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Estradiol and cortisol interactions in youth externalizing psychopathology

Jennifer L. Tackett[a,*], Kathleen W. Reardon[a], Kathrin Herzhoff[a], Elizabeth Page-Gould[b], K. Paige Harden[c], Robert A. Josephs[c]

[a] *Department of Psychology, University of Houston, 126 Heyne Building, Houston, TX 77204, United States*
[b] *Department of Psychology, University of Toronto, 100 St. George Street, Toronto, Ontario M5S 3G3, Canada*
[c] *Department of Psychology, The University of Texas at Austin, 108 E. Dean Keeton Stop A8000, Austin, TX 78712-1043, United States*

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Summary Growing evidence has indicated that gonadal and stress hormones interact to shape socially dominant behavior and externalizing psychopathology; however, such work to date has focused exclusively on the testosterone—cortisol interaction, despite expectations that estradiol should be associated with similar behavioral outcomes to testosterone. Here, we present the first empirical test of the hypothesis that adolescent males and females ($N = 105$, ages 13—18) with high estradiol and low cortisol concentrations are at highest risk for externalizing problems, but — replicating previous work — *only* among adolescents high on pathological personality traits. Parents reported on youth psychopathology and personality, and hormone concentrations were measured via passive drool. Results confirmed the hypothesis: high estradiol was associated with more externalizing behaviors, but *only* when cortisol was low *and* personality traits of disagreeableness and emotional instability were high. Further, these associations held when controlling for testosterone concentrations. These findings provide the first empirical evidence of a hypothalamic pituitary adrenal (HPA) × hypothalamic pituitary gonadal (HPG) axis interaction that extends the ''dual hormone'' hypothesis beyond testosterone.
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* Corresponding author. Tel.: +1 713 743 4863.
E-mail addresses: jltackett@uh.edu (J.L. Tackett), kwreardon@uh.edu (K.W. Reardon), kherzhoff@uh.edu (K. Herzhoff), liz@psych.utoronto.ca (E. Page-Gould), harden@psy.utexas.edu (K.P. Harden), bob.josephs@utexas.edu (R.A. Josephs).

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1. Introduction

A growing literature provides evidence for the ''dual hormone hypothesis'' (DHH) that social dominance is jointly regulated by the hypothalamic pituitary gonadal (HPG) and hypothalamic pituitary adrenal (HPA) axes (Mehta & Josephs, 2010; van Honk et al., 2010). Specifically, testosterone (T) predicts high levels of social dominance, particularly when levels of cortisol (C) are low, a pattern consistent with a body of research showing stress-evoked suppression of reproductive hormones (e.g., Johnson et al., 1992; Tilbrook et al., 2000). This growing literature has extended these findings to multiple phenotypes, including clinically relevant constructs such as physical aggression and delinquent rule-breaking behaviors, all behaviors which are typically referred to as *externalizing behaviors* (Dabbs et al., 1991; Popma et al., 2007).

Some barriers to establishing the generality of these effects have emerged. For example, research examining clinical phenotypes such as aggression have primarily focused on forensic male adolescent samples (Dabbs et al., 1991; Popma et al., 2007), raising questions regarding the applicability of these findings to mixed-gender non-clinical populations. One recent study, conducted with the sample examined in the current investigation, offered the first evidence of DHH effects in a mixed-gender community sample of youth, demonstrating that T × C interactions on externalizing problems emerged in the context of disordered personality traits (Tackett et al., 2014a). Specifically, DHH effects emerged for youth who were high on those personality disorder traits that are primarily associated with externalizing problems: disagreeableness and emotional instability. Furthermore, no evidence for DHH effects emerged when personality moderation was ignored. This work suggests that personality represents a key consideration when testing hormone-behavior associations, particularly in non-clinical samples.

