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The effects of sex, age and commensal way of life on levels of fecal glucocorticoid metabolites in spiny mice (*Acomys cahirinus*)

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ABSTRACT

We studied levels of fecal glucocorticoid metabolites (GCM) in a social rodent — Egyptian spiny mouse. As breeding adults are socially dominant over subadults, and adolescent males are driven away by the dominant males, we addressed the question whether animals within extended families are stressed differently depending upon their social category. In addition, we evaluated whether there are differences between non-commensal (outdoor) and commensal (adapted to human settlements) populations. Concentrations of fecal GCM were assessed from samples collected in a special cage that allowed continuous individual sampling of undisturbed mice housed as a semi-natural social unit. First we performed an ACTH challenge test to validate two enzyme immunoassays (EIA): a 5α-pregnane-3β,11β,21-triol-20-one EIA and an 11-oxoetiocholanolone EIA to measure a group of fecal GCM in this species. Next we monitored concentrations of fecal GCM in 68 individuals belonging to 10 family groups and two populations. Commensal spiny mice showed higher fecal GCM levels than non-commensal ones. No effect of age (i.e., social dominance) and only a small effect of sex (in the commensal population only, with males exhibiting lower values) on fecal GCM levels were found. On the other hand, considerable variations in measured fecal GCM between family groups were revealed, indicating that the social settings of the particular group play an important role.

1. Introduction

Studies comparing glucocorticoid (GC) levels in socially dominant and subordinate mammals reveal controversial results. Contrary to the expectations, subordinates are not always stressed in mammalian societies (for reviews see [1–3]). In some highly social species, such as cooperatively breeding marmosets [e.g., 4,5] and carnivores [e.g., 6,7], socially dominant breeders exhibit higher levels of circulating GC than family members sacrificing their immediate reproductive success and engaging in helping. In contrast, subordinate individuals have markedly elevated GC levels in social arrangements where they experience more stressors without the possibility to obtain social support from their kin ([1] and references therein). Obviously, it is the way in which dominance is achieved and maintained that may play a key role. Accordingly, interspecific variation in the ratio of GC levels in dominants and subordinates was successfully explained by the costs of social status [3]. As these costs differ considerably among species, sexes and social arrangements, they provide a functional link between social behavior and hormonal status.

Rodents, as a group of different species exhibiting a wide range of social arrangements, may serve as a proper model for comparative studies of such kind. Surprisingly, only limited comparative data concerning these relationships have been published for this group. It was repeatedly demonstrated in laboratory mice that future subordinates [8] and/or losers of social interactions [e.g., 9,10] have elevated GC levels and may develop GC resistance [11]. Similar results have also been reported in other laboratory rodents, such as hamsters [12] and Mongolian gerbils [13]. Nevertheless, studies monitoring GC levels within natural [14–18] or semi-natural [19] social units are fairly exceptional.

As a model species we selected Egyptian spiny mouse *Acomys cahirinus* (Desmarest, 1819), a nocturnal [20] and desert dwelling [21] rodent from North Africa. Currently, this species (or its closest relative *A. dimidiatus* that is also often erroneously labeled as *A. cahirinus*) is widely used as an experimental model in both physiological [e.g., 22,23] and behavioral studies [e.g., 24]. Spiny mice, even those recently captured in the field, exhibit no behavioral signs of stress under standard laboratory conditions and breed well. In the Nile

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Valley, some populations of *A. cahirinus* have adopted a commensal lifestyle [21]. Commensal spiny mice are dark (instead of sandy light) colored, and differ from non-commensal ones also in some behavioral traits. They are fast movers, better jumpers and climbers, and more anxious in laboratory tests (Benkovičová and Frynta, unpublished data).

Spiny mice are social animals that should be kept in families consisting of an adult male, multiple females and their descendants [25]. Their societies are not anonymous, and individual recognition was demonstrated [26]. Spiny mice, females in particular, spend the most time in close body contact with familiar conspecifics. Social interactions are selective and strongly depend on familiarity [27], kinship [28], and sibling recognition [29]. Mothers are able to recognize their own offspring. However, at least in groups consisting of related individuals, communal care for the young comprising allosuckling is frequent [30]. Interestingly, male sires participate in parental care and clearly discriminate between own and alien young [31].

