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One size does not fit all: Monitoring faecal glucocorticoid metabolites in marsupials

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ABSTRACT

Marsupial research, conservation, and management can benefit greatly from knowledge about glucocorticoid (GC) secretion patterns because GCs influence numerous aspects of physiology and play a crucial role in regulating an animal's response to stressors. Faecal glucocorticoid metabolites (FGM) offer a non-invasive tool for tracking changes in GCs over time. To date, there are relatively few validated assays for marsupials compared with other taxa, and those that have been published generally test only one assay. However, different assays can yield very different signals of adrenal activity. The goal of this study was to compare the performance of five different enzyme immunoassays (EIAs) for monitoring adrenocortical activity via FGM in 13 marsupial species. We monitored FGM response to two types of events: biological stressors (e.g., transport, novel environment) and pharmacological stimulation (ACTH injection). For each individual animal and assay, FGM peaks were identified using the iterative baseline approach. Performance of the EIAs for each species was evaluated by determining (1) the percent of individuals with a detectable peak 0.125–4.5 days post-event, and (2) the biological sensitivity of the assay as measured by strength of the post-event response relative to baseline variability (Z-score). Assays were defined as successful if they detected a peak in at least 50% of the individuals and the mean species response had a $Z \geqslant 2$. By this criterion, at least one assay was successful in 10 of the 13 species, but the best-performing assay varied among species, even those species that were closely related. Furthermore, the ability to confidently assess assay performance was influenced by the experimental protocols used. We discuss the implications of our findings for biological validation studies.

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1. Introduction

Marsupials exhibit several unique physiological, reproductive, and ecological traits compared with other mammals, making them important for understanding basic endocrine function. Indeed, the potential influence of hormones on individual fitness is well illustrated in dasyurids, which provide a particularly unique example of endocrine-mediated life history strategy – the complete male die-off at the end of the breeding season. This die-off is a result of a failure in the negative feedback system of the hypothalamic–pituitary–adrenal axis resulting in extended, elevated GC production which becomes deleterious to the health of the animal (Bradley, 2003; Naylor et al., 2008). However, aside from a few well-studied examples, there is still relatively little known about basic endocrinology and particularly adrenal function for many marsupial species (Hing et al., 2014; McDonald, 1977). Furthermore, more than 30% of Australian mammals are classified as threatened (Woinarski et al., 2014), and nearly 50% of recent mammalian extinctions have occurred in Australia (Short and Smith, 1994). Conservation efforts are key to species survival, and relocation to predator-free sanctuaries and captive breeding programs have become important management practices to help sustain dwindling marsupial populations (Selwood and Cui, 2006; Sheean et al., 2012).

Glucocorticoids (GCs) are secreted by the adrenal glands and regulate several aspects of physiology, including stress physiology. Because GCs play a broad role in energy regulation and homeostasis (Sapolsky et al., 2000), their characterisation as only a "stress" hormone is a misnomer. At baseline levels, GCs help regulate circadian and circannual rhythms (Dallmann et al., 2006), promote healthy reproductive function (Whirledge and Cidlowski, 2013), and facilitate immune function (Padgett and Glaser, 2003). These diverse roles are illustrated by the dramatic physiological effects of chronic GC elevation, including reproductive suppression, impaired immune function, muscle wasting, and decreased cognitive function (McEwen, 1998; Sapolsky, 2002). Individual differences in GC have also been associated with variation in behaviour (Carere et al., 2010; Koolhaas et al., 2010), and may underlie individual differences in fitness or life history strategies. Therefore, monitoring patterns of adrenocortical activity in marsupials could facilitate the evaluation of conservation efforts on health, welfare, and reproductive success.

