

## CHAPTER 5

# FACTORS MODIFYING HYPOTHALAMIC PITUITARY ADRENAL ACTIVITY IN CAPTIVE LION-TAILED MACAQUES (*Macaca silenus*)

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### 5.1 Introduction

The hypothalamic-pituitary-adrenal (HPA) axis is a conserved axis and thus involved in many physiological processes (Moberg, 2000). HPA activity is commonly measured via the glucocorticoid cortisol (Smith, 2004). There are a number of biological factors that can modify cortisol levels (Honess et al., 2005; Moberg, 2000) which include time of day (Coe & Levine, 1995; Honess & Marin, 2006a; McCallister et al., 2004; Raminelli et al., 2001; Smith & French, 1997a), species (Crockett et al., 2000), genetics (Kulberg et al., 2002), temperament (Anestis, 2005; Anestis & Bribiescas, 2004; De Palma et al., 2005; Ray & Sapolsky, 1992; Sapolsky, 2005), social status (Sapolsky, 2005), reproductive condition (Owen et al., 2004; Raminelli et al., 2001; Wingfield et al., 1994), developmental history (Crockett et al., 2000; Dettling et al., 2002; Paulk et al., 1977; Pryce et al., 2002), age (Pryce et al., 2002), physical health (Heath & Dufty, 1998; Smith et al., 1994), social environment (Ahola et al., 2006), diet (Erwin et al., 1973), season (McKenzie & Deane, 2003; Owen et al., 2005; Reyes et al., 1997) and climate (Marai et al., 2003). Ideally all of these factors should be controlled for when studying HPA activity but this is not always possible. Cortisol as a reflection of HPA activity should be interpreted with caution.

The interpretation of cortisol level results can be difficult both due to the above factors and because of the variation in stress response and basal cortisol levels between different individuals of the same species (Honess &

Marin, 2006a). Beyond individual differences in response some non-stressful stimuli for example locomotion, have been known to increase cortisol levels (Smith et al., 1998) while some known stress responses have not resulted in a cortisol increase (Moberg, 2000). It is thought that these different responses are reflective of the role of cortisol in energy production (Honeess & Marin, 2006a). High cortisol levels may therefore not be an indicator of psychological stress. Further, low cortisol levels can be equally misleading as chronically stressed primates have been known to exhibit hypocortisolism (Mendoza et al., 2000). Hypocortisolism, a biological condition common to all animals, is a disease of the adrenal gland cortex that leads to the under production of cortisol (Boden, 1998). Chronically stressed animals can also have low cortisol levels as a result of apathy, unresponsiveness (Broom & Johnson, 1993; Carlstead, 1996), or allostatic overload (McEwen, 1998; McEwen & Wingfield, 2003).

Previous research (which will be discussed below) suggests the variables: institution (Crockett et al., 1993), age (Capitanio et al., 2005) and sex (Ahola et al., 2005) may have an effect on modifying cortisol levels in several species. These variables will be examined in detail in this chapter to see the role they play in modifying cortisol levels in the lion-tailed macaque.

### ***5.1.1 Effect of institution on cortisol levels***

The social and physical environment of an animal may modify its cortisol levels (Honeess & Marin, 2006a). As mentioned in the previous chapter the current study will focus on lion-tailed macaques housed in zoological gardens which can vary greatly by institution. The institutions included in the study varied in group size, the amount of space provided, visitor numbers and the way in which the animals were managed (see chapter 4, table 1). All of these variables have been shown to modify hypothalamic-pituitary-adrenal (HPA) activity in primates (Baker & Aureli, 1997; Paulk et al., 1977). The effect of overall cage size (Draper & Bernstein, 1963; Paulk et al., 1977) and its complexity (Buchanan-Smith et al., 2004; Wolfensohn & Honeess, 2005) has a clear effect on the psychological well-being of primates. Any resulting

performance of abnormal behaviours may affect cortisol levels. For example, leopards pace more when confined to indoor off-exhibit enclosures than when on-exhibit outdoors in more natural enclosures (Mallapur & Chellam, 2002). The only study that appears to have been published on the effect of cage size on cortisol levels (Crockett et al., 1993) showed no effect of laboratory cage size on long-tailed macaque urinary cortisol levels but these animals were singly housed. Enclosure size and the number of animals may affect the individual's cortisol levels by crowding (Baker & Aureli, 1997). To further complicate the matter, some studies have shown the presence of familiar social partners can reduce or eliminate the cortisol response to various stressors by social buffering (Levine et al., 1993; Smith & French, 1997b). Social housing conditions can therefore influence HPA activity in a complicated manner.