Despite the broad hormonal basis for the DHH, suggesting involvement of other reproductive and/or stress hormones, to date, research has exclusively focused on T and C. In the current study, we tested the hypothesis that estradiol (E2) — another key gonadal hormone — may also interact with C, consistent with previous DHH findings for T. In adolescent females, E2 is produced in the ovaries and drives breast development and the accumulation of body fat (e.g., de Ridder et al., 1992). Although often considered a female hormone, average secretion of E2 also increases in males over the course of pubertal development, as it is produced directly in the testes and is also synthesized via aromatization of circulating T (de Ronde et al., 2003). E2 has important effects on male fertility and growth, including impact on metabolic processes, cardiovascular function, and bone mass achievement and maintenance (de Ronde et al., 2003). Although it is called the dual-hormone hypothesis, the hypothesis is truly about the underlying neuroendocrine axes. It states that the HPA moderates the relationship between the HPG and human behavior. Testosterone is the most widely studied hormone of the HPG axis, but it is not the only hormone in the HPG axis; E2 is also an end product of the HPG, as both testosterone and estradiol are synthesized from HPG-mediated release of androstenedione. Despite this fact, the DHH has been solely tested by measuring testosterone and cortisol in humans. If the DHH is correct, then any hormone that reflects activation of the HPG should demonstrate the same moderation by the HPA of its relationship with behavior.

Like T, higher E2 has been linked to risk-taking and dysregulated behavioral phenotypes (Vermeersch et al., 2008, 2009; de Water et al., 2013) and socially dominant behavior (Stanton & Schultheiss, 2007; Stanton & Edelstein, 2009). However, associations between higher levels of E2 and higher aggressive or risk-taking behaviors have been infrequently examined and often only in females (much as research on T has often been conducted only with males (Balzer et al., 2015); but see also for evidence of associations between E2 and risky tendencies in both male and female adolescents (Vermeersch et al., 2009)). Vermeersch et al. (2008) demonstrated that serum E2, but NOT serum T, was associated with both aggressive and risk-taking behaviors in a sample of adolescent girls. Furthermore, this pattern was especially strong in girls with deviant peer relations, pointing to the power of potential moderators at elucidating the nature of these associations. Such findings point to potential sex specificity of T and E2 associations with behaviors, although evidence for sex-differentiated thresholds does not necessitate differential mechanisms across males and females. In addition, the extent to which many previous studies do not measure both T and E2 in mixed-sex samples have limited the extent to which such relationships may be properly teased apart. Another recent study found endogenous administration of E2 in female rhesus monkeys to increase motivated sexual behavior, but only in lower-stress animals (i.e., the effect was not found in animals exposed to chronic stress in the form of social subordination; Reding et al., 2012). Taken together, these studies demonstrate the need to explicitly examine potential DHH effects for E2 in mixed-sex samples, and alongside measurement of T.

Such findings set the stage for the current investigation, and suggest that it is reasonable to expect E2 to show similar patterns of association to T in a DHH framework and in the context of externalizing behaviors, specifically. Despite the importance of E2, it has not received the attention given to T in relation to hormonal associations with risky behavior phenotypes. Further, we are aware of no empirical investigations to date that have sought to extend the DHH to E2. This represented the primary goal of the current study. Furthermore, we restricted analyses to the traits of disagreeableness and emotional instability, given existing rationale for their particular relevance for youth externalizing psychopathology (Tackett et al., 2014b), and to maintain consistency with our previous work (Tackett et al., 2014a). Specifically, we hypothesized that:

1. Higher levels of E2 would be associated with higher levels of externalizing psychopathology, but *only* when C levels were low, and *only* for youth high in pathological personality traits.
2. Such effects would remain even while controlling for concurrent T, indicating unique effects for E2.

2. Material and methods

2.1. Participants

Participants were 105 adolescents (55% female) between the ages of 13 and 18 ($M = 16.00$ years, $SD = 1.29$ years) and one of their parents (95 mothers and 10 fathers). Participants were initially recruited from an urban area in southern Ontario, Canada as part of a larger, longitudinal study using a community-based recruitment strategy. The overall

follow-up response rate was 83%, and 72% of all responders came in to the lab to provide saliva samples (the rest provided questionnaires through the mail). Exclusion criteria for the original study were lack of English fluency in either the parent or the adolescent, and a history of autism, mental retardation, or schizophrenia in the adolescent. For further information regarding the demographics of this sample, please see Tackett et al. (2014a). Informed consent and assent were obtained from parents and adolescents, respectively. Parents were compensated with 50 Canadian dollars for participation in the lab visit, and adolescents were compensated with a 25-dollar gift card for their participation. The university Research Ethics Board approved all study methods and materials.

