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Relationships between cortisol and urinary androgens in female titi monkeys (*Plecturocebus cupreus*)

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Abstract

Steroid hormones are critical to the regulation of sociosexual behavior. Their role in the formation of pair bonds is complicated by the relative scarcity of this social system in mammals, as well as species and taxonomic differences in endocrine systems. In the present study, we experimentally manipulated the hypothalamic-pituitary-adrenal axis in female titi monkeys (*Plecturocebus cupreus*), a neotropical monkey studied for its strong, selective pair bonds. We validated an assay for plasma and urinary cortisol in this species, showing a strong suppression of cortisol following dexamethasone injection, and a significant but somewhat blunted response to adrenocorticotrophin hormone (ACTH) stimulation. Urinary androgens did not change in response to dexamethasone or ACTH. Plasma and urinary cortisol were moderately correlated, whereas urinary cortisol and androgens were only correlated when extreme cortisol values were included. In this study, we laid groundwork for studying the role of glucocorticoids and androgens (and eventually, their interactions with peptides) in the behavioral endocrinology of pair bonds in female titi monkeys.

Graphical Abstract

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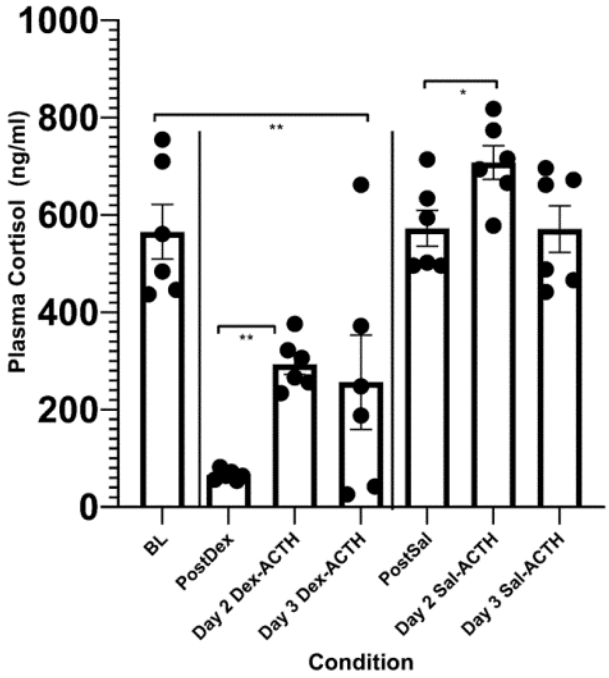
Lynea R. Witczak: Conceptualization, Formal analysis, Investigation, Writing-Original Draft, **Rocío Arias del Razo**: Investigation, Writing-Review & Editing, **Alexander Baxter**: Investigation, Writing-Review & Editing, **Alan J. Conley**: Conceptualization, Methodology, Investigation, Writing-Review & Editing, **Rebecca Cotterman:** Methodology, Investigation, **Madison Dufek**: Investigation, Project administration, Writing-Review & Editing, **Leana R. Goetze**: Investigation, Project administration, Writing-Review & Editing, **Allison R. Lau**: Investigation, Writing-Review & Editing, **Sally P. Mendoza**: Conceptualization, Writing-Review & Editing, **Logan E. Savidge**: Investigation, Writing-Review & Editing, and **Karen L. Bales**: Conceptualization, Methodology, Formal analysis, Investigation, Resources, Writing-Original Draft, Visualization, Supervision, Funding acquisition



Female coppery titi monkey plasma cortisol is significantly suppressed following a dexamethasone injection, and remains lower than baseline two days later. Plasma cortisol is significantly elevated following ACTH stimulation, but this response is somewhat blunted. Measures of urinary cortisol follow this same pattern, suggesting urine samples are a valid, non-invasive measure in this species. BL = baseline blood draw; PostDex = first blood draw the morning after females received Dexamethasone; Day 2 Dex-ACTH = blood draw after females received ACTH following the PostDex blood draw; Day 3 Dex-ACTH = blood draw day after ACTH injection for females treated with Dexamethasone on Day 1; PostSal = first blood draw the morning after females received saline; Day 2 Sal-ACTH = blood draw after females received ACTH following PostSal blood draw; Day 3 Sal-ACTH = blood draw day after ACTH injection for females treated with saline on Day 1.

Keywords

Social monogamy; pair bonds; steroid/peptide theory

1. Introduction

Steroid hormones are critical to our understanding of social behavior across species. In both their direct actions, and in their interactions with peptide hormones, they coordinate sexual behavior, reproduction, social relationships and parenting behaviors (Carter and Perkeybile, 2018; van Anders et al., 2011). While social monogamy and its associated behaviors (pair bonding, mate-guarding, male parenting) have likely re-evolved multiple times (Lukas and Clutton-Brock, 2013), it is reasonable to expect that similar mechanisms may have been co-opted repeatedly by evolution. Indeed, a common characteristic of these species is reduced sexual dimorphism related to a reduction in the effects of androgen hormones (Carter and

Perkeybile, 2018; Kleiman, 1977; Roberts et al., 1998). Glucocorticoids have also been linked to modulatory roles in the formation and maintenance of pair bonds (DeVries et al., 1996; Hinde et al., 2016).

Titi monkeys (*Plecturocebus cupreus*) are a socially monogamous, neotropical primate that forms strong pair bonds and displays high levels of male parenting behavior (Mendoza and Mason, 1997, 1986). They display a strong preference for their familiar partner (Carp et al., 2016; Rothwell et al., 2020) and a suite of other pair-bonding behaviors like distress upon separation from their partner (Hinde et al., 2016), mate-guarding behavior (Fisher-Phelps et al., 2016; Witczak et al., 2018), social buffering of their partner (Mendoza et al., 2000), and "jealousy" (Maninger et al., 2017b). They are also amenable to pharmacological manipulation (Larke et al., 2016; Ragen et al., 2015; Rothwell et al., 2019) and imaging experiments (Bales et al., 2007; Hinde et al., 2016; Hostetler et al., 2017; Maninger et al., 2017a,b), and as such are a strong animal model for the neurobiology of pair-bonding behavior (Bales et al., 2017).