Until recently, blood samples were needed to monitor GC patterns, but advances in methods for monitoring faecal glucocorticoid metabolites (FGM) have made it possible to measure GC repeatedly in the same free-living individuals without the need for capture or restraint (Sheriff et al., 2011). Since changes in hormone concentrations (not just absolute values) are a key determinant of endocrine function, FGM monitoring allows us to develop an understanding of critical elements of endocrinology that are difficult to monitor using traditional techniques. In addition, FGM provide a pooled estimate of circulating hormone concentrations (Möstl and Palme, 2002; Sheriff et al., 2011; Wielebnowski and Watters, 2007), thereby reducing the pulsatile patterns present in serum and offering a more integrated measure of adrenal function. These features arguably make FGM more useful for answering large-scale questions about conservation biology, individual variation, and animal welfare (Palme, 2012; Sheriff et al., 2011).

While FGM monitoring has great potential, it also presents several methodological challenges deriving from the fact that circulating GCs are metabolized into a diverse array of molecules by liver and gut bacteria prior to excretion (Palme et al., 2005; Touma and Palme, 2005; Wielebnowski and Watters, 2007). Steroid metabolism can differ as a function of species, sex, diet, age, and season (Goymann, 2012), so one must first ensure that the FGM detected by a specific assay reflect biologically relevant changes in adrenocortical activity (Touma and Palme, 2005). Typically, this biological validation is achieved by stimulating adrenocortical GC production pharmacologically (ACTH injection) or biologically (environmental stressor), and monitoring changes in FGM following this event. Assay selection is an important but often undervalued aspect of biological validation (Palme, 2005). Assay performance varies based on the ligand against which the antibody was raised, the part of the ligand recognised by the antibody, the range of FGM to which the antibody binds, cross-reactivity with metabolites of other (particularly gonadal) hormones, and the match between the antibody binding and a species' FGM profile (Möstl et al., 2005; Palme et al., 2005). In recent years, an increasing number of studies have sought to biologically validate an assay for monitoring FGM in a specific marsupial species (Davies et al., 2013; Hogan et al., 2011, 2012; Johnston et al., 2013; Keeley et al., 2012; McKenzie and Deane, 2005; Narayan et al., 2014, 2013; Oates et al., 2007), but none have directly compared the performance of multiple assays across species.

In this study, we aimed to compare the performance of five enzyme immunoassays (EIAs) for monitoring adrenocortical activity in 13 species representing three orders of Australian marsupials. We first identified overall similarities and differences in the performance of the five assays, controlling for species and individual differences. We then assessed EIA performance within each species by (1) identifying the percent of individuals who had a detectable post-event peak in each assay, (2) quantifying the strength of the post-event response (signal) relative to the amount of variation (noise) in the baseline, and (3) evaluating timing of peaks relative to the event. Finally, we discuss which assays were most effective for each species and consider the pattern of assay performance across taxonomic categories. Our data facilitate discussion of key aspects of study design that influence the probability of detecting a peak and we outline strategies to follow when biologically validating FGM assays.

2. Materials and methods

2.1. Study species and stressors

This study included a total of 44 individuals from 13 marsupial species representing three Marsupialia Orders (see Table 1 for details, including the number of males and females of each species). All animals were permanently housed in captivity except for western grey kangaroos (wild-caught and temporarily held in holding pens) and the northern hairy-nosed wombat (undergoing rehabilitation). All animals were adults except the northern hairy-nosed wombat, which was a juvenile. All procedures were approved by the appropriate ethics committees and review boards.

For seven species, an ACTH challenge was used to pharmacologically stimulate the adrenal cortex (see Table 1 for manufacturer and dose). ACTH was administered as a single intramuscular injection of Synacthen (25 IU/ml) or Synacthen Depot (100 IU/ml; Novartis, Basel, Switzerland) or Corticotropin (80 IU/ml; Wedgewood Pharmacy, New Jersey, USA). This injection was administered under anaesthesia to two species (Tasmanian devils and koalas) and without anaesthesia to five species (numbats, bilbies, woylies, eastern grey kangaroo, and western grey kangaroo).

For the remaining six species, pre-scheduled biological stressors were used (Table 1). Three species were transferred from one institution to another within Australia and sample collection was initiated as soon as they arrived at the new institution. For yellow-bellied gliders, and southern bettongs, transit time was 5–6 h via plane and automobile. For the long-nosed potoroo, transit time was approximately 1 h via automobile. In these cases, peak values

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