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Stress monitoring in captive vervet monkeys (*Chlorocebus pygerythrus*) co-housed with domestic cats (*Felis silvestris catus*).

By

John Kudakwashe Chipangura

A dissertation submitted in partial fulfilment of the requirements for the degree of:

Master of Veterinary Medicine (Laboratory Animal Science)

Supervisor:

Prof Vinny Naidoo

Co-supervisor:

Prof Andre Ganswindt

Department of Paraclinical Veterinary Science

University of Pretoria

Republic of South Africa

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DECLARATION

I declare that the dissertation hereby submitted to the University of Pretoria for the degree Master of Veterinary Medicine (Laboratory Animal Science) has not been previously submitted by me for a degree at this University or any other University, that it is my own work in design, execution and that all assistance in the study has been duly acknowledged

John Kudakwashe Chipangura



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Thank you all.



ABSTRACT

Housing conditions for laboratory animals can be improved by ensuring that animals are given an opportunity to perform species-specific behaviour. However, in most institutions space is a limiting factor because housing systems have been designed based on economic and ergonomic aspects without considering environmental needs of animals used in research. Vervet monkeys (*Chlorocebus pygerythrus*) are one common non-human primate species used in biomedical research. Despite the extensive use of vervets in research there is paucity of data describing the environmental enrichment of this species.

The current study sought to ascertain the compatibility between domestic cats and vervet monkeys, to allow for better utilisation of limited laboratory space. The idea was based on the cohabitation and stress alleviation effect of horses housed with goats. The study used a habituation method whereby the domestic cats were slowly introduced to the vervet monkeys. Domestic cats were selected as they were already housed within the same research centre, under semi-controlled conditions.

While the aim of the experiment was to ascertain the compatibility of cohousing cats with vervets, the possibility of the animals responding adversely to the interaction could not be overruled and hence common methods for monitoring stress in animals were used, which were behavioural changes, changes in faecal glucocorticoid metabolite concentrations and weight changes over time, to evaluate the situation. Faecal samples were collected for six days prior to introduction of domestic cats as baseline. After introducing the domestic cats, faeces were collected for another six days and the concentrations of faecal glucocorticoid metabolites (fGCM) determined using enzyme immunoassays. Behavioural observations were analysed to



check for significant changes before and during cohousing using the Kruskal-Wallis One Way Analysis of Variance on Ranks and multiple comparisons using Tukey post hoc test. The paired sample t-test was used to compare alterations in weight and fGCM before and during cohousing.

On the first day of cohousing, the animals while inquisitive kept their distance. The vervets housed in cages that were closest to the domestic cats were the most active. During the first one minute, the vervets looked at the cats, climbed up to the top of the cage and made alarm calls. During the same time, the cats were moving and sniffing around the new housing. Overall average fGCM levels before cohousing was 0.24 $\mu\text{g/g DW}$ for the cats and 95.22 ng/g DW for the vervets while during cohousing the average was 0.34 $\mu\text{g/g DW}$ for the cats and 125.77 ng/g DW for the vervets. For both species, the fGCM levels were elevated a day after introduction, thereafter the levels started to decline.

The results from this study provides evidence that vervets and domestic cats can be cohoused without inducing prolonged additional stress and this can be used as a way of utilising limited laboratory animal space.



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LIST OF ABBREVIATIONS

ACTH: Adrenocorticotrophic Hormone

AEC: Animal Ethics Committee

AIDS: Acquired Immunodeficiency Syndrome

ANOVA: one-way analysis of variance

CNS: Central nervous system

CRH: Corticotrophin releasing Hormone

EIA: Enzyme Immunoassay

fGCM: Faecal Glucocorticoid Metabolites

FIV: Feline Immunodeficiency Virus

GC: Glucocorticoids

HIV: Human Immunodeficiency Virus

HPA: Hypothalamic Pituitary Adrenocortical

LAS: Laboratory Animal Science

NHP: Non-Human Primate

RIA: Radioimmunoassay

SIV: Simian Immunodeficiency Virus

UPAEC: University of Pretoria Animal Ethics Committee

UPBRC: University of Pretoria Biomedical Research Centre

NIH: National Institute of Health

NRC: National Research Council



CHAPTER 1: INTRODUCTION

1.1 Background

The main objective of an experiment involving animals is to collect reliable data from which high quality scientific information can be obtained. However, in some instances, it is not possible to obtain reliable data as Hubrecht and Kirkwood (2010) acknowledged that animals used in research are exposed to stressful conditions (e.g. experimental procedures and poor housing conditions). Stressful conditions can confound research data by altering the physiological status of the animal (NRC, 2010). Since laboratory animals are always in captivity, poor environmental conditions are the most frequent cause of stress. To avoid stressing research animals, good animal welfare advocates for the full implementation of the 3Rs (reduction, replacement and refinement) (NRC, 2010).

Refinement defines methods used to minimise the severity of inhumane handling of animals in research with a goal of improving animal welfare and reducing any stressful conditions (Newberry, 1995; Olsson and Dahlborn, 2002; NRC, 2010). Refinement involves all aspects of animal use in research, from housing, husbandry to the scientific procedures performed. Examples of refinement techniques include gentle handling and restraint, use of appropriate anaesthesia, postoperative care and analgesia, and providing animals with appropriate housing that allows for the expression of species-specific behaviours (Newberry, 1995; Olsson and Dahlborn, 2002). Environmental enrichment is an essential component of refinement that focuses on ways of improving the laboratory animal's environment (housing and husbandry). Housing conditions for laboratory animals can also be improved by ensuring that animals are given an opportunity to perform species-specific behaviour (NRC, 2010). However,



in most institutions space is always a limiting factor because housing systems have been designed based on economic and ergonomic aspects without taking into consideration on all environmental needs of animals used in research (Hubrecht and Kirkwood 2010).

Vervet monkeys (*Chlorocebus pygerythrus*) are one common non-human primate species (NHP) used in biomedical research (Jorgensen et al., 2017). Despite their widespread use in biomedical research, there is limited information available describing the environmental enrichment of vervets. With vervets in the wild living in large social groups, it is not surprising that these animal social needs require attention when in captivity to prevent behavioural problems while at the same time maintaining the integrity and quality of research data (Seelig, 2007). In contrast, the rhesus macaque (*Macaca mulatta*) that represents the most commonly used NHP species in research has numerous published studies on their optimal enrichment in captivity (Novak et al., 1998; Reinhardt, 1999; Weed et al., 2003). As a result, enrichment methods developed for macaques are often adopted for vervet monkeys. While these interventions may be successful, generalizations on husbandry of NHPs can lead to further problems, since the way the animals' psychological response to said interventions may be very species-specific.

The University of Pretoria Biomedical Research Centre (UPBRC), has housed a number of adult vervet monkeys over a period of ten years, for which numerous environmental enrichment techniques have been tried on the colony. For this study, we evaluate the success of housing vervet monkeys next to domestic cats, separated by a physical barrier between the two groups, as a novel method of enrichment. The study also sought to ascertain the compatibility between domestic cats and vervets, to allow for better utilisation of limited laboratory space. The idea was based on the cohabitation and stress alleviation effect of horses housed with goats



(Winter, 1996). The study used a habituation method whereby the domestic cats were slowly introduced to the vervets. Domestic cats were selected as they were already housed within the same research centre, under semi-controlled conditions. Furthermore, the housing needs of domestic cats are not substantially different to primates in captivity.

Similar to NHP in captivity, the health and welfare of domestic cats may be affected by their surroundings if the environment is not designed to meet their species-specific needs. Domestic cats in captivity retain their natural investigatory and communication behaviours (e.g., climbing, scratching, chewing and elimination) and when denied enough vertical and horizontal space they display undesirable behaviours (NRC, 2010). Housing for domestic cats in captivity must be designed in such a way that it will provide mental stimulation and allow the cats to express a wide range of normal behaviours including climbing, jumping, stretching, exploring and hiding. Like NHP, domestic cats also need social contact but the success of providing social contact depends on the social compatibility of the cats, their ability to manage distance from one another, and easy access for all group members to comfortable resting places, food, water, and litter trays (NRC, 2010). The primary mechanism for domestic cats to avoid conflict is by ensuring that they have enough social distance.

1.2 Hypothesis

The cohousing of vervet monkeys and domestic cats in adjacent pens results in a general reduction of stress of laboratory housed primate species and domestic cats.



1.3 Benefits arising from the project

The following benefits will arise from the study:

- Expansion of the evidence based literature on vervet monkey housing, social enrichment with non-conspecifics and maximal use of limited laboratory space
- This study will contribute towards a mini dissertation for the completion of a Master of Veterinary Medicine (LAS) degree.

1.4 Aim

The aim of this study was a first step towards evaluation of the benefits of housing vervet monkeys (*Chlorocebus pygerythrus*) next to domestic cats (*Felis silvestris catus*), with the two-species separated by a physical barrier for safety reasons.

1.5 Objectives

The following objectives were used to meet the main aim of this study

- To monitor behavioural changes in vervet monkeys housed adjacent to domestic cats, under similar captive conditions.
- To monitor stress-related steroid alterations in vervet monkeys housed adjacent to domestic cats using faecal glucocorticoids metabolite measurement.
- To monitor changes in weight in both species prior and during cohousing.



