Within-Person Changes in Salivary Testosterone and Physical Characteristics of Puberty Predict Boys' Daily Affect

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Abstract

Recent investigations highlighted the role of within-person pubertal changes for adolescents' behavior. Yet, little is known about effects on adolescents' daily affect, particularly regarding the hormonal changes underlying physical changes during puberty. In a study with 148 boys aged 10 to 20 years, we tested whether within-person physical and hormonal changes over eight months predicted everyday affect fluctuations, measured with experience sampling. As expected, greater within-person changes in testosterone (but not in dehydroepiandrosterone) were associated with higher affect fluctuations in daily life. Additionally, greater physical changes predicted higher affect fluctuations for individuals in the beginning of puberty. The findings demonstrate the relevance of physical and hormonal changes in boys' affective (in)stability.

Keywords: affect fluctuations; experience-sampling method; pubertal tempo; within-person physical and hormonal changes; testosterone and dehydroepiandrosterone; adolescence and puberty; boys; GLLA

Within-Person Changes in Salivary Testosterone and Physical Characteristics of Puberty Predict Boys' Daily Affect

Adolescents are widely assumed to experience rapid mood swings. Empirical findings indeed demonstrate a tendency for amplified affect in adolescence as compared to late childhood and adulthood (e.g., Larson, Moneta, Richards, & Wilson, 2002; Sallquist et al., 2009; Weinstein, Mermelstein, Hankin, Hedeker, & Flay, 2007; for an overview see Zimmermann & Iwanski, 2014). Pubertal, as compared to prepubertal, adolescents showed more intense, more varying, and more negative affective experiences (e.g., Berenbaum, Beltz, & Corley, 2015; Buchanan, Eccles, & Becker, 1992; Steiner, Dunn, & Born, 2003). Findings are less clear when it comes to whether different stages of pubertal development (beginning, middle, or advanced puberty) are particularly prone to elevated affective responding (e.g., Gunnar, Wewerka, Frenn, Long, & Griggs, 2009; Hunt, 1999; Sumter, Bokhorst, Miers, Van Pelt, & Westenberg, 2010). We propose that findings remain inconclusive because most prior studies on adolescents' affective experiences have taken a cross-sectional approach, that is, compared different physical characteristics or hormonal levels, and thus were unable to capture within-person *changes* during puberty.

Employing a within-person approach, investigations on behavior problems and psychopathology highlighted the potential importance of the amount of pubertal changes adolescents experience during a particular time. This perspective has been subsumed by using the term pubertal tempo (e.g., Dorn & Biro, 2011; Mendle, 2014). With regard to everyday affective experiences, however, investigations on the role of within-person pubertal changes are lacking. It has been argued that quicker pubertal maturation may demand quicker adaption to biological and social transitions (Dorn & Biro, 2011; Mendle & Ferrero, 2012) and reflect more pronounced hormonal changes (e.g., Dorn & Biro, 2011; Shirtcliff, Dahl, & Pollak, 2009; Spielberg, Olino,

Forbes, & Dahl, 2014). Particularly hormonal changes in puberty are widely assumed to have implications for affect and behavior (e.g., Amin, 2006; Balzer, Duke, Hawke, & Steinbeck, 2015; Buchanan et al., 1992; Mendle, 2014; Walker, Sabuwalla, & Huot, 2004). The vast majority of these studies, however, stem from adolescent girls and adult women (Balzer et al., 2015; Steiner et al., 2003). Very little is known about associations between sex-steroids and affect in boys (for reviews, see Duke, Balzer, & Steinbeck, 2014; Mendle & Ferrero, 2012).

In the present paper, we attempt to contribute to a better understanding of the role of puberty in boys' affect since the evidence is still inconclusive. We propose that it is the amount of pubertal and especially hormonal changes that influence the stability of individuals' affective experiences, with greater amount of changes in a given time resulting in more intense and more quickly fluctuating daily affective experiences. Using a multi-method investigation, we studied boys' within-person changes in self-reported physical development as well as their within-person changes in sex-steroid hormone levels. We focused on within-person affect fluctuations (i.e., how quickly and intensely an individual's affect occurs and diminishes, thus fluctuates, throughout the course of the day), as affect fluctuations constitute a central characteristic of affective experiences (e.g., Eid & Diener, 1999; Wang, Hamaker, & Bergeman, 2012) and psychological health (e.g., Bowen, Baetz, Hawkes, & Bowen, 2006; Gruber, Kogan, Quioidbach, & Mauss, 2013; Klinkman, 2007). We use the terms adolescence or puberty when prior evidence refers to both boys and girls, and otherwise identify findings as solely referring to boys or girls.

The Role of Pubertal Changes in Affective Experiences

Different hypotheses have been discussed in the literature on how puberty impacts adolescents' affective experiences (for review, see Ge & Natsuaki, 2009). First, hormonal changes in puberty have long been assumed to be relevant for affective experiences (e.g., Buchanan et al., 1992; Duke et al., 2014; Steiner et al., 2003). Second, physical changes in

puberty might elicit feelings of distress or insecurity because of others' reactions or own perceptions of the changes (e.g., for a review, see Mendle & Ferrero, 2012), especially when physical changes occur rapidly (e.g., Mendle, Harden, Brooks-Gunn, & Graber, 2010).

Physical development in puberty (e.g., genital or pubic hair growth) results from hormonal changes, primarily androgens, such as testosterone in boys and estrogens in girls (Ellison et al., 2012). Androgens exert stimulating effects on the central nervous system and result in structural changes (e.g., Brouwer et al., 2015; Schulz, Molenda-Figueira, & Sisk, 2009; Smith, Adams, Schmidt, Rubinow, & Wassermann, 2002; Van Wingen, Ossewaarde, Bäckström, Hermans, & Fernández, 2011). Specifically, sex steroids activate androgen and estrogen receptors that are particularly prevalent in regions involved in the processing of affective information, such as the hypothalamus, amygdala, septal nucleus, and hippocampus (e.g., Brouwer et al., 2015; Van Wingen et al., 2011). Through receptor activity, large changes in sex steroids can stimulate affect-related brain structures (for further readings on mechanisms stemming from human and animal studies, see Celec, Ostatníková, & Hodosy, 2015). This likely results in unstable affective experiences in both boys and girls (e.g., Amin, 2006; Schulz et al., 2009; Walker et al., 2004).

Hormonal changes during puberty, and especially in beginning[1] stages of puberty (e.g., Ankarberg-Lindgren & Norjavaara, 2004; Khairullah et al., 2014), exceed most hormonal changes that occur later in life (e.g., testosterone decline in older men or menopause in women; Schulz et al., 2009; Walker et al., 2004). Hence, it seems plausible to assume that hormonal changes in puberty contribute to elevated affective experiences in pubertal adolescents. Importantly, greater amount of hormonal changes in boys (Ankarberg-Lindgren & Norjavaara,

[1] Throughout the manuscript, we chose the terminology "beginning" and "advanced" stages of puberty as opposed to the terminology "early" and "late" status of puberty to avoid confusion with the concept of early and late pubertal timing. Pubertal timing denotes the age at which changes in primary and secondary sex characteristics appear relative to peers, that is, earlier, concurrently, or later than same-aged peers.

2004; Khairullah et al., 2014) and girls (Alonso & Rosenfield, 2002) were observed in beginning as compared to more advanced stages of physical puberty. In addition, changes in beginning stages of physical puberty might be particularly stressful to individuals because they experience these changes for the first time (e.g., Ge & Natsuaki, 2009). We therefore predicted that (a) greater amount of hormonal changes and (b) physical changes especially during beginning stages of physical puberty are related to higher affect fluctuations.

Next, we discuss the available empirical evidence on the association between puberty and affective experiences with regard to the role of within-person changes in puberty. Because the majority of studies focused on physical development in puberty, we first review studies that investigated physical development in puberty in relation to affective experiences. Next, we review the few studies that directly investigated underlying hormonal changes in puberty in relation to affective experiences.

Empirical Evidence on Associations between Physical Development and Affect in Puberty

Longitudinal studies on associations between physical changes in puberty and affective experiences are rare. Most prior studies on adolescents' affective experiences used a cross-sectional approach. In some of these studies, adolescents in beginning and middle puberty, as compared to those in advanced puberty, were prone to amplified affective experiences (e.g., Hunt, 1999; Steiner et al., 2003). Other studies found lower affect reactivity in middle as compared to advanced puberty (Sumter et al., 2010) or no differences in affective experiences between levels of physical development in puberty (Gunnar et al., 2009; Yim, Quas, Cahill, & Hayakawa, 2010). Differences in sample selectivity (Shirtcliff et al., 2009), in the assessment of physical development in puberty (e.g., Gunnar et al., 2009; Sumter et al., 2010), or in the investigated facet of affective experiences (e.g., Oldehinkel, Verhulst, & Ormel, 2011; Silk et al.,

2009) impede comparisons across studies. However, inconclusive findings may also be due to one-time cross-sectional assessments that did not capture the temporal characteristics of the pubertal process.

