General and Comparative Endocrinology 195 (2014) 68–79

Contents lists available at ScienceDirect

General and Comparative Endocrinology

journal homepage: www.elsevier.com/locate/ygcen

Evaluating capture stress in wild gray mouse lemurs via repeated fecal sampling: Method validation and the influence of prior experience and handling protocols on stress responses

Anni Hämäläinen [a,b,*], Michael Heistermann [c], Zo Samuel Ella Fenosoa [d], Cornelia Kraus [a,b,e]

[a] Department of Sociobiology/Anthropology, University of Göttingen, Kellnerweg 6, 37077 Göttingen, Germany
[b] Behavioral Ecology and Sociobiology Unit, German Primate Center DPZ, Kellnerweg 4, 37077 Göttingen, Germany
[c] Endocrinology Laboratory, German Primate Center DPZ, Kellnerweg 4, 37077 Göttingen, Germany
[d] Department of Animal Biology, Faculty of Sciences, University of Antananarivo, BP 906, Antananarivo 101, Madagascar
[e] Courant Research Centre Evolution of Social Behaviour, Kellnerweg 6, 37077 Göttingen, Germany

ARTICLE INFO

Article history:
Received 21 June 2013
Revised 17 October 2013
Accepted 21 October 2013
Available online 5 November 2013

Keywords:
Physiological stress response
Cortisol
Fecal glucocorticoids
Capture-and-handling
Prosimian
Enzymeimmunoassay

ABSTRACT

Reliable measurements of physiological stress are increasingly needed for eco-physiological research and for species conservation or management. Stress can be estimated by quantifying plasma glucocorticoid levels, but when this is not feasible, glucocorticoid metabolites are often measured from feces (FGCM). However, evidence is accumulating on the sensitivity of FGCM measurements to various nuisance factors. Careful species- and context-specific validations are therefore necessary to confirm the biological relevance and specificity of the method. The goals of this study were to: (1) establish and validate sampling methods and an enzymeimmunoassay to measure FGCM in the gray mouse lemur (*Microcebus murinus*); (2) explore causes of variability in the FGCM measurements, and; (3) assess the consequences of capturing and handling for free-living individuals by quantifying their stress responses via repeated fecal sampling within capture sessions. We further assessed the influence of different handling protocols and the animals' previous capture experience on the magnitude of the physiological response. Our validations identified the group-specific measurement of 11ß-hydroxyetiocholanolone as the most suitable assay for monitoring adrenocortical activity. The sample water content and the animal's age were found to significantly influence baseline FGCM-levels. Most captured animals exhibited a post-capture FGCM-elevation but its magnitude was not related to the handling protocol or capture experience. We found no evidence for long-term consequences of routine capturing on the animals' stress physiology. Hence the described methods can be employed to measure physiological stress in mouse lemurs in an effective and relatively non-invasive way.

1. Introduction

The assessment of physiological stress is increasingly used in many contexts of evolutionary biology, ecology and conservation to assess the health and coping of individuals or populations with environmental challenges (Ricklefs and Wikelski, 2002; Romero, 2004; Wikelski and Cooke, 2006). To this end, the capturing of animals is often necessary for physical examinations or sample collection. However, capturing and handling themselves are known to cause a stress response (Fletcher and Boonstra, 2006; Romero and Reed, 2005) that may introduce significant bias to studies of other phenomena if unaccounted for (Reeder and Kramer, 2005). The level of invasiveness of the handling procedures can influence the level of capture stress experienced by the animal (Bennett et al., 2012; Garcia et al., 2000) and within an individual, the magnitude of the physiological response to capture may change over subsequent captures via habituation or sensitization to the stressor (Boonstra, 2013; Dickens et al., 2013; Fletcher and Boonstra, 2006; Garcia et al., 2000; Lynn et al., 2010; Romero, 2004; Walker and Dee, 2006). Relatively little is known about the impact of capturing and handling procedures on animals despite the potential consequences for the research outcome and the welfare of the animals involved.

