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Gonadal Steroid Modulation of the Limbic–Hypothalamic–Pituitary–Adrenal (LHPA) Axis Is Influenced by Social Status in Female Rhesus Monkeys

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Chronic stress can have a deleterious effect on the reproductive axis that, for females, is manifested in an increased incidence of infertility. However, gonadal steroids may, in turn, affect a female's response to stress as measured by activity within the limbic–hypothalamic–pituitary–adrenal (LHPA) axis. What is not clear is whether a history of exposure to stress modifies the effect of gonadal steroids on LHPA responsivity. Rhesus monkeys present a unique opportunity to assess LHPA responsivity when housed socially in groups. Under these situations, monkeys exhibit a rich network of affiliation and have established social status hierarchies. Previous work indicates that socially subordinate macaque females are hypercortisolemic due to diminished glucocorticoid negative feedback. The present study tested the hypothesis that estradiol (E_2) would decrease glucocorticoid negative feedback, assessed from a dexamethasone (DEX) suppression test, and increase the response to corticotropin releasing factor (CRF) and that these effects would be attenuated by co-treatment with P_4. In addition, we also determined whether E_2 and P_4 would differentially affect LHPA responsiveness to pharmacological challenge in socially dominant compared with subordinate females. Endogenous gonadal hormone secretion in female rhesus monkeys (n = 7) was suppressed by continuous treatment with a sustained release formulation of the GnRH analog leuprolide acetate (Lupron Depot). The response to a combined DEX suppression–CRF stimulation test was assessed using a counterbalanced design during a placebo (control) treatment condition and during E_2, P_4, and E_2 + P_4 replacement therapy. Females who were members of a large breeding group of 140 adults and juveniles of both sexes, were classified as dominant (n = 4) or subordinate (n = 3) based on the relative social dominance positions within the group. Plasma levels of cortisol were significantly higher during E_2 replacement compared to the other treatment conditions following DEX suppression and stimulation with CRF. Escape from glucocorticoid negative feedback, assessed as the increase in cortisol following maximum suppression by DEX and prior to stimulation by CRF, was enhanced by E_2. Plasma ACTH was also significantly higher during E_2 replacement following DEX suppression, an effect that was attenuated by co-treatment with P_4. The evaluation of the influence of social status indicated that the decrease in glucocorticoid negative feedback on cortisol and ACTH release induced by E_2 was exacerbated in socially subordinate females. Overall, cortisol and ACTH decreased less in response to DEX and increased more in response to CRF in socially subordinate females compared with dominant females. Taken together, these data indicate that E_2 increases the responsiveness of the LHPA axis in female rhesus monkeys and this response in enhanced by social subordination.

Key Words: Estradiol; progesterone; LHPA axis; glucocorticoid negative feedback; social status.

Introduction

Chronic stress can have a deleterious effect on the reproductive axis *(1)* that, for females, is manifested in reduced pulsatile gonadotropin secretion *(2)* and increased incidence of ovulatory abnormalities and infertility *(3,4)*. However, gonadal steroids may, in turn, affect a female's response to stress. Subtypes of the estrogen receptor (ER) and progesterone receptor are expressed in critical areas involved in the regulation of the limbic–hypothalamic–pituitary–adrenal (LHPA) axis, including the hypothalamic paraventricular nucleus, limbic system, and raphe nuclei in the brain stem *(5–11)*.

Although these anatomical data suggest a functional role for gonadal steroids in the regulation of a female's response to stress, given the range of approaches used and the multiple control points that affect LHPA responsivity, a unifying conclusion about the influence of gonadal steroids does not easily emerge from the number of model systems examined. For example, daytime cortisol and ACTH in monkeys

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(12–15), rats *(16,17)*, and women *(18,19)* can be enhanced by estradiol (E_2). Under non-stressful, basal conditions, E_2 increases hypothalamic corticotropin releasing factor (CRF) mRNA expression and protein content in monkeys *(20,21)* and rats *(22–24)* as well as increasing immunoreactivity for arginine vasopressin (AVP) in the PVN of rats *(25)*. Ovariectomy reduces CRF-induced ACTH but not cortisol release in women *(26)*, while E_2, as well as progesterone (P_4), increases CRF expression in the bed nucleus of the stria terminalis (BNST) but not the PVN in ewes *(27)*. Studies from rats suggest that the effect of E_2 on CRF expression may be mediated by a change in glucocorticoid negative feedback as E_2 reduces mineralocorticoid receptor (MR) but not glucocorticoid receptor (GR) expression in the hippocampus, an effect reversed by co-treatment with progesterone *(28,29)*. Other studies show that E_2 reverses the increase in GR in the PVN following ovariectomy while simultaneously increasing GR mRNA in the amygdala *(30)*. In contrast to these data, other studies in rodent models show that E_2 increases adrenal glucocorticoid release and glucocorticoid negative feedback *(22)* as well as attenuating the expression of CRF mRNA in the PVN and proopiomelanocortin mRNA in the pituitary *(31)*. Along these same lines, studies of adrenalectomized rats have shown that ovariectomy decreases the expression of GR in the hippocampus *(29)*. The foregoing suggests that most but not all data indicate that E_2 may facilitate LHPA responsiveness under basal conditions.

