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**Probing Hormones and Personality Underlying the Emergence of
Adaptive and Maladaptive Behaviors in Adolescence**

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Dedication

To the companions who provided love and comfort during this journey but did not live to see the completion. Kenzie, Bravo, and of course my sweet girl Dog: you are missed, always.

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Abstract

Probing Hormones and Personality Underlying the Emergence of Adaptive and Maladaptive Behaviors in Adolescence

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Adolescence is uniquely characterized by alterations on multiple levels, such as biological changes associated with puberty, shifts in personality and behavior, and fluctuations in social contexts. Aspects of adolescent-specific development may set the stage for teens to achieve adult levels of competence, but also place them under increased stress resulting in deleterious outcomes. Adolescent increases in sensation seeking have been observed across cultures and species, suggesting functional significance rooted in basic biological maturation that occurs during puberty. Gonadal hormones have effects on neurobiological processes, implicating puberty-related hormonal change as a mechanism for changes in adolescent behavior, such as risk-taking and psychopathology. Researchers have speculated that hormones are responsible for behavioral associations with puberty; however, research on hormone-behavior associations in adolescence has been largely cross-sectional and has yielded inconsistent results. Informed by the animal literature on the timing-dependent action of hormones, we hypothesize that measuring intra-individual change in hormones is essential to understanding their sex-specific

effects on behavior. The proposed dissertation will use data from an on-going, population-representative study of children and adolescents, capitalizing on the large cross-sectional sample size ($N > 1000$), as well as longitudinal assessments of hormones and behaviors to investigate puberty-related processes underlying adolescent behavior. The overarching goal of this dissertation is to evaluate the degree to which typical adolescent development, as marked by sensation seeking and rises in pubertal hormones, relates to both adaptive and maladaptive behaviors in a normative adolescent sample.

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Paper 1: Is all risk-taking bad? Mixed methods validation of a positive risk-taking scale and evidence for genetic links to personality

INTRODUCTION

Society lauds adventurers: explorers, entrepreneurs, pioneers of geographical and outer space, of technology, culture, and human understanding. From first person in space Yuri Gagarin, to Malala Yousafzai's education activism, people are honored for taking risks that benefit society. Many of those who take societally-approved risks, however, might also be the same people who engage in illegal or antisocial risks. Take, for example, Steve Jobs, who claimed that his use of illegal psychedelics was one of the most important experiences of his life.

The current paper explores the construct of *positive risk-taking*, defined here as behaviors that have varied potential outcomes, including potential harm, failure, or rejection, but that do not violate societal norms or the rights of others. We conceptualize this set of behaviors not as categorically separate from negative, or antisocial forms of risk-taking, but rather as another expression of risk-taking tendencies that are potentially shaped by shared personality roots. In particular, we focus on the personality trait of *sensation seeking*, which captures a person's tendency to seek out and prefer novel, exciting and stimulating experiences despite potentially hazardous consequences (Zuckerman, 1994). Further, given the increased orientation of adolescents towards social relatedness (Somerville, 2013), we consider the important influence of personality traits reflecting social orientation such as *extraversion* (Fishman, Ng, & Bellugi, 2011), as well as the importance of peer contexts. We seek to illuminate not only the common sources of risk-taking behaviors generally, but also personality and peer environmental factors that may differentially influence expressions of risk-taking.

Sensation Seeking and Risk-Taking

During adolescence, average levels of sensation seeking rise dramatically (Harden & Tucker-Drob, 2011), as do rates of maladaptive risk-taking (Duell et al., 2018). Indeed, much of the current research on adolescence has focused on sensation seeking's association with maladaptive health and social risks, such as unwanted sex (Donohew et al., 2000), drunk driving (Jonah, 1997), or criminal behaviors (Newcomb & McGee, 1991; reviewed in Roberti, 2004). Sensation seeking prospectively predicts future engagement in antisocial risk-taking and mediates a substantial portion of its genetic etiology (Harden, Quinn, & Tucker-Drob, 2012; Mann et al., 2016, 2017).

Yet despite its associations with negative risk-taking, conceptualizing sensation seeking entirely as a vulnerability factor for psychopathology overlooks its potential adaptive value. Sensation seeking shows normative mean-level increases during adolescence (Harden & Tucker-Drob, 2011; Harden et al., 2012; Steinberg, 2013), a pattern that is seen across cultures (Steinberg et al., 2017) and across species (Vetter-O'Hagen & Spear, 2012). This universality suggests that increases in sensation-seeking may have particular functional significance during this period. Moreover, this pattern of mean-level developmental change is consistent with a broader corpus of research demonstrating that adolescence and young adulthood is a time of personality plasticity, reflected in both mean-level changes and rank-order instability (Briley & Tucker-Drob, 2014, 2017; Josef et al., 2016; Srivastava, John, Gosling & Potter, 2003).

In theoretical work, the developmental increase in sensation seeking has been hypothesized to be adaptive for adolescents as they navigate the complex tasks of separating from primary caregivers, establishing identities, forming intimate relationships, and exploring new interests that might fuel adult pursuits (Crone & Dahl, 2012). However, empirical evidence regarding the potential adaptive value of sensation

seeking has been limited, as few studies have attempted to measure positive risk-taking. Limited previous research has assessed positive risk-taking with participation in physically-risky sports or performance activities and has shown these activities are correlated with sensation seeking (Hansen & Breivik, 2001; Wood, Dawe, & Gullo, 2013). This focus on physical risk, however, neglects interpersonal risks that adolescents may take in everyday situations, such as initiating a new friendship. Given the importance of navigating social relationships and salience of social motivations in adolescence (Crone & Dahl, 2012), the social nature of risk-taking behaviors may be key to capturing tendencies toward positive risk-taking during adolescence.

Social Orientation and Interpersonal Risk in Adolescence

During adolescence, teenagers experience increasing autonomy to shape themselves and their own environments. At this time, adolescents begin to spend less time in the family context, and more time with their peers (Barnes, Hoffman, Welte, Farrell, & Dintcheff, 2007; Larson & Richards, 1991), and peer influence begins to take a greater role in shaping teenage behavior and decision-making (Brechwald & Prinstein, 2011; Gardner & Steinberg, 2005; Giordano, 2003; Steinberg & Monahan, 2007). Concerns about peer acceptance and rejection are heightened in adolescence, and peer evaluations have greater implications for how adolescents view their own self-worth and identity compared to other developmental periods (Kloep, 1999; O'Brien & Bierman, 1988; Pfeifer et al., 2009). Overall, adolescence has been characterized by an importance placed on social relationships.

Neurodevelopmental changes during this period sensitize adolescents to interpersonal and social dynamics (Foulkes & Blakemore, 2016; Guyer, Silk, & Nelson, 2016; Nelson, Leibenluft, McClure, & Pine, 2005). Non-human animals also show

increased social engagement during adolescence, suggesting that alterations in social sensitivity may facilitate the acquisition of adult levels of social competence (Douglas, Varlinskaya, & Spear, 2004; Meng, Li, Han, Shao, & Wang, 2010; Primus & Kellogg, 1989). The increased salience of social connection is reflected in increased attention and emotion related to processing social information (Crone & Dahl, 2012; Sommerville, 2013). Socially-sensitive adolescents show increases in emotional reactivity to cues of inclusion and exclusion, heightened attention to real or perceived social evaluation, and more complex considerations of the thoughts and feelings of others (Sommerville, 2013).

Heightened attunement to the social environment may intensify the potential rewarding or threatening consequences of interpersonal interactions or activities that may elicit social evaluation. Compared to adults, adolescents experience greater affective costs such as decreased mood and increased anxiety in response to experimentally-manipulated peer rejection (Sebastian, Viding, Williams, & Blakemore, 2010). Conversely, adolescents – but not young adults – respond to social rewards as strongly as they do to monetary rewards (Ethridge et al., 2017). Furthermore, studies of the neural processing of social rejection suggest that similar brain areas are activated in social pain as in physical pain (Eisenberger, 2012, 2015; Masten et al., 2009), while acceptance has been related to areas relevant for processing of rewards (Guyer, Choat, Pine, & Nelson, 2011). Even engaging in activities such as joining a sports team or interacting with unfamiliar peers may evoke perceptions of potential rejection, failure, or evaluation that imbue these behaviors with an element of riskiness. At the same time, the potential for gains in social status, inclusion, or positive peer perceptions through engaging in these behaviors may be especially motivating and rewarding.

Individual differences in sociability and interpersonal competence affect processing and engagement in social interactions. Extraversion is a developmentally

stable facet of personality that taps into an individual's proclivity towards social and activity participation, and positive affect (John, Naumann, & Soto, 2008), and reflects engagement in behaviors that attract social attention as well as reward responsivity (Ashton, Lee, & Paunonen, 2002; Lucas, Diener, Grob, Suh, & Shao, 2000). Extraverted individuals participate more in social interactions (Selfhout et al., 2010; Srivastava, Angelo, & Vallereux, 2008), show increased processing of social stimuli (Fishman et al., 2011), and enjoy social interactions that gain them attention, are prosocial, and involve group interactions (Ashton et al., 2002; Foulkes, Neumann, Roberts, McCrory, & Viding, 2017). Furthermore, in response to rejection, teens rated higher in interpersonal competence by their parents demonstrated higher levels of emotional reactivity but also greater emotion regulation processing suggesting an increased ability to tolerate the distress accompanied by rejection (Masten et al., 2009). As such, higher levels of extraversion may facilitate the selection of activities that result in positive social rewards, and provide a buffer from the negative toll of the consequences of rejection.

Peer Contexts and Risk-Taking in Adolescence

The peer context might be a critical determinant of whether tendencies toward risk-taking are manifest in negative or positive ways. Affiliation with deviant peers (e.g., those who skip school, use substances, commit petty crimes) is a robust correlate of antisocial behaviors (Thornberry, Lizotte, Krohn, Farnworth, & Jang, 1994). Additionally, studies involving laboratory tasks of risky decision-making demonstrate that teens are more susceptible to peer influence when it comes to engaging in risks (Gardner & Steinberg, 2005; Peake, Dishion, Stormshak, Moore, & Pfeifer, 2013). However, peer influence might also contribute to adolescents' decisions to engage in positive or prosocial behaviors (Barry & Wentzel, 2006; van Hoorne, van Dijk,

Meuwese, Rieffe, & Crone, 2016). For instance, teens who see their peers as having prosocial values and receive positive encouragement from their friends endorse higher self-esteem, empathy, and social initiative (Padilla-Walker & Bean, 2009).

In correlational studies, such as the current analysis, similarities between peer groups and individual behavior reflects both selection (predispositions to associate with similar peers) and socialization (peer influence that operates to make the individual more similar to his/her peers). Both selection and socialization effects have been documented with regards to the association between sensation seeking and negative risk-taking: Sensation seeking predicts association with deviant peers, which in turn partially accounts for the relationship between sensation seeking and engaging in problematic risks (Mann, Kretsch, Tackett, Harden, & Tucker-Drob, 2015). But even after controlling for selection effects using a co-twin control design, teenagers higher in sensation seeking were still more susceptible to negative influence from deviant peers (Mann et al., 2016). Insofar as sensation seeking acts as a personality mechanism in the development of risk-taking behavior, parallel positive peer selection and socialization effects may be present with sensation seeking and positive risk-taking.

The Current Study

The aims of the current study are three-fold. First, we aim to validate a measure of positive risk-taking. Second, we estimate the relationship of sensation seeking to both positive and negative risk-taking, as well as examine the presence of shared genetic associations among all three. Third, we evaluate the role of individual differences in extraversion and differential peer contexts in determining whether adolescents engage in positive or negative risks. To accomplish the first aim, we generated a pool of potential items tapping into activities and behaviors that adolescents engage in that may constitute

positive risks, and qualitatively assessed whether these behaviors were described as risks in a sample of late adolescents and emerging adults. Next, we examined the psychometric properties and further refined the resulting measure of positive risk-taking in a large, population representative sample of adolescent twins. To further examine the relationship between underlying personality, peer contexts, and both forms of risk-taking, we estimated phenotypic and genetic associations in this second sample. Based on previous theoretical literature positing that sensation seeking has functional significance in adolescence (e.g., Crone & Dahl, 2012), we hypothesized that sensation seeking would be equally strongly associated with positive and negative manifestations of risk, and that common genetic influence would partially account for this association, as has been found previously for sensation seeking and negative risk-taking. Finally, we explored whether extraversion was uniquely associated with risk-taking after accounting for effects of sensation seeking, and the mediational effects of positive and negative peer environments.

MATERIALS AND METHODS

Study 1. Qualitative Validation of Positive Risk-Taking

Data Collection

To validate a battery of positive risk-taking items, a sample of 75 undergraduate students (68.7% female, 29% male, 22.4% not reporting gender; age range: 18-28 years, $M = 20.1$, $SD = 1.7$), who were enrolled in an online introductory psychology course at a large southwestern university, completed a one-question survey through which they provided an open-ended, typed response. Responses were provided via learning

management software (Canvas). Student participants were asked to respond to the following prompt:

“In this writing assignment, please write about a time in your life when you took a risk. This could have been a small risk or a big one. It could have turned out well, or turned out badly, or just turned out different than you expected. It could be something that you did that was obviously dangerous or that broke the rules. Or, maybe the risk you took only felt risky for you personally. In your writing, you might think about some of these issues: What is your definition of risky? Why did you take that risk? What did it feel like to take that risk? What were you hoping would happen? What were you afraid would happen? What role did other people in your life -- your parents, friends, boyfriend/girlfriend -- play in the experience you are writing about. Write continuously for the full 15 minutes. Really let go and analyze your thoughts and feelings.”

This prompt was intended to tap into specific adolescent risk-taking experiences and as well as adolescent perceptions of risk-taking. Having students provide a typed response via online classroom software—as opposed to verbal, hand written, or emailed responses—allowed for greater participant anonymity and efficient data collection (Braun & Clarke, 2013). The open-ended format also allowed participants the freedom to answer however they want, without word limits (Terry & Braun, 2017).

Analytic Plan

We used thematic analysis of participants’ writing samples, in order to identify, analyze, and report patterns within and across the responses to the above prompt (Braun & Clarke, 2006). We began with an initial set of theory-driven, *a priori* items generated

to assess interpersonal interactions and activities that adolescents might engage in and that might result in consequences of failure or rejection (Table 1.1). The first and second authors then independently hand-coded ten risk-taking responses from a random subset of Study 1 participants. *A priori* themes identified in this item set, along with sub-themes and individual items are provided in Table 1.1. The first and second author also took notes on additional themes and subthemes that emerged from familiarizing and coding these ten responses.

Table 1.1. *A Priori* and Emergent Themes and Item-Specific Qualitative Coding Structure

Risk-Taking Domain	Broad Themes	Sub-Themes (where applicable)	Item-Specific Codes (where applicable)
Negative Risks	Physical Harm to Self Physical Harm to Others Breaking Rules/Laws <i>Emotional Harm (emergent)</i>		
Positive Risks	Disclosure		“Taken a risk and trusted someone?” [27] “Told a secret about yourself?” [21]
	Autonomy		“Ended a friendship?” [26] “Stood up for what you believe is right, either socially or academically?” [18] “Told someone the truth even if they didn’t want to hear it?” [16] “Broken up with somebody?” [24]
	Novelty	Interpersonal	“Initiated a new friendship?” [7] “Talked to someone who seemed very different from you?” [9] “Hung out with a new group of people?” [22] “Gone to a social event where you didn’t know anyone?” [23] “Asked someone on a date?” [11] “Gone on a blind date?” [12]
		Non-Interpersonal	“Taken a class in an area you know nothing about?” [13] “Played a sport you’re not good at?” [6] “Tried a new hairstyle or fashion trend?” [14] “Tried a new food?” [15] “Started a new diet or fitness plan?” [17] “Traveled someplace new?” [25]
	Joining In	Sports Related	“Played soccer, basketball, football, or some other team sport?” [4] “Played a sport with the opposite sex?” [1] “Tried out for a team...” [3]
		Non-Sports Related	“Run for office in school or in some other organization?” [10] “...joined a new club or activated, or auditioned for a play?” [3] “Volunteered?” [20] “Gone camping?” [2] <i>Education related activity, e.g. selecting a major (emergent)</i>
	Perceived Physical Danger		“Snow skied or snowboarded?” [5] “Ridden a rollercoaster?” [8] “Been skateboarding or biking without a helmet?” [19]
	<i>Prosociality (emergent)</i>		

Note. Emergent themes are italicized. Corresponding item numbers to the positive risk-taking measure are identified in brackets for the item-specific codes.

After this first round of coding, the first and second authors reconvened to discuss suitability, mutual exclusivity, and exhaustiveness of the initial set of *a priori* themes and subthemes to the data (Weber, 1990). The authors then used notes taken during the first-round of coding to determine an additional set of emergent themes and subthemes. Within positive risk-taking, one emergent theme was *Prosociality*, and an emergent subtheme was *Education-related risks* under the superordinate *Joining In* theme. Within negative risk-taking, one emergent theme was *Emotional Harm*. For a full set of superordinate themes and subthemes, see Table 1.1.

Given this updated list of *a priori* and emergent themes and subthemes, the authors then recoded the first ten risk-taking responses and continued to code the remaining risk-taking responses using MAXQDA (Version 12.1.3) qualitative coding software (Stemler, 2001).

After coding each risk-taking response using the modified set of emergent and *a priori* themes and subthemes, the first two authors came together to compare coding on all risk-taking responses as a quality control check (Stemler, 2001). Where there were coding discrepancies, the authors discussed and reached an agreement on the appropriate codes, resulting in 100% consensus.

Study 2: Psychometric Properties of Positive Risk-Taking and Associations with Personality

Participants

The sample for the primary analyses was drawn from the Texas Twin Project (Harden, Tucker-Drob, & Tackett, 2013), an ongoing twin study based in central Texas. The current sample ($N = 1249$) ranged in age from 13.5 to 20.1 years ($M = 16.0$, $SD = 1.3$), and was 49.0% female. Of these, $N = 875$ contributed data on positive risk-taking, as this measure was not administered until the second year of data collection (*i.e.*, data

missing completely at random). Participants were identified from public high school rosters and recruited for an in-laboratory assessment. Surveys were administered via the computer and in separate rooms to protect the participants' privacy. All study procedures received approval from the University of Texas Institutional Review Board.

The Texas Twin Project is a racially, ethnically, and socioeconomically diverse population-based sample. Of the individuals who self-reported race/ethnicity (1.0% did not report), 55.1% were White, 14.9% Hispanic/Latino, 11.5% African American, 5.8% other, and 11.6% multiple race/ethnicity. Forty-two percent of the sample reported receiving some means-tested public assistance (*e.g.*, food stamps) since the birth of the twins.

Genetic analyses included 571 twin pairs (382 pairs with positive risk-taking data, 3 with a missing co-twin), with 15 triplet sets contributing three pair-wise combinations each, and one quadruplet set contributing six pair-wise combinations.

Measures

Sensation Seeking. The UPPS-Impulsive Personality Sensation Seeking subscale (Whiteside & Lynam, 2001) was used to measure sensation seeking. Twelve items assessed self-reported agreement (1 – *Disagree Strongly* to 4 – *Agree Strongly*) with statements such as, “I generally seek new and exciting experiences and sensations,” and “I will try anything once.”

Negative Risk-Taking. Thirty-six items assessing frequency of lifetime engagement in delinquent behaviors rated on a 3-point scale (0 – *Never*, 1 – *Once*, 2 – *More Than Once*) was used to measure antisocial risk-taking (Huizinga, Esbensen & Weiher, 1991). Items ranged in severity from “driven a car very fast?” to “carried a hidden weapon (a knife or a gun)?”

Positive Risk-Taking. Twenty-seven items were administered to assess positive risk-taking by rating the frequency in the last year of positive risks on a 6-point scale (0 – *Never*, 1 – *1-2 times*, 2 – *3-5 times*, 3 – *6-10 times*, 4 – *11-15 times*, 5 – *16 times or more*). Item selection and construction of scaled score was informed by results of Study 1 and confirmatory factor analysis in Study 2 (see “Positive Risk-Taking Psychometrics” section of the Results). For an overview of the steps in scale creation see Figure 1.1, and all items are located in Table 1.2. The score was normally distributed and had good internal reliability (Table 1.3).