We argue that the omission of temporal characteristics of puberty may be crucial, as adolescents with comparable levels of pubertal maturation may well differ in how fast pubertal changes occurred. Thus, pubertal changes may be more important for affective experiences than current maturation levels. In support of this notion, Hunt (1999) found more varying and more intense affective experiences when adolescents experienced physical changes in puberty over six and twelve months, as compared to when they did not experience physical changes. Further support for the potential importance of investigating within-person pubertal changes stems from studies on adolescents' psychopathology and behavior problems (for a review, see Mendle, 2014). These studies showed that individuals experiencing more physical changes in puberty during a fixed time period displayed higher depressive and other internalizing symptoms, substance abuse, and social difficulties (e.g., Beltz, Corley, Bricker, Wadsworth, & Berenbaum, 2014; Marceau, Ram, Houts, Grimm, & Susman, 2011). By relying on physical changes in self-reported puberty rather than directly measuring hormonal changes, these studies only provide indirect evidence on the importance of hormonal changes for individual differences in affect fluctuations.

Empirical Evidence on Associations between Hormonal Development and Affect in Puberty

There is only limited direct evidence for associations between sex-steroids and affective experiences in adolescence, which almost entirely stems from cross-sectional studies on girls. A recent systematic literature review found nine studies on the effects of girls' estradiol concentrations on affect, of which seven studies were older than 20 years. The authors concluded

that estrogen showed consistent associations with depression and affect variability (Balzer et al., 2015). In one study, the effect of estrogen was stronger than the effect of self-reported physical pubertal characteristics, emphasizing the role of hormonal characteristics of puberty in affect (Angold, Costello, Erkanli, & Worthman, 1999). Boys' primary sex-steroids, such as testosterone, have not been studied in relation to affective experiences in adolescence (Duke et al., 2014). In their systematic review, Duke and colleagues (2014) concluded that, to date, testosterone levels can only be associated with aggressive behavior. With respect to aggressive behavior, these studies provide further support for the hypothesis that changes in hormone levels in adolescence might be more important than the current level of hormones. This is the case because aggressive behavior often decreases across adolescence despite testosterone levels remaining high (Duke et al., 2014).

One study directly investigated longitudinal change in testosterone concentration across a two-year study period in young adolescents in relation to affective processing. Results showed that higher changes in testosterone were related to greater brain activity in emotion-relevant brain regions in response to emotional stimuli in a mixed-sex sample as well as in a boys-only sample (Spielberg et al., 2014). To date, longitudinal investigations that directly assess within-person hormonal changes in relation to different aspects of daily affective experiences in adolescence have not been conducted.

Current Study

In short, the purpose of the current study was to investigate whether within-person pubertal changes are related to boys' affect fluctuations in daily life. We hypothesized that more pronounced within-person changes in boys' sex-steroid hormones (testosterone and dehydroepiandrosterone) are associated with higher affect fluctuations. As sex-steroid changes manifest in physical characteristics of puberty, we additionally tested whether changes in

physical puberty are associated with affect fluctuations. Because prior studies indicated that sex-steroid changes were comparatively larger in beginning stages of puberty (e.g., Alonso & Rosenfield, 2002; Khairullah et al., 2014) and because beginning stages of puberty might be perceived as particularly stressful (e.g., Ge & Natsuaki, 2009), we hypothesized that changes in physical puberty are more strongly related to higher affect fluctuations in beginning than advanced stages of puberty.

We investigated our predictions using data from a larger project on the development of adolescent boys (e.g., Klipker, Wrzus, Rauers, & Riediger, 2017). In contrast to most previous studies, we investigated pubertal development as a within-person process by following up on participants' hormonal and physical development after approximately eight months. We chose this time frame as measurement intervals of 6 to 12 months have been shown to capture pubertal changes in other studies (Hunt, 1999; Marceau, Ram, & Susman, 2015; Mendle et al., 2010).

Further advancing previous studies, within-person fluctuations in adolescents' affective experiences were assessed during an experience-sampling phase with multiple assessments of momentary everyday affect. We operationalized affect fluctuations using derivative estimates of affect ratings, which reflect how rapidly affective experiences change during the day (e.g., Deboeck, Montpetit, Bergeman, & Boker, 2009; Wang et al., 2012).

Method

Participants

We investigated a sample of 158 adolescent boys ranging in age from 10 to 20 years (M = 14.69; SD = 2.68), of which 148 completed all study parts. Participants lived with both (69%) or one of their parents (30%) in Berlin, Germany. One participant lived alone and one participant lived in a shared apartment. Of the participants, 95% attended school (primary school: 11%; secondary school with higher school track: 65%; secondary school with lower school track: 2%;

secondary school with combined school track: 21%), 4% attended college and 1% was in vocational training. Only participants without hormone dysfunctions and chronic medications were recruited. Information on socio-economic status (income and education) were reported by 78% of participants' parents. Most parents were highly educated (highest degree in the family: 73% university degree, 11% university entrance diploma, 16% high school diploma). Family net incomes ranged from EUR 750 to 20,000 per month (M = 3,727, SD = 2,310).

Procedure

Study part 1. For the first part of the study (T1), participants came to the laboratory, received information about the study, declared their informed consent (for participants under the age of 18 years, participants and one of their legal guardians provided their informed consent), and then answered questionnaires amongst others on physical development. Participants received detailed instructions on taking morning and evening saliva samples on four consecutive regular school-days. Participants were not allowed to brush their teeth, chew gum, smoke, eat or drink anything besides water 30 minutes prior to saliva sampling. For each saliva sample, participants recorded the time and filled out a questionnaire typically used in the context of hormone assessments (e.g., Schultheiss & Stanton, 2009). Participants or their parents received a reminder SMS for the evening saliva sampling at 7:30 pm. This also included a reminder on the morning saliva sampling as soon as they woke up as well as a reminder to store the sample in the refrigerator. After four consecutive weekdays of saliva sampling, participants placed all eight saliva samples into a prepared box and sent it to the laboratory for hormone analysis (Labor Krone, Bad Salzuflen, Germany). For the first part of the study, participants received a reimbursement of EUR 20.

Study part 2. After a period of M = 8.02 months (SD = 0.71, MIN = 6.24, MAX = 9.43), participants returned for the second part of the study (T2) where they again reported on their

current physical development in puberty, among other things. The saliva sampling procedure of T2 followed the protocol of T1. After detailed instructions, participants received specially programmed mobile phones (Nokia E50) as assessment instruments for an experience-sampling period of two weeks. The mobile-phone based experience-sampling phase comprised three cycles of three assessment days followed by two rest days each. On assessment days, participants were prompted six times a day approximately two hours apart to answer questions on the mobile phone using the phone's joy stick (for further details see Klipker et al., 2017). If participants failed to answer at least five of the six daily assessments, the three-day assessment period was prolonged by one day. On average, participants completed 45.60 mobile phone assessments (SD = 11.33, r_{age} = -.02, p = .80) over the two-week experience-sampling phase. For T2, participants received a reimbursement of EUR 70 that was increased to EUR 80 if they had responded to more than 80% of assessments in the experience-sampling phase. The ethics committee of the Max Planck Institute for Human Development approved the study prior to data collection.

Measures

Physical change in puberty (T1 and T2). Physical development in puberty was assessed using two self-report rating scales, both of which were administered at T1 and at T2: the Pubertal Developmental Scale (PDS, Petersen, Crockett, Richards, & Boxer, 1988), and a modified version of the Tanner scales, using schematic drawings of boys' genital development (Taylor et al., 2001). The PDS for boys includes questions regarding growth of pubic hair, facial hair, and voice changes. The modified Tanner scale includes drawings indicating five stages of genital and pubic hair development from pre- to postpuberty. Individuals checked the drawing that looked most similar to themselves. PDS and Tanner scales were highly correlated (r = .88, p < .05). We standardized both scale scores across T1 and T2 to yield a combined z-score measure of physical puberty ranging from -1.91 (prepuberty) to 1.38 (postpuberty). Within-person change in physical

characteristics of puberty across the study period was obtained by calculating the individual difference between puberty *z*-scores from T1 and T2. Change scores ranged from 0, indicating no change, to an increase of 1.16 standard deviations ($M = 0.35$, $SD = 0.32$).

Hormonal change in puberty (ambulatory assessment at T1 and T2). Free testosterone and dehydroepiandrosterone (DHEA) concentrations were measured as the two main sex-steroids relevant in boys' puberty (e.g., Shirtcliff et al., 2009). Hormone values were obtained from saliva using the IBL SalivaCap kit with solid phase enzyme-linked immunosorbent assay (ELISA). ELISA is particularly recommended for the assessment of steroid hormones and showed excellent coefficients of variations (9.2% and 5.8% for testosterone and 5.7% and 4.2% for DHEA intra- and inter-assay precision, respectively), assay sensitivity (determined by subtracting two standard deviations from the mean of 20 replicate analyses at the 0 pg/ml level: 2.00 pg/ml for testosterone and 2.20 pg/ml for DHEA), and method accuracy (determined by recovery and linearity: 101.2% and 106.3% for testosterone and 103.7% and 96.0% for DHEA). To get a reliable estimate of the overall concentration level we assayed samples on four consecutive regular school-days after awakening and at 7:30 pm, resulting in eight saliva assays at T1 as well as at T2. Within-person change in sex-steroids across the study period were obtained by calculating the individual difference between average concentration levels from T1 and T2 (see Hormone Analysis).