Results of studies examining the effects of gonadal steroids on LHPA responsivity during stress are also mixed. The increase in ACTH and corticosterone following restraint stress *(9)* or exposure to a novel environment *(28)* is enhanced by E_2 secondary to a decrease in hippocampal MR expression. Furthermore, E_2 potentiates the increase in serum ACTH and corticosterone following restraint stress in rats, and this occurs coincident with an augmentation of stress-induced expression of *c-fos* and CRF but not AVP in the PVN *(32)*. Other studies using restraint stress in rats indicate that the potentiation of stress induced corticosterone and ACTH release by E_2 is associated with an increase AVP but not CRF expression *(33)*. However, E_2 reduces the restraint stress–induced increase in ACTH but not corticosterone in female rats *(34)* and attenuates both ACTH and cortisol increase following a cognitive challenge in women *(35)*. Moreover, the expression of *c-fos* in the PVN, medial amygdala, and lateral septum in response to an acute restraint stress is attenuated during proestrus and estrus compared to diestrus females *(36)*. *c-fos* activation in the PVN following restraint stress is also attenuated by E_2 *(37,38)* but is enhanced by E_2 in the hippocampus, an effect reversed by P_4 *(37)*. Finally, the response to dexamethasone, as an assessment of glucocorticoid negative feedback, is attenuated in ovariectomized compared to diestrus rats associated with a significant reduction in GR expression in the PVN and hippocampus *(39)*.

Thus, it is clear from this summary that E_2 can both potentiate and attenuate the responsivity of the LHPA axis to a stressor and that P_4 can block the action of E_2. However, given the fairly consistent observation that females show a greater response to stress than do males *(9,32)*, it seems likely that estrogens are important for the enhanced activation the LHPA axis. What is not clear is whether a history of exposure to stress modifies the effect of gonadal steroids on LHPA responsivity. Socially housed rhesus monkeys present a unique opportunity to assess how gonadal steroids may interact with a history of stress exposure to influence LHPA responsivity. Under group housing situations, rhesus monkeys exhibit a rich network of affiliation and have established social status hierarchies *(40)* that function to maintain group stability, not through contact aggression but rather through harassment and the continuous threat of aggression *(41)*. Importantly, social subordination also functions to delay access to available resources, including food and use of physical space, even in provisioned groups. Thus, socially subordinate females have little control of their social and physical environment *(42,43)*. A key characteristic of socially subordinate macaque females is hypercortisolemia due to diminished glucocorticoid negative feedback *(42,43)*, an indicator of chronic stress exposure *(39)*. The present study was designed to further understand how E_2 and P_4 affect the responsivity of the LHPA axis in rhesus monkeys. We tested the hypothesis that E_2 would decrease glucocorticoid negative feedback, assessed from a dexamethasone suppression test, and increase the response to CRF and that these effects would be attenuated by cotreatment with P_4. Furthermore, we predicted that the facilitating of E_2 on LHPA responsiveness were be exacerbated by social subordination.

Results

Gonadal Steroid Treatments

Illustrated in Table 1 are hormone values during each treatment phase and the following washout period. Plasma E_2 was below the sensitivity of the assay (<5 pg/mL) during the placebo and P_4-only treatment conditions, but was increased significantly during the E_2-only and $E_2 + P_4$ treatment phases ($F_{3, 15} = 25.60$, $p < 0.001$). Levels of E_2 were similar during the E_2 only and E_2–P_4 combination treatments. Plasma P_4 was low and unvarying during the placebo and E_2-only treatment periods, but increased significantly during the P_4 replacement periods ($F_{3, 15} = 11.27$, $p < 0.001$). Plasma E_2 and P_4 were below or near the sensitivity of the assay, respectively, during the washout period.

