EFFECTS OF CORTISOL ADMINISTRATION ON COOPERATIVE BEHAVIOUR IN MEERKAT HELPERS

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ABSTRACT

Although the ultimate causes for variation in contributions to helping in cooperative breeders are increasingly well understood, the underlying physiological mechanisms remain largely unknown. Recent work has suggested that glucocorticoids (GC) may play an important role in the expression of cooperative behaviour. Here, we present the first experimental test of the effects of GCs on helper behaviour in a cooperative breeder. GC levels of adult female and male meerkat helpers were elevated with an intra-muscular injection of cortisol (hydrocortisone 21hemi-succinate sodiumsalt) dissolved in saline, whilst matched controls simultaneously received an injection of saline. There were no changes in pup feeding or sentinel behaviour, but females spent more time in close proximity to pups and less time foraging when GC levels were elevated. These results provide no evidence that GCs affect cooperative behaviour, but suggest that there may be an effect on affiliation with pups and foraging effort.

INTRODUCTION

One remaining challenge in the study of cooperative breeding is to understand why individuals vary in their contributions to cooperative activities (Cockburn 1998; Komdeur 2006). Although the ultimate causes underlying this variation are increasingly well understood and have been shown to be associated with factors such as sex, age, condition and relatedness to offspring (e.g. Cockburn 1998), the underlying physiological mechanisms remain largely unknown. Initial studies examining a physiological basis for cooperative behaviour focussed primarily on the hormones prolactin and testosterone (Schoech 2004), but recently the role of glucocorticoids has attracted more attention (Soares et al. 2010). Glucocorticoids (GC) are a class of hormones, produced by activation of the hypothalamic-pituitary-adrenal axis in response to stressors, of which cortisol is the primary type in mammals (Sapolsky et al. 2000; Landys et al. 2006). As social factors are the primary stressors in social species (Abbott et al. 2003; Creel 2001), understanding the behavioural effects of GCs is crucial to understand how individuals adapt their behaviour in response to social factors.
Recent work on cooperative breeders suggests that GCs may be an important factor underlying individual variation in contributions to cooperative activities. In subordinate male meerkats (*Suricata suricatta*), pup provisioning rates were positively correlated with GC levels at the start of the breeding attempt, and males who were on sentinel duty frequently had higher GC levels than those who were on sentinel duty infrequently (Carlson et al. 2006; Tatalovic 2008). Such increases in contributions to cooperative activities may result from the direct effects of GCs on cooperative behaviour, or indirectly from the effects of GCs on foraging rates and food intake (Koch et al. 2002; Dallman 2004), attention and vigilance (Chapotot et al. 1998) and interactions with offspring (Fleming et al. 1997), which may facilitate cooperative behaviour. To date, however, no studies have experimentally tested the effects of GCs on behaviour of helpers in cooperative breeders, and it remains unclear whether previously reported correlations between GC levels and contributions to cooperative activities represent causal relations.

Our aim was to examine the effects of elevated GC levels on contributions to pup feeding and sentinel behaviour, as well as on foraging, vigilance and affiliation with pups. Meerkats live in groups of up to 50 individuals with a dominant breeding pair that largely monopolises reproduction and helpers of both sexes who do not normally breed (Griffin et al. 2003). Litters of up to six pups are produced two to four times per year by the dominant pair and raised cooperatively by the group (Clutton-Brock et al. 1999a). Typically, the group leaves the sleeping burrow after sunrise to forage for the day and returns to the burrow around sunset. During these foraging trips, helpers engage in two distinct forms of cooperative behaviour. First, after the pups have started joining the group on foraging trips at around 30 days of age up until they reach nutritional independence at around 90 days, adults provision them with invertebrate and small vertebrate prey items, a behaviour referred to as ‘pup feeding’ (Clutton-Brock et al. 2002, 2003). Second, group members occasionally cease foraging to scan for predators from an elevated position, and to alert the other group members upon detecting a predator, a behaviour referred to as ‘sentinel duty’ (Clutton-Brock et al. 1999b).