In laboratory conditions, aggressive interactions between unfamiliar spiny mice are frequent. Porter [32] has reported that females tested in their home cage were dominant over unfamiliar males, but not in the males' home cage. This author has never observed spiny mice to adopt a noticeable stereotype submissive posture, when attacked by a conspecific. They simply moved away from the aggressor, often emitting high-pitched squeals. These observations suggest that the function of aggressive behavior in this species is probably exclusion of the attacked individual from the social unit and/or territory rather than the establishment of a dominance hierarchy.

According to our experience, adolescent males (sons or kin) are usually driven away by a territorial male while females are tolerated. Occasionally such conflicts may result in apparent social tension within the whole group and immediate suspension of reproduction. In affected groups, mice start to bite each other. As the tails are most vulnerable, this aggression results in frequent tail losses, a phenomenon also reported in 12% of males and 25% of females from natural populations [33]. It is highly apparent that the degree of tolerance towards subordinates and thus the level of social tension varies greatly from group to group and from time to time. This predetermines spiny mice as a proper model for the study of social stress.

The aim of this study was to (1) validate enzyme immunoassays (EIAs) for the measurement of fecal GCM in spiny mice; (2) assess levels of fecal GCM in individual mice kept in family groups; (3) compare these levels in mice of different functional categories (gender, adult/subadult) and between families; (4) evaluate possible differences in fecal GCM between the commensal and non-commensal populations.

2. Materials and methods

2.1. Studied animals

We studied two laboratory colonies of spiny mice further referred to as the non-commensal and commensal populations (labeled according to the ecological strategy of their wild ancestors). The former colony was founded by a dozen of spiny mice captured in the vicinity of Abu Simbel, an archaeological site in southern Egypt. The breeding stock founding the other colony (kindly donated by O. Barome) was originally derived from animals captured in Cairo (N Egypt; see [34] for genetic characteristics). In our laboratory, both colonies were maintained outbred in numbers exceeding 50 pairs for about ten generations. According to morphological and molecular (mitochondrial DNA sequences) analyses (unpublished results), animals of both colonies belong without any doubt to Egyptian spiny mouse (*A. cahirinus*) in a narrow sense. As has been previously reported, cortisol is the main GC in circulating blood in this species [35].

The animals were kept in glass breeding cages (600×500×400 mm) with sliding front doors and wire-mesh ventilation in the upper part of the backside. Wood shavings were used as bedding material, a flowerpot with lateral opening served as a shelter, and some branches for climbing and gnawing were provided as environmental enrichment. The light schedule in the animal housing room corresponded to the outdoor light cycle. As the experiments were performed in March, it was very close to 12L:12D. The room was maintained under standard laboratory conditions (temperature 22±1 °C, relative humidity 37±5%). Food (standard diet for rats and mice ST1; VELAZ, Czech Republic) occasionally supplemented with a mixture of grains, bread, mealworms, apples and herb leaves and water were available *ad libitum*. During the experiment (and two weeks before) the diet was standardized (solely ST1 diet). Spiny mice were kept in family groups consisting of one male, two females (sisters) and their offspring.

Ethical note: Any harm to experimental animals was avoided and only non-invasive methods for sample collection were used. The experiments were performed in accordance with Czech law and corresponding EU regulations and were approved by the Institutional Animal Care and Use Committee.

2.2. Experimental cage

The experimental cage was the same size as the breeding one. It was constructed from glass and subdivided into five compartments — one single central and four lateral ones in 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 was adjusted in each compartment. The space below the grid was freely accessible and was covered with clean filter paper allowing collection of fecal samples. For details see [36]. After each sampling interval, the paper was thoroughly removed and changed for a new sheet. Feces were collected in Eppendorf cups (feces contaminated by urine were 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, a branch, food and water.