Momentary affect (experience-sampling phase at T2). On each measurement occasion during the two-week experience-sampling phase, participants rated a total of 12 mood adjectives to report on the intensity of their current positive and negative affective experiences using a 7-point Likert scale ranging from 0 *(not at all)* to 6 *(very much)*. Answers on how happy, energetic, enthusiastic, relaxed, content, and secure participants currently felt (original German items: *froh, energiegeladen, begeistert, entspannt, zufrieden, sicher*) were averaged to represent positive

affective experiences. Answers on how angry, stressed, irritable, sad, unhappy, and disappointed participants currently felt (original German items: *ärgerlich, angespannt, gereizt, traurig, unglücklich, enttäuscht*) were averaged to represent negative affective experiences. On each measurement occasion, negative affect scores were subtracted from positive affect scores yielding an affect-balance measure (e.g., Green & Salovey, 1999) with a theoretical range from -6 (high intense negative and no positive momentary affect) to +6 (high intense positive and no negative momentary affect). The obtained time series of such momentary measures of affect balance were then used to characterize participants' time-dependent fluctuations in affective experiences, taking both positive and negative portions of affect fluctuations into consideration. More precisely, we calculated first-order derivative estimates of the time series of momentary affect-balance measures (see Data Analysis section). First-order derivative estimates express the rate of change with which participants' reported affect changed over the course of the experience sampling phase (e.g., Deboeck et al., 2009).

Covariate perceived stress (T2). Participants' overall *perceived stress* during the two-week experience-sampling phase was assessed after the experience-sampling phase, using a shortened version of the Adolescence Stress Questionnaire (ASQ, Byrne, Davenport, & Mazanov, 2007). We selected 33 items based on the factor loadings (reported by Byrne et al., 2007) of the original scale. The ASQ addresses different areas of stress, for example at home, in school, with romantic relationships, regarding physical appearance, or from peer pressure. For each item, participants indicated their perceived stress on a scale from 0 (not at all stressful) to 5 (very stressful). Participants' mean scores were used as indicators of their overall life stress (M = 1.25, SD = 0.70, MIN = 0.03, MAX = 3.64).

Data analysis

Hormone Analysis

Across all individuals, 2.52% of all testosterone and 7.28% of DHEA values could not be analyzed by the laboratory due to values outside detection limits (testosterone: 2−760 pg/ml; DHEA: 2.20−1440 pg/ml), insufficient amount of saliva, or viscous saliva samples. Data was screened for potential outliers separately for morning and evening samples and within puberty groups according to Tanner (Taylor et al., 2001). According to the outlier labeling method (Hoaglin & Iglewicz, 1987), 2.44% of the testosterone and 0.75% of the DHEA values were statistical outliers and were set to their respective cutoff values of 2.2 times the interquartile range above the 75th percentile of their morning or evening values in their respective puberty group.

For each day of hormone assessments, we applied an area under the curve formula to estimate the overall level (AUCg: area under the curve with respect to ground concentrations of zero, e.g., Pruessner, Kirschbaum, Meinlschmid, & Hellhammer, 2003) of participants' testosterone and DHEA concentrations (see Appendix A for detailed information on the computation of the area under the curve).

To take individual differences in the time between daily morning and evening saliva sampling into consideration (M = 12.76 hours; SD = 1.18), we calculated the area under the curve for a fixed time period of twelve hours, with $AUC_{12h} = (AUC \times 12)/t_{(evening–morning)}$, where t represents the time between the morning and evening saliva sample. This allowed us to compare measures within and between individuals and was crucial for calculating within-person change in hormone concentrations across the study period. Thus, within-person change in sex-steroids across the study period was obtained by subtracting the average time-corrected area under the curve at T1 from the average time-corrected area under the curve at T2.

Analysis of Affect Fluctuations

We were interested in within-person affect fluctuations, that is, the rate at which participants' affective experiences changed over the course of the experience-sampling phase. First order derivative estimates of the time series of momentary affect-balance ratings were obtained using generalized local linear approximation (GLLA, Boker, Deboeck, Edler, & Keel, 2010). GLLA is a time-delay embedded convolution filter method, used to calculate approximate derivatives of a differential equation from time series data, and is defined as:

$$Y = X^D W \text{ ; with: } W = L(L'L)^{-1}.$$

Matrix X^D is a reorganization of an individual's observed time series with an embedding dimension D, and is called an embedded matrix. D describes the number of measurement occasions used to calculate each derivative estimate. Matrix L is the loading matrix that produces weights (matrix W) that express the relationship between X^D and Y. As a result, matrix Y contains the least squares estimates of the displacement and derivatives of the data (Boker et al., 2010). Following Deboeck and colleagues (2009), we used an embedding dimension D of 3 measurement occasions, yielding a maximum of four derivative estimates per day. Since time delay embedding is robust to sampling interval misspecification (Boker, Tiberio, & Moulder, in press), all available data from each daily burst was able to be used in the analysis (see Appendix B for detailed information on applying GLLA to the present study's time-series data). On average, 4.56 observations were reported per participant per day (SD=1.23), yielding an average of 45.60 observations per participant (SD=11.33). We used the *gllaWMatrix()* function in the statistics software *R* provided in Boker and colleagues (2010) to calculate the W matrix.

The within-person mean of the absolute value of within-day derivative estimates is a good estimator of the variability in affect ratings over short periods of time (e.g., Deboeck et al., 2009). We used this indicator as an estimate of individuals' affect fluctuations in further analysis because we were interested in such daily affect fluctuations. Individuals' affect fluctuation scores ranged

from 0.25 to 2.03 (M = 0.75, SD = 0.32), that is, individuals with the lowest affect fluctuation score showed a mean within-person change of 0.25 on the affect rating scale from one assessment occasion to the next. For individuals with the highest affect fluctuations score, within-person change on the affect rating scale was eight times higher, namely 2.03 points on the affect rating scale from one assessment occasion to the next. Four exemplary time-series of participants' affect ratings as well as the corresponding affect fluctuations score (i.e., the within-person mean of the absolute value of participants' first-order derivative estimates) are shown in Figure 1.

Results

We hypothesized that greater amount of hormonal and physical changes in puberty are associated with more pronounced affect fluctuations in daily life. We tested these predictions in two sets of multivariate regression analyses. In the first set of analyses, we investigated whether within-person hormonal changes predicted subsequent affect fluctuations. In the second set of analyses, we investigated whether within-person changes in physical puberty predicted affect fluctuations especially in beginning stages of puberty. The dependent variable in these analyses was individuals' within-person affect fluctuations during the two-week experience-sampling period at T2 (i.e., the absolute mean of within-day derivatives). Because we predicted that greater testosterone change was associated with higher affect fluctuations, we first tested a model with the main effect testosterone change as predictor variable in the testosterone model. Since we know from previous studies that hormonal change is particularly high in the beginning of physical puberty rather than in advanced physical puberty, we predicted that change in physical puberty was associated with higher affect fluctuations particularly in the beginning stages of puberty. The first model on physical puberty paralleling the testosterone model therefore includes an interaction between physical pubertal status and physical pubertal change. The remaining models for testosterone and physical puberty further examine the respective main models by

including important covariates in addition to the hypothesized predictor variables. Independent variables were centered to their respective sample mean. Descriptive statistics on all central study variables as well as zero-order correlations of all central study variables are depicted in Tables 1 and 2 respectively.

Hormonal Changes and Affect Fluctuations

As expected, individuals with higher testosterone changes across the study period showed significantly higher affect fluctuations than individuals with lower testosterone changes (Model 1 in Table 3, R^2 = 3.2 %; $F(1,132) = 4.32$; $p < .05$). To account for individual differences in the time elapsed between the first and the second study part, we included the time elapsed and its interaction with testosterone changes as independent variables in the model. The main effect of testosterone changes on affect fluctuations remained stable.

Because prior cross-sectional studies investigated adolescents' hormone levels, as opposed to within-person hormone changes in relation to affect and behavior (e.g., Balzer et al., 2015; Duke et al., 2014), we next controlled for participants' testosterone levels at T1. Additionally, we controlled for individual differences in adolescents perceived stress during the experience-sampling and hormone-sampling period at T2 (Model 2 in Table 3). In this final model, the main effect of testosterone changes on affect fluctuations remained stable ($\beta = 0.26$; $p < .05$) and a significant interaction with the time elapsed between study parts ($\beta = -0.19$; $p < .05$) denoted that the effect of testosterone change on affect fluctuations was more pronounced when change occurred in a shorter time. In addition to testosterone changes, higher perceived stress, but not higher testosterone levels, were associated with higher affect fluctuations (Model 2 in Table 3). Further interactions between testosterone change and the respective covariates did not reach significance ($p > .05$ for each) and were therefore not included in the final model. In other words, there was no evidence that the effect of higher affect fluctuations for individuals with higher

testosterone changes depended on adolescents' initial testosterone levels or differences in current perceived stress.

The results provided no evidence for associations between affect fluctuations and DHEA changes across the period of study (R^2 < 0.1 %; $F(1,130) = 1.12$; $p = .29$, see also Table 2).

