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The effects of a long term psychosocial stress on reproductive indicators in the baboon

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Abstract

Psychosocial stress is thought to negatively impact fecundity, but human studies are confounded by variation in nutrition and lifestyle. Baboons offer a useful model to test the effect of prolonged mild stress on reproductive indicators in a controlled setting. Following relocation from social groups to solitary housing, a previously documented stressful event for non-human primates, daily urine samples, tumescence and menstrual bleeding were monitored in twenty baboons (*Papio* sp.) for 120–150 days. Specimens were assayed for estrone conjugates (E1C), pregnanediol-3-glucuronide (PDG), follicle-stimulating hormone (FSH), and cortisol. Linear mixed effects models examined 1) the effects of stress on frequency of anovulation, hormone levels, tumescence and cycle length, and 2) the relationship of cortisol with reproductive indicators. Despite cortisol levels indicative of stress, anovulation was negligible (1% in 102 cycles). PDG, FSH, cycle length, and tumescence declined during the first four cycles, but began recovery by the fifth. Cortisol was negatively associated with FSH but not associated with PDG, E1C or tumescence. Ovulation, E1C, and luteal phase length were not affected. Tumescence tracked changes in FSH and PDG, and thus may be a useful indicator of stress on the reproductive axis. Elevated cortisol was associated with reduced FSH, supporting a model of cortisol action at the hypothalamus rather than the gonad. After 4–5 menstrual cycles the reproductive indicators began recovery, suggesting adjustment to new housing conditions. In conclusion, individual housing is stressful for captive baboons, as reflected by cortisol and reproductive indicators, although ovulation, a relatively direct proxy for fecundity, is unaffected.

Keywords

reproductive ecology; cortisol; ovarian steroids; FSH; tumescence

Acute and chronic stressors from psychosocial, metabolic, physiologic, and environmental sources have been shown to disrupt the menstrual cycle in humans and animal models (Ferin 1999; Ferin 2006). Understanding changes in the reproductive axis in response to stress is an important endeavor as hormonal changes, in addition to their potential effects on fecundity and fertility, may have health consequences, including affecting the risk of cardiovascular

disease, reproductive cancer, and osteoporosis (Kaplan and Manuck, 2004). Additionally, understanding how robust or labile the reproductive axis is with respect to stress is important for animal management and breeding, and for insight into the life history and evolution of the reproductive axis in female primates (Caperton et al., 2006; Dobson and Smith, 2000; Kaplan and Manuck, 2004).

A number of studies have shown that social support networks play an important role in the survival and health of humans (e.g., Berkman and Syme, 1979; Uchino, 2006) and other primates (Sterck et al., 1997), including baboons (Silk et al., 2009). Several studies have examined stress induced by removing individuals from their social groups, and found that the move to individual housing represents a significant stress for many primates (Caperton et al., 2006; Laudenslager et al., 1999; Maestripieri et al., 2008; Rowell, 1970).

Several hypotheses have emerged over the last several decades suggesting adaptive suppression of the reproductive axis during times of hardship and stress (Ellison, 2003; Wasser, 1996; Wasser and Barash, 1983). With a basis in life history theory, these hypotheses suggest that during nutritional, immunological, social, or psychological duress, the female reproductive axis down-regulates, preventing reproduction, or even causing spontaneous abortion, conserving energy for survival to a more advantageous reproductive environment (Ellison, 2003; Nepomnaschy et al., 2006; Thompson and Wrangham, 2008; Wasser, 1996; Wasser and Barash, 1983). This study offers an in-depth examination of reproductive hormones for an extended period of socially isolated stress, while simultaneously measuring a hormonal stress indicator. These detailed data make it possible to study psychosocial stress as a potential signal for down regulation of the female reproductive system.

Non-human primate models have several advantages for the study of stress effects on the reproductive axis. Using captive primates allows for control of the confounding effects of metabolic stress and enables experimental protocols not feasible in humans (Edozien, 2006; Ellison et al., 2007). Macaques and baboons have menstrual cycle characteristics, including menstruation, similar to humans (Hendrick and Dukelow, 1995; Stevens et al., 1970). The bulk of experimental research on stress and reproductive hormones in primates has been undertaken in captive macaques, which tend to be seasonal breeders and thus have reproductive axes sensitive to environmental cues (Bercovitch and Goy, 1990). Baboons typically have regular menstrual cycles without seasonal breaks (Stevens et al., 1970), and may thus be a better model for understanding human reproduction and stress, yet few studies have examined baboons (Caperton et al., 2006; Norman et al., 1994a; Rowell, 1970).