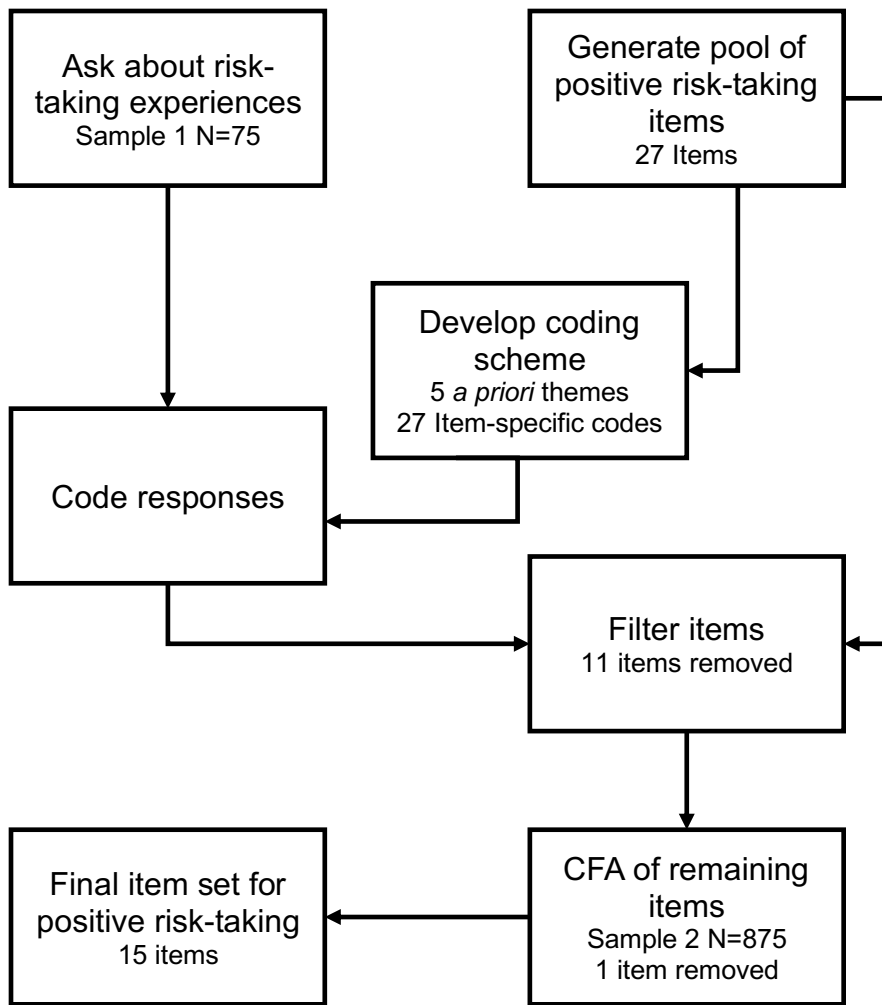


Figure 1.1. Pipeline of qualitative and quantitative methods applied to create and validate the positive risk-taking measure.

Table 1.2. Positive Risk-Taking Items Qualitative Endorsement and CFA Factor Loadings

Item	Endorsements	% of Total Responses	CFA Factor Loadings (SE)
3. Tried out for a team, joined a new club or activity, or auditioned for a play?	16	21%	.50 (.04)
4. Played soccer, basketball, football, or some other team sport?	1	1%	.36 (.04)
6. Played a sport you're not good at?	1	1%	.42 (.04)
7. Initiated a new friendship?	10	13%	.59 (.03)
9. Talked to someone who seemed very different from you?	8	11%	.63 (.03)
10. Run for office in school or in some other organization?	3	4%	.35 (.04)
11. Asked someone on a date?	9	12%	.35 (.04)
12. Gone on a blind date?	2	3%	--
13. Taken a class in an area you know nothing about?	3	4%	.40 (.04)
18. Stood up for what you believe is right, either socially or academically?	21	28%	.52 (.03)
21. Told a secret about yourself?	2	3%	.63 (.03)
22. Hung out with a new group of people?	18	24%	.63 (.03)
23. Gone to a social event where you didn't know anyone?	7	9%	.58 (.04)
25. Traveled someplace new?	14	19%	.56 (.03)
26. Ended a friendship?	2	3%	.38 (.04)
27. Taken a risk and trusted someone?	6	8%	.60 (.03)
1. Played a sport with the opposite sex?	0		
2. Gone camping?	0		
5. Snow skied or snowboarded?	0		
8. Ridden a rollercoaster?	0		
14. Tried a new hairstyle or fashion trend?	0		
15. Tried a new food?	0		
16. Told someone the truth even if they didn't want to hear it?	0		
17. Started a new diet or fitness plan?	0		
19. Been skateboarding or biking without a helmet?	0		
20. Volunteered?	0		
24. Broken up with somebody?	0		

Note. Endorsements is the number of unique responses identified with this item-specific code, and % of total responses is the percentage of this unique code in the total 75 written responses. CFA factor loading are reported from the unidimensional factor model, dropping item 12, with standard errors reported in parentheses. All factor loadings were significant at $p < .001$.

Peer Environment. Measures of negative and positive peer environments were adapted from a peer deviance scale (Thornberry et al., 1994). Twins rated how many of their peers engaged in certain behaviors on a 4-point scale (1 – *None of Them*, 2 – *One or Two*, 3 – *Most of Them*, 4 – *All of Them*). Ten items assessed negative peer behaviors, such as “Lied, disobeyed, or talked back to adults?” and “Sold drugs?”. Twelve items assessed positive peer behaviors, such as “Took part in family activities?” and “Were considered good citizens?”.

Zygosity. All opposite-sex twin pairs were classified as dizygotic (DZ). Zygosity of same-sex twin pairs was determined through Latent Class Analysis (LCA) of similarity ratings administered independently to a parent, each twin, and two trained research assistants. Nine items assessed physical similarity (e.g. facial appearance, hair color and texture) and whether the twins were mistaken for one another. LCA classified 207 monozygotic (MZ) twin pairs, and 364 DZ pairs (171 opposite-sex). LCA of similarity ratings has greater than 99% concordance with zygosity classification through genotyping (Heath et al., 2003).

UPPS Impulsive Personality. UPPS subscales for Negative Urgency, Lack of Premeditation, and Lack of Perseverance were included in convergent and divergent validity correlations (Whiteside & Lynam, 2001). Participants rated agreement on a 4-point scale, similar to sensation seeking. Twelve-items measured negative urgency, such as “When I am upset I often act without thinking.” Lack of premeditation was measured by eleven reverse coded items, such as: “I usually think carefully before doing anything.” Lack of perseverance was measured by 10 items such as “I tend to give up easily,” and 8 of which were reverse coded (e.g. “I finish what I start.”).

Big Five Personality. Normative personality was assessed using The Big Five Inventory (John et al., 2008) to measure five broad dispositional dimensions:

Extraversion (8 items, e.g. “is full of energy”), Agreeableness (9 items, e.g. “is helpful and unselfish with others”), Conscientiousness (9 items, e.g. “is a reliable worker”), Openness to Experience (10 items, e.g. “is curious about many different things”), and Neuroticism (8 items, e.g. “can be moody”). Participants rated agreement with each item on a 5-point scale from 1 – *Strongly Disagree*, to 5 – *Strongly Agree*.

Parental Monitoring. Fifteen items assessed parental monitoring—that is, how much parents know about their children’s friends and whereabouts, as well as rules parents’ have (Capaldi & Patterson, 1989). Adolescents rated their parents’ knowledge on a three-point scale (1 – *They Don’t Know* to 3 – *They Know a Lot*) for items such as, “What you do with your free time?” They also rated frequency of certain rules, such as “If you have been out past curfew, do your parents require an explanation?” on a three-point scale (1 – *Never* to 3 – *Always*).

Friendship Quality. Peer relationship quality was measured with 25 items rated on a 7-point scale from 1 – *None of the time* to 7 – *All of the time* (Hudson, Nurius, Daley, & Newsome, 1990). Positively coded items included: “I get along very with my peers” and reverse coded items included, “My peers act like they don’t care about me.”

Relational Aggression. Relational (non-physical) aggression towards peers was rated on nine items on a 5-point scale from 1 – *Never True* to 5 – *Almost Always True* (5 positively coded, 4 reverse coded; Crick & Grotpeter, 1995). Positively coded items included: “I spread rumors or gossip about other teens,” and reverse coded items included: “I say supportive things to other teens.”

IQ. The Wechsler Abbreviated Scale of Intelligence-II (WASI-II; Wechsler, 1999) was used to assess general cognitive ability. This assessment consists of four tasks: Block Design and Matrix Reasoning constitute the Perceptual Reasoning Index score, and Vocabulary and Similarities combine to form the Verbal Comprehension Index. Using

the WASI-II, Perceptual Reasoning and Verbal Comprehension Index scales are combined to form Full-Scale IQ (FSIQ) ($M = 102.3$, $SD = 13.1$). The WASI-II is normed for ages 6 through 89 and has a high test-retest correlation ($r = .91$; Wechsler, 1999).

Descriptive statistics for all measures are reported in Table 1.3.

Table 1.3. Descriptive Statistics, and Convergent and Divergent Correlations with Psychosocial Measures

Measures	Range	Mean (SD)	α	Correlations															
				1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	12.	13.	14.	15.	16.
1. PRT	0.00 – 4.80	1.57 (.07)	.83																
2. E	1.25 – 5.00	3.17 (0.74)	.84	.28															
3. A	1.33 – 5.00	3.69 (0.54)	.76	.07	.18														
4. C	1.44 – 5.00	3.32 (0.61)	.79	.05	.03	.36													
5. N	1.00 – 5.00	2.89 (0.70)	.81	.03	-.19	-.27	-.16												
6. O	1.80 – 5.00	3.79 (0.51)	.74	.14	.11	.15	.07	.04											
7. Urg	1.00 – 4.00	2.18 (0.59)	.87	.10	.03	-.37	-.35	.48	-.01										
8. Prem	1.00 – 3.64	2.04 (0.47)	.83	.07	.22	-.24	-.48	.01	-.14	.27									
9. Pers	1.00 – 3.70	2.01 (0.51)	.84	-.12	-.13	-.29	-.70	.19	-.17	.30	.44								
10. SS	1.00 – 4.00	2.83 (0.59)	.86	.24	.21	.04	-.03	-.17	.04	.11	.24	-.07							
11. IQ	57 – 146	103 (13.5)		-.01	-.04	.02	.05	-.04	.12	-.10	-.01	-.05	.02						
12. PMon	1.07 – 3.00	2.59 (0.31)	.81	.08	.06	.15	.17	.002	.06	-.18	-.13	-.15	-.15	.13					
13. FQual	1.24 – 7.00	5.61 (0.93)	.94	.06	.25	.34	.21	-.30	.04	-.34	-.09	-.28	.10	-.003	.18				
14. RAgg	1.00 – 3.89	1.87 (0.47)	.72	-.05	-.08	-.55	-.27	.11	-.29	.34	.24	.27	.07	-.15	-.19	-.34			
15. NRT	0.00 – 1.56	0.20 (0.21)	.87	.18	.09	-.26	-.28	.10	-.07	.38	.28	.19	.32	-.12	-.39	-.22	.31		
16. Sex				-.01	.03	-.08	-.10	-.30	-.09	-.01	.05	.03	.18	.03	-.13	.002	.19	.19	
17. Age	13.5 – 20.1	16.0 (1.35)		.04	-.04	.09	.07	.02	.07	.01	-.11	-.08	.10	-.03	-.26	-.02	-.11	.31	-.02

Note. Descriptive statistics are reported on raw variables all transformations. Correlations are reported using z-scored variables post-transformations, including log transforming delinquency, parental monitoring, and friendship quality, and positive risk-taking item-level sex regression. Significant correlations of $p_{two-tailed} < .001$ in bold. PRT = Positive Risk-Taking; E = Extraversion; A = Agreeableness; C = Conscientiousness; N = Neuroticism; O = Openness; Urg = Negative Urgency; Prem = Lack of Premeditation; Pers = Lack of Perseveration; SS = Sensation Seeking; PMon = Parental Monitoring; FQual = Friendship Quality; RAgg = Relational Aggression; NRT = Negative Risk-Taking. Sex coded 0 = female, 1 = male. Age centered at 16.

Planned Analyses

Negative risk-taking and negative peer environment measures were log-transformed to correct for positive skew. In phenotypic analyses, age, sex, African-American and Other race, and Hispanic/Latino ethnicity were included as covariates. In behavior genetic analyses, the same covariates were regressed out of the focal variables, and standardized residuals were used. We conducted three sets of analyses: (1) evaluating the psychometrics of the positive-risk taking scale; (2) estimating phenotypic and genetic associations across sensation seeking and risk-taking; and (3) differentiating personality and peer contextual factors in positive and negative risk-taking.

In the first set of analyses, which examined the psychometric properties of the positive risk-taking scale, confirmatory factor analyses (CFA) were conducted with the items endorsed in the qualitative responses. To determine if there were measurement differences across males and females, we also tested for measurement invariance across sex. Convergent and discriminant validity of the resulting positive risk-taking measure was evaluated by estimating correlations with sex, age, IQ, personality traits, parent and peer relationship variables, and negative risk-taking.

Our second set of analyses investigated the phenotypic and genetic associations between sensation seeking and risk-taking. We first assessed the phenotypic relationship between sensation seeking and both forms of risk-taking, allowing for a residual correlation between positive and negative risks. Next, we evaluated shared genetic and environmental variance unique and shared between sensation seeking and both forms of risk-taking. Classic twin models are parameterized by decomposing the variance in a given trait into standardized latent factors of additive genetic (A), non-shared environmental (E), and either dominance genetic (D) or shared environmental (C)

influence, given the different genetic relatedness between MZ (100% shared genes) and DZ twins (on average 50% shared genes) raised together in the same home. Dominance genetic effects and shared environmental effects cannot be assessed within the same model, and previous literature was used to guide model selection. In these models, A and D correlate at 1 for MZ twins, and .5 and .25 respectively for DZ twins, and C correlates at 1 for all twin pairs. E is uncorrelated between twin pairs and includes measurement error. Based on previous behavior genetic analyses of sensation seeking in this sample, an AE model was fit for sensation seeking, such that shared environmental influences (C) were constrained to zero (Mann et al., 2016). A univariate ACE model was fit to the positive risk-taking measure to determine the composition of genetic and environmental variance.

Next, to assess shared and unique genetic and environmental effects between both forms of risk-taking, we used a bivariate Cholesky decomposition, such that positive risk-taking was regressed on the ACE factors of negative risk-taking. Finally, we included sensation seeking as the initial predictor in a trivariate Cholesky decomposition to determine whether genetic and environmental variance on sensation seeking accounted for variance in both forms of risk-taking. In this model, variance in sensation seeking was decomposed into AE factors, with cross paths to negative and positive risk-taking (e.g., A in sensation seeking predicting variance in negative risk-taking), variance in negative risk-taking net of sensation seeking was decomposed into ACE with cross paths to positive risk-taking, and the unique variance in positive risk-taking was decomposed into ACE .

Our third set of analyses investigated whether extraversion and peer contexts differentiated between positive and negative risk-taking. First, we included extraversion as a predictor of both forms of risk-taking in our phenotypic model, accounting for the

correlation between sensation seeking and extraversion. Next, we evaluated the mediation of personality effects on risk-taking through positive and negative peer environments, allowing for a residual correlation between peer environments. Finally, to determine whether genetic and environmental effects on extraversion influenced risk-taking after accounting for common etiology with sensation seeking, a Cholesky decomposition including extraversion was used. Consistent with previous work in this sample, an *ADE* model was fit for extraversion (Mann, Briley, Tucker-Drob, & Harden, 2015). In this model, sensation seeking *AE* factors predicted variance in extraversion, and both forms of risk-taking, *AE* factors for extraversion net of sensation seeking predicted both forms of risk-taking, and *ACE* factors for negative risk-taking net personality measures predicted positive risk-taking, with the remaining unique variance in positive risk-taking was modeled as *ACE* factors.

RESULTS

Study 1: Qualitative Analysis Results

Within the 75 written responses, 93% of responses contained positive risk-taking themes, and 21% of responses contained negative risk-taking themes, with two responses failing to identify a risk-taking behavior (<3%). Within responses describing risk-taking behavior, 17% fell into both positive and negative risk-taking themes.

Of the emergent themes that were identified, only 3% of responses coded as positive risk-taking were identified within the Prosociality theme, and all of these responses fell within one or more of the *a priori* positive risk-taking thematic codes. For the emergent theme for Education-related risks, 37% of positive risk-taking responses were identified with this code; however, only one response did not include any *a priori* positive risk-taking themes. Within negative risk-taking, an emergent broad theme for

“Emotional harm to self/others” was identified, and accounted for 13% of responses including negative risk-taking (2 total).

All positive risk-taking responses included at least one *a priori* thematic code (Table 1.2). A total of 123 item-specific codes were assigned, accounting for 53% of total positive risk-taking codes. Item-specific codes with at least one endorsement were included in subsequent quantitative analyses, resulting in 11 initially-generated items failing to be endorsed as risks within this sample (Table 1.2). Of the 16 responses identifying negative risk-taking, 2 responses were coded as only physical harm to self (13%), 10 responses as breaking rules/laws (63%), and 2 responses (13%) with both of these negative risk-taking themes. No responses were coded as physical harm to others.

Study 2

Structural equation modeling (SEM) was conducted in *Mplus* version 7.2, using the complex sampling option to account for non-independence of twins within families for phenotypic analyses, and for multiple pair-wise combinations for sets of multiples in behavior genetic analyses. Triplet pair-wise combinations were weighted by .5, and quadruplet pair-wise combinations by .33 to account for representation of individuals within more than one pair. Full information maximum likelihood was used to account for missing data in phenotypic and behavior genetic SEMs, such that the entire sample ($N = 1249$) was included, even for participants with missing positive risk-taking data. Analyses were restricted to the positive risk-taking subset ($N = 875$) only when determining the psychometric properties of the positive risk-taking items.

Psychometrics of the Positive Risk-Taking Scale

Confirmatory factor analysis. Using the 16 items endorsed in the qualitative analysis, we tested a unidimensional confirmatory factor model. Model fit was good ($\chi^2 = 313.16$, $df = 104$, $p < .001$, RMSEA = .048, AIC = 40018.88). In this CFA, only one item did not significantly load on the latent factor at $p < .001$ (“Gone on a blind date”, $\lambda_{12} = .08$, $p = .07$). Dropping this item did not significantly deteriorate model fit ($\Delta\chi^2 = 2.93$, $\Delta df = 1$, $p = .09$), and the resulting model retained good fit ($\chi^2 = 316.09$, $df = 105$, $p < .001$, RMSEA = .048, AIC = 40021.08). The final CFA of these items produced factor loadings that were all significant and moderate in magnitude (Table 1.2).

Measurement invariance between males and females. A series of measurement invariance models were fit to determine whether the positive risk-taking factor was invariant across sex. We first compared a *configural invariance* model, in which an equivalent factor model was fit in a two-group model, with item means and factor loading allowed to differ between sexes, to a *metric invariance* model, in which factor loadings were fixed to equality for boys and girls. The metric invariance model did not result in a decrement of fit compared to the configural invariance model, indicating that items were equally good indicators of the latent positive risk-taking factor (Table 1.4). The metric invariance model was then compared to a *scalar invariance* model, in which both factor loadings and item-specific means were constrained to equality across groups. The scalar invariance model fit significant worse than the metric invariance model, indicating a difference in the item means for boys and girls (Table 1.4). This demonstrated that items were equally good indicators of positive risk-taking for both boys and girls but occurred with different frequency across sexes. Six item means were not invariant across sex: means in the metric invariance model for items 4, 6, 11, and 27 were greater in boys than in girls, and for item 26, greater in girls than in boys.

Table 1.4. Measurement Invariance Model Comparisons

Model	Chi-square (df)	RMSEA	CFI/TLI	Model comparison	Chi-square difference (Δ df)
Configural	411.09*** (180)	.054	.886/.867		
Metric	421.40*** (194)	.052	.888/.878	Metric vs. Configural	13.58 (14)
Scalar	535.834*** (209)	.060	.838/.838	Scalar vs. Metric	124.69*** (15)

Note. *** = $p < .001$.

To account for sex differences in the means when computing composite scores, sex was first regressed out of each item, and then the mean of the item-specific residuals was calculated. Resulting values were standardized, and these scores were used in subsequent analyses.

Convergent and discriminant validity. Convergent and discriminant validity was explored by examining correlations between positive risk-taking and Big Five personality, UPPS Impulsive Personality, IQ, parental monitoring, peer friendship quality, relational aggression, and negative risk-taking (Table 1.3). Prosocial risk-taking was not isomorphic with established personality or contextual variables. For Big Five personality traits, positive risk-taking showed significant, small to moderate correlations with extraversion and openness, but was unrelated to agreeableness, conscientiousness, or neuroticism. For UPPS Impulsive Personality subscales, positive risk-taking showed a small to moderate correlation with sensation seeking, and a small negative correlation with lack of perseverance, but was unrelated to negative urgency, or lack of premeditation. Positive risk-taking was unrelated to IQ, parental monitoring, peer friendship quality, or relational aggression. Negative risk-taking was positively correlated with positive risk-taking, consistent with our hypothesis that these share personality roots.

Phenotypic and Genetic Associations Between Sensation Seeking and Risk-Taking

Main effects of sensation seeking and on risk-taking. An initial phenotypic SEM tested the associations of sensation seeking with both positive and negative risk-taking, with a residual correlation between forms of risk-taking. This model demonstrated adequate fit to the data ($\chi^2 = 47.70$, $df = 10$, $p < .001$, RMSEA = .055, CFI = .89, AIC = 17971.44). Sensation seeking was significantly positively associated with both forms of

risk-taking (positive risk-taking: $\beta = .24, p < .001$; negative risk-taking $\beta = .27, p < .001$). After accounting for common effects of sensation seeking, positive and negative risk-taking maintained a small positive residual correlation ($r = .10, p < .001$).

Positive risk-taking univariate model. To determine the genetic and environmental influence on positive risk-taking, a univariate *ACE* model was used to decompose the variance in positive risk-taking into additive genetic, shared environmental and non-shared environmental variance. Additive genetic and non-shared environmental estimates were significant ($a_{PRT} = .54, SE = .07$; $e_{PRT} = .83, SE = .05$), such that 30% of variance in positive risk-taking was accounted for by additive genetic influences, and the remaining 70% were accounted for by non-shared environment. There were no estimated effects of the shared environment on positive risk-taking.