Zoo management procedures can have an effect on cortisol levels as demonstrated previously in at least three species in captivity, pandas, *Ailuropoda melanoleuca* (Owen et al., 2005), leopards, *Neofelis nebulosa* (Wielebnowski et al., 2002) and elephants, *Loxodonta africana* (Wilson et al., 2004). When pandas were given the choice of access to two enclosure areas they showed fewer signs of behavioural agitation and lower levels of urinary cortisol than when their access was controlled by humans (Owen et al., 2005). The authors proposed that cortisol levels may have been related to husbandry factors such as enclosure height and type of keeper interaction. Faecal cortisol levels in clouded leopards were found to vary across individuals and institution facilities (Wielebnowski et al., 2002). A study on elephants showed that strict zoo management procedures increased stereotyped swaying but did not effect cortisol levels (Wilson et al., 2004).

The research discussed demonstrates that zoo management procedures may affect cortisol levels in lion-tailed macaques. It was therefore predicted that lion-tailed macaques housed in different institutions would have different cortisol levels.

### 5.1.2 Effect of age on cortisol levels

Although an individual's age may modify its levels of cortisol, there is controversy regarding the effect of age on the activity of the HPA system (Sapolsky, 1991). Age effects in cortisol levels have been found in several primate species including common marmosets, *Callithrix jacchus* (Pryce et al., 2002) rhesus macaques, *Macaca mulatta* (Capitanio et al., 2005), mountain, *Gorilla gorilla beringei* and western lowland gorillas (*Gorilla gorilla gorilla*) (Robbins & Czekala, 1997; Stoinski et al., 2002). In all of the latter species younger animals had higher cortisol concentrations than older animals. In some cases this age difference in cortisol levels may be explained by the role of cortisol in energy metabolism (Sapolsky, 1991), as younger animals often have higher levels of activity or energy expenditure than older animals (Robbins & Czekala, 1997; Stoinski et al., 2002). It is also possible that in some cases younger animals find certain events more stressful than older animals due to lack of experience or their rearing history (Capitanio et al., 2005). Stoinski et al (2002) found an increase in cortisol levels within different age groups of young individuals (from sub-adult to young adult male western lowland gorillas). The authors feel this difference may reflect increased stress levels in captive young males.

Some studies in rats have shown elevation in basal plasma corticosterone concentration of aged rats (Scaccianoce et al., 1990) while others have found no difference in cortisol levels in young or aged rats (Brett et al., 1983; Lorens et al., 1990; Van Eekelen et al., 1991). Dogs have been shown to exhibit increased basal urinary free cortisol concentrations with aging (Reul et al., 1991). Similar results have been found in tree shrews, *Tupaia belangeri* (Van Kampen & Fuchs, 1998) and golden hamsters (Wommack et al., 2005) where baseline glucocorticoid levels gradually increase throughout puberty. It is important to understand that age effects of cortisol may be confounded by levels of activity, social status and reproductive status (Sapolsky, 2005). Although not straight forward the above research illustrates how an animal's age can affect cortisol levels.

It was predicted from the results of the research above that young captive lion-tailed macaques would have higher cortisol levels than older individuals.

### **5.1.3 Effect of sex on cortisol levels**

Reproductive hormones influence HPA activity and cortisol production (Honeess & Marin, 2006a) in both male and female animals (Rivier & Rivest, 1991). There seems to be a direct link between some reproductive hormones such as oestrogen and cortisol. For example, cortisol levels in oestrus female savannah baboons (*Papio hamadryas ursinus*) are lower than levels found in individuals at other reproductive stages (Weingrill et al., 2004). Cortisol levels in female ring-tailed lemurs (*Lemur catta*) and black tufted-ear marmosets (*Callithrix kuhli*) during late gestation are significantly higher than those during early gestation (Cavigelli, 1999; Smith & French, 1997b). Circulating glucocorticoid levels were significantly elevated in lactating female yellow-pine chipmunks (*Tamias amoenus*) compared to those that were not (Kenagy & Place, 2000). The influence of reproductive hormones on cortisol is also observed in males. For example, male squirrel monkeys (*Saimiri sciureus*) have higher cortisol levels during the mating season compared to other times (Coe & Levine, 1995). In contrast to the above research, reproductive stage does not influence faecal glucocorticoid levels in captive red deer, *Cervus elaphus* (Huber et al., 2003).

