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Family Affairs and Experimental Male Replacement Affect Fecal Glucocorticoid Metabolites Levels in the Egyptian Spiny Mouse *Acomys cahirinus*

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Marcela Fraňková, Rupert Palme, and Daniel Frynta (2012) Family affairs and experimental male replacement affect fecal glucocorticoid metabolites levels in the Egyptian spiny mouse *Acomys cahirinus*. *Zoological Studies* **51**(3): 277-287. In social animals, a temporary intrusion of an unfamiliar adult male into an established family group and/or male replacement may be accompanied by social stress. We performed experiments involving non-invasive monitoring of fecal glucocorticoid metabolites (GCMs) in 10 manipulated family groups (50 individuals in total) of the Egyptian spiny mouse *Acomys cahirinus*. Study animals were kept in special apparatuses enabling individual collection of fecal samples without considerable disturbance to family group members. The results of this study revealed only limited effects of experimental male intrusion and/or removal on GCM levels. Nevertheless, GCM levels of adult males sharply decreased following immigration into a new family group which provided them new breeding and social opportunities. We also found that there was no effect of age and only a partial effect of sex (males exhibited lower values) on baseline GCM levels. In contrast, there were considerable and consistent differences in GCM levels among individual family groups. This finding suggests that differences in social settings among these groups were a more important source of variation in social stress. Thus, we recommend that group identity should be taken into account in performing behavioral and physiological studies in social rodents. http://zoolstud.sinica.edu.tw/Journals/51.3/277.pdf

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Living in social groups has many advantages, such as cooperation and social support, but it also has disadvantages, such as social conflicts and increased competition between individuals (Krause and Ruxton 2002, Goymann and Wingfield 2004). Relationships among individuals living in groups vary from being nearly anonymous in some species to highly social, especially in cooperative breeding species (mole rats, marmosets, and social carnivores; Clarke and Faulkes 1997, Saltzman et al. 1998, Creel 2005). Compared to solitary animals, socially living animals are exposed to additional types of stressors such as dominance hierarchies, increased mating competition, and integration into unfamiliar societies (Sapolsky 1982, Goymann et al. 2001, Abbott et al. 2003, Creel 2005, Sapolsky 2005). Beginning with Christian (1950), considerable attention has been paid to the role of social stress in determining fitness and population processes of small mammals, such as small rodents. Stressful stimuli activate the hypothalamic-pituitary-adrenal (HPA) axis, which causes the release of glucocorticoid (GC) hormones into the bloodstream. Thus, stress levels have traditionally been monitored by measuring blood GC concentrations, which may

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change within minutes after a stressful stimulus is introduced (Möstl and Palme 2002, Romero 2004, Sheriff et al. 2011).

Most studies dealing with social stress were aimed at investigating the relationship between the group dominance hierarchy and GC levels, which represents a highly complex physiological and behavioral phenomenon (Creel 2001, Sapolsky 2005), and/or seasonal changes in hormonal profiles connected to reproduction (Boonstra et al. 2001, Franceschini et al. 2007, Schradin 2008). Less attention has been paid to responses of group members to changes in the social composition of a group, such as immigration and/or expulsion of an individual. Most of the current data on this topic have come from long-term studies of large and/or easily observable species such as primates, hyenas, and meerkats (Alberts et al. 1992, Goymann et al. 2003, Beehner et al. 2005, Young et al. 2008). Studying this phenomenon in muroid rodents, which are generally small species with hidden and/or nocturnal activity, is complicated and often impossible under natural conditions (Schradin 2008, Woodruff et al. 2010). Currently, the only feasible method for collecting information about the behavior and physiology of these animals is to keep them under seminatural or laboratory conditions (Sachser et al. 1998, Scheibler et al. 2004; but see Wolff 2003 for the limited applicability of studies performed in captivity).

In the last few decades, assessment of fecal GC metabolites (GCMs) has become a widely used method for monitoring stress responses (Miller et al. 1991, Palme 2005, Touma and Palme 2005, Sheriff et al. 2011). This non-invasive method allows repeated sampling with no manipulation of experimental subjects (after previous live-trapping or careful observation due to individual marking). Nevertheless, applying this technique may still cause some difficulties in small, socially living rodents. It is hard to sample feces of known individuals without stressful procedures such as handling and isolation. For this purpose, we recently introduced use of a special experimental apparatus that allows repeated fecal sampling without notable disturbance to studied animals. This technique permits continuous maintenance of study animals in a familiar environment with uninterrupted communication between group members (Frynta et al. 2009).

In the present study, we chose as a study subject the spiny mouse *Acomys cahirinus*, a nocturnal desert-dwelling rodent from North Africa (Osborn and Helmy 1980). *Acomys* is widely used as a model species, both in physiological (Dickinson et al. 2007, Nováková et al. 2008, Wube et al. 2008) and behavioral studies (Ilany and Eilam 2008, Deacon 2009, Nováková et al. 2010, Frynta et al. 2011), and it exhibits no behavioral signs of stress under standard laboratory conditions (Dieterlen 1962).

Spiny mice are social animals that should be kept in families consisting of an adult male, multiple females, and their descendants (Young 1976). They spend most of their time in close body contact with familiar conspecifics. Social interactions between spiny mice are strongly dependent on familiarity (Porter et al. 1984), kinship (Porter et al. 1983), and sibling recognition (Porter and Wyrick 1979). Mothers are able to recognize their own offspring, but communal care of the young in the form of allosuckling was frequently observed in groups consisting of related individuals (Porter et al. 1980). Interestingly, male sires participate in parental care and clearly discriminate between their own and alien young (Makin and Porter 1984).