Bivariate genetic associations between positive and negative risk-taking. In order to determine whether the relationship between forms of risk-taking arose from genetic or environmental influences, we used a bivariate Cholesky decomposition to determine etiological cross-paths from negative to positive risk-taking (Figure 1.2a; Table 1.5). Additive genetic, shared environmental, and non-shared environmental effects on negative risk-taking were each significant, and accounted for 49.7%, 11.4%, and 38.9% of the total variance respectively. For positive risk-taking, additive genetic and non-shared environmental influences were largely unique and accounted for 29.6% (85% unique) and 70.5% (98.3% unique) of the total variance respectively. The phenotypic association between negative and positive risk-taking was primarily accounted for by shared additive genetic effects, which accounted for 78.5% of their correlation.

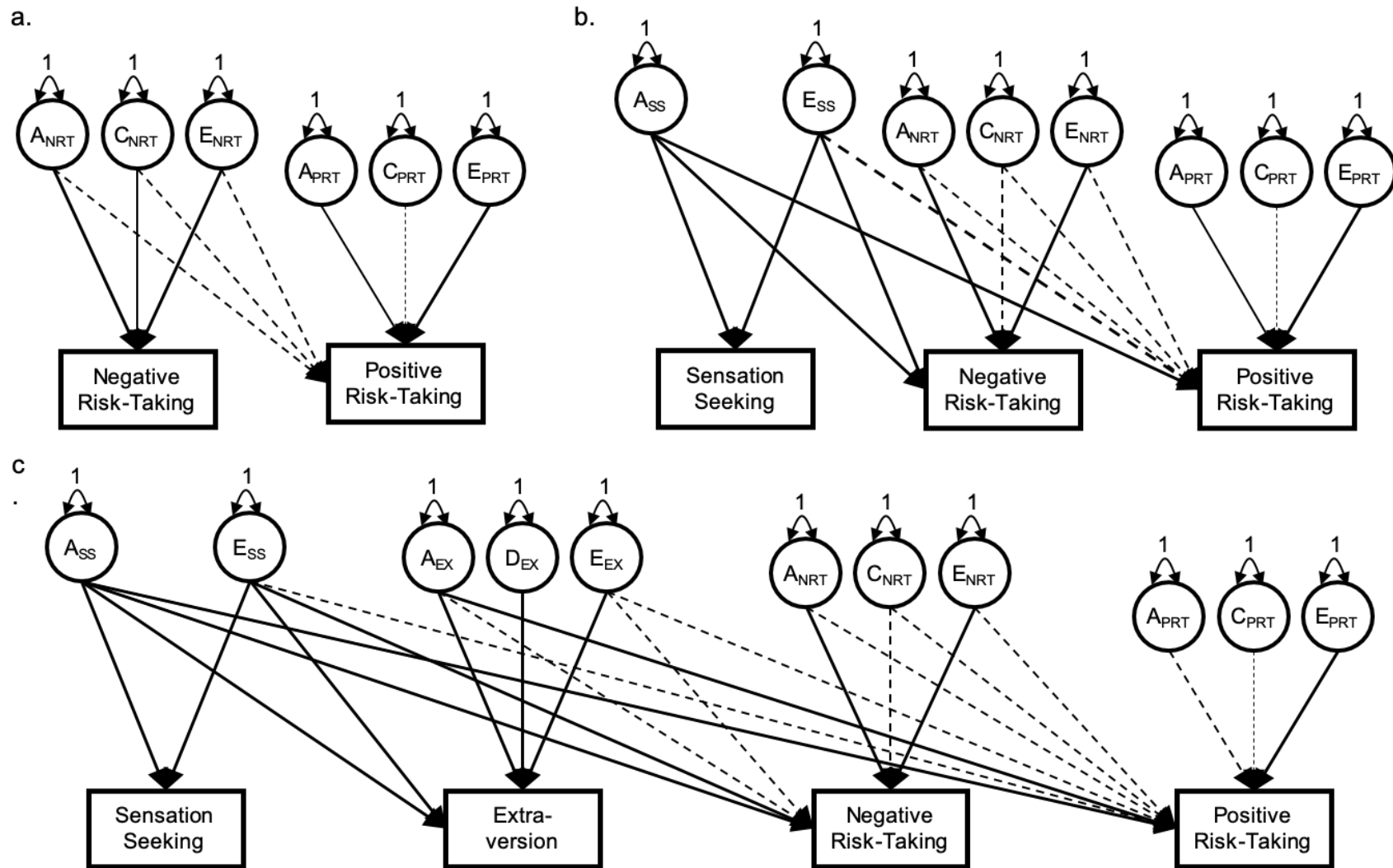


Figure 1.2a. Bivariate Cholesky decomposition of negative and positive risk-taking. 1.2b. Trivariate Cholesky decomposition of sensation seeking, negative, and positive risk-taking. 1.2c. Multivariate Cholesky decomposition of sensation seeking, extraversion, negative and positive risk-taking. Solid paths significant at $p < .05$. Estimates provided in Table 1.6.

Table 1.5. Model Estimates of the Multivariate Cholesky

		Sensation Seeking	Extraversion	Negative Risk-Taking	Positive Risk-Taking
Bivariate Cholesky					
Negative Risk-Taking					
	ANRT			.79*** (.09)	.21 (.11)
	CNRT			.33* (.15)	-.02 (.14)
	ENRT			.61*** (.03)	.11 (.06)
Positive Risk-Taking					
	APRT				.50*** (.09)
	CPRT				0.000 (.00)
	EPRT				.83*** (.05)
Trivariate Cholesky					
Sensation Seeking					
	ASS	.71*** (.04)		.28*** (.05)	.28*** (.07)
	ESS	.69*** (.03)		.11** (.04)	.06 (.06)
Negative Risk-Taking					
	ANRT			.65*** (.10)	.09 (.11)
	CNRT			.27 (.17)	-.02 (.17)
	ENRT			.60*** (.04)	.10 (.06)
Positive Risk-Taking					
	APRT				.46*** (.08)
	CPRT				0.000 (.00)
	EPRT				.82*** (.05)
Multivariate Cholesky					
Sensation Seeking					
	ASS	.71*** (.04)	.22*** (.06)	.28*** (.05)	.29*** (.06)
	ESS	.68*** (.03)	.11* (.05)	.11** (.04)	.05 (.06)
Extraversion					
	AEX		.34*** (.07)	.11 (.11)	.49*** (.07)
	DEX		.56*** (.07)	--	--
	EEX		.72*** (.04)	-.001 (.04)	.05 (.05)
Negative Risk-Taking					
	ANRT			.64*** (.10)	.01 (.09)
	CNRT			.27 (.17)	-.03 (.10)
	ENRT			.60*** (.04)	.11 (.06)
Positive Risk-Taking					
	APRT				.000 (.000)
	CPRT				.000 (.000)
	EPRT				.80*** (.04)

Note. * = $p < .05$, ** = $p < .005$, *** = $p < .001$. Unstandardized estimates of standardized variables reported, standard error in parentheses. Models depicted in Figure 1.2.

Sensation seeking, negative risk-taking, and positive risk-taking genetic associations. In order to investigate whether genetic and environmental influences on sensation seeking accounted for the shared variance in negative and positive risk-taking, a trivariate Cholesky decomposition was fit. Sensation seeking, negative and positive risk-taking were all moderately to strongly heritable, and additive genetic influence on sensation seeking significantly predicted both negative and positive risk-taking (Figure 1.2b; Table 1.5). Additive genetic influences accounted for 51.4% of the total variance in sensation seeking. Including genetic cross-paths, additive genetic influences explained 53.0% of the total variance in negative risk-taking, and 29.4% of the total variance in positive risk-taking. Genetic cross-paths from sensation seeking were significant for both forms of risk-taking, such that 15.7% and 27% of total additive genetic influences on negative and positive risk-taking respectively were accounted for by additive genetic effects on sensation seeking. Additionally, there were significant genetic influences on negative risk-taking net of sensation seeking, but these unique genetic influences did not significantly predict positive risk-taking. Finally, significant unique genetic influences on positive risk-taking accounted for 21.5% of the total variance in positive risk-taking.

There were minimal effects of shared environment on negative or positive risk-taking (7.7% and <0.01% of their respective total variance). Additionally, shared environmental effects common across risk-taking phenotypes were negligible. The non-shared environment contributed significantly to sensation seeking, accounting for 48.6% of total variance; negative risk-taking, accounting for 39.3% of total variance (3.3% of which was shared with sensation seeking); and positive risk-taking accounting for 69.7% of total variance (98.0% of which was unique).

Extraversion and Peer Context Pathways to Risk-Taking

Main effects of extraversion on risk-taking. In order to assess the effects of extraversion on risk-taking, extraversion was added to the initial phenotypic model, such that paths from extraversion to both forms of risk-taking, and a correlation between extraversion and sensation seeking, were added to this model. This model also had adequate fit to the data ($\chi^2 = 47.70$, $df = 10$, $p < .001$, RMSEA = .055, CFI = .91, AIC = 21417.44). Extraversion was significantly positively associated with positive risk-taking ($\beta = .24$, $p < .001$), but not with negative risk-taking ($\beta = .04$, $p = .19$). Accounting for a significant correlation between extraversion and sensation seeking ($r = .21$, $p < .001$), the relationship between sensation seeking and positive risk-taking remained significant ($\beta = .20$, $p < .001$), as did the association between sensation seeking and negative risk-taking ($\beta = .26$, $p < .001$) and the residual correlation between forms of risk-taking ($r = .10$, $p < .001$).

Indirect effects of personality on risk-taking by peer contexts. A final phenotypic SEM additionally estimated the mediating effects of positive and negative peer environments (Figure 1.3). This model adequately fit the data ($\chi^2 = 47.701$, $df = 10$, $p < .001$, RMSEA = .055, CFI = .97, AIC = 27464.33). The main effects of sensation seeking on both negative and positive risk-taking, of extraversion on positive risk-taking, and correlations between personality variables and between risk-taking measures remained significant. Sensation seeking was associated with negative peer environments, but not positive peer environments. Conversely, extraversion was associated with positive peer environments, but not negative peer environments. There was a strong negative residual correlation between positive and negative peer environments. Furthermore, negative peer environments predicted *both* positive and negative risk-taking, such that there was a statistical indirect effect of sensation seeking on both forms of risk-taking through

negative peer environments. On the other hand, a slight negative association was detected between positive peer environments and negative risk-taking, consistent with a protective effect of positive peer associations against negative behaviors. Additionally, a positive association was detected between positive peer environments and positive risk-taking. The statistical indirect effect of extraversion through positive peer environments was significant for both forms of risk-taking. Associations with covariates in this final model are depicted in Table 1.6.

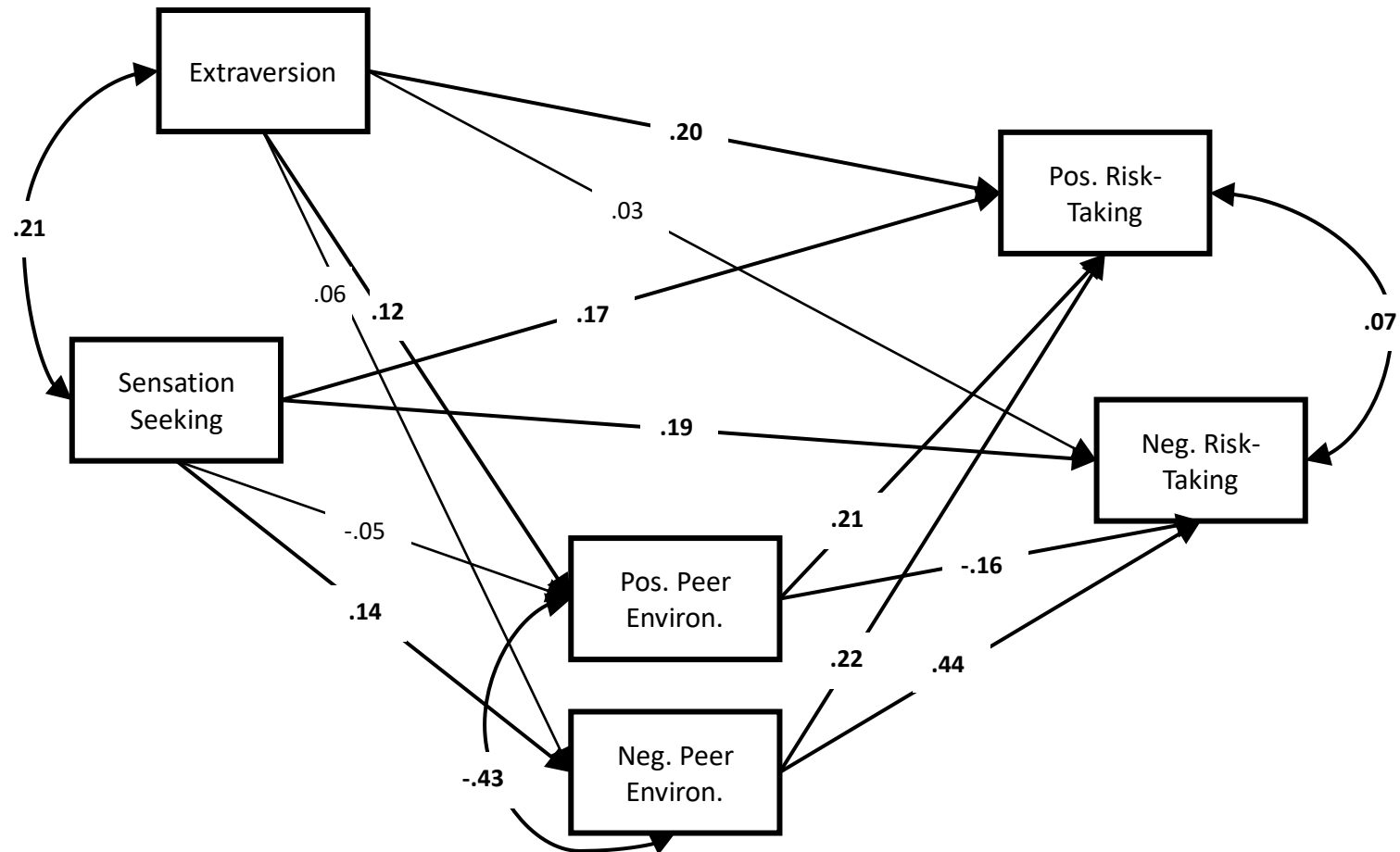


Figure 1.3. Peer mediation model evaluating sensation seeking and extraversion associations with risk-taking. Age, sex, and race included in the model but not depicted. Bolded paths significant at $p < .05$.

Table 1.6. Model Estimates of Covariates in Peer Mediation Model

	Age	Sex	African-American	Hispanic/Latino	Other
Sensation Seeking	.07*** (.02)	.35 (.06)***	-.05 (.09)	.05 (.08)	-.01 (.12)
Extraversion	-.02 (.02)	.05 (.06)	.07 (.08)	-.10 (.07)	-.08 (.11)
Prosocial Risk-Taking	-.01 (.03)	-.06 (.06)	.06 (.11)	-.14 (.08)	.01 (.12)
Antisocial Risk-Taking	.15*** (.02)	.17*** (.05)	.20** (.07)	-.02 (.08)	-.03 (.08)
Positive Peer Environments	-.01 (.02)	-.25*** (.03)	-.24* (.10)	-.19* (.08)	.32*** (.09)
Negative Peer Environments	.14*** (.02)	.18** (.06)	.24* (.11)	.01 (.08)	-.32*** (.09)

Note. * = $p < .05$, ** = $p < .005$, *** = $p < .001$. Unstandardized estimates of standardized variables reported, standard error in parentheses.

Sensation seeking, extraversion, and risk-taking genetic associations. To determine whether genetic and environmental influences on extraversion accounted for variance in risk-taking after accounting for common effects of sensation seeking, extraversion was added to the Cholesky decomposition. This allowed us to determine common genetic and environmental influences between extraversion and risk-taking, net of the influence from sensation seeking. This model is illustrated in Figure 1.2c, with model estimates reported in Table 1.5. Additive genetic influence on sensation seeking was significantly associated with extraversion, as were non-shared environmental effects of sensation seeking. Additionally, additive genetic influences on extraversion unique from sensation seeking were significantly associated with positive risk-taking, but not with negative risk-taking. Consistent with the previous model, total heritability for sensation seeking was estimated at 51.1%, and non-shared environmental influence at 48.9%. Including the genetic cross-paths, total additive genetic influence accounted for 28.4% of variance in extraversion (38.9% of which was shared with sensation seeking). Dominance genetic effects on extraversion accounted for 28.7% of variance, and total non-shared environmental effects accounted for 43.0% (13.5% shared with sensation seeking).

For negative risk-taking, total additive genetic influence including all cross paths accounted for 51.5% of variance, with significant genetic influence shared with sensation seeking (27.3%) consistent with the previous model. Additionally, 11% of the total additive genetic influence on negative risk-taking was accounted for by additive genetic influence unique to extraversion; however, this estimate was not statistically significant. Sixty-two percent of additive genetic influence on negative risk-taking was unique of additive genetic influence on sensation seeking and extraversion. Shared environmental influence was estimated as greater than in the previous model (13.4%) but remained not

statistically significant. Total non-shared environmental influence on negative risk-taking accounted for 35.1% of the variance, with 15.2% shared with sensation seeking.

For positive risk-taking, total additive genetic influence accounted for 46.5% of the variance, higher than in previous estimates. Of this, 37.2% was shared with sensation seeking, and 62.8% was accounted for by additive genetic influences unique to extraversion. No additive genetic influences unique to positive risk-taking remained after accounting for variance shared with sensation seeking and extraversion. Shared environmental influences remained negligible, as in previous models. Total non-shared environmental variance accounted for the remaining 53.5% of variance in positive risk-taking, 89.3% of which was unique (5.3% shared with sensation seeking; 5.6% shared with extraversion).

DISCUSSION

Adolescents take risks. Developmental research has attempted to predict and devise new ways to protect adolescents from the problematic outcomes associated with adolescent risk-taking. Yet, adolescents' ability to face uncertain choices involving risk of danger, failure, or rejection can also be celebrated. To appreciate the virtuous side of adolescent risk-taking, one need only turn toward young adult novels, or the blockbuster movies based on them. Risk-taking protagonists, such as Harry Potter or Katniss Everdeen, are not only popular – they are celebrated. And, the risks these heroes and heroines take span harmful or antisocial risks and positive or prosocial risks, an observation that concords with our thesis that positive and negative forms of risk-taking do not exist as opposite sides of a continuum but are rather part of a whole.

The positive side of risk taking is gaining prominence in some emerging theoretical perspectives on adolescent development (Steinberg, 2014; Yeager, Dahl, &

Dweck, 2018), but predominant perspectives continue to conceptualize adolescent risk-taking as exclusively pathological. To advance the study of positive risk-taking, the current study validates a self-report measure of behaviors that place adolescents at risk for negative outcomes such as failure or rejection, but also afford them the potential for greater success, gains in social status, interpersonal connection, and self-discovery. Engaging in these behaviors, regardless of the favorable or unfavorable consequences, may benefit adolescents as they navigate their changing social contexts, explore novel relationships, and take on new roles. Supporting the validity of this self-report scale, the quantitatively-scored items asked about behaviors that were independently generated by late adolescents when they responded to an open-ended prompt on risks they have taken in their life. Further, the items formed a unidimensional construct, demonstrated good internal reliability, and consistent convergent and discriminant validity with a host of personality and contextual factors in a large, population-representative sample of teens.

Sensation seeking has been found to increase from childhood to adolescence, across the globe and across species, suggesting a potential role in evolutionary advantage for this period of development (Doremus-Fitzwater & Spear, 2016; Harden, Quinn, & Tucker-Drob, 2012; Steinberg et al., 2017). This study provides the first empirical evidence that sensation seeking is equivalently associated with both positive and negative risk-taking. And, the association between positive risk-taking and sensation seeking persisted even after accounting for the related personality trait of extraversion. Our behavioral genetic findings further suggest that a genetically-based disposition for sensation seeking is as likely to be expressed in taking adaptive risks as it is to taking maladaptive risks. That sensation seeking predicts such positive risks indicates that these risks do indeed tap into a drive for novel sensations and experiences—that is, adolescents driven by a desire for thrill are just as likely to engage in positive as in antisocial risks.

Individual differences in extraversion additionally accounted for unique phenotypic and genetic variance in positive risk-taking, after accounting for effects of sensation seeking, but extraversion did not show a similar relationship with negative risk-taking. Being sociable, outgoing, and cheerful may differentiate the types of risks that adolescents choose to engage in. Socially risky activities, such as trying out for a school play or approaching an unfamiliar peer, may both appear potentially more rewarding and more dangerous to adolescents than to adults, given the salience of peer relationships during this period of life. Social rewards and consequences may therefore have important influences on adolescent behaviors, such as risk-taking and goal pursuits (Crone & Dahl, 2012). For individuals higher in extraversion, these social rewards may be more motivating than for less extraverted teens. At the same time, higher levels of extraversion may provide a buffer from the potential consequences of rejection or social evaluation, resulting in the cost-benefit exchange to value the gains from positive risks disproportionately.

Understanding intermediary traits between genetic propensity and complex human behaviors such as risk-taking may aid identification of the genetic variants involved. Identification of genes under a disease model attempts to detect mutations or variants that negatively affect functioning. In contrast, personality genetics offers a different perspective that is more consistent with the hypothesis that risk-taking itself is not inherently negative: that genetic variants that give rise to risk-taking affect adaptive as well as maladaptive functioning. That teens engage in a wide range of risk-taking behaviors demonstrates that risk-taking during adolescence is an expression of typical, and at times adaptive functioning. Indeed, rises in risk-propensity during adolescence may reflect an adaptation to evolutionary pressures operating at this point in the lifespan.