Cortisol Response

The effect of gonadal hormone replacement and social status on plasma cortisol is shown in Figs. 1 (top panel) and 2 (left panels), respectively. Cortisol concentrations during all treatments varied significantly as a function of time from

Table 1
Mean ± SEM Plasma Estradiol (E_2) and Progesterone (P_4) at D 10 During Each Treatment Phase[a]

	D 10		D 25	
Treatment Phase	E_2 (pg/mL)	P_4 (ng/mL)	E_2 (pg/mL)	P_4 (ng/mL)
Placebo (placebo)	<5[a]	0.16 ± 0.03[a]	<5	0.15 ± 0.05
E_2	64 ± 8[c]	0.14 ± 0.03[a]	<5	0.15 ± 0.02
P_4	<5[a]	3.19 ± 0.90[b]	<5	0.16 ± 0.05
E_2 plus P_4	60 ± 10[c]	3.97 ± 1.10[b]	<5	0.12 ± 0.01

[a]Also shown are plasma levels at 25 d following the initiation of each treatment condition. Within each hormone comparison, values with different superscripts are significantly different from one another ($p \leq 0.05$).

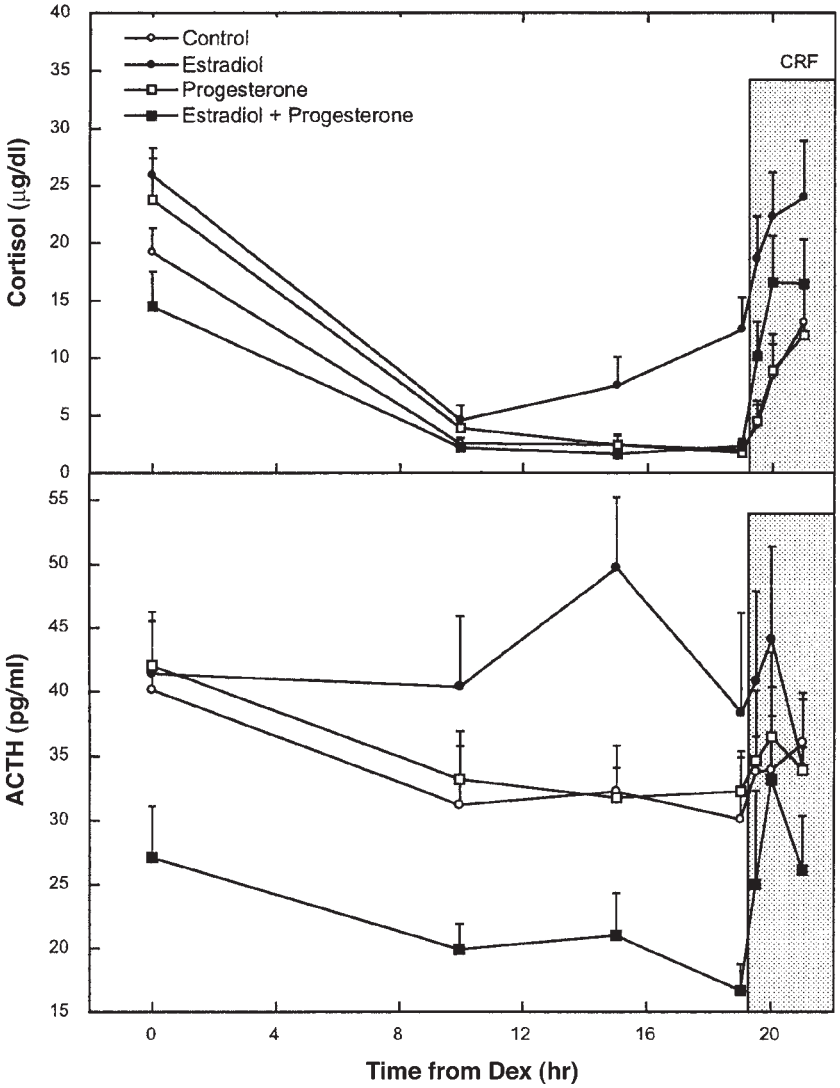

Fig. 1. Mean ± SEM plasma concentrations of ACTH (lower panel) and cortisol (upper panel) for females during placebo, E_2, P_4, and E_2 plus P_4 treatment conditions prior to and following DEX at time 0 (2100 h) and CRF at time 19 h (1600 h the following day). The shaded are refers to the times following CRF administration. The results of the statistical analyses of these data are shown in Table 2.

DEX and CRF administration ($F_{6, 30} = 31.53$, $p < 0.001$, Fig. 1, top panel). However, plasma cortisol concentrations in response to DEX suppression and CRF stimulation were significantly affected by the prevailing steroid replacement condition ($F_{3, 15} = 15.99$, $p < 0.001$, Fig. 1, top panel) and social status ($F_{6, 90} = 2.89$, $p = 0.024$, Fig. 2 top panel).