2.3. ACTH challenge test

To physiologically validate the non-invasive method for assessment of adrenocortical activity in *A. cahirinus* by measuring fecal glucocorticoid metabolites (GCM), we performed an ACTH challenge test. We used subadult animals (aged about 90 days, i.e., sexually immature) from the non-commensal population. At the beginning, spiny mice were locked in lateral compartments at 0700 h and fecal samples were collected at 1000, 1200, 1400, 1600, 1800 and 2200 h to obtain comparable baseline values. Between 0615 and 0645 h of the next day each animal was injected intraperitoneally with 0.1 ml of a solution. The experimental group (five males and five females) received ACTH (Synacthen, Ciba-Geigy AG, Basel, Switzerland) dissolved in isotonic saline. We used a dosage of 60 μg Synacthen/100 g body weight (according to [37]). In the control group (five males and five females) isotonic saline was administered to evaluate the effect of the injection procedure itself. All manipulations of the animals (i.e., catching the individual in the compartment, fixation, injection and returning the animal back to the cage) were completed within 3 min. After the injection, fecal samples were collected at the same time points as on the previous day (i.e., 3, 5, 7, 9, 11, 15 h after injection). Next day samples were collected at 1000, 1400, and 1600 h (i.e., 27, 31 and 33 h after injection).

2.4. Levels of fecal GCM in family groups

Five family groups from each population (37 and 31 individuals from the non-commensal and the commensal population,

respectively) were studied. Adults were aged 7–11 months and subadults 60–90 days (i.e., sexually immature). Animals were marked by painting small spots on the dorsal pelage for individual identification. At the beginning of the experiment, a family group was transferred from the breeding cage to the experimental one. All four metallic doors were left open to allow free movements of the animals throughout the compartments. After an initial adaptation period (7 days) the sampling procedure began. An animal voluntarily entering an empty (no other conspecifics) lateral compartment was locked and thereby separated inside and left for 4 h to collect fecal samples. Then the metallic door was unlocked and the animal could rejoin the group. Each individual was sampled repeatedly, three times in the morning (from 0800 to 1200 h) to assess concentrations of fecal GCM, reflecting circulating cortisol levels at the end of the respective night, and three times in the afternoon (from 1800 to 2200 h) to assess GCM levels corresponding to afternoon cortisol levels.

2.5. Measurement of fecal glucocorticoid metabolites (GCM)

The samples were frozen immediately after collection and stored at −20 °C until analysis. Each sample was well homogenized with mortar and pestle, a portion of 0.05 g was weighed into an Eppendorf cup (1.5 ml) and 1 ml of 80% methanol was added as described by Touma et al. [38]. Samples were shaken on a multivortex for 15 min and centrifuged (11,500 ×g) for 2 min (Eppendorf Microcentrifuge 5415 C). The supernatant (800 µl) was transferred to new titre tubes. Aliquots of the supernatant were diluted with assay buffer (1:10), transferred to new titre tubes and frozen at −20 °C until analysis. For the determination of the amounts of fecal GCM we used two already established group-specific enzyme immunoassays (EIA). The first one was a 5α-pregnane-3β,11β,21-triol-20-one EIA (further 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-reactions of different steroids, see [37,38]). Due to high concentrations of fecal GCM, a 1:100 dilution has to be used. The other assay was an 11-oxo-etiocholanolone EIA (further referred to as EIA2) for GCM with a 5β-3α-hydroxy-11-one structure (first developed for ruminants by Möstl et al. [39]). The intra- and interassay coefficients of variation for EIA1 were 9.1 and 14.0% and for EIA2 10.2 and 12.5%, respectively. The results of both EIAs were correlated (see under Results), but evaluated separately, because the EIAs detect different groups of fecal GCM.

2.6. Statistical analysis

In the case of the ACTH challenge test, GCM levels were evaluated by non-parametric statistics: Mann–Whitney *U*-test (further referred to as MW) and Wilcoxon matched paired test. The a priori prediction that ACTH administration and/or injection causes increase in GCM levels during the first experimental day (3–15 h after the injection) allowed us to use one-tailed tests in comparisons concerning this period. To test temporal dynamics, we computed Friedman ANOVA by ranks and Kendall coefficients of concordance from the dataset in which missing values (e.g., not enough fecal pellets obtained) were substituted by the mean values computed for the given animal and part of the experiment (before versus after the injection procedure).