CHAPTER 2: LITERATURE REVIEW

2.1 The vervet monkey

The vervet monkey (*Chlorocebus pygerythrus*), is an old-world primate of the family Cercopithecidae that is native to Africa and found mostly in eastern and southern Africa (Figure 1). Currently there are six species officially recognised in the family, which are *Chlorocebus aethiops* (grivet), *Chlorocebus pygerythrus* (vervet), *Chlorocebus tantalus* (tantalus), *Chlorocebus sabaesus* (green monkey), *Chlorocebus cynosures* (malbrouck), and *Chlorocebus djamdjamensis* (Bale Mountain vervet) (Ayoub et al., 2015). The fur colour and length vary from species to species, as does the presence or absence of a distinguished band of fur on the brow (Grooves, 2001). Vervets look similar to the grivet, malbrouck and tantalus monkeys. Their abdomen has bluish skin while their faces, hands and feet are all black skinned (Figure 2). They have a yellow to greenish-brown coat with white undersides and white fur on their brows and cheeks. All six species have white undersides (Grooves, 2001). The green monkey (*C. sabaesus*) is different from the other members of the family in that it has golden-green fur, does not have the white band on the brow and lacks the black skinned hands and feet (Ayoub et al., 2015). *Chlorocebus djamdjamensis* has longer darker brown fur, with a white beard and a very slight white brow (Ayoub et al., 2015). Vervets prefer to inhabit the savannah, open woodland and forest-grasslands close to rivers but they are also adaptable and versatile species that can live in both rural and urban environments.

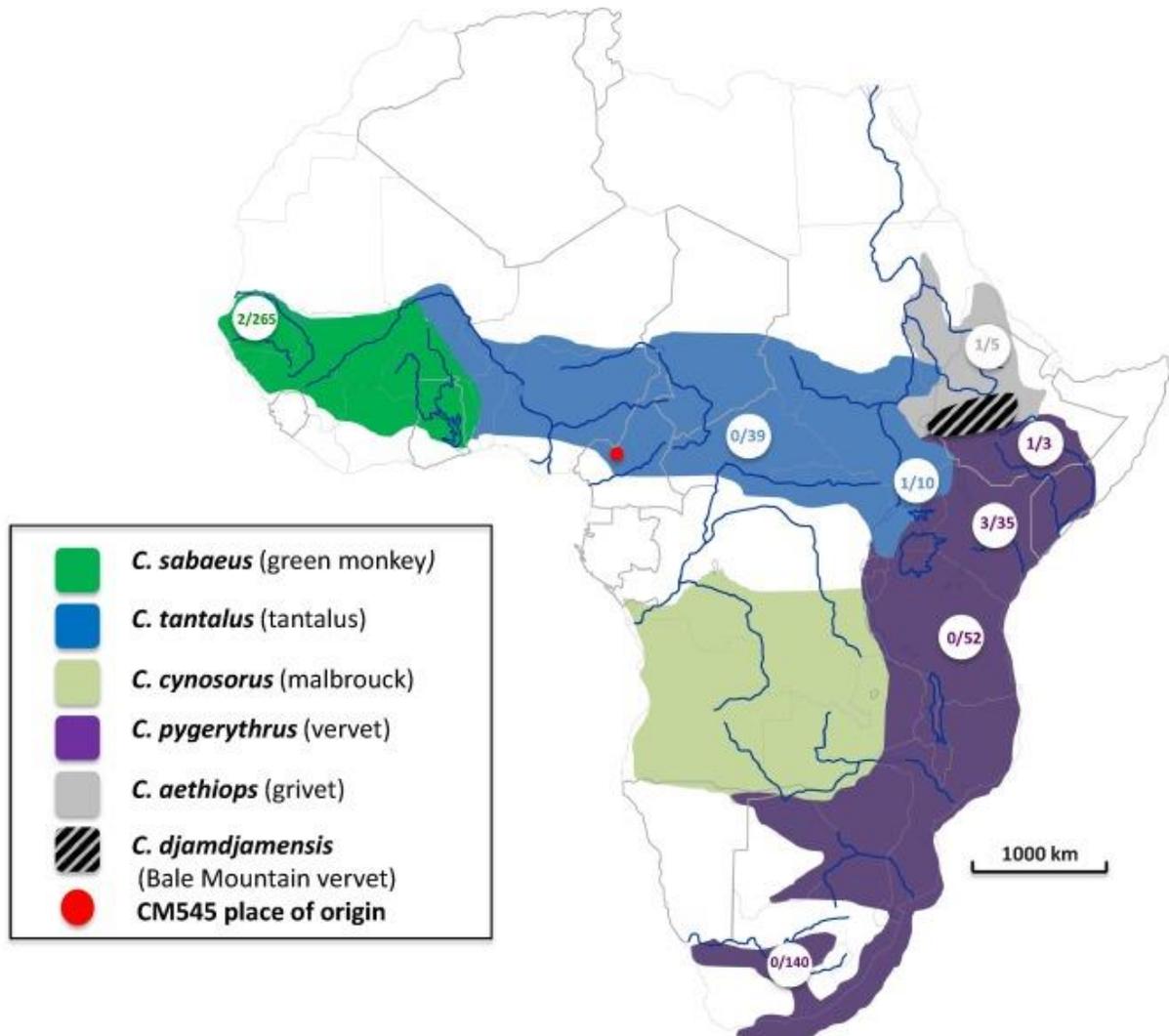


Figure 1: The natural distribution of primates in the family Cercopithecidae, with the *Chlorocebus pygerythrus* habitat shown as the purple area (Ayoub et al., 2015).



Figure 2: Vervet monkeys grooming

Vervets exhibit sexual dimorphism with males being larger in weight and body length (Fedigan and Fedigan, 1988). Adult males have an average weight of 5.5 kg and body length of 49cm, from head to base of tail. Adult females have an average weight of 4.1 kg and length of 42.6 cm. Males have the typical bright blue scrotal areas that contrasts with red penises (Fedigan and Fedigan, 1988).

As most primates, vervets are social animals, naturally living (travel, feed and sleep) in large groups of 10-50 individuals in the wild (Struhsaker, 1976; Harrison, 1983). The group composition usually includes 1-7 adult males and 2-10 adult females plus their offspring (Harrison, 1983). In most of the cases, females remain in their original colonies for the entire life, with the result that groups tend to comprise of closely related females and their offspring. Males will migrate from their natal groups when they reach sexual maturity (\pm 5 years of age)



and they can possibly change social groups several times throughout their lifetimes (Baldellou and Henzi, 1992). Male migration may be a way to avoid fights with the dominant males. A linear dominance hierarchy exists within groups with high-ranking females (mothers, sisters and daughters) being the most sought-after grooming partners (Isbell et al., 1999). High-ranking females are the first to access high quality food, which greatly increases their food intake compared to lower ranking females (Whitten, 1983). The preferred access to high quality food increases their health and subsequently result in higher reproductive success. Vervets are generally herbivorous surviving mainly on fruits, leaves and seeds; however, they are not strict herbivores, as they also eat grasshoppers, termites and bird eggs.

The study of vervet vocal communications by Seyfarth et al., (1980) laid an important foundation to the better understanding of the complexity of vervet communication. Vervet calls are grouped into three categories: wanting, aggression and alarm. Wanting calls include medium-intensity gargles by mothers to attract their infants, while aggression or irritation is signified by chattering (Seelig, 2007). Anti-predatory alarm calls are important for primates since they use them to alert other members of the colony of approaching danger and they are typically high frequency sounds (Cheney and Seyfarth, 1985). The advantage of high frequency sound is that they are not easily localised by predators while at the same time can be picked up as a warning by other members of the troop, together with the location of the caller being identifiable (Cheney and Seyfarth, 1985; Baldellou and Henzi, 1992). With an individual being able to successfully alert its colony of approaching danger without being heard by the predator, significantly decreasing the chance of the colony from predators.

The three most documented vervet predator-specific alarm calls are for leopard, martial eagle and python (Seyfarth et al., 1980). Leopard calls are short tonal calls produced in a series



of inhalations and exhalations, while eagle calls are low-pitched grunt and python calls are high-pitched chutters. Different calls evoke different responses from individuals that heard the call and in most instances; the first reaction of vervets is to look in the direction of the caller (Seyfarth et al., 1980). Looking in the direction of the caller gives vervets an idea why the call was made. The direction that the caller is facing also reveals the direction of the approaching predator. On hearing leopard calls, the vervets would respond by climbing up into the tree to avoid being attacked and then sit on the branches furthest from the tree trunk as these branches cannot support the weight of a leopard (Cheney and Seyfarth, 1985). When an eagle alarm call is given, vervets would look up and run to the nearest bush to avoid an approaching aerial attack whilst they respond to a python alarm call by standing on hind legs and looking down on the ground (Seyfarth et al., 1980). The ability to discriminate between predators starts during infancy and alarm calls are instinctual in vervet monkeys (Seyfarth et al., 1980).

Vervets also use visual communication to signal the presence of predators and this type of communication is commonly used in crop raiding. Vervet visual communication involves all members of the group getting into a cultivated field to feed, leaving only one guard animal that positions conspicuously while the rest of the group forages (Cheney and Seyfarth, 1985). When the guard animal sees a predator, instead of giving an alarm call, it will simply move from its position, that way signalling members of the colony of imminent danger (Gerald, 2001). Silent signalling will not draw the attention of the predator to the rest of the colony. The guard animal will only vocalise in the event of being detected by the predator before it has the chance to pass the signal to other colony members (Cheney and Seyfarth, 1985). Another form of visual communication seen in male vervets is the splay legged and red, white and blue displays that is used by male vervets as a sign of aggression (Baldellou and Henzi, 1992; Gerald, 2001).