Research on stress in non-human primate models has focused on short duration, moderate-to-severe stressors, such as tethering, repeated injections or blood draws (e.g., Bethea et al., 2005; Caperton et al., 2006; Norman et al., 1994a; Norman et al., 1994b; Xiao et al., 1998; Xiao et al., 1999; Xiao et al., 2002; Xiao et al., 1996; Xiao et al., 2007). Studies in humans and non-human primates suggest that a stress must be fairly severe in order to produce chronic anovulation or amenorrhea (Cameron, 1997; Ellison et al., 2007; Ferin, 1999; Nepomnaschy et al., 2004). Few studies have monitored in detail the longer term effects of stress exposure on the reproductive axis (Rowell, 1970; Sopelak et al., 1983; Watson et al., 1998) or focused on less severe stress (Williams et al., 2007). Additionally, most studies in non-human primates have used experimental designs to examine the role of stress (as indicated by cortisol elevation) in suppression of the HPG axis (Hayashi and Moberg, 1990; Norman et al., 1994a; Van Vugt et al., 1997; Xiao et al., 1998; Xiao et al., 1999; Xiao et al., 2002; Xiao et al., 1996; Xiao et al., 2007). To date, there have been no studies in non-human primates using non-invasive and observational methods to examine the association of cortisol with reproductive indicators.

The mechanisms by which stressors disrupt the hypothalamic-pituitary-gonadal (HPG) axis are not well understood. Activation of the hypothalamic-pituitary-adrenal (HPA) axis, which culminates in the production of the glucocorticoid hormone cortisol, in conjunction with suppression of the HPG axis has been demonstrated in several studies in non-human primates (e.g. Cameron, 1997; Ferin, 1999; Hayashi and Moberg, 1990; Xiao et al., 1998; Xiao et al., 1999; Xiao et al., 2002; Xiao et al., 1996; Xiao et al., 2007). However, there are also studies reporting HPA axis response without HPG suppression in some or all animals (Cameron, 1997; Van Vugt et al., 1997). Other studies find evidence suggesting that corticotrophin-releasing hormone (CRH) modulated β -endorphins, or other aspects of the autonomic nervous system, particularly the locus ceruleus norepinephrine system (LC/NE) may cause down regulated hypothalamic gonadotropin-releasing hormone (GnRH) secretion (Chrousos and Gold, 1998; Ferin, 1999). While it is generally accepted that one of the chief impacts of stress on the HPG axis involves reduced secretion of GnRH in the hypothalamus and consequent reduced secretion of luteinizing hormone (LH) and FSH from the anterior pituitary (Ferin, 1999; Tilbrook et al., 2002), some evidence suggests that stress may act upon the HPG axis by direct inhibition via glucocorticoids at the level of the gonads instead of via the hypothalamus (Figure 1) (Tsigos and Chrousos, 2002).

The current study examines the effects of a mild but prolonged psychosocial stress on reproductive indicators in 20 baboons monitored prospectively in individual cages for up to 5 menstrual cycles. Our objectives were to use a baboon model to 1) examine the effects of a low-level psychosocial stress—individual caging—on reproductive hormones, tumescence, ovulatory status and cycle length across a prolonged period of observation (4–5 months); and 2) examine the association of a measure of stress, cortisol, with indicators of reproductive function.

MATERIALS AND METHODS

Animals and experimental protocol

At the Southwest Foundation for Biomedical Research in San Antonio, Texas, twenty female baboons (*Papio* sp.) aged 15–19 years were moved from outdoor social cages housing ten to twenty baboons each into indoor individual cages. Thirteen females came from groups with a vasectomized male, three from breeding groups (one intact male, multiple females), and four from all female groups with no male.

Animals retained visual and auditory contact while individually caged. Sixteen animals were individually caged for 150 days, and four for 120 days. Based on tumescence and menstrual bleed patterns assessed every other day, ten baboons were moved indoors during the luteal phase (LPM-luteal phase move) and ten during the follicular phase (FPM-follicular phase move). The move entailed immobilizing the animals (10 mg/kg ketamine, IM) and anesthesia with isoflurane for muscle biopsy. Other data collections at the time of transfer included blood and urine collection and dual energy X-ray absorptiometry. The animals were sedated 10–14 days later for suture removal at the biopsy sites. No other procedures were performed on the animals throughout the remainder of the study protocol. The animals had unlimited access to water, were fed about 1 pound Teklad (17% protein) per day (routine diet for both gang and individual cages) and were kept on a 6 am to 6 pm light cycle. The baboons in this study had parities ranging from 1 to 11 offspring.