2.2. Measures

2.2.1. DIPSI — Dimensional Personality Symptom Item pool (DIPSI; Tackett & De Clercq, 2009)

Adolescents' personality pathology traits were assessed by parent-report on the English language DIPSI, which is a 172-item questionnaire. The DIPSI consists of four higher-order dimensions of personality pathology, two of which are theoretically relevant for externalizing problems (Disagreeableness and Emotional Instability). These dimensions are comprised of 12 and 9 facets, respectively. Sample items of Disagreeableness include ''always demands other children to listen'', ''always makes decisions in a very inconsiderate way'', and ''feels no emotion when other children get hurt'' and of Emotional Instability include ''needs someone around all the time'' and ''is very sensitive to stress''. Parents rated each item on a scale from 1 (*not characteristic*) to 5 (*highly characteristic*). Internal consistencies (Cronbach's alphas) for the higher-order dimensions were .97 (Emotional Instability) and .98 (Disagreeableness), and for the lower-order facets ranged from .56 (Insecure Attachment) to .92 (Inflexibility).

2.2.2. CBCL — Child Behavior Checklist (CBCL—6-18; Achenbach & Rescorla, 2001)

Adolescents' externalizing problems were assessed by parent-report on the CBCL, which is a 118-item questionnaire. Sample items of CBCL Externalizing Problems include ''lies, cheats'', ''destroys others' things'', and ''threatens others''. Parents rated each item on a scale from 0 (*not true (as far as you know)*) to 2 (*very true or often true*) for the past 6 months. As clinical research on the DHH has demonstrated predictive validity for externalizing problems, we focused on the Externalizing Problems scale. Internal consistency (Cronbach's alpha) in the present sample was .90. The Externalizing Problems scale correlated with Disagreeableness at $r = .78$, $p < .001$ and with Emotional Instability at $r = .51$, $p < .001$.

2.2.3. Hormone Assays — E2, T, and C assays

After providing assent, adolescents rinsed out their mouths and drank a small cup of water. Participants drooled through a sanitary straw into a 2-ml IBL vial 30 min after arrival to the lab following a period of sedentary activity (questionnaire completion). Saliva samples were frozen at −20 °C before being shipped on dry ice to Clemens Kirschbaum's laboratory at the Technical University of Dresden. They were thawed and centrifuged at 3000 rpm for 5 min, which produced a clear supernatant of low viscosity. Salivary hormone concentrations were measured using highly sensitive, commercially available chemiluminescence-immunoassays (IBL International, Hamburg, Germany). The average intra- and interassay coefficients for C, E2, and T were each below 8%.

2.3. Procedure

Parents completed informed consent documents and questionnaires upon arrival to the lab. Given the diurnal variation of the relevant hormones (Kirschbaum & Hellhammer, 1994), all saliva samples were collected between noon and sundown (range: 11:59:40 to 19:15:50; $M = 14{:}11{:}03$, $SD = 2{:}07{:}00$), and the exact time of sundown on the day of the assessment was determined by consulting online references. Female participants were scheduled within the first ten days of the menstrual phase (follicular cycle) when hormone levels are most stable (Worthman et al., 1990; Liening et al., 2010). For boys, and for girls who were not yet cycling, participants were scheduled according to which date and time was most convenient for the family's schedule, in order to maximize follow-up participation. Participants were instructed not to eat or drink for 2 h and not to smoke for 4 h before their assessment to prevent contamination of the saliva samples.

