Three-month cumulative exposure to