Physical Changes and Affect Fluctuations

We predicted that within-person changes in physical signs of puberty are related to higher affect fluctuations, particularly during beginning stages of physical puberty. Consistent with our hypothesis, the interaction between the initial level of physical puberty at T1 and the amount of physical changes in puberty across the study period significantly predicted higher affect fluctuations at T2 (Model 1 in Table 4). In other words, the association between physical changes in puberty and affect fluctuations depended on how physically advanced in puberty individuals were at T1. For adolescents, whose initial physical puberty stage was low (e.g., individuals proceeding from pre- to beginning of physical puberty), greater physical changes were associated with higher affect fluctuations. In contrast, for adolescents with more advanced physical puberty (e.g., individuals proceeding from mid-puberty to advanced puberty), greater physical changes were not associated with affect fluctuations. Figure 2 illustrates model-predicted values for the interaction effect for the average level of physical puberty at T1 and for one standard deviation below the average level. Region of significance analyses (Bauer & Curran, 2005; Preacher, Curran, & Bauer, 2006) showed that the positive association between physical changes in puberty and subsequent affect fluctuations reached statistical significance in individuals who had an initial physical puberty *z*-score lower than -1.24, and thus were in beginning of puberty at T1.We did not plot model-predicted values for one standard deviation above the average level of physical puberty at T1, that is for individuals who were in advanced to post-puberty at T1. We

refrained from plotting these values because for individuals in advanced or post-puberty medium to high physical changes in puberty did not occur.

Parallel to our analyses on hormonal changes, we controlled for individual differences in the time elapsed between the first and the second study part, by including the time elapsed and its interaction with physical puberty changes as independent variables in the second model (Table 4). There was no significant interaction between time elapsed and physical changes in puberty. Due to reasons of model parsimony, this non-significant interaction effect was excluded from the third and final model.

Because hormonal changes accompany physical development in puberty, we included testosterone changes as covariates into the final model. To control for differences in adolescents' perceived stress during the experience-sampling period at T2, we additionally controlled for perceived stress in the final model. The interaction effect of initial physical puberty and within-person change in physical puberty in predicting affect fluctuations remained stable (see Model 3 in Table 4). In addition to the physical puberty interaction, both higher perceived stress and higher testosterone changes were associated with higher affect fluctuations in the final model, which mirror results in Table 3.

Discussion

The present study aimed at better understanding the role of pubertal development for adolescents' elevated daily affect fluctuations by examining physical and hormonal changes that occur during puberty. We used a within-person study design to investigate changes in boys' pubertal processes. In addition to within-person changes in self-reported physical puberty, our study is the first to investigate changes in boys' sex-steroids during puberty in relation to affective experiences. Findings from the present study support the hypothesis that boys' within-

person pubertal change processes are associated with individual differences in daily affect fluctuations.

Hormonal Changes and Affect Fluctuations

In line with our first hypothesis, our results showed that a higher within-person increase in individuals' testosterone concentrations across the study period was followed by higher affect fluctuations at T2. So far, prior studies on the role of testosterone in adolescence have investigated the level of testosterone, finding that higher testosterone levels were related to higher aggressive behavior (e.g., Duke et al., 2014). It has been argued that the effect of higher testosterone levels on behavior in adolescence might reflect a sudden increase in testosterone concentration across puberty (e.g., Duke et al., 2014). Our results support this argument because boys' within-person changes in testosterone concentrations predicted subsequent affect fluctuations regardless of individuals' initial level of testosterone concentrations.

To control for social influences on testosterone concentrations that might have confounded the results, we tested whether stressful life circumstances alter the association between changes in testosterone and affect fluctuations. The effects of testosterone remained stable after controlling for adolescents' stress (Byrne et al., 2007), containing situations related to social dominance, self-image, and romantic interests, all of which have been associated with testosterone concentrations (for a review, see Duke et al., 2014).

There was no significant association between within-person changes in DHEA and affect fluctuations in our sample. DHEA is considered a weak androgen, therefore the observed changes in DHEA at this age might be insufficient to affect the emotion system (e.g., Blakemore, Burnett, & Dahl, 2010). The initial rise in DHEA concentrations, that might influence affective experiences, happens as early as 7 to 9 years of age (e.g., Dorn & Biro, 2011) and was not captured in our sample.

476 **Physical Changes and Affect Fluctuations**

477 On the level of physical changes in puberty, we predicted that within-person changes in
478 physical puberty would be related to higher affect fluctuations, especially in beginning stages of
479 physical puberty. As predicted, an increase in the level of physical puberty across the study period
480 was associated with higher affect fluctuations only for individuals who had not or who had just
481 begun showing signs of physical puberty. We had predicted that within-person changes in
482 beginning stages of physical puberty show stronger associations with affect fluctuations because
483 beginning stages have been associated with greater hormonal changes (e.g., Ankarberg-Lindgren
484 & Norjavaara, 2004; Khairullah et al., 2014). Therefore, when statistically controlling for
485 testosterone changes, physical changes in puberty should predict affect fluctuations similarly for
486 beginning and advanced stages of puberty. Unlike expected, the interaction effect remained stable
487 when controlling for testosterone changes.

488 This finding might partly be due to the operationalization of physical puberty as self-rated
489 and thus perceived physical puberty rather than physician-rated physical puberty. Self-reported
490 physical puberty might be more closely related to difficulties in coping with the perceived
491 physical changes associated with puberty than physician-rated physical puberty is. Such
492 difficulties have been associated with adolescents' affect and behavior in prior studies (see
493 Mendle, 2014; Mendle & Ferrero, 2012). Changes in beginning stages of puberty (i.e. entering
494 puberty) might be particularly demanding for adolescents and their social context because these
495 changes are likely to initiate deviations from former behavior structures (e.g., Eccles, Templeton,
496 Barber, & Stone, 2003). Adapting to physical changes in more advanced puberty might be easier,
497 in comparison, because the experience of previously accomplished adaptation to preceding
498 changes could make adolescents and their social surroundings more flexible. We therefore
499 controlled for adolescents' perceived stress, which included situations reflecting difficulties in

adjusting to physical changes. We found that more perceived stress was associated with higher affect fluctuations. Again, this was the case in addition to the interaction effect, reflecting that physical changes in beginning stages of puberty predicted affect fluctuations regardless of perceived life stress. Therefore, additional processes, as for example less effective emotion regulation skills (e.g., Silvers et al., 2012), might have contributed to elevated affect fluctuations in this group. Overall, our results emphasize the unique contribution of changes in self-reported physical puberty and testosterone changes to higher affect fluctuations in adolescent boys.

Differentiating Effects of Pubertal Changes in Boys and Girls

The present study focused on boys and study results cannot be generalized to puberty-affect relations in girls. Pubertal development in girls is accompanied by different hormonal changes. As opposed to boys, testosterone concentrations in girls increase only very little across puberty. Instead, estrogen concentrations (i.e., girls' primary sex-steroid) show largest increases in girls' puberty. However, whereas testosterone concentrations increase 10-fold across puberty in boys, estrogen concentrations only increase two-fold across puberty in girls, and a monthly cycle develops (e.g., Walker et al., 2004).

Despite these differences, similar effects of sex-steroids on affective experiences in boys and girls have been found. Our study found associations between boys' testosterone changes and affect fluctuations. Prior studies with adolescent girls have confirmed associations between estrogen levels and affect fluctuations (see Balzer et al., 2015). In fact, similar effects of testosterone and estrogen in puberty are plausible because both androgen (i.e. testosterone, DHEA) and estrogen receptors are especially dense in brain regions associated with affective processing (e.g., Brouwer et al., 2015; Van Wingen et al., 2011). In addition, models integrating animal and human data demonstrated that the mechanisms on how estrogen and testosterone influence these receptors are overlapping (e.g., Celec et al., 2015; Sotomayor-Zárate, Cruz,

Renard, Espinosa, & Ramírez, 2014) and testosterone can convert to estrogen in the brain, especially in the amygdala, which is involved in affective processing (for further reading on estrogen syntheses stemming from human and animal studies, see Roselli, Liu, & Hurn, 2009). In fact, both testosterone in boys and estrogen in girls were associated with structural growth (e.g., Goddings et al., 2014; Herting et al., 2014) and increased functional activity (e.g., Spielberg et al., 2014) of brain regions associated with affective processing (e.g., the amygdala), likely resulting in increased affective responding (e.g., Blakemore et al., 2010; Smith et al., 2002). Additionally, testosterone and estrogen effects are not necessarily limited to one gender: Higher testosterone changes in both adolescent boys and girls were associated with greater amygdala activity for emotional stimuli (Spielberg et al., 2014).

Nevertheless, boys' and girls' primary sex-steroids might result in differential effects on adolescents' affect and behavior. Although boys' and girls' sex steroids are associated with amygdala growth (e.g., Goddings et al., 2014; Herting et al., 2014) and increased amygdala reactivity (e.g., Spielberg et al., 2014), girls' sex-steroids increase its coupling with prefrontal brain regions, likely promoting rumination, whereas boys' sex steroids decrease coupling, likely promoting impulsivity (Van Wingen et al., 2011). Further studies on how sex-steroids predict affect and behavior are warranted and may provide intriguing insight into the emergence of sex-differences in psychopathology that are observed with pubertal development (e.g., Kessler et al., 2005).

Limitations and Outlook

The present study was designed to specifically investigate pubertal development as a within-person process in adolescent boys. The operationalization of pubertal changes based on two study parts, demands careful interpretation. Most prior research investigated pubertal change as the time between pubertal onset and offset (e.g., Pantsiotou et al., 2008), masking possible

548 non-linear effects within pubertal development. By studying 8-months longitudinal excerpts in
549 the pubertal process, the present study followed the trend to investigate developmental processes
550 in smaller intervals (Marceau et al., 2015; Mendle et al., 2010). However, the present study
551 cannot provide insight into the process of pubertal changes during the period of eight months. A
552 challenging task for future studies would be to investigate pubertal change processes at more
553 frequent intervals over a longer period. Such research would be fundamental to confirm the
554 interpretation of the current study, namely that within adolescents, periods with greater amounts
555 of pubertal changes are associated with higher affect fluctuations than periods with lower amount
556 of pubertal changes.