2.4. Statistics

Variable frequencies were examined and one case with an extreme value for C (167 SDs above the mean) was dropped before analyses. Then, C, T, and E2 variables were log-transformed to reduce skew. E2 and T values were next standardized (z-scored) within sex, consistent with previous research examining gonadal hormones in mixed-gender samples (Mehta & Josephs, 2010). Prior to analyses, all remaining predictor variables (age, gender, time of waking, C, and personality variables) were standardized (z-scored) to facilitate interpretation of results. Regression analyses were conducted to examine evidence for the primary study hypothesis. Regression models included covariates: age, gender, and time of waking; main effects: E2, C, and either DIPSI Disagreeableness or Emotional Instability; two-way interaction terms between E2 and DIPSI Disagreeableness or Emotional Instability and C and DIPSI Disagreeableness or Emotional Instability; and the three-way interaction term between E2, C, and DIPSI Disagreeableness or Emotional Instability. As with the higher-order analyses, regressions were then run separately for each facet comprising Disagreeableness and Emotional Instability. Models included covariates: age, gender, and time of waking; main effects: E2, C, and the DIPSI facet; two-way interaction terms between E2 and the DIPSI facet and C and the DIPSI facet, and the three-way interaction term between E2, C, and DIPSI facet. CBCL externalizing behavior score was the outcome variable for all regressions. Significant interactions were probed using simple slope analyses at C and PD levels 1 SD above and below the mean using Hayes' (2013) PROCESS modeling Model 3.

3. Results

Descriptive statistics (mean values and standard deviations) for all variables and zero-order correlations between the hormones, personality traits, and externalizing behavior in both the mixed-sex full sample and in boys and girls separately are presented in Table 1.

Table 1 Means, standard deviations, and correlations between personality pathology traits, externalizing problems, and cortisol, estradiol, and testosterone based on the full sample and separately by sex.

	M	SD	Correlations								
			Cortisol[a]			Estradiol[b]			Testosterone[a]		
			Full	Female	Male	Full	Female	Male	Full	Female	Male
Age	16.00	1.29									
Cortisol (nmol/L)	5.59	3.57									
Estradiol (pg/ml)	2.62	1.53	−.01	.06	−.09						
Testosterone (pg/ml)	20.26	25.27	.14	.16	.13	.39***	.38**	.41**			
Externalizing problems	6.64	7.24	−.01	−.08	.06	.06	−.04	.14	.04	−.06	.11
DIPSI higher-order factors											
Disagreeableness	1.69	.59	−.06	−.27*	.13	.07	−.05	.20	.01	−.08	.11
Emotional instability	1.51	.56	.01	−.28*	.24	.06	−.08	.18	−.03	−.07	.01
DIPSI lower-order factors											
Hyperexpressive traits	1.63	.72	−.07	−.40**	.21	.11	.00	.22	.01	.00	.02
Hyperactive traits	1.83	.72	−.02	−.22	.19	.14	.03	.27	−.01	−.10	.08
Dominance—egocentrism	1.74	.71	−.13	−.34**	.06	.08	−.03	.18	.05	.09	.01
Impulsivity	1.63	.87	.04	−.08	.17	.05	−.06	.17	.08	−.02	.18
Irritable—aggressive	1.61	.75	.01	−.16	.17	.01	−.07	.09	−.02	−.08	.04
Disorderliness	2.23	.95	−.08	−.19	.05	.01	−.15	.21	.02	−.15	.23
Distraction	1.55	.71	−.06	−.15	.03	.11	−.09	.32*	.12	−.01	.26
Risk behavior	1.56	.62	−.12	−.22	−.02	−.08	−.19	.04	−.08	−.15	.01
Narcissistic traits	1.92	.67	−.07	−.26*	.13	.05	−.02	.12	−.10	−.12	−.08
Affective lability	1.76	.86	.02	−.24	.24	−.01	−.07	.06	.02	−.08	.11
Resistance	1.39	.63	−.18	−.23	−.13	−.01	−.16	.14	−.03	−.12	.07
Lack of empathy	1.37	.53	−.09	−.29*	.11	.05	.03	.07	−.03	−.05	−.01
Dependency	1.36	.63	−.01	−.26*	.24	.03	−.08	.15	−.02	−.08	.04
Anxious traits	1.52	.72	.03	−.20	.25	.07	−.05	.20	.03	−.01	.07
Lack of Self-confidence	1.51	.63	.00	−.29*	.24	−.05	−.19	.06	−.10	−.16	−.05
Insecure attachment	1.58	.64	.02	−.18	.22	.04	−.03	.12	−.11	−.16	−.06
Submissiveness	1.52	.57	.01	−.18	.19	.13	−.02	.29*	.07	.12	.02
Ineffective coping	1.90	.86	.06	−.13	.25	.01	−.11	.15	−.01	−.08	.07
Separation anxiety	1.27	.64	.03	−.33*	.31*	.07	−.04	.17	−.02	−.07	.02
Depressive traits	1.45	.66	−.01	−.18	.14	.06	.02	.09	.00	−.01	.01
Inflexibility	1.53	.73	−.10	−.29*	.06	.11	.03	.19	−.02	.02	−.07