The role of steroid hormones in monogamy have primarily been investigated in rodent models. In monogamous prairie voles (*Microtus ochrogaster*), an elevation in circulating corticosterone is necessary for male pair bond formation (DeVries, DeVries, Taymans, and Carter, 1996) but blocks pair bond formation in females (DeVries, DeVries, Taymans, and Carter, 1995). The role of glucocorticoids has not been studied in a similar fashion in monogamous New World monkeys. In general, neotropical monkeys (not specifically monogamous monkeys) often display high levels of glucocorticoid steroids and associated glucocorticoid resistance (Chrousos et al., 1982). However, titi monkeys display levels of cortisol closer to that of cercopithecine primates and humans, and a robust feedback response to dexamethasone (DEX; Mendoza and Moberg, 1985). Titi monkeys are both especially sensitive to novelty in their environment and yet buffered against this novelty by the presence of their partner (Hennessy et al., 1995), potentially suggesting interesting interactions between the hypothalamic-pituitary-adrenal (HPA) axis and other socially relevant hormones like oxytocin (DeVries et al., 2003). Further, no previous studies in titi monkeys have explored the response of the titi monkey HPA axis to adrenocorticotropin hormone (ACTH) stimulation or investigated its interactions with gonadal hormones. While androgens in titi monkeys have been measured in the context of puberty (Arias Del Razo et al., 2019), jealousy (Maninger et al., 2017b), and prenatal exposure (Baxter et al., 2019), they have also otherwise been little studied in the context of titi monkey pair bonds (and have never been studied in non-pregnant titi monkey females).

The regulation of steroid hormones may differ in certain ways in socially monogamous species compared to non-monogamous species (Carter and Perkeybile, 2018), and there remain a large number of important questions regarding the role of steroid hormones, and interactions between steroid hormones, in titi monkey pair bonding. For many years, a commercial assay was used for assaying cortisol in titi monkeys, but that assay was discontinued and is no longer sold, thus necessitating a new assay. The goals of the present study were to set the stage for these studies by endeavoring to: a) biologically validate a new assay for plasma cortisol in titi monkeys, using DEX and ACTH challenge; b) validate urinary cortisol as a non-invasive measure reflective of plasma cortisol; c) examine

the effect of HPA axis manipulation (DEX and ACTH challenge) on urinary androgens; and d) measure correlations between urinary cortisol and urinary androgens. We expected treatments of DEX to decrease circulating cortisol in urine and plasma samples and expected ACTH treatments to elicit a rise in circulating cortisol in our samples. We did not expect treatments to directly impact androgens; however, given how interconnected the HPA and Hypothalamic-Pituitary-Gonadal (HPG) axes are, we predicted these treatments may have an indirect effect on circulating androgens (Viau, 2002).

2. Materials and Methods

2.1 Subjects

Subjects were six female titi monkeys, all born and raised at the California National Primate Research Center (CNPRC). A 6:00 am to 6:00 pm light-dark cycle is maintained year-round. All were housed with male pair mates, except for one that was still housed in her natal group with her parents. Subjects were not on hormonal birth control, but were confirmed non-pregnant either because of a previously vasectomized mate or a negative ultrasound. Subject ages ranged from 3.11 to 16.17 years, with a mean of 9.82 (± S.E.M. 2.19) years at the beginning of testing, which took place over a two-week period. As female titi monkeys are capable of reproduction by 18 months (Valeggia et al., 1999), all of the females in our study were considered fully adult. This study was approved by the IACUC of the University of California, Davis (protocol #19641); and complied with legal requirements of the United States and the policies of the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

2.2 Study design and sample collection

On Day 1, we collected a baseline blood and urine sample from each female at approximately 6:00 am, with urine samples being collected prior to blood samples (Figure 1). Lights turn on at 6:00 am so this sample was the first morning void of subjects, representing an overnight urine sample. At 5:00 pm of the same day, subjects were treated with either saline or dexamethasone (DEX; 500 ug/kg, intramuscular). Half of the females received DEX the first week, and half received saline. This dosage was based on a previous study in titi monkeys, in which it had been shown to nearly completely suppress basal levels of plasma cortisol in titi monkeys (Mendoza and Moberg, 1985).

On Day 2 at 6:00 am, another set of blood and urine samples were collected, immediately followed by an intramuscular injection of 2.5 IU ACTH (Sorenson et al., 2013; Vandeleest et al., 2013). Since an ACTH challenge had not previously been carried out in this species, we used the dose used to assess HPA axis responsiveness in infant rhesus monkeys (Sorenson et al., 2013). At 6:30 am, another blood sample was collected. Urine is voided periodically by unrestrained animals and can be collected reliably only once or twice per day; therefore, we were unable to collect a second urine sample at 6:30 am when the second blood sample was collected.

On Day 3 at 6:00 am, final blood and urine samples were collected. Each female underwent this sequence for a second week; however, those females that received DEX the first week received saline the second week, and vice versa.

For urine collection, experimenters entered subjects' home cages at lights-on and waited until the subject naturally urinated. Urine was caught free-fall in a collection cup and taken back to the laboratory, where it was aliquoted and frozen at −20° until assay. Subjects habituated to urine collection procedures, which have occurred multiple times weekly in the colony since 2005. Importantly, this collection represented first void urine but not a 24-hour summed urine collection. 24-hour collection would have required separation of the female from her family group and would have constituted a significant stressor, potentially obscuring other effects in the study.

For blood collection, subjects were trained to jump into transport boxes (0.31 by 0.31 by 0.33 m). They were then quickly removed by an experimenter and held for blood collection from the femoral vein. Blood collection times averaged 3.6 ± 0.26 minutes from first disturbance until the end of collection and did not differ between treatments ($F_{4,47} = 1.54$, $p = 0.234$). This window of time from disturbance to blood collection is fast enough to ensure that plasma cortisol measured in the sample was not influenced by the catching and handling procedures (Hennessy et al., 1995). Blood samples were frozen at −80° until assay.