557 Additional study limitations pertain to our assessment of physical and hormonal
558 development in puberty. We used self-report instruments to obtain individuals' physical puberty
559 because explanatory models on the role of pubertal changes in affective experiences have
560 highlighted the role of how adolescents perceive their physical changes (Mendle et al., 2010).
561 Future studies might consider also including physicians' reports on pubertal development to
562 investigate whether a more objective measure of pubertal changes or self-reported and thus
563 perceived pubertal changes altered stability in adolescents' affective experiences (Dorn & Biro,
564 2011). On the level of hormone assessments, the present study also faces limitations. First,
565 testosterone and DHEA do not act in isolation and future studies might want to assess further
566 hormones and neurotransmitters. Endocrine development in puberty comprises a complex
567 interplay of different hormones inhibiting or stimulating the release of other hormones (e.g.,
568 Ellison et al., 2012). The complex properties with which hormonal changes are thought to affect
569 brain and behavior are poorly understood (see Celec et al., 2015; Peper & Dahl, 2013 for an
570 integration of animal and human data). Besides stimulating and organizational effects via sex-
571 steroid receptors in brain areas associated with emotional processing, there is an increasing

number of animal and human studies investigating the role of neurotransmitters (e.g., serotonin, dopamine) as agents in hormone-affect relations (e.g., Sotomayor-Zárate et al., 2014). Second, hormone concentrations are also influenced by factors other than puberty. In the present study, we controlled for the largest effects on hormone concentrations (e.g., time of day, blood contamination, food intake, social situations, perceived stress, etc.). Other factors, such as possible seasonal effects, might alter hormone concentrations as well. However, evidence for seasonal effects on testosterone are weak and inconsistent (for review, see Smith, Coward, Kovac, & Lipshultz, 2013). Further studies examining the interplay between different hormones and such context effects in adolescents' maturing brains are needed to better understand the mechanisms of hormone-affect relations.

It would have been desirable to have an additional measure of affect fluctuations at the first study part to control for participants' baseline affect. Our conclusions on puberty-affect associations are therefore limited to between-person comparisons of within-person pubertal changes. Further longitudinal studies are necessary to address the question on whether the reported increase in testosterone concentrations is associated with a within-person increase in affect fluctuations.

Conclusion

The investigation of intraindividual change processes is important to understand the heterogeneity of affective experiences in adolescence. Using a within-person approach to study puberty-affect relations, the present study showed that pubertal changes predicted individual differences in boys' daily affect fluctuations. This effect is present on the level of both hormonal and physical development in puberty: Boys with higher changes in testosterone concentrations showed elevated affect fluctuations in daily life. In addition to testosterone changes, physical changes in beginning stages of puberty (e.g., individuals entering puberty) were related to

596 elevated affect fluctuations. Thus, both hormonal and physical changes are relevant for
597 understanding adolescents' mood swings in daily life, especially when adolescents enter puberty.
598

Appendix A

Computation of the Area under the Curve

The formula for the area under the curve (AUC) is based on the trapezoid formula (Cohen, Lee, & Sklar, 2010) and is calculated with the information of the hormone concentrations at the particular measurement occasions and the time distance between measurement occasions. AUC can be used with any number of repetitions and is independent of the total number of measurements (e.g., Pruessner et al., 2003). With two measurement occasions m at time distance t *(in hours)*, the formula for the AUC_g reduces to $AUG_g = [(m_{evening} + m_{morning}) \times t_{(evening–morning)}]/2$.

Appendix B

Application of GLLA to Unequally-Spaced Data

The sampling schedule of the present study has several unique characteristics that need to be taken into account before applying GLLA to calculate derivative estimates of affect ratings: (1) Same day measurement occasions occurred pseudo-randomly in six two-hour time frames with an average time of two hours between two same-day assessment occasions (*SD* = 0.50; *MIN* = 0.78; *MAX* = 3.22), and (2) the interval between the last assessment occasion of one day and the first assessment of the following measurement day varied between 12.77 and 23.87 hours. This complex sampling schedule with randomly occurring within- and between-day intervals resulted in an unevenly spaced data structure. With an irregular sampling interval, the loading matrix *L* specifies time incorrectly because the coefficients assume equal intervals between measurement occasions. However, given that the random sampling interval is drawn from a symmetric distribution, the sampling intervals will be of equal length, on average.

(Add 1) Results from simulation studies have shown that in symmetric distributions, where time is only slightly misspecified, derivative estimates are essentially equivalent to derivative estimates derived from equally spaced data (see Boker et al., in press; Tiberio, 2008 on the issue of unequally spaced data in GLLA). Within-person Shapiro-Wilk tests indicated that individuals' sampling schedules in the present study followed a symmetric normal distribution when assessment intervals that did not occur within the same day (intervals between the last and the first measurement of consecutive measurement days) were dismissed.

(Add 2) No derivatives were calculated for moving sequences that included intervals between measurements that spanned across days and hence violated an assumption of only slightly misspecified time intervals. The construction of matrix X^D from our time series $X_{p.q,t}$ with

$t = 1,...,6$ observations on each assessment day $q = 1,...,A$ for each individual $p = 1,...,N$ is as follows.

$$X^{(3)} = \begin{pmatrix} X_{(1.1,1)} & X_{(1.1,2)} & X_{(1.1,3)} \\ X_{(1.1,2)} & X_{(1.1,3)} & X_{(1.1,4)} \\ X_{(1.1,3)} & X_{(1.1,4)} & X_{(1.1,5)} \\ X_{(1.1,4)} & X_{(1.1,5)} & X_{(1.1,6)} \\ X_{(1.2,1)} & X_{(1.2,2)} & X_{(1.2,3)} \\ X_{(1.2,2)} & X_{(1.2,3)} & X_{(1.2,4)} \\ . & . & . \\ . & . & . \\ X_{(N.1,1)} & X_{(N.1,2)} & X_{(N.1,3)} \\ X_{(N.1,2} & X_{(N.1,3)} & X_{(N.1,4)} \\ . & . & . \\ . & . & . \\ X_{(N.A,4)} & X_{(N.A,5)} & X_{(N.A,6)} \end{pmatrix}$$

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645

References

Alonso, L. C., & Rosenfield, R. L. (2002). Oestrogens and puberty. *Best Practice and Research: Clinical Endocrinology and Metabolism, 16*, 13-30. doi: 10.1053/beem.2002.0177

Amin, Z. (2006). *Effects of hormonal variation on neural correlates of cognitive-affective processing.* Amin, Zenab: State U New York At Stony Brook, US. Available from Ovid Technologies PsycINFO database.

Angold, A., Costello, E. J., Erkanli, A., & Worthman, C. (1999). Pubertal changes in hormone levels and depression in girls. *Psychological Medicine, 29*, 1043-1053.

Ankarberg-Lindgren, C., & Norjavaara, E. (2004). Changes of diurnal rhythm and levels of total and free testosterone secretion from pre to late puberty in boys: testis size of 3 ml is a transition stage to puberty. *European Journal of Endocrinology, 151*, 747-757. doi: 10.1530/eje.0.1510747

Balzer, B. W. R., Duke, S. A., Hawke, C. I., & Steinbeck, K. S. (2015). The effects of estradiol on mood and behavior in human female adolescents: A systematic review. *European Journal of Pediatrics, 174*, 289-298. doi: 10.1007/s00431-014-2475-3

Bauer, D. J., & Curran, P. J. (2005). Probing interactions in fixed and multilevel regression: Inferential and graphical techniques. *Multivariate Behavioral Research, 40*, 373-400. doi: 10.1207/s15327906mbr4003_5

Beltz, A. M., Corley, R. P., Bricker, J. B., Wadsworth, S. J., & Berenbaum, S. A. (2014). Modelinig pubertal timing and tempo and examininig links to behavior problems. *Developmental Psychology*, 50, 2715-2726. doi: 10.1037/a0038096

Berenbaum, S. A., Beltz, A. M., & Corley, R. (2015). The importance of puberty for adolescent development: conceptualization and measurement. *Advances in Child Development and Behavior, 48*, 53-92. doi: 10.1016/bs.acdb.2014.11.002

Blakemore, S. J., Burnett, S., & Dahl, R. E. (2010). The role of puberty in the developing adolescent brain. *Human Brain Mapping, 31*, 926-933. doi: 10.1002/hbm.21052

Boker, S. M., Deboeck, P. R., Edler, C., & Keel, P. K. (2010). Generalized local linear approximation of derivatives from time series. In S.-M. Chow & E. Ferrar (Eds.), *Statistical Methods for Modeling Human Dynamics: An Interdisciplinary Dialogue* (pp. 161-178). Boca Raton, FL: Taylor & Francis.

Boker, S. M., Tiberio, S. S.,& Moulder, R. G. (in press). Robustness of time delay embedding to sampling interval misspecification. In K. v. Montfort, J. Oud, & M. Voelkle (Eds.), *Continuous Time Modeling in the Behavioral and Related Sciences.* Berlin: Springer.

