tamarin species. It was found that marmosets took longer to respond positively to the trainers than tamarins. However, once this occurred, they typically learned new behaviours within ten, 10-15 minute training sessions, which in turn was found to be the most appropriate duration of training periods. These results are in accord with those reported in Chapter 6.

Another interesting result was the considerable differences between species. While marmosets responded best when training was conducted twice daily in 10-15 minute sessions, tamarins lost interest more quickly and responded better during frequent short training sessions. While pale-headed saki monkeys *(Pithecia pithecia)* readily approached the trainers from the start of the programme, Bolivian gray titi monkeys *(Callicebus donacophilus)* would only do so following the introduction of less timid marmosets and tamarins that were already participating in the training process (Savastano *et al.*, 2003). These observations illustrate, not only the need for a flexible approach and the effects of minor variations on basic techniques, but also the need for species-specific information across the primate order.

8.9 Final comments

The recent development in both laboratories and zoos suggest that, in the UK, training is an idea whose time has come. Given the potential contribution that training could make to welfare this is a reason for considerable optimism regarding the psychological well-being of captive animals. However, the current enthusiasm for training also presents cause for concern. It may be more accurate to say that training is an idea whose time has come *again.* The history of animals kept in captivity shows that training was used extensively in the past. However, cruel training techniques and attempts to train inappropriate behaviours caused the practice to fall into disrepute and created the very reservations and attitudes that are currently being re-considered. Without the organised educational programmes and wider organisational structure that exists in the US there is a danger that training in the UK will be implemented by well-meaning but ill-informed individuals who decide to 'have a go' based on a one-day seminar on the subject. The first 'animal welfare' book I ever read contained a chapter designed to show that animals can suffer as much through mistakes arising from a lack of knowledge as they can through deliberate cruelty, a point best illustrated by the following extract;

"Only ignorance? How *can you talk about* ***only*** *ignorance? Don't you know that it is the worst thing in the world, next to wickedness? – and which does the most mischief, heaven only knows."*

Anna Sewell, Black Beauty

However, identifying a potential problem is the first step to averting it and in the years following the decline of training as a management tool there have been enormous advances in our understanding of the ways in which animals learn and the factors that are important in promoting their welfare. In addition, there are now numerous techniques that can be used to evaluate and refine the techniques that are

used when working with animals. The mere fact that the importance of promoting both physiological and psychological well-being is being recognised by increasing numbers of people within the research community is in itself reason for hope. With these factors in place, there is a real possibility that the potential that training represents is fulfilled and that many of the current practices that cause such distress to the animals in our care are replaced with increasingly better alternatives.

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Appendix

Appendix to Chapter 4 – Effects of training on the behaviour of stump-tailed macaques.

As reported in Chapter 4, there were a number of limitations that arose from the data collection methods used to record the behaviour of the study animals outwith the actual training sessions. The rationale behind the selection of these methods is presented here.

The most obvious problem with regard to the macaques was the small sample size. In addition, during pre- and post-training observations, behaviour was recorded using scan sampling and, as the behaviour of each animal was likely to have been affected by other group members, observations cannot be considered to be independent (Martin & Batson, 1993). Ideally, analysis should have used means for the group rather than individuals, producing a mean activity budget for the group as a whole, the procedure used when analysing the marmoset data (Chapter 7). However, this would have resulted in one figure per condition, making further analysis impossible.

A considerable number of studies attempt to increase a limited data set by analysing multiple observations on the same animals independently rather than calculating means, this techniques is subject to the 'pooling fallacy' and increases the probability of a Type 1 error - rejecting a true null hypothesis (Machlis, Dodd & Fentress, 1985). The use of focal sampling was considered but due to the limited opportunities for observations available, it was felt that this technique would not generate a sufficient data set. In addition, following each session, the immediate post-training reactions of four of the five study animals would be missed and this was of particular concern as the actual duration of the training period was unknown at the

start of the study. While it would have been possible to have conducted five consecutive six minute focal observations within the 30 minute observation period, this technique would have generated fewer data and such closely conducted focal observations are arguably no more independent that those collected using scan sampling (Martin & Batson, 1993). While not ideal, the methods used were chosen as the best compromise available given the limited opportunities for data collection available.