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Validation of a Method for Measuring the Fecal Glucocorticoid Metabolites in the Midday Gerbil (*Meriones meridianus* Pallas 1773, Muridae, Rodentia): Biological and Physiological Approaches

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Abstract—Estimating the level of stress in wild populations opens opportunities for studying various populational processes, including population dynamics, range expansions, and colonization. Over the past two decades, methods for a noninvasive assay of the glucocorticoid levels have been actively developed and widely implemented in various biological fields. They are most suitable for assessing long-term stress, but require a validation procedure for each new animal species studied. We have conducted biological and physiological validation of a noninvasive method for determination of fecal glucocorticoid metabolites (FGMs) in the midday gerbil, *Meriones meridianus*. Both methods showed a significant increase in the concentration of FGMs after a similar time after manipulations (4–5 hours after stressful procedures or the administration of ACTH), and peak values were two times the baseline. Saline injection (control group) did not cause a significant increase in the FGM concentration, thus confirming that the noninvasive measuring of a smoothed level of FGMs is more suitable for assessing the long-term stress, in contrast to the assay of glucocorticoids in the blood. The biological validation was as effective as the physiological method, which is commonly considered more reliable. Given the low invasiveness of the biological method, the high effectiveness expands the potential for its application.

Keywords: rodent, physiology, stress, noninvasive study

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INTRODUCTION

In the modern world, wild populations are subject to many external influences, mainly anthropogenic ones. Studying the response of organisms to external stressors can provide insight into the sensitivity of populations to changes in the external environment. For example, it has been shown that anthropogenic disturbance of habitats can lead to increased stress (Kuznetsov et al., 2004; Josserand et al., 2017). Stress is considered one of the important factors in population regulation, since a positive relationship between the level of stress hormones and density is noted (Christian, 1950; Shilov, 1984; Boonstra and Boag, 1992; Novikov and Moshkin, 1998; Shang et al., 2022), although the data are contradictory on this point (Creel et al., 2013). Finally, stress reactivity can determine the proactive or reactive life strategy of individuals and, accordingly, the tendency to disperse (Koolhaas et al., 1999; Cote et al., 2010), thereby influencing the processes of colonization of new territories, range expansion, and invasions (Clobert et al., 2009; Chuang and Peterson, 2016).

External factors (stressors) activate a complex of physiological reactions that increase adaptation and, ultimately, the survival of individuals in new conditions (Selye, 1960). A central place in the regulation of physiological processes in animals in response to stress is occupied by the hypothalamic–pituitary–adrenal axis (HPA). Glucocorticoids (GCs) are one of the main hormones produced by this system in response to stress; therefore, the blood level of GCs (mainly cortisol or corticosterone, depending on the species of animal) is often used to assess the stress of organisms (Möstl and Palme, 2002). However, in the past two decades, non-invasive methods for determining GC levels have become increasingly widespread (Palme et al., 2005; Palme, 2019). GCs circulate unchanged in the blood, while excretory products contain mainly their metabolites, and the level of stress can be determined by the concentration of these metabolites. This is most often done by collecting fecal samples, which, compared to taking blood samples, has a number of advantages when studying wild populations. Unlike taking blood, collecting feces does not stress the animal at all or causes little stress; this procedure does not require intervention in the body, which in itself is a strong stress factor, and most often does not require immobilization or even capture of the animal. Fecal

samples can be collected an unlimited number of times at any time and in any physiological state of the animal, whereas blood samples cannot be collected too frequently in small animals. In addition, feces provide a smoothed level of FGMs for a certain period of time prior to sampling—this level is less dependent on short-term stressful events and is better suited for assessing long-term effects (Touma and Palme, 2005).

To measure the serum and plasma concentration of GCs, many ready-made, relatively inexpensive, and easy-to-use enzyme-linked immunosorbent assay (ELISA) kits have been developed, but they cannot be used to determine the concentration of FGMs as an indicator of stress without a validation procedure (Gerlinskaya et al., 1993; Möstl and Palme, 2002; Touma and Palme, 2005; Palme, 2019), since feces practically do not contain the hormones themselves in their native form in feces, but only their metabolites (Stead et al., 2000; Chen et al., 2017; Palme, 2019). Cross-reactivity of ELISA kits for native GCs in blood to their metabolites has been noted repeatedly, but each kit requires validation for each new species (Abelson et al., 2016).

The leading role in deciding whether to use a particular set of ELISA reagents for noninvasive assessment of the stress level based on FGM concentrations is played by biological and physiological validation (Palme, 2019). Analytical validation (including the "parallelism test") (Kolosova et al., 2008) is used additionally to assess the accuracy, sensitivity, and specificity of the analysis. There are many cases in which analytical validation has passed, but biological or physiological validation has failed to show a stress response (e.g., Fanson et al., 2017).