The importance of peer influence on risk-taking may occur both with regard to contexts in which teens engage in risky behavior, as well as motivations behind the decision to do so. Peer influence also has notable effects on adolescent risk-taking, both unique of and within the context of personality factors. Consistent with previous work (Mann et al., 2015; Mann et al., 2016; Padilla-Walker & Bean, 2009), results of the current study indicated that highly sensation seeking individuals were more likely to associate with negative peers, which partially mediated the effect of sensation seeking on both forms of risk-taking. Conversely, highly extraverted individuals were more likely to associate with positive peer groups, which partially accounted for extraversion's relationship with positive risk-taking. This association with positive peers buffered against engaging in negative risk-taking, with a significant indirect effect from extraversion to negative risk-taking through positive peer associations. This indicates that any protective influence from extraversion on taking negative risks operates through the peer group. It appears that either selection or socialization effects from personality to peer affiliation partially account for the type of risk-taking that teenagers participate in.

However, both positive and negative peer affiliation predicted positive risk-taking suggesting that engaging in these risks occurs across peer contexts. Along with the residual correlation between both forms of risk-taking, this also suggests that many individuals do not engage in either solely antisocial or solely positive risks, but engage in both forms of risk-taking. Furthermore, though positive and negative peer environments were negatively correlated, this did not show a perfect inverse relationship. That is, although teens who tend to have positive peers are less likely to have negative peers and vice versa, this is not always the case. This further supports the perspective that propensity for risk-taking occurs across a variety of activities and contexts that include both negative and positive behaviors.

Although the current study provides promising initial evidence regarding how to measure and conceptualize the advantageous risks that adolescents take, limitations do need to be acknowledged. Foremost, this study is cross-sectional and correlational, and is therefore inadequate to draw causal conclusions regarding the associations between personality, peer environments, and risk-taking, or in understanding changes in how these processes work across development.

Additionally, all measures in the current study were derived from adolescent self-report. Our measure of positive risk-taking included many items on interpersonal risks, which, although noted as a strength above, may also limit the generalizability of this scale to non-interpersonal positive risk-taking. Antisocial risk-taking was measured using a scale of delinquency, which does not include some health-risk behaviors (e.g. unprotected sex) that are considered maladaptive risk-taking. Furthermore, behavioral and self-report measures of risk-taking tend to have low correlations (Harden et al., 2017), however, mean-level changes in both measures over time tend to demonstrate similar patterns (Duell et al., 2017; Josef et al., 2016). Furthermore, indicators of peer environment reflect adolescent perceptions of their peers' behavior, which may be inaccurate representations of the peer context (Helms et al., 2014). However, perceptions of peers, as well as indirect influences from peers such as social norms and direct peer pressure, have been associated with likelihood to engage in risk-taking (Padilla-Walker & Bean, 2009; van Hoorn et al., 2016; van Hoorn, Crone, & Leijenhorst, 2017).

Conceptualizing risk-taking behavior, particularly during adolescence, as encompassing a wide range of adaptive to maladaptive behavior may provide potential direction for the identification of genetic and neurobiological roots underlying risk-taking behavior. Additionally, understanding risk-taking as not solely negative in adolescents has implications for intervention and prevention work as well. Promoting positive risk-

taking in youth may be a beneficial way to decrease consequences of antisocial risk-taking. Rather than attempting to mitigate negative peer associations and negative risk behaviors, adolescents should be encouraged to engage with positive peers, and be provided opportunities and encouragement to take prosocial risks. The results of the current study provide initial evidence for the presence of positive risk-taking behavior in adolescence, that is at least partially driven by adolescents' drive to seek out exciting experiences and extraversion, both uniquely and in combination with peer associations.

Paper 2: Developmental Differences in Gonadal Hormones in Hair and Saliva, and Associations with Pubertal Maturation

INTRODUCTION

The onset of puberty commonly marks the beginning of adolescence, as individuals initiate the process of becoming reproductively mature. During this time a number of biological, neurological, and psychosocial changes occur, one of which is that gonadal hormones increase in both sexes. Individual differences in the timing of onset and the rate of pubertal maturation have consistent links with adverse psychological outcomes as well as consequences for health more broadly (Day, Elks, Murray, Ong, & Perry, 2005; Mendle, 2014a). Most research on the health consequences of early and rapid puberty has used self-report measures of pubertal development, which are noninvasive and easily administered in large-scale epidemiological research. Self-reports have been criticized, however, as potentially biased by adolescent's skewed self-perceptions and ignorance regarding how their bodies compare to others (Dorn, Dahl, Woodward, & Biro, 2006). The ability to measure puberty-related hormones cheaply and noninvasively, such as through saliva and hair, has galvanized interest in using hormonal measures as a putatively more valid and biologically more proximal measure of an adolescent's maturation. Surprisingly little research, however, has examined the correspondence between self-report and hormonal measures of pubertal development, and the few studies that have largely relied on cross-sectional measures of hormones rather than assessment of concordance between maturational processes (Harden, Kretsch, Moore, & Mendle, 2014). The current study addresses this gap in the literature, using longitudinal data on pubertal hormones collected from saliva and hair across two time-points, in order evaluate the relationship between within-person hormonal change and self-reported pubertal maturation.

Hormonal Changes at Puberty: Adrenarche and Gonadarche

Pubertal development occurs in two biological phases: adrenarche and gonadarche. First, adrenarche occurs around ages 5-7 with the maturation of the zona reticularis within the adrenal gland, which secretes dehydroepiandrosterone (DHEA) and dehydroepiandrosterone-sulfate (DHEA-S; Parker, Sack, Fisher, & Odell, 1978; Remer, Boye, Hartmann, & Wudy, 2005). This increase in androgens results in physiological changes such as body and pubertal hair growth, and body odor (Auchus & Rainey, 2003). Adrenarche, which is only observed in humans and a few other primate species, is thought to protect synaptic plasticity and extend human neural development, based in part on parallel timing of androgen increases and cortical maturation (Campbell, 2006; 2010). Importantly, some research in adults suggests that DHEA and DHEA-S may have an effect on mood, and have been positively associated with well-being and self-esteem, and negatively associated with depression and anxiety (reviewed in Campbell, 2006). This literature provides evidence for the importance of considering adrenarche as a potential sensitive period in neurobiological development with impacts on mood and behavior (Byrne et al., 2017).

Second, gonadarche marks the maturation of the hypothalamic-pituitary-gonadal (HPG) axis that underlies the development of secondary sex characteristics, and occurs approximately ages 8-13 and 9-14 for girls and boys respectively (Cameron, 2004; Witchel & Topaloglu, 2017). The reawakening of the HPG axis is initiated by pulses of gonadotropin-releasing hormone (GnRH), resulting in secretion of hormones that stimulate the production of sex-specific gonadal steroids: estradiol in the ovaries for girls, and testosterone in the testes in boys. These gonadal steroids promote reproductive maturation, namely breast development and menarche in girls, and in boys, testicular

development and secondary sex characteristics such as deepening of voice and muscle development.

Importantly, both gonadal hormones are present in both sexes, though in different concentrations (Cameron, 2004). Throughout puberty girls experience low level testosterone increases, which is produced in both the ovaries and the adrenal gland (Braams et al., 2015; Burger, 2002). In boys, estrogens are also produced in the testes (Carreau et al., 2003), and testosterone can be converted to estradiol with aromatase; in fact, many effects of testosterone may be mediated through aromatization to estradiol (Cameron, 2004). Receptors for both androgens (such as testosterone) and estrogens (such as estradiol) are located in the brain and peripheral tissues of both sexes (Cameron, 2004).

Considering Longitudinal Change in Hormonal Concentrations

The neurobiological consequences of rising hormone levels have been hypothesized to be key mechanisms for psychosocial and behavioral development during adolescence (Blakemore, Burnett, & Dahl, 2010). Notably, several lines of research suggest that the effects of a particular hormonal concentration depend on its developmental timing and the extent to which it represents a change from the organism's usual hormonal milieu. Animal research on the organizational and activation effects of hormones on subsequent behavior note the specificity of the pubertal window for the effects of hormones on behavior (Schulz & Sisk, 2016). For instance, late testosterone treatments in castrated male hamsters did not result in the same development of sexual behaviors as early or on-time treatments (Schulz, Zehr, Salas-Ramirez, & Sisk, 2009). Research on hormone replacement in women during the menopausal transition, as well as animal models of menopause, additionally provide evidence of the importance of timing-

dependent actions of hormones, given the contrasting health effects depending on the hormonal environment at the time of hormone administration (Bhupathiraju & Manson, 2014; Garcia, Depena, Yin & Gore, 2016). This research suggests that it is important to consider intra-individual change in the hormonal environment, rather than a single snapshot of hormonal concentrations at one time.

Yet almost no research has considered how longitudinal change in hormonal concentrations corresponds with self-report measures of puberty. Cross-sectional studies have demonstrated some modest associations between both testosterone and estradiol measured in saliva and physician ratings and self-reports of pubertal maturation (Shirtcliff, Dahl, & Pollack, 2009; Huang et al., 2012), though the evidence is weaker for estradiol in girls (Shirtcliff et al., 2009). For testosterone only, saliva testosterone levels have previously demonstrated increases over the period of pubertal maturation (Butler et al., 1989; Granger, Shirtcliff, Booth, Kivlighan, & Schwartz, 2004). Though both these studies used longitudinal assessments of saliva testosterone, they either did not assess self-reported pubertal maturation, instead using Tanner staging of genital and pubic hair growth (only in boys; Butler et al., 1989), or evaluated stability in the associations between testosterone and pubertal maturation rather than correspondence between within-person changes in development (Granger et al., 2004). Overall these studies provide some evidence that salivary hormones may contemporaneously correspond with an individual's level of pubertal maturation, yet it is unclear whether developmental rises in hormones may serve as an indicator of commensurate changes in pubertal maturation.

Furthermore, salivary measures of hormones are complicated by diurnal variability, and in girls, differences across the menstrual cycle, which additionally undergo changes during puberty (Butler et al., 1989; Dorn & Biro, 2011). In contrast, the measurement of sex steroids in hair is a relatively novel procedure, and captures hormone

accumulation over a period of months to provide a stable measurement of hormone concentrations (Noppe et al., 2015; Smith, Johnson, Whittle, Allen, & Simmons, 2018). This stable accumulation of hormones could potentially surmount problems of variability present with saliva by tapping into person-specific hormonal environments. The concordance between hair hormones and measures of puberty has had relatively little attention in the literature, though a previous cross-sectional examination in this sample reported a slight correlation between hair testosterone and self-reported pubertal maturation in boys only (Grotzinger et al., 2018). No previous studies have assessed hair testosterone over time along with self-reported pubertal maturation.

The Current Study

The current study considers hormones related to gonadarche (specifically, testosterone and estradiol), while recognizing that the window of pubertal development begins earlier in development with adrenarche. Furthermore, hormonal changes begin early on in puberty, and understanding the within-person arc of these developmental trajectories may be key to understanding pubertal hormone influences on behavior. In a longitudinal sample of children and adolescents, the current study sought to capture developmental differences across two different sampling methods, saliva and hair, for testosterone, as well as salivary estradiol. In both saliva and hair, concordance of hormone change with pubertal development has yet to be established. To characterize the developmental trajectory in each hormone measurement as well as self-reported pubertal maturation, we estimated linear and non-linear differences across age. Furthermore, to understand the relationship between within-person changes in hormones and pubertal maturation, we evaluated the contribution of hormone change over time to pubertal development, controlling for person-level hormone average and age.

MATERIALS AND METHODS

Participants

Our sample included participants from the two in-laboratory arms of the Texas Twin Project, a population-based sample of middle childhood and adolescent twins in the central Texas area. Participants attended an in-person visit to the University of Texas at Austin, where they provided saliva samples for hormonal assays, completed self-report measures on a wide variety of measures, including pubertal maturation, and participated in behavioral and cognitive tasks. This sample is racially and socioeconomically diverse, with approximately 35% of families reporting having received some kind of means tested public assistance since the children's birth. Of participants who reported race/ethnicity, 60% of participants self-reported their race as White, 13% Hispanic/Latino, 7% African American, 5% identified as another race or ethnicity, and 15% identified as more than one race/ethnicity. For descriptive data visualization, the full sample was retained with number of complete observations reported by measure in Table 2.1. Longitudinal analyses were restricted to individuals with at least two time-points, with missing data patterns and final sample size reported in Table 2.2.

Table 2.1. Descriptive Statistics for Complete Observations in the Full Sample

Sex	Wave 1		Wave 2		Wave 3		Wave 4	
	M	F	M	F	M	F	M	F
PDS								
N =	928	938	360	338	54	55	4	3
M (SD)	2.2 (0.8)	2.5 (1.0)	2.6 (0.7)	3.1 (0.8)	2.8 (0.6)	3.2 (0.8)	3.5 (0.4)	3.7 (0.1)
Age: M (SD)	12.7 (2.9)	12.6 (3.0)	14.5 (2.3)	14.4 (2.3)	15.3 (1.9)	15.5 (1.9)	17.7 (0.4)	17.4 (.24)
Range	7.8 - 19.5	7.8 - 20.1	8.8 - 19.0	8.8 - 19.0	10.7 - 18.9	10.7 - 18.9	17.2 - 18.1	17.2 - 17.6
Saliva								
logT								
N =	678	653	158	140	9	12	0	0
M (SD)	3.7 (1.3)	2.8 (1.0)	3.9 (1.1)	2.8 (0.9)	4.7 (0.3)	2.6 (0.8)	--	--
Age: M (SD)	14.3 (2.4)	14.4 (2.5)	15.9 (2.3)	16.0 (2.2)	17.8 (0.5)	17.8 (0.5)	--	--
Range	8.1 - 19.5	8.0 - 20.1	10.1 - 19.0	10.3 - 19.0	17.2 - 18.9	17.2 - 18.9		
logE								
N =	460	459	127	118	9	13	0	0
M (SD)	1.2 (0.7)	1.3 (0.6)	1.2 (0.5)	1.4 (0.5)	1.3 (0.3)	1.2 (0.3)	--	--
Age: M (SD)	15.6 (1.3)	15.6 (1.4)	16.8 (1.3)	16.8 (1.2)	17.8 (0.5)	17.8 (0.5)	--	--
Range	13.5 - 19.5	13.5 - 20.1	14.0 - 19.0	14.0 - 19.0	17.2 - 18.9	17.2 - 18.9		
Hair								
logT								
N =	540	539	201	171	17	19	0	2
M (SD)	0.3 (1.4)	-0.2 (1.0)	0.4 (1.1)	-0.1 (1.3)	0.4 (0.6)	-0.1 (1.4)	--	-0.01 (1.4)
Age: M (SD)	12.1 (3.0)	12.0 (2.9)	14.3 (2.0)	14.2 (2.2)	15.3 (1.7)	15.3 (1.5)	--	17.4 (0.3)
Range	7.9 - 19.5	7.9 - 19.5	8.8 - 18.8	8.8 - 19.0	12.7 - 18.9	10.7 - 17.5		17.2 - 17.6

Note. Wave 1 is comprised of the first time point each variable was measured, with subsequent waves the next time point at which that variable was assessed. Complete observations after accounting for participants missing, excluded, or below detection limits reported. The full sample above was used only for data visualization in Figures 2.1a – 2.4a. Reported hormones are the log raw (pg/ml) values prior to residualizing for covariates, standardization, and winsorizing. M = male; F = female. T = testosterone; E = estradiol

Table 2.2. Patterns of Missing and Excluded Data and Final Effective Sample Size for Longitudinal Mixed Effects Models

Sex	PDS		Saliva T		Saliva E		Hair T	
	M	F	M	F	M	F	M	F
Total N=	369	345	174	155	109	108	254	312
Missing								
Wave 1	11	14	3	1	7	1	2 ^c	2 ^f
Wave 2	9	7	5 ^a	3	4	1	5 ^d	4 ^g
Both	1	0	1	0	0	0	0	0
Excluded								
Wave 1	--	--	4 ^b	1	1	1	2 ^e	2
Wave 2			1	0	0	0	1	1 ^h
Both			1	0	0	0	0	2
Below DL								
Wave 1	--	--	8 ^a	1	0	0	46 ^d	61 ^{g h}
Wave 2			6 ^b	7	0	0	33 ^{c e}	84 ^f
Both			1	5	0	0	14	50
% obs. PDS	--	--	97%	96%	97%	98%	96%	92%
Final N=	348	324	141	132	94	103	149	101
Lag: M	2.6 years (1.0)		2.2 years (0.8)		2.4 years (0.8)		2.9 years (1.0)	
(SD)								

Note. Wave 1 is comprised by the first assessment of that measure, and Wave 2 the next following time-point at which that measure was assessed. Percent observations for pubertal development is the amount of complete observations for contemporaneous puberty for both time-point. Lag is the time delay between assessments. Participants were excluded due to either reported endocrine issues or missing covariates of BMI or time since waking. M = male; F = female. T = testosterone; E = estradiol.

^a = 1 missing Wave 2, below detection limit at Wave 1

^h = 1 excluded at Wave 2, below detection limit at Wave 1

^b = 1 below detection limit Wave 2, excluded Wave 1

^c = 1 missing Wave 1, below detection limit at Wave 2

^d = 2 missing Wave 2, below detection limit at Wave 1

^e = 1 excluded at Wave 1, below detection limit at Wave 2

^f = 1 missing at Wave 1, below detection limit at Wave 2

^g = 2 missing at Wave 2, below detection limit at Wave 1

Measures

Puberty. Puberty was assessed using self-report on the Pubertal Development Scale (PDS; Petersen, Crockett, Richards, & Boxer, 1988). Participants rated their growth in height, changes in body hair and skin, as well as male and female secondary sex characteristics: facial hair and deepening of voice for boys, and breast growth and menarche in girls. Items were presented on a 4-point scale. To be consistent with this scale, menarche was coded as 1 = No and 4 = Yes. The mean score across the five items was used to index pubertal status. To control for the effect of race/ethnicity on pubertal development, African American race, Latino ethnicity, and other race/ethnicity were regressed on PDS, with white as the reference group. Standardized residuals were used in subsequent analyses. Figure 2.1 depicts pubertal maturation across the full sample.

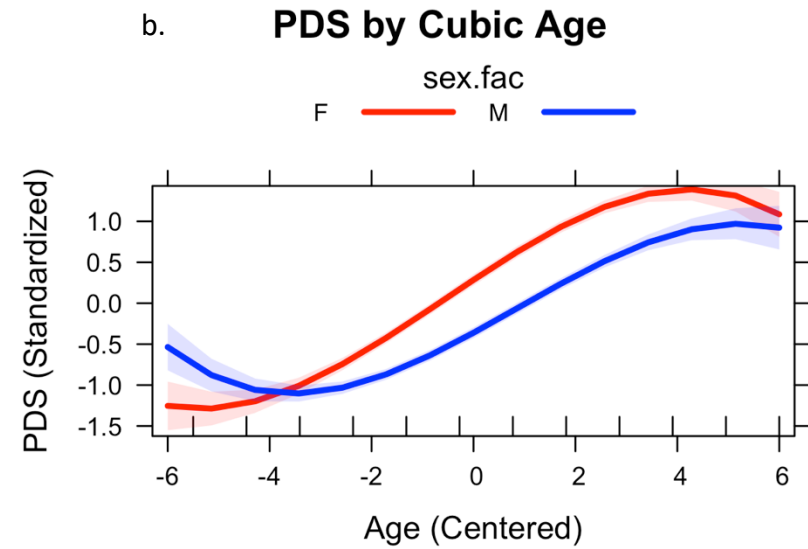
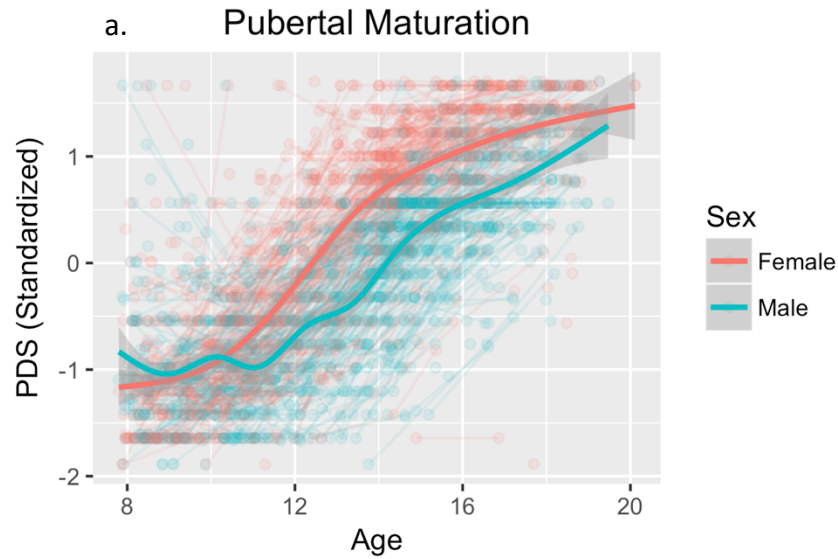


Figure 2.1a. Pubertal maturation plotted separately by sex over age including values at each time-point (data points), and for individuals over time (person-specific slopes), loess smoothing curves with 95% confidence intervals. *2.1b.* PDS for males and females as a function linear, quadratic, and cubic effects of age from the longitudinal mixed effects Model 3. Age is centered at 13.2.