In this study, we first validated the use of intra-muscular injections of cortisol as a method to elevate circulating GC levels in meerkat helpers. This method had previously been used successfully to elevate GC levels in common marmoset females (*Callithrix jacchus*, Saltzman & Abbott 2009). We then used this method to elevate
GC levels and performed 80-minute behavioural observations, starting 15 minutes after the injections, in order to test the effects of elevated GC levels on pup feeding, sentinel behaviour, foraging, vigilance and association with pups. In addition, we performed an experiment where the meerkats were supplementary provisioned with scorpions two hours after the injection, in order to specifically examine the effects of elevated GC levels on pup feeding behaviour.

**METHODS**

*Study site and population*

The study was conducted in the southern Kalahari Desert, South Africa (26°58′ S, 21°49′ E). The study site consists of the dry riverbeds of the Kuruman River, herbaceous flats, and vegetated dunes. The ecological conditions and climate of the study site have been described elsewhere (Clutton-Brock et al. 1999a, 1999c; Russell et al. 2002). One meerkat in each group is fitted with a radio collar as part of the ongoing research, thereby allowing groups to be easily located. All individuals are habituated to the presence of researchers and can be identified by a unique pattern of dye marks on their fur, which are maintained while individuals are resting without the need to disturb or capture them. Groups are visited at least once every three days and the age of all individuals is therefore known to within a few days. Dominance status of females can be readily identified, as the dominant female is the primary breeder in the group (Clutton-Brock et al. 2001c; Griffin et al. 2003), whilst all subordinate females are behaviourally submissive to her (Clutton-Brock et al. 1999c; O’Riain et al. 2000). Dominant status of males can be identified by their enlarged anal glands and scent-marking behaviour, as well as by aggressive behaviour of the dominant male towards subordinate males (Spong et al. 2008). Individuals are referred to as adults when they are older that 12 months of age and have the potential to reproduce (Brotherton et al. 2001).
In May 2010 and May 2011, trials were performed to examine the effects of intra-muscular injection of cortisol on circulating GC levels. For the treatment, two adult female helpers and three adult male helpers received an injection of 10 µg cortisol (hydrocortisone 21-hemisuccinate sodiumsalt, Sigma Aldrich, Buchs, Switzerland) dissolved in 0.05 ml physiological saline solution. For the control, two adult female helpers and two adult male helpers received an injection of 0.05 ml saline only. Injections were performed while the meerkats were sunning in the morning before leaving the burrow to forage. Because the meerkats are fully habituated to the presence of human observers, this could be done without the need to capture or restrain them. Injections were done intra-muscularly in the thigh with a 1 ml syringe with a 25G needle. Meerkats typically sprang away from the injection, but resumed sunning within seconds and did not become wary towards the observers, nor were there any observable long term effects of the injections. Meerkats responded to the injection in a similar manner as to bites by ants that they naturally encounter whilst foraging (P. Santema 2010, personal observation). Two hours (mean: 121 min. ± 4.3 SE) after the injection, a blood sample was taken for subsequent analyses of serum GC levels. For this, meerkats were captured by gently lifting them by the tail base and immediately placing them in a cotton bag (Jordan et al. 2007). Anaesthesia was induced and maintained using isoflurane (Isofor; Safe Line Pharmaceuticals, Johannesburg, South Africa) in air which was administered through a face mask (Jordan et al. 2007). Blood (0.5 to 1.5 ml) was drawn from the jugular vein using a 25G needle and a 2 ml syringe (Drewe 2009). All blood samples were collected within 5 minutes from the moment of capture (mean: 3.46 min. ± 0.29 SE) and 60% of the samples were collected within 3 minutes from the moment of capture. These are among the shortest obtained for free living vertebrates, therefore minimising the potential effects of capture stress on circulating GC levels (Schoech et al. 1996; Romero & Reid 2005). Capture-to-bleed times were comparable for individuals that received an injection of cortisol (mean: 3.33 min. ± 0.32 SE) and individuals that received an injection of saline (mean: 3.57 min. ± 0.52 SE). Following sampling, meerkats were placed on a layer of sand in a dark plastic box to allow recovery from anaesthesia. Individuals regained normal locomotory ability after one to seven minutes, after which they remained in the recovery box for a further five minutes to ensure complete recovery (Jordan et al. 2007).
Meerkats were then released to the centre of the foraging group. Blood samples were kept cool on wet ice until centrifugation at the research station which occurred within one hour. Serum was pipetted off and frozen at -20 °C. Serum samples were then shipped to the University of Pretoria where they were analysed for GC levels using standard methodology (more info needed from Bennett).