Basal cortisol levels have been shown to differ between the sexes, probably as a result of sex differences in reproductive hormones. Male mice have been shown to excrete higher cortisol levels than females (Touma et al., 2003), whereas faecal cortisol is higher in female clouded leopards (*Neofelis nebulosa*) and blue foxes (Ahola et al., 2005), than in males (Wielebnowski et al., 2002). Sex differences in cortisol levels have also been found following stressful events. Females show a higher cortisol response than males to various stressors in long-tailed macaques, *Macaca fascicularis* (Crockett et al., 1993), rhesus macaques (Capitanio et al., 2005) marmosets, *Callithrix kuhli* (Smith & French, 1997a) and rats (Critchlow et al., 1963; Handa et al., 1994; Kitay, 1961). The opposite was found in squirrel monkeys where

males showed a higher response than females (Coe et al., 1978). As mentioned above female and male glucocorticoid levels are linked directly to reproductive hormone levels so sex differences in reproductive hormones may explain sex differences in cortisol levels. Sex variation may also reflect underlying differences in steroid metabolism, excretion routes and pituitary responsiveness (Handa & McGivern, 2000).

Several other theories on sex differences in cortisol suggest that when females have a higher average glucocorticoid concentration than males it could be argued that the difference is due to an evolutionary adaptation in females to increased watchfulness. The increased 'fight or flight' response is assumed to assist in rearing young and protecting them from predation (Buirski et al., 1978) and avoiding aggression from dominant males (Vandenheede & Bouissou, 1993). There is however, limited data to support these theories.

It is predicted from this research that sex will modify cortisol levels in captive lion-tailed macaques.

#### **5.1.4 Effect of behaviour on cortisol levels**

Certain behaviours have been shown previously to be linked with cortisol levels (Honest et al., 2004; Morimoto et al., 1993; Smith et al., 1998). Some behaviours are linked with anxiety and therefore may also be linked with the HPA response and cortisol, for example auto-grooming and yawning (Aureli & Schino, 2004; Castles et al., 1999). Behaviours that may modify cortisol levels will be discussed below.

Increased locomotion in response to a stressor has been observed in primates as a result of separation (Levine et al., 1993), novelty (Hennesey, 1995), and fear stimuli (Coe et al., 1982). Levels of locomotion and cortisol were found to increase in marmosets when housed alone in a novel cage (Smith et al., 1998) and rats (Morimoto et al., 1993) placed in a novel cage, showing a direct positive correlation between cortisol and locomotion. A

rapid increase in corticosterone levels has been found to trigger locomotion behaviour in passerine birds (Astheimer et al., 1992). The opposite relationship between cortisol and locomotion has been found in elephants (*Elaphas maximus*) and cows (Schmid et al., 2001; Van Reenen et al., 2005), where a negative correlation between locomotion and cortisol levels has been found. The latter research suggests a link between locomotion and cortisol levels.

Some studies have shown an association between aggression and cortisol levels. Injected cortisol has resulted in higher levels of aggression in golden hamsters (Hayden-Hixson & Ferris, 1991) and rats (Haller et al., 2000). Aggression is associated with increased cortisol in captive marmosets (Smith & French, 1997a) and timber wolves, *Canis lupus* (McLeod et al., 1996). In contrast, some studies have shown that aggression does not always correlate with cortisol levels (Beehner et al., 2005; Ginther et al., 2001; Saltzman et al., 1996; Sands & Creel, 2004).

Grooming behaviours have been associated with decreased cortisol in olive baboons, *Papio anubis* (Ray & Sapolsky, 1992). In this species high levels of social affiliation (grooming and positive interactions) with females were associated with low basal cortisol concentrations (Ray & Sapolsky, 1992). The same results have been found in rhesus macaques (Gust et al., 1993). Heart rate has also been shown to decrease when pigtail and rhesus macaques receive grooming (Bahr et al., 1999; Boccia et al., 1989). Beehner et al (2005) however found allo-grooming and cortisol levels in baboons (*Papio hamadryas ursinus*) to be unrelated. High levels of affiliative social behaviour and close proximity to other individuals may be potential social strategies to reduce HPA function (Smith et al., 1998).

