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# Hypothalamic–pituitary–adrenal (HPA) axis function in the California mouse (*Peromyscus californicus*): Changes in baseline activity, reactivity, and fecal excretion of glucocorticoids across the diurnal cycle

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ABSTRACT

The California mouse, *Peromyscus californicus*, is an increasingly popular animal model in behavioral, neural, and endocrine studies, but little is known about its baseline hypothalamic–pituitary–adrenal (HPA) axis activity or HPA responses to stressors. We characterized plasma corticosterone (CORT) concentrations in *P. californicus* under baseline conditions across the diurnal cycle, in response to pharmacological manipulation of the HPA axis, and in response to a variety of stressors at different times of day. In addition, we explored the use of fecal samples to monitor adrenocortical activity non-invasively. California mice have very high baseline levels of circulating CORT that change markedly over 24 h, but that do not differ between the sexes. This species may be somewhat glucocorticoid-resistant in comparison to other rodents as a relatively high dose of dexamethasone (5 mg/kg, s.c.) was required to suppress plasma CORT for 8 h post-injection. CORT responses to stressors and ACTH injection differed with time of day, as CORT concentrations were elevated more readily during the morning (inactive period) than in the evening (active period) when compared to time-matched control. Data from $^{3}$H-CORT injection studies show that the time course for excretion of fecal CORT, or glucocorticoid metabolites, differs with time of injection. Mice injected in the evening excreted the majority of fecal radioactivity 2–4 h post-injection whereas mice injected during the morning did so at 14–16 h post-injection. Unfortunately, the antibody we used does not adequately bind the most prevalent fecal glucocorticoid metabolites and therefore we could not validate its use for fecal assays.



## 1. Introduction

The hypothalamic–pituitary–adrenal (HPA) stress response has been characterized in many vertebrates [31,38] and is activated when an organism is presented with an actual or potential threat, resulting in the release of glucocorticoids (GCs) from the adrenal cortex. The principal glucocorticoid produced by humans and other primates is cortisol, whereas many rodents, including *Peromyscus californicus*, primarily produce corticosterone [64]. Despite the fact that GCs are often referred to as "stress hormones", the HPA axis is continuously active and GCs at baseline levels have important daily functions [72]. Baseline levels of GCs exhibit a sinusoidal pattern over the course of the day, with the highest concentrations occurring just prior to the onset of waking in most species [reviewed by 33,54,57,58]. Glucocorticoids help organisms respond to and recover from stressors, and aid in regulation of inflammation and immune function, gluconeogenesis, brain function, cardiovascular activity, various behaviors, and numerous other processes [19,24,25,60,69,72,77,78].

The California mouse, *P. californicus*, is a monogamous, biparental rodent that is becoming an increasingly popular animal model. Not only is this species useful for studying parental behavior and its neural and hormonal correlates [4,7,12,29,30,41,55,84], but it is also used in studies on aggression [6,76,85,86] and immune function [36,61,62], as well as in studies investigating relationships between stress and behavior [3,15,46,87]. California mice survive and breed well in captivity, and parental behavior by both sexes has been well characterized [6,12,29,30,39]. Since both males and females invest heavily in their offspring [40,42], *P. californicus* provides a valuable model for studying the effects of stress on parental care, as well as the effects of parental status on stress responsiveness in both mothers and fathers.

Despite the increasing use of California mice in behavioral, neural, and endocrine studies, little is known about their baseline HPA activity or their HPA response to stressors. Although

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many aspects of the HPA axis are conserved across vertebrates, several parameters, including circulating GC concentrations, circadian dynamics, and temporal responses to and recovery from stressful stimuli, differ markedly among species [9,28,34,54,68,95] and even within strains of a single species [32,73]. Thus, before we are able to fully understand and interpret behavioral or physiological data in the context of stress in California mice, we must first characterize normative activity and reactivity of the HPA axis.

Determination of circulating GC concentrations via plasma or serum assay provides the most direct measure of GC levels available to the tissues and can provide important information on immediate HPA responses to perturbations; however, this approach presents several problems. Handling and blood collection are stressful to many animals and can alter GC levels, as well as other physiological and behavioral measures, over the short-term and potentially the long-term, which complicates characterization of basal GC levels. Collection of repeated blood samples is compromised by the small body size of many animals and the limited volume of blood that can be removed without adverse effects. To avoid these problems, many researchers are turning to fecal hormone assays, especially in small and/or free-living animals. Fecal hormone levels are not as sensitive to minor disturbances as are hormone concentrations measured in the circulation, and reflect circulating hormone levels over a period of hours due to the processes of metabolism and excretion [81–83]. Fecal measures can thus provide more time-integrated hormone data, thereby yielding a more comprehensive measure of hormone levels over a period of time [see 63]. Glucocorticoids or their metabolites have been measured from fecal samples in numerous mammals, birds and reptiles [13,37,52,63,82,92]; however, proper and extensive assay validation for each species is essential to confirm that biologically relevant GCs or GC metabolites can be measured accurately [63,66,81–83]. Although fecal GC measures in California mice have been reported previously [3], a fecal GC assay has not been validated for this species, and GC or GC metabolite excretion patterns have not been characterized.