2.2 The vervet monkey as a model in biomedical research

Nonhuman primates are a commonly used model in biomedical research due to their similar physiology to humans. According to Carlsson et al., (2004), vervets are the most commonly used NHPs in biomedical research. Vervets have gained in popularity in biomedical research due to their relative abundance, their status of being free of Herpes B virus and being a financially more viable model than macaques (Baulu et al., 2002; Jasinska et al., 2013). The use of vervets range from toxicity testing to the study of disease and manner of curing them. Vervets are an ideal neurobehavioral model because they are an intermediate model between humans and rodents, which can elucidate human cognitive, processes for example schizophrenia (James et al., 2007). NHPs can be used in pharmacokinetic and toxicokinetic studies when there is enough scientific justification that the dog is not an appropriate model of the study (Chapman et al., 2007) for example the testing of biotechnology-derived pharmaceuticals e.g. monoclonal antibodies. Vervet monkeys are increasingly used in the study of human immunodeficiency virus (HIV) because when infected with simian immunodeficiency virus (SIV) they do not develop signs of clinical disease while the Caribbean vervet which is free of SIV, elicits an immune response on initial infection (Goldstein, 2006). The Caribbean model is also a useful model to understand how the immune system prevents disease progression (Goldstein, 2006; Pandrea, 2006). Vervet monkeys have gained popularity as a model of metabolic disease since they can survive on a human diet, making them useful in research aimed at screening and heritability analysis for markers of metabolic syndrome (Kavanagh et al., 2007). Isolated vervet tissues have found use



in the investigation of hepatic lipid metabolism in cholesterol-induced non-alcoholic fatty liver disease (Kavanagh et al., 2007).

2.3 The domestic cat as a model in biomedical research

Domestic cats are used as models in biomedical research because they spontaneously develop a number of conditions similar to human diseases such as feline asthma, obesity and diabetes (Hoenig, 2006; Leemans et al., 2012). The feline immunodeficiency virus (FIV) that causes clinical pathology of immunodeficiency in cats that is similar to human AIDS, has structural and biochemical similarities to the Human Immunodeficiency Virus (HIV) (Elder et al., 2010), making the domestic cat an important model for research on viral neuropathology (Fletcher et al., 2008), and antiretroviral therapies (Van Rompay, 2010). Disease progression of feline asthma is similar to the immunological and pathological changes found in human asthma (Reinero, 2011), making the domestic cat a valuable model for allergic asthma allowing further research into the treatment and diagnosis of the condition (Leemans et al., 2012). Domestic cats are also prone to conditions of impaired metabolism such as obesity and type 2 diabetes mellitus that also affect people (Hoenig 2006). Research into the pathogenesis of feline type 2 diabetes mellitus has made known the similarities between the feline and the human disease (Henson and O'Brien 2006). Cats have also been used as models for interstitial cystitis since they spontaneously develop inflammations of the lower urinary tract (Westropp and Buffington 2002).



2.4 Stress

Stress is a term that is currently not well defined, but being widely used to describe the biological response to a threat or to describe the actual threat (Romero, 2004) or as a synonym to describe an inability to cope with a situation. For this study, we define stress as the biological response exhibited by an animal to cope with a threat to its homeostasis (Kranner et al., 2010), which could be either physical, physiological, or behavioural. Dantzer et al., (2014) defined a stressor as a predictable or unpredictable environmental perturbation that results in the unconscious activation of physiological responses in the animal. As this response cannot last for long periods, the body tries to bring back the physiological conditions to stability in a process called homeostasis (Davis, 2006). Homeostasis keeps set points that are essential for life, such as body temperature and glucose levels (Lupien et al., 2006). Allostasis is the process by which homeostasis is achieved; it is the process of attaining stability through physiological or behavioural changes (McEwen and Wingfield, 2003). Allostasis allows modification of the above-mentioned set points through changes of intrinsic and extrinsic factors, to maintain the body in stable conditions during physical, psychological, or environmental challenges (McEwen, 2002; Dantzer et al., 2014). Animals can cope with stress through stress responses, which imply both physiological changes (e.g. secretion of glucocorticoids (GCs)) and behavioural changes (e.g. fight or flight response). In cases of acute stress (e.g. predator attack) GCs may increase temporarily (Dantzer et al., 2014), while persistence of a stressor over longer periods (e.g. poor housing conditions) may manifest as chronic stress responses (Dantzer et al., 2014) resulting in continuously elevated GC levels, as the body may not be able to bring back the physiological conditions to homeostasis between recurring stressors.



From the stress model (Figure 3), it is evident that the stress response is a cascade of biological events with respective feedback loops, and it can be broadly divided into three stages, namely the recognition of a stressor, the biological defence against this stressor and the consequences of the stress response (Figure 3) (Moberg, 2000). The stress response begins with the central nervous system (CNS) perceiving a potential threat and then mounts a biological response that consists of the four general biological responses (behavioural, autonomic, neuroendocrine or immune responses) (Moberg, 2000).

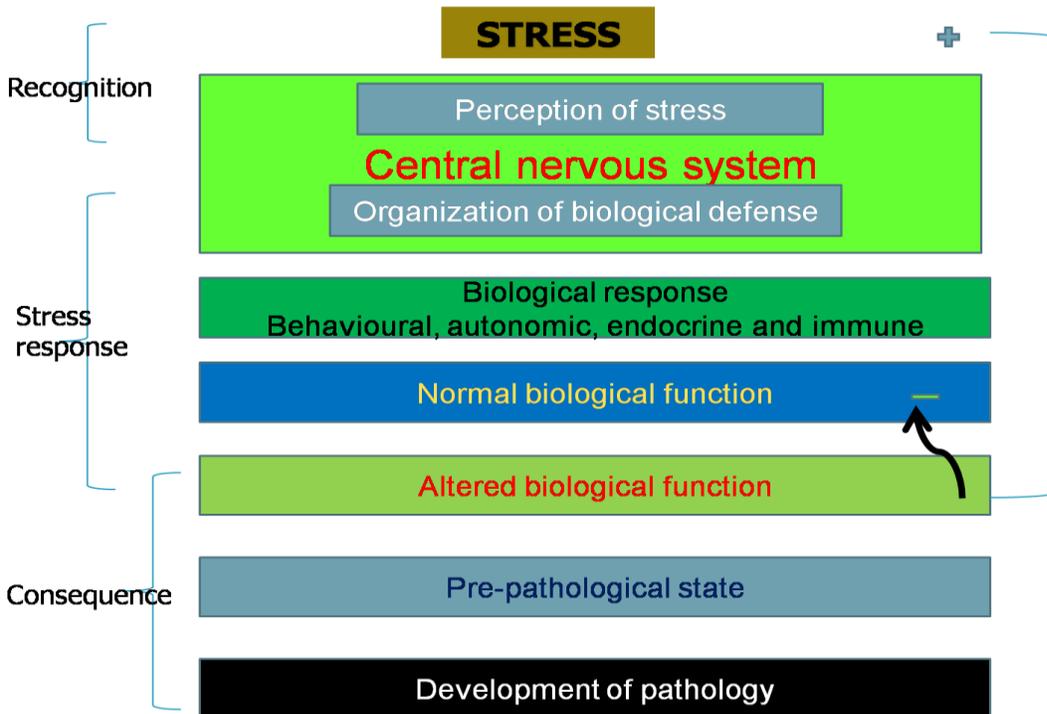


Figure 3: A model of the biological response of animals to stress (adapted from Moberg, 2000). The model is a cascade of biological events with respective feedback loops.

- Behavioural response: When faced with a stressor the most common and immediate biological response is for the animal to move away from the stressor. For example, an animal moving away to avoid a predator or seeking shade when an animal's body



temperature is elevated. However, in some instances behavioural responses may not be expressed because the animal is in a situation where its options are limited for example animals in captivity might not have enough room to express the behavioural response (escape from predators).

- **Autonomic response:** The autonomic nervous system is the second line of defence for animals faced with a stressor. The autonomic nervous system is the basis for the flight or fight response during stress and has the function of prioritising certain physiological functions to optimise survival in a stressful situation such as the interaction with a predator. A stressed animal will trigger the autonomic nervous system to exert its effect on various biological systems, such as the cardiovascular system, gastrointestinal system, the exocrine glands and the adrenal medulla with consequent changes in carbohydrate metabolism, heart rate, blood pressure and gastrointestinal activity. These changes caused by the autonomic nervous system are the physical signs that are normally associated with stress. Since the autonomic nervous system results in biological effects that are short lived, it can be argued that the autonomic nervous system does not have a significant impact on an animal's long-term welfare unless constantly stimulated.
- **Neuroendocrine response:** The neuroendocrine response begins when cognitive brain centres perceive stress leading to the release of corticotrophin-releasing hormone (CRH) (Figure 4). The CRH is then transported to the anterior pituitary gland where it stimulates the hypothalamic-pituitary-adrenocortical (HPA) axis to release



adrenocorticotrophic hormone (ACTH) (Narayan and Williams, 2016). The increased ACTH then stimulates the release of glucocorticoids, such as cortisol, from the adrenal cortex. The glucocorticoids affect a wide range of biological functions within the body, such as carbohydrate metabolism and immune function (Narayan and Williams, 2016). The main catecholamines are adrenaline and noradrenaline, which play essential roles in an animal's short-term reaction to stress.