As can be seen in Fig. 1 (Table 2, results of post hoc tests), plasma cortisol was significantly higher during both

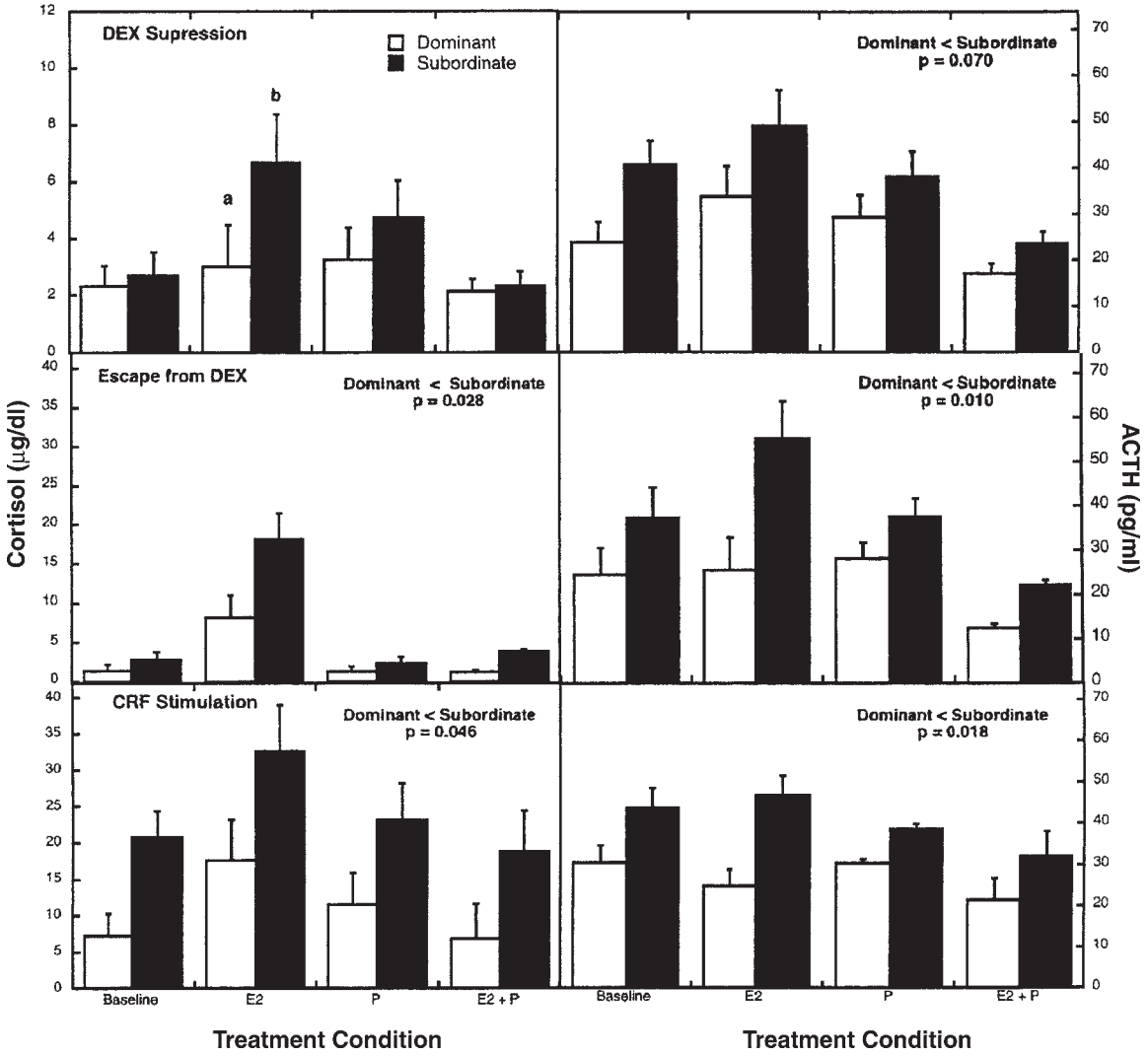

Fig. 2. Mean ± SEM of plasma concentrations of cortisol (left panels) and ACTH (right panels) for socially dominant (open bars) and subordinate females (closed bars) during placebo, E_2, P_4, and high E_2 plus P_4 treatment conditions representing dexamethasone suppression, escape from dexamethasone suppression, and increase to CRF stimulation. Inclusion of a "*p* value" refers to the probability of the result of the main effect of social status. Bars with different letters indicate a significant interaction between social status and treatment condition ($p \leq 0.05$). Note that the cortisol scale for "Dex suppression" (upper panel) is different than the scale for "Escape from Dex" and "CRF stimulation."

the E_2 and P_4 conditions compared with placebo and the E_2–P_4 treatment combination at time 0 and 10 h following DEX. However, at 15 and 19 h following DEX, cortisol levels were significantly higher during the E_2 compared to the other conditions. Following the CRF injection at 19 h post-DEX, plasma cortisol was significantly higher at 30 and 60 min during E_2 compared with E_2–P_4, which, in turn, was significantly higher than values observed during the placebo and progesterone-only conditions. Plasma cortisol was still significantly higher at 120 min during E_2 treatment compared to the other three conditions.