Bowen, R., Baetz, M., Hawkes, J., & Bowen, A. (2006). Mood variability in anxiety disorders. *Journal of Affective Disorders, 91*, 165-170. doi: 10.1016/j.jad.2005.12.050

Brouwer, R. M., Koenis, M. M. G., Schnack, H. G., van Baal, G. C., van Soelen, I. L. C., Boomsma, D. I., & Hulshoff Pol, H. E. (2015). Longitudinal development of hormone levels and grey matter density in 9 and 12-year-old twins. *Behavior Genetics, 45*, 313-323. doi: 10.1007/s10519-015-9708-8

Buchanan, C. M., Eccles, J. S., & Becker, J. B. (1992). Are adolescents the victims of raging hormones: Evidence for activational effects of hormones on moods and behavior at adolescence. *Psychological Bulletin, 111*, 62-107. doi: 10.1037/0033-2909.111.1.62

Byrne, D. G., Davenport, S. C., & Mazanov, J. (2007). Profiles of adolescent stress: The development of the adolescent stress questionnaire (ASQ). *Journal of Adolescence, 30*, 393-416. doi: 10.1016/j.adolescence.2006.04.004

Celec, P., Ostatníková, D., & Hodosy, J. (2015). On the effects of testosterone on brain behavioral functions. *Frontiers in Neuroscience, 9*, 1-17. doi: 10.3389/fnins.2015.00012

693 Cohen, D., Lee, T. B., & Sklar, D. (2010). *Precalculus: a problems-oriented approach* (6th ed.).
694 Belmont, CA: Brooks/Cole.
695 Deboeck, P. R., Montpetit, M. A., Bergeman, C. S., & Boker, S. M. (2009). Using derivative
696 estimates to describe intraindividual variability at multiple time scales. *Psychological*
697 *Methods, 14*, 367-386. doi: 10.1037/a0016622
698 Dorn, L. D., & Biro, F. M. (2011). Puberty and its measurement: A decade in review. *Journal of*
699 *Research on Adolescence, 21*, 180-195. doi: 10.1111/j.1532-7795.2010.00722.x
700 Duke, S. A., Balzer, B. W. R., & Steinbeck, K. S. (2014). Testosterone and its effects on human
701 male adolescent mood and behavior: A systematic review. *Journal of Adolescent Health,*
702 *55*, 315-322. doi: 10.1016/j.jadohealth.2014.05.007
703 Eccles, J. S., Templeton, J., Barber, B., & Stone, M. (2003). Adolescence and emerging
704 adulthood: The critical passage ways to adulthood. In M. H. Bornstein, L. Davidson, C. L.
705 M. Keyess & K. A. Moore (Eds.), *Well-being. Positive development across the life course*
706 (pp. 383-406). New Jersey: Lawrence Erlbaum Associates.
707 Eid, M., & Diener, E. (1999). Intraindividual variability in affect: Reliability, validity, and
708 personality correlates. *Journal of Personality and Social Psychology, 76*, 662-676. doi:
709 10.1037/0022-3514.76.4.662
710 Ellison, P. T., Reiches, M. W., Shattuck-Faegre, H., Breakey, A., Konecna, M., Urlacher, S., &
711 Wobber, V. (2012). Puberty as a life history transition. *Annals of Human Biology, 39*,
712 352-360. doi: 10.3109/03014460.2012.693199
713 Ge, X., & Natsuaki, M. N. (2009). In search of explanations for early pubertal timing effects on
714 developmental psychopathology. *Current Directions in Psychological Science, 18*, 327-
715 331. doi: 10.1111/j.1467-8721.2009.01661.x

Goddings, A. L., Mills, K. L., Clasen, L. S., Giedd, J. N., Viner, R. M., & Blakemore, S. J. (2014). The influence of puberty on subcortical brain development. *NeuroImage, 88*, 242-251.

Green, D. P., & Salovey, P. (1999). In what sense are positive and negative affect independent? A reply to Tellegen, Watson, and Clark. *Psychological Science, 10*, 304-306. doi: 10.1111/1467-9280.00158

Gruber, J., Kogan, A., Quioidbach, J., & Mauss, I. B. (2013). Happiness is best kept stable: Positive emotion variability is associated with poorer psychological health. *Emotion, 13*, 1-6. doi: 10.1037/a0030262

Gunnar, M. R., Wewerka, S., Frenn, K., Long, J. D., & Griggs, C. (2009). Developmental changes in hypothalamus-pituitary-adrenal activity over the transition to adolescence: Normative changes and associations with puberty. *Development and Psychopathology, 21*, 69-85. doi: 10.1017/S0954579409000054

Herting , M. M., Gautam, P., Spielberg, J. M., Kan, E., Dahl, R. E., & Sowell, E. R. (2014). The role of testosterone and estradiol in brain volume changes across adolescence: A longitudinal structural MRI study. *Human Brain Mapping, 35*, 5633-5643. doi: 10.1002/hbm.22575

Hoaglin, D. C., & Iglewicz, B. (1987). Fine tuning some resistant rules for outlier labeling. *Journal of the American Statistical Association, 82*, 1147-1149. doi: 10.1080/01621459.1987.10478551

Hunt, D. P. (1999). *Emotion in early puberty: Sex differences in affect patterns across pubertal phases from a developmental neuropsychology perspective*. Unpublished doctoral dissertation thesis. University of Michigan. Ann Arbor, MA.

739 Kessler, R. C., Berglund, P., Demler, O., Jin, R., Merikangas, K. R., & Walters, E. E. (2005).
740 Lifetime prevalence and age-of-onset distributions of DSM-IV disorders in the National
741 Comorbidity Survey Replication. *Archives of General Psychiatry*, 593-602. doi:
742 10.1001/archpsyc.62.6.593
743 Khairullah, A., Cousino Klein, L., Ingle, S. M., May, M. T., Whetzel, C. A., Susman, E. J., &
744 Paus, T. (2014). Testosterone trajectories and reference ranges in a large longitudinal
745 sample of male adolescents. *PLoS ONE, 9*. doi: 10.1371/journal.pone.0108838.g003
746 Klinkman, M. S. (2007). A whole new world: Complexity theory and mood variability in mental
747 disorders. *Primary Care Companion to the Journal of Clinical Psychiatry, 9*, 180-182.
748 doi: 10.4088/PCC.v09n0302
749 Klipker, K., Wrzus, C., Rauers, A., & Riediger, M. (2017). Hedonic orientation moderates the
750 association between cognitive control and affect reactivity to daily hassles in adolescent
751 boys. *Emotion*, 17(3), 497-508. doi: 10.1037/emo0000241
752 Larson, R., Moneta, G., Richards, M. H., & Wilson, S. (2002). Continuity, stability, and change
753 in daily emotional experience across adolescence. *Child Development, 73*, 1151-1165.
754 doi:10.1111/1467-8624.00464
755 Marceau, K., Ram, N., Houts, R. M., Grimm, K. J., & Susman, E. J. (2011). Individual
756 differences in boys' and girls' timing and tempo of puberty: modeling development with
757 nonlinear growth models. *Developmental Psychology*, 47, 1389-1409. doi:
758 10.1037/a0023838
759 Marceau, K., Ram, N., & Susman, E. J. (2015). Development and lability in the parent-child
760 relationship during adolescence: Associations with pubertal timing and tempo. *Journal of*
761 *Research on Adolescence,* 25, 474-489. doi: 10.1111/jora.12139

762 Mendle, J. (2014). Beyond pubertal timing: New directions for studying individual differences in
763 development. *Current Directions in Psychological Science, 23*, 215-219. doi:
764 10.1177/0963721414530144
765 Mendle, J., & Ferrero, J. (2012). Detrimental psychological outcomes associated with pubertal
766 timing in adolescent boys. *Developmental Review, 32*, 49–66. doi:
767 10.1016/j.dr.2011.11.001
768 Mendle, J., Harden, K. P., Brooks-Gunn, J., & Graber, J. A. (2010). Development's tortoise and
769 hare: Pubertal timing, pubertal tempo, and depressive symptoms in boys and girls.
770 *Developmental Psychology, 46*, 1341-1353. doi: 10.1037/a0020205
771 Oldehinkel, A. J., Verhulst, F. C., & Ormel, J. (2011). Mental health problems during puberty:
772 Tanner stage-related differences in specific symptoms. The TRAILS study. *Journal of*
773 *Adolescence, 34*, 73-85. doi: 10.1016/j.adolescence.2010.01.010
774 Pantsiotou, S., Papadimitriou, A., Douros, K., Priftis, K., Nicolaidou, P., & Fretzayas, A. (2008).
775 Maturational tempo differences in relation to the timing of the onset of puberty in girls.
776 *Acta Paediatrica, 97*, 217-220. doi: 10.1111/j.1651-2227.2007.00598.x
777 Peper, J. S., & Dahl, R. E. (2013). The teenage brain: Surging hormones - brain-behavior
778 interactions during puberty. *Current Directions in Psychological Science, 22*, 134-139.
779 doi: 10.1177/0963721412473755
780 Petersen, A. C., Crockett, L., Richards, M., & Boxer, A. (1988). A self-report measure of
781 pubertal status: Reliability, validity, and initial norms. *Journal of Youth and Adolescence,*
782 *17*, 117-133. doi: 10.1007/BF01537962
783 Preacher, K. J., Curran, P. J., & Bauer, D. J. (2006). Computational tools for probing interaction
784 effects in multiple linear regression, multilevel modeling, and latent curve analysis.