Facial displays such as yawning have been linked to anxiety in olive baboons (Castles et al., 1999). Mixed context facial displays in lion-tailed macaques include raise brow/flatten ears, purse lips, lipsmack and yawn but there is controversy about the function of these facial displays in this species (Johnson, 1985; Skinner & Lockard, 1979). Facial displays like these have

been used in threat, appeasement and reassurance (Johnson, 1985). As the exact function of the facial displays is unknown they may be associated with cortisol levels, especially if they signal aggression or anxiety.

As mentioned earlier in this chapter the social environment can influence an individual's cortisol response (Levine et al., 1993; Smith & French, 1997a). The social buffering theory suggests close proximity of familiar social partners can reduce the HPA response to stress. Cortisol levels in young rhesus macaques were negatively correlated with proximity to others (Laudenslager et al., 1995), that is as proximity increases, cortisol levels decreased. Solitary male western lowland gorillas have been found to have higher cortisol levels than their socially housed counterparts (Stoinski et al., 2003). Therefore the current study predicted that high amounts of time spent in arms reach proximity to other individuals would result in lower cortisol levels than high amounts of time spent solitary by lion-tailed macaques.

The behavioural variables: locomotion, agonistic behaviour, mixed-context facial displays, allo-grooming, auto-grooming, time spent in "arms-reach" proximity to another individual and time spent solitary were examined to see if they were predictors of cortisol levels in captive lion-tailed macaques. Successful behavioural predictors of cortisol levels would enable non-invasive measures of HPA activity; therefore this study has important welfare implications.

### **5.1.5 Aims**

This chapter aimed to examine factors modifying hypothalamic-pituitary-adrenal (HPA) activity in captive lion-tailed macaques (*Macaca silenus*). The variables that were examined included the animal's institution, age, sex and behaviour (locomotion, agonistic behaviour, mixed-context facial displays, allo-grooming, auto-grooming, time in "arms-reach" proximity and time spent solitary). It was important to understand how these life history and environmental traits modify cortisol levels, if cortisol is to be used to assess



welfare. The research also aimed to develop non-invasive measures of HPA activity.

## **5.2 Methods**

Behavioural data and faecal samples were collected from 29 captive lion-tailed macaques (12 males, 17 females) housed in three institutions, Chester Zoo, Bristol Zoo Gardens and The Assiniboine Park Zoo. A total of 170 hours of behaviour data and 259 faecal samples were analysed. An additional 18 faecal samples, collected over a week period from a fourth institution (San Diego Wild Animal Park) from eight subjects (1 male, 7 females) were also used in the analysis. Details on subjects, housing, sample collection and data collection are outlined in chapter 2 sections 2.1-2.3. The faecal extraction and EIA procedure are described in chapter 2 section 2.4.

### **5.2.1 Data Analysis**

Data were not normally distributed so instantaneous and one-zero behavioural data were arcsine transformed while all occurrence behaviour and cortisol levels were log transformed following the addition of 1 for all occurrence behaviour (Sokal & Rohlf, 1995). The effect of the independent variables institution, age and sex (using all four institutions) on cortisol levels were examined using a 3-Factor between groups ANOVA. Tukey post-hoc analysis was performed on the significant data. The relationship between behaviour data (locomotion, agonistic behaviour, mixed context facial displays, auto-grooming, allo-grooming, time in "arms-reach" proximity and time spent solitary) and cortisol from three institutions (Chester, Bristol and Assiniboine) was examined using stepwise multiple regression analysis. An association between cortisol and behaviour was examined. The behaviours were selected as they were predicted to have an effect on cortisol levels on the basis of previous research (see introduction above). For behavioural category definitions refer to chapter 2 section 2.2.1