These observations suggest that social relationships within family groups of spiny mice are individualized. Thus, we hypothesized that any change of a group's composition in this species is likely to represent an important stressor. Specifically, we focused on the effect of replacing the breeding male. This is a process that occasionally occurs in polygynous families which is associated with considerable social tension or even infanticidal acts in most mammalian taxa (Hausfter and Hrdy 1984, Parmigiani and vom Saal 1994).

The aim of this study was to assess the effects of the following experimental manipulations on fecal GCM levels: (1) the addition of an unfamiliar male into an established family group and (2) removal of a familiar male followed by (3) its replacement by an unfamiliar male.

MATERIALS AND METHODS

Study animals

The spiny mice that we used in this study are descendants of a dozen founders that were captured in the vicinity of the Abu Simbel archaeological site in southern Egypt. The laboratory colony was maintained through outbreeding in numbers exceeding 50 pairs for approximately 10 generations. The animals were

kept in glass breeding cages (60 × 50 × 40 cm) with a sliding front door and wire-mesh ventilation in the upper part of the back side. Wood shavings were used as bedding material, a flowerpot with a lateral opening served as a shelter, and branches were provided for climbing and gnawing as a form of environmental enrichment. The light schedule in the animal housing room corresponded to the outdoor light cycle. The room was maintained under standard laboratory conditions (at a temperature of 22 ± 1°C and relative humidity of 37% ± 5%). A standard diet for rats and mice (ST1; Velaz, Únětice, Czech Republic) was supplemented with a mixture of grains, bread, mealworms, apples, and fresh seasonal herb leaves, and water was available ad libitum. For 2 wk before and during the experimental period, the diet was restricted solely to the ST1 diet (as in our previous work; Nováková et al. 2008). Spiny mice were kept in family groups consisting of 1 adult male, 2 adult females (sisters), and their offspring (subadults). We tested 10 family groups (a total of 50 individuals). Adults were aged 7-11 mo, and subadults were aged 60-90 d (i.e., sexually immature). Animals were marked by painting small spots on the dorsal pelage for individual identification.

Ethical notes

Steps were taken to avoid any harm to the experimental animals, and only non-invasive methods for sample collection were used. All experiments were performed in accordance with Czech law and corresponding EU regulations and were approved by the Institutional Animal Care and Use Committee (no. 27671/2007-30).

Experimental cage

The experimental cage was the same size as the breeding cage. It was constructed of glass and subdivided into 5 compartments: 1 central and 4 lateral ones at each corner of the cage. The partitions separating the central compartment from the lateral parts were constructed from wire mesh to allow uninterrupted communication between the animals, and each compartment was equipped with a metallic slide door operated from the outside. Above the glass bottom, a wire-mesh grid bottom could be adjusted in each compartment. The space below the grid was freely accessible and was covered with clean filter paper allowing collection of fecal samples (Frynta et al. 2009). After each sampling interval, the paper was completely removed and replaced with a clean sheet. Feces was collected in Eppendorf tubes (feces contaminated by urine was not used) and immediately frozen at -20°C for subsequent analyses. As in the breeding cage, each compartment of the experimental cage was supplied with a shelter, an enrichment branch, food, and water.

Experimental design

At the beginning of the experiment, a family group was transferred from a breeding cage to an experimental cage. After an initial adaptation period of 14 d, manipulative procedures began. We performed 3 types of manipulations that involved dominant adult males. Baseline GCM values were assessed between each experimental manipulation to obtain control values for all experimental animals. All fecal samples were collected at the same time interval of 10:00-14:00. Manipulative procedures were temporally designed to take into account the 5-7 h time lag between when GCs are released into the bloodstream and when they become detectable in feces to allow for detection of prospective changes in GCM levels (Nováková et al. 2008).

First, we obtained fecal samples from all animals on days 7 and 14 after their transfer to the experimental apparatus (hereafter referred to as the accommodation period). Following this accommodation period, we began a series of experimental manipulations on the mice. Each individual was sampled on 3 consecutive days (days 23, 24, and 25) to obtain a relatively robust baseline value of GCM levels in each animal (hereafter referred to as baseline 1). On day 27, we performed the 1st manipulative experiments in which an unfamiliar dominant adult male was introduced for a short time interval (the male intruder was added to the group at 04:00 in the morning and removed at 10:00) to a family group. A 6-h interval was chosen as an average of 5-7 h, i.e., the peak of GCM excretion in feces detected during the adrenocorticotropin (ACTH) test, which corresponds to the gut passage time (Nováková et al. 2008). Direct contact between all animals was allowed, and each family group was observed for 2 periods of 30 min each after the intruder had been introduced, to record possible aggressive interactions between animals. Contrary to expectations (Čížková et al. 2011), we failed to record any strong agonistic interactions

between the male intruder and residents. After 6 h, the intruder was removed, and the family group was sampled. Samples were collected at 10:00-14:00, which reflected values of circulating GC at 04:00-08:00, i.e., during contact with the intruder (hereafter referred to as the addition of a male intruder). No samples were collected for the following 10 d to allow the family groups to recover from the intrusion by an unfamiliar individual. All individuals were sampled again on 3 consecutive days (days 37, 38, and 39) to obtain the next baseline value (baseline 2). After this sampling interval, the next experimental manipulation was performed on day 39. The adult male from each family group was removed and housed in a separate cage. The family group without an adult male was then monitored for 3 d (days 40, 41, and 42, hereafter referred to as male removal). The final manipulation was carried out on day 43 at 04:00 (the same time schedule as during the male intruder experiment, see above), when the adult male that had been temporarily placed in a separate cage was introduced to an unfamiliar family group. Samples were collected from all individuals on days 43, 44, 45, and 46 (hereafter referred to as male replacement) and then on days 53, 73, and 74 (that is, 1 wk and approximately 1 mo after the replacement, hereafter referred to as the stabilization period). For the experimental design see also table 1.