The data concerning fecal GCM levels in family groups were naturally log-transformed to achieve normality prior to further analyses (Kolmogorov–Smirnov tests: all $P>0.05$). First, we applied the General Linear Models (GLM) procedure in which concentration of fecal GCM was given as a dependent variable, Animal identity as a random factor and Time of sample collection as a fixed factor. As homogeneity of variance was violated in the models including both the commensal and non-commensal populations (Bartlett test: EIA1: $P<0.0001$; EIA2: $P=0.0032$), further analyses were performed for each population separately. The procedure confirmed significance of between-individual variation in fecal GCM levels ($P<0.001$ for each combination of EIA and population). Surprisingly, in the case of EIA1 in the non-commensal population, the effect of Time of sample collection was also highly significant ($F_{1,13}=40.3$, $P<0.0001$).

To characterize fecal GCM levels in each particular animal, we pooled the morning and afternoon data (allowed due to the balanced experimental design: each animal was sampled three times in the morning and three times in the afternoon), and computed both means and medians (which are robust with respect to outliers) of the log-transformed values. In both EIAs, means and medians were highly correlated ($r=0.975$ and 0.987 for EIA1 and EIA2, respectively). Consequently, the means were used instead of the original data for further analyses. They exhibited a normal distribution (Shapiro–Wilk $W=0.98$ and 0.97, $P=0.6259$ and 0.0702 for EIA1 and EIA2, respectively). Thus they were further treated by parametric GLM performed separately for the commensal and non-commensal populations. In these models, Sex and Age (adult versus subadult) were used as fixed factors, while Family group as a random factor.

As the identity of the family group and sex considerably contributed to the fecal GCM levels (see under the Results), the family means computed for animals of the same sex were used when the studied populations were compared. Such comparisons were carried out by *t*-tests. Statistica 6.0 (StatSoft, Tulsa, OK, USA) was used for all statistical analyses.

3. Results

3.1. ACTH challenge test

During the day preceding ACTH or saline injection we analyzed the effect of time of sampling on fecal GCM levels by Friedman ANOVA on Ranks ($N=20$, $df=5$). The results revealed a significant effect only in the case of values obtained by EIA1 ($\chi^2=12.29$, $P=0.0309$, Kendall coefficient of concordance=0.1229; EIA2: $\chi^2=10.24$, $P=0.0687$, Concordance=0.1079).

In the period following ACTH injection, fecal GCM levels measured by both EIAs exhibited almost the same dynamics, involving an apparent peak 5–7 h after administration (i.e., between 1200–1400 h) followed by a gradual decrease that continued next day into a compensatory drop below baseline values (see Figs. 1 and 2). Consequently, Kendall coefficients of concordance increased sharply to 0.3766 and 0.4095 (for EIA1 and EIA2, respectively) in ACTH injected group, but only moderately in saline injected group (EIA1: 0.2302, EIA2: 0.1345).

In both EIAs, fecal GCM values at the time of the peak were significantly higher in the ACTH treated group than in the saline injected one (MW test, EIA1: $z=-1.89$, $P=0.0288$, EIA2: $z=-1.79$, $P=0.0362$). These values were also significantly higher, when compared with control values collected at the same time interval during the previous day (Wilcoxon test, EIA1: $z=2.31$, $P=0.0104$, EIA2: $z=1.68$, $P=0.0464$). In the case of EIA1, also the time interval 3–5 h after ACTH injection (i.e., between 1000 and 1200 h) was significantly different ($z=1.75$, $P=0.0398$), and that following peak (7–9 h after injection, i.e., 1400–1600 h) approached significance ($z=1.52$, $P=0.0640$). Also the comparison concerning 11–15 h after injection (1800–2200 h) was formally significant ($z=2.36$, $P=0.0089$), although this difference is not obvious from mean plot. In contrast to the ACTH injected group, no such comparisons were significant in the saline injected group.

A drop of fecal GCM levels below the baseline was found during the second day following ACTH injection. When compared with values assessed during corresponding time intervals in the day before injection, two-tailed Wilcoxon test revealed significant differences ($P<0.05$) for the interval 31–33 h after injection (i.e., 1400–1600 h) in both EIAs.