Note: DIPSI, Dimensional Personality Symptom Item pool (Tackett & De Clercq, 2009).
[a] Correlations for cortisol are based on log-transformed values.
[b] Correlations for estradiol and testosterone are based on values log-transformed and then standardized within gender.
* $p < .05$.
** $p < .01$.
*** $p < .001$.

3.1. E2 × C interaction effects moderated by DIPSI traits

Consistent with the DHH, analyses revealed significant E2 × C × PD interactions associated with externalizing behavior. Specifically, both Disagreeableness ($\beta = -.22$, $t(94) = -2.94$, $p = .004$) and Emotional Instability ($\beta = -.33$, $t(94) = -2.74$, $p = .007$) moderated the E2 × C interaction in relation to Externalizing Behavior (see Table 2). The E2 × C interaction was *not* significant at low levels of Disagreeableness ($b = 1.66$, $t(94) = 1.65$, $p = .102$) or Emotional Instability ($b = 1.13$, $t(94) = 1.01$, $p = .315$; see Fig. 1). At high levels of PD traits, however, the E2 slope was significant only at low levels of C for Disagreeableness ($b = 2.41$, $t(94) = 2.23$, $p = .028$). The E2 slope was not significant at high levels of C for Disagreeableness ($b = -.58$, $t(94) = -.79$, $p = .434$), nor for high or low levels of C for Emotional Instability: high ($b = -.58$, $t(94) = -.55$, $p = .587$); low ($b = 3.02$, $t(94) = 1.92$, $p = .058$. Main effects were $\beta = .76$ for Disagreeableness, $\beta = .50$ for Emotional Instability, and ranged from $\beta = .00$ to $\beta = .04$ for E2 and C. Proportion of variance explained was $R^2 = .07$ for Step 1 (covariates), $R^2 = .63$ (Disagreeableness main effects) or $R^2 = .32$ (Emotional Instability main effects), and $R^2 = .64$ (Disagreeableness two-way interactions) or $R^2 = .34$ (Emotional Instability two-way interactions).

Next, we repeated these analyses adding T and T × C as covariates, to test the robustness of the interaction effect for specific variance in E2. The pattern of results was the same: Disagreeableness ($\beta = -.24$, $t(92) = -3.35$, $p = .001$) and Emotional Instability ($\beta = -.33$, $t(92) = -2.82$, $p = .006$)

Table 2 Moderated regression analyses predicting Child Behavior Checklist externalizing behavior scores from E2, baseline C, and DIPSI traits.