Gonadal Hormones. Participants provided a saliva sample for hormonal assays via passive drool in a 2-ml vial. Saliva samples were collected after the consenting process, during the first hour of one of three appointment times: 09:00-10:00, 12:00-13:00, or 14:00-15:00. Participants were provided instructions to avoid eating or drinking anything in the 2 hours prior to their appointment, to avoid dairy or flossing that morning, to avoid smoking cigarettes for 4 hours prior. Collected saliva samples were stored on site at $\leq -30^{\circ}\text{C}$ for a maximum of 12 months prior to being shipped to Dr. Clemens Kirshbaum's laboratory at the Technical University of Dresden to be assayed. Concentrations of testosterone and estradiol were determined via commercially available chemiluminescence immunoassays (IBL International, Hamburg, Germany). For testosterone, the lower limit of sensitivity for the assays was 1.8pg/ml, and for estradiol 0.3 pg/ml. Extremely high values for both were estimated from standard curves. Intra-assay and inter-assay coefficients for both hormones were $<8\%$ and $<11\%$ respectively.

Participants additionally provided a hair sample during their in-lab visit. A hair sample 3 cm in diameter and at least 3 cm in length was taken from the posterior vertex position at the back of the head. Hair samples were not collected if the participant declined, or had hair less than 3 cm in length. Participants were instructed not to use leave-in hair products the day of the appointment, though hair hormone levels have previously demonstrated consistency across potential confounds such as birth control use, cigarette smoking, and hair washing (Dettenborn et al., 2012). Procedures for hormonal assay have been described in detail in previous work (Gao et al., 2013; Grotzinger et al., 2018). Testosterone concentrations were assessed from the closest 3 cm of hair to the scalp representing the most recent 3-month interval prior to sampling. Following procedures described elsewhere (Gao et al., 2013), whole non-pulverized hair was washed in isopropanol and subject to steroid extraction. The lower detection limit for

testosterone in hair was 0.1 pg/ml, and testosterone inter- and intra-assay coefficients have been assessed at <10% in previous work (Gao et al., 2013). Previous work conducted in a subsample of the current study demonstrated good reliability across duplicate samples and monozygotic twins (Grotzinger et al., 2018).

Hormonal data was excluded for participants who reported the presence of an endocrine disorder, suspected pregnancy, or were missing covariates. Following the procedures detailed in previous work using testosterone and estradiol measures in this sample (Grotzinger et al., 2018; Harden et al., 2018), all hormone concentrations were log-transformed due to positive-skew. Log-hormone values were then corrected for body mass index, race/ethnicity, analytic batch, menstrual cycle phase, birth control use, use of steroid medication in the past six months, use of hormonal medication in the last six months, and study of participation. To account for established diurnal variation, salivary hormones were additionally corrected for time since waking. Standardized residuals were then subject to a winsorizing procedure to account for extreme values, which were replaced with the highest value within 3 standard deviations. Figures 2 through 4 depict salivary testosterone, salivary estradiol, and hair testosterone respectively across the full sample.

Analyses

Our first set of analyses investigated linear and non-linear age-related trends in PDS, salivary testosterone and estradiol, and hair testosterone. A series of mixed effects models with time-points nested within participants was used account for longitudinal time-points, with individuals additionally nested within families to account for non-independence of twin data. The variance of the random intercept for families was allowed to vary by zygosity. For each measure, the first model assessed linear effects of age, the

second quadratic effects of age, and the third cubic effects of age, with each model allowing sex moderation on all age effects. Model comparisons were used to determine the best fit model for each study variable.

Second, in order to examine whether hormone change accounted for variation in pubertal maturation, we assessed the effects of hormone change in saliva and hair on PDS after accounting for the effects of age and person-specific hormone average. Rate of hormone change was calculated as the deviation of hormone concentration at each time point from person-level average as a ratio by lag time.

$$\text{hor. rate}_{ijkl} = \frac{(\text{hor}_{ijkl} - \overline{\text{hor}}_{jkl})}{\text{Lag}_{jkl}}$$

Mixed effect models were again used to account for longitudinal, twin-level data. Fixed effects of age, hormone deviation, and hormone average, as well as sex moderation on each effect were estimated. Separate models were fit for each hormone: salivary testosterone, salivary estradiol, and hair testosterone.

RESULTS

In accordance with the procedures described above, covariates for PDS and hormones measures were controlled for prior to analyses. Across the full sample, PDS demonstrated a steep increase during early adolescence that leveled off by later adolescence in females, and variability in early adolescence that continued to increase into later adolescence in males (Figure 2.1a).

For salivary testosterone, there appeared to be a largely consistent increases in males across childhood into adolescence, whereas for females, there was a small rise and fall during late childhood into early adolescence, as well as a gradual increase during later adolescence (Figure 2.2a). Female levels of testosterone were greater than male levels in childhood, with male levels of testosterone surpassing female levels around age

12.5. Child-age samples were not assayed for salivary estradiol due to circulating concentrations of estradiol in children being largely below the detection limit. As such, estradiol appeared to show a small increase during early adolescence in males that leveled off in mid adolescence, and an overall small increase in females (Figure 2.3a). Hair testosterone showed relatively little change across childhood and adolescence in either males or females but was greater in males across the age range (Figure 2.4a).

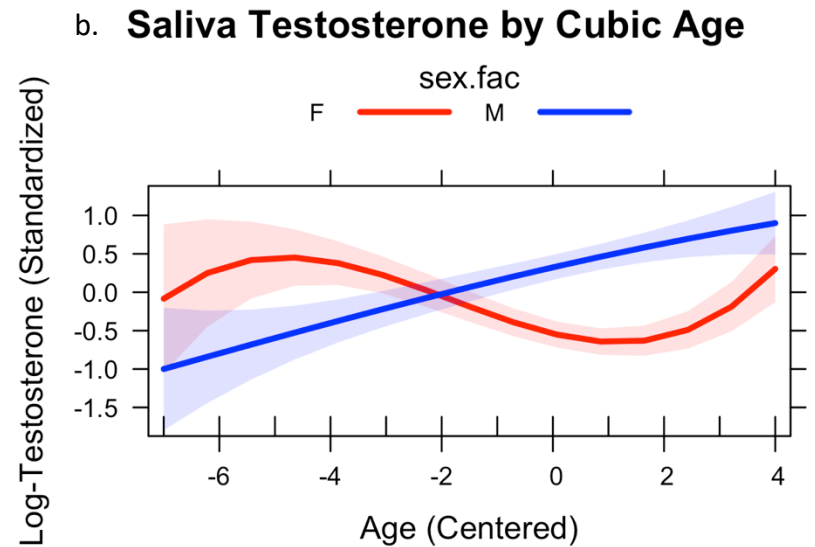
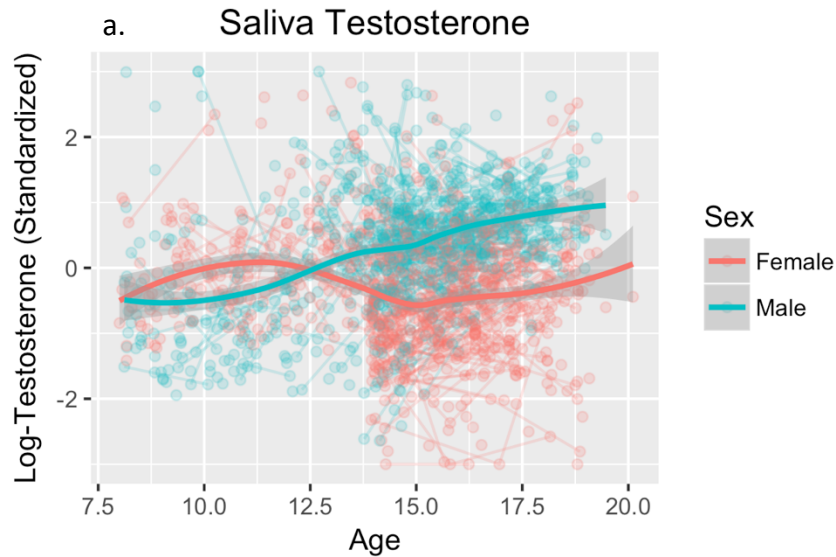


Figure 2.2a. Salivary testosterone plotted separately by sex over age including values at each time-point (data points), and for individuals over time (person-specific slopes), loess smoothing curves with 95% confidence intervals. *2.2b.* Salivary testosterone for males and females as a function linear, quadratic, and cubic effects of age from the longitudinal mixed effects Model 3. Age is centered at 15.

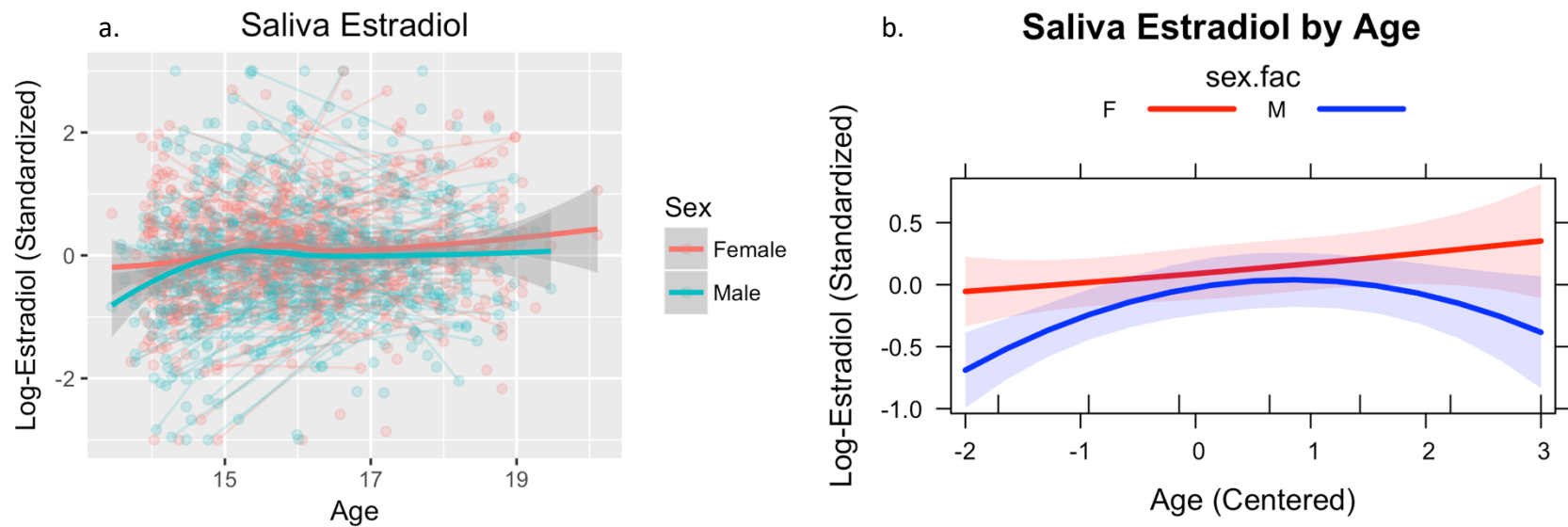


Figure 2.3a. Salivary estradiol plotted separately by sex over age including values at each time-point (data points), and for individuals over time (person-specific slopes), loess smoothing curves with 95% confidence intervals. *2.3b.* Salivary estradiol for males and females as a function linear, and quadratic effects of age from the longitudinal mixed effects Model 2. Age is centered at 16.

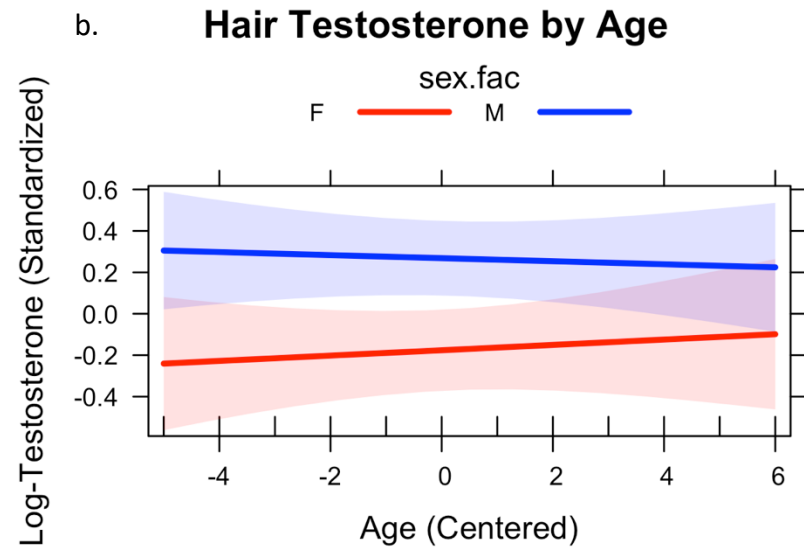
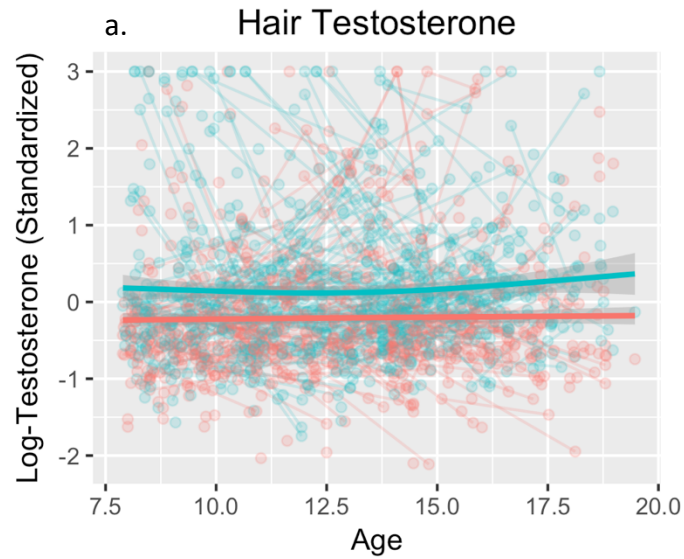


Figure 2.4a. Hair testosterone plotted separately by sex over age including values at each time-point (data points), and for individuals over time (person-specific slopes), loess smoothing curves with 95% confidence intervals. 2.4b. Hair testosterone for males and females as a function linear effects of age from the longitudinal mixed effects Model 1. Age is centered at 13.

Developmental Differences as a Function of Age

A series of mixed effects models estimating the linear, quadratic, and cubic effects of age by sex predicting pubertal development were fit to data with two complete time-points for PDS. Model 3 estimating all three effects demonstrated superior fit to the data than either earlier model (Model 3 vs. Model 2: log-likelihood χ^2 difference = 89.96, $\Delta df = 2$, $p < .001$; Model 2 vs. Model 1: log likelihood χ^2 difference = 13.10, $\Delta df = 2$, $p < .01$), and is depicted in Figure 2.1b, with model estimates provided in Table 2.3. This model demonstrated curvilinear differences in pubertal development across age such that PDS rose sharply during middle adolescence and relatively little change occurring in the ends of the age range, with the onset of this change occurring earlier in females.

Table 2.3. Fixed Effects of the Best Fit Model for Longitudinal Differences Across Age

	Sex		Age		Age × Sex		Age ²		Age ² × Sex		Age ³		Age ³ × Sex	
	Est.	SE	Est.	SE	Est.	SE	Est.	SE	Est.	SE	Est.	SE	Est.	SE
PDS	-.65***	.05	.41***	.02	-.07**	.02	-.01***	.003	-.03***	.004	-.01***	.001	<.001	.001
Saliva T	.86***	.10	-.17***	.04	.34***	.06	.06***	.01	-.06***	.01	.01***	.003	-.01**	.004
Saliva E	-.11	.14	.08	.04	.07	.06	.003	.03	-.09*	.04				
Hair T	.44***	.10	.01	.03	-.02	.03								

Note. Estimates and standard error of standardized variables reported. Sex is coded 0 = female, 1 = male. Age is centered at 13.2 for pubertal development, 15.0 for salivary testosterone, 16.0 for salivary estradiol, and 13.0 for hair testosterone. T = testosterone; E = estradiol. $p < .05 = *$, $p < .01 = **$, $p < .001 = ***$

In the series of models predicting salivary testosterone as a function of the linear and exponential effects of age separately by sex, Model 3 estimating the linear, quadratic, and cubic effects was the best fit model (Model 3 vs. Model 2: log-likelihood χ^2 difference = 13.15 $\Delta df = 2$, $p < .01$; Model 2 vs. Model 1: log likelihood χ^2 difference = 13.10, $\Delta df = 2$, $p < .01$; Table 2.3). In females, a curvilinear effect was estimated such that rises in testosterone occurred in late childhood and late adolescence, with decreases during middle adolescence. In males, largely linear increases in testosterone were detected across the age range (Figure 2.2b).

For the models predicting salivary estradiol, the model estimating both the linear and quadratic effects of age by sex was the best fit model, as estimating the cubic effects of age did not significantly improve model fit (Model 3 vs. Model 2: log-likelihood χ^2 difference = 1.99, $\Delta df = 2$, $p = .37$; Model 2 vs. Model 1: log likelihood χ^2 difference = 8.35, $\Delta df = 2$, $p < .05$; Table 2.3). As shown in Figure 2.3b, estradiol estimates showed an inverted-U shape in males, with the highest estimates around ages 16 to 17 years. For females, relatively little variation across age was detected.

The model estimating only the linear effects of age by sex was the best fit to the data predicting hair testosterone (Model 3 vs. Model 2: log-likelihood χ^2 difference = 0.16, $\Delta df = 2$, $p = .92$; Model 2 vs. Model 1: log likelihood χ^2 difference = 3.92, $\Delta df = 2$, $p = .14$; Table 2.3). However, the effect of age was non-significant, and only a main-effect of sex was detected (Table 2.3; Figure 2.4b).

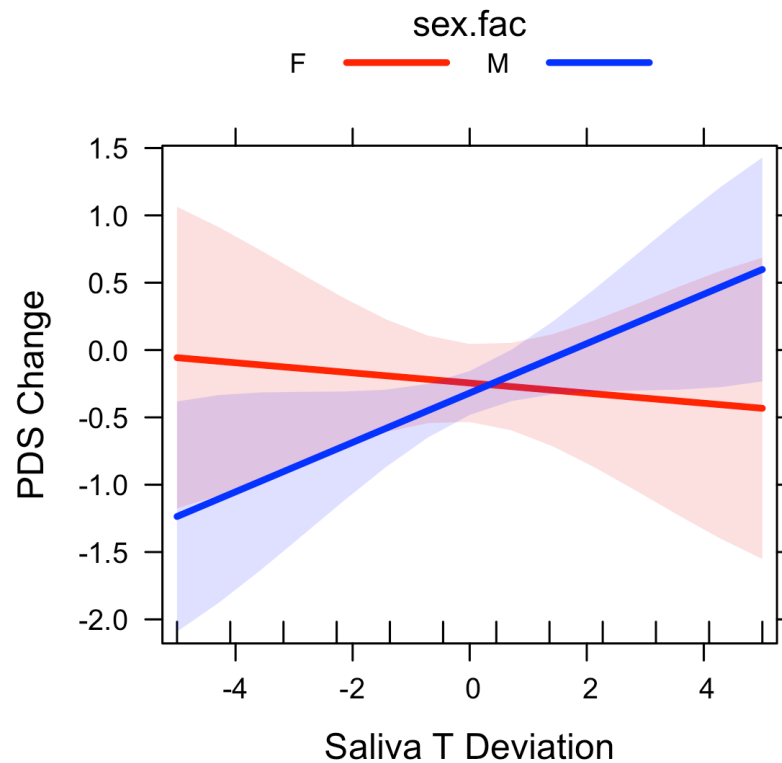
Hormone Change Predicting Pubertal Maturation

Separate mixed effect models estimating the sex differentiated effects of age, person-specific hormone average, and hormone deviation from average over time

between measurements predicting pubertal maturation were fit for salivary testosterone, salivary estradiol, and hair testosterone. Estimates for these models are provided in Table 2.4.

Change in none of the hormonal variables was significantly associated with self-reported pubertal development on the PDS. Due to the large age range, and non-linear variation in salivary testosterone changes across development, we conducted an exploratory analysis fitting models separately to individuals who had barely started puberty at Wave 1 ($PDS \leq 2$, $N = 69$) versus individuals who had already started puberty by Wave 1 ($PDS \geq 2$, $N = 204$). This exploratory analysis found a significant effect of testosterone deviation only in the pubertally matured group, along with the main effects of age and sex, such that *decreases* in testosterone were related to greater pubertal maturation (Table 2.4; Figure 2.5).

a. **Pre- and Peri-Pubertal at Wave 1**
PDS by Saliva Testosterone Deviation



b. **Pubertal at Wave 1**
PDS by Saliva Testosterone Deviation

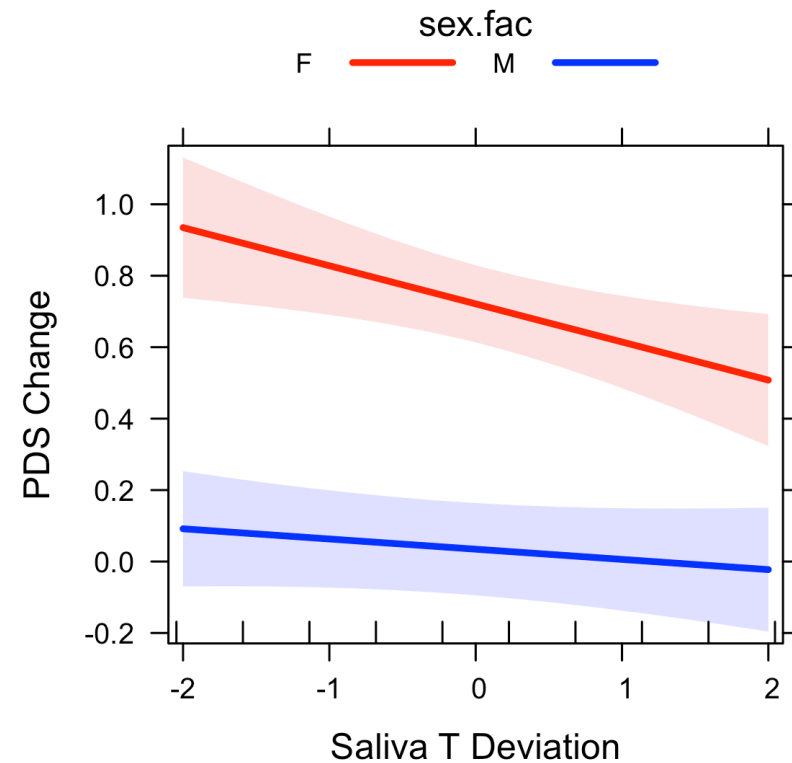


Figure 2.5a. Sex differentiated, non-significant effect of testosterone deviation predicting PDS in participants who self-reported not beginning, or barely beginning puberty at Wave 1. *2.5b.* Sex differentiated effect of testosterone deviation predicting PDS in participants who self-reported having begun puberty at Wave 1. Significant at $p < .01$.