- Immune response: Stress can depress the immune system and render animals vulnerable to infection and disease. Stressors significantly activate hormones (e.g., glucocorticoids) that have direct receptor-mediated interactions with the immune system, leading to an immune compromised state exposing the animal to disease. Stressors known to have such immune suppressive effects include those associated with husbandry practices e.g., movement stress or isolation stress.

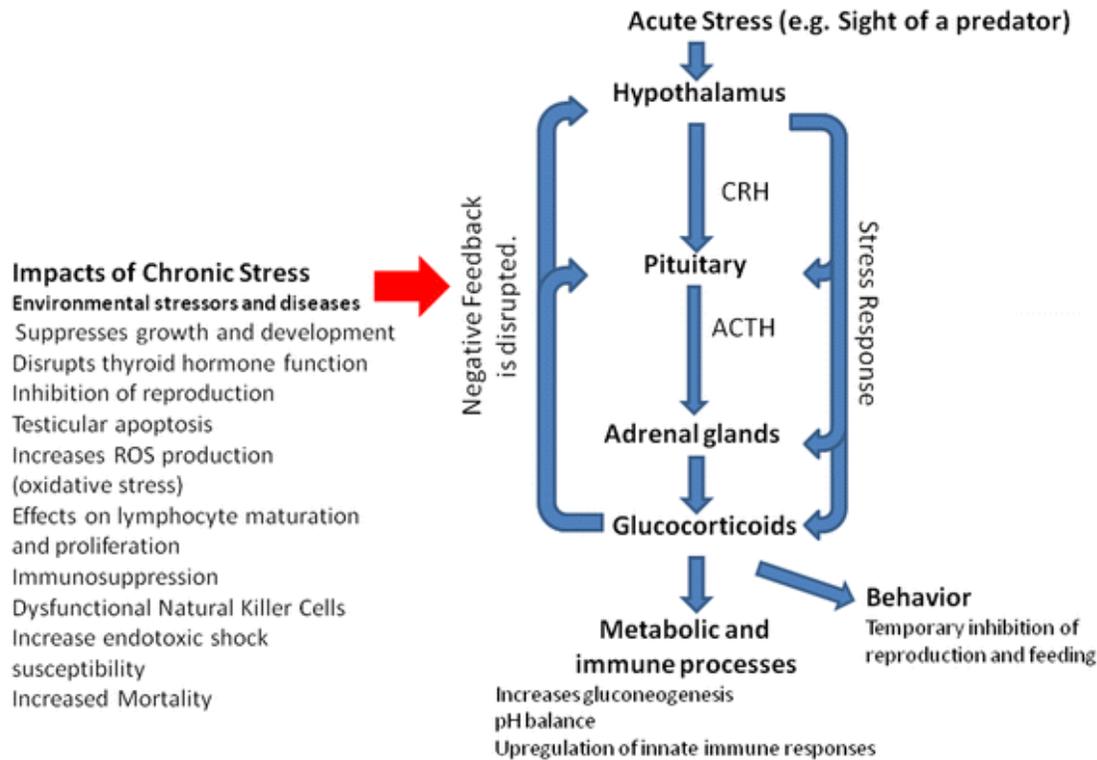


Figure 4: The hypothalamic–pituitary–adrenal axis and the effects of acute and chronic stressors on biological functions (Narayan and Williams 2016).

During prolonged or severe stress, the biological cost is significant and the work of stress becomes a significant burden to the body. It is during chronic or severe stress that the animal enters the next stages of stress: prepathology and pathology (Figure 3). The prepathological state occurs when the stress response alters biological function sufficiently to place the animal at risk of developing pathologies. The change in biological function occurring during a stress response may suppress immune competence, rendering the animal susceptible to pathogens that may be present in the environment. If the animal succumbs to these pathogens and becomes ill, it enters a pathological state. For example, respiratory disease in animals being transported is attributed to a suppression of the immune system caused by the transportation stress. The longer an animal is



stressed the longer the animal is in a prepathological state and the greater is the opportunity for a pathology to develop (Moberg, 2000).

2.4.1 Stress in laboratory animals

Stressors confronting the laboratory animal fall into two general categories, stressors associated with experimental procedures and stressors associated with husbandry practices (NRC, 2010). Experimental procedures are regarded as the main cause of stress in laboratory animals and are given much scrutiny on ethical approval, however there are other factors that can contribute to stress in laboratory animals like the research environment (feeding, housing, noise and lighting). The housing system can induce stress if it is designed in such a way that it does not take care of the nocturnal or diurnal sleeping patterns of most laboratory animals and the animal's circadian rhythms (NRC, 2010). Background environmental noise such as the opening and closing of doors, ventilation systems, talking workers and equipment sounds can also induce stress (Poole, 1997; NRC, 2010). It is noteworthy that for laboratory animals, noise does not necessarily mean a stimulus that is heard but it can be any factor affecting the production of the signal that the animals are used and acclimatised to. In addition, rodents, dogs and smaller primates can perceive high frequency noises such as ultrasound (Poole, 1997) causing stress from the constant background noise.

Animal housing that does not take care of the species-specific animal requirements provokes stress and can be a constant confounder on research data (NRC, 2010). This therefore makes it crucial for maintaining a constant experimental environment that will allow for reliable research results and ultimately the reduction of animals used for research. Stress alleviation



should thus not only be for the purposes of getting ethical approval but also to ensure the research is reputable.

2.4.2 Stress monitoring in laboratory animals

The three most commonly used methods for monitoring stress in laboratory animals are the monitoring of changes in behaviour, glucocorticoid concentrations, and changes in weight:

- Monitoring changes in behaviour is an appropriate way of monitoring stress in laboratory primates because it is non-invasive (Mench, 1998); but it has a challenge in that we lack understanding of animal behaviour as it relates to stress (Rushen, 2000). Accepting behaviour as an indicator of stress requires that one be able to correlate behavioural changes with stress-induced physical / physiological changes. During stress-related behaviour monitoring the following categories are often monitored; vocalising, stereotypic behaviour, and isolation.
- Stressed animals generally have a poor appetite leading to decreased food intake with a failure to either maintain their body weight, gain weight or even lose weight (NRC, 2010). Even if the animal maintains a normal appetite during stress, the underlying causes of stress may increase energy expenditure contributing to a net loss of energy that will subsequently lead to weight loss (NRC, 2010). Since many laboratory animal species are group housed it can be difficult to detect changes in food and water intake, thus making loss of body weight an important indirect measure of a decrease in food and water intake.



- Monitoring alterations in GC concentrations is an accurate and well-known way of assessing stress because it directly monitors the responses of the neuroendocrine, autonomic and immune systems to potential stressors (Touma et al., 2003). This method has challenges in that the act of restraining animals and drawing blood is a stressor, which causes significant changes in circulating neuroendocrine hormones or autonomic nervous system activity. Furthermore, while in larger animals it may be possible to obtain serial blood samples via a cannula placed some time before the first sampling, this approach is not practical for stress monitoring in small laboratory animals and inquisitive animals like NHP nor does it eliminate the stress of handling the animal. In contrast to determining GC levels in blood, faecal glucocorticoid metabolite concentration can be measured. This method has benefit over blood sampling, in that the procedure is non-invasive i.e. samples collected with minimal disturbance to the animal, and the method also allows for re-sampling the same individuals without bias from sampling disturbance (Millspaugh et al., 2002; Palme et al., 2013; Touma et al., 2003). Plasma and faecal sampling also differ in that plasma samples represent a snapshot of circulating hormones at a specific point in time while faecal samples represent an accumulation of metabolized and excreted hormones over a given time period (Palme and Möstl, 1997). Steroid hormones undergo metabolism and conjugation in the liver and if they are not recycled through the enterohepatic cycle, they will be excreted via bile into the digestive system. The period that hormones and their metabolites pass through the digestive system before being excreted in faeces is called the temporal lag phase (Anestis, 2010). Thus, whilst plasma measures provide the option to ascertain the maximum concentrations which



circulating hormones may reach following a stimulus, faecal samples represent an integrated measure of the hormonal response to a stimulus (Romero, 2004).

2.4.2.1 Faecal hormone metabolite measurement

Faecal steroid analysis has an advantage in that sample collection is easy and does not require the isolation of animals thereby avoiding the disruption of the animal's natural behaviour. It also uses a small faecal sample and in most cases 1-3g of faeces being sufficient (Palme et al., 2013). To be able to identify faecal samples to a particular individual, animals can be fed with coloured food items e.g. edible food colorants or different sorts of seeds/grains that are not easily digested (Stavisky et al., 2001). For steroid analysis, prior to collection the sample must be homogenised because steroids might unevenly distribute in faeces. Water and urine contamination should be avoided as this can alter steroid hormone levels (Wasser et al., 1988). Faecal hormone metabolite analysis is the preferred non-invasive method in primates as some primate species excrete insignificant amounts of hormones via urine (Ziegler et al., 1996).