	DV: externalizing behavior						
Variable	β	b	SEB	95% CI	R^2	ΔR^2	F
DIPSI higher-order factors							
Disagreeableness × E2 × C	**−.22**	**−1.58**	**.54**	**[−2.64, −.51]**	**.67**	**.03****	**19.02**
Emotional Instability × E2 × C	**−.33**	**−1.53**	**.56**	**[−2.63, −.42]**	**.39**	**.05****	**5.98**
DIPSI disagreeableness facets							
Hyperexpressive traits × E2 × C	−.24	−1.49	.60	[−2.69, −.29]	.49	.03*	9.10
Hyperactive traits × E2 × C	**−.26**	**−1.64**	**.62**	**[−2.87, −.42]**	**.45**	**.04****	**7.64**
Dominance-Egocentrism × E2 × C	−.21	−1.96	.72	[−3.39, −.54]	.53	.04**	10.42
Impulsivity × E2 × C	−.25	−1.56	.54	[−2.63, −.49]	.59	.04**	13.64
Irritable-aggressive traits × E2 × C	−.02	−.14	.70	[−1.52, 1.24]	.57	.00	12.68
Disorderliness × E2 × C	−.15	−.97	.59	[−2.13, .19]	.48	.02	8.57
Distraction × E2 × C	**−.37**	**−2.29**	**.56**	**[−3.39, −1.19]**	**.54**	**.08*****	**11.06**
Risk taking × E2 × C	−.24	−2.60	1.04	[−4.66, −.54]	.51	.03*	9.75
Narcissistic traits × E2 × C	−.13	−1.13	.88	[−2.87, .61]	.22	.01	2.70
Affective lability × E2 × C	.02	.14	.64	[−1.12, 1.40]	.54	.00	10.82
Resistance × E2 × C	−.17	−2.17	1.29	[−4.74, .40]	.57	.01	12.31
Lack of Empathy × E2 × C	**−.26**	**−2.66**	**.86**	**[−4.36, −.96]**	**.51**	**.05****	**9.84**
DIPSI emotional instability facets							
Dependency × E2 × C	**−.45**	**−2.13**	**.57**	**[−3.27, −.99]**	**.39**	**.09*****	**6.02**
Anxious traits × E2 × C	−.21	−1.13	.73	[−2.58, .31]	.25	.02	3.18
Lack of self-confidence × E2 × C	−.25	−1.17	.54	[−2.25, −.10]	.39	.03*	6.02
Insecure attachment × E2 × C	−.16	−.93	.75	[−2.41, .55]	.19	.01	2.19
Submissiveness × E2 × C	−.31	−1.54	.64	[−2.80, −.27]	.26	.05*	3.34
Ineffective coping × E2 × C	−.14	−.91	.54	[−1.98, .16]	.51	.02	9.82
Separation anxiety × E2 × C	−.33	−1.26	.53	[−2.31, −.20]	.20	.05*	2.55
Depressive traits × E2 × C	−.19	−1.12	.72	[−2.55, .31]	.24	.02	3.10
Inflexibility × E2 × C	−.23	−1.33	.73	[−2.27, .11]	.32	.02	4.49

Note: CI, confidence interval (values in square brackets denote 95% confidence intervals of unstandardized regression coefficients); DIPSI, Dimensional Personality Symptom Item pool (Tackett & De Clercq, 2009); E2, estradiol; C, cortisol.
Full regressions with three-way interactions included age, sex, and time of waking as covariates, main effects for E2, C, and the personality pathology trait, the two-way interaction terms between E2, C, and the personality pathology trait, and the three-way interaction term between E2, C, and the personality pathology trait. For trait regressions, bold indicates significant at $p < .05$; for facet regressions, bold indicates significant at $p < .01$. For ΔR^2, *$p < .05$; **$p < .01$; ***$p < .001$.

continued to moderate the E2 × C interaction in relation to Externalizing Behavior.

Finally, facet-level (i.e., lower-order personality trait) moderation of the E2 × C interaction was explored to extend these findings beyond the higher-order personality trait level. Hyperactive Traits ($\beta = -.26$, $t(94) = -2.67$, $p = .009$), Dominance/Egocentrism ($\beta = -.21$, $t(94) = -2.75$, $p = .007$), Impulsivity ($\beta = -.25$, $t(94) = -2.88$, $p = .005$), Distraction ($\beta = -.37$, $t(94) = -4.13$, $p = .000$), Lack of Empathy ($\beta = -.26$, $t(94) = -3.11$, $p = .002$), and Dependency ($\beta = -.45$, $t(94) = -3.18$, $p = .000$) moderated the E2 × C interaction in predicting parent-reported