Noninvasive assessment of stress is usually validated using physiological and less often biological approaches (Touma and Palme, 2005; Palme et al., 2005; Palme, 2019), each of which has its own advantages and disadvantages. The biological approach is aimed at studying the dynamics of FGMs after stressful effects on the animal often associated with capture, immobilization, transportation, and other manipulations that lead to changes in the activity of the HPAS (Goymann et al., 1999; Touma and Palme, 2005; Palme, 2019). For example, in the Amur Leopard, transportation caused an increase in FGM, which indicated the validity of a non-invasive assessment of stress (Ivanov et al., 2014). Biological validation is relatively simple (samples for subsequent analysis can be collected in the field) and is less invasive. However, successful biological validation shows that a non-invasive method for determining stress levels can detect biologically significant changes in the activity of the HPA (Palme, 2019). However, the biological method cannot be standardized because it is difficult, if not impossible, to standardize the level of stress exposure.

The physiological approach to validation is considered more reliable, but more complex (Touma and Palme, 2005). Unlike the biological one, the physiological approach involves the creation of standard laboratory conditions and mandatory intervention in the animal's body. One of the most accurate and widespread physiological methods is to test animals by the administration of adrenocorticotropic hormone (ACTH)—a pituitary hormone (Goymann et al., 1999; Touma and Palme, 2005; Pavlova and Naidenko, 2008; Palme, 2019). ACTH travels through the bloodstream to the adrenal cortex, where it stimulates the production of GCs, which are then metabolized and enter feces as metabolites. If, after injection, the validated ELISA kit detects an increase in FGM levels in fecal samples, then the validation can be considered successful and the kit can be used to assess stress noninvasively in a specific species. Thus, both approaches are important for validating a noninvasive method for determining stress levels (Palme, 2019), and a comparative analysis of their results represents an independent scientific task, allowing their effectiveness and applicability to be evaluated in practice (see, for example, Ivanov et al., 2014).

The midday derbil is a keystone species of rodents in the pasture ecosystems of southern Kalmykia. The use of this species as a model object in wild populations makes it possible to determine the degree of influence of external factors, including anthropogenic transformation of habitats, on the population and on the ecosystem as a whole (Kuznetsov et al., 2004; Surkova et al., 2019; Tchabovsky et al., 2016, 2019). Due to an increase in pasture load and the onset of a new desertification cycle, the midday gerbil population in Kalmykia is currently expanding its range, opening up opportunities for studying the dispersal syndrome (Surkova et al., 2022; Tchabovsky et al., 2023) and, in particular, the stress among colonists. Validation of a noninvasive method for determining stress based on FGMs will open the possibility of studying this issue. The goal of our study is to validate a method for noninvasive assessment of stress in the midday gerbil (*Meriones meridianus*) and compare the effectiveness of the biological and physiological approaches.

MATERIALS AND METHODS

Biological Validation

Biological validation was carried out under field conditions in Kalmykia (from April 29 to May 3). Capturing was carried out according to a standard procedure using mesh live traps designed by Shchipanov (1987); sunflower seeds were used as bait (for details, see, for example, Tchabovsky et al., 2016). Traps were placed directly at the exit of a burrow at the beginning of activity, around 20:00, and were checked at least once every hour (so that the potentially stressful capture procedure would not have time to affect the FGM concentration in the collected samples). A hygro-

scopic white synthetic fabric was placed under the mesh bottom of the trap to absorb urine and protect feces from sand. Midday gerbils are seed-eating desert rodents and produce little urine, so the likelihood of fecal contamination with urine was extremely low; however, in rare cases of doubt, the feces were discarded.

To conduct the experiments, we used four adult animals (two females and two males). We collected the first fecal sample after an animal fell into a trap (on average, around 22:00) and considered the FGM concentration in this sample to be basic, reflecting the level of stress before capture and the start of stressful manipulations. The next sample was taken at 3:00, five hours after capture, during which the animal was subjected to stressful influences, including the capture itself, standard manipulations with the animal (determination of sex, age, body weight, marking, etc.), behavioral tests, taking DNA samples, and transportation to the field laboratory. Next, samples were collected every four hours (7:00, 11:00, 15:00, 19:00, 23:00, 3:00) for three days in order to assess not only the reaction to the stress factor, but also daily fluctuations in the FGM concentration. The animals were kept individually in mesh cages (30 × 15 × 15 cm); each cage stood on filter paper in a separate ventilated cardboard box under a canopy that provided protection from the sun, wind, and cold. Food (sunflower seeds, oats, carrots, and apples) was added after each sample collection, and the paper under the cage was also changed at the same time to avoid mixing feces and urine. All feces (without exception) were collected in paper bags, which were stored under the same conditions in a dry, ventilated place to prevent molding, at a temperature of 15–25°C until the end of the field season for 10–14 days. The territory of Kalmykia is characterized by an arid climate, so the samples did not require additional drying. Upon completion of biological validation, the animals were immediately released into their burrows. After transporting samples from the field to the laboratory, they were dried to constant weight at a temperature of 60°C for 12–16 h and stored in tightly closed tubes at –20°C until extraction (if extraction was not carried out immediately after drying).

Physiological Validation

Physiological validation was carried out in laboratory conditions. For the experiment, we used nine animals (five males and four females) captured in May in Kalmykia. Before the start of the experiment, the animals were acclimatized for two months in the vivarium. The gerbils lived alone in plastic cages with a mesh lid (50 × 30 × 20 cm); inside each cage there was a house for shelter and objects that the gerbil could gnaw (apple tree branches, cones without traces of resin). Grain food (a mixture of millet and oats) was constantly available in the cage, sunflower seeds and succulent food (carrots, apples, zucchini) were given twice a week. The gerbils were not given water, since in nature gerbils do not drink and obtain moisture from their food.