Daily twenty-four hour urines were collected in pans, underneath the individual cages, covered with a double layer of fine wire mesh. Urine was collected in containers surrounded by ice. A 30 mL aliquot was frozen and shipped to the lab for assay. Tumescence and menstrual bleeding were recorded daily. Tumescence was indexed (TI) from 0 to 4 for

swelling size relative for each specific female, where 0 indicated absence of swelling and 4 represented maximal swelling (Shaikh et al., 1982).

All procedures were approved by the Institutional Animal Care and Use Committee of the Southwest Foundation for Biomedical Research, San Antonio, TX.

Assays

Urine specimens were assayed in duplicate using enzyme immunoassays for estrone conjugates (E1C, urinary metabolites of estradiol), pregnanediol-3-glucuronide (PDG, a urinary metabolite of progesterone), intact FSH and cortisol. The E1C enzyme immunoassay (EIA) cross reacts 100% with estrone-3-glucuronide, free estrone, and estrone-3-sulfate, and less than 1% with other estrogens. (O'Connor et al., 2003). The E1C EIA had a lower limit of detection of 129 pg/mL, and the inter-assay and intra-assay coefficients of variation for a high control were 3.6% and 8.2%, respectively.

The PDG EIA used the polyclonal R13904 antibody (C. Munro, UC Davis) which cross reacts 100% with pregnanediol-3-glucuronide, 45% with 20 α hydroxy-4-pregnen-3-one, and less than 10% with other progestins. The assay format is identical to a PDG EIA using the Q330 monoclonal antibody (O'Connor et al., 2003) but has a lower limit of detection (5 ng/mL) better suited to baboon urine specimens. Slight non-parallelism (parallelism was defined as slope = 0 for a range of dilution corrected hormone concentrations plotted against urine volume) was demonstrated for 6 serially diluted (from neat to 1:32) baboon urine specimens (slope = $.075 \pm .014$, $p < .0001$); no correction was made for this non-parallelism as the CV's averaged only 16% across dilutions. Recovery averaged 92 % across low, medium and high spiked specimens from 6 baboons. The inter-assay and intra-assay coefficients of variation for a high control across 20 plates were 14% and 4.5%.

A solid phase two site sandwich immunoassay (IEMA) was used to estimate the urinary levels of intact FSH. The assay used a primary monoclonal antibody directed against human FSH (Brindle et al., 2006), in an assay format identical to a human β FSH IEMA (Brindle et al., 2006), but had a secondary polyclonal anti-recombinant baboon FSH antibody (BIOQUAL 67190; from A.F. Parlow, NIDDK). Slight but significant non-parallelism was demonstrated for 7 serially diluted baboon urine specimens (slope = $-.017 \pm 0.04$, $p = .0003$). This non-parallelism did not affect our results as all specimens were run undiluted. Recovery of BioRad Immunoassay Controls Levels 1, 2 and 3 averaged 97% in urine specimens from six baboons. The baboon intact FSH IEMA cross reacted less than 1% with baboon β FSH, intact LH and LH α and β subunits. The inter- and intra-assay coefficients of variation for a high control tested on 20 plates were 5.8% and 8.2%, respectively. The FSH IEMA had a lower limit of detection of 0.38 pg/mL; twenty-five percent of our specimens were below this limit and these were coded as missing for statistical analyses, but assigned a value of 0.38 pg/mL for the graphs.

Cortisol was measured using an EIA with the polyclonal antibody R4866 (Morrow et al., 2000; Munro and Stabenfeldt, 1985; Munro, 1985). The assay cross-reacts 100% with cortisol and less than 10% with other steroids tested. Inter- and intra-assay CVs were 13.9 % and 4.6% for the high control (n=20 plates for each). The minimal detectable dose was 302 pg/mL. Recovery in human urine specimens averaged 94% across spiked low, medium and high doses. Parallelism of a standard curve with 7 serially diluted (1:4 to 1:32) human urine specimens was demonstrated (slope = $.061 \pm .035$, $p = .11$).