2.3 Plasma and urinary cortisol assay

Plates (Thermo Nunc Maxisorp 442404P) were coated at a concentration of 1:20,000 with antisera R4866 (produced by UC Davis Endocrinology Laboratory) in 50 ul coating buffer (0.05M carbonate, pH 9.6) per well, overnight, and stored at 4^{O} C until use. The same antisera has been previously used to measure fecal and urinary cortisol in other neotropical primates such as marmosets and tamarins (Bales et al., 2005; Cantarelli et al., 2017; McCallister, Smith, and Elwood, 2004; Smith and French, 1997; Ziegler, Scheffler and Snowdon, 1995) and chipmunk feces (Montiglio et al., 2012) and exhibits the following cross-reactivities; cortisol, 100%; prednisolone, 9.9%; prednisone, 6.3%; cortisone, 5%; corticosterone, 0.7%, all other steroids tested, <0.3% (Munro and Stabenfeldt, 1985). A pair of blank wells not coated were used for non-specific binding. Plasma and controls were not extracted, but were diluted 1:1000 in EIA buffer (0.1M phosphate buffer, pH 7.0, 0.1% bovine serum albumin), in microtubes. Serial dilution of titi monkey plasma was parallel to the standard curve (Supplementary Figure 1). Plasma was charcoal stripped and spiked with cortisol (500, 250, 125, 62.5, 31.3, 15.6, 7.8, 3.9, 1.95, and 0.98pg) and assayed with an average recovery of 115 ± 2%). Spiked samples were also parallel to the standard curve (Supplementary Figure 2). The above recovery data indicated a sensitivity of at least 1pg/well.

Before use, antibody coated plates were washed 4 times in EIA wash solution (0.15 M NaCl, 0.05% Tween 20), using a Biotek 405LS plate washer, blotted, then immediately blocked for 20 minutes by adding 50 ul of EIA buffer per well. A solution of Cortisol-3-CMO conjugated to a horseradish peroxidase label (UC Davis Endocrinology Laboratory) was diluted in EIA buffer to a concentration of 1:200,000. A standard curve of cortisol (Sigma-Aldrich H4001) ranging from 0.98 to 500 pg/50 ul was prepared in EIA buffer, and

transferred to microtubes. Two tubes for total binding (B_0) were included that do not contain standard.

After the 20-minute blocking period, 50 ul of standards and samples were added to the plate using a multichannel pipette. Immediately, 50 ul of cortisol:HRP solution was added to the wells. Plates were shaken for 5 minutes on a plate shaker, then incubated at 4^o overnight. The next day, plates were washed again, then 100 ul of a 0.05M citrate buffer, with 0.4 mM ABTS, and H_2O_2 substrate solution were added to each well.

Plates were monitored until the B_0 (total binding) wells reach an optical density of approximately 1.0 on a plate reader (Versamax), then read at 405/570 dual wavelength. Results were analyzed using Softmax software with a 4-place fit using concentration vs. a percent binding calculation of the standard curve. Urine samples were diluted at 1:1000 in water rather than buffer, and final values were adjusted for creatinine. The samples from each female before and after treatments were assayed side by side and all were run in a single assay.

Intra-assay CVs were 3.95% for the plasma cortisol assay; and 5.97% for the urinary cortisol assay.

2.4 Urinary androgen assay

Titi monkey urinary immuno-reactive androgen assays were performed using antibody R156/7 (UC Davis Endocrinology Laboratory) diluted in EIA buffer to a concentration of 1:15,000 which had been used in previous studies in fecal androgen in golden lion tamarins (Bales et al., 2006). Samples were hydrolyzed with hydrochloric acid and reconstituted in EIA buffer. Samples of hydrolyzed urine were used at a dilution of 1:20, and corrected for creatinine, as described in previous studies on male and female titi monkeys (Arias Del Razo et al., 2020 and Baxter et al., 2019). In order to evaluate parallelism, we hydrolyzed male and female titi monkey urine for 20 minutes in boiling water, then serially diluted male urine 1:2 into female urine. Serial dilution of titi monkey urine was parallel to the standard curve (Supplementary Figure 3). The exact identity of immuno-reactive androgens excreted in urine of titi monkeys is not known and the recovery of conjugated androgen was not determined We also performed a form of biological validation by analyzing samples on males from age 6 months to 3.5 −4.5 years, thus spanning puberty and young adulthood, and saw the expected rise in androgens by age (Supplementary Figure 4). The sensitivity was 4pg/ml and inter- and intra-assay CVs were 3.92% and 7.81%.

2.5 Data analysis

Data were analyzed by general linear mixed models, with female identity as the random factor and treatment condition as the fixed effect. Models therefore had two levels: subjects nested within treatment groups. Residuals were checked for normality using the Kolmogorov-Smirnov test. While residuals were normal for the plasma study, the distributions for urinary cortisol and androgen values were non-normal and were not transformable to normality when we included outliers. Given our small sample size (n = 6) it is not surprising that our data were non-normally distributed. It is possible some of the data points were outliers, which may drive spurious effects; however, it is also possible

that all values collected are representative of the population. Therefore, we carried out the cortisol analyses twice; once with outliers, and once eliminating outliers for urinary cortisol, one of which was also an outlier for urinary androgens (two samples). When we removed outliers, our data were normally distributed. Post-hoc tests were carried out by least-squared means upon a significant result from the overall ANOVA. Pearson correlations were used to analyze correlations between urinary and plasma cortisol as well as urinary cortisol and urinary androgens. All tests were two-tailed and were considered significant if $p < 0.05$. Baseline levels from week 1 and 2 were analyzed first, and did not differ. The two baseline levels were therefore combined for further analyses. Statistical analysis was carried out in SAS 9.4 using PROC GLM (SAS Institute, Cary, NC). Data for this study are available via Open Access (https://doi.org/10.5281/zenodo.4386444).