GC levels were measured using a coat-a-count Cortisol kit (Diagnostic products Corporation). A series of known calibrators of cortisol were assayed to set up a standard curve. Serum samples (25 µL) and 125I-labelled cortisol (1000 µL) were added to assay tubes in duplicate and briefly vortexed. Assay tubes were then incubated in a water bath (37º C) for 45 minutes. Bound and free 125I-labelled cortisol were separated by decanting the excess label from the assay tubes, which were then counted in a gamma counter for one minute. A calibration curve was used to convert the counts into cortisol concentrations. A serial double dilution of a sample containing a high concentration of cortisol paralleled the standard curve, validating the assay (ANCOVA, F=1.34, P<0.05). The sensitivity of the assay was 5.5 nmol/L and the intra-assay coefficient of variation was 7.0%. The assay has previously been validated for use in meerkats by Carlson et al. (2004).

**Behavioural observations**

Between October 2011 and March 2012, 24 trials were performed on 12 female and 12 male pairs in 8 different groups. All trials were carried out when pups between 40 and 75 days of age were present in the group. Meerkat groups were visited in the morning and treatments were carried out after the meerkats had emerged from their burrow, but before they started foraging. One of two previously selected same-sex littermates was injected with 10 µg cortisol dissolved in 0.05 ml physiological saline, whilst the other received an injection with 0.05 ml saline only. Injections were performed double blind, so that the person injecting the meerkats did not know which treatment was being administered, and the observers did not know which individual had received what treatment. In two of the trials, no same sex litter mate was available as a control, and an individual of the same sex and no more than six months difference in age was selected as a control.
Behavioural observations started 15 minutes after the injections, or, if the group was not foraging yet after 15 minutes, as soon as the group started foraging. This resulted in an average of 26 minutes (± 2 SE) between the injections and the onset of the observations. Four 20 minute focal observations (Altmann 1974) were performed on each focal individual, resulting in a total observation time of 80 minutes for each meerkat. Behavioural observations were performed on both focal animals simultaneously, with two observers who switched between focal individual after each 20-minute observation. Behavioural data were entered directly onto a handheld Psion organiser (Psion Teklogix Inc., Ontario, Canada), programmed as a data logger that allows recording of behaviours to the nearest second. During focal observations, all food items found, including their size category and whether or not they were fed to a pup, were recorded, as well as all instances of sentinel behaviour. In addition, the amount of time spent foraging, the amount of time spent within 2 m of a pup and the number of times vigilant was recorded. Following previous studies, food items were categorised as tiny, small, medium or large. The biomass was then estimated using the average mass of the food items in each size category (tiny= 0.05, small= 0.11 g, medium = 0.58 g, large = 2.86 g; Thornton 2008). An individual was said to be vigilant when it interrupted foraging to adapt a bipedal position to scan the environment. Observations were paused if foraging was interrupted for more than one minute (e.g. by alarm calls) and continued when foraging had been resumed by at least 50 % of the group.

Scorpion provisioning

Following the behavioural observations, an experiment was performed where scorpions were supplementary provisioned to each of the focal individuals in order to specifically test the effects of elevated GC levels on pup feeding behaviour. The experiment started two hours (mean: 128 min. ± 3 SE) following the administration of the injections. Four scorpions (Opistophthalmus sp.) were provisioned at five minute intervals to each of the focal individuals. Scorpions were presented when no other meerkat was within two metres of the focal individual, in order to avoid the potential for interruption by other individuals. For each individual, the number of scorpions that was given to a pup was recorded.
RESULTS

GC treatment validation

Males that received an injection of 10 µg cortisol dissolved in saline (n = 3) had an average of 429 ng GC / ml serum (± 3 SE) two hours after the injection, whereas males that received an injection of saline only (n= 4) had an average of 38 ng GC / ml serum (± 19 SE), representing an 11-fold increase (Fig. 1a). In comparison, untreated males (n = 21) have an average of 48 ng GC / ml serum (± 8 SE) (P. Santema 2010, unpublished data, Fig 1a).