The validation procedure began at 15:00 with placing the gerbils in new cages made of metal mesh with cells of 1 cm (30 × 15 × 15 cm), and filter paper was put under the cage. The mesh structure of the cells simplified the collection of samples and the feeding procedure without unnecessary disturbance to the animals. In addition, since midday gerbils are nocturnal animals, we covered the cages with fabric to prevent light from causing stress. The first fecal sample was collected after four hours (at 19:00), and the level of FGM in this sample was considered baseline. Next, samples were collected every four hours throughout the day (23:00, 3:00, 7:00, 11:00, 15:00, 19:00). One day later, after collecting the sample at 15:00, all gerbils were given an injection of synthetic ACTH (Synacthen, Germany) at a dosage of 40 μg per 100 g of weight (average dosage for the ACTH test, Palme, 2019). Because the injection procedure itself could be stressful, we repeated the procedure described above for three gerbils, except that saline (control) was injected instead of ACTH. After injection, samples were collected for another two days at the same time intervals (23:00, 3:00, 7:00, 11:00, 15:00, 19:00). The samples were immediately dried to constant weight at a temperature of 60°C for 12–16 h and stored in tightly closed tubes at –20°C until extraction (if extraction was not carried out immediately after drying).

Extraction of Metabolites

Extraction of GC metabolites from feces was carried out according to the standard procedure with minor modifications: feces were crushed using a porcelain mortar and pounder, aliquots of crushed feces weighing 0.05 g were measured using a scale with an accuracy of 0.001 g on an Ohaus scale (Scout SPX 123) and transferred to a 1.5 mL microcentrifuge tube for further extraction. Next, 0.9 mL of 80% methanol (the most suitable concentration for the extraction of GC metabolites in mammals, Palme et al., 2013) was added to each tube and extraction was performed for 30 min in a rotary shaker (BioSan Bio RS-24). The extracts were then centrifuged for 10 min at 1500 g (Eppendorf, Centrifuge 5424), and 400 μL of the resulting supernatant was transferred into clean tubes. 400 μL of distilled water was added to the resulting extract and stored at –20°C until ELISA was carried out.

Enzyme Immunoassay

Enzyme immunoassay was carried out at the Living Collection of Wild Species of Mammals Center for Collective Use, Institute of Ecology and Evolution, Russian Academy of Sciences, using a Thermo Scientific Multiskan FC spectrophotometer. We used

ready-made XEMA commercial kits for the determination of cortisol in the blood serum (Moscow) in accordance with the instructions. Since we were working not with blood serum, but with methanol-based fecal extracts, we diluted the calibration samples from the kit with methanol. Each measurement of the FGM concentration in each sample was performed twice to determine the coefficient of variation, and the average value was taken for further analysis. If the coefficient of variation exceeded 10% for a particular sample, the analysis was redone until the value was less than 10%. In the vast majority of cases, this figure was less than 5% (132 of 150, 88%), averaging 2.1% (median = 1.9) and ranging from 0.1 to 9.8%.

Data Processing and Statistical Analysis

The biological test samples met normality criteria (Shapiro–Wilk, $p > 0.05$), but the physiological validation data only approximated a normal distribution and were highly variable, so we logarithmized them to achieve a normal distribution.

Not all gerbils excreted excrements within a four-hour interval during the ACTH test, especially during the daytime hours (midday gerbils are nocturnal rodents). Therefore, to make a statistical analysis of physiological validation data, we used samples taken at eight-hour intervals. In order to assess the change in the FGM concentration in feces after ACTH administration, we chose four consecutive measurements (intervals) for comparison: at 07:00–15:00 (the period immediately before the administration), 15:00–23:00 (after ACTH administration), 23:00–07:00 and 15:00–23:00 (the next day after ACTH administration). The interval 07:00–15:00 on the day after administration was excluded because not all gerbils excreted excrements during this period. Comparisons of the FGM concentrations across these four consecutive measurements were performed using Repeated Measures ANOVA.

To control for the possible effect of diurnal fluctuations in GC (Palme, 2019), we compared fecal FGM concentrations at the same time of day (15:00–23:00) one day before the administration, on the day of the administration, and one day after the ACTH administration (ANOVA for linked data). Comparisons of the FGM levels between ACTH-treated midday gerbils and saline-injected control animals were performed using the *t* test (Student's test).

RESULTS

Biological Validation

Stress exposure to midday gerbils as a result of capture, subsequent manipulations in the field, and transportation to the field laboratory (period from 22:00 to 03:00) caused a significant increase in the level of FGMs in samples taken five hours after the start of manipulations and an increase to peak values in samples taken after nine hours. The peak was followed by a decline and return to baseline values (about 350 ng/g) one day after the start of the test (Fig. 1). The level of FGMs varied significantly during the first day of the experiment (from control samples taken around 22:00 before the start of manipulations until 23:00 the next day; ANOVA for related data: $F_{3.15} = 9.1$, $p = 0.0004$), and its values in samples taken five and nine hours after stress exposure significantly exceeded the baseline level (1.6 and 1.8 times, respectively; Fig. 1).