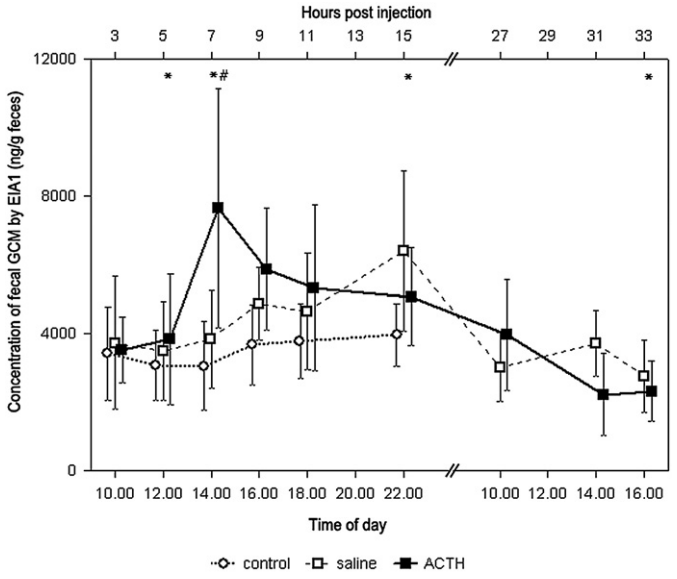

Fig. 1. Mean and 90% confidence intervals for concentrations of fecal GCM (ng/g) measured by EIA1 during the ACTH challenge test. ACTH ($n=10$; solid line with filled squares) or saline ($n=10$; broken line with open squares) was injected intraperitoneally at 0630 h. Control values (dashed line with open circles) were obtained at the same time from the same animals ($n=20$) during the previous day (* indicates significance levels between ACTH and control group, values 27–33 h after the injection were compared with those assessed during corresponding time intervals in the day before; # indicates significance levels between saline and control group; all $P<0.05$).

3.2. Effects of social factors

Mean fecal GCM levels split according to population, sex and age are provided in the Table 1. In the non-commensal population, there was a significant effect of Family group on fecal GCM levels in EIA2 only (EIA1: $F_{4,29}=0.95$, $P=0.4514$; EIA2: $F_{4,29}=4.3$, $P=0.0073$). No effects

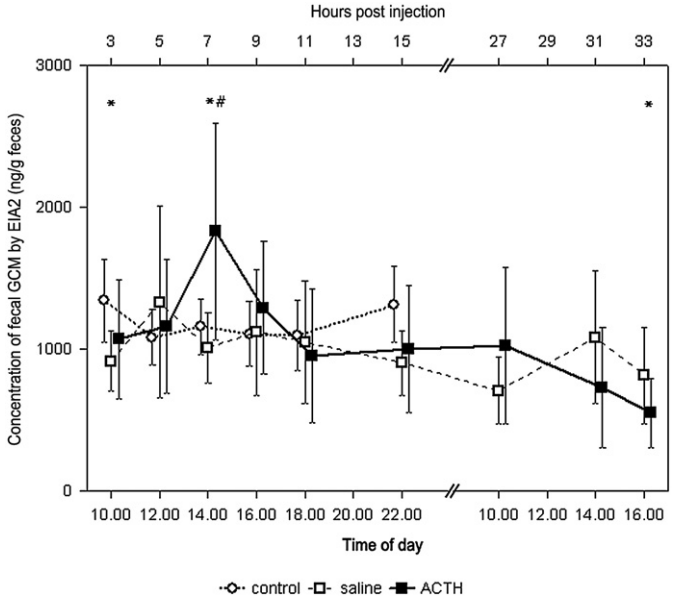

Fig. 2. Mean and 90% confidence intervals for concentrations of fecal GCM (ng/g) measured by EIA2 during the ACTH challenge test. ACTH ($n=10$; solid line with filled squares) or saline ($n=10$; broken line with open squares) was injected intraperitoneally at 0630 h. Control values (dashed line with open circles) were obtained at the same time from the same animals ($n=20$) during the previous day (* indicates significance levels between ACTH and control group, values 27–33 h after the injection were compared with those assessed during corresponding time intervals in the day before; # indicates significance levels between saline and control group; all $P<0.05$).