Measurement of fecal GCMs

Samples were frozen immediately after collection and stored at -20°C until being analyzed. Each sample was homogenized using a mortar and pestle. Then, 0.05 g of the sample was weighed into an Eppendorf tube (1.5 ml), and 1 ml of 80% methanol was added, as described by Touma et al. (2003). Samples were shaken on a multivortex for 15 min and centrifuged at 11,500 xg for 2 min (Eppendorf microcentrifuge 5415 C, Westbury, NY, USA). The supernatant (800 μl) was transferred to new titer tubes, and aliquots were diluted 1:10 with assay buffer, then transferred to new titer tubes, and frozen at -20°C until being analyzed. To determine the amounts of fecal GCM, we used 2 previously established group-specific enzyme immunoassays (EIAs). The 1st of these EIAs was a 5α-pregnane-3β,11β, 21-triol-20-one EIA (hereafter referred to as EIA1), which recognizes GCM with a 5α-3β,11β-diol structure (developed for laboratory mice; for details of the EIA, including cross-reactivity of different steroids, Touma et al. 2003 2004). Due to high concentrations of fecal GCM, a 1:100 dilution of the supernatant was used for this assay. The other assay was an 11-oxoetiocholanolone EIA (hereafter referred to as EIA2) for GCMs with a 5β-3α-hydroxy-11-one structure (first developed for ruminants by Möstl et al. 2002). Both EIAs were previously successfully validated for measuring adrenocortical activity in spiny mice and exhibited comparable effect sizes after ACTH stimulation (Nováková et al. 2008). The use of 2 EIAs was further justified by the fact that these assays covered partially different spectra of GCMs, and thus this approach increased the sensitivity of the results (Möstl et al. 2005). The intra- and interassay coefficients of variation for EIA1 were 9.1% and 13.3% and for EIA2 were 9.7% and 15.0%, respectively.

Table 1. Basic experimental design including sampling periods and the order of sample collection

Sample no.	Sampling period	Experimental day
1, 2	Accommodation	7, 14
3-5	Baseline 1	23, 24, 25
6	Male intruder	27
7-9	Baseline 2	37, 38, 39
10-12	Male removal	40, 41, 42
13-16	Male replacement	43, 44, 45, 46
17-19	Stabilization	53, 73, 74

Statistical analyses

GCM levels were log-transformed (ln(x)) to improve the normality of the distribution of within-group residuals, and 6 obvious outliers were omitted (those data points of < 500 ng/g for EIA1 and < 100 ng/g for EIA2; these values corresponded to a complete EIA reaction failure), and these data were further analyzed. General linear mixed-effects (LME) models, including fixed factors of the individual and family group identity (whenever applicable) as random factors (random intercept terms), were calculated using a restricted maximum likelihood (REML) method with the *lme* function as implemented in *nlme* of the R package, vers. 2.10.0 (R Development Core Team 2009). The significance of the family group identity was tested by comparison of the models (including and excluding the family group) by a log-likelihood ratio test. Variance components of random models were computed to quantify the contributions of

random factors.

Initially, we performed an analysis of GCM levels collected exclusively during periods of no experimental manipulation (accommodation, baseline 1, and baseline 2) and implemented the period, sex, age, and sex × age interaction as fixed factors. These partial analyses revealed no effect of period (see below under "Results"); we thus pooled the accommodation, baseline 1, and baseline 2 periods into a baseline for further analyses.

Next, we included the periods of experimental manipulations and divided the entire dataset into those concerning adult males and remaining family group members. These 2 subsets were further analyzed separately because of (1) different numbers of records (males were not sampled during their experimental removal which included transfer to a novel environment and a change in the social environment), (2) male exchanges between family groups possibly corrupting the hierarchical arrangement of the random factors, and (3) different social roles of adult males and other family members.

The experimental manipulation (at the levels of baseline, intruder, replacement, and stabilization periods) was the only fixed factor analyzed in the adult male subset; family group identity was not applicable to this analysis, and individual identity was the sole random factor.

The experimental manipulation (at the levels of baseline, intruder, removal, replacement, and stabilization periods), sex/age (at the levels of adult females, subadult females, and subadult males), and their interaction were introduced as fixed factors in the analyses of the subset of remaining family members.

RESULTS

Accommodation and baseline periods

We compared GCM levels assessed in the periods in which the composition of the groups was not experimentally manipulated. The results confirmed the stability of GCM levels throughout the sampling periods and also revealed variations among family groups and partial differences between the sexes (Table 2).