Females that received an injection of 10 µg cortisol dissolved in saline (n = 2) had an average of 97 ng GC / ml serum (± 63 SE) two hours after the injection, whereas females that received an injection of saline only (n = 2) had an average of 8 ng GC / ml serum (± 8 SE), representing a 12-fold increase (Fig. 1b). No data of untreated females was available.

![Figure 1](image_url). Serum GC levels of a) males that were untreated, males that were injected with saline only and males that were injected with 10 µg cortisol dissolved in saline, and of b) females that were injected with saline only and females that were injected with 10 µg cortisol dissolved in saline. Figures represent mean ± SE.
**Behavioural observations**

Intra-muscular cortisol administration did not affect the total biomass that was fed to pups by females (Wilcoxon signed rank-test: $V = 36, P = 0.41$; Fig 2a) or by males (Wilcoxon signed rank-test: $V = 17.5, P = 0.59$; Fig 3a), nor did it affect the proportion of biomass that was fed to pups relative to the amount of biomass found by females (Wilcoxon signed rank-test: $V = 42, P = 0.45$) or by males (Wilcoxon signed rank-test: $V = 34, P = 0.51$).

**Figure 2.** Effects of intra-muscular cortisol administration to female meerkats helpers on a) amount of food given to a pup, b) sentinel behaviour, c) time spent foraging, d) amount of food found, e) vigilance and f) time spent near a pup. Figures represent mean per 80 minute observation ± SE.
rank-test: V = 24, P = 0.76). Cortisol administration did also not affect the number of times individuals went on sentinel duty in females (Wilcoxon signed rank-test: V = 5, P = 1; Fig 2b) or in males (Wilcoxon signed rank-test: V = 27.5, P = 0.20; Fig 3b).

Cortisol administration resulted in a reduction in the amount of time spent foraging by females (Wilcoxon signed rank-test: V = 1, P < 0.001; Fig 2c), but not by males (Wilcoxon signed rank-test: V = 30, P = 0.52; Fig 3c). It did not, however, affect the total biomass found by females (Wilcoxon signed rank-test: V = 46, P =

**Figure 3.** Effects of intra-muscular cortisol administration to male meerkats helpers on a) amount of food given to a pup, b) sentinel duty, c) time spent foraging, d) amount of food found, e) vigilance and f) time spent near a pup. Figures represent mean per 80 minute observation ± SE.
0.62; Fig 2e) or by males (Wilcoxon signed rank-test: V = 29, P = 0.47; Fig 3d). The reduction in foraging time was not because of an increase in vigilance, as this was unaffected by treatment in both females (Wilcoxon signed rank-test: V = 29, P = 0.76; Fig 2e) and males (Wilcoxon signed rank-test: V = 31.5, P = 0.93; Fig 3e). Cortisol treatment increased the amount of time spent within 2 m of pups by females (Wilcoxon signed rank-test: V = 68, P = 0.021; Fig 2f), but not by males (Wilcoxon signed rank-test: V = 43, P = 0.79, Fig 3f).

**Scorpion provisioning**

Female helpers that were injected with cortisol fed on average 1.3 scorpions (± 0.4 SE) to pups out of the four that were provided, and female helpers that were injected with saline solution fed on average 1.1 scorpions (± 0.4 SE) to pups. This difference was not statistically significant (Wilcoxon signed rank-test: V = 13.5, P = 0.59; Fig 4a). Male helpers that were injected with cortisol fed 1.6 scorpions (± 0.4 SE) to pups out of the four that were provided and male helper that were injected with saline fed 1.2 scorpions (± 0.2 SE) to pups. This difference was also not statistically significant (Wilcoxon signed rank-test: V = 19, P = 0.44; Fig 4b).

![Figure 4](image_url)

**Figure 4.** Effects of intra-muscular cortisol administration on the number of scorpions out of a total of four provisioned to a pup for a) female helpers and b) male helpers. Figures represent mean ± SE.
DISCUSSION

We provide, the first experimental test of the effects of glucocorticoids (GC) on helping behaviour in a cooperative breeder. Using intra-muscular injections, we elevated circulating GC levels in female and male meerkat helpers to assess the effects of GCs on pup feeding and sentinel behaviour. Intramuscular injection effectively elevated circulating GC levels. Two hours after the treatment was administered, circulating GC levels of all individuals that received an injection with GCs were higher than those of all individuals that received injected with saline solution. Circulating GC levels of individuals injected with saline were comparable to GC levels of untreated individuals. Intra-muscular cortisol injection did not affect contributions to pup feeding or sentinel behaviour in either females or males. Female helpers that were injected with cortisol did, however, show an increase in the time spent close to pups and a decrease in the time spent foraging, but there were no changes in the amount of food found or the level of vigilance. Male helpers showed no changes in any of the examined behaviours. For both females and males, there was no significant effect of cortisol administration on the number of scorpions that were fed to pups when scorpions were experimentally provisioned, although there was a trend for both sexes towards more pup feeds when GC levels were elevated.