Various extraction methods have been described and the most common extraction method involves extracting into solutions of ethanol or methanol (40–80%) by simple shaking (e.g. Heistermann et al., 1995). This method allows for direct measurement of hormones from the supernatant following centrifugation of the faecal suspension. (Ziegler et al., 1996). Faecal hormone assays are usually designed to measure the unconjugated portion, although the use of conjugate assays can also be successful, particularly when the antibody involved shows a substantial cross-reactivity with the unconjugated steroid metabolites excreted (Daspre et al., 2009; Heistermann et al., 2001). The most commonly used immunoassays are the



radioimmunoassay (RIA) and enzyme immunoassays (EIA). The RIAs make use of radiolabelled hormones as the competitive tracer in the quantification process, while the EIAs use enzyme labelled preparations (Hodges et al., 2010).

2.4.2.2 Immunoassay Validation

Glucocorticoid metabolism and excretion differs between species, even from those that are closely related (Palme, 2005) making it crucial to reliably validate the method for each species before using faeces as a hormone matrix for monitoring adrenocortical activity. The adrenal gland differs between species and different species often secrete different compositions of glucocorticoids in various quantities, with cortisol being the main glucocorticoid secreted in primates, carnivores and ungulates, whereas corticosterone is the main circulating GC in most rodents, birds and reptiles (Palme, 2005; Touma and Palme, 2005). Glucocorticoid secretion is affected by complex processes that include circadian and circannual rhythms; hepatic metabolism and conjugation (Palme, 2005; Reeder and Kramer, 2005). These processes differ between species and the sex of a given species (Palme, 2005). The validation should also evaluate the specificity of the measurement when immunological detection methods are used and not only to be validated for demonstration that the GC measurement detects adrenocortical endocrine activity in response to stress (Goymann, 2012). The immunological detection is of utmost importance since glucocorticoids and other steroids (e.g. testosterone) can be structurally very similar (Ganswindt et al., 2003); enabling antibody cross-reactivity. Such cross-reactions can have major and distorting effects on the results obtained (Ganswindt et al., 2003; Goymann, 2012). Glucocorticoid metabolism can even differ between sexes within a given species (Baltic



et al., 2005), making comparisons of GC levels between males and females potentially problematic and meaningless unless the immunological specificity of the assay used is demonstrated (Goymann, 2012).

2.5 Stress alleviation in laboratory vervets

In order to manage stress in laboratory vervets, one has to be able to recognise that stress is occurring, and this requires that species typical behaviour that has been discussed in previous sections is well understood. Stress that results from painful procedures can be alleviated by either removing the inciting cause or pharmacologically by administering analgesic drugs. The use of tranquilisers can also help laboratory animals adapt to changes associated with staying in captivity but this alone is not sufficient (Hawkins, 2002). Non-pain induced stress, in most cases, is not amenable to pharmacologic treatment alone (Swindle et al., 2002) and this makes it important to address environmental factors that are normally regarded as the main causes of stress in captive vervets. Refinement is the attempt to enhance animal welfare and control extraneous variables that may stress research animals and it is of key importance in improving the well-being of laboratory animals (Newberry, 1995; Olsson and Dahlborn, 2002).

2.5.1 Environmental enrichment

Environmental enrichment is a term used in laboratory animal science that refers to the provision of stimuli that promote the expression of species specific behavioural and mental activities (Newberry, 1995), aimed at creating an environment that is as close as possible to the animal's real life in nature. Environmental enrichment is of utmost importance to the welfare of laboratory



animals as they spend most of their time in captivity. Housing conditions of laboratory animals can be enriched by providing opportunities for the animals to perform species-specific behavioural repertoire. Environmental enrichment must meet the animal's needs, be practical, inexpensive and pose no risk to laboratory staff, the animals and to the experiment (NRC, 2010). Environmental enrichment improves the animal's well-being, whilst animals in captivity and deprived of enrichment have poorly developed brains for example animals with disturbed motor function have increased arborisation of dendrites when provided with enrichment (Mohammed et al., 2002). Animals from enriched housing conditions make representative animal models because they are physiologically and psychologically stable (NRC, 2010). Besides improving animal welfare and the quality of scientific results, environmental enrichment also has a benefit in that animals become easier to handle. Environmental enrichment can be further classified into physical, social, nutritional, occupational and sensory enrichment (Newberry, 1995; Olsson and Dahlborn, 2002; Wells, 2009).

2.5.1.1 Physical enrichment

Physical enrichment includes additions to the physical environment of the animal with the aim of promoting species-specific manual manipulation and locomotor patterns, and providing visual barriers for privacy (Newberry, 1995). Physical enrichment includes manipulable and structural enrichment.

- **Manipulable Enrichment:** Manipulable enrichment can be in the form of big manipulable toys that animals can manipulate and the toys must be durable objects such as plastic balls and toys (NRC, 2010). If smaller toys are to be used, they must be attached to the



enclosure with short chains to provide opportunities for manipulation without the possibility of them being swallowed. Manipulanda must be sanitized regularly, as they can be a source of infection for the animals. Mirrors (plastic or stainless steel) can also be used.

- **Structural Enrichment:** Cages can be divided horizontally or vertically to promote climbing behaviour and allow for variation in cage configuration. Cages can be constructed with at least one permanently fixed perch or alternatively tree branches can be used if enough space is available. If group housing NHP is not possible, cages must be equipped with view boxes that allows NHP to see other animals and provide a different perspective of their environment (NRC, 2010). Cages must also be constructed in such a way that they have solid partitions to which animals can use to avoid conflict.

2.5.1.2 Social Enrichment

The ancestors of NHPs, commonly used in biomedical research, evolved in complex social groups (Sapolsky, 2005) and social groups are important in the evolutionary history of primate species (Seyfarth et al., 1980). Approximately 10% of singly caged rhesus macaques develop self-injurious behaviour, regurgitation, and locomotor and other stereotypies. (Novak et al., 1998), and because of this, welfare and regulatory agencies recommending that “social housing” be the default housing arrangement for all laboratory animals including nonhuman primate species. When group housing primates it is necessary to be aware of the welfare issues surrounding the implementation of such a strategy for example animal aggression and dominance over food. Social partners are the most basic environmental variable as they can provide



constantly changing stimuli, which can be stimuli to the animal's social and cognitive functioning. Forming social pairs of NHPs is not without risks; however, the benefits of social housing usually outweigh the risks. It is also important to allow sufficient room for group housed animals so that they can move away from one another. If group or pair housing is not possible, all singly housed primates should have auditory, visual, and olfactory contact with conspecifics and, occasionally, provided with tactile contact via grooming/contact bars. Humans can also provide a positive interaction, which is important to develop good relations with those primates that are constantly being handled.

2.5.1.3 Nutritional Enrichment

Nutritional enrichment includes novel food items presented in a variety of ways that increase the diversity of the animals' diets (Olsson and Dahlborn, 2002). Food enrichment is provided if it does not interfere with the study or health of the animal. Primates can be supplied with whole pieces of fresh fruit (e.g. oranges) or vegetables with peels still intact so that the animals get the same experience of manipulation and processing prior to consumption that a primate would have to exhibit in the wild. A diverse assortment of novel food items should be supplied to increase the variety of the animals' diets, including but not limited to: peanuts, dried fruits and vegetables.

2.5.1.4 Occupational Enrichment

Occupational enrichment stimulates problem-solving, motor skills, and coordination e.g. puzzle feeders (Laule, 2003). Puzzle feeders filled with grain or treats are put into the primate's cage. Positive reinforcement can be used to shape a primate's behaviour and encourage cooperation in



research procedures (Laule, 2003). Animals are rewarded for performing desired behaviours, which builds a more positive relationship with the caregiver and provides goal directed, enriching activities. Training provides a sense of control and predictability for the animals, minimizes environmental stressors, and reduces time and labour for staff caring for the animals.

2.5.1.5 Sensory Enrichment

Sensory enrichment is aimed at promoting auditory and visual stimulation, for example use of television and music (Wells, 2009). Volume should be kept at a reasonable level and can be played for one to eight hours per day and turned off at the end of the working day.

2.6 Co-housing under laboratory conditions

In biomedical research, the need for cohousing animals of different species can be due to factors such as space limitations, sharing of limited laboratory equipment and consolidating animal feeding and cleaning. Co-housing also reduces operating expenses, for example electricity, cleaning and disinfectant materials. However, cohousing of different species must be implemented taking into consideration the size of the animals being cohoused since larger animals could trample and injure smaller ones, diseases status of the animals as there could be transfer of interspecies parasites and diseases. Considering the above-mentioned factors, cohousing of different species may be acceptable if there is need and the species to be cohoused are of a similar pathogen status and behaviourally compatible.

The following species have been successfully cohoused in the same room but in different cages under laboratory condition; rats and mice, gerbils and hamsters, guinea pigs and mice



(Buchanan, 2000; Apfelbach et al., 2005). However, it should be noted that the literature on the cohousing of mice and rats is equivocal because of their predator/prey relationship. Some studies have shown that co-housed mice and rats exhibit signs of acute and chronic stress (Greene et al., 2014; Calvo-Torrent et al., 1999; Buchanan, 2000.) while other studies have found negligible stress related effects (Arndt et al., 2010). Arndt et al., (2010) argued that the influence of rats on mice could be reduced or even prevented if mice are habituated to the presence of rats throughout their lives. Zhang et al., (2003) noted that predator odour affects prey more profoundly than previously believed, and the impacts are not always negative since chronically cat-odour-exposed mice become more aggressive making their urine more attractive to female mice (Zhang et al., 2003), which is beneficial and positive for the female mice. Naturally, dominant mice have increased aggression, are more attractive to females and have lower anxiety levels than subordinate mice (Bartolomucci et al., 2001).