Journal of Educational and Behavioral Statistics, 31, 437-448. doi: 10.3102/10769986031004437

Pruessner, J. C., Kirschbaum, C., Meinlschmid, G., & Hellhammer, D. H. (2003). Two formulas for computation of the area under the curve represent measures of total hormone concentration versus time-dependent change. *Psychoneuroendocrinology, 28*, 916-931. doi: 10.1016/S0306-4530(02)00108-7

Roselli, C. E., Liu, M., & Hurn, P. D. (2009). Brain aromatization: Classical roles and new perspectives. *Seminars in Reproductive Endocrinology, 27*, 207-217. doi: 10.1055/s-0029-1216274

Sallquist, J. V., Eisenberg, N., Spinrad, T. L., Reiser, M., Hofer, C., Zhou, Q., . . . Eggum, N. (2009). Positive and negative emotionality: Trajectories across six years and relations with social competence. *Emotion, 9*, 15-28. doi: 10.1037/a0013970

Schultheiss, O. C., & Stanton, S. J. (2009). Assessment of salivary hormones. In E. Harmon-Jones & J. S. Beer (Eds.), *Methods in Social Neuroscience* (pp. 17-44). New York: The Guilford Press.

Schulz, K., Molenda-Figueira, H., & Sisk, C. (2009). Back to the future: The organizational-activational hypothesis adapted to puberty and adolescence. *Hormones and Behavior, 55*, 597-604. doi: 10.1016/j.yhbeh.2009.03.010

Shirtcliff, E. A., Dahl, R. E., & Pollak, S. D. (2009). Pubertal development: Correspondence between hormonal and physical development. *Child Development, 80*, 327-337. doi: 10.1111/j.1467-8624.2009.01263.x

Silk, J. S., Siegle, G. J., Whalen, D. J., Ostapenko, L. J., Ladouceur, C. D., & Dahl, R. E. (2009). Pubertal changes in emotional information processing: Pupillary, behavioral, and

subjective evidence during emotional word identification. *Development and Psychopathology, 21*, 7-26. doi: 10.1017/S0954579409000029

Silvers, J. A., McRae, K., Gabrieli, J. D. E., Gross, J. J., Remy, K. A., & Ochsner, K. N. (2012). Age-related differences in emotional reactivity, regulation, and rejection sensitivity in adolescence. *Emotion, 12*, 1235-1247. doi: 10.1037/a0028297

Smith, M. J., Adams, L. F., Schmidt, P. J., Rubinow, D. R., & Wassermann, E. M. (2002). Effects of ovarian hormones on human cortical excitability. *Annals of Neurology, 51*, 599-603. doi: 10.1002/ana.10180

Smith, R. P., Coward, R. M., Kovac, J. R., & Lipshultz, L. I. (2013). The evidence for seasonal variations of testosterone in men. *Maturitas, 74*, 208-212. doi: j.maturitas.2012.12.003

Sotomayor-Zárate, R., Cruz, G., Renard, G. M., Espinosa, P., & Ramírez, V. D. (2014). Sex hormones and brain dopamine functions. *Central Nervous System Agents in Medicinal Chemistry, 14*, 62-71. doi: 10.2174/18715249146661412261051 37

Spielberg, J. M., Olino, T. M., Forbes, E. E., & Dahl, R. E. (2014). Exciting fear in adolescence: Does pubertal development alter threat processing? *Developmental Cognitive Neuroscience, 8*, 86-95. doi: 10.1016/j.dcn.2014.01.004

Steiner, M., Dunn, E., & Born, L. (2003). Hormones and mood: From menarche to menopause and beyond. *Journal of Affective Disorders, 74*, 67-83. doi: 10.1016/S0165-0327(02)00432-9

Sumter, S. R., Bokhorst, C. L., Miers, A. C., Van Pelt, J., & Westenberg, P. M. (2010). Age and puberty differences in stress responses during a public speaking task: Do adolescents grow more sensitive to social evaluation? *Psychoneuroendocrinology, 35*, 1510-1516. doi: 10.1016/j.psyneuen.2010.05.004

Taylor, S. J. C., Whincup, P. H., Hindmarsh, P. C., Lampe, F., Odoki, K., & Cook, D. G. (2001). Performance of a new pubertal self-assessment questionnaire: A preliminary study. *Paediatric and Perinatal Epidemiology, 15*, 88-94. doi: 10.1046/j.1365-3016.2001.00317.x

Tiberio, S. S. (2008). *The effects of misspecified measurement intervals in multivariate latent differential equation models.* (Unpublished doctoral dissertation thesis), University of Virginia, Charlottesville, VA.

Van Wingen, G. A., Ossewaarde, L., Bäckström, T., Hermans, E. J., & Fernández, G. (2011). Gonadal hormone regulation of the emotion circuitry in humans. *Neuroscience, 191*, 38-45. doi: 10.1016/j.neuroscience.2011.04.042

Walker, E. F., Sabuwalla, Z., & Huot, R. (2004). Pubertal neuromaturation, stress sensitivity, and psychopathology. *Development and Psychopathology, 16*, 807-824. doi: 10.1017/S0954579404040027

Wang, L., Hamaker, E., & Bergeman, C. S. (2012). Investigating inter-individual differences in short-term intra-individual variability. *Psychological Methods, 17*, 567-581. doi: 10.1037/a0029317

Weinstein, S. M., Mermelstein, R. J., Hankin, B. L., Hedeker, D., & Flay, B. R. (2007). Longitudinal patterns of daily affect and global mood during adolescence. *Journal of Research on Adolescence, 17*, 587–600. doi: 10.1111/j.1532-7795.2007.00536.x

Yim, I. S., Quas, J. A., Cahill, L., & Hayakawa, C. M. (2010). Children's and adults' salivary cortisol responses to an identical psychosocial laboratory stressor. *Psychoneuroendocrinology, 35*, 241-248. doi: 10.1016/j.psyneuen.2009.06.014

Zimmermann, P., & Iwanski, A. (2014). Emotion regulation from early adolescence to emerging adulthood and middle adulthood: Age differences, gender differences, and emotion-

855 specific developmental variations. *International Journal of Behavioral Development, 38*,
856 182-194. doi: 10.1177/0165025413515405
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Table 1

Descriptive Statistics of Age, Physical Puberty, and Hormone Concentrations.

	T1 Physical puberty[a]				
	Q1	**Q2**	**Q3**	**Q4**	**Q5**
	M (SD)	*M (SD)*	*M (SD)*	*M (SD)*	*M (SD)*
T1 age (in years)	11.55 (0.90)	13.12 (1.03)	15.06 (1.48)	16.97 (1.46)	17.85 (1.34)
T2 age (in years)	12.22 (0.90)	13.81 (1.04)	15.72 (1.46)	17.63 (1.44)	18.50 (1.33)
Physical puberty[b]					
T1 *z*-score	-1.53 (0.26)	-0.64 (0.33)	0.06 (0.16)	0.55 (0.17)	1.09 (0.17)
T2 *z*-score	-1.20 (0.41)	-0.10 (0.38)	0.53 (0.36)	0.86 (0.28)	1.19 (0.17)
T1 Tanner score	1.65 (0.42)	2.74 (0.43)	3.78 (0.28)	4.23 (0.41)	4.82 (0.24)
T2 Tanner score	2.04 (0.58)	3.57 (0.48)	4.39 (0.48)	4.65 (0.38)	4.93 (0.18)
T1 PDS score	1.22 (0.23)	2.01 (0.43)	2.51 (0.26)	3.06 (0.37)	3.60 (0.33)
T2 PDS score	1.52 (0.38)	2.38 (0.45)	2.91 (0.38)	3.32 (0.45)	3.71 (0.28)
Hormone concentrations[c]					
T1 testosterone	323.25 (183.00)	770.19 (558.43)	1528.42 (528.15)	1813.14 (616.76)	2032.22 (877.24)
T2 testosterone	510.52 (352.58)	1293.53 (747.14)	1755.26 (760.30)	2078.78 (1189.66)	2218.44 (1093.60)
T1 DHEA	2561.68 (1468.32)	4084.37 (2168.67)	5356.99 (2211.18)	6078.25 (2944.79)	7146.67 (2602.48)
T2 DHEA	2968.33 (1837.38)	4568.33 (2894.16)	6415.89 (2353.66)	6417.62 (3313.98)	6974.33 (2479.66)

[a] Puberty groups based on quantiles of the combined puberty *z*-score from verbal and picture ratings of physical puberty at time 1 (T1) with Q1 to Q5 referring to quantiles 1 through 5 respectively.

[b] Physical puberty is based on the combined puberty *z*-score from verbal and picture ratings of physical puberty at time 1 (T1) and time 2 (T2) respectively.

[c] Hormone concentration levels are depicted as the mean area under the curve with respect to the ground (AUC_g) for time 1 (T1) and time 2 (T2) respectively.

Table 2

Zero-Order-Correlations of Age, Physical and Hormonal Characteristics in Puberty, and Affect Fluctuations.

	Pubertal status			**Pubertal change** [c]			**Affect fluctu-ations**
	Physical puberty[a]	Testos-terone[b]	DHEA[b]	Physical signs	Testos-terone	DHEA	
Age (in years)	.88*	.72*	.61*	- .20*	- .05	- .12	- .12
Pubertal status							
Physical puberty[a]	-	.75*	.58*	- .26*	- .04	- .07	- .08
Testosterone[b]	-	-	.59*	- .13	- .19*	- .06	- .08
DHEA[b]	-	-	-	- .21*	- .14	- .38*	- .08
Pubertal change							
Physical puberty	-	-	-	-	. 09	.17	.04
Testosterone	-	-	-	-	-	.13	.18*
DHEA	-	-	-	-	-	-	.09

[a] Combined puberty *z*-score from the Tanner and Pubertal Development Scale at T1.