3. Results

3.1 Plasma cortisol

Plasma cortisol varied significantly across conditions ($F_{6,47} = 37.95$, $p < 0.0001$; $\eta^2 = 0.86$; Figure 2). Female identity also significantly predicted cortisol levels ($F_{5,47} = 7.61$, $p < 0.0001$, $\eta^2 = 0.51$). The morning following dexamethasone administration (Day 2, “PostDex”), there was a significant reduction in plasma cortisol when compared to baseline (Day 1, “BL”, $t = -10.68$, $p < 0.0001$), and when compared to saline administration (Day 2, “PostSal”, $t = -9.38$, $p < 0.0001$). The subjects were then given ACTH, resulting in a significant increase in plasma cortisol if they had previously received dexamethasone (Day 2, “Dex-ACTH” compared to “PostDex”, $t = -4.22$, $p = 0.0002$), as well as if they had previously received saline (Day 2, “Sal-ACTH” compared to “PostSAL”, $t = -2.50$, $p = 0.017$). Plasma cortisol was also significantly higher with the Sal-ACTH condition compared to the Dex-ACTH condition both on Day 2 ($t = 5.702$, $p < 0.0001$) and Day 3 ($t = 4.330$, $p < 0.0001$). In the last sample taken (Day 3), the subjects which had gotten dexamethasone and ACTH continued to have lower cortisol levels than baseline (Day 3 “Dex-ACTH” compared to “BL”, $t = -6.603$, $p < 0.0001$), while subjects which had gotten saline and ACTH (Day 3 “Sal-ACTH”) had returned to cortisol levels indistinguishable from baseline ($t = 0.117$, $p = 0.907$). Day 3 plasma samples for titi monkeys given dexamethasone and ACTH were also still higher than plasma samples taken on Day 2 post-dexamethasone (Day 3 “Dex-ACTH” compared to “PostDex”, $t = -2.628$, $p = 0.012$).

3.2 Urinary cortisol

Urinary cortisol also varied significantly across condition, both with ($F_{4,35} = 6.37$, $p = 0.001$; $\eta^2 = 0.50$) or without ($F_{4,33} = 32.61$, $p < 0.0001$; $\eta^2 = 0.85$) the inclusion of outliers (Figure 3). With the outliers in the analysis, female identity was not statistically significant ($F_{6,35} = 1.61$, $p = 0.187$; $\eta^2 = 0.28$), however, without outliers this term was significant ($F_{6,35} = 3.40$, $p = 0.015$; $\eta^2 = 0.47$). Here we report the values without outliers, except when inclusion changes the statistical significance of a result.

The morning following dexamethasone administration (Day 2, “PostDex”), there was a significant reduction in urinary cortisol when compared to baseline (PostDex; $t = -5.845$, $p < 0.0001$), and when compared to saline administration (PostSal; $t = 8.463$, $p < 0.0001$).

In fact, without inclusion of outliers, Day 2 post-saline was significantly elevated compared to baseline ($t = 3.57$, $p = 0.002$), whereas it was not significantly different when outliers were included. The final morning (Day 3), the post-dexamethasone and ACTH urinary cortisol remained significantly lower than baseline (Dex-ACTH; $t = -6.965$, $p < 0.0001$). Additionally, urinary cortisol was significantly lower in the Dex-ACTH condition than the Sal-ACTH condition ($t = -4.957$, $p < 0.0001$). In contrast to the plasma cortisol results, the Day 3 urine samples post dexamethasone and ACTH were not significantly higher than the Day 2 post-dexamethasone urinary cortisol levels. Day 3 urinary cortisol in subjects that had received saline and ACTH did not differ from baseline (Sal-ACTH; $t = -0.295$, $p = 0.770$). We should reiterate here that urinary cortisol was first void but not a 24-hour urine collection.

Plasma and urinary cortisol were moderately positively correlated either with ($r = 0.648$, $p < 0.0001$; Figure 4) or without outliers ($r = 0.783$, $p < 0.0001$).

3.3 Urinary Androgens

Urinary androgens did not vary significantly across condition either with ($F_{4,35} = 1.11$, $p = 0.376$; $\eta^2 = 0.15$) or without the samples that had been outliers for cortisol ($F_{4,33} = 0.88$, $p = 0.491$; $\eta^2 = 0.13$; Figure 5). In both cases, female identity was not a significant predictor of urinary androgens.

3.4 Correlations between hormones

With outliers, urinary androgens and cortisol were positively correlated (Figure 6; $r = 0.412$, $p = 0.012$). Without outliers, urinary androgens and cortisol were not significantly correlated ($r = 0.048$, $p = 0.786$).

4. Discussion

This study set out to examine cortisol and immuno-reactive androgens, and correlations between these two hormones in a socially monogamous neotropical primate. Directionality of the cortisol response to DEX and ACTH challenge was as expected, although the DEX response was extended, lasting at least until our final timepoint (37 hours later), even in females that had subsequently received ACTH. Only one other study has examined the effects of DEX on titi monkeys (Mendoza & Moberg, 1985) and this study only examined changes in plasma cortisol 15 minutes post-administration; therefore, it is unclear whether this prolonged blunted response is unusual for this species. It appears that studies examining the effects of DEX on mammals, particularly platyrrhines, do not typically collect blood samples past 12 hours, making this an exciting area for future investigation (see Table 1 in Desantis et al., 2018 for a summary of post-DEX blood draw timelines across several studies conducted in mammals). In contrast, the ACTH response was relatively muted with a less than 20% increase following SAL and ACTH. It is possible that this relatively low response was due to social buffering by the partner (Mendoza et al., 2000) or by the father in the case of one female which was still in her natal group (Hoffman et al., 1995; Mendoza and Mason, 1986). To examine the role of social buffering during a DEX and ACTH challenge, future

studies could test females with and without their attachment figures and compare changes in cortisol in response to treatment conditions.