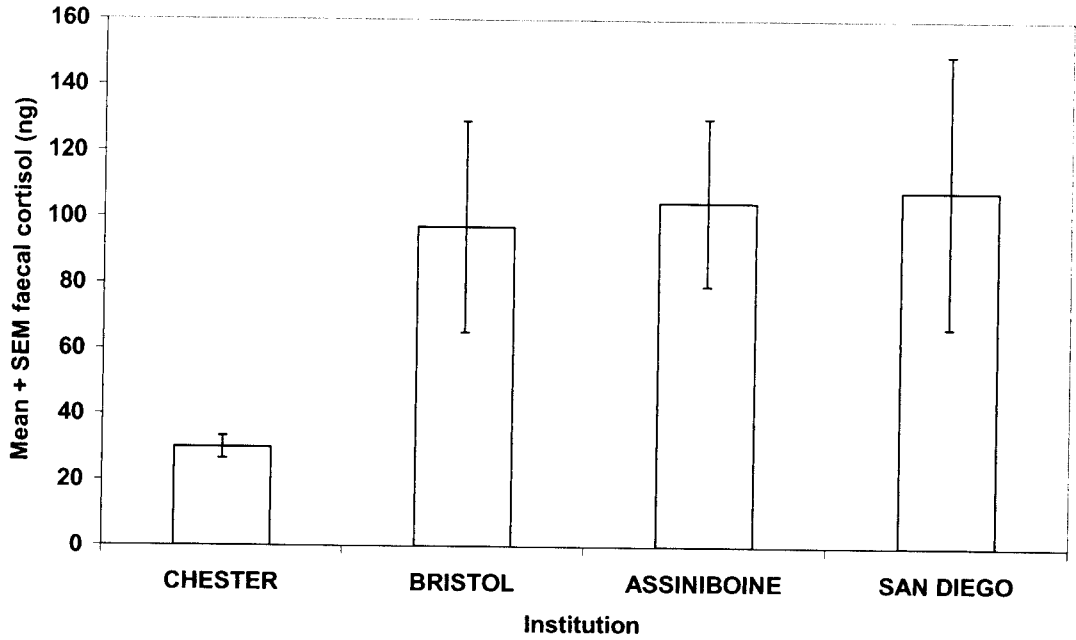
### 5.3 Results

Cortisol levels were significantly influenced by the institution in which the subjects were housed ( $F [3, 18] = 3.34, P < 0.05$ ). Age and sex did not significantly modify cortisol levels. There were no interaction effects (table 5.1).

**Table 5.1** – Table showing results of a 3-Factor ANOVA assessing the variables (institution, age and sex) modifying cortisol levels. \*indicates a significant result.

Dependent variables	Cortisol (Independent variable)
<b>Institution</b>	$F (3, 18) = 3.34, p < 0.05^*$
<b>Age</b>	$F (2, 18) = 1.54, NS$
<b>Sex</b>	$F (1, 18) = 0.36, NS$
<b>Institution * Age</b>	$F (4, 18) = 0.30, NS$
<b>Institution * Sex</b>	$F (3, 18) = 0.64, NS$
<b>Age * Sex</b>	$F (1, 18) = 0.03, NS$
<b>Institution * Age * Sex</b>	$F (2, 18) = 0.00, NS$

Mean cortisol levels varied across institution (figure 5.1). Chester Zoo had the lowest mean cortisol levels ( $30 \text{ ng} \pm 3.4$ ) and San Diego Wild Animal Park the highest ( $108.07 \text{ ng} \pm 41.29$ ). Tukey post hoc analysis showed cortisol levels at Chester were significantly different to levels at Assiniboine ( $p < 0.05$ ) and San Diego Wild Animal Park ( $p < 0.05$ ) but not Bristol.

**Figure 5.1** – Mean cortisol levels  $\pm$  SEM per institution.

Levels of locomotion, agonistic behaviour, mixed context facial displays, allo-grooming, auto-grooming, time spent in contact and time spent solitary did not significantly predict cortisol levels (table 5.2).

**Table 5.2** – Table showing results of stepwise multiple regression analysis assessing the behaviour variables modifying cortisol.

Behaviour	Cortisol
Locomotion	$r = -0.05$ , $n = 26$ , NS
Agonistic	$r = -0.08$ , $n = 26$ , NS
Auto-grooming	$r = -0.11$ , $n = 26$ , NS
Allo-grooming	$r = 0.24$ , $n = 26$ , NS
Mixed-context facial display	$r = 0.18$ , $n = 26$ , NS
Time spent in arms-reach contact	$r = 0.08$ , $n = 26$ , NS
Time spent solitary	$r = -0.01$ , $n = 26$ , NS

### **5.3.1 Results Summary**

- The institution in which lion-tailed macaques were housed significantly modified cortisol levels.
- The age of individuals did not modify cortisol levels.
- The sex of individuals did not modify cortisol levels.
- The behaviour of individuals did not modify cortisol levels.

## **5.4 Discussion**

The cortisol levels of captive lion-tailed macaques were significantly modified by the institution in which the animal was housed. The age and sex of lion-tailed macaques did not significantly modify cortisol levels. Behaviour variables assessed in this study did not significantly predict cortisol levels.