Physiological Validation (ACTH Test)

The FGM concentration changed noticeably over the course of three days of the experiment (Fig. 2a). On the first day (before ACTH administration), the FGM concentration varied slightly, remaining approximately at the same level—about 400 ng/g. After ACTH administration, there was a sharp increase in the concentration (approximately twofold) with two peaks observed in samples taken after four hours (at 19:00) and 16 hours (at 07:00 the next day) and a slight decline between them. The FGM concentration returned to the baseline level only one day after the administration.

Analysis of variance for related measures (ANOVA) for eight-hour intervals showed that the FGM concentration varied significantly throughout the day after administration (between the first interval, 07:00–15:00, before ACTH administration, and the last interval, 15:00–23:00, one day after the administration; $F_{3.24} = 5.1$, $p = 0.007$, Fig. 2b). Moreover, the FGM concentrations in the intervals 15:00–23:00 and 23:00–07:00 after the administration (after 4–8 and 12–16 hours, respectively) were higher than the FGM concentration before the administration (07:00–15:00, Tukey's test, the differences are close to significant: $p = 0.06$ in both cases, Fig. 2b) and one day after the administration (15:00–23:00 the next day, the differences are significant: $p = 0.04$ in both cases), but did not differ from each other ($p = 1.0$, Fig. 2b).

Comparison of concentrations at the same time of day (15:00–23:00) the day before ACTH administration, on the day of the administration, and one day after the administration showed significant differences (ANOVA for linked data: $F_{2.16} = 5.4$, $p = 0.02$). The FGM concentration after ACTH administration was significantly higher than at the same time of day the day before the administration and one day after the administration (Tukey's test: $p = 0.04$ and 0.02, respectively). No differences were found in the concentrations the day before the administration and one day after the administration (Tukey's test: $p = 0.9$). Thus, the increase in the FGM concentration after ACTH administration (Fig. 2) is obviously not associated with diurnal fluctuations in the hormone levels.

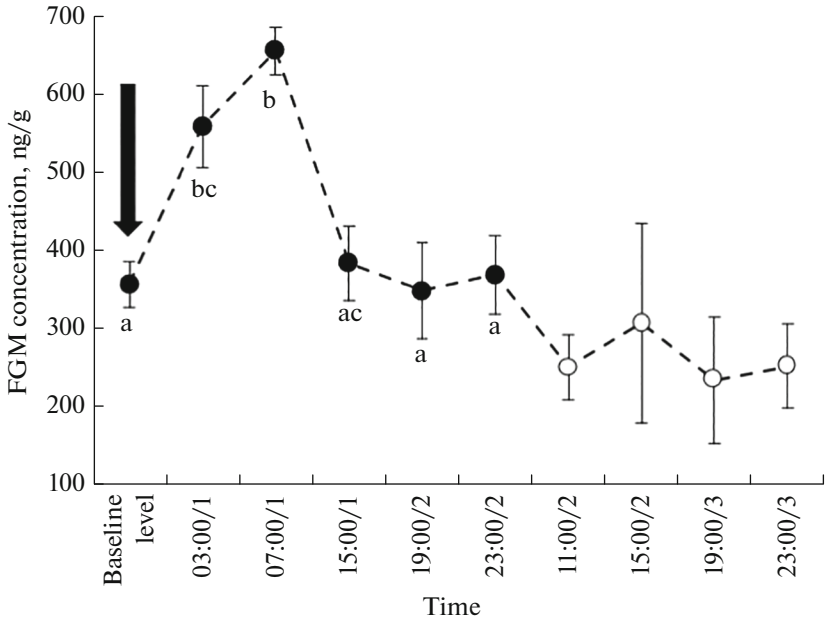

Fig. 1. Dynamics of the FGM level (mean ± error of the mean) after stress exposure in midday gerbils in the field. Black circles indicate samples for the first 24 hours after stress exposure (down arrow) used in analysis of variance (ANOVA for linked data). Identical Latin letters indicate that there are no significant differences ($p > 0.05$, Tukey's test). Baseline is samples taken around 22:00 before the start of midday gerbil manipulation. The numbers after the time intervals show the serial number of the day of the experiment.

In control midday gerbils that received an injection of saline instead of ACTH, no noticeable fluctuations in the FGM level were detected over three days. The FGM concentration in the midday gerbils that received an ACTH injection was significantly higher in the interval from 15:00 to 23:00 after injection than in control animals (Student's t test: $t = 2.8$, $p = 0.03$, Fig. 3). Judging by the nontransformed data, the FGM level in the midday gerbils from the control group was more than two times lower compared to the same indicator in the midday gerbils from the experimental group (329.6 and 810.7 ng/g, respectively). Thus, the increase in the FGM concentration after ACTH administration is not associated with possible stress as a result of manipulations with the animal. In general, we can conclude that the increase in FGM levels adequately reflects the increase in GC in the blood of midday gerbils in response to the action of ACTH; i.e., it serves as a reliable indicator of stress.