Cats and ferrets have been found to be compatible living in the same room that has a visual barrier to decrease possible anxiety (Hillyer et al., 2004). Cats have also been successfully transported with other species such as ferrets, horses and cattle in compartmentalized areas with a physical barrier to prevent direct bodily contact (NIH, 2013). Historically, different species of farm animals have been housed in the same pastures and in separate pens, with goats used as companion animals for horses, donkeys housed with sheep to minimize predators (Blecha, 2007). It has been proven that pastures are more efficiently utilized when sheep and cattle share the same pastures (Blecha, 2007). A precedent has been set for housing different species together and has proved to improve the welfare of animals; however, to our knowledge no systematic research on this topic has been undertaken for vervets. For this study, we investigate the potential of co-housing cats and vervets as a manner of stress alleviation. This idea arose following a visit



to a sanctuary in South Africa, where the vervets were housed in groups in large outdoor pens.

An unexpected finding was that a group of feral cats had also made the vervet enclosure their home without any fear of the vervets. More interestingly, the vervets appeared to tolerate the presence of the cats extremely well.



CHAPTER 3: MATERIALS AND METHODS

3.1 Animal ethics

The University of Pretoria Animal Ethics Committee (UPAEC) approved the use of vervets and the domestic cats that were resident at the University of Pretoria Biomedical Research Centre (UPBRC) for this study (Protocol Number V118/15).

3.2 Animals used in the study

3.2.1 Vervets

The vervets used in this study were resident at the UPBRC, and were not part of any other research study at the time of this experiment. The colony was unique in that the individual history of the animals was unknown including their age and relationship to each other. In 2003, using the data obtained from the registered hunter who supplied the vervets, the colony was estimated to have an average age of nine years. The colony comprised of six females and four males. The colony was kept in heterosexual pairs or groups of three in ceiling height type cages, with an indoor and outdoor component since 2011 (Figure 5 and Figure 6), with both enclosures having artificial trees therein. The cages were cleaned at least once a day. The vervets were fed twice daily, in the morning with fruits and primate biscuits (wheat bran, maize meal, PVM primate supplement (PVM Nutritional Sciences, South Africa) and vitamin C (Junglevites Chewey C, PharmaNatura, South Africa) and in the afternoon (fruits and vegetables). Potable municipal water was provided ad libitum. All male vervets were neutered in 2011 prior to



heterosexual pair housing. Room temperature for the indoor cages was maintained at 20-25 °C with a relative air humidity of approximately 50% and a 12-hour artificial light/dark cycle. Food enrichment was provided in the form of raisins, rusks, sunflower seeds and nuts; and environmental enrichment by providing hard plastic toys, balls, foraging containers, plastic crates, climbing wooden logs, puzzle feeders, swing ropes and tyres.



Figure 5: Ceiling height type cages for vervet monkeys at UPBRC, indoor enclosure (5.7m²).



Figure 6: Ceiling height type cages for Vervet monkeys at UPBRC, outdoor enclosure (9.7m²) with environmental enrichment.

3.2.2 Domestic cats

Eight domestic cats that were part of another long study were used in this study (Protocol number V006/15). All cats underwent elective neutering before the study. The colony comprised of four females and four males. Before cohousing, the cats were housed in a standardised indoor enclosure equipped with a water bowl, food bowl, litter tray, cat beds and soft blankets. The cages were cleaned at least once a day. The cats were fed twice a day with a commercial cat diet and water was provided ad libitum. Environmental enrichment included scratch poles, tree stumps, hiding places and toys (Figure 7). Expanded metal grids that were thirty centimetres apart separated the outside enclosure for the cats and vervets.



Figure 7: Outdoor enclosure for the cats with associated environmental enrichment

3.3 Experimental procedures

3.3.1 Behavioural observations

To monitor predator avoidance behaviour, the vervets were kept in their usual social groups, during the introduction of the domestic cats as next door neighbours that were brought to the enclosure next to the vervets. No changes were made to the animal's diets and water was provided ad libitum. The cats were allowed outdoor access to the enclosure next to the vervets during the day (8am – 2pm), while they were returned to their indoor housing at night. After one month, the cats were left permanently in the enclosure next to the vervets, with a free choice of inside or outside access. To prevent injuries, the cats and vervets were never in direct contact, with a double expanded-metal mesh separating them always, but they could see and smell each



other via the cage fencing. To minimise confounding variables, only personnel whom the primates were accustomed to, could work in the unit. Staff, responsible for monitoring the animals, had at least three years of experience working with the vervets. During the first six days of introduction, the domestic cat and vervet behaviour were monitored on an hourly basis, which we decreased to twice daily thereafter. Animals were also monitored for the full duration by closed circuit video capture, for predator avoidance behaviour. The following predator avoidance behaviours were chosen for analysis, as described by Seyfarth et al (1980) as relevant for primates facing a potential predator:

1. Looking in direction of predator
2. Alarm calls (short tonal calls produced in a series of inhalations and exhalations)
3. Climbing high up tree branches

3.3.2 Faecal sample collection

Faecal samples were collected for six days prior to introduction of domestic cats as baseline. After introducing the domestic cats, faeces were collected for another six days. The enclosures were inspected three times a day, in the morning (7:00 - 10:00), at noon, and afternoon (14:00 - 15:00) and all faeces were collected into individually labelled plastic bottles. Samples were homogenised using a wooden spatula before placing into plastic bottles. A new spatula was used for each sample. All available samples, including night samples were collected. All samples were stored in the $-20\text{ }^{\circ}\text{C}$ freezer within one hour of collection (to avoid microbial degradation of the hormones and their metabolites) until analysis.



For the vervets, individual sampling was possible because they stayed in small groups (2 to 3 individuals) and they were fed a biscuit that was mixed with different food colorants (Robertsons) so that their faeces would be coloured. The domestic cats were using communal litter trays and the faecal sample could only be identified to a particular cat when the cat was observed defecating. Three hundred and eleven samples were collected for the vervets and seventy-nine for the cats.

3.3.3 Faecal steroid extraction

The frozen faecal samples were then sent to the Endocrine Research Laboratory at the Faculty of Veterinary Science, University of Pretoria, where they were lyophilised (CHRIST Alpha 1-2LD plus freeze dryer) at -50°C and 0.96mbar for 2-3 days until dry. Thereafter the samples were pulverised using a pestle and mortar. After pulverising the samples were then sifted through a wire-mesh strainer separating dried faecal powder from undigested material. From the collected faecal powder, between 0.10 g and 0.11g from each sample was then transferred into test tubes where 80 % (v/v) ethanol (3ml) was added and then vortexed for 15 min and centrifuged (1500g for 10 min). The supernatant (1.5ml) was then transferred into labelled Eppendorf safe-lock micro test tubes and stored at -20°C until further analysis for hormone determination.

3.3.4 Enzyme-immunoassays

The concentration of glucocorticoid metabolites was determined using a cortisol EIA following the description by Palme and Möstl (1997). The assay for cats (Schatz and Palme, 2001) and wild vervets (Young et al., 2017) has already been validated and successfully used for



monitoring changes in adrenocortical activity. The fGCMs were determined using a 50 μ l aliquot standards, quality controls and diluted faecal extracts (from 1:10 to 1:1000) which were pipetted into coated microtiter wells. Thereafter, biotin label solution (50 μ l) and respective antibody (50 μ l) were added before incubating the plates overnight at 4°C. The plates were then washed four times after incubation (Biotek Instruments, EL 405 LS) and blotted dry. Streptavidin-peroxidase (150 μ l) was then added to each well before further incubating at 4°C for 45 min in the dark. After incubating, the plates were washed again four times and blotted dry, thereafter tetramethylbenzidine peroxidase substrate solution (150 μ l) was added and the plates incubated in the dark for a further 30 – 60 min at 4°C. The reaction was then stopped by adding sulphuric acid (50 μ l) and the optical density of each well measured at dual wavelength of 450 nm and 620 nm using a plate reader (BioTek Instruments Elx 800 and Gen v5.00 software).

For the vervet samples the coefficient for intra-assay variance ranged from 4.8% to 5.8%, and the coefficient for inter-assay variance ranged from 8.1% to 12.6%. The sensitivity of the EIA used was 0.6 ng/g dry weight (Cortisol EIA). For the cats, the coefficient for intra-assay variance was 4.0% - 4.8%, and the coefficient of variance for inter-assay variance was 12.7% - 14.7%. The sensitivity of the EIA used was 2.4 ng/g DW (11, 17-DOA EIA).

3.4 Statistical analysis

The behavioural observations of the vervets were analysed for a period of one minute prior to cat introduction, for one minute immediately post introduction and for one minute after three days of cohousing. To check for significant changes in behaviour before and during cohousing the data was checked for normality using the Shapiro-Wilk test and analysed using the Kruskal-Wallis



One Way Analysis of Variance on Ranks and multiple comparisons using Tukey post hoc test was used to isolate the group or groups that differ from others (Sigma Plot for Windows software, ver. 12.5).

To check if cohousing influenced animal weight, the animals were weighed before and six days during cohousing. The weights were then checked for normality using the Shapiro-Wilk test and then analysed using paired t-test (Sigma Plot for Windows software, ver. 12.5) to check for any significant changes.