The effect of social status on this response in cortisol was consistent across treatments (no status by treatment interaction: $F_{3,15} = 0.66$, $p = 0.592$) and the overall main effect of social status approached significance ($F_{1,5} = 5.00$, $p = 0.076$), despite the reduced sample size (dominant $n = 4$ and subordinate $n = 3$). Plasma cortisol at time 0 (just prior to DEX administration) did not differ significantly between dominant and subordinate females across the treatments ($F_{1,5} = 3.24$, $p = 0.132$, data not shown). The effect of social status and treatment condition was further evaluated on DEX suppression, escape form DEX suppression, and CRF stimulation (Fig. 2, left panels). The effect of gonadal hormone treatments on the suppression of plasma cortisol by DEX differed significantly between dominant and subordinate females ($F_{3,15} = 3.62$, $p = 0.038$), as plasma cortisol was significantly higher 10 h following DEX in subordinate compared with dominant females during the E_2 but not the other treatment conditions. In addition, the escape from DEX suppression ($F_{1,5} = 9.37$, $p = 0.028$) and the increase

Table 2
Statistical Analyses (Simple Main Effects and Post Hoc Comparisons)[a]

	Cortisol		ACTH	
Time point	$F_{3, 15}$	Post-hoc	$F_{3, 15}$	Post hoc
0 (pre DEX)	7.43, $p = 0.003$	$E_2 = P > C > E_2\ P_4$	8.24, $p = 0.002$	$E_2\ P < C = E_2 = P_4$
10 h	8.79, $p = 0.001$	$E_2 = P > C > E_2\ P_4$	8.72, $p = 0.001$	$E_2 > C = P > E_2\ P_4$
15 h	3.98, $p = 0.029$	$E_2 > P = C > E_2\ P_4$	14.54, $p < 0.001$	$E_2 > C = P > E_2\ P_4$
19 h (pre CRF)	23.64, $p < 0.001$	$E_2 > P = C > E_2\ P_4$	6.21, $p = 0.006$	$E_2 > C = P > E_2\ P_4$
19.5 h	16.79, $p < 0.001$	$E_2 > E_2\ P > C = P_4$	1.12, $p = 0.372$	$C = E_2 = P = E_2\ P_4$
20 h	17.37, $p < 0.001$	$E_2 > E_2\ P > C = P_4$	0.56, $p = 0.65$	$C = E_2 = P = E_2\ P_4$
21 h	9.42, $p = 0.001$	$E_2 > C = P = E_2\ P_4$	1.79, $p = 0.192$	$C = E_2 = P = E_2\ P_4$

[a]Statistical analyses (simple main effects and post hoc comparisons) of the effects of DEX suppression and CRF stimulation on plasma cortisol and ACTH levels during placebo (C), estradiol (E_2), progesterone (P_4), and estradiol plus progesterone (E_2P_4) conditions at each time point shown graphically in Fig. 1. An inequality sign indicates the hormone value is significantly ($p < 0.05$) higher (">") or lower ("<") between two treatments whereas an equal sign ("=") indicates the difference is not significant.

following CRF ($F_{1, 5} = 6.93$, $p = 0.046$) in plasma cortisol was significantly greater in subordinate compared with dominant female across all four-treatment conditions.

ACTH Response

The effect of gonadal hormone replacement and social status on plasma ACTH is shown in Figs. 1 (bottom panel) and 2 (right panels), respectively. Overall, DEX significantly suppressed while CRF significantly increased plasma levels of ACTH ($F_{6, 30} = 6.94$, $p < 0.001$, Fig. 1). As observed with cortisol, the pattern of plasma ACTH in response to DEX suppression and stimulation by CRF (see Fig. 1, Table 2 for results of post hoc tests) was significantly affected by steroid treatment for ACTH ($F_{18, 90} = 3.62$, $p < 0.001$). Furthermore, plasma ACTH was significantly and consistently higher in socially subordinate compared with dominant females ($F_{1, 5} = 62.72$, $p = 0.001$, Fig. 2, right panels).

As can be seen in Fig. 1 (Table 2, results of post hoc tests), plasma ACTH was significantly lower during the combined E_2–P_4 condition compared with the other conditions at time 0, prior to DEX. However, at 10, 15, and 19 h following DEX, ACTH was significantly higher during the E_2 compared with the placebo and P_4 conditions, which, in turn, were higher than the combined E_2–P_4 condition. Following the CRF injection at 19 h post-DEX, plasma ACTH did not vary significantly between treatment conditions.