The primary objectives of this study were to characterize plasma corticosterone (CORT) concentrations in *P. californicus* (1) under baseline conditions across the diurnal cycle, (2) in response to pharmacological manipulation of the HPA axis, and (3) in response to a variety of stressors at different times of day. In addition, we explored the use of fecal samples as a means to monitor adrenocortical activity non-invasively in this species.

## 2. Materials and methods

### 2.1. Animals

We used California mice that were born and maintained in our breeding colony at the University of California, Riverside. They were descended from individuals purchased from the Peromyscus Genetic Stock Center, University of South Carolina (Columbia, SC) in 2007. The colony was kept on a 14:10 light:dark cycle, with lights-on at 0500 h and lights-off at 1900 h. Ambient temperature was approximately 23 °C with humidity of about 65%. Mice were housed in standard shoe-box-style, polycarbonate cages ($44 \times 24 \times 20$ cm) lined with aspen shavings; cotton wool was provided for nesting material. Food (Purina 5001 rodent chow) and water were provided *ad libitum*. Cages were cleaned once per week unless otherwise noted. In our colony, siblings are never mated with one another, and first-cousin matings are avoided whenever possible. Animals were weaned at 27–32 days of age (prior to the birth of younger siblings), ear-punched for individual identification, and housed in same-sex groups of 2–4 mice. Mice either remained in same-sex groups or were paired with an individual of the opposite sex after 90 days of age.

We used a total of 147 mice (69 males and 78 females) from either same-sex (virgin) or male–female pairs. We were very interested at obtaining basic information about the HPA axis in a representative sample of California mice as this species is becoming increasingly popular animal model. Additionally, data from our lab show that baseline CORT concentrations do not differ between males and females (see Section 3.1), and that baseline and stress-induced CORT levels do not vary across differing reproductive conditions [15;unpub.data] or adult ages [unpub. data]. Therefore, mice of both sexes and of various ages and reproductive conditions were used for our HPA-characterization experiments; whenever possible, however, we avoided the use of pregnant and possibly pregnant females (6 out of 78 females were housed with a reproductively mature adult male, and might have been pregnant during testing). Additionally, some mice were used for more than one data set (diurnal rhythms, stress tests, pharmacological manipulation, or fecal collection); the mean ± SE number of data sets to which each mouse contributed data was 1.02 ± 0.05, range 1–3. Different experiments on the same animal were separated by at least 1 week to allow recovery. The sex, number, and housing condition of mice used for each experiment are listed in the description of each experiment, below. UCR has full AAALAC accreditation, and all procedures were approved by the UCR IACUC and conducted in accordance with the *Guide for the Care and Use of Laboratory Animals*.

### 2.2. Blood collection and analysis

Mice were anesthetized with isoflurane, and blood (70–140 μl) was collected from the retro-orbital sinus using heparinized microhematocrit tubes. Time from disturbance or end of the preceding test to collection of the blood sample was always less than 4.6 min (mean ± SE: 89 ± 2.61 s; range 22–279 s); 97% of samples were collected in under 3 min and 84% in 2 min or less. Blood samples were centrifuged for 12 min (13,300 rpm, 4 °C), and plasma was removed and stored at −80 °C until assay.

Plasma was assayed in duplicate for CORT using an $^{125}$I double-antibody radioimmunoassay (RIA) kit (#07–120102, MP Biomedicals, Costa Mesa, CA) previously validated for this species [15]. Samples from each experiment were analyzed in the same assay if possible, or balanced evenly across multiple assays; however, an individual mouse's samples from a given experiment were always analyzed in a single assay run. The standard curve ranged from 12.5 ng/ml (91% bound) to 1000 ng/ml (20% bound), and plasma samples were assayed using dilutions ranging from 1:50 to 1:1600 depending on anticipated CORT concentrations. Inter- and intra-assay coefficients of variation (CVs) were 11.2% and 4.7%, respectively ($N = 45$ assays).

### 2.3. Characterization of diurnal rhythm

A total of 6 plasma samples (1200, 1600, 2000, 2400, 0400, 0800 h) were collected from 8 virgin male and 7 virgin female California mice (all housed in same-sex pairs) under undisturbed conditions to characterize the diurnal pattern of circulating CORT concentrations. At least 7 days elapsed between collection of successive samples from the same animal, and the order of sampling time points was approximately balanced across individuals within each sex.

### 2.4. Pharmacological stimulation of adrenal activity

We used adrenocorticotropic hormone (ACTH) to stimulate the production of CORT. Synthetic ACTH (Cosyntropin, Penn Veterinary, Lancaster, PA) was diluted to 25 μg/ml with sterile saline