Table 1
Fecal GCM levels (ng/g) assessed by EIA1 and EIA2 (mean and range are given)

	Commensal population		Non-commensal population	
	EIA1 (ng/g)	EIA2 (ng/g)	EIA1 (ng/g)	EIA2 (ng/g)
Adult females	5303 (3177–8983)	1311 (636–2421)	2989 (1287–6610)	434 (261–563)
Subadult females	5281 (3351–10601)	1331 (1032–2175)	2383 (1496–3874)	461 (280–818)
Adult males	4515 (2413–8582)	1158 (724–2009)	2995 (2400–4029)	420 (267–625)
Subadult males	3842 (1928–7547)	1015 (661–1322)	2503 (1462–4603)	403 (233–684)

of Sex (EIA1: $F_{1,29}=0.19$, $P=0.6637$; EIA2: $F_{1,29}=0.19$, $P=0.6592$), Age (EIA1: $F_{1,29}=2.84$, $P=0.1026$; EIA2: $F_{1,29}=0.11$, $P=0.7463$) and its interaction (EIA1: $F_{1,29}=0.01$, $P=0.9137$; EIA2: $F_{1,29}=0.38$, $P=0.5438$) on GCM levels were found.

In the commensal population, we also found a considerable effect of Family group (EIA1: $F_{4,23}=10.12$, $P<0.0001$; EIA2: $F_{4,23}=5.75$, $P=0.0023$). Moreover, there was significant effect of Sex in both EIAs (EIA1: $F_{1,23}=6.97$, $P=0.0146$; EIA2: $F_{1,23}=6.75$, $P=0.0161$), males exhibiting lower values of fecal GCM than females (for means see below). Similarly as in the non-commensal population, no effects of Age (EIA1: $F_{1,23}=1.24$, $P=0.3765$; EIA2: $F_{1,23}=0.23$, $P=0.6345$) and Sex–Age interaction (EIA1: $F_{1,23}=1.64$, $P=0.2135$; EIA2: $F_{1,23}=2.45$, $P=0.1316$) were recorded.

Fecal GCM levels assessed by EIA1 (mean=3451 ng/g, range 1288–10601 ng/g, $n=68$ animals) were markedly higher than those assessed by EIA2 (mean=688 ng/g, range 233–2421 ng/g). Nevertheless, values measured by these assays were correlated $r=0.82$ ($r^2=0.67$; $P<0.0001$), when non-commensal and commensal population were treated separately, r was 0.60 and 0.79, respectively.

3.3. Comparison of studied populations

Fecal GCM levels assessed by both EIAs were apparently higher in commensal than in non-commensal population (Fig. 3). In females, two-tailed t-tests applied on family means revealed highly significant difference between populations in both EIAs (EIA1: 2594 versus

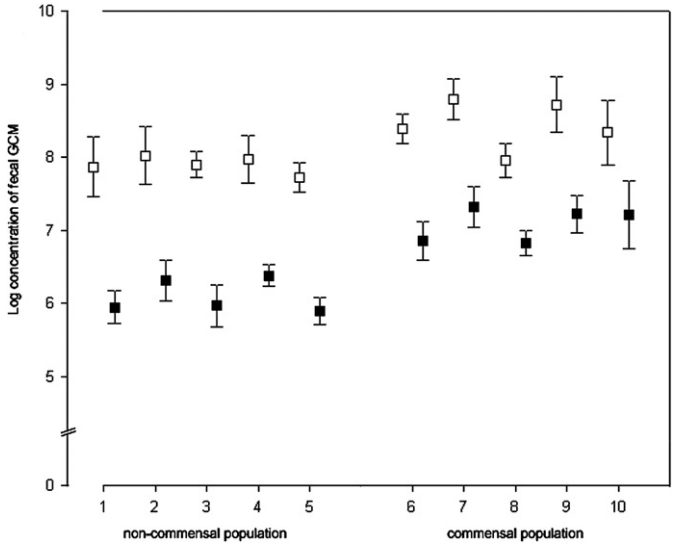

Fig. 3. Family means and 95% confidence intervals of log-transformed levels of GCM in the feces (ng/g) assessed by EIA1 (open squares) and EIA2 (filled squares). Details of the EIAs are given in the Materials and methods section. Family groups 1–5 correspond to the non-commensal population, while 6–10 to the commensal population of Egyptian spiny mouse.