For both EIAs, comparisons using LME models, including family group and individual identity with those considering only individual identity as random factors, revealed that inclusion of the random intercept term for family groups significantly improved the fit of the models (EIA1:

Table 2. Fecal glucocorticoid metabolites levels (μg/g WW (wet weight)) assessed by enzyme immunoassay (EIA): EIA1 and EIA2 (mean and 95% confidence intervals are given) during the experiment

	Experimental period						
	Accommodation	Baseline 1	Male intruder	Baseline 2	Male removal	Male replacement	Stabilization
EIA1 (μg/g WW)							
Adult females	1.90	2.28	2.30	1.77	1.83	2.08	2.27
	(1.37-2.63)	(1.59-3.26)	(1.80-2.95)	(1.41-2.22)	(1.56-2.14)	(1.69-2.55)	(1.73-2.97)
Subadult females	2.47	2.08	2.09	2.18	2.27	2.45	1.96
	(1.95-3.12)	(1.80-2.39)	(1.67-2.61)	(1.71-2.78)	(1.75-2.94)	(1.94-3.09)	(1.59-2.42)
Adult males	2.49	2.48	2.56	2.25		2.25	1.62
	(1.82-3.42)	(1.67-3.67)	(1.81-3.64)	(1.82-2.78)		(1.60-3.17)	(1.25-2.12)
Subadult males	2.09	2.29	2.20	1.76	2.24	2.13	2.02
	(1.51-2.89)	(1.72-3.04)	(1.60-3.02)	(1.31-2.36)	(1.69-2.97)	(1.69-2.69)	(1.60-2.56)
EIA2 (μg/g WW)							
Adult females	0.90	0.89	0.86	0.69	0.70	0.73	0.85
	(0.67-1.20)	(0.66-1.18)	(0.65-1.14)	(0.53-0.92)	(0.56-0.87)	(0.54-0.99)	(0.58-1.24)
Subadult females	0.77	0.81	0.92	0.73	0.67	0.74	0.64
	(0.60-0.98)	(0.62-1.06)	(0.63-1.36)	(0.53-1.01)	(0.48-0.93)	(0.56-0.98)	(0.49-0.84)
Adult males	0.59	0.73	0.8	0.68		0.54	0.41
	(0.51-0.68)	(0.49-1.09)	(0.57-1.12)	(0.52-0.91)		(0.43-0.69)	(0.27-0.63)
Subadult males	0.45	0.52	0.62	0.45	0.54	0.55	0.48
	(0.37-0.56)	(0.39-0.69)	(0.43-0.90)	(0.32-0.64)	(0.40-0.71)	(0.46-0.66)	(0.35-0.65)

AIC = 321.43 vs. 329.096, c = 9.64, $p = 0.0019$; EIA2: AIC = 454.76 vs. 462.85, c = 10.10, $p = 0.0015$; Fig. 1).

With the EIA1, none of the fixed factors was significant (period: $F_{(2,335)} = 1.09$, $p = 0.3375$; sex: $F_{(1,37)} = 0.69$, $p = 0.4124$; age: $F_{(1,37)} = 0.13$, $p = 0.7159$; and the sex × age interaction: $F_{(1,37)} = 2.01$, $p = 0.1642$). Therefore, we removed the fixed effects from the model and performed a random model (i.e., a model with just the intercept and random intercept terms for family group and individual identity) to evaluate the relative contributions of the random factors; family group contributed 27.6% and individual identity 36.6% to the variation.

With the EIA2, the only significant factor was sex ($F_{(1,37)} = 17.38$, $p = 0.0002$; females had higher GCM values than males), while the effects of period ($F_{(2,335)} = 0.17$, $p = 0.8441$), age ($F_{(1,37)} = 1.01$, $p = 0.3222$), and the sex × age interaction ($F_{(1,37)} = 1.36$, $p = 0.2513$) remained non-significant (Table 3). The random factors family group and individual identity respectively contributed 22.6% and 26.1% to the variation.

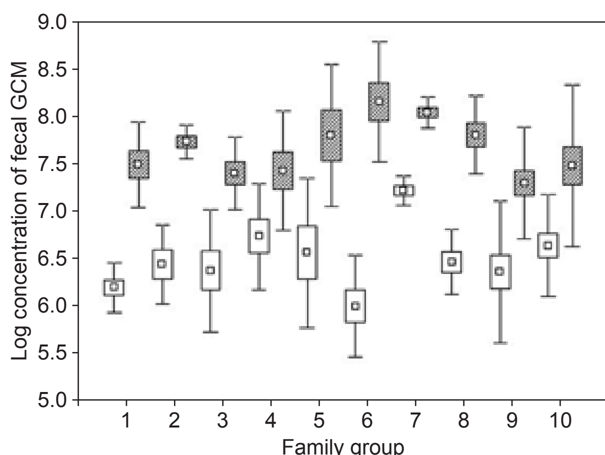

Fig. 1. Family means (mean ± S.E., whiskers represent 95% confidence intervals) of log-transformed levels of glucocorticoid metabolites (GCM) in the feces (ng/g) assessed by enzyme immunoassay (EIA): EIA1 (dashed box plots) and EIA2 (open box plots) in non-manipulated periods of the experiment, i.e., accommodation, baseline 1, and baseline 2.

The absence of an effect of period allowed us to pool the accommodation, baseline 1, and baseline 2 periods and then use these data as a reference level to test the effects of the experimental manipulations in further analyses.

Male addition, removal, and replacement

Adult males

An analysis of variance (ANOVA) of the LME models revealed strong effects of the experimental manipulations on GCM levels (EIA1: $F_{(3,139)} = 9.05$, $p = 0.0001$; EIA2: $F_{(3,139)} = 6.75$, $p = 0.0003$).

The presence of an adult male intruder had no substantial effect on GCM levels of the resident adult male. No such effect was revealed by the EIA1 ($t = 0.08$, $p = 0.9349$), while an increase approaching significance was detected in the case of the EIA2 (Beta = 0.255, SE = 0.133; $t = 1.92$, $p = 0.0571$).