Hormone concentrations were estimated from optical density with a Dynatech MR7000 Plate Reader (test wavelength 405 nm, reference wavelength 570 nm) and a four parameter logistic model (Rodbard 1974) in Bioline 1.0 Software (Dynex Laboratories, Inc., Chantilly,

VA). Commercial standards for steroid metabolites (estrone- β -D-glucuronide, Sigma Catalog No. E1752; 5 β -pregnane-3 α , 20 α -diol glucuronide, Sigma Catalog No. P3635; 4-pregnen-11 β , 17, 21-triol-3, 20-dione, Steraloids, Catalog No. Q3880), baboon recombinant FSH calibrators (AFP6944A, from A.F. Parlow, NIDDK), and in-house urine controls were used in the assays.

To adjust for hydration status, urinary hormone values were corrected by specific gravity using a population mean specific gravity of 1.010 (Miller et al., 2004).

Statistical methods

Menstrual cycle day was computed using the first day of menstrual bleeding as cycle day 1, where the bleed episode had to consist of at least 1 day of bleeding and be preceded by at least 5 consecutive days of no bleeding to be considered menses. Censored and complete cycles were tested for the presence and day of ovulation, using FSH, PDG and E1C data in a hierarchical, multi-method algorithm (O'Connor et al., 2006). Day of ovulation was used to divide cycles into follicular and luteal phases. We used pre-move bleeding and tumescence data collected every other day (except on weekends) to calculate cycle length just prior to the move. A linear mixed effects model was used to compare the length of the pre-move cycle to cycle length immediately following the move.

For each cycle, total PDG area under the curve (AUC), FSH AUC, E1C AUC, cortisol AUC and tumescence AUC were calculated using the trapezoidal method. Linear mixed effects models were used to estimate how reproductive indicators and cortisol varied across successive cycles, differed between the FPM and LPM baboons, and whether reproductive indicators were associated with cortisol levels. These models control for repeated measures by estimating a separate error term due to variability among subjects in addition to the residual error term representing within subject variability. Fixed effects were cycle number, modeled as a 5 category factor (1 thru 4 and >4) to capture non-linear trends, and cycle phase at time of move (PM). Interactions between cycle number and PM were added to models to assess if the relationship between AUC and cycle number differed by PM. All models of AUC were adjusted for cycle length to control for differences among and within animals in cycle length, and to control for the presence of right and left censored cycles at the start and end of the study. As the variability of AUC tended to increase with increasing mean, a variance function was added to achieve homogeneity of variances. To determine if the variability of AUC measures differed by PM group, a model allowing for different variances by PM group was compared against a model with no variance differences, using likelihood ratio tests (Pinheiro and Bates 2000). Analyses were carried out using STATA 11.1 (College Station, TX) and R 2.6.1 (Team 2007).

RESULTS

One-hundred fifty days of daily specimens were collected from 7 FPM animals and 9 LPM animals, with the remaining 3 FPM and 1 LPM animals contributing 120 daily specimens. A total of 102 cycles from 20 animals, including right-censored, left-censored and complete cycles were examined in this study. The age distribution for LPM vs. FPM animals is shown in Table 1.

Figures 2 panels A–D show individual-level changes in steroid, gonadotropin and cortisol patterns across the observation period for two LPM (panels A and B) and two FPM (panels C and D) animals; these provide illustrative examples of the statistical trends discussed below.

Ovulation

Of 75 complete cycles for which both presence and day of ovulation could be evaluated, 74 were ovulatory and 1 was anovulatory (1.3% rate of anovulation). Adding 7 left censored cycles, which had 20 or more days in the censored segment where ovulation day could be estimated by the algorithm, still identifies only 1 anovulatory cycle (1/82 cycles = 1.2% rate of anovulation). There were 8 right censored cycles with insufficient information for assessing whether ovulation occurred or not, as these cycles were censored in the early follicular phase. There were 10 left censored cycles cut off in the luteal phase (ranging from 4 to 15 days of observation); of these, 7 had clear PDG peaks and descents to menses, whereas three had indeterminate ovulation status on the basis of PDG or any other indicator. On the whole, there was no evidence that individual caging for up to 4–5 cycles affected ovulatory status.

Cycle length

Sufficient pre-move cycle length information was available for only 18 of the 20 animals. For these 18 animals, there was no difference in the length of the pre-move cycle and the cycle encompassing the move ($p=0.557$; pre move cycle, average length = 33.38 (SD 11.05) days; post-move cycle, average length = 35.11 (SD 20.99) days). Post-move cycle lengths, and follicular and luteal phase lengths (mean, SD) by take-in group (LPM, FPM), and for the total sample for complete cycles only, are shown in Table 2. In a linear mixed effects model, cycle length did not differ between the LPM and FPM animals ($p=0.6$). Cycle length decreased with increasing cycle number (i.e., increasing time spent in individual housing) ($p<0.001$), by an average of 1 day for each additional cycle; this trend did not differ between LPM and FPM animals ($p=0.9$) (Figure 3a). There was no association of follicular or luteal phase length with take-in phase, but, as with total cycle length, there was a decline in follicular phase length, by about 1 day, with each increasing cycle number ($p<0.01$); this association did not differ by take-in group ($p=0.8$).