5268 ng/g, $t=-4.389$, $P=0.0023$; EIA2: 442 versus 1340 ng/g, $t=-5.935$, $P=0.0003$). In males, corresponding differences were highly significant in EIA2 (427 versus 1031 ng/g, $t=-5.348$, $P=0.0007$), but not in case of EIA1 (2695 versus 3865 ng/g, $t=-1.912$, $P=0.0923$).

4. Discussion

The results of the performed ACTH tests demonstrated that both EIAs are suited for monitoring of fecal GCM levels in spiny mice. Such a successful validation is an obligatory prerequisite for any further application of non-invasive methods for measuring GC metabolites in the feces of any previously unstudied species [40,41]. Moreover, we utilized a special apparatus allowing repeated collection of fecal samples from individuals housed in a social group. Thus our sampling method was not only non-invasive, in accordance with the general trend in studies on small rodents, but also allowed to minimize the disturbance associated with sample collection.

Results of both group-specific EIAs were mutually correlated, and there is no use suggesting which EIA would be the method of choice. EIA1 was developed for fecal corticosterone metabolites in mice where corticosterone is the dominant GC. Nevertheless, the only difference between corticosterone and cortisol is an additional 17α-hydroxy group present in the latter. The group-specific EIA1 [38] detects steroids with a 5α-3β,11β-diol structure, which can be derived from both, corticosterone and cortisol. As groups of fecal GCM detected by the used EIAs are not identical, the observed nearly five-fold differences in assessed concentrations are neither much surprising nor indicative of the quality of the assays.

Our experiments with spiny mice further provided good evidence that this species is a really suitable experimental model for evaluating the influence of social factors on the stress response. Spiny mice spend the most time in body contact and social interactions are frequent. Fecal sample collection is further facilitated by the fact that as desert rock dwellers and good climbers they easily accept a grid floor, which resembles the coarse surface of desert stones. Moreover, they do not build nests from soft material and accept artificial shelters (e.g., flowerpots) as nest cavities.

In spiny mice, GC levels were measured in several studies; however, there is no agreement among the authors as to baseline values. The reported mean baseline levels of cortisol in circulating blood are fairly high when compared with other rodents and vary greatly from 40 to 830 ng/ml [35,42–44]. This range approaches the values reported in highly social monogamous voles (*Microtus ochrogaster*) that are GC resistant [45,46]. Nevertheless, as collection of blood itself is stressful, it can easily elevate cortisol levels within a few minutes and thus the values reported in spiny mice may not always represent a real baseline level. On the other hand, there are experiments demonstrating that spiny mice respond well by elevated circulating cortisol when exposed to stressful stimuli such as owl calls [43] or intruders [42].

The time lag between the increase of GC in circulating blood and its reflection in GCM levels depends on the gut transit time in the studied species [40,47,48]. As wild spiny mice consume a high proportion of invertebrate food items [49], we expected this time lag to be somewhat shorter than that in the house mice. Accordingly, we detected peak concentrations of fecal GCM 5 to 7 h after administration of ACTH, i.e., about 3 h earlier than in laboratory mice [37,38]. The detected peak was, however, less apparent than that reported in laboratory mice [37]. We are inclined to think that the ACTH dosage adopted from studies on laboratory mice should be increased. In spiny mice the peak was followed by a compensatory decrease in fecal GCM levels the next day (Figs. 1 and 2). If such a compensatory effect also occurs after responses to natural stimuli, mean GCM levels may be a bit closer to baseline than expected even in animals affected by temporary stress. The injection of saline itself had no effect on fecal GCM levels, thus probably only mild stress induced by injection was not successfully detected by our EIAs. This may be attributed to smooth temporal dynamics of GCM in fecal samples reducing sensitivity to short stress bouts.

Unexplained individual variation in fecal GCM levels in our study seems to be high; nevertheless, as reported in the recent review [50], variation of such a magnitude is fairly common in endocrinological studies and may be viewed as a typical rather than suspect phenomenon.