The significant effect of social status on the response in ACTH to DEX and CRF (Fig. 2, right panels) that was consistent across treatments [no social status by treatment interaction: $F_{3, 15} = 0.57$, $p = 0.642$) and time (no status by time interaction: $F_{6, 90} = 0.56$, $p = 0.753$)]. Plasma ACTH at time 0 (just prior to DEX administration) did not differ significantly between dominant and subordinate females across the treatments ($F_{1, 5} = 4.21$, $p = 0.095$, data not shown). However, suppression of plasma ACTH by DEX was consistently less in subordinate compared with dominant females across the four treatments although the difference only approached significance ($F_{1, 5} = 5.25$, $p = 0.07$, see Fig. 2, right panels). In contrast, the eventual escape from DEX suppression ($F_{1, 5} = 16.05$, $p = 0.010$) and the increase in response to CRF ($F_{1, 5} = 11.86$, $p = 0.018$) was significantly greater in subordinate compared with dominant females across all four-treatment conditions.

Discussion

The present analysis indicates that, in female monkeys, gonadal steroids significantly affect the responsiveness of the LHPA axis to pharmacological challenge and that this can be modulated by social dominance status. E_2 replacement to gonadally suppressed female rhesus monkeys most consistently increased plasma cortisol and ACTH throughout the DEX suppression and CRF stimulation tests, an effect that was attenuated by co-treatment with P_4. In addition to these effects of E_2, females of low status also had significantly higher concentrations of plasma cortisol and ACTH following dexamethasone and CRF administration.

The model employed, a GnRH analog–induced suppression of the pituitary–ovarian axis, effectively reduced ovarian steroid concentrations in circulation to ovariectomy-like levels *(44)*. E_2 replacement elevated plasma levels to those typically observed during the early follicular phase, while P_4 treatment produced levels comparable to the mid-luteal phase *(45)*. Although GnRH receptors are found in the adrenal cortex *(46)* and corticotropes *(47)*, it is unlikely that Lupron had any direct effects on LHPA responsiveness as plasma levels of cortisol and ACTH during the placebo condition were similar to those observed in non-steroid replaced, ovariectomized monkeys *(48)*.

Our observations that plasma cortisol and ACTH are higher throughout the assessment period during E_2 replacement, support previous reports indicating that E_2 increases morning levels of cortisol and ACTH *(12–19)* and influences gene expression in the hypothalamic or limbic areas

that would produce an enhanced LHPA response *(20–24)*. In addition, our data show that P_4 attenuates the facilitating effects of E_2 on both cortisol and ACTH release, an observation that is consistent with other observations *(28,29,37)*. Although previous studies have suggested that P_4 alone may increase certain parameters of HPA responsivity *(27)*, the data from the present analyses show that plasma cortisol and ACTH in response to the DEX–CRF tests are similar during the placebo, no replacement condition, and P_4-only period. Since E_2 upregulates the progestin receptors in brain areas that govern LHPA responsivity *(9,10)*, it is not unexpected that an elevation of luteal phase-like concentrations of P_4 may produce little, if any, effects in the absence of E_2. However, what is not clear is how P_4 attenuates the effect of E_2. Potentially this inhibition could be induced through allopregnanolone, an A-ring reduced metabolite of P_4. Since allopregnanolone acts as a positive allosteric modulator of GABAA receptors *(49,50)* and intrahypothalamic GABAergic neurons exert a major inhibitory effect on CRF-secreting cells in the PVN *(51,52)*, this neuroactive metabolite may block E_2 induced release of CRF through a non-genomic mechanism.

Although plasma levels of cortisol and ACTH were significantly higher during the E_2 replacement condition, the most striking effect was the escape from glucocorticoid negative feedback following the administration of DEX. Cortisol was least suppressed by DEX during the E_2 and P_4 conditions whereas ACTH was least suppressed during E_2 treatment. However, escape from glucocorticoid negative feedback, which was defined as the increase in plasma concentrations from maximal suppression, was significantly greater for both cortisol and ACTH during the E_2 condition. In response to CRF, cortisol but not ACTH was higher during E_2 suggesting that E_2 may act primarily through mechanisms that regulate glucocorticoid negative feedback and not responsivity to CRF, specifically reducing GR and/or MR expression or binding in pituitary, hypothalamic, and limbic areas. Indeed, previous data indicate that E_2 reduces MR expression in the hippocampus *(28)* and GR expression in the PVN *(30)* as well as the pituitary *(9)*. The diminution in glucocorticoid negative feedback by estradiol could result in a greater increase in CRF and stimulation of pituitary ACTH and adrenal cortisol secretion. Our approach cannot rule out a direct effect of E_2 on cortisol secretion from the adrenal *(13,22)*. However, given the significant effect of E_2 on pituitary ACTH secretion it is likely that estradiol acts primarily through the limbic–hypothalamic–pituitary axis to diminish glucocorticoid negative feedback.