DISCUSSION

Although analytical characterization of the pool of glucocorticoid metabolites in midday gerbil feces has not been performed (Palme, 2019), the biological and physiological validation methods have demonstrated the suitability of commercial serum cortisol kits for measuring FGM levels and provided biologically relevant information regarding the HPA activity in midday gerbils. Both methods showed similar results, and the effectiveness of biological validation was no worse than that of the physiological method, which is generally considered more reliable. Considering the low invasiveness of the biological method, this expands the potential for its application.

The FGM level during the day preceding the ACTH administration (about 400 ng/g, Fig. 2a) was relatively stable and comparable to the FGM concentration in the wild population (about 350 ng/g, Fig. 1). This suggests that the FGM concentrations before the administration reflect well the baseline stress levels and that manipulations with midday gerbils prior to the ACTH test did not produce a significant stressor effect. Moreover, we found no effect of saline injection on fecal FGM levels. The injection itself may be a stressor, increasing FGM levels, making it difficult to interpret results from physiological validation of noninvasive stress assessments (Palme, 2019; Navarro-Castilla et al., 2021). The lack of response to injection as such in our experiments confirms the fact that short-term stress does not introduce significant changes in the FGM level, which thus reflects a smoothed, baseline GC level, i.e., stress level (Touma and Palme, 2005). This increases the validity of the results of our ACTH test for the midday gerbil, thereby allowing the long-term stress level to be assessed in wild populations, which is very important for this type of research (Touma and Palme, 2005; Palme, 2019).

The jump in the FGM concentration in both tests occurred (taking into account the sensitivity of the experiments) after approximately the same time: after

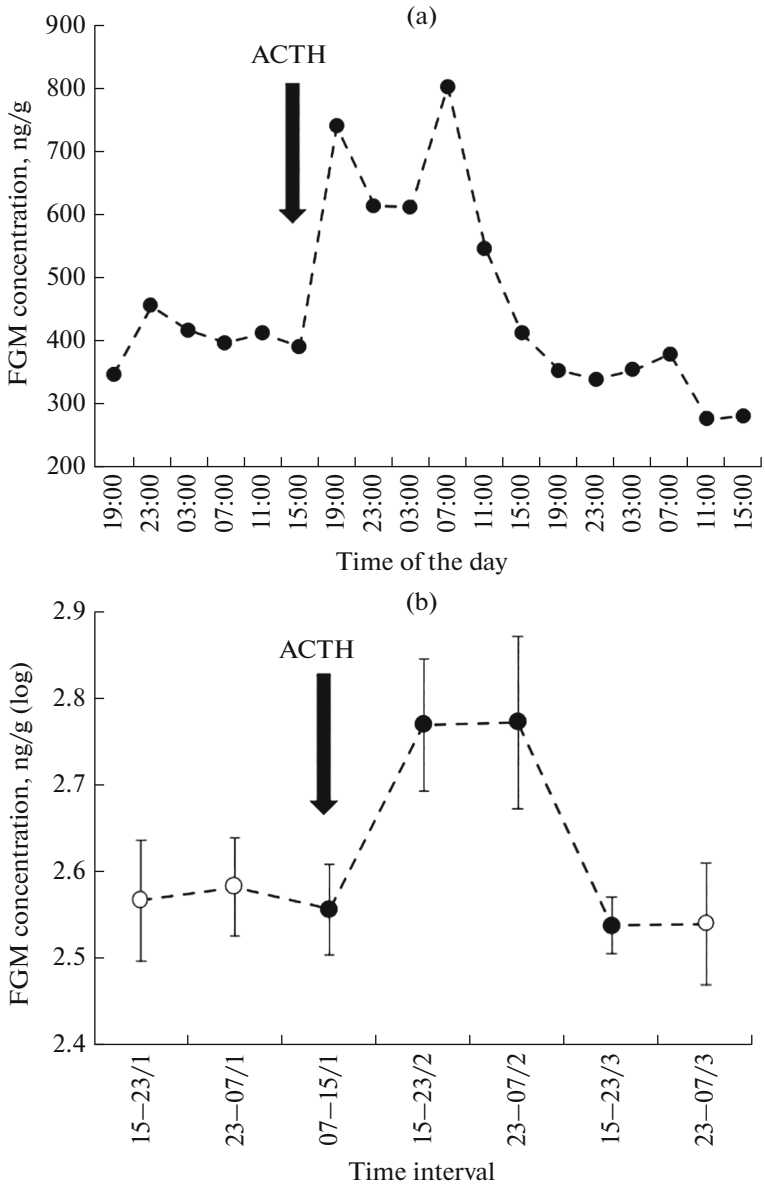

Fig. 2. Dynamics of the FGM concentration in the feces of midday gerbils in the ACTH test: (a) initial, not transformed average values for each four-hour interval for three days; (b) logarithmized average values (±error of the mean) for eight-hour intervals (periods in which samples were not obtained for all animals within an eight-hour interval are excluded: from 11:00 to 19:00 on the second and third days of the experiment). Black circles indicate intervals used in the analysis of variance (ANOVA) to compare FGM concentrations before and after ACTH administration. The numbers after the time intervals show the serial number of the day of the experiment.

five hours in the biological test and after four hours in the physiological test. This indicates that the FGM concentration in feces similarly reflects the rate of response of the HPA to both stressful manipulations and the ACTH administration, which directly stimulates the release of GCs into the blood.