Table 2.4. Fixed Effects of Longitudinal Models of Hormone Change and Hormone Average on PDS

	Saliva T		Saliva E		Hair T		Saliva T Wave 1 PDS ≤ 2		Saliva T Wave 1 PDS ≥ 2	
	<u>Est.</u>	<u>SE</u>	<u>Est.</u>	<u>SE</u>	<u>Est.</u>	<u>SE</u>	<u>Est.</u>	<u>SE</u>	<u>Est.</u>	<u>SE</u>
Sex	-.55***	.06	-.60***	.07	-.37***	.06	-.25	.16	-.62***	.07
Age	.26***	.02	.16***	.02	.33***	.02	.20***	.04	.16***	.02
Age × Sex	-.02	.02	.08**	.03	-.11***	.02	-.06	.05	.05	.03
Rate	-.07	.04	.03	.05	.03	.08	-.04	.11	-.11**	.04
Rate × Sex	.08	.05	-.01	.06	-.02	.10	.22	.14	.08	.05
Average	-.06	.05	.09	.06	.09	.07	.31	.17	.004	.04
Avg. × Sex	.12	.08	-.01	.08	-.12	.09	-.35	.18	.06	.07

Note. Estimates and standard error of standardized variables reported. Hormone rate was calculated as the deviation at each time-point as a ratio over lag between time-points, and hormone average was the mean between the two time-points. Sex is coded 0 = female, 1 = male. Age is centered at 15.0 for salivary testosterone, 16.0 for salivary estradiol, and 13.0 for hair testosterone. T = testosterone; E = estradiol. $p < .05 = *$, $p < .01 = **$, $p < .001 = ***$

DISCUSSION

Individual differences in measures of puberty and in trajectories through pubertal development have been implicated as factors underlying adolescent behavioral changes. Self-reports of pubertal changes are used widely in the literature, but their ability to accurately reflect biological maturation has been questioned. In this, particular interest has been paid to the role of pubertal hormones as both markers for pubertal development as well as physiological proximal mechanisms in the pathway from biological development to behavioral manifestations. However, the precise correspondence between pubertal hormone changes and physical changes captured in self-reported puberty measures has received little validation in the literature. In the current study, we sought to fill this gap in the literature by examining hormone *change* during puberty across multiple methods of hormone sampling in conjunction with self-reported puberty. Self-reported puberty demonstrated expected age-related trends across our sample of children and adolescents. Yet, in contrast to our expectations, hormonal change was not robustly associated with variation in pubertal maturation over time, suggesting that these measures may capture somewhat independent manifestations of puberty.

To characterize self-reported pubertal development within our sample, we first visually inspected pubertal maturation changes across the full sample, and modeled variation in pubertal development across two time-points as a function of linear and non-linear increases in age. As anticipated, pubertal maturation followed a non-linear pattern of development in both boys and girls, with an earlier onset in females. In our sample, more rapid change in pubertal development occurred for girls largely between the ages of 10 and 14. Though some later changes in physical developmental as assessed by the PDS were detected, pubertal maturation had largely leveled off by age 15. For boys, pubertal

maturation was more variable at younger ages, with the sharpest changes in physical development reported in early to middle adolescence around the ages 13 to 15. Later in adolescence, continued changes in physical development were apparent and pubertal maturation did not quite appear to level off at the upper end of the age range.

However, developmental patterns of hormone differences across age did not match these age-related trends in self-reported puberty. In our evaluation of developmental differences in salivary estradiol, we detected relatively little increase across age in females. An increase in salivary estradiol was only detected in males prior to the age of 16. Overall, hair testosterone appeared stable over the course of development, and hair testosterone concentrations were higher in males than in females, as expected.

The largest amount of variation over development was detected for salivary testosterone, which demonstrated a sex-differentiated pattern that indicated developmental increases across adolescence for males, and a curvilinear pattern of development in females. Though a dramatic rise in testosterone was not detected for boys, the steady increases in testosterone appear somewhat consistent with the continued increases in pubertal maturation across our sample. A developmental peak in testosterone was detected for females such that salivary testosterone rose from childhood into early adolescence, peaking between ages 10 to 12, followed by falling testosterone levels until approximately age 15 with steady rises thereafter. Though the timing of this surge in salivary testosterone in females appears to precede the sharp increases in physical development following this period, we did not anticipate declines in testosterone in mid-adolescence. Taken together, these results suggest that salivary testosterone is sensitive to developmental changes in both sexes, and that these changes are more pronounced early on or prior to the onset of change in physical maturation.

Yet, within-person hormone change was not related to changes in self-reported pubertal maturation for any hormonal measurement. That neither developmental trends in hormones nor within-person changes corresponded to pubertal maturation may seem surprising given that gonadal hormones catalyze the physical changes assessed by self-report puberty measures. This appears to indicate that rather than serving as a biologically proximal indicator of pubertal maturation, developmental hormone changes and self-reported physical changes instead capture somewhat differentiated manifestations of pubertal maturation. First, previous literature has demonstrated only modest associations between self-reported puberty and salivary hormone concentrations (Dorn, 2011; Shirtcliff et al., 2009; Huang et al., 2012). Moreover, hormone concentrations that correspond to stages of pubertal development vary between individuals (Huang et al., 2012; Harden et al., 2011).

Second, both self-reported puberty and hormonal measures are complicated by measurement error. For adolescent self-reports, teens tend to over- or underestimate their own pubertal development in favor of rating themselves similar to others of the same age (Shirtcliff et al., 2009). That is, adolescents who are at a lower stage in comparison to same age peers overestimate their own development, and vice versa for those who are at a higher stage relative to same age peers. Though more biologically proximal, hormone concentrations reflect variation across a number of factors, such as time of day, incidental occurrences such as injury or stress, and for females, menstrual cycle phase. An individual's hormone concentration may therefore reflect a number of factors beyond simply their hormonal milieu.

Though no relationship was estimated between saliva testosterone change and pubertal maturation, given the wide age-range and variability in testosterone changes for females across development, we conducted an additional exploratory analysis

differentiating individuals who had not started or barely started puberty, and those who had already begun to see physical changes associated with puberty at the time of the first assessment. Only after separating these groups did we detect a relationship between salivary testosterone change and pubertal maturation, such that in pubertal males and females, testosterone decreases were associated with pubertal maturation. Though there appeared to be a relationship between greater increases in salivary testosterone in boys who were pre- or peri-pubertal at Wave 1 and greater pubertal maturation across time-points, this failed to reach significance. The large standard errors estimated in this model may suggest that we were underpowered in the pre- and peri-pubertal group to detect an effect. Given the exploratory nature of this analysis, these findings should be interpreted with caution prior to replication.

It should be noted that estradiol was only assayed in an older age range with the lower bound at 13.5 years. That substantial puberty related increases in estradiol were not detected in our age range suggests that developmental studies should concentrate on earlier age ranges in order to detect estradiol changes. However, this is complicated by the lack of established reliability of salivary estradiol measurement in pre- and peri-pubertal individuals (Dorn & Biro, 2011; Shirtcliff et al., 2000). Furthermore, salivary estradiol measurements may underestimate the contribution of hormones on behavioral outcomes in comparison to estradiol concentrations from blood spot or serum analysis (Shirtcliff et al., 2000). Until improvements in technology establish a reliable method for estimating estradiol levels in younger individuals from saliva, developmental researchers should carefully consider whether this method of estradiol assessment is adequate to capture developmental changes in accordance with puberty.

Steroid concentrations derived from hair sampling methods estimate average levels over a period of months. In our sample, we estimated hormone concentrations from

the 3cm closest to the scalp, approximating testosterone concentrations over the three months prior to assessment. The average time between hair testosterone measurement was approximately 3 years, indicating that average accumulations were non-overlapping. However, accumulation over a period of months may smooth over the pulses of gonadal hormones that catalyze pubertal maturation, resulting in relatively little variation over development. Though this method is robust against diurnal and menstrual cycle variation present in hormones measured in saliva or blood, the average accumulation captured in hair appears to represent a more stable, trait-like measurement of testosterone.

In addition to the noted constriction of our age range and under sampling of pre- and peri-pubertal youth, further limitations must be acknowledged. As discussed, salivary hormone concentrations show diurnal fluctuations over the course of the day. Though we statistically controlled for time since waking, saliva samples were collected at the beginning of three different appointment times throughout the day. As such, variation in hormones within individuals may be partially influenced by differences in collection time. Collection of salivary hormones at a single time in the day, such as just after waking, would provide a more robust control for diurnal fluctuations. Additionally, salivary hormones were assessed by a single saliva sample at each time point. Previous studies indicate that averaging across multiple saliva samples taken on separate days increases reliability (Dabbs, 1990).

For post-menarcheal females, hormones also vary over the course of the menstrual cycle. We attempted to schedule female participants who were currently cycling during the first two weeks of their cycle, capturing the menstruating and follicular phases, however only 63% of females reporting a regular cycle were within this window. Furthermore, irregular menstruation and variable cycle lengths are common in adolescence, particularly early on after menarche (Dorn et al., 2006). Though we

statistically controlled for menstrual cycle phase, irregular menses, and use of hormonal contraception that may impact hormone variation, the impact of this on individual variability is unclear.

Given the evidence of the current study, self-reported physical changes and hormonal changes during the window of pubertal development reflect separate indexes of maturation. We detected expected developmental differences in self-reported puberty, however differences in hormones did not capture puberty related changes. Though we detected some continued increases in physical changes associated with puberty as well as salivary testosterone in both sexes after age 15, these changes were relatively slight in magnitude. However, we detected rises in testosterone as early as ages 8 to 10 in females. As such, researchers should carefully sample pre- and peri-pubertal children and adolescents in order to capture key developmental rises in testosterone. Surges of pubertal hormones may not yield the valid, biologically proximal marker of pubertal development anticipated by researchers. Developmental researchers should carefully consider methods of hormone measurement and age of study sample to optimize study design tailored to particular developmental questions.

Paper 3: Associations between within-person gonadal hormone changes and psychopathology in children and adolescents

INTRODUCTION

Adolescence is a developmental period characterized predominantly by change. These changes occur both inside the individual, such as the marked developments in sexual maturation that occur with puberty, as well as outside the adolescent, such as shifts in context and structure from changing school, family and social environments. Confronting the challenges that result from these pronounced fluctuations allow adolescents the opportunity for growth and learning, but this process may also be rife with turmoil as adolescents find their cognitive and emotional resources not quite equipped to meet these demands. This turmoil may underlie that notable rises in psychopathology that occur in adolescence.

Pubertal increases in gonadal hormones such as testosterone and estradiol have been specifically implicated as influencing behavioral changes via their effects on neurobiological development (Blakemore, Burnett, & Dahl, 2010; Forbes & Dahl, 2010; Peper & Dahl, 2013). These dynamic neurodevelopmental changes putatively result in transformation of adolescent motivation, ranging from increased goal-direction in pursuit of thrilling experiences, as well as inhibition and withdrawal due to the increased salience of emotional and social cues (Crone & Dahl, 2012; Gladwin, Figner, Crone, & Wiers, 2011; Guyer, Silk, & Nelson, 2016; Nelson, Leibenluft, McClure, & Pine, 2005). Together these changes are hypothesized to set the stage for adolescents to achieve key developmental goals of social competence and complex learning, but also leave them vulnerable to stress, negative affect, peer influence, and conflictual expectations that give rise to sexually dimorphic increases in psychopathology (Blakemore et al., 2010; Byrne et al., 2017; Dahl et al., 2018; Somerville, Jones, & Casey, 2010).

These pathways may culminate in increases in *externalizing* psychopathology, such as rule-breaking, aggression, and delinquency, and *internalizing* psychopathology such as anxiety and depression (Costello, Copeland, & Angold, 2011; Merikangas et al., 2010). These forms of psychopathology show consistent sex differences, such that boys demonstrate greater rates of externalizing and behavioral problems, and girls higher rates of internalizing problems (Zahn-Waxler, Shirtcliff, & Marceau, 2008). In fact, during adolescence prevalence rates for depressive disorders in girls rise dramatically, two to three times that of boys (Cyranowski, Frank, Young, & Shear, 2000). The current study uses a longitudinal sample of children and adolescents to investigate the relationship between pubertal increases in testosterone and estradiol and developmental changes in both internalizing and externalizing psychopathology.

The Role of Puberty in Sensation Seeking and Psychopathology

Developmental increases in psychopathology are associated with *puberty* above and beyond the effects of chronological age (Mendle, 2014). Individual differences in multiple aspects of puberty have been consistently associated with internalizing and externalizing symptoms across sexes. Associations with the timing of the onset of puberty has received the most attention in the literature. Early pubertal timing has been associated with increased anxiety and depressive symptoms as well as increased risk for substance use and delinquency across both boys and girls (Mendle Turkheimer, & Emery, 2007; Mendle & Ferrero, 2012). Furthermore, research considering the impact of pubertal tempo, or the rate at which an individual progresses through puberty, indicates that tempo is a superior predictor of psychopathology than level of pubertal maturation (Mendle, Harden, Brooks-Gunn, & Graber, 2010; Mendle, 2014). More rapid pubertal tempo has also been uniquely associated with development of a broad range of psychopathological

symptoms across disorders and across sexes (Beltz, Corley, Bricker, Wadsworth, & Berenbaum, 2014; Marceau, Ram, Houts, Grimm, & Susman, 2011). The mechanisms by which these associations arise is not as well understood, but a complex interplay between biological maturation and psychosocial changes is hypothesized to underlie the interrelationship between puberty and psychopathology (Ge & Natsuaki, 2009; Mendle 2014).

Adolescent-specific trajectories of externalizing psychopathology have received particular attention given the substantial impact on mortality and morbidity. During this period of the lifespan, rates of law-breaking, substance use, risky sex, and violence are all on the rise, and teenagers are more likely to die from accidental injury due to reckless and risky behaviors than any other cause (Kann et al., 2018; Steinberg, 2008; Steinberg, 2013). Adolescence increases in appetitive behavior towards novel and exciting experiences, or *sensation seeking*, has been identified as a predisposing factor for risk-taking during this period (Harden & Tucker-Drob, 2011; Steinberg et al., 2008). As a result, adolescents are thought to be vulnerable to the short-term rewards of exciting but potentially harmful risks (e.g. substance use, risky sex), but also sensitive to the high affective and social rewards of positive risks (e.g. artistic endeavors, new relationships). Sensation seeking is robustly associated with engagement in a variety of externalizing behaviors such as substance use, risky-sex, gambling, and delinquency (reviewed in Roberti, 2004).

Multiple lines of evidence implicate puberty rather than chronological age per se in the alterations in approach and avoidance of risk that occurs during this developmental period (Crone & Dahl, 2012; Forbes & Dahl, 2010). First, the curvilinear trend evincing a peak of sensation seeking in adolescence is better accounted for by measures of pubertal development than by chronological age (Castellanos-Ryan, Parent, Vitaro, Tremblay, &

Séguin, 2013; Martin et al., 2002), though some studies found this relationship only in boys (Alarcón, Cservedka, & Nagel, 2017; Steinberg et al., 2009). Second, evidence of sex differences in risk related behaviors and processes implicate pubertal mechanisms. Consistent with greater externalizing behaviors in boys, sensation seeking tends to be higher in boys than in girls (Cross, Cyrenne, & Brown, 2013; Quinn & Harden, 2013; Shulman, Harden, Chein, & Steinberg, 2015). Furthermore, Shulman and colleagues (2015) found that female sensation seeking peaked earlier in adolescence than in boys, commensurate with earlier timing of puberty in females. Sex differences have also been detected in reward related neural response (Alarcón et al., 2017), as well as puberty-related structural changes (Wieranga et al., 2018). Over all, this suggests sexually dimorphic mechanisms, such as the rise in sex steroid hormones that occur in puberty, may play a role in adolescent-specific rises in sensation seeking and externalizing behaviors such as delinquency.

Biology to Behavior

Both testosterone and estradiol rise in both sexes during puberty, and act as the primary gonadal hormone for boys and girls respectively. Importantly, receptors for testosterone and estradiol are located within the brain and nervous system in both sexes, including areas related to reward processing, and threat processing (Sarkey, Azcoitia, Garcia-Segura, Garcia-Ovejero, & DonCarlos, 2008; Simerly, Chang, Marumatsu, & Swanson, 1990). The reawakening of the HPG axis and the dynamic cascade of hormonal changes that occur in puberty initiate a period of reorganization and activation within the brain (Schulz & Sisk, 2016). The human and animal developmental neuroscience literature has recently characterized many of these effects (Blakemore et al., 2010; Crone & Dahl, 2012; Peper & Dahl, 2013; Schulz & Sisk, 2016). Alterations in the ventral and

dorsal striatum, and prefrontal cortex affect adolescent processing of rewards and integrative learning (Braams et al., 2015; Chambers, Taylor, Potenza, 2003; Galvan et al., 2006; Van Leijenhorst et al., 2010). Furthermore, developmental differences in limbic regions, such as the amygdala, are related to processing of emotional and threatening cues (Hare et al., 2008; Spielberg, Olino, Forbes, & Dahl, 2014), and higher order cortical areas, such as the anterior cingulate cortex, play a role in social-affective maturation (Burnett, Sebastian, Kadosh, & Blakemore, 2011; De Lorme, Bell & Sisk, 2013).

This hypothesized pathway from hormonal changes in puberty to commensurate neurodevelopmental changes are thought to undergird many behavioral changes that occur around the onset of puberty in early adolescence. However, much of the research directly relating gonadal hormones to complex behaviors has been conducted in adults or relied on single time-point measures of hormones, limiting the developmental conclusions. To further complicate matters, evidence for associations between hormones and behavioral processes is made difficult by the complex effects of not only hormonal concentrations, but also the overall hormone environment, including timing and fluctuations, which may have particular significance during puberty (Berenbaum, 1998). Limited research into the processes underlying psychopathology in adolescent samples suggest effects of gonadal hormones, however results have been mixed.

Testosterone Associations with Sensation Seeking, and Externalizing Behavior

Testosterone affects multiple underlying neurobiological systems that may have important down-stream effects on psychopathology, such as reward sensitivity, emotion regulation, and stress response. Studies on reward and risk-taking related processes in relation to gonadal hormones in adolescence have primarily focused on underlying

processes rather than behavioral manifestations. Testosterone concentration in boys has been associated with striatal activation during behavioral reward tasks in boys (Alarcón et al., 2017; Forbes et al., 2010; Op de Macks et al., 2011). However, results for girls are more mixed with both similar (Alarcón et al., 2017), attenuated (Op de Macks et al., 2011), or negligible effects detected in girls (Forbes et al., 2010). However, in both sexes, testosterone has shown negligible or negative associations with self-reported sensation or fun seeking, or approach of rewards (Alarcón et al., 2017; Harden et al., 2017; Vermeersch, T'sjoen, Kaufman, & Vincke, 2009).

A few key studies have been conducted evaluating longitudinal changes in testosterone and its effect on reward and threat related neural activation. In a sample of around 250 adolescents, Braams and colleagues (2015) found that change in testosterone was related to reward-related neural response such that higher testosterone levels over time demonstrated linear increases in neural activity in response to rewards. However, this effect was not detected when effects of age were entered into the regression model, and was not moderated by sex, despite different patterns of testosterone increase over time for boys and girls. Additionally, Spielberg and colleagues (2014) investigated changes in testosterone over time in relation to both reward related and threat related activation to emotional stimuli. Change in testosterone, but not average levels of circulating testosterone, was related to increases in both amygdala and nucleus accumbens activity. This suggests that adolescents begin to process threatening stimuli in more complex ways, potentially experiencing the arousal as rewarding, and that this increased complexity is related to testosterone increases in puberty. Importantly, when considering boys and girls separately, the effect did not reach significance in girls though was trending in the expected direction. This suggests that effects of testosterone change

on reward related processes is likely similar across sex in the expected pattern, but attenuated in magnitude in girls.