In contrast, adult males showed consistently lower GCM levels after the experimental immigration and acceptance into a new family group (replacement and stabilization periods). The model coefficients for male replacement and subsequent stabilization periods were highly significant for both the EIA1 (Beta = -0.181 and -0.329, S.E. = 0.066 and 0.068; $t = -2.75$ and -4.87, $p < 0.0067$ and = 0.0001, respectively) and EIA2 (Beta = -0.276 and -0.181, S.E. = 0.082 and 0.085; $t = -3.34$ and -2.13, $p = 0.0011$ and 0.0351, respectively; Table 4).

Table 3. Coefficients of the linear mixed-effects model (Akaike information criterion = 454.8) assessing the effects of accommodation and baseline periods on glucocorticoid metabolites levels in the family group members assessed by enzyme immunoassay EIA2 (with individual identity included as a random factor)

	Beta	Standard error	*d.f.*	*t*-value	*p* value
Baseline 1	-0.001	0.050	335	-0,02	0.9873
Baseline 2	-0.023	0.050	335	-0.47	0.6373
Males	-0.436	0.118	37	-3.70	0.0007
Adults	-0.024	0.128	37	-0.19	0.8540
Adult males	0.204	0.175	37	1.17	0.2513

Adult females and subadults

The effect of the experimental manipulation on GCM levels of remaining family members was demonstrable only in the data subset assessed by the EIA2.

For the EIA1, the ANOVA of the LME models revealed no effects of fixed factors: experimental manipulation ($F_{(4,663)}$ = 1.28, p = 0.2747), sex/age (at the levels of adult females, subadult females, and subadult males; $F_{(4,663)}$ = 1.28, p = 0.2747), or their interaction ($F_{(8,663)}$ = 1.08, p = 0.3722) on GCM levels. For the EIA2, the ANOVA of the LME models revealed significant effects of the experimental manipulation ($F_{(4,664)}$ = 3.90, p = 0.0039) and sex/age (at the levels of adult females, subadult females, and subadult males; $F_{(2,29)}$ = 10.77, p = 0.0003), but not their interaction ($F_{(8,664)}$ = 1.49, p = 0.1562) on GCM levels. Males (i.e., subadult males) exhibited lower levels than females as already demonstrated above in data from unmanipulated periods (subadult males: Beta = -0.407, S.E. = 0.101; t = -4.04, p = 0.0004). Although significant effect of the experimental manipulation were detected, mice showed only a tendency to decrease their GCM levels after removal of the adult male (removal period: Beta = -0.154, S.E. = 0.079; t = -1.95, p = 0.0514; Table 5), while neither the addition of an adult male intruder nor male replacement affected their GCM levels.

Table 4. Coefficients of the linear mixed-effects model (Akaike information criteria for enzyme immunoassay (EIA): EIA1 and EIA2 = 130.3 and 193.7, respectively) assessing the effects of experimental manipulation period on glucocorticoid metabolites levels in adult males assessed by the EIA1 and EIA2

	Beta	Standard error	*d.f.*	*t*-value	*p* value
EIA1					
Male intruder	0.009	0.106	139	0.08	0.9346
Male replacement	-0.181	0.066	139	-2.75	0.0067
Stabilization period	-0.329	0.068	139	-4.87	< 0.0001
EIA2					
Male intruder	0.255	0.133	139	1.92	0.0571
Male replacement	-0.276	0.083	139	-3.34	0.0011
Stabilization period	-0.181	0.085	139	-2.13	0.0351

Table 5. Coefficients of the linear mixed-effects model (Akaike information criterion = 881.3) assessing the effects of experimental manipulation period on glucocorticoid metabolites levels in remaining family group members (adult females, subadult females, and subadult males) assessed by the enzyme immunoassay EIA2 (individual identity was included as a random factor)

	Beta	Standard error	*d.f.*	*t*-value	*p* value
Male intruder	0.044	0.123	664	0.36	0.7204
Male removal	-0.154	0.079	664	-1.95	0.5140
Male replacement	0.041	0.072	664	0.56	0.5728
Stabilization period	-0.105	0.081	664	-1.28	0.1994
Subadult females	0.024	0.114	29	0.21	0.8366
Subadult males	-0.407	0.101	29	-4.04	0.1114
Male intruder × subadult females	0.068	0.182	664	0.37	0.7105
Male removal × subadult females	0.068	0.116	664	-0.58	0.5621
Male replacement × subadult females	-0.090	0.104	664	-0.87	0.3860
Stabilization × subadult females	-0.068	0.121	664	-0.56	0.5753
Male intruder × subadult males	0.160	0.160	664	1.00	0.3180
Male removal × subadult males	0.238	0.107	664	2.31	0.0210
Male replacement × subadult males	-0.035	0.096	664	-0.37	0.7110
Stabilization × subadult males	-0.007	0.107	664	-0.07	0.9480

DISCUSSION

We found that the experimental manipulation of an existing family group of spiny mice by the addition of an adult male intruder, or removing or replacing an adult male resident did not cause substantial increases in GCM levels in family members. Particularly in the case of simulated immigration into an established family group, we expected that the simultaneous presence of an intruder and the resident male in a family group would lead to conflict, resulting in elevated GCM levels in all family members, particularly in the resident adult male. Nevertheless, the only result conforming to this expectation was a nearly significant increase in GCM levels in EIA2 values measured in adult male residents.

It was previously reported that aggressive interactions between unfamiliar spiny mice of the same sex are frequent (Porter 1976). In laboratory experiments performed to simulate adult male intrusion into families of spiny mice, the adult male resident interacted with and attacked the intruder more than did other family members (Čížková et al. 2011). Moreover, according to our experience with spiny mice, adult males are usually unable to permanently tolerate even their adolescent sons, which they eventually attack and drive away. Stressful conflicts over social dominance were also reported in other socially living rodents, usually leading to antagonistic interactions that are connected to elevated GC levels. Finally, these conflicts may result in suppression of reproduction in affected individuals and/or even their expulsion from the group (gerbils: Rogovin et al. 2003, Scheibler et al. 2004; guinea pigs: Sachser et al. 1998, Hennessy et al. 2006).