Reproductive hormones

Figure 3b shows the composite trend of PDG AUC across the time period of observation for the 20 animals as estimated by a linear mixed effects model, adjusting for cycle length. PDG AUC and cycle number were inversely associated ($p<0.01$) up to the fourth cycle. In both take-in groups PDG appears to begin recovery by the fifth cycle. Overall PDG AUC did not differ between take-in groups ($p=0.6$) and there was no difference in variance (assessed by SD) in PDG AUC between the take-in groups ($p=0.8$).

FSH AUC had a similar association with cycle number ($p<0.0001$): decreasing from the first to the fourth cycle, and then increasing (Figure 3c). FSH AUC did not differ between take-in groups ($p=0.5$), but the variance of FSH AUC was higher in the FPM animals by about a factor of 1.5 ($p=0.016$), even after excluding a cycle in the FPM group with a large FSH AUC.

E1C AUC did not vary by cycle number ($p=0.4$) (Figure 3d), and this null association was the same for both the FPM and LPM animals ($p=0.5$). E1C AUC was higher in FPM than LPM animals by about 20,000 pg/mL, on average ($p=0.022$), and the variance in E1C AUC was also higher in FPM than LPM animals by a factor of about 1.9 ($p<0.001$), as variance tended to increase with increasing mean.

Tumescence

Analyses of tumescence (TI) and cycle number excludes the first (censored) cycle for the LPM baboons, as tumescence can only increase from the late luteal phase in the first cycle to a full second cycle. Tumescence AUC was significantly associated with cycle number ($p<$

0001); it declined from the first to second cycle in the FPM baboons, and then for both groups it remained statistically unchanged from the second to fourth cycles and increased in the fifth cycle. (Figure 3e).

The association of cortisol and reproductive indicators

Cortisol AUC had a significant relationship with cycle number, ($p < .0001$) with cortisol increasing up to the third cycle and then falling thereafter (Figure 4a). There was a slight difference in this pattern by take-in group ($p = .029$) with cortisol AUC peaking higher and dropping more steeply in the FPM group. The FPM group also had higher mean cortisol AUC ($p = .018$) and higher variability ($p < .0001$) than the LPM group (Figure 4b).

Adjusting for cycle number and take in group, cortisol AUC was not associated with PDG AUC, E1C AUC or tumescence AUC ($p > .1$), but was negatively associated with FSH AUC ($p = .0085$).

DISCUSSION

The SFBR baboons usually live in gang cages with established dominance and social hierarchies. In this study, changes in reproductive and glucocorticoid hormones characteristic of responses to stress (Ferin, 1999) were evident in animals kept in individual cages for up to 150 days, supporting that individual housing was stressful for most of the animals. Ovulation, luteal phase length and E1C AUC were not affected, but PDG, FSH, tumescence and follicular phase and total cycle lengths declined from the first to the fourth cycles, and showed evidence of recovery by the fifth cycle (Figure 3, Table 2), suggesting that approximately 4–5 cycles are needed before the animals adapt to their new housing conditions. Despite evidence of stress from elevated cortisol and depressed PDG and FSH, ovulation was not impaired.

Few previous studies have examined effects on the reproductive axis of a move from social to individual cages. Similar to our findings, Rowell (Rowell, 1970) monitored baboons for 4–5 menstrual cycles and reported that the early phase of the menstrual cycle shortened in baboons moved from group to individual cages; this was attributed to reduced social stress from fighting and aggression. In contrast, a study of macaques moved from group to single housing for up to 12 months found no effect on luteal phase progesterone levels (sampled three times per week throughout the study), despite heart rate evidence that social isolation was stressful (Watson et al., 1998).

A surprising finding was that cycle length significantly declined across the study, by about 1 day with each successive cycle; this was entirely due to a shortening of the follicular phase. This is similar to the findings of Rowell (Rowell, 1970), but in contrast to stress studies that report a lengthening of the cycle from a prolonged follicular phase (Caperton et al., 2006; Todd et al., 1999; Williams et al., 2007; Xiao et al., 1998; Xiao et al., 2002), or no change in cycle length (Xiao et al., 1999; Xiao et al., 2007). In our study, cycle lengths began to recover by the fourth or fifth cycle (Figure 3).