Our results provide no support for the notion that GCs affect contributions to cooperative activities in meerkat helpers. This contrasts with previous studies on meerkats that showed positive correlations between GC levels and contributions to pup feeding and sentinel behaviour (Carlson et al. 2006; Tatalovic 2008). A number of other studies have investigated a role of GCs on the expression of cooperative behaviour, but the results of these are inconclusive. In the cooperative breeding cichlid *Neolamprologus pulcher*, cortisol levels are unrelated to contributions to brood care for both helpers and breeders (Bender et al. 2008), and in the cotton top tamarin (*Saguinus oedipus*), GC levels were unrelated to levels of infant carrying by both male helpers and fathers (da Silva Mota 2006).

Our results do suggest, however, that GCs may affect affiliative behaviour towards pups, as female helpers that were injected with GCs spent more time close to pups. Similar effects of GCs have been reported in non-cooperative breeders. In humans, mothers with higher GC levels showed more affectionate behaviour towards offspring (Fleming et al. 1987, 1997). However, in common marmosets (*Callithrix*...
jacchus), mothers carried their infants less when GC levels were experimentally elevated (Saltzman & Abbott 2009), and in western lowland gorillas (Gorilla gorilla) and savannah baboons (Papio hamadryas) individuals with higher GC levels spent less time in contact with their infants (Bahr et al. 1998; Bardi et al. 2004). Our results also suggest that GCs may influence foraging effort, as female helpers that were injected with GCs spent less time foraging. This contrasts with previous findings that showed a positive relation between GC levels and food intake (Koch et al. 2002; Dallman et al. 2004). Finally, our results suggest that behavioural responses to GC elevation may be sex-specific, as only females, but not males, showed behavioural changes in response to GC elevation.

The most obvious conclusion from this overview is that there is little consistency in the effects of GCs on helping behaviour in cooperative breeders, or in the behavioural effects of GCs in general. This may be partly because behavioural responses to GC elevation are likely to be dependent on a range of factors. First, experiences earlier in life may affect behavioural responses to GC elevation. For example, female rats that had previously reproduced show increased maternal behaviour in response to GC elevation, whereas virgin females show a reduction in maternal behaviour (Rees et al. 2004, 2006). Second, life history characteristics of a species may affect behavioural responses to GC elevation. For instance, GC elevations induces behavioural modifications in sparrows with multiple breeding attempts per year, but not in closely related sparrows with only one reproductive attempt per year (Wingfield & Sapolsky 2003). Third, GC elevation may have divergent behavioural effects depending on the duration of GC elevation and the level of increase (Sapolsky 1992), and relations between GC levels and behavioural responses may be non-linear and even U-shaped (Mateo 2008). These complexities in the relationship between GC levels and the expression of behaviour make it difficult to make generalisations about the behavioural effects of GCs across different contexts.

In summary, we successfully elevated circulating glucocorticoid (GC) levels in meerkat helpers using intra-muscular injections. Experimental elevation of GC levels had no effect on pup feeding or sentinel behaviour in either females or males, but females spent more time in close proximity to pups and less time foraging when GC levels were elevated. Males showed no behavioural changes in response to GC elevation. These results suggest that affiliation with pups and foraging effort may be
affected by GC levels, and that there may be sex differences in behavioural responses to GCs. The available evidence from different species, however, suggest that behavioural responses to GC elevation may be affected by a range of factors, and more targeted experiments are needed to illuminate the complex relations between GCs and the expression of cooperative behaviour.

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REFERENCES


Romero L. M., Reid, J. M. 2005. Collecting baseline corticosterone samples in the field: is under 3 min good enough? Comparative Biochemistry and Physiology 140, 73-79.