Plasma cortisol was moderately correlated with urinary cortisol, and followed the expected pattern of change in response to DEX and ACTH challenges. Therefore, urine samples are able to capture changes in circulating cortisol from one day to the next. This finding validates the use of a more non-invasive method to dive deeper into questions related to changes in cortisol over time in titi monkeys. One caveat is that the urinary cortisol did not completely mirror the plasma cortisol data with regards to the Day 3 response to ACTH. In plasma samples, we observed a rise in cortisol for monkeys that received dexamethasone and ACTH, whereas in urinary samples cortisol levels remained blunted compared to baseline but did not rise in comparison to the Day 2 post-dexamethasone levels. Therefore, plasma samples may be better at capturing some of these more dynamic responses to dexamethasone and ACTH. This limitation relates to the potential time-lag between changes in cortisol in blood and the appearance and detection of the hormone in urine. This has not been studied extensively across primate species but the peak of urinary cortisol after injection has been estimated to occur within 5.5 hrs in one study of chimpanzees, long-tailed macaques and marmosets (Bahr et al., 2000), less than 3 hrs in bonobos (Verspeek et al., 2021) and within 1 hr in human subjects where the delay in reaching peak urinary concentrations was about 30 mins relative to plasma. (Jung et al., 2014). Urinary androgen excretion appears to be equally rapid (Mohle et al., 2002). Logistical constraints notwithstanding, more frequent sampling always improves resolution regardless of species. Nevertheless, our data support the conclusion that morning urinary cortisol can detect changes in adrenocortical secretion from the previous day in titi monkeys. Another limitation of our design is that we did not collect additional urine samples to assess female cyclic status, though treatment effects were still detected. Whether or not stage of the cycle influences adrenocortical response is unknown and should be investigated perhaps in future studies.

While plasma and urinary cortisol concentrations were moderately related, we found limited correlations between androgens and either urinary or plasma cortisol. With urinary cortisol, there was a positive correlation with androgens only when extreme outliers were included. While these results may not be biologically meaningful, they could also suggest that conditions that elicit an extreme cortisol reaction also result in a higher androgen response. We would not expect ACTH and DEX to directly affect the HPG axis; however, given how interconnected the HPG and HPA axes can be, it may be indirectly impacted by downstream HPA axis activity (Viau, 2002). Androgens can inhibit the HPA-axis response (Zuloaga et al., 2020); therefore, this concurrent rise in androgens may function to reduce the physiological stress response. In an experimental manipulation meant to provoke “jealousy” in male titi monkeys, the jealousy condition elicited a rise in both plasma cortisol and plasma testosterone (Maninger et al., 2017b); similar data do not exist for female titi monkeys. For the present study, it is possible that either the urine sample was unable to capture rapid changes in androgens in response to cortisol manipulations, or our paradigm was not strong enough to elicit changes in androgens. In the future, it would be helpful to measure plasma androgens as well. It would also be interesting to test this prediction in other socially relevant situations, such as social separations or intruder encounters, as social

challenges have been shown to increase testosterone in male titi monkeys (Maninger et al., 2017b) and humans (for a review, see Cheng and Kornienko, 2020).

Social context can significantly impact physiology, and interactions between steroid hormones and peptides may explain paradoxical relationships between physiological profiles and social behaviors (van Anders et al., 2011). Given that gonadal steroid hormones can impact oxytocin receptor binding in rodents and both testosterone and oxytocin can have inhibitory effects on the HPA-axis (Zuloaga et al., 2020), it would be interesting to study how interactions between cortisol, androgens, and oxytocin mediate the stress response. While we were not able to incorporate peptides into the current study, we hope to use it to set the stage for studying these interactions in future.

5. Conclusions

The current study provided important groundwork for these efforts in studying the behavioral endocrinology of pair bonding in female titi monkeys. Both the plasma and urinary cortisol data supported the biological validity of the cortisol assay. Urinary cortisol was also moderately correlated with plasma cortisol and therefore represents an accurate, non-invasive measure for use in this species. While the DEX and ACTH challenge did not appear to affect circulating androgen concentrations, future studies incorporating social challenges may elucidate the role of androgens and glucocorticoids in female titi monkey responses to social stressors. By studying interactions between steroid hormones as well as neuropeptides, future studies will be able to disentangle the complex physiological processes underlying behaviors important for pair bonding.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

ACTH	adrenocorticotropin hormone
DEX	dexamethasone
HPA	hypothalamic-pituitary-adrenal
HPG	hypothalamic-pituitary-gonadal

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Highlights

- Dexamethasone injection strongly suppresses female titi monkey cortisol
- Females exhibited a significant but blunted response to ACTH stimulation following treatment with dexamethasone
- An assay for titi monkey plasma and urinary cortisol was newly validated
- Plasma and urinary cortisol values were moderately correlated

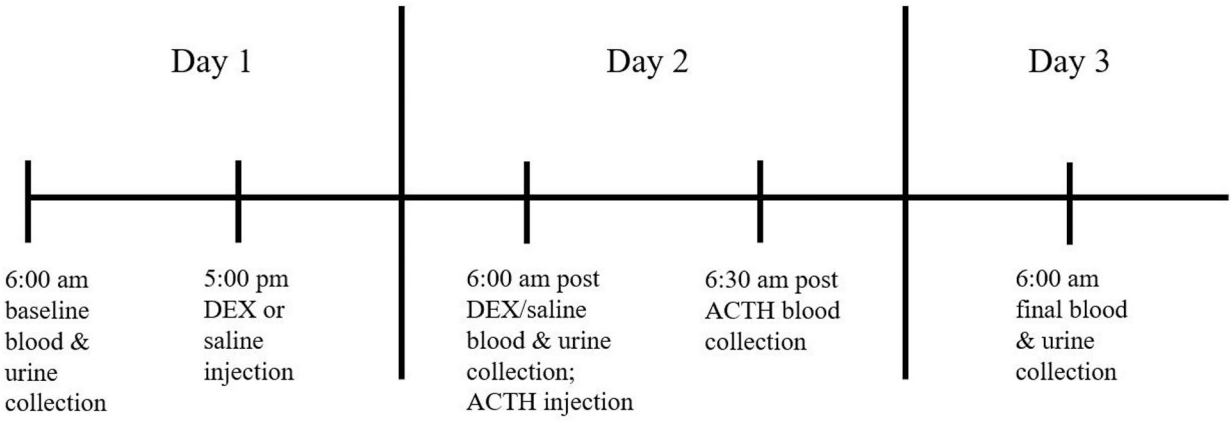

Figure 1.