To check for significant changes in fGCM concentrations before and during cohousing, daily medians for each individual was calculated where more than one sample per day was collected. This was done for the day prior to introduction, of introduction, and day 1 & 2 post-introduction. The fGCM data was checked for normality using the Shapiro-Wilk test and then analysed using the one-way analysis of variance (ANOVA) (Sigma Plot for Windows software, ver. 12.5). The significance level was set at 0.05.



CHAPTER 4: RESULTS

4.1 Behavioural observations

On the first day of cohousing, the animals were inquisitive (looking in direction of cats, pacing up and down), kept their distance. Based on the subjective assessment of the observer, the vervets (Rex, Maisie, Suzi & Oom Piet) housed in cages that were closest to the domestic cats were the most active. During the first one minute, the vervets looked at the cats, climbed up to the top of the cage and made alarm calls (Table 1). During the same time, the cats were moving and sniffing around the new housing. The calls were made whilst looking in the direction of the cats with the other vervets in the cages furthest from the cats responding by looking at the direction of the caller. None of the vervets showed redirected aggression to cage mates. In contrast, the cats moved around and explored their new environment and did not seem to be perturbed by the presence of the primates.

When comparing the number of alarm calls made one minute before cats were introduced, the time when cats were introduced and three days after cat introduction, the difference in the median values (2; 31 and 15 respectively) among the groups were greater than would be expected by chance ($P = 0.001$) and the pairwise multiple comparison test showed evidence of a statistically significant difference only for one minute before cats were introduced and at the time the cats were introduced. For the number of vervets looking in the direction of the cats, the difference in the median values among the groups were greater than would be expected by chance ($P = 0.029$) and the pairwise multiple comparison test showed evidence of a statistically significant difference only for one minute before cats were introduced and at the time the cats were introduced.



Table 1: Frequency of recorded responses of the vervets

Respondent location	Number of leopard alarm calls recorded *			Number of vervets looking in direction cats *			Number of vervets climbing up the tree branches		
	One min before cats introduced	Cats introduced	Three days after cats introduced	One min before cats introduced	Cats introduced	Three days after cats introduced	One min before cats introduced	Cats introduced	Three days after cats introduced
Cage 1	0	43	20	1	2	1	1	2	2
Cage 2	3	37	12	2	2	2	2	2	2
Cage 3	1	25	18	1	3	0	0	3	1
Cage 4	6	12	7	0	3	1	2	3	2

The response with an asterisk (*) had a statistically significant difference after performing the ANOVA test (leopard alarm calls, (p = 0.001); looking in direction of cats, p = 0.029)

4.2 Weight alterations related to changes in housing conditions

There was a slight non-significant ($P = 0.832$) increase in average weight for the vervets from $5.5\text{kg} \pm 0.82\text{kg}$ (mean \pm SD) before cohousing to $5.6\text{kg} \pm 0.81\text{kg}$ during cohousing (Table 2). In contrast, there was a significant increase ($p < 0.001$) in weight gain for the cats during cohousing with the overall average weight of $3.7\text{kg} \pm 0.72\text{kg}$ prior compared to $3.9\text{kg} \pm 0.73\text{kg}$ during cohousing (Table 3).



Table 2: Vervet weight before and during cohousing

Vervet ID	Sex	Weight before cohousing	Weight during cohousing	Cage
Rex	Male	5.8	6.0	Cage 1
Meisie	Female	5.8	5.6	
Suzy	Female	6.8	6.6	Cage 2
Oom Piet	Male	5.8	5.8	
Ouma	Female	4.9	4.8	Cage 3
Agie	Female	5.0	5.0	
Janneman	Male	6.8	6.8	Cage 4
Walker	Male	5.6	5.4	
Sweety	Female	4.0	4.2	
Magogo	Female	5.1	5.0	

Table 3: Domestic cats' weight before and during cohousing

Cat ID	Sex	Weight before cohousing	Weight during cohousing
Happy	Female	3.4	3.6
Walter	Male	4.1	4.3
Toby	Female	4.6	4.8
Sylvester	Male	4.8	5.0
Rae	Female	3.0	3.2
Paige	Female	3.1	3.2
Cabe	Male	3.0	3.2
Ralph	Male	3.9	4.1

4.3 Glucocorticoid alterations related to changes in housing conditions

The average fGCM levels before cohousing was 95.22 ± 43.04 ng/g DW (mean \pm SD) for the vervets while the average during the six days of cohousing was 125.77 ± 61.69 ng/g DW.

Although there was an overall increase in fGCM during cohousing, the differences were not significant ($p = 0.257$) (Table 4).



Table 4: Average faecal glucocorticoid metabolites (fGCM) concentration in vervets before and during cohousing

Day	N	Mean	Std Dev	SEM
Prior introduction	10	70,996	17,004	6,012
Introduction	10	97,314	63,850	20,191
Day 1 post introduction day	10	127,553	121,528	38,431
Day 2 post introduction day	10	124,616	62,609	19,799

For the period of cohousing, the vervets in the cage that was closest to the domestic cats had an average fGCM of 127.7 ng/g DW while those that were furthest had an average of 123.77 ng/g DW. For the individual animals (Figure 8), fGCM levels were mostly elevated 24hrs after domestic cat introduction with the exception for Ouma, Sweetie and Meisie. Four vervets (Magogo, Ouma, Sweetie and Walker) had a distinct peak in fGCM values prior to domestic cat introduction.

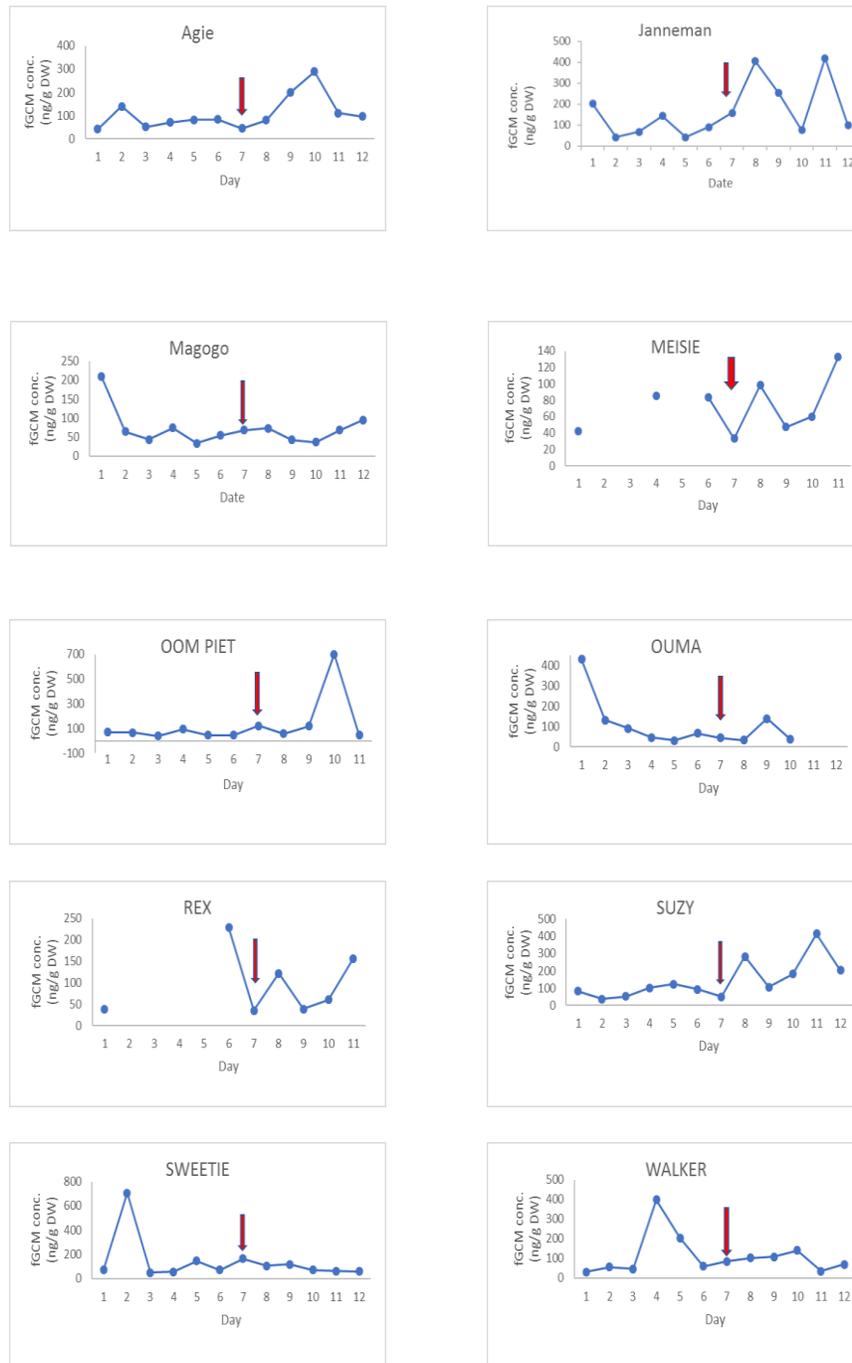


Figure 8: Individual faecal glucocorticoid (fGCM) concentration in vervets before and during cohousing with domestic cats.

The red arrow shows the time point when the cats were introduced



The pooled average fGCM levels before cohousing was 0.24 ± 0.06 ng/g DW (mean \pm SD) for the cats while during cohousing the average was 0.34 ± 0.10 μ g/g DW. Although there was an overall increase in fGCM during cohousing, the differences in the median values among the groups (Table 5) were not significantly different ($p = 0.42$).