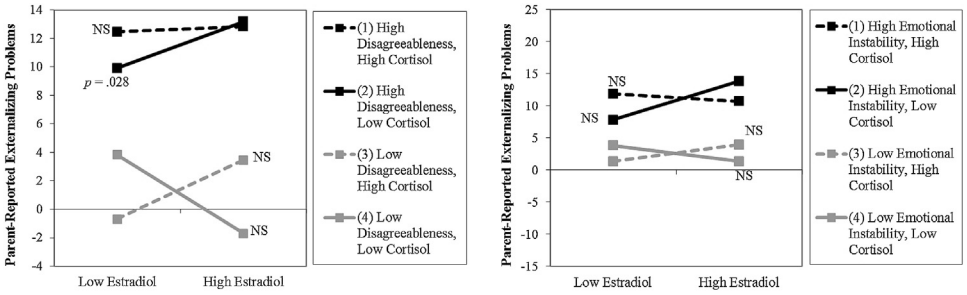

Figure 1 Linear interactions between baseline cortisol and DIPSI Disagreeableness traits (left panel) and baseline cortisol and DIPSI Emotional Instability traits (right panel) in predicting CBCL Externalizing Problems.

Externalizing Problems at a more stringent alpha level of .01[1].

4. Discussion

These results provide support for the first empirical extension of the DHH to the gonadal hormone, E2. Specifically, we demonstrated a three-way interaction between E2, C, and personality pathology on youth externalizing problems. In a mixed-gender community sample of adolescents, we found that higher levels of E2 predicted more externalizing psychopathology, but *only* when C levels were low and disagreeableness and emotional instability were high. These results were robust across both negative affectivity traits (emotional instability) and dysregulation and social dominance traits (disagreeableness), which reflect primary personality associations with externalizing psychopathology. In addition, these results remained consistent when controlling for concurrent levels of T, suggesting a specific association for E2 that is not fully accounted for by its covariation with T.

These findings represent an important and novel extension of a growing literature examining HPG-HPA axis regulation of socially dominant, competitive, and aggressive phenotypes (Dabbs et al., 1991; Popma et al., 2007; Mehta & Josephs, 2010). Mechanisms underlying the DHH have been proposed with some empirical support, including suggestions that high levels of C inhibit or suppress high levels of T at both neurological and behavioral levels of analysis (Popma et al., 2007; Mehta & Josephs, 2010; Zilioli and Watson, 2012; Marceau et al., 2014; Sinclair et al., 2014). Furthermore, the multi-level suppressive effects (i.e., the direct and multiple indirect pathways through which glucocorticoids exert their influence on HPG functioning) occur across multiple species (Kirby et al., 2009). Despite broader conceptualization of HPA axis activity suppression of the HPG axis in animal work (Kirby et al., 2009), investigations of these effects in humans have thus far been limited to T. The relevance of E2 and other gonadal hormones for adolescent development further points to potential mechanisms related to the rise in gonadal hormones across pubertal development. For example, increasing levels of E2 may exert influence on behavior via E2 modulation of genetic expression in neural systems or activation of organized neuroarchitecture (i.e., the ''organizational-activational hypothesis''; Peper & Dahl, 2013; Marceau et al., 2014; Sinclair et al., 2014; Balzer et al., 2015). Advances in focused research on these hypotheses will undoubtedly shed more light on DHH effects such as those examined here.

It remains an important future direction to better understand mechanisms underlying DHH effects, and to further determine the extent to which such effects may differ for T and E2. For example, some research has suggested that the aromatization of T into E2 may result in stronger associations between E2 and externalizing phenotypes, perhaps particularly in boys (Vermeersch et al., 2009; de Water et al., 2013). Parallel findings between behavioral associations with T and E2 should be examined more critically to determine both similarities and differences in these effects, and do so against the broader context of gender and pubertal development (Peper & Dahl, 2013; Koolschijn et al., 2014; Marceau et al., 2014; Sinclair et al., 2014). In line with established research demonstrating E2 links to aggressive and socially dominant behavior (e.g., Stanton & Schultheiss, 2007; Stanton & Edelstein, 2009; Vermeersch et al., 2008), links which parallel findings with T, the broader literature indicated support for the hypotheses tested in the present study as a first test of DHH effects for E2.