Study timeline. Half of the females received DEX on Day 1 and half received saline. This process was repeated one week later, and females who received DEX on week one were given saline the second week, and vice versa. DEX, dexamethasone; ACTH, adrenocorticotropin hormone

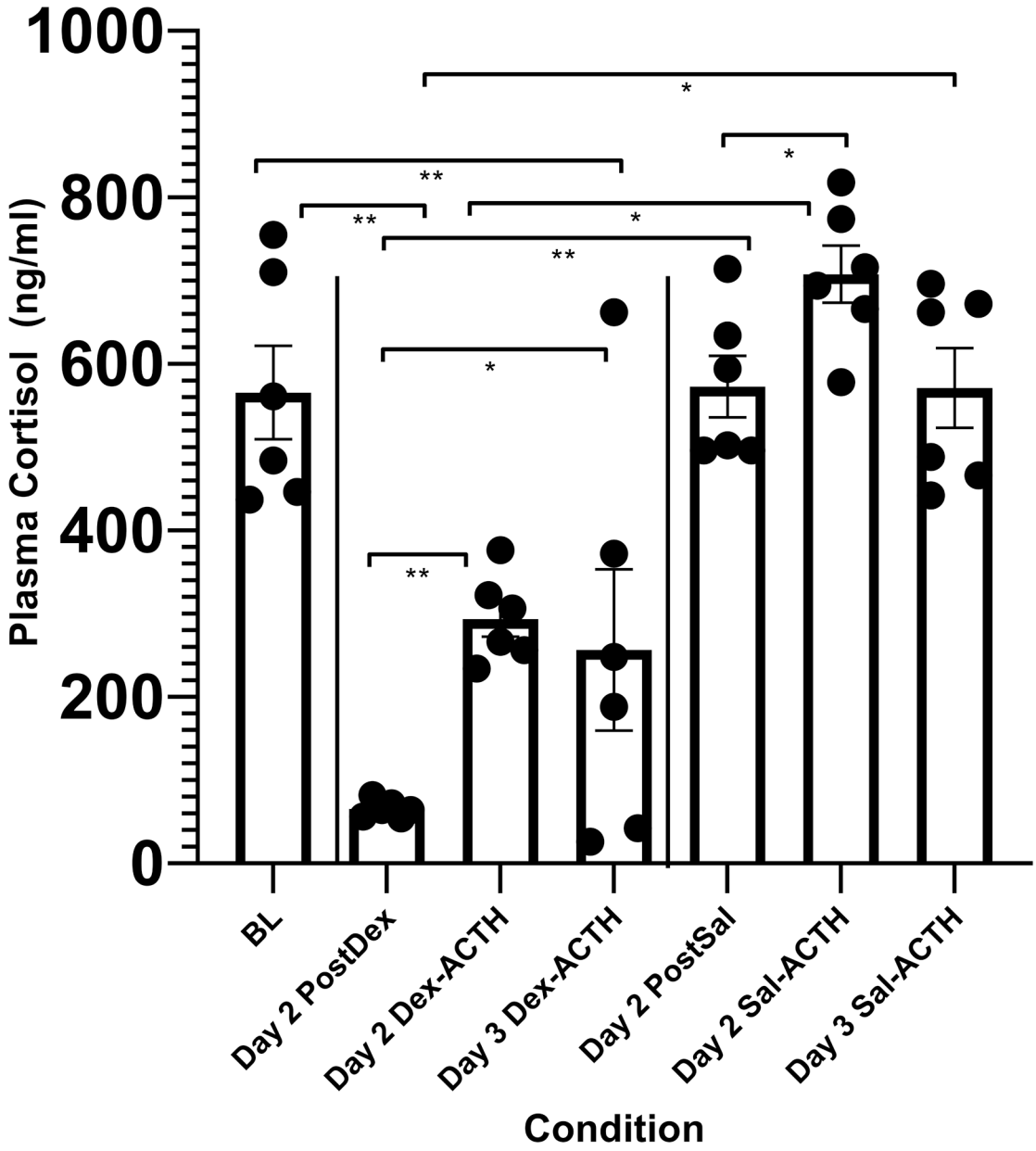

Figure 2.

Plasma cortisol differs significantly by condition ($F_{6,47} = 37.95$, $p < 0.0001$; $\eta^2 = 0.86$). *, $p < .05$; **, $p < .001$; BL, baseline; PostDex, Post-dexamethasone injection; Dex-ACTH, post-dexamethasone and adrenocorticotropin hormone injection; PostSal, post-saline injection; Sal-ACTH, post-saline and adrenocorticotropin hormone injection