Experiments in which adult males are replaced fundamentally differ from male-addition experiments (Čížková et al. 2011). The resident adult male was removed 3 d before the addition of a newcomer male, and the remaining resident family members were thus not confronted with the conflict of loyalty to a particular dominant male. Immigrating adult males received an opportunity to mate with new, unrelated sexual partners (Hausfather and Hrdy 1984, Van Horn et al. 2008), which could explain why their GCM levels consistently and considerably decreased in the replacement and stabilization phases of our experiments. We found no such changes in GCM levels of resident females or subadult males. However, for resident subadult males, it would be expected that the presence of at least a temporarily tolerant father would be more advantageous than the presence of an unrelated adult male newcomer, and thus, they would be expected to show increased stress levels.

Females of many rodent species are known to increase aggressive behaviors during lactation (Vom Saal et al. 1995, Heise and Lippke 1997, Ebensperger 1998, Gammie and Nelson 2005), probably in anticipation of an elevated risk of infanticide during this time (Palanza and Parmigiani 1994, Stevens 1998, Ebensperger et al. 2000). For ethical reasons, our experiments were performed with family groups that did not possess pups or weanlings. Thus, male addition and/or replacement were not associated with the risk of infanticide by this unfamiliar individual. This may also explain why we did not observe strong agonistic interactions of female group members with the "intruder" as well as explaining the absence of a GCM response.

We found that baseline GCM levels considerably differed among unmanipulated family groups kept under standardized conditions. This variation sharply contrasts with relatively mild effects of experimental manipulations of disturbing the family group composition. This suggests that specific group settings for social relations determine GCM levels in a comparable manner as individual variations and even experimental transfers of adult males. We speculated that these settings predominantly depend on social relationships between females, and that the role of adult males is limited. This speculation is consistent with the above-mentioned results of Porter (1976), who concluded that females are sometimes socially dominant over males in spiny mice societies.

Steroid metabolism may be sex dependent, and thus it is not very surprising that strong sexual differences in GCM levels were previously reported in some other laboratory animals (mice: Touma et al. 2004, rats: Lepschy et al. 2007 2010). When we used the EIA2 to measure GCM levels, we found significant sex differences in GCM levels during both the baseline and experimental manipulation periods, although the differences were considerably smaller than those reported for other species. In spiny mice, these mean GCM values were 1.5-times higher in females (810 ng/g) than in males (541 ng/g). Nevertheless, this finding is not fully consistent with our previous results, which suggests that such sex differences occur only in the case of the commensal population of *A. cahirinus* from Cairo (Nováková

et al. 2008). In contrast to the EIA2 results, we failed to demonstrate any sex differences using the EIA1. This may be attributed to different spectra of GCM covered by the 2 EIAs (Möstl et al. 2005). However, as both EIAs performed comparably well in the validation experiment (Nováková et al. 2008), there is no justification for preferring 1 EIA over the other. This explanation and interpretation are also applicable to other discrepancies between the effects of the experimental manipulations revealed by both EIAs, including: (1) the contrast between the nearly significant and non-significant effects in the same direction in the case of resident males exposed to intruders and (2) the contrast between the significant and non-significant effects of experimental manipulations (male addition, removal, and replacement) on adult females and subadults. Nevertheless, in particular, the former disagreement may be alternatively explained by purely statistical reasons: a limited power of the analysis due to the small number of adult males.

In conclusion, we demonstrated that GCM levels may be affected by manipulating the social composition of a group of spiny mice. Nevertheless, only a minority of experimental manipulations resulted in notable effects. In particular, GCM levels of adult males decreased following their immigration into a new family group composed of females and subadults. We also found that GCM levels substantially differed between family groups of spiny mice. This result is in agreement with our previous findings that some families of spiny mice occasionally suffer from social conflicts. However, even though only families of comparable size that exhibited no obvious signs of social conflict were included in our experiments, GCM levels still varied from group to group. Thus, we recommend considering the effect of social settings on any behavioral or physiological research performed in group-living animals including laboratory-maintained murids such as mice, rats, and gerbils.

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REFERENCES

Abbott DH, EB Keverne, FB Bercovitch, CA Shively, SP Mendoza, W Saltzman et al. 2003. Are subordinates always stressed? A comparative analysis of rank differences in cortisol levels among primates. Horm. Behav. **43:** 67-82.

Alberts SC, RM Sapolsky, J Altmann. 1992. Behavioral, endocrine, and immunological correlates of immigration by an aggressive male into a natural primate group. Horm. Behav. **26:** 167-178.

Beehner JC, TJ Bergman, DL Cheney, RM Seyfarth, PL Whitten. 2005. The effect of new alpha males on female stress in free-ranging baboons. Anim. Behav. **69:** 1211-1221.

Boonstra R, AH Hubbs, EA Lacey, CJ McColl. 2001. Seasonal changes in glucocorticoid and testosterone concentrations in free-living arctic ground squirrels from the boreal forest of the Yukon. Can. J. Zool. **79:** 49-58.

Christian JJ. 1950. The adreno-pituitary system and population cycles in mammals. J. Mammal. **31:** 247-259.

Čížková B, R Šumbera, D Frynta. 2011. A new member or an intruder: How do Sinai spiny mouse (*Acomys dimidiatus*) families respond to a male newcomer? Behaviour **148:** 889-908.