Consistent with the findings relating testosterone to processes underlying psychopathology, meta-analysis shows a weak but positive relationship between testosterone and aggressions in males (Archer, Graham-Kevan, Davies, 2005). This is evident in the mixed evidence of testosterone and aggression relationships in the literature. Some evidence demonstrates a small relationship between testosterone and aggression in adolescent boys (Peper, de Reus, Heuvel, & Schutter, 2015), while other evidence demonstrates testosterone contributions to aggression only in mid, rather than early or late, adolescence (van Bokhoven et al., 2006). However, context and other hormonal concentrations appear to be important moderators of this effect (Carré, McCormick, & Hariri, 2011). In the presence of positive peers, testosterone was associated with social dominance rather than antisocial behaviors (Rowe, Maughan, Worthman, Costello, & Angold, 2004), and the relationship between testosterone and aggression appears stronger in the presence of low cortisol, an endocrine marker of the stress regulatory system (Grotzinger et al., 2018; Mehta & Josephs, 2010; Popma, et al., 2007). The testosterone relationship with aggression appears negligible in girls (Maras et al., 2003; Peper et al., 2015; Vermeersch, T'sjoen, Kaufman, & Vincke, 2008a).

Further, other studies show testosterone as related to non-aggressive (such as rule-breaking, delinquency and risk-taking) rather than aggressive externalizing behaviors in adolescent boys (Maras et al., 2003; Vermeersche, T'sjoen, Kaufman, & Vincke, 2008b; Vermeersche, T'sjoen, Kaufamn, Vincke, & Van Houtte, 2010). Higher testosterone levels have been associated with adolescent criminal records (van Bokhoven et al., 2006), and rule-breaking behaviors in boys (Fang et al., 2009), particularly in the face of negative peer associations (Rowe et al., 2004; Vermeersch et al., 2008a), and poor parent-

child relationships (in both sexes; Booth, Johnson, Granger, Crouter, & McHale, 2003). Largely, in girls, testosterone does not appear to have the same relationship with rule-breaking and risk-taking, with both null and negative effects reported (Fang et al., 2009; Vermeersch et al., 2008a). Though, in a recent study, Op de Macks and colleagues (2016) found that testosterone was related to both self-reported and task-related risk-taking in a sample of adolescent girls.

Estradiol Associations with Sensation Seeking and Externalizing Behavior

Less research has investigated the effects of estradiol on risk-taking, externalizing psychopathology, and related processes, and the few extent studies were cross-sectional. Reward-related striatal activity has demonstrated negligible associations with estradiol in boys, and small to negligible effects in girls (Alarcón, et al., 2017; Op de Macks, 2011), which may be complex: in a sample of girls, an indirect effect of estradiol through increased nucleus accumbens activation related to decreases in task-related risk-taking (Op de Macks et al., 2016). In boys, higher estradiol levels have been related to levels of fun seeking, but were unrelated to other measures of behavioral reward approach or inhibition, or reported risk-taking behaviors (Vermeersch et al., 2008b; 2009). In girls, estradiol has not been significantly associated with performance in computerized tasks of rewards and risks (Cardoos et al., 2017; Op de Macks et al., 2016), though there are mixed findings regarding associations with self-reported sensation or fun seeking, demonstrating both positive and negligible associations (Alarcón, et al., 2017; Vermeersch et al., 2009).

Vermeersch and colleagues (2008a) found that higher estradiol was associated with both aggressive and non-aggressive forms of risk-taking in girls, however they did not find an association with either in boys (Vermeersch et al., 2008b). In girls, self-

reported risk-taking and increased alcohol use have been associated with estradiol (Martin et al., 1999; Op de Macks et al., 2016). In a study of both sexes, externalizing behavior was associated with high levels of estradiol only in adolescents with high pathological personality traits when cortisol levels were low (Tackett et al., 2015). Overall, the literature suggests that estradiol may be positively associated with both internalizing and externalizing forms of psychopathology, primarily in girls, with mixed associations with different forms of risk-related processes and behaviors in both sexes.

Testosterone, Estradiol, and Internalizing Psychopathology

For internalizing psychopathology, testosterone appears to have a protective effect. Increases in testosterone were associated with positive changes in psychosocial adjustment in adolescent girls (Slap, Khalid, Paikoff, Brooks-Gunn, & Warren, 1994), and higher levels of testosterone have been associated with self-esteem in boys (Vermeersch et al., 2010). Consistent with this, lower levels of testosterone have been associated with internalizing symptoms and negative affect in boys, though not in girls (Granger et al., 2003; Susman, Dorn & Chrousos, 1991). Conversely, higher testosterone has been related to depression and negative affect in adolescent girls (Angold, Costello, Erkanli, & Worthman, 1999), though other studies have demonstrated no association between testosterone and internalizing symptoms in girls (Granger et al., 2003). Overall, the literature largely supports positive association between testosterone and externalizing behaviors and negative associations with internalizing psychopathology, though evidence for these effects are mixed and stronger in boys relative to girls.

Associations between puberty and internalizing symptoms in girls have long implicated female sex steroids that lead to pubertal changes as playing a role in anxiety and depression (Angold, Costello, & Worthman, 1998; Joinson, Heron, Araya, & Paus,

2012; Parry & Newton, 2001). However, the overall evidence for this link is somewhat mixed, and studies have primarily focused on female samples. Largely, estradiol appears to show a positive association with negative affect and depressive symptoms in girls (Angold et al., 1999; Graber, Brooks-Gunn, & Warren, 2006; reviewed in Balzer, Duke, Hawke, & Steinbeck, 2015), though some studies show no association (Rapkin et al., 2006; Slap et al., 1994), and others non-linear effects such that estradiol showed greatest association with depressive symptoms during mid-puberty relative to pre- or post-pubertal levels of estradiol (Brooks-Gunn & Warren, 1989; Warren & Brooks-Gunn, 1989). One study evaluating negative affect across sex detected no association with estradiol in boys (Susman et al., 1991). Evidence with regard to anxiety symptoms is even more limited, with some evidence of estradiol associations with state anxiety across sex (Alarcon et al., 2017), and other demonstrating a negative relationship between estradiol and anxiety symptoms in girls (only when estradiol level was trichotomized; Rapkin et al., 2006).

Disentangling Within-Person Effects Using Longitudinal Change

This heterogeneity in part may reflect the reliance of single time-point measures of testosterone and estradiol, which further limits ability to demonstrate the role of hormones in developmental processes. Additionally, controlling for the effects of pubertal development may mask the puberty related developmental changes in gonadal hormones. Both longitudinal measurement of hormonal and behavioral change and consideration of differential effects by sex may be necessary for illuminating the connection between hormones and processes underlying adolescent rises in risk-taking and psychopathology. Understanding the relationships between hormones and these

distinct but related constructs may be key to understanding the down-stream effects of hormones on behavior.

Associations of individual and developmental differences alone are not sufficient to illuminate commensurate developmental processes and are only suggestive of developmental effects. Rapid increases in hormonal levels during transitions into and out of puberty have been associated with changes in negative affect, depression, impulse control, and psychopathology above and beyond the effects of pubertal stages and age, highlighting the importance for considering hormonal change rather than concentration or physical characteristics (Brooks-Gunn & Warren, 1989; Warren & Brooks-Gunn, 1989). As noted by Peper and Dahl (2013), “the biological signals created by a given hormone can sometimes have little to do with the *level* of the hormone...as opposed to the timing or pattern of hormone secretion” (p. 137). Given these converging lines of evidence, we hypothesize that measuring hormone change may be key for examining the effects of gonadal hormones on psychopathology.

The Current Study

In order to investigate the association between within-person changes in pubertal hormones and psychopathology, we assessed salivary testosterone and estradiol and a host of self-report measures across two time points in a large sample of children and adolescents. For our first set of analyses, we examined the association between hormone change and externalizing and internalizing symptoms relevant across children and adolescents using the Youth Self-Report of the Child Behavior Checklist in a sample of >300 youth ranging in ages from 8 - 19. Second, in a subsample of adolescents (N ~200), we assessed the contribution of hormones to changes in adolescent-specific externalizing behaviors and sensation seeking. Given the sex differences of hormonal concentrations,

as well as predominant sex differences apparent in the limited literature examining hormone-behavior associations, we anticipate greater associations between outcomes and testosterone in boys, and estradiol and girls. Further, we anticipate greater associations between testosterone and externalizing behaviors, with negative associations with internalizing. With estradiol, we anticipate positive effects on internalizing symptoms. Associations with sensation seeking in both hormones, as well as externalizing with estradiol are largely exploratory given mixed findings in the past literature.

MATERIALS AND METHODS

Participants

This sample for this study comprises in-laboratory participants of the Texas Twin Project with two waves of data. Ages at each wave, total sample size, and patterns of missing hormone data are reported in Table 3.1, and effective sample sizes for each measure are reported in Table 3.2. Twins and multiples were recruited from public school rosters in the Austin, Texas metropolitan area and were invited to participate in one of two in laboratory studies depending on age eligibility: 3rd – 8th grade (approximately aged 8-13) in a study on cognitive ability and achievement, and 9th – 12th grade (approximately aged 14-18) in a study on adolescent risk-taking. Participants completed self-report measures of personality and psychopathology, as well as saliva samples to determine hormone levels, at each wave. The Texas Twin Project is a population representative sample, with 58% of participants self-reported their race as White, 9% Hispanic/Latino, 11% African American, 9% identified as another race or ethnicity, and 12% identified as multiple races/ethnicities.

Table 3.1. Patterns of Missing, Excluded Hormones, and Ages for Final Sample

	Testosterone				Estradiol			
	Wave 1		Wave 2		Wave 1		Wave 2	
	M	F	M	F	M	F	M	F
Total N=	174	155	174	155	109	108	109	108
Missing	4 ^a	1	6 ^{ab}	3	7	1	4	1
Excluded	5 ^{ac}	1	2 ^a	0	1	1	0	0
Below DL	9 ^{ab}	6 ^d	7 ^{ac}	12 ^d	0	0	0	0
Final N=	141	132	141	132	94	103	94	103
Age: M (SD)	13.9 (1.8)		16.1 (2.0)		14.8 (0.7)		17.1 (1.0)	
Age Range	8.1 – 16.6		10.1 – 19.0		13.6 – 16.6		15.3 – 19.0	
Lag: M (SD)	2.2 years (0.8)				2.4 years (0.8)			

^a = 1 missing, excluded, or below detection limit for both waves, counted in each wave

^b = 1 missing Wave 2 and below detection limit at Wave 1, counted in each

^c = 1 below detection limit Wave 2 and excluded Wave 1, counted in each

^d = 5 below detection limit for both waves, counted in each wave

Table 3.2. Effective Sample Size for Each Outcome Measure

	Testosterone		Estradiol	
	M	F	M	F
Full Sample N =	141	132	94	103
Internalizing				
Anxiety				
% obs.	96%	99%	100%	100%
Final N	136	131	94	103
PDS $\leq$ 2	42	22	15	5
Below DL	10	10	0	0
Depression				
% obs.	96%	98%	100%	99%
Final N	136	130	94	102
PDS $\leq$ 2	42	21	15	4
Below DL	10	10	0	0
Externalizing				
Rule-Breaking				
% obs.	96%	99%	100%	100%
Final N	136	131	94	103
PDS $\leq$ 2	42	22	15	5
Below DL	10	10	0	0
Aggression				
% obs.	97%	98%	100%	99%
Final N	137	130	94	102
PDS $\leq$ 2	42	21	15	4
Below DL	10	10	0	0
Adolescent				
Subsample N =	100	101	94	103
Delinquency				
% obs.	99%	100%	99%	100%
Final N	99	101	93	103
PDS $\leq$ 2	15	4	14	5
Below DL	0	3	0	0
Sensation Seeking				
% obs.	100%	100%	100%	100%
Final N	100	101	94	103
PDS $\leq$ 2	16	4	15	5
Below DL	0	3	0	0

Note. Full sample after accounting for missing, excluded and samples below the detection limit as reported in Table 3.1. Below DL = number of participants assigned at the detection limit for testosterone in robustness check models. % observations of the adolescent subsample reported for delinquency and sensation seeking.

Measures

Psychopathology. Externalizing and internalizing psychopathology were assessed using an abbreviated version of the Youth Self-Report of the Achenbach Child Behavior Checklist (CBCL; Achenbach, 1991; Thornberry et al., 1994). Children and adolescents provided self-reports on their agreement with items assessing rule-breaking, aggression, anxious, and depressive behaviors on a 3-point scale.

Adolescent Delinquency. Forms of adolescent-specific externalizing behaviors were assessed using the 36-item delinquency measure used to capture negative risk-taking in Paper 1 (pp. 17; Huizinga, Esbensen & Weiher, 1991). As in Paper 1, scores were log-transformed to account for positive-skew.

Sensation Seeking. As in Paper 1, the sensation seeking subscale of the UPPS-Impulsive Personality inventory was used to assess self-reported sensation seeking in our adolescent subsample (pp. 16; Whiteside & Lynam, 2001).

Salivary Hormones. Testosterone and estradiol concentrations across two time-points were assessed via passive drool saliva samples. Hormone collections, storing, assaying, and data processing procedures are as described in Paper 2 (pp. 59-60).

Pubertal Development. Self-reported pubertal development was assessed by the Pubertal Development Scale, as in Paper 2 (pp. 57; PDS; Petersen, Crockett, Richards, & Boxer, 1988). PDS at Wave 1 was dichotomized to differentiate individuals who had barely begun puberty at the first time-point ($PDS \leq 2$) and those who had already begun puberty at the first assessment ($PDS \geq 2$).

Across the full sample, salivary testosterone, and CBCL internalizing and externalizing symptoms were assessed. In the adolescent subsample, salivary estradiol,

sensation seeking, and adolescent delinquency were additionally measured. Example items for self-report measures, and descriptive statistics are provided in Table 3.3. Outcomes were residualized for African American and other race, and Hispanic/Latino ethnicity, and standardized residuals were used.

Table 3.3. Example Items and Descriptive Statistics for Self-Report Measures

Example Item		Mean (SD)		Range
		Wave 1	Wave 2	
Internalizing				
Anxiety	“I am nervous or tense.”	0.67	0.71	0 – 2
	“I worry a lot.”	(0.5)	(0.5)	
Depression	“I am unhappy, sad, or depressed.”	0.35	0.42	0 – 1.8
	“I cry a lot.”	(0.4)	(0.4)	
Externalizing				
Rule-Breaking	“I break rules at school, home or elsewhere.”	0.25	0.28	0 – 2
	“I hang around with kids who get in trouble.”	(0.2)	(0.2)	
Aggression	“I physically attack people.”	0.40	0.39	0 – 1.6
	“I am mean to others.”	(0.3)	(0.3)	
Adolescent Delinquency	“Driven a car very fast?”	0.13	0.20	0 – 0.9
	“Carried a hidden weapon (a knife or a gun)?”	(0.1)	(0.2)	
Sensation Seeking	“I generally see new and exciting experiences and sensations.”	2.79	2.90	1.3 – 4
	“I will try anything once.”	(0.6)	(0.6)	

Note. Internalizing and externalizing across the full sample assessed using the CBCL. Adolescent delinquency and sensation seeking assessed in an adolescent subsample. Log-delinquency scores reported. Raw scores prior residualizing for race for all variables reported here, standardized residuals used in analyses.

Analyses

We used a multilevel regression framework to model the between- and within-person effects of age, person-specific hormone average, and rate of hormone change moderated by sex on internalizing and externalizing psychopathology, taking into account the time-varying nature of the data. A Bonferonni correction for testing of six outcomes was applied such that our threshold of significance was specified at $p < .008$ ($.05/6 = .008$). Our modeling procedures followed three broad steps: Model 1, including the main effects of age, sex, hormone rate, and hormone average on the dependent variable; Model 2a-2c, adding an interaction with sex separately for each age (2a), hormone average (2b), and hormone rate (2c); and Model 3, including sex moderation on all three predictor variables. This modeling procedure was conducted separately for each hormone for six dependent variables: rule-breaking, aggression, anxiety, and depression, and in an adolescent subsample, adolescent delinquency and sensation seeking. For testosterone and psychopathology assessed using the CBCL in children and adolescents, a fourth model (Model 4) was additionally fit including moderation by level of pubertal development at Wave 1 on all predictors. PDS at Wave 1 was dichotomized as $PDS \leq 2$ (barely begun puberty) and $PDS \geq 2$ (begun puberty). However, due to adolescent delinquency and sensation seeking outcome measures and estradiol only available for the adolescent subsample, lower rates of participants with $PDS \leq 2$ contraindicated fitting this model with these measures (Table 3.2). As a robustness check, all testosterone models were additionally fit including participants with testosterone below the detection limit (1.8 pg/ml) assigned at the detection limit. No participants were below the detection limit for estradiol (Table 3.1). Effects estimated in each model for testosterone and estradiol are summarized in Table 3.4.

Table 3.4. Fixed Effects Estimated by Model and Hormone

	Model 1	Model 2			Model 3	Model 4
		a	b	c		
Age	T, E	T, E	T, E	T, E	T, E	T
Sex	T, E	T, E	T, E	T, E	T, E	T
Hor. Avg	T, E	T, E	T, E	T, E	T, E	T
Hor. Rate	T, E	T, E	T, E	T, E	T, E	T
Age × Sex		T, E			T, E	T
Hor. Avg × Sex			T, E		T, E	T
Hor. Rate × Sex				T, E	T, E	T
PDS W1						T
Age × PDS W1						T
Sex × PDS W1						T
Hor. Avg × PDS W1						T
Hor. Rate × PDS W1						T
Age × Sex × PDS W1						T
Hor. Avg × Sex × PDS W1						T
Hor. Rate × Sex × PDS W1						T

Note. Fixed effects for each modeling step fit separately for testosterone (T) and estradiol (E) reported above. Hor. Avg = person-specific hormone average; Hor. Rate = deviation from hormone average over time lag between assessments; PDS W1 = dichotomized grouping variable of pubertal development at first assessment, 0 = pre- and peri-pubertal, 1 = begun puberty.

Three random intercepts were estimated in each model for participants nested within families, families nested within twin zygosity, and zygosity in order to account for nesting of multiple time points within individuals, as well as non-independence of data within families. Allowing for random variation at the between-person level accounts for the potential effects of person-level characteristics, while simultaneously assessing within-person variation between the two time points. Person-level (non-time varying) characteristics include sex and average level of hormones, which were entered into the model as fixed effects. Fixed effects of time-varying characteristics include age and hormone rate, as well as cross-level interactions with sex.

The mixed effects regression equation for the simplest model containing only main effects (Model 1) is as follows:

Level 1 (time-varying):

$$DV_{ijkl} = \beta_{0jkl} + \beta_1 \text{Age}_{ijkl} + \beta_2 \text{hor.rate}_{ijkl} + \varepsilon_{0ijk}$$

Level 2 (persons nested within families):

$$\beta_{0jkl} = \beta_{0kl} + \beta_3 \text{Sex}_{jkl} + \beta_4 \overline{\text{hor}}_{jkl} + u_{0jkl}$$

Level 3 (families nested within zygosity):

$$\beta_{0kl} = \beta_{0l} + u_{0kl}$$

Level 4 (zygosity):

$$\beta_{0l} = \beta_0 + u_{0l}$$

RESULTS

Internalizing Psychopathology

Anxiety. Only the effect of sex was significant in Models 1-3 for either testosterone or estradiol, such that anxiety symptoms were higher in females (Table A1 &

B1 in Appendices A & B). In Model 4, which estimated the effects of testosterone dependent on level of pubertal development at the first assessment, no significant effects were present. When participants were assigned at the detection limit for testosterone, consistent sex effects were detected in Models 1-3, and in Model 4 there was an interaction between sex and PDS at Wave 1 ($\beta = -.63$, $SE = .32$, $p = .05$), such that higher PDS at Wave 1 was positively associated with anxiety symptoms in females, and negatively associated in males (Table C1 in Appendix C). However, this effect did not meet the threshold of significance given correction multiple testing and should be interpreted in caution, given that it was not detected in earlier models.

Depression. For depression symptoms predicted by either testosterone or estradiol, in Models 1, 2b, and 2c, only the main effects of age and sex were significant, and in Models 2a and 3 the interaction between age and sex was additionally detected (Table A2 & B2 in Appendix A & B), such that depression symptoms increased with age and were more prevalent in girls, with stronger age associations in females. However, for testosterone, the main effect of age in models without an interaction by sex (Models 1, 2b, and 2c) did not reach the threshold for significance specified by multiple testing ($p = .02$), and for estradiol, the same was true for age by sex interactions in Models 2c and 3 ($p = .02$).

In Model 4, which was only estimated for testosterone, the effects of age, sex, and their interaction were no longer significant, and effects of testosterone rate by sex ($\beta = .41$, $SE = .18$, $p = .03$), and a three-way interaction of testosterone by sex and Wave 1 PDS ($\beta = -.45$, $SE = .21$, $p = .03$) were estimated. However, neither of these effects were significant at our corrected p-value.

Although these effects should be interpreted with caution, the higher-order interaction effect of testosterone rate by both sex and PDS at Wave 1 is depicted in

Figure 3.1. This may suggest a small effect such that increases in testosterone for males with lower PDS at Wave 1 were associated with *higher* depression symptoms, with the opposite effect detected in girls. For both males and females higher in PDS at Wave 1, there were negligible effects of testosterone rate on depression symptoms.