A limitation of our study is lack of pre-stress hormonal information so it is not clear whether the first cycle or subsequent cycles represent low or high stress cycles. This is a common limitation of studies examining stressors in non-human primates, including studies of primate housing (Laudenslager et al., 1999; Maestriperieri et al., 2008); obtaining pre-stress hormonal data in a manner that does not itself constitute a stress is usually nearly impossible. Pre-stress information on tumescence or cycle length has been used in some housing studies (Caperton et al., 2006; Rowell, 1970). We found no difference in cycle length between the pre-move cycle and immediate post-move cycle. Subsequent cycle

lengths declined from the immediate post-move cycle. One interpretation of this is that the impact of changed housing on cycle length is not immediate, but delayed. Without pre-move data we are not able to assess if hormonal indicators or tumescence follow a similar pattern. Regardless of the lack of pre-move data, our data suggest what the effects of a change from social to individual housing might be, for example, for studies wishing to examine longitudinal age-related changes in reproductive parameters in individual animals. For such studies it is crucial to have some understanding of how experimental conditions such as single housing might affect the reproductive parameters in question. Our study suggests that some but not all hormonal and cycle level parameters are in flux for several months under these conditions.

The steady decline in FSH and PDG across the first few cycles in our study indicates that we are capturing common responses to stress (Ferin, 1999). Although cortisol is elevated in most animals at the beginning (Figure 2), the trend in cortisol does not suggest continual stress or a decline in stress (Figure 4a). Instead, cortisol increased from the first to the third cycle, and then declined through the fourth and fifth cycles. This pattern was influenced by a period of elevated cortisol in the third to fourth cycles of 12 of the animals (e.g., Figure 2). This date-linked (beginning around July 25–29) increase lasted about 30 days and was present in equal numbers of FPM and LPM baboons, with overall higher levels of cortisol in the FPM baboons (Figures 4a and 4b). It is not clear what caused this period of elevated cortisol. We could not ascertain if there was a change in lab personnel or some other disruptive event occurring during this time.

Like other studies in non-human primates (Bethea et al., 2005; Cameron, 1997), we found individual heterogeneity in cortisol response (e.g., Figure 2, Figure 4b) to both the initial individual caging, and the unknown event occurring mid-study. We do not have social rank data, so cannot examine its effect on cortisol variation. For lower ranking animals individual caging could have lowered stress, due to reduced threats and aggression directed toward them (Rowell, 1970), and improved access to food (Silk, 1987). Generally, however, social isolation is reported to be stressful (Caperton et al., 2006; Laudenslager et al., 1999; Maestripieri et al., 2008; Rowell, 1970). Some of the animals in our study had previous experience with individual caging (though never for as long as this study) which may have moderated their stress responses.

We found that FSH significantly and monotonically declined for up to four cycles following the housing change (Figure 3), and cortisol and FSH were significantly inversely associated. Together, these results support the HPA axis as a modulator of FSH secretion. We did not have sufficient power to assess whether animals with higher cortisol were the ones with suppressed FSH. While inspection of individual graphs tentatively suggests that this is not the case (Figure 2), further research with a larger sample of animals is needed to adequately address this question. This study design examined the effects of HPA activation on reproductive indicators, but did not examine other potential stress pathways, such as autonomic nervous system actions, or peripheral HPA effects. Some studies in non-human primates reported suppression of gonadotropins following a stress (Hayashi and Moberg, 1990; Norman et al., 1994a; Norman et al., 1994b), but others have either noted a rise in FSH at the time of, or immediately following, stress (Todd et al., 1999; Xiao et al., 1998), or found no effect of stress on FSH levels at the time of stress or after the stress (Williams et al., 2007; Xiao et al., 1999; Xiao et al., 2002; Xiao et al., 2007). One explanation for these disparate results is that stress effects on the HPG axis may depend on the severity, length and type of stress (Ellison et al., 2007; Ferin, 1999; Xiao et al., 2007).