Peak values were approximately twice the initial value in both tests, which corresponds to a 2- to 3.5-fold increase in the FGM concentrations recognized as valid for the ACTH test in other gerbil species (St. Juliana et al., 2014, 2019; Navarro-Castilla et al., 2021). Differences in the dynamics of the FGM concentrations between biological and physiological tests are manifested in the time parameters of peak values: after manipulations with midday gerbils in the field, the peak occurred later (after nine hours) and was one, while the ACTH test caused two peaks: after four and 16 hours. The two peaks in the ACTH test reflect inter-individual variability in the response rate: in other gerbils reaching the peak varied within the same species from six to 24 hours (*Gerbillus gerbillus*) (Navarro-Castilla et al., 2021), and *in G. andersoni* it

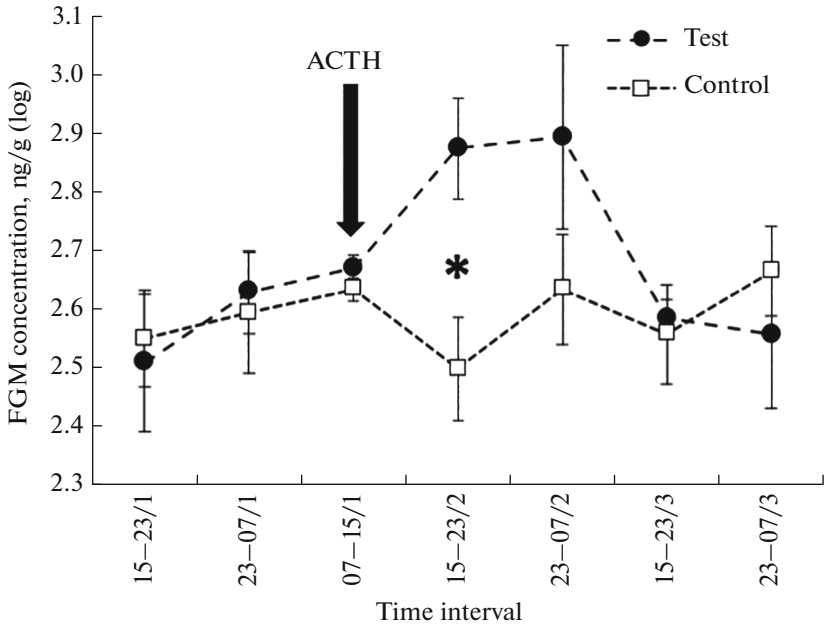

Fig. 3. Dynamics of the FGM concentration (mean ± error of the mean, logarithmized data) in control midday gerbils (saline injection) and experimental midday gerbils (ACTH injection). An asterisk indicates significant differences (Student's *t* test). The numbers after the time intervals show the serial number of the day of the experiment.

varied from six up to nine hours (St. Juliana et al., 2014, 2019). The return to the baseline level occurred in midday gerbils within 24 hours in both tests, whereas it occurred only after three days in gerbils of the genus *Gerbillus*. Thus, midday gerbils, in comparison with gerbils of the genus *Gerbillus*, demonstrate similarly strong, but faster dynamics of both the speed of response to the stressor and the speed of return to the initial level. It can be assumed that such stress reactivity is a species characteristic of midday gerbils, which is consistent with their high behavioral reactivity (Goltzman et al., 1994).

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ETHICS APPROVAL AND CONSENT TO PARTICIPATE

All applicable international, national, and/or institutional guidelines for the use and care of experimental animals were followed. All experimental procedures with animals were approved by the Bioethics Commission of the Institute of Ecology and Evolution of the Russian Academy of Sciences (protocol no. 58).

CONFLICT OF INTEREST

The authors of this work declare that they have no conflicts of interest.

REFERENCES

Abelson, K.S.P., Kalliokoski, O., Teilmann, A.Ch., and Hau, J., Applicability of commercially available ELISA kits for the quantification of faecal immunoreactive corticosterone metabolites in mice, *In Vivo*, 2016, vol. 30, no. 6, pp. 739–744.
https://doi.org/10.21873/invivo.10989

Boonstra, R. and Boag, P.T., Spring declines in *Microtus pennsylvanicus* and the role of steroid hormones, *J. Anim. Ecol.*, 1992, vol. 61, no. 2, pp. 339–352.
https://doi.org/10.2307/5326

Chen, H., Yao, H., Yang, W., Fan, P., and Xiang, Z., Assessing the utility of urinary and fecal cortisol as an indicator of stress in golden snub-nosed monkeys (*Rhinopithecus roxellana*), *PeerJ*, 2017, vol. 5, p. e3648.
https://doi.org/10.7717/peerj.3648

Christian, J.J., The adreno-pituitary system and population cycles in mammals, *J. Mammalogy*, 1950, vol. 31, no. 3,

pp. 247–259.
https://doi.org/10.2307/1375290

Chuang, A. and Peterson, Ch.R., Expanding population edges: Theories, traits, and trade-offs, *Global Change Biol.*, 2016, vol. 22, no. 2, pp. 494–512.
https://doi.org/10.1111/gcb.13107