* Corresponding author at: Department of Sociobiology/Anthropology, Johann-Friedrich-Blumenbach Institute for Zoology and Anthropology, University of Göttingen, Kellnerweg 6, 37077 Göttingen, Germany.
E-mail addresses: anni.m.hamalainen@gmail.com (A. Hämäläinen), mheistermann@dpz.eu (M. Heistermann), zoellafenosoa@gmail.com (Z.S.E. Fenosoa), cornelia.kraus@gwdg.de (C. Kraus).

0016-6480
http://dx.doi.org/10.1016/j.ygcen.2013.10.017

The physiological stress response – reflected by an increase in circulating glucocorticoid (GC) levels – facilitates appropriate reactions to and recovery from diverse challenges (Sapolsky et al., 2000). Capturing may cause an acute elevation in stress hormone output, but it might also alter the long term stress physiology of the animal (Boonstra, 2013; Clinchy et al., 2011) if a return to normal state is not achieved between capture events. While chronic stress may be adaptive in natural conditions in some circumstances (Boonstra, 2013; Crespi et al., 2013), chronically elevated GC levels are typically associated with compromised health, reproduction and survival of individuals (reviewed in e.g. Bonier et al., 2009; Romero, 2004). Therefore, capturing may adversely affect especially those individuals that already have high GC levels prior to the capture (Collins, 2001; Matthews et al., 2001) or have an impaired feedback system (e.g. due to old age) to facilitate the return back to baseline GC (Sapolsky et al., 1986).

The physiological stress response to capture can be measured via repeated blood sampling (e.g. Lynn et al., 2010; Romero and Reed, 2005, see also (Fletcher and Boonstra, 2006) or, alternatively, non-invasively using feces (Taylor, 1971). Fecal levels of GC metabolites (FGCM) reflect the baseline GC level at a delay of several hours and provide an integrated measurement of physiological stress levels over a period reflecting at minimum the animal's gut passage time (Harper and Austad, 2000; Sheriff et al., 2010; Touma and Palme, 2005). FGCM may also better indicate the levels of biologically active, unbound GCs than total GC levels measured from blood (Breuner et al., 2013; Sheriff et al., 2011, 2010; Touma and Palme, 2005).

Even though these advantages have led to the widespread use of FGCM in studies of natural populations, several caveats have recently been raised due to the sensitivity of FGCMs to potentially confounding factors (Goymann, 2012). The assessment of stress via FGCM measurements can be influenced by e.g. the ecological season (Huber et al., 2003; Romero, 2002), the animal's diet (Goymann, 2005), metabolic rate and gut bacterial community (Goymann, 2012) and, not least importantly, the treatment of the samples and the analyses performed (Goymann, 2005; Heistermann et al., 2006; Huber et al., 2003; Millspaugh and Washburn, 2004; Möstl et al., 2005; Sheriff et al., 2011; Shutt et al., 2012). Furthermore, the baseline GC level and the intensity of the endocrine stress response are known to vary among individuals based e.g. on their sex (Kudielka and Kirschbaum, 2005), age (Goncharova and Lapin, 2002; Sapolsky et al., 1987), or prior experiences (Fletcher and Boonstra, 2006; Garcia et al., 2000; Lynn et al., 2010; Walker and Dee, 2006). As a result of these complex interactions, FGCM data tends to be "noisy" and, to draw meaningful conclusions, it is necessary to conduct careful species-specific validations of the methods and an evaluation of factors potentially confounding FGCM levels (Buchanan and Goldsmith, 2004; Millspaugh and Washburn, 2004; Möstl and Palme, 2002; Romero, 2004; Sheriff et al., 2011; Touma and Palme, 2005).