### **5.4.1 Effect of institution on lion-tailed macaque cortisol levels**

The institution had a significant effect on lion-tailed macaque cortisol levels, possibly due to a number of factors. The size of the enclosures varied quite considerably between institutions. The institution with the lowest cortisol levels (Chester) has the second largest enclosure of the three institutions. However the highest cortisol levels were seen in San Diego, which had the largest enclosure overall. Previous research has shown the opposite in pigs and buffalo (Jarvis et al., 2002; Tripaldi et al., 2004) with lower cortisol levels being associated with larger space. Cortisol levels from this research can not therefore be explained by enclosure size (Crockett et al., 1993) and probably not crowding (Baker & Aureli, 1997; De Waal et al., 2000). Cortisol levels are likely to be as a result of enclosure complexity (Buchanan-Smith et al., 2004) and management (Hosey, 2005; Owen et al., 2005; Wielebnowski et al., 2002; Wilson et al., 2004).

The San Diego group were not reproducing at the time of sample collection and did not have any young. The male was relatively new to the group, having only been introduced 4 months previously (after being housed nearby

with visual and olfactory contact for 12 months), so cortisol levels may have been affected by the novelty of the male (Grandin, 1997). As a result of the changes in the group, the group also did not have a defined alpha female at the time of the study (Kathy De Falco, Personal communication); therefore the dominance hierarchies were unstable. The lack of a defined hierarchy may have therefore resulted in high cortisol levels (Sapolsky, 2005). The group at San Diego, unlike the other three groups were off show to the public, yet still had the highest cortisol levels (although not significantly different to Bristol or Assiniboine), therefore high cortisol levels cannot be attributed to visitors (Davis et al., 2005).

It should be noted that cortisol levels at Bristol, Assiniboine and San Diego were not significantly different. It may therefore be the case that cortisol levels at Chester are low for lion-tailed macaques, levels at Bristol, Assiniboine and San Diego being more usual in this species. Cortisol levels have not been measured in wild lion-tailed macaques so these levels can not be compared. Further research is required in both captivity and the wild to determine mean basal cortisol levels in this species.

#### ***5.4.2 Effect of age on lion-tailed macaque cortisol levels***

The age group of lion-tailed macaques had no significant effect on cortisol levels. Similar results have been found in some studies on marmosets (Smith & French, 1997a) and rats (Brett et al., 1983; Lorens et al., 1990; Van Eekelen et al., 1991) so this may not be a surprising result. The non-significant effect of age on cortisol levels may however have been due to confounding variables of captivity and a different result may have been found if wild lion-tailed macaques had been studied. Simply being raised and living in captivity can effect animals in various ways, which can produce confounding variables for research. Perhaps most importantly animals housed in captivity are not totally free to make their own choices but are instead managed by humans. Social structure of the group, space allowance and feeding is all controlled by human caretakers. Captive animals usually have easy access to food and often do not spend the same amounts of time

searching for food as they would in the wild, resulting in behavioural and possibly physiological changes. There may not be the same amount of mate choice in captivity as in the wild and group structure may be changed at unnatural times or in unnatural ways. For example, in the case of lion-tailed macaques, where males naturally migrate from the group, human caretakers may take males from the group either earlier or later than would be preferred. In some collections females, as opposed to males may even be moved from the group which is unnatural for the species and again may result in behavioural and physiological changes. Most zoological institutions are open to the public and therefore have visitors as a major variable that may confound their captive animal behaviour. The design of enclosures to reduce interference from visitors and noise levels are important in minimising the amount of animal behaviour that is modified, however the impact of these factors can not be controlled for throughout institutions.

It is also possible that the length of study period may have been too short to highlight significant differences in cortisol levels between different age groups. The small sample size as well as variability in social groups may have also masked significant results. The effect of age on lion-tailed macaque cortisol has not previously been published. Although it is possible that age does not affect cortisol levels in this species, to confirm this more longitudinal research is needed.