Clarke FM, CG Faulkes. 1997. Dominance and queen succession in captive colonies of the eusocial naked mole-rat, *Heterocephalus glaber*. Proc. R. Soc. Lond. B Biol. Sci. **264:** 993-1000.

Creel S. 2001. Social dominance and stress hormones. Trends Ecol. Evol. **16:** 491-497.

Creel S. 2005. Dominance, aggression, and glucocorticoid levels in social carnivores. J. Mammal. **86:** 255-264.

Deacon RMJ. 2009. Burrowing: a sensitive behavioural assay, tested in five species of laboratory rodents. Behav. Brain Res. **200:** 128-133.

Dickinson H, DW Walker, EM Wintour, K Moritz. 2007. Maternal dexamethasone treatment at midgestation reduces nephron number and alters renal gene expression in the fetal spiny mouse. Am. J. Physiol. Regul. Integr. Compar. Physiol. **292:** R453-R461.

Dieterlen F. 1962. Geburt und Geburtshilfe bei der Stachelmaus, *Acomys cahirinus*. Z. Tierpsychol. **19:** 191-222.

Ebensperger LA. 1998. The potential effects of protected nests and cage complexity on maternal aggression in house mice. Aggress. Behav. **24:** 385-396.

Ebensperger LA, C Botto-Mahan, RH Tamarin. 2000. Nonparental infanticide in meadow voles, *Microtus pennsylvanicus*: the influence of nutritional benefits. Ethol. Ecol. Evol. **12:** 149-160.

Franceschini C, C Lutz, R Palme, E Millesi. 2007. Seasonal changes in cortisol and progesterone secretion in common hamsters. Gen. Compar. Endocrinol. **152:** 14-21.

Frynta D, M Fraňková, B Čížková, H Skarlandtová, K Galeštoková, K Průšová et al. 2011. Social and life history correlates of litter size in captive colonies of precocial spiny mice (*Acomys*). Acta Theriol. **56:** 289-295.

Frynta D, M Nováková, H Kutalová, R Palme, F Sedláček. 2009. A new apparatus for the collection of faecal samples in undisturbed spiny mice living in a complex social group. J. Am. Assoc. Lab. Anim. **48:** 196-201.

Gammie SC, RJ Nelson. 2005. High maternal aggression in dwarf hamsters (*Phodopus campbelli* and *P. sungorus*). Aggress. Behav. **31:** 294-302.

Goymann W, ML East, B Wachter, OP Höner, E Möstl, H Hofer. 2003. Social status does not predict corticosteroid levels in postdispersal male spotted hyenas. Horm. Behav. **43:** 474-479.

Goymann W, ML East, B Wachter, OP Höner, E Möstl, TJ Van't Hof, H Hofer. 2001. Social, state-dependent and environmental modulation of faecal corticosteroid levels in free-ranging female spotted hyenas. Proc. R. Soc. Lond. B Biol. Sci. **268:** 2453-2459.

Goymann W, JC Wingfield. 2004. Allostatic load, social status and stress hormones: the costs of social status matter. Anim. Behav. **67:** 591-602.

Hausfter GI, SB Hrdy, eds. 1984. Infanticide: comparative and evolutionary perspectives. New York: Aldine, 598 pp.

Heise S, J Lippke. 1997. Role of female aggression in prevention of infanticidal behavior in male common voles, *Microtus arvalis* (Pallas, 1779). Aggress. Behav. **23:** 293-298.

Hennessy MB, G Hornschuh, S Kaiser, N Sachser. 2006. Cortisol responses and social buffering: a study throughout the life span. Horm. Behav. **49:** 383-390.

Ilany A, D Eilam. 2008. Wait before running for your life: defensive tactics of spiny mice (*Acomys cahirinus*) in evading Barn Owl (*Tyto alba*) attack. Behav. Ecol. Sociobiol. **62:** 923-933.

Krause J, GD Ruxton, eds. 2002. Living in groups. Oxford, UK: Oxford Univ. Press, 240 pp.

Lepschy M, C Touma, R Hruby, R Palme. 2007. Non-invasive measurement of adrenocortical activity in male and female rats. Lab. Anim. **41:** 372-387.

Lepschy M, C Touma, R Palme. 2010. Faecal glucocorticoid metabolites: how to express yourself-comparison of absolute amounts versus concentrations in samples from a study in laboratory rats. Lab. Anim. **44:** 192-198.

Makin JW, RH Porter. 1984. Paternal behavior in the spiny mouse (*Acomys cahirinus*). Behav. Neural. Biol. **41:** 135-151.

Miller MW, NT Hobbs, MC Sousa. 1991. Detecting stress responses in Rocky Mountain bighorn sheep (*Ovis canadensis canadensis*): reliability of cortisol concentrations in urine and feces. Can. J. Zool. **69:** 15-24.

Möstl E, JL Maggs, G Schrotter, U Besenfelder, R Palme. 2002. Measurement of cortisol metabolites in faeces of ruminants. Vet. Res. Commun. **26:** 127-139.

Möstl E, R Palme. 2002. Hormones as indicators of stress. Domest. Anim. Endocrinol. **23:** 67-74.

Möstl E, S Rettenbacher, R Palme. 2005. Measurement of corticosterone metabolites in birds' droppings: an analytical approach. Ann. N. Y. Acad. Sci. **1046:** 17-34.

Nováková M, H Kutalová, R Palme, L Janský, D Frynta. 2008. The effects of sex, age and commensal way of life on baseline glucocorticoid levels in spiny mice (*Acomys cahirinus*). Physiol. Behav. **95:** 187-193.