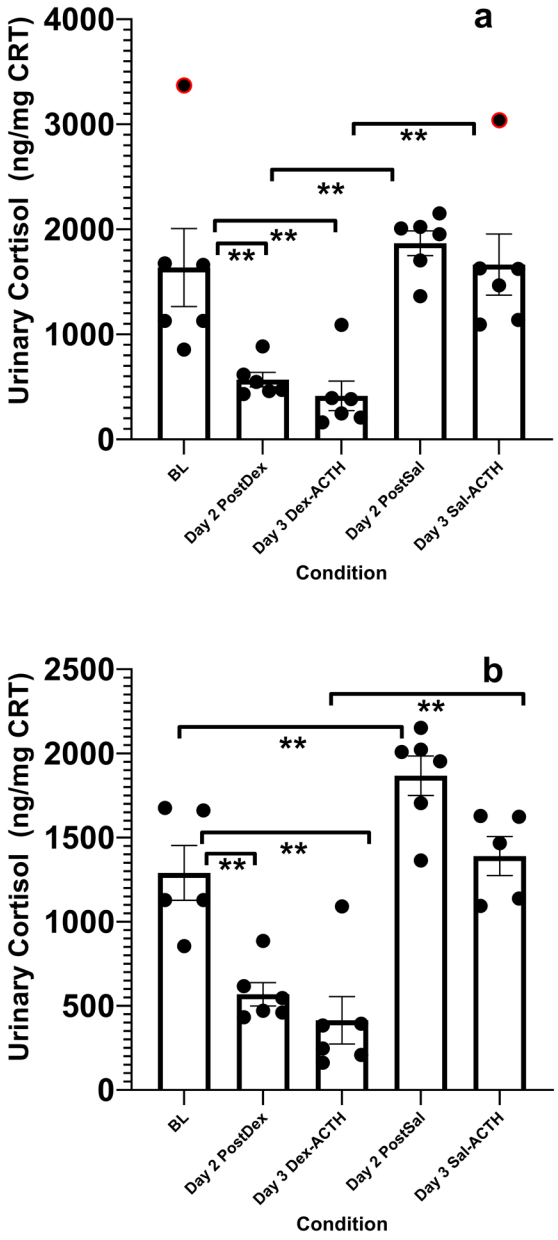

Figure 3.

Urinary cortisol varied significantly across conditions, both with outliers (panel a: $F_{4,35}$ = 6.37, p = 0.001; η^2= 0.50) or without outliers (panel b: $F_{4,33}$ = 32.61, p < 0.0001; η^2= 0.85). Outliers were further than two standard deviations from the mean and are noted in the graph with a red circle around the dot. **, p < .001; BL, baseline; PostDex, Post-dexamethasone injection; Dex-ACTH, post-dexamethasone and adrenocorticotropin hormone injection; PostSal, post-saline injection; Sal-ACTH, post-saline and adrenocorticotropin hormone injection

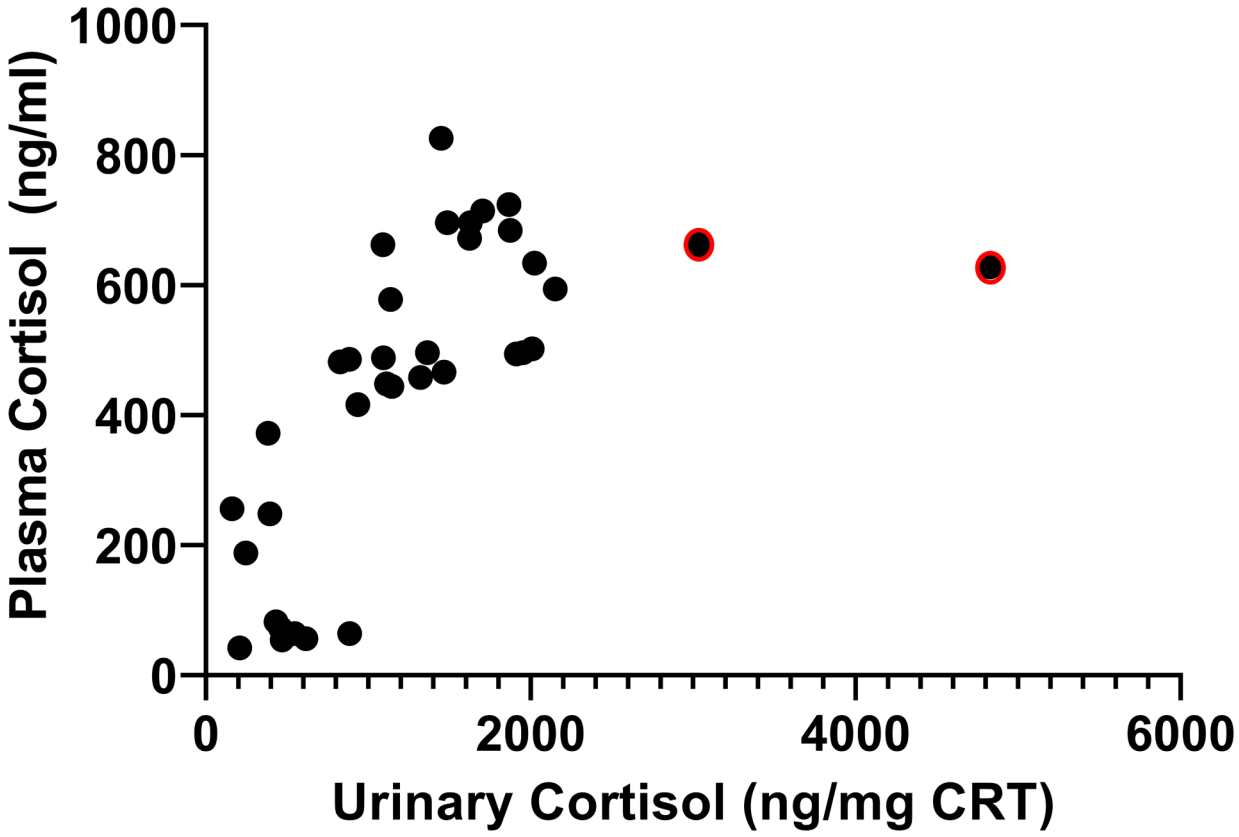

Figure 4.

Plasma and urinary cortisol were moderately positively correlated ($r = 0.648$, $p < 0.0001$). Outliers were further than two standard deviations from the mean and are noted in the graph with a red circle around the dot.