It is important to point out that, although the dexamethasone suppression test is widely used clinically in psychiatric populations *(53)* and experimentally to assess LHPA responsivity *(39,54)*, it likely illustrates changes in glucocorticoid negative feedback at the level of the pituitary more so than in limbic–hypothalamic areas *(55,56)*. Low doses of dexamethasone administered peripherally may be less potent centrally given reduced penetration of the blood–brain barrier *(57)*. Thus, while we cannot rule out that some of the effect $E_2 \pm P_4$ on dexamethasone suppression does not occur at limbic–hypothalamic sites, it is likely that these steroids modulate glucocorticoid negative feedback at the pituitary. Studies using graded doses of GR and/or MR receptor antagonists that readily cross the blood–brain barrier *(58,59)* could address how gonadal steroids affect pharmacologically induced decreases glucocorticoid negative feedback.

Inclusion of the subject's social status also showed that these effects of gonadal steroids are modified by the psychobiological state of the individual. This analysis, of course, is similar to approaches used to determine how steroids influence the response to acute and chronic stress *(29,39)*. Previous work has shown that socially subordinate female macaques are less sensitive to glucocorticoid negative feedback *(42,43)*. Studies with nonprimate models have described the neurobiological circuits that define the loss of glucocorticoid negative feedback in response to chronic stress. With continual exposure to stressors, the upregulation of CRF in the amygdala and BNST *(60–62)* and AVP in the PVN *(63–65)* results from the downregulation of GR and MR in limbic and hypothalamic areas *(63–69)*. Thus, the behavioral *(42,43)* and physiological consequences *(42, 70–72)* of low social status in macaque females implies that these females are under chronic, albeit low, stress. Our results support this hypothesis, as hormone levels in response to the pharmacological challenges were consistently different in socially dominant females, regardless of gonadal steroid replacement. However, given the small number of subjects, our analysis of the interactive effects of social status and gonadal steroid replacement on LHPA responsivity was underpowered and an analysis of more animals may show significant interactions between social status and gonadal steroid replacement. Nevertheless, our data indicate that E_2 exacerbates the reduced efficacy of glucocorticoid negative feedback and accelerates the escape of this suppression in socially subordinate females. The data appear to suggest that E_2 synergizes with the neurobiological changes resulting from the experience of being socially subordinate to affect the regulation of the LHPA axis.

The observation that E_2 enhances LHPA dysregulation, manifested in altered glucocorticoid negative feedback, would suggest that this steroid could exacerbate stress-related disorders mediated by CRF and AVP such as depression and anxiety *(54,73)*. The higher incidence of depression in women compared with men is thought to be estrogen-dependent *(74)*. However, this notion appears at odds with E_2's known facilitating effects on serotonergic neurotransmission *(75–77)* and its use with serotonin reuptake inhibitors in the treatment of anxiety *(78–80)*. Serotonin's role in the stress response is complex as, on the one hand, it is integral to the activation and limitation of the stress response

(81–86). However, with repeated stress, CRF projections from the amygdala to the raphe attenuate serotonergic inhibition of neuronal activity in limbic targets *(87,88)*. Thus, it is possible that E_2 administration to females exposed to a chronic stressor, such as the socially subordinate females in the present study, is not able to augment serotonin neurotransmission given the central inhibitory activity of CRF.

This implies that the effects of E_2 depend on the neurobiological milieu prevailing at that time and underscores the need to better define how E_2 enhances LHPA dysregulation in stressed females and increases vulnerability of this dysregulation in females not exposed to chronic stressors.

Methods

Subjects were seven adult, gonadally intact, female rhesus monkeys (*Macaca mulatta*) housed in a social group at the Yerkes National Primate Research Center Field Station. The group (n = 140 animals) contained 2 adult males, 30 additional adult females, and their juvenile and infant offspring. Animals were fed commercial monkey chow (Lab Diet, number 5038, Purina Mills International) *ad libitum* twice daily and received a daily supplement of fresh fruit and vegetables. Subjects were chosen to represent a range of dominance positions (see below). Animals were trained for conscious venipuncture as described previously *(89)*. Animals readily habituate and show minimal arousal to the procedures *(90)* that have no adverse consequences on reproductive parameters or neuroendocrine responsivity *(48, 89)*. The protocol was approved by the Emory University Institutional Animal Care and Use Committee in accordance with the Animal Welfare Act and the US Department of Health and Human Services "Guide for Care and Use of Laboratory Animals."