Nováková M, B Vašáková, H Kutalová, K Galeštoková, K Průšová, P Šmilauer et al. 2010. Secondary sex ratios do not support maternal manipulation: extensive data from laboratory colonies of spiny mice (Muridae: *Acomys*). Behav. Ecol. Sociobiol. **64:** 371-379.

Osborn DJ, I Helmy. 1980. The contemporary land mammals of Egypt (including Sinai). Fieldiana Zool. **5:** 1-579.

Palanza P, S Parmigiani. 1994. Functional analysis of maternal aggression in the house mouse (*Mus musculus domesticus*). Behav. Process. **32:** 1-16.

Palme R. 2005. Measuring fecal steroids - guidelines for practical application. Ann. NY Acad. Sci. **1046:** 75-80.

Parmigiani S, FS vom Saal, eds. 1994. Infanticide and parental care. Chur, Switzerland: Harwood Academic Publishers, 493 pp.

Porter RH. 1976. Sex-differences in the agonistic behavior of spiny-mice (*Acomys cahirinus*). Z. Tierpsychol. **40:** 100-108.

Porter RH, SA Cavallaro, JD Moore. 1980. Developmental parameters of mother-offspring interactions in *Acomys cahirinus*. Z. Tierpsychol. **53:** 153-170.

Porter RH, JA Matochik, JW Makin. 1983. Evidence for phenotype matching in spiny mice (*Acomys cahirinus*). Anim. Behav. **31:** 978-984.

Porter RH, JA Matochik, JW Makin. 1984. The role of familiarity in the development of social preference in spiny mice. Behav. Process. **9:** 241-254.

Porter RH, M Wyrick. 1979. Sibling recognition in spiny mice (*Acomys cahirinus*): influence of age and isolation. Anim. Behav. **27:** 761-766.

R Development Core Team. 2009. R: a language and environment for statistical computing. R Foundation for Statistical Computing. Available at www.R-project.org

Rogovin K, JA Randall, I Kolosova, MP Moshkin. 2003. Social correlates of stress in adult males of the great gerbil, *Rhombomys opimus*, in years of high and low population densities. Horm. Behav. **43:** 132-139.

Romero LM. 2004. Physiological stress in ecology: lessons from biomedical research. Trends Ecol. Evol. **19:** 249-255.

Sachser N, M Durschlag, D Hirzel. 1998. Social relationships and the management of stress. Psychoneuroendocrinology **23:** 891-904.

Saltzman W, NJ Schultz-Darken, FH Wegner, DJ Wittwer, DH Abbott. 1998. Suppression of cortisol levels in subordinate female marmosets: reproductive and social contributions. Hormones and Behavior **33:** 58-74.

Sapolsky RM. 1982. The endocrine stress-response and social status in the wild baboon. Horm. Behav. **16:** 279-292.

Sapolsky RM. 2005. The influence of social hierarchy on primate health. Science **29:** 648-652.

Scheibler E, R Weinandy, R Gattermann. 2004. Social categories in families of Mongolian gerbils. Physiol. Behav. **81:** 455-464.

Schradin C. 2008. Seasonal changes in testosterone and corticosterone levels in four social classes of a desert dwelling sociable rodent. Horm. Behav. **53:** 573-579.

Sheriff MJ, CJ Krebs, R Boonstra. 2011. From pattern to process: How fluctuating predation risk impacts the stress axis of snowshoe hares during the 10 year cycle. Oecologia **166:** 593-605.

Stevens SD. 1998. High incidence of infanticide by lactating females in a population of Columbian ground squirrels (*Spermophilus columbianus*). Can. J. Zool. **76:** 1183-1187.

Touma C, R Palme. 2005. Measuring fecal glucocorticoid metabolites in mammals and birds: the importance of validation. Ann. NY Acad. Sci. **1046:** 54-74.

Touma C, R Palme, N Sachser. 2004. Analyzing corticosterone metabolites in fecal samples of mice: a noninvasive

technique to monitor stress hormones. Horm. Behav. **45:** 10-22.

Touma C, N Sachser, E Möstl, R Palme. 2003. Effects of sex and time of day on metabolism and excretion of corticosterone in urine and feces of mice. Gen. Compar. Endocrinol. **130:** 267-278.

Van Horn RC, HE Watts, KE Holekamp. 2008. Do female hyaenas choose mates based on tenure? Nature **454:** E1.

Vom Saal FS, P Franks, M Boechler, P Palanza, S Parmigiani. 1995. Nest defense and survival of offspring in highly aggressive wild Canadian female house mice. Physiol. Behav. **58:** 669-678.

Wolff JO. 2003. Laboratory studies with rodents: facts or artifacts? BioScience **53:** 421-427.

Woodruff JA, EA Lacey, G Bentley. 2010. Contrasting fecal corticosterone metabolite levels in captive and free-living colonial tuco-tucos (*Ctenomys sociabilis*). J. Exp. Zool. A Ecol. Genet. Physiol. **313:** 498-507.

Wube T, F Fares, A Haim. 2008. A differential response in the reproductive system and energy balance of spiny mice *Acomys* populations to vasopressin treatment. Compar. Biochem. Physiol. **151:** 499-504.

Young AJ, SL Monfort, TH Clutton-Brock. 2008. The causes of physiological suppression among female meerkats: a role for subordinate restraint due to the threat of infanticide? Horm. Behav. **53:** 131-139.

Young DAB. 1976. Breeding and fertility of the Egyptian spiny mouse, *Acomys cahirinus*: effect of different environments. Lab. Anim. **10:** 15-24.