These findings also demonstrate the importance of measuring personality when testing associations between endocrine activity and clinically relevant phenotypes (Tackett et al., 2014a). Specifically, relevant personality traits potentially moderate the threshold at which hormone-behavior associations are detected, elucidating such findings in broader, more representative samples such as that used in the current study. Such moderators may help explain previous evidence suggesting sex differentiation of T and E2 associations with socially dominant and externalizing phenotypes — including personality moderation may allow a clearer understanding of whether such mechanisms truly function differently in men and women, or whether differences primarily exist in overall mean levels of sex-differentiated hormones. For example, personality characteristics reflecting sensation-seeking risk tendencies were associated with E2 in both male and female adolescents, demonstrating the potential generalization of broadly distributed personality traits across gender groups (Vermeersch et al., 2009). In addition, comparison of these findings with those for T in Tackett et al. (2014) indicate potential differences in associations at the facet level. It is important to note that, without using a more stringent alpha level (e.g., if examining all effects significant at $p < .05$), few differences emerge. However, future replication of these findings is needed to better understand potential lower-order trait differences in HPA-HPG interactions. This work underscores the potential relevance of personality trait measures for researchers working in this area, and the opportunity for enhancing generalizability of such associations across age, sex, and clinical status.

This work also provides an important extension to existing evidence for the DHH, which to date has only been demonstrated for T. Building on evidence that E2 may show similar associations with externalizing-relevant phenotypes (Stanton & Schultheiss, 2007; Vermeersch et al., 2008; Stanton & Edelstein, 2009), these findings demonstrate the larger role played by the HPG axis in potential DHH effects. Nonetheless, much more work examining the DHH is needed. For example, explorations of dynamic interactions between HPG and HPA hormones would help test temporal hypotheses about such hormonal associations. One recent investigation found that increases in T to a social stressor were highest for participants who had the lowest levels of basal C (Bedgood et al., 2014). Other research has examined state-level fluctuations of T and C relative to one another and relative to daily environmental stressors (Mehta & Josephs, 2010; Denson et al., 2013). Such work stands to move the field toward a more nuanced understanding of how HPA—HPG axes cross-talk over time.

Salient limitations of the current study represent important questions in future research. It will be necessary to replicate the current evidence for E2-C interplay in larger samples and across different externalizing-relevant phenotypes. The use of single samples for hormonal measurement is a limitation that should be addressed in studies with access to multiple measurement points, and across different data collection methods (e.g., plasma and hair).

[1] We replicated our analyses using youth-reported personality pathology traits. We also replicated our analyses in each sex analyzed separately. In both instances, we find the same pattern of results. For example, the slope of the interaction term for Disagreeableness is $\beta = -0.08$ for girls and $\beta = -0.25$ for boys and for Emotional Instability is $\beta = -0.40$ for girls and $\beta = -0.57$ for boys. Detailed findings are available from the first author on request.

Additionally, the present study gathered information about hormonal birth control use for its female participants, but did not include detailed information regarding those forms of contraception which are most relevant to E2 levels. Similarly, although efforts were made to schedule all female participants within the follicular phase, there was still some variation in cycle phase in the present study. Previous investigations have noted differential associations between E2 and externalizing behaviors in adolescent girls, depending on their phase in the menstrual cycle (Vermeersch et al., 2008, 2009), suggesting that the results presented here (primarily reflecting the earlier phase of the cycle) may represent underestimates of true effect sizes for these associations. Nevertheless, the current study provides an exciting and novel extension of the DHH, demonstrating the extended relevance of the HPG axis for understanding the potential influence of the HPA stress axis on socially dominant and dysregulated phenotypes, such as externalizing psychopathology.

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Contributors

The first author (J.L.T.) was responsible for the overall conceptualization and execution of this manuscript, data collection, data analysis and writing the majority of the manuscript. The second author (K.W.R.) was responsible for data analysis and writing portions of the manuscript. The third author (K.H.) assisted with data analysis and writing portions of the manuscript. The fourth—sixth authors (E.P.-G., K.P.H., and R.A.J.) were responsible for conceptual contributions, hypothesis development, interpretation of findings, contributing to analytic decisions, and editing the final manuscript. The fourth author (E.P.-G.) additionally participated in data collection efforts.

We confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed.

We further confirm that the order of authors listed in the manuscript has been approved by all of us.

Conflict of interest

The authors of this manuscript confirm that there are no known conflicts of interest associated with this publication.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.psyneuen.2015.02.014.

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