#### **5.4.3 Effect of sex on lion-tailed macaque cortisol levels**

The sex of lion-tailed macaques had no significant effect on cortisol levels. Although sex differences have been found in many animals (Capitanio et al., 2005; Coe et al., 1978; Crockett et al., 1993; Handa et al., 1994; Honess & Marin, 2006a; Touma et al., 2003; Wielebnowski et al., 2002), there was no sex difference found in cortisol levels in the common diving petrel (*Pelecanoides urinatrix*) after capture and handling restraint (Smith et al., 1994). Again this non-significant result in the current research may have been due to confounding variables of captivity, sample size, social group variability or the length of the study period. The effect of sex on lion-tailed

macaque cortisol has not previously been published. It is possible that sex does not affect cortisol levels in this primate species but as mentioned above this is unlikely and further research is needed before this can be confirmed. The reproductive status of females was not controlled for in this study, which ensured a general picture of female cortisol levels were obtained, unbiased by reproductive state. It is possible however that male and female lion-tailed macaques have different cortisol levels at different stages of reproduction, such as oestrus (Weingrill et al., 2004) and gestation (Cavigelli, 1999; Smith & French, 1997b). It is important for future work to examine cortisol levels at each reproductive stage in lion-tailed macaques. This was unfortunately not possible in the current study as time restraints meant that reproductive hormones could not be analysed.

#### ***5.4.4 Effect of behaviour on lion-tailed macaque cortisol levels***

Levels of locomotion, agonistic behaviour, auto-grooming, allo-grooming, time in contact and time spent solitary did not significantly modify cortisol levels in lion-tailed macaques. These variables are therefore not predictors of cortisol levels in this species and should not be used alone to indicate well-being.

It is surprising that cortisol levels were not modified by levels of locomotion as many studies have found cortisol and locomotion levels to be correlated (Astheimer et al., 1992; Morimoto et al., 1993; Schmid et al., 2001; Smith et al., 1998; Van Reenen et al., 2005). Levels of aggression have been found to be uncorrelated with cortisol levels in studies on cotton-top tamarins, wolves and baboons (Beehner et al., 2005; Ginther et al., 2001; Sands & Creel, 2004), which is consistent with this research. However, aggression has been correlated with cortisol in other studies (McLeod et al., 1996; Smith & French, 1997a). It is also surprising that the occurrence of grooming and time spent in contact did not significantly modify cortisol levels as many other studies have found significant relationships between these variables (Gust et al., 1993; Laudenslager et al., 1995; Ray & Sapolsky, 1992). However, Beehner et al (2005) found no correlation between cortisol and allo-grooming

in baboons. Individuals are known to respond to challenging situations in different ways in all species (Van Reenen et al., 2005), therefore the lack of association between cortisol levels and the various behaviours explored may represent the different coping styles found in individual lion-tailed macaques. It is possible that an inefficient number and frequency of samples were collected to demonstrate a link between cortisol and certain behaviours. If a higher number of samples were collected it is possible that a link between these variables would have been found. Cortisol levels have not however been previously measured in lion-tailed macaques so there is a possibility that the result is accurate and there is no association between cortisol levels and the behaviours measured in this primate species or in these particular groups. However if other behaviour variables were examined associations may have been found and it is always possible that other factors or confounding variables are masking an association between HPA activity and behaviour.

The interpretation of animal physiology is difficult as previously mentioned, especially with regard to stress and welfare. Physiological state is not necessarily exhibited behaviourally and unless a reduction in stress levels are achieved, behavioural changes are not necessarily advantageous. It is also possible that the link between cortisol and behaviour is different in captive and wild lion-tailed macaques because of environmental restraints such as enclosure size. There will also be individual variation in behavioural and physiological responses as well as species differences and due to a lack of research in this area the current results are difficult to interpret. It is possible that a different method of cortisol measurement may have provided different results. For example the groups of lion-tailed macaques studied had relatively high levels of locomotion and perhaps by measuring faecal cortisol discreet changes in cortisol levels were not detected that may have been detected through the measurement of plasma.

The results from this study indicate that cortisol is significantly modified by fundamental factors (the institution in which the individual is housed) that may be outside of the stress response in this species of macaque. These



results suggest that lion-tailed macaques are sensitive to their environment which could be a concern for wild individuals in fragmented habitats. Fragmented groups may have different cortisol levels and therefore need to be considered on an individual basis for successful conservation. Thus caution should be exercised when using cortisol as an indicator of well-being. The glucocorticoid cortisol may only provide a reliable indicator of well-being for those species in which we have a thorough understanding of the multiple factors that modify its metabolism and release. Due to a lack of information on cortisol levels in wild or other captive lion-tailed macaques, it can not be assessed whether cortisol levels in these captive groups lie within the normal healthy limits for the species.