[b] Testosterone and DHEA concentrations as the mean area under the curve with respect to the ground (AUC_g) at T1.

[c] Pubertal change in physical puberty [a], testosterone[b] and DHEA[b] as the within-person change from T1 to T2.

[d] Affect fluctuations calculated as the within-person mean of the absolute value of within-day derivative estimates of affect-balance ratings during the experience-sampling phase at T2.

* $p < .05$.

Table 3

Predicting Affect Fluctuations (Dependent Variable) From Within-Person Changes in Testosterone: Results From Multivariate Regression Analysis

	Affect fluctuations	
	Model 1	*Model 2*
Model Parameters	β	β
Testosterone change [a]	0.23 *	0.26 *
Covariates		
Timespan [b]	0.01	-0.06
Testosterone (T1) [c]		0.03
Perceived stress (T2)		0.26 *
Interaction effect		
Testosterone change × timespan	- 0.18 †	- 0.19 *
Adjusted R^2	4.0 %	10.4%
Model fit	$F(3,130)$ = 2.83 *	$F(5,126)$ = 4.04 *

Note. Standardized regression coefficients (β) are reported.

[a] The difference between the mean area under the curve (AUC_g) from T1 and T2 was used as indicator of testosterone change.

[b] Time elapsed between T1 and T2.

[c] Testosterone concentrations as the mean area under the curve with respect to the ground (AUC_g) at T1.

* $p < .05$; † $p = .05$

Table 4

Predicting Affect Fluctuations (Dependent Variable) From Within-Person Changes in Physical Signs of Puberty: Results From Multivariate Regression Analyses

	Affect fluctuations		
	Model 1	*Model 2*	*Model 3*
Model Parameter	β	β	β
Physical puberty change [a]	-0.06	-0.06	-0.07
Covariates			
Physical puberty (T1) [b]	-0.16	-0.17	-0.15
Timespan [c]		0.01	-0.05
Perceived stress (T2)			0.25 *
Testosterone change [d]			0.20 *
Interaction effect			
Physical puberty change × Physical puberty (T1)	-0.24 *	-0.27 *	-0.19 *
Physical puberty change × timespan		-0.08	
Adjusted R^2	3.8%	2.9%	9.6%
Model fit	$F(3,141) = 2.87$ *	$F(5,139) = 1.87$	$F(6,124) = 3.30$ *

Note. Standardized regression coefficients (β) are reported.

[a] The difference between puberty *z*-scores from T1 and T2 was used as indicator of physical puberty change.

[b] Combined puberty *z*-score from the Tanner and Pubertal Development Scale at T1.

[c] Time elapsed between T1 and T2.

[d] The difference between the mean area under the curve (AUC_g) from T1 and T2 was used as indicator of testosterone change.

* $p < .05$.

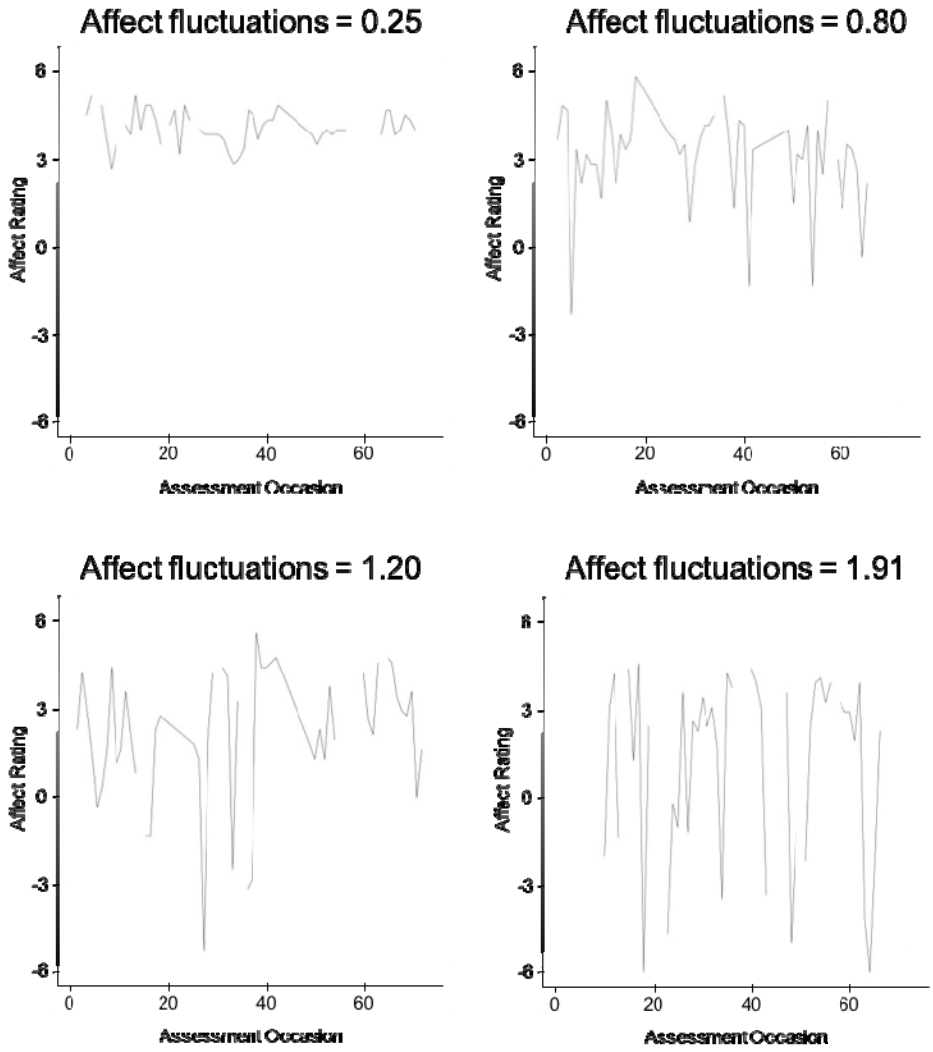

Figure 1. Exemplary time-series of four participants' affect ratings with the corresponding first-order derivative estimate (i.e. the within-person mean of the absolute value of within-day derivative estimates), characterizing participants' affect fluctuations.

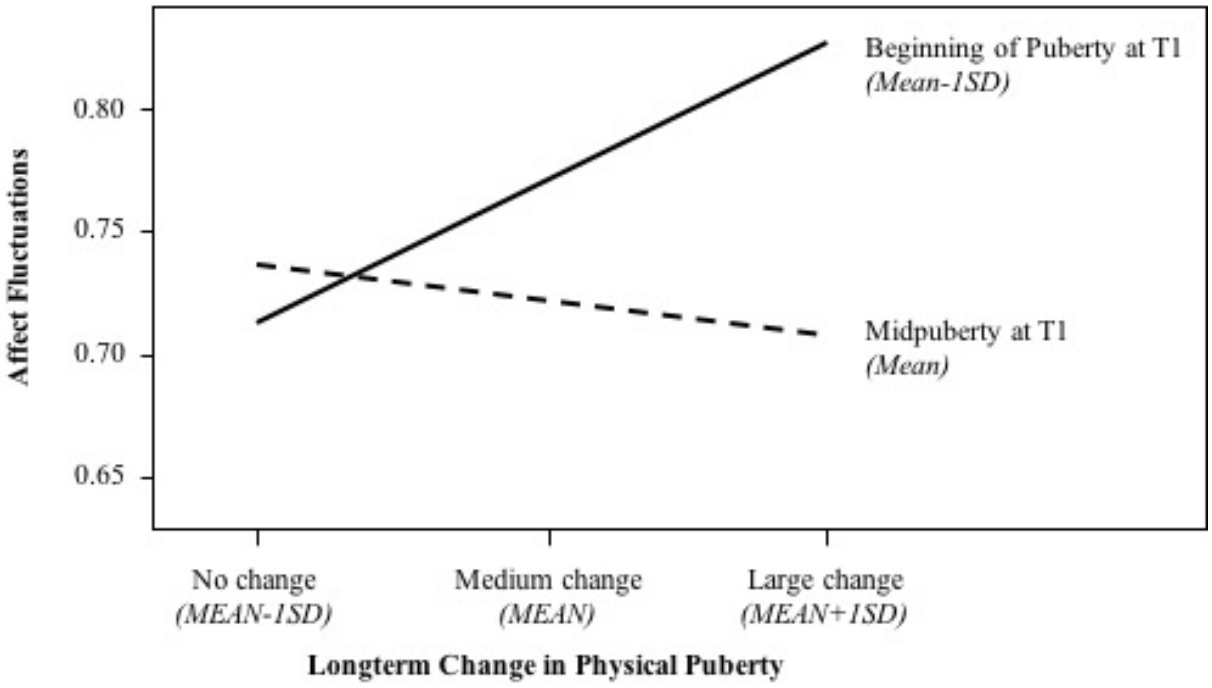

Figure 2. Model-predicted associations between within-person changes in physical puberty (x-axis) and affect fluctuations at T2 (y-axis) for beginning and middle physical puberty at T1. No model-predicted associations were plotted for one standard deviation above the average level of physical puberty at T1 (i.e., advanced to post-puberty) because for individuals in advanced or post-puberty medium to high physical changes in puberty did not occur. *SD* = standard deviation.