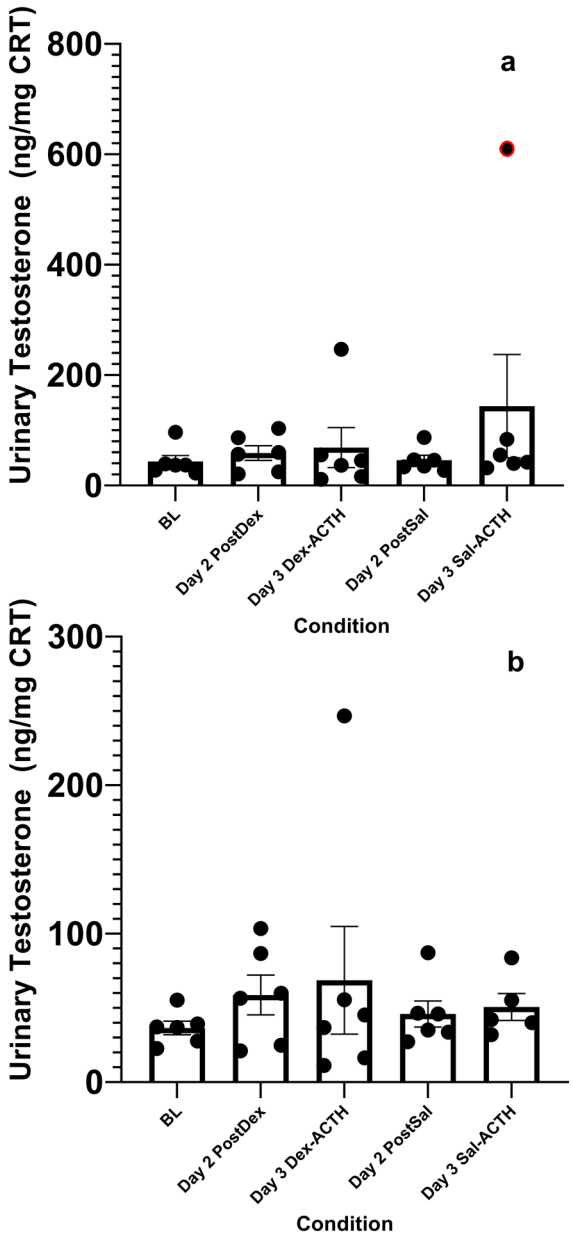

Figure 5.

Urinary androgens did not vary significantly across conditions either with (panel a) or without (panel b) samples that had been outliers for urinary cortisol. Outliers were further than two standard deviations from the mean and are noted in the graph with a red circle around the dot. BL, baseline; PostDex, Post-dexamethasone injection; Dex-ACTH, post-dexamethasone and adrenocorticotropin hormone injection; PostSal, post-saline injection; Sal-ACTH, post-saline and adrenocorticotropin hormone injection

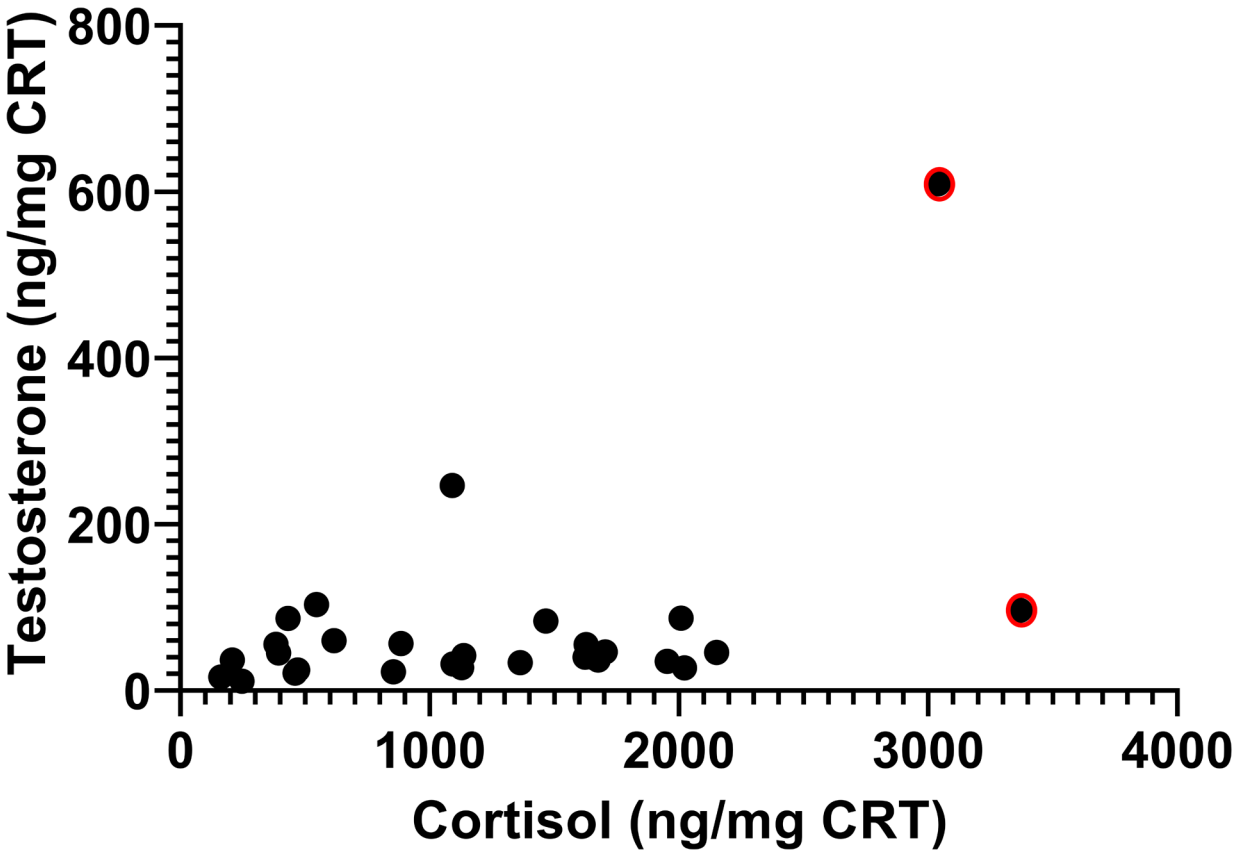

Figure 6.

Urinary androgens and cortisol were positively correlated (r = 0.412, p = 0.012), but only when we included outliers. Outliers were further than two standard deviations from the mean and are noted in the graph with a red circle around the dot.