Table 5: Average faecal glucocorticoid metabolites (fGCM) concentration in domestic cats before and during cohousing

Day	N	Median	25%	75%
Prior introduction	7	0,297	0,205	0,461
Introduction	4	0,276	0,115	0,406
Day 1 post introduction day	5	0,417	0,253	0,888
Day 2 post introduction day	5	0,417	0,253	0,888



CHAPTER 5: DISCUSSION

5.1 Introduction

This study was aimed at evaluating the success of housing vervet monkeys (*Chlorocebus pygerythrus*) next to domestic cats (*Felis silvestris catus*) separated by a physical barrier between the two groups. The idea was based on the cohabitation and stress alleviation effect of horses housed with goats (Winter, 1996) and an observation made in the field. The study also sought to ascertain the compatibility between domestic cats and vervets, to allow for better utilisation of laboratory space. While the benefits of cohousing are important in biomedical research because of limited laboratory space, this evaluation was undertaken knowing that NHP do not interact well with wild cats, as under natural conditions NHP and leopards share a predator-prey relationship. However, for this study, it was assumed that the smaller size of the domestic cat in relation to the leopard would not result in adverse behaviour in the vervets. Another negative effect that was considered in this study was the potential for the vervets to have redirected aggression and start fighting with resultant injuries to cage mates. This arrangement also had potential benefits to the cats as they were allowed more space and were able to express species-specific behaviour e.g. innate need to climb and seek refuge up high. While the aim of the experiment was to ascertain the value of cohousing cats with vervets, the possibility of the animals responding adversely to the interaction could not be overruled and hence the animals had to be monitored for stress. The study made use of the most common methods for stress monitoring in animals, which are the monitoring of changes in behaviour, monitoring faecal glucocorticoids and monitoring changes in weight.



5.2 Effect on behaviour

On the first day of cohousing, both vervets and domestic cats were inquisitive although they kept their distance. No aggression was evident in any of the cage groupings. The monkeys (Rex, Maisie, Suzi & Oom Piet) housed in the cages that were closest to the domestic cats were the most active. Our findings are similar to the findings by Jorgensen et al., (2017); who noted that vervets rarely show overt aggression on the day they are first pair housed. The vervets did however respond in a similar way reported for animals in the wild when they encounter leopards by looking at the cats, climbing up to the top of the cage and making alarm calls, as described by Seyfarth et al., (1980). The climbing behaviour of the vervets was similar to what has been previously described by Seyfarth et al., (1980) that in the wild vervets climb and sit on the branches furthest from the tree trunk with the speculation that they chose these branches since they are not able to support a leopard's weight.

Despite alarm calls being specific to a particular type of threat, they pose a challenge when used to measure the severity of different types of stressors (Moberg, 2000), because identification and interpretation of these depends on a solid foundation of knowledge on animal behaviour and may likely require special training of relevant personnel. In this study, all the personnel that were responsible for monitoring the animals had at least three years of experience of working with vervets in captivity. Moberg (2000) also states that interpretation of behavioural responses to stress is not easy, and highlights the need for understanding the processes and pathways through which the underlying behaviour is brought into being, for one to be able to use behavioural indices of stress with any confidence. Based on Moberg's (2000) suggestion we also used other methods of assessing stress other than monitoring behaviour changes only. From



studies on wild vervets, in addition to the specific vocal calls, another behaviour shown is for the animals to point out the direction of the potential danger by looking directly at the threat, so that other members may also visualise the said threat (Seyfarth et al., 1980). For this study, in addition to the vocal alarm raised, the vervets made it clear that their concern was due to the cats, as they looked directly at the cats, with the animals in the furthest enclosure looking at the direction of the caller. While the behaviour shown by the animals was consistent with that of wild animals, this was an unexpected finding as the vervets have been in captivity for 15 years. This supports previous findings that alarm calls are a learned behaviour during infancy that later become instinctual thereafter (Seyfarth et al., 1980).

The inquisitive behaviour of the cats as they explored the new housing was not unexpected as naturally cats are territorial animals that use their urine and faeces to mark their territories (Linda, 2003). The cat excretions contain organic compounds that function as signals for conspecific recognition between cats (Linda, 2003; Nakabayashi et al., 2012) and interspecies communication with other prey animals (Hegab et al., 2014). Naturally after exploring the environment, cats will urine mark their new territory, however in this study none of the cats was seen urine marking which we could attribute to the fact that the cats were accustomed to using a litter tray and that they were neutered.

5.3 Changes in weight

Another important parameter in the monitoring of animal welfare is change in body weight or ability to maintain it. Under laboratory housing conditions where animals are group housed it is not always easy to ascertain individual food intake hence body weight measurement becomes an important parameter since it can be individualised. This thus makes it very important to closely



monitor body weight for animals undergoing experimental interventions that have a high risk to cause stress. For this study, no significant changes in weight (average of 5.5kg before cohousing and 5.6kg during cohousing) were evident for the vervets, which would indicate that the animals were not severely stressed during the study. The pooled average weight before cohousing for the cats was 3.7kg while during cohousing was 3.9kg. While the weight of the cats was significantly different before and during cohousing, this was not seen as an adverse response in the study, as all the cats gained weight during the monitoring period. The significant weight gain for the cats can thus be attributed to the normal growth in healthy cats of their age.

Under stressful conditions, it is common for animals to lose weight (Poole, 1997). In the immediate period of the stressful incident, the animals can stop eating or have reduced food and water intake. As a result, they are unable to maintain their weight or lose body weight. Even when the animal maintains a normal appetite during stress, the underlying causes of stress may increase energy expenditure contributing to a net loss of energy that will subsequently lead to weight loss (Poole, 1997; NRC, 2010). Since all the animals in this study did not lose weight it can thus be concluded that cohousing vervet monkeys and domestic cats is not stressful to both species.

5.4 Glucocorticoid response

Several studies have reported the use of fGCM for assessment of stress in other NHP (Girard-Buttoz et al., 2009) and other mammals such as the African (*Loxodonta africana*) and Asian (*Elephas maximus*) elephant (Ganswindt et al., 2002; Ghosal et al., 2013) and the sun bear (*Helarctos malayamus*) (Schwarzenberger et al., 2004). Following cohousing, an increasing



trend was seen for both species with a mean group fGCM level of 95.22 ± 43.04 ng/g DW (mean \pm SD) before cohousing and 125.77 ± 61.69 ng/g DW at six days of cohousing for the vervets. Mean group fGCM level was 0.24 ± 0.06 ng/g DW before cohousing and 0.34 ± 0.10 ng/g DW at six days of cohousing for the cats. The differences in fGCM concentrations seen were neither significant for the vervets ($P=0.26$) nor for the cats ($P=0.42$). This response was short lived, with the fGCM concentration returning to baseline concentration for the period before the domestic cats were introduced. The elevation in average fGCM after introducing the domestic cats can be attributed to the study relying on excretory kinetics i.e. the hormones undergoing metabolism before subsequent faecal excretion (Anestis, 2010). The lag time from first plasma increase to excretion has previously been described to be in the range of 20 - 48 hours in mammals (Anestis, 2010; Heistermann et al., 2006). On average, the results showed a peak in fGCMs concentrations twenty-four hours after cohousing has been initiated. Based on the excretory kinetics, it would appear that the animals were stressed for the first one to two days after exposure, after which time then concentrations decreased. The finding that the average fGCM peaked a day after cohousing is not too dissimilar to the findings of a study that showed that fGCMs in non-habituated (2188 ng/g) orangutans unfamiliar with people showed higher concentrations than those of habituated (1367 ng/g) to human contact (Muehlenbein et al., 2012).

Based on the short increase in GC, it would appear that the vervets while initially stressed by the exposure to the domestic cats, this was not a major stressful event with the animals appearing to adapt to their new housing conditions. Nonetheless, the findings of this study should be interpreted with care, as differences in fGCMs do not always indicate a stress response (Breuner et al., 2013). As an example, high levels of fGCMs may just signify a normal response to stimuli that may not mean the animal is stressed (Treves, 2005), since different animals may



have different basal levels of stress hormones (Shutt et al., 2012), as has been noted that some vervets had elevated fGCM levels before cat introduction. It should also be noted that fGCM differences may be due to other causes like season, diet and life history differences (Romero, 2004). With the animals in the current study being housed under the same conditions and fed the same diet, we believe these to be unlikely reasons for the evident differences in fGCMs. Furthermore, the GC responses of individual vervets to cohousing with domestic cats could have also been influenced by differences in animal temperament (Muehlenbein et al., 2012).

5.5 General conclusion

The results from this study provide first step information in demonstrating that vervets and domestic cats could be cohoused and this can be used as a way of utilising limited laboratory animal space. Although the cohousing of vervet monkeys and domestic cats in adjacent pens did not result in a general increase in the stress in either species it should be noted that the behaviour of vervets is different from other NHP used in biomedical research and therefore these results must be extrapolated to other NHP species with utmost caution. However, we believe that the findings of this study are going to help address the problem of limited laboratory animal facility space faced by many laboratory animal facilities. The findings of this study will also help policy makers review current legislation on the requirements for laboratory animal housing since there are differing opinions on the most appropriate refinement methods to use for different laboratory animals. This study is a first step in the refinement of animal housing by way of cohousing vervets and domestic cats separated by a barrier fence and future studies will explore the possibilities of cohousing vervets and cats in the same enclosure.



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