Laboratory mice and rats exhibit considerable sex differences in levels of fecal GCM. As demonstrated by radiometabolism studies, this phenomenon is mainly attributed to differences in the metabolism and/or excretion of GCs [38,51]. Nevertheless, distinct sexual differences are also reported in plasma GC levels of these rodent species [52,53]. These differences are consistent with the polygynous/promiscuous social system with clearly distinct gender roles. In spiny mice we found no sex differences in fecal GCM of the magnitude comparable to that reported in laboratory mice and rats. Sexual differences in circulating cortisol levels reported in spiny mice also lack consistency: Penefsky and Diamond [44] detected higher baseline levels in males than in females, while Dickinson et al. [54] observed the inverse relationship. Similar values in fecal corticosterone metabolites in males and females were also reported in Mongolian gerbils [19]. We speculate that the absence of sex differences in GC levels could result from undifferentiated social roles of the sexes (but see the sex difference in GC levels in socially monogamous prairie voles [55]). Mongolian gerbils are social animals with tight monogamous pair bonds and almost equal gender roles [56]. Although spiny mice are not monogamous, they also exhibit male parental care [30]. It should be mentioned here that according to current molecular phylogenies, gerbils (and not murids as true rats and house mice belonging to the subfamily Murinae) are the close relatives of spiny mice [57].

Contrary to our expectations we found no significant differences between fecal GCM levels in adults and subadults, although adults were apparently socially dominant. It is especially remarkable that subadult males had not elevated fecal GCM levels. Later on they are subjected to serious attacks of the dominant male leading even to their eviction from the family group. One would expect that subadult males suffer from social stress even in the period preceding the beginning of apparent violence. In accordance with our results, no differences were found between fecal cortisol levels in juvenile and adult Mongolian gerbils [58] and in GC levels between dominant and subordinate male guinea pigs [59].

In some species, not subordinates but rather adult family founders are victims of social tension. In Mongolian gerbils, GC levels of adults are elevated during the aggressive period when adults attempt to expel a subadult family member [19]. On the other hand, GC levels in territorial male marmots correlates negatively with the number of subadult sons present in the group (*Marmota marmota* [14]).

Surprisingly, fecal GCM levels in the commensal and partially (when assessed by EIA2) also in the non-commensal populations of Egyptian spiny mice varied significantly among family groups. It suggests that the production of GC is probably controlled by the particular social settings of each group affecting all family members. This interpretation is a challenge for further research attempting to explain this phenomenon by physiological and social factors. On the other hand, there is an alternative explanation attributing variation in fecal GCM between family groups to genetic factors as high heritability of HPA axis activity/reactivity has been repeatedly reported in diverse vertebrate taxa [e.g., 60–62]. Sharp differences between the studied populations discussed below support high heritability of fecal GCM levels. Nevertheless, genetic variation within our colonies was unintentionally but unavoidably reduced by small effective population size and our protocol followed strict outbreeding in formation of breeding units. Taken together, these facts favor the social settings over genetic factors as the more likely explanation for variation in fecal GCM between family groups belonging to the same population.

Moreover, the non-commensal population that was less affected by genetic bottleneck in the laboratory exhibited less variation between the family groups.

Behavioral and physiological differences between the commensal and non-commensal populations were extensively studied in house mouse [63–66]. In a striking parallel to our results, commensal house mice were reported to have higher levels of plasma corticosterone than their non-commensal conspecifics [65]. Adrenocortical response to novelty was, however, higher in non-commensal house mice [66]. Higher fecal GCM levels in commensal Egyptian spiny mice (Fig. 3) correspond well with the behavioral profile of the animals from this population. These spiny mice exhibit extraordinary long latencies to enter novel space in free-exploration tests (Frynta and Benkovičová, unpublished results), suggesting that they are much more anxious than their non-commensal conspecifics. It may be interpreted as a behavioral adaptation to putatively increased predation pressure inside the buildings (presence of cats, aggregated distribution of both resources and mice, high densities) than in the desert habitats where low rodent densities make predation unprofitable and poor environmental conditions are the main source of pressure. As life inside the buildings and stores instead of natural habitats was adopted by commensal populations only a few thousands years ago when permanent agricultural settlement was established in the Near East region, peculiarities of commensals may serve as an evidence for rapid evolutionary change of behavioral and physiological traits.

Interestingly enough, differences comparable to those between commensal and non-commensal spiny-mice have been reported between lines of laboratory mice and rats that underwent artificial selection for such behavioral traits as attack latency, anxiety or tameness [67–69]. Also in this case, apparent differences in behavioral and endocrine traits may be acquired after a few generations of selection.

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