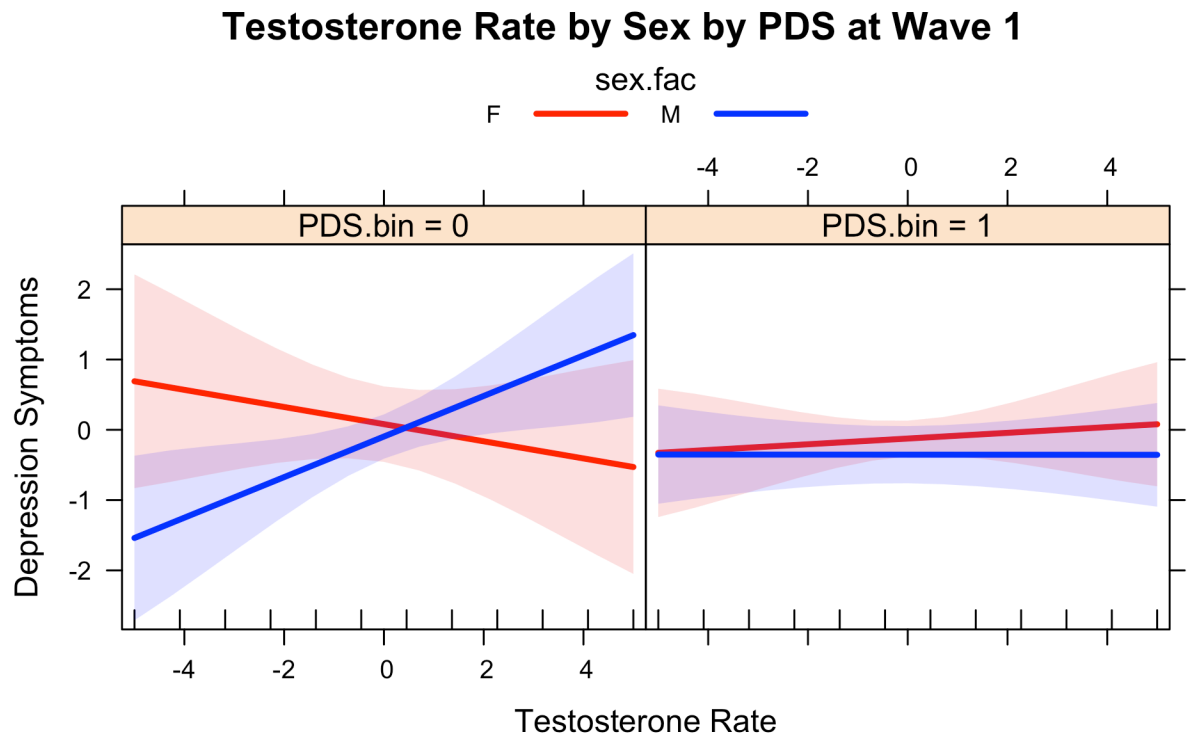


Figure 3.1. Sex differentiated effect of testosterone rate by PDS at Wave 1 predicting depression symptoms, non-significant after correction for multiple testing ($p = .03$). Individuals who were pre- and peri-pubertal at the first assessment are in PDS.bin = 0; individuals who had begun puberty at the first time-point are in PDS.bin = 1.

In the models in which participants were assigned at the detection limit for testosterone, effects of age, sex, and their interaction in Models 1-3 were consistent with the previous testosterone models (Table C2 in Appendix C). In Model 4, the effects of testosterone rate by sex ($\beta = .25$, $SE = .14$, $p = .07$), and testosterone rate by sex by Wave 1 PDS ($\beta = -.33$, $SE = .17$, $p = .05$) were attenuated and no longer significant even at the less stringent threshold of $p < .05$.

Overall, neither testosterone or estradiol average or rate of change were associated with anxiety symptoms or depression symptoms after accounting for multiple testing, and in models including participants below the detection limit.

Externalizing Psychopathology

Rule-Breaking. For rule-breaking symptoms predicted by testosterone rate and average, only the main effect of age was significant in Models 1-3 (Table A3 in Appendix A), however this effect did not surpass the threshold for multiple testing in models estimating an age by sex interaction (Model 2a $p = .02$; Model 3 $p = .03$). Consistent with this, when estradiol was entered as a predictor, only the main effects of age were significant in Models 1, 2b, and 2c, however these were not significant after correction for multiple testing ($ps = .009$; Table B3 in Appendix B).

In the testosterone only Model 4, in which three-way interactions with dichotomized pubertal development at Wave 1 were included, age was no longer significant, and three other effects were estimated: testosterone rate ($\beta = -.33$, $SE = .14$, $p = .02$), testosterone rate by sex ($\beta = .40$, $SE = .18$, $p = .03$), and sex by Wave 1 PDS ($\beta = .76$, $SE = .35$, $p = .03$). None of these effects withstood correction for multiple testing, and should be interpreted with caution. These slight effects indicated that overall, higher rates of testosterone change were related to *lower* rule-breaking symptoms, an effect that

was primarily driven by females. For females, lower PDS at Wave 1 was related to rule-breaking, whereas for males, higher PDS at Wave 1 was related to rule-breaking. These lower order interactions are best depicted by plotting the higher-order three-way interaction between testosterone rate, sex and PDS at Wave 1 (Figure 3.2), although this effect failed to reach significance ($\beta = -.35$, $SE = .21$, $p = .10$).

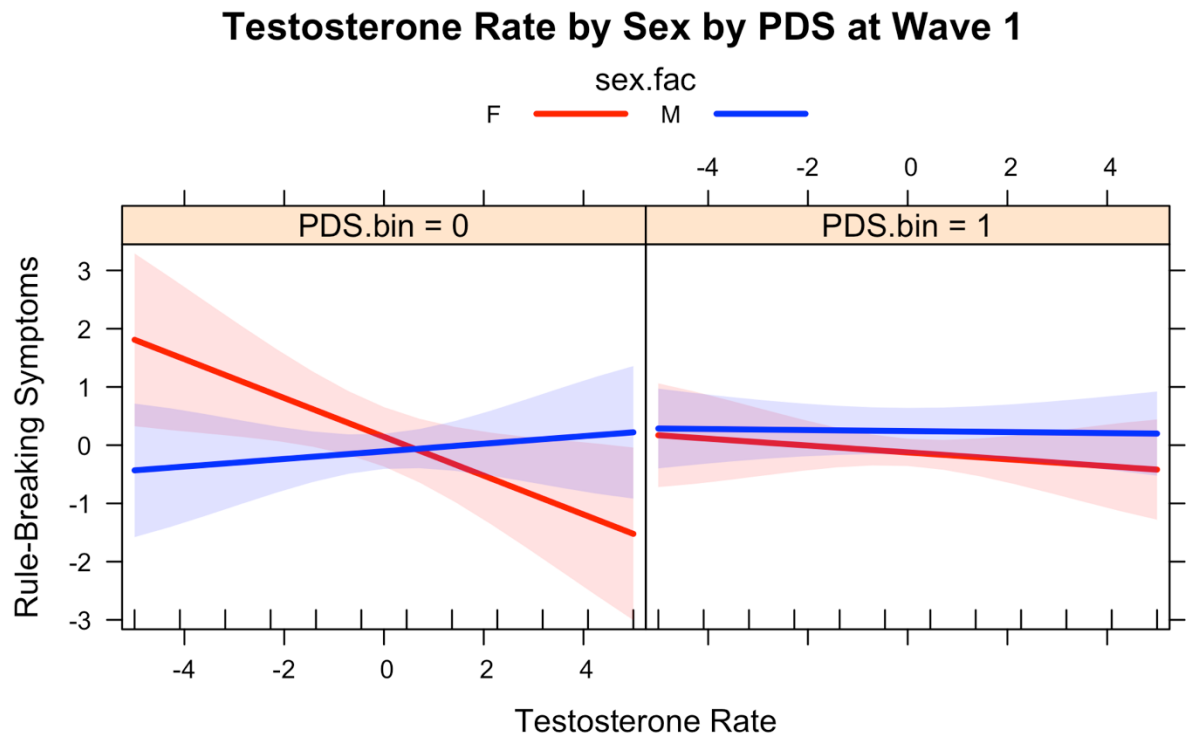


Figure 3.2. Sex differentiated effect of testosterone rate by PDS at Wave 1 predicting rule-breaking symptoms. Three-way interaction was non-significant ($p = .10$). Main effects of testosterone rate, and lower order interaction effects of testosterone rate by sex, and sex by PDS at Wave 1 non-significant after correction for multiple testing ($ps = .02-.03$). Individuals who were pre- and peri-pubertal at the first assessment are in PDS.bin = 0; individuals who had begun puberty at the first time-point are in PDS.bin = 1.

In our robustness check with participants below the detection limit for testosterone assigned at 1.8 pg/ml, only the effect of age was significant in predicting rule-breaking in Models 1-3, consistent with the previous testosterone models (Table C3 in Appendix C). In Model 4, only the effect of sex by PDS at Wave 1 remained significant ($\beta = .61$, $SE = .30$, $p = .05$) but this was considered null under the correction for multiple testing. No significant effects of testosterone were detected, even at a less stringent significance threshold.

Aggression. With testosterone as a predictor, no significant effects were detected in Models 1, 2a, 2b, and 4 (Table A4 in Appendix A). In Model 2c, an effect of testosterone rate on aggression symptoms was detected, and this effect remained in Model 3 when interaction terms with sex were included for age, testosterone average, and testosterone rate ($\beta = -.15$, $SE = .07$, $p = .02$ in both models). However, neither of these effects were significant after correction for testing of multiple outcomes. These slight effects should be interpreted with caution, but may suggest an association between lower rates of testosterone change and *higher* aggression symptoms, an effect that was primarily driven by females (Figure 3.3), though the moderation by sex failed to reach significance ($\beta = .15$, $SE = .08$, $p = .06$). Furthermore, in our robustness check assigning participants at the detection limit for testosterone, no significant effects were detected in any model (Table C4 in Appendix C).

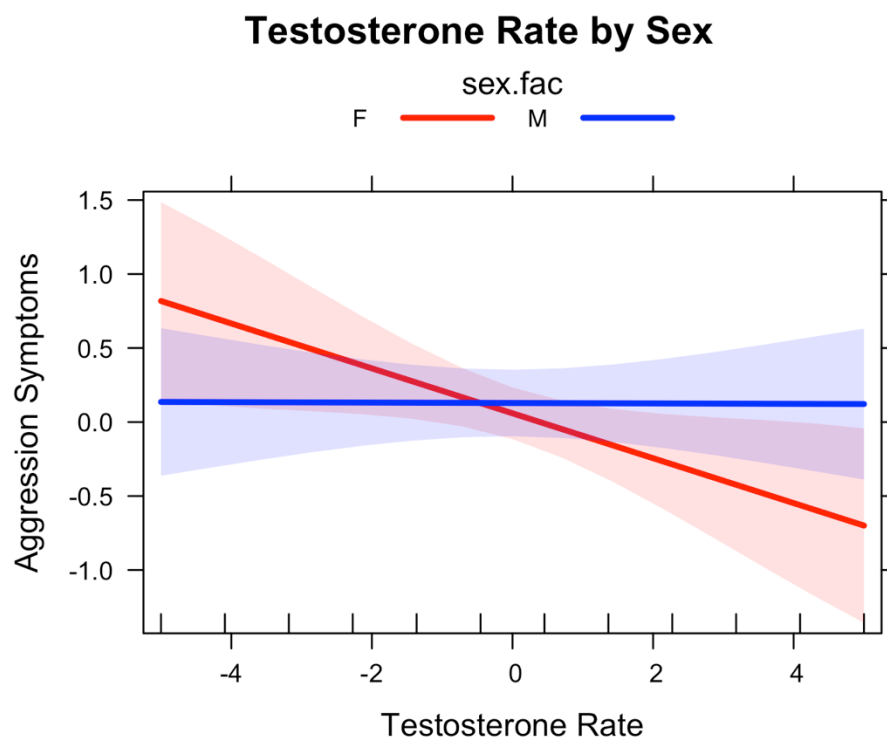


Figure 3.3. Sex differentiated effect of testosterone rate predicting aggression symptoms, non-significant after correction for multiple testing ($p = .02$).

For aggression predicted by estradiol, no significant effects were detected in Models 1, 2a, or 2c (Table B4 in Appendix B). In Models 2b and 3, where an interaction between sex and estradiol average was estimated, there was a main effect of average levels of estradiol predicting aggression symptoms ($\beta = -.25$, $SE = .11$, $p = .02$ in both models). Once again, this did not meet the threshold of significance under multiple testing. Similar to the effect of testosterone change, this may suggest a small effect such that lower levels of average estradiol were associated with *higher* aggression symptoms (Figure 3.4), an effect that was driven primarily by females, though the interaction with sex did not reach significance (Model 2b: $\beta = .21$, $SE = .14$, $p = .15$; $\beta = .22$, $SE = .14$, $p = .13$).

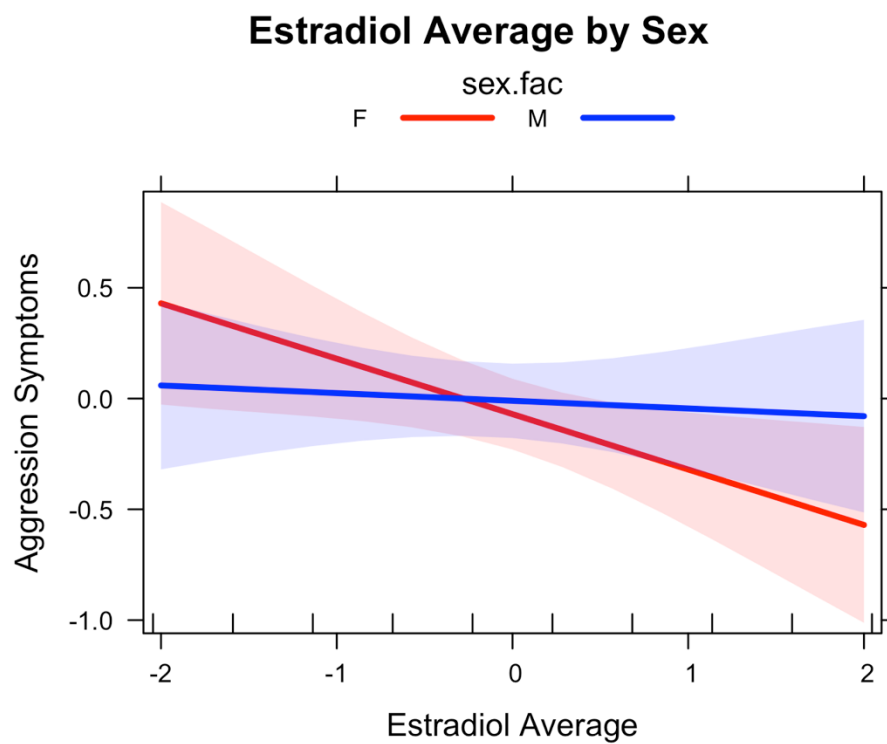


Figure 3.4. Sex differentiated effect of estradiol average predicting aggression symptoms, non-significant after correction for multiple testing ($p = .02$).

Across models for externalizing behaviors relevant for both children and adolescents, neither testosterone or estradiol average or change was associated with rule-breaking or aggressive behavior after accounting for the testing of multiple outcome variables, nor in robustness checks including participants below the detection limit.

Adolescent-Specific Externalizing and Sensation Seeking

Delinquency. Within the adolescent subsample, in Models 1, 2b, and 2c predicting delinquency by either testosterone or estradiol, only the main effects of age and sex were significant, along with age by sex interaction in Models 2a and 3 (Table A5 & B5 in Appendix A & B), indicating that increases in delinquency were related to older age and were higher in boys, with this relationship stronger in males. However, for both testosterone and estradiol models, the age by sex interaction in Model 2a and 3 failed to reach significance at $p < .008$ ($ps = .02 - .04$). No significant effects of testosterone were detected, and models including individuals assigned at the detection limit for testosterone were consistent with these estimates (Table C5 in Appendix C).

Sensation Seeking. In all models for either testosterone or estradiol, only age and sex significantly predicted sensation seeking, such that higher sensation seeking was found for older age and for males (Table A6 & B6 in Appendix A & B). For testosterone, in Models 2a and 3, the main effects of age were no longer significant after correcting for multiple testing ($ps = .02$) and was similarly attenuated when estimated with estradiol ($ps = .009$). No significant effects of testosterone rate or average were detected. Models including participants below to detection limit for testosterone were consistent with prior models (Table C6 in Appendix C).

Overall, no hormone effects were detected in our adolescent specific subsample for either delinquency or sensation seeking.

DISCUSSION

Much of the theory surrounding the sexually dimorphic rise in internalizing and externalizing psychopathology in adolescence implicates hormones as a mechanism in the pathways from biological pubertal maturation to behavioral symptoms (Blakemore, Burnett, & Dahl, 2010; Forbes & Dahl, 2010; Peper & Dahl, 2013). However, the literature with regard to both psychopathological outcomes and intermediary neurobiological processes has yielded mixed and inconsistent results. Relying on single time-point measures of hormones may contribute to the inconsistent findings across prior studies. Motivated by theories of timing-dependent action of hormones that suggest potentially greater relevance of hormonal change relative to circulating levels of hormones, we estimated the relationship between testosterone and estradiol changes and psychopathology across two time-points. Overall, estimates of hormone contribution to rises in psychopathology were minimal, suggesting that effects of hormones on behavior are relatively small, even with leveraging differences across time.

None of our self-report psychopathology or personality measures were associated with hormones after correcting for multiple testing. Prior to correction, we detected several effects of testosterone change with externalizing and internalizing symptoms dependent on sex and pubertal development moderators. However, these effects were small, and did not withstand inclusion of participants below the detection limit included in analyses. We additionally estimated a slight effect of estradiol average on aggression symptoms. Largely, these effects suggested that decreases in testosterone, and lower levels of average estradiol were related to aggression only in females, and decreases in testosterone to rule-breaking only in females who were pre- or peri-pubertal at the first assessment. For pre- and peri-pubertal males, increases in testosterone were associated with greater depression symptoms, with the opposite trend in females. These slight

effects should be interpreted with caution given that they were not robust enough to withstand correction for multiple testing, or inclusion of participants below the detection limit. Given these results as well as the inconsistent findings in the previous literature, it is likely that any effects of hormones on behavior are very small, and detecting these effects robustly likely requires larger samples and consideration of developmental moderators.

The slight effects of testosterone on externalizing and internalizing symptoms that were detected prior to correction for multiple testing were found in relation to testosterone change rather than person-level testosterone average. This may suggest that the hypothesis that hormonal change is more greatly related to behavior than concentrations alone is not entirely misguided; however, effects remain minimal. Many of these small effects were found only for participants who were pre- or peri-pubertal at the outset of the study. These participants were under represented in our full sample such that the younger end of our age range may be underpowered to detect these effects. The small effect of estradiol average on aggression should be considered in light of the relatively little change in estradiol in this sample, as discussed in previous work (Paper 2). Careful consideration of sample age ranges may suggest focusing on late childhood and early adolescence, when the action of hormones may have a greater impact.

That we did not find large effects of hormones on psychopathological outcomes is not entirely inconsistent with the prior literature. Many of the associations between pubertal hormones and externalizing and internalizing behaviors have been detected only in the presence of psychosocial moderators such as peer groups (Rowe et al., 2004), personality characteristics (Reardon, Herzhoff, & Tackett, 2015), and parent-child relationship factors (Booth et al., 2003; Chen, Raine, & Granger 2018). Furthermore, a robust literature on dual-hormone theories suggests that hormone effects on behavior are

dependent on interactions between the HPG and HPA axes (Mehta & Josephs, 2010; Mehta & Prasad, 2015). Indeed, considering both psychosocial moderators and cross-axis hormone interactions may be necessary to further illuminate hormone effects on psychopathology (Tackett et al., 2015). Finally, it is possible, given the small sample sizes and investigator degrees of freedom that have characterized much of the hormone literature, that reported positive associations represent false positives.

As in prior work utilizing salivary hormone measurement in this sample, limitations due to hormone collection procedures must be acknowledged. At each time-point, hormone concentrations were assayed from a single saliva sample, collected at three different appointment times over the day. Variance in hormone levels may capture both an individual's trait hormone levels as well as state-specific variation due to incidental factors, and diurnal variability. Furthermore, for females who have begun menstruation, varying levels of hormones over the menstrual cycle play a role in hormone concentration levels. Additionally, previous work suggests differential associations between hormone levels and psychopathology dependent on menstrual cycle phase (Vermeersch et al., 2008a). Though we statistically controlled for time since waking and menstrual cycle phase, collection at specific times within the day or specific days in the menstrual cycle would provide a more rigorous control for these potential sources of variation.

The clear story of pubertal hormones giving rise to behavioral changes during adolescence appears overly simple, at best. The impact of hormones on behavior are likely very slight and may be complicated by localized action in particular times in development. Just as candidate genes were initially heralded as biologically proximal markers for complex human behavior, but ultimately did not have replicable associations, perhaps the impact of hormones on behavior has been over-estimated in our theories.

Single hormone effects are likely inadequate to account for a substantial amount of variation in the development of psychopathology. Careful consideration of interaction effects between hormonal axes and psychosocial moderators appears necessary to quantify the downstream contribution of hormones on behavioral outcomes.

Appendix A

Tables A1 – A6 reporting models of testosterone change predicting psychopathology are contained in an attached excel document titled Appendix A.

Appendix B

Tables B1 – B6 reporting models of estradiol change predicting psychopathology are contained in an attached excel document titled Appendix B.

Appendix C

Tables C1 – C6 reporting models of testosterone change including participants below the detection limit assigned at 1.8 pg/ml predicting psychopathology are contained in an attached excel document titled Appendix C.

References

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