Reduced luteal phase progesterone is a fairly consistently observed HPG response to stress, but little is known about the pathways leading to this (Tilbrook et al., 2002; Xiao et al.,

2002). Studies in non-human primates find reduced progesterone in response to stress, associated with reduced luteinizing hormone (LH), often lasting well past the cycle in which the stress occurred (Hayashi and Moberg, 1990; Williams et al., 2007; Xiao et al., 1998; Xiao et al., 1999; Xiao et al., 2002; Xiao et al., 2007). Inadequate gonadotropin support is one proposed mechanism for reduced secretion of progesterone from the corpus luteum (Dobson and Smith, 2000); another is direct inhibition by glucocorticoids at the level of the gonads (Figure 1) (Tsigos and Chrousos, 2002). In our study there was no association between cortisol and PDG levels, suggesting that reduced gonadotropin support may explain our findings of decreased PDG. It is interesting that despite an effect on FSH, PDG and cortisol were not associated. We did not measure LH, the principal gonadotropin regulator of luteal phase progesterone (see Figure 1) (Gard, 1998). Other possible explanations include 1) the effects of cortisol on PDG are lagged because PDG is far downstream from the effects of cortisol at the hypothalamus, or 2) secretion of PDG occurs in the luteal phase, while FSH secretion is dominant in the follicular phase.

Insufficient gonadotropin support is also hypothesized as the cause of delayed follicular growth, reduced follicular phase E2 and prolonged follicular phases in response to stress (Dobson and Smith, 2000). Although we found suppressed FSH, which is the principal gonadotropin regulator of estradiol (Gard, 1998), we did not find any effect on E1C levels, nor did we find any association between cortisol and E1C. Moreover, we found that follicular phase length shortened with increasing time spent in individual housing. There is no clear consensus in the non-human primate literature regarding the effect of stress on estrogen; studies have reported no effect on pre-ovulatory peak E2 (Xiao et al., 1999; Xiao et al., 2002; Xiao et al., 2007), a decrease in peak E2 and follicular phase E2, (Hayashi and Moberg, 1990; Williams et al., 2007; Xiao et al., 1998), an increase in peak E2 (Xiao et al., 1998) or a delayed E2 peak (Xiao et al., 2002). Our analyses differ from others by focusing on total rather than peak or phase-specific levels of estrogen; and together with our cycle length findings they might suggest that follicular growth is not significantly delayed or impaired by the stress of long term individual housing. However, we note our immunoassay measures multiple forms of estrogen metabolites, and thus may not be sensitive enough to detect small but meaningful physiological changes in circulating estradiol. Moreover, the decrease in tumescence in our data supports a decline in circulating estradiol.

Tumescence is thought to be initiated and maintained by estrogen (Gesquiere et al., 2007; Zuckerman and Parkes, 1939) while de-tumescence is stimulated by progesterone (Gillmann, 1940). Tumescence AUC tracked the trend in PDG and FSH across the study period (Figure 3e). Tumescence may thus be a useful indicator of the effects of stress on the menstrual cycle of baboons. Rowell (Rowell, 1970) also found that the amount of time spent in tumescence declined when baboons were singly housed for an extended period.

The endocrine environment at the time of a stress may modulate the effect of stress on the HPG axis (Ferin 1999). The decline and recovery pattern of FSH, PDG, cycle length and tumescence in our study did not differ between FPM and LPM baboons; but we did find higher means and variance in E1C and cortisol AUC and higher variance in FSH AUC in FPM animals, indicating that the follicular phase may be more sensitive than the luteal phase to stress. Our results concur with most studies finding that the follicular phase is more sensitive to stressors than the luteal phase (Todd et al., 1999; Xiao et al., 1998; Xiao et al., 1999; Xiao et al., 2002). Xiao and colleagues (Xiao et al., 1999; Xiao et al., 2002) also measured cortisol, but unlike our study they did not find any difference in cortisol response between macaques stressed in the luteal and follicular phases. Our finding of greater cortisol responsiveness in FPM animals is heavily influenced by the unexplained stress event occurring mid-study, which occurred in 6 FPM and 6 LPM animals (Figure 4a).