In this study, we use FGCM measurements to quantify physiological stress in a small primate, the gray mouse lemur (*Microcebus murinus*). As GC excretion into feces has not been previously studied in the species, it was necessary to first select the most suitable assay for recording HPA axis activity and to validate the method. Biological validations have been suggested as a method alternative to an ACTH-challenge for quantifying the hormonal stress response (Goymann, 2005; Sheriff et al., 2011; Touma and Palme, 2005). Therefore we measured GC levels of wild and captive mouse lemurs before and after a known stressful event in three independent experiments. Based on these data, we selected an assay, assessed the lag-time to peak GC elevation and examined the influence of sample processing protocols on the FGCM measurements.

Following these validations, we examined stress responses to capture and handling of wild animals via repeated FGCM measurements. In a long-term monitored, routinely captured population of gray mouse lemurs (*Microcebus murinus*) (e.g. Dammhahn, 2012; Dammhahn and Kappeler, 2009; Kraus et al., 2008) some individuals voluntarily enter a trap up to 20 times per year and may be handled more than six times per year. The fact that mouse lemurs are easily re-trapped might suggest that the procedure is only minimally stressful to the individuals involved or that they habituate easily to trapping, in which case routine capturing may have few long-term consequences on their stress physiology. We hypothesized that the stress response to routine handling should be attenuated in animals with relatively frequent capture experiences and, if repeated capturing evokes chronic stress, this could translate to elevated baseline FGCM levels in the animals that are captured often. Additionally, the magnitude of the hormonal response should depend on the invasiveness of the handling regime the individual is subjected to (Bennett et al., 2012; Pitman et al., 1988). To evaluate these effects, baseline FGCM and the change from baseline to response level FGCM were measured via repeated fecal sampling during capture sessions.

2. Methods

2.1. The study population

The gray mouse lemur (*M. murinus*) is a small-bodied primate (average body mass ~60–80 g) that inhabits dry deciduous forests in Western Madagascar. The species is nocturnal, arboreal, sexually monomorphic and solitary living. The study population in the forest segment "N5" in Kirindy forest, Western Madagascar, has been intensively monitored since year 2001 for the purposes of long-term data collection (see e.g. Dammhahn, 2012; Dammhahn and Kappeler, 2009; Kraus et al., 2008). All individuals of the study population are individually marked with a subcutaneous transponder chip (Trovan). The animals are trapped using live-catch Sherman traps baited with banana. Routine capturing is conducted on three consecutive nights ("capture session") monthly in March–May and September–November, in addition to which smaller scale captures may take place for the purposes of specific research projects.

2.2. Fecal sampling and field extraction

Upon each capture, fresh fecal samples were collected into polypropylene tubes from previously cleaned traps or when animals defecated during handling. The freshness of the feces was assessed based on the presence of a glossy surface on the pellets, since in the dry season the feces dry quickly after defecation. Because in most primates, a larger proportion of glucocorticoids are excreted via urine than via feces (Bahr et al., 2000; Wasser et al., 2000), any feces where urine contamination was suspected was not sampled. The time of day of collection and time lags to processing and extraction were recorded for each sample. Samples were extracted into ethanol in the field within 4 h of sample collection adapting a protocol described by Ziegler and Wittwer (2005) and modified by Shutt et al. (2012). Briefly, the freshest collected fecal pellets (total fecal mass of 0.15–0.8 g) were homogenized in a collection tube or on a petri dish with a metal rod, then a subsample of approximately 0.2 g (to the nearest 0.001 g) was weighed into an extraction tube and mixed with 2 ml of ~90% ethanol. For logistic reasons, the fecal suspensions were left to stand for 5–12 h, then vortexed for 2 min. Samples were finally centrifuged using a manually operated centrifuge (Hettich GmbH & Co. KG Tuttlingen, Germany) for 2 min (Shutt et al., 2012). The supernatant was poured into a 2 ml polypropylene tube, sealed with parafilm and stored in a dark container at slightly below ambient temperatures until export to Germany, where samples were stored at −20 °C until