Clobert, J., Le Galliard, J., Cote, J., Meylan, S., and Massot, M., Informed dispersal, heterogeneity in animal dispersal syndromes and the dynamics of spatially structured populations, *Ecol. Lett.*, 2009, vol. 12, no. 3, pp. 197–209.
https://doi.org/10.1111/j.1461-0248.2008.01267.x

Cote, J., Clobert, J., Brodin, T., Fogarty, S., and Sih, A., Personality-dependent dispersal: Characterization, ontogeny and consequences for spatially structured populations, *Philos. Trans. R. Soc. B: Biol. Sci.*, 1560, vol. 365, no. 1560, pp. 4065–4076.
https://doi.org/10.1098/rstb.2010.0176

Creel, S., Dantzer, B., Goymann, W., and Rubenstein, D.R., The ecology of stress: Effects of the social environment, *Funct. Ecol.*, 2013, vol. 27, no. 1, pp. 66–80.
https://doi.org/10.1111/j.1365-2435.2012.02029.x

Fanson, K.V., Best, E.C., Bunce, A., Fanson, B.G., Hogan, L.A., Keeley, T., Narayan, E.J., Palme, R., Parrott, M.L., Sharp, T.M., Skogvold, K., Tuthill, L., Webster, K.N., and Bashaw, M., One size does not fit all: Monitoring faecal glucocorticoid metabolites in marsupials, *Gen. Comp. Endocrinol.*, 2017, vol. 244, pp. 146–156.
https://doi.org/10.1016/j.ygcen.2015.10.011

Gerlinskaya, L.A., Moshkin, M.P., and Evsikov, V.I., Methodical approaches to estimation of stress level in the wild animals, *Ekologiya*, 1993, no. 1, pp. 97–100.

Gol'tsman, M.E., Popov, S.V., Tchabovskii, A.V., and Borisova, N.G., The sociality syndrome. A comparative study of the behavior of gerbils, *Zh. Obshch. Biol.*, 1994, vol. 55, no. 1, pp. 49–69.

Goymann, W., Möstl, E., Van't Hof, T., East, M.L., and Hofer, H., Noninvasive fecal monitoring of glucocorticoids in spotted hyenas, *Crocuta crocuta, Gen. Comp. Endocrinol.*, 1999, vol. 114, no. 3, pp. 340–348.
https://doi.org/10.1006/gcen.1999.7268

Ivanov, E.A., Sidorchuk, N.V., Rozhnov, V.V., and Naidenko, S.V., Noninvasive estimation of the hypothalamic-pituitary-adrenal system activity in the Far East leopard, *Dokl. Biol. Sci.*, 2014, vol. 456, no. 1, pp. 165–168.
https://doi.org/10.1134/s0012496614030120

Josserand, R., Dupoué, A., Agostini, S., Haussy, C., Le Galliard, J.-F., and Meylan, S., Habitat degradation increases stress-hormone levels during the breeding season, and decreases survival and reproduction in adult common lizards, *Oecologia*, 2017, vol. 184, no. 1, pp. 75–86.
https://doi.org/10.1007/s00442-017-3841-4

Kolosova, I.E., Rogovin, K.A., and Moshkin, M.P., The capacity and limitations of noninvasive evaluation of stress based on the fecal glucocorticoid assay in the great gerbil (*Rhombomys opimus*), *Zool. Zh.*, 2008, vol. 87, no. 1, pp. 104–113. https://elibrary.ru/ibxddb.

Koolhaas, J.M., Korte, S.M., De Boer, S.F., Van Der Vegt, B.J., Van Reenen, C.G., Hopster, H., De Jong, I.C., Ruis, M.A.W., and Blokhuis, H.J., Coping styles in animals: Current status in behavior and stress-physiology, *Neurosci. Biobehav. Rev.*, 1999, vol. 23, no. 7, pp. 925–935.
https://doi.org/10.1016/s0149-7634(99)00026-3

Kuznetsov, V.A., Tchabovsky, A.V., Kolosova, I.E., and Moshkin, M.P., Effect of habitat type and population density on the stress level of midday gerbils (*Meriones meridianus* Pall.) in free-living populations, *Biol. Bull.*, 2004, vol. 31, no. 6, pp. 628–632.
https://doi.org/10.1023/b:bibu.0000049736.02912.e2

Möstl, E. and Palme, R., Hormones as indicators of stress, *Domest. Anim. Endocrinol.*, 2002, vol. 23, nos. 1–2, pp. 67–74.
https://doi.org/10.1016/s0739-7240(02)00146-7

Navarro-Castilla, Á., Garrido, M., Hawlena, H., and Barja, I., Non-invasive monitoring of adrenocortical activity in three sympatric desert gerbil species, *Animals*, 2021, vol. 11, no. 1, p. 75.
https://doi.org/10.3390/ani11010075

Novikov, E. and Moshkin, M., Sexual maturation, adrenocortical function and population density of red-backed vole, *Clethrionomys rutilus* (Pall.), *Mammalia*, 1998, vol. 62, no. 4, pp. 529–540.
https://doi.org/10.1515/mamm.1998.62.4.529