Despite elevated cortisol and suppressed FSH, PDG, tumescence and follicular phase lengths in our study, the frequency of anovulation was only about 1%. Our results indicate that ovulation is robust to mild and moderate stress. On the whole, our findings are consistent with much of the literature: mild to moderate stress is associated with reduced gonadotropins and progesterone but has little to no effect on ovulation. This includes inflammatory challenges (Xiao et al., 1998; Xiao et al., 1999; Xiao et al., 2007), tethering (Caperton et al., 2006; Sopelak et al., 1983; Xiao et al., 2002); housing changes (Caperton et al., 2006; Watson et al., 1998; Xiao et al., 2002), and multiple stressors occurring simultaneously (Williams et al., 2007; Xiao et al., 2002). It is not clear whether reduced hormone levels, including luteal phase deficiency, are indicative of compromised fecundity. Some research suggests that fecundity varies with ovarian steroid levels (Ellison, 1990; Ellison, 1994; Ellison, 2003). While two studies suggest that luteal progesterone is higher in conceptive versus non-conceptive cycles (Baird et al., 1999; Stewart et al., 1993), most studies find no evidence of progesterone differences between conceptive and non-conceptive cycles (Li et al., 2001; Lipson and Ellison, 1996; Venners et al., 2006; Vitzthum et al., 2006; Vitzthum et al., 2004). There is minimal support for estrogen mediated fecundity; several studies report higher estrogen in conceptive cycles (Lipson and Ellison, 1996; Stewart et al., 1993; Venners et al., 2006), while other larger studies find no difference between estrogen in conceptive and non-conceptive cycles (Baird et al., 1999; Li et al., 2001). Whether insufficient luteal progesterone or shortened luteal phases, commonly known as luteal phase deficiency or defect, are associated with infertility is controversial (Shivapathasundram et al., 2010).

CONCLUSIONS

The stress of extended individual housing significantly reduced FSH, PDG, tumescence, and follicular phase length, but did not affect ovulation or luteal phase length. Tumescence tracked the changes in FSH, PDG, and cycle length, and may therefore be a useful external indicator of the impact of stress on the HPG axis. Our findings indicated that cortisol AUC was directly associated with pituitary (FSH) but not ovarian (E1C, PDG) hormone levels, supporting the role of cortisol as a mediator of stress on the reproductive axis at the level of the hypothalamus or pituitary. Finally, reproductive indicators showed evidence of recovery by the fourth or fifth cycle, suggesting that the animals were beginning to adapt to the new housing conditions. We conclude that for captive baboons, individual housing is stressful, as reflected by both cortisol and reproductive indicators, although ovulation, a relatively direct proxy for fecundity, was unaffected.

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Fig. 1.

Stress can down regulate the hypothalamic-pituitary-gonadal axis at several locations, resulting in suppression of circulating reproductive hormones. GnRH, gonadotropin releasing hormone; FSH, follicle stimulating hormone; LH, luteinizing hormone. A color version of this figure may be viewed in the online issue, which is available at <http://onlinelibrary.wiley.com/>.

Figure 2A

Figure 2B

Figure 2C

Figure 2D

Fig. 2.

Illustrative examples of urinary cortisol, estrone conjugates (E1C), pregnanediol-3-glucuronide (PDG), follicle stimulating hormone (FSH), and tumescence index (TI) across the observation period for two luteal phase move (LPM) (panels A and B) and two follicular phase move (FPM) baboons (panels C and D). SG adj., specific gravity adjusted.

Fig. 3.

Estimates of average reproductive indicator by cycle number and phase move group for cycle length (panel A), pregnanediol-3-glucuronide (PDG) area under the curve (AUC) (panel B), follicle stimulating hormone (FSH) AUC (panel C), estrone conjugates (E1C) AUC (panel D), and tumescence index (TI) AUC (panel E) by cycle number from linear mixed effects models adjusting for cycle length (panels B through E).

Fig. 4.

Estimates of average cortisol area under the curve (AUC) by cycle number and phase move group from a linear mixed effects model adjusting for cycle length (panel A). Cortisol AUC (divided by cycle length) by cycle number: left side follicular phase move (FPM) group, right side luteal phase move (LPM) group (panel B).

Table 1

Ages of LPM and FPM animals

	LPM (luteal phase move)	FPM (follicular phase move)
number of animals	10	10
average age (years)	17.74	17.71
min age	15.8	16.5
max age	19.2	18.6

Cycle and phase length characteristics for LPM (luteal phase move) and FPM (follicular phase move) animals; based on complete cycles only

Table 2

	FPM Group		LPM Group		Total Sample	
	Mean # Days (SD)	N (cycles)	Mean # Days (SD)	N (cycles)	Mean # Days (SD)	N (cycles)
Total Cycle Length	33.0 (3.7)	26	31.8 (3.8)	38	32.3 (3.8)	64
Follicular Phase Length	15.7 (4.2)	31	14.9 (3.2)	41	15.3 (3.6)	72
Luteal Phase Length	16.6 (3.5)	34	16.9 (2.9)	39	16.8 (3.2)	73