In order to assess the effects of $E_2 \pm P_4$ on LHPA responsivity, the pituitary–gonadal axis was suppressed by treating females continuously with the long acting GnRH agonist, Lupron Depot (Tap Pharmaceuticals, Deerfield IL) at a dose (200 μg/kg/28 d) that completely arrests precocious puberty in girls *(91,92)*. Treatment efficacy was verified by hormonal analyses (see *Results*). Lupron Depot treatments were begun 6 wk prior to the initiation of the experimental protocol to ensure the pituitary–gonadal axis was completely suppressed. The LHPA axis was assessed under placebo (no steroid treatment), E_2 (8 μg/kg/d), P_4 (440 μg/kg/d), and E_2 given in combination with P_4. Steroid hormone replacement was accomplished by subcutaneous placement of 21-d, sustained-release pellets (Innovative Research of America, Sarasota FL). Each 21-d steroid treatment was separated by a 21-d washout period. The order of hormone treatment was randomly determined.

The responsivity of the LHPA axis was assessed on d 10 and 11 of each treatment period. A combined dexamethasone (DEX) suppression test–CRF stimulation test was performed *(93)*. Following the plasma sample collected at 2100 h, females received dexamethasone (DEX) at a dose of 0.50 mg/kg, im and plasma samples were obtained at 10, 15, and 19 h thereafter. Following the +19 h sample, females received CRF at a dose of 1.0 μg/kg, iv and samples were collected at +30, +60, and +120 min thereafter. This dose of DEX has been used previously to assess glucocorticoid negative feedback in monkeys *(43)* and the dose of CRF stimulates ACTH release in women *(94)*. All samples were assayed for cortisol and ACTH.

Social dominance ranks were determined from the outcome of dyadic interactions between the subject females themselves and other group members *(40)*. Behavior was recorded in two 1- h sessions each week randomly distributed between the morning and afternoon throughout the study (including both treatment and washout periods). Using a standard rhesus monkey Ethogram *(95)*, an animal was categorized as subordinate if she emitted unequivocal submission gestures to another animal, including avoidance to an approach, flee from a chase, grimace from an approach or stare, and squeal from a threat. Females were selected as subjects to represent of range of dominance ranks. Excluding infants, subjects were ranked 3, 18, 23, 54, 74, 76, and 100 out of 115 possible animals. Following previously established protocols *(96)*, those subjects whose rank was in the upper half of the dominance hierarchy (n = 4) were considered dominant and those in the bottom half (n = 3) subordinate.

Assays were performed in the Endocrine Core Laboratory at the Yerkes National Primate Research Center. Serum estradiol was determined using a modification *(97)* of a commercially available radioimmunoassay [Diagnostic Products Corporation (DPC), Los Angeles CA]. Prior to assay, samples (250 μL) were extracted twice with 5 mL of anesthesia grade ether. Following evaporation of the solvent, samples were reconstituted with 250 μL of zero calibrator and 100 μL aliquots were assayed in duplicate. The assay has a sensitivity of 5 pg/mL using 100 μL of extracted serum, with an intra- and interassay coefficient of variation (CV) of 5.8% and 11.7%, respectively. Sample values of estradiol were corrected for extraction efficiency, which exceeded 95%. Plasma P_4 was determined by a commercially available RIA (DPC) following extraction with ether *(98)*. The assay has a sensitivity of 0.10 ng/mL using 20 μL of serum with an intra- and interassay CV of 5.13% and 8.33%, respectively. Plasma cortisol was determined by radioimmunoassay of 25 μL duplicates using commercially available reagents (Diagnostic Systems Laboratory). The assay has a sensitivity of 0.02 μg/dL and an intra- and interassay CV of 3.1% and 7.6%, respectively. Plasma ACTH was determined by radioimmunoassay of 100 μL duplicates using commercially available reagents (DiaSorin, Stillwater, MN). The assay has a sensitivity of 4.5 pg/mL and an intra- and interassay CV of 7.1% and 12.4%, respectively.

Data were summarized as mean ± SEM and were analyzed with analysis of variance models for repeated measures, with rank (high vs low, see *Results*) as a categorical main effect, treatment and time as repeated main effects, and hormone concentrations as dependent measures. Specific means were compared using least significant difference post hoc tests. Using ANOVA, the effect of treatment condition (placebo, E_2, P_4, and E_2 plus P_4) and social status on glucocorticoid negative feedback was evaluated by comparing hormone values from samples collected 10 h following DEX as an index of suppression. Escape from glucocorticoid negative feedback was evaluated by comparing hormone values from samples collected at 15 h (ACTH) or 19 h (cortisol) from DEX. The response to CRF was evaluated by comparing hormone values from samples collected at 60 min (ACTH) or 120 min (cortisol) following the CRF injection (19 h following DEX). The rationale for using later time points for the cortisol analysis compared to the ACTH analysis is based on feedforward and feedback relationship between these hormones. Statistical tests were performed with SPSS (v11) and results with a probability of $p \leq 0.05$ were considered significant.

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