Palme, R., Non-invasive measurement of glucocorticoids: Advances and problems, *Physiol. Behav.*, 2019, vol. 199, pp. 229–243.
https://doi.org/10.1016/j.physbeh.2018.11.021

Palme, R., Rettenbacher, S., Touma, C., El-Bahr, S.M., and Möstl, E., Stress hormones in mammals and birds: Comparative aspects regarding metabolism, excretion, and noninvasive measurement in fecal samples, *Ann. N. Y. Acad. Sci.*, 2005, vol. 1040, no. 1, pp. 162–171.
https://doi.org/10.1196/annals.1327.021

Palme, R., Touma, C., Arias, N., Dominchin, M.F., and Lepschy, M., Steroid extraction: Get the best out of faecal samples, *Wien. Tierarztl. Monatsschr.*, 2013, no. 100, pp. 238–246.

Pavlova, E.V. and Naidenko, S.V., Noninvasive monitoring of glucocorticoids in feces of the bengal cat (*Prionailurus bengalensis euptilurus*), *Zool. Zh.*, 2008, vol. 87, no. 11, pp. 1375–1381. https://elibrary.ru/jskmpf.

Selye, H., *The Story of Adaptation Syndrome (Told in the Form of Informal, Illustrated Lectures)*, Montreal: Acta, Inc., Medical Publ., 1950.

Shang, G., Du, Sh., Yang, Ya., Wu, Ya., Cao, Yi., and Bian, J., Is negative density-dependent reproduction regulated by density-induced stress in root voles? Two field experiments, *Ecol. Evol.*, 2022, vol. 12, no. 5, p. e8927.
https://doi.org/10.1002/ece3.8927

Shchipanov, N.A., Universal live trap for small mammals, *Zool. Zh.*, 1987, vol. 66, no. 5, pp. 759–761.

Shilov, I.A., Stress as an environmental phenomenon, *Zool. Zh.*, 1984, vol. 63, no. 6, pp. 805–812.

St. Juliana, J.R., Bryant, J.L., Wielebnowski, N., and Kotler, B.P., Physiological validation of a non-invasive method to evaluate adrenocortical activity and the time course for the excretion of stress hormones in the feces of three species of desert gerbils, *Isr. J. Ecol. Evol.*, 2019, vol. 65, nos. 1–2, pp. 21–27.
https://doi.org/10.1163/22244662-20191043

St. Juliana, J.R., Khokhlova, I.S., Wielebnowski, N., Kotler, B.P., and Krasnov, B.R., Ectoparasitism and stress hormones: Strategy of host exploitation, common host-parasite history and energetics matter, *J. Anim. Ecol.*, 2014, vol. 83, no. 5, pp. 1113–1123.
https://doi.org/10.1111/1365-2656.12217

Stead, S.K., Meltzer, D.G.A., and Palme, R., The measurement of glucocorticoid concentrations in the serum and faeces of captive African elephants (*Loxodonta africana*) after ACTH stimulation : Research communication, *J. S. Afr. Vet. Assoc.*, 2000, vol. 71, no. 3, pp. 192–196.
https://doi.org/10.4102/jsava.v71i3.712

Surkova, E., Popov, S., and Tchabovsky, A., Rodent burrow network dynamics under human-induced landscape transformation from desert to steppe in Kalmykian rangelands, *Integr. Zool.*, 2019, vol. 14, no. 4, pp. 410–420.
https://doi.org/10.1111/1749-4877.12392

Surkova, E.N., Kulik, A.A., Kuznetsova, E.V., Bazykina, S.G., Savinetskaya, L.E., and Tchabovskii, A.V., Chyornye Zemli of Kalmykia: Is the desert returning back?, *Priroda*, 2022, no. 8, pp. 13–20.
https://doi.org/10.7868/S0032874X22080026

Tchabovsky, A.V., Surkova, E.N., Savinetskaya, L.E., and Kulik, A.A., Range expansion and population patterns on the wave of colonization: The midday gerbil (*Meriones meridianus* Pallas 1773, Muridae, Rodentia) in Kalmykia taken as a model, *Biol. Bull.*, 2023, vol. 50, no. 9, pp. 2552–2560.
https://doi.org/10.1134/S1062359023090078

Tchabovsky, A.V., Savinetskaya, L.E., Surkova, E.N., Ovchinnikova, N.L., and Kshnyasev, I.A., Delayed threshold response of a rodent population to human-induced landscape change, *Oecologia*, 2016, vol. 182, no. 4, pp. 1075–1082.
https://doi.org/10.1007/s00442-016-3736-9

Tchabovsky, A., Savinetskaya, L., and Surkova, E., Breeding versus survival: Proximate causes of abrupt population decline under environmental change in a desert rodent, the midday gerbil (*Meriones meridianus*), *Integr. Zool.*, 2019, vol. 14, no. 4, pp. 366–375.
https://doi.org/10.1111/1749-4877.12372

Touma, Ch. and Palme, R., Measuring fecal glucocorticoid metabolites in mammals and birds: The importance of validation, *Ann. N. Y. Acad. Sci.*, 2005, vol. 1046, no. 1, pp. 54–74.
https://doi.org/10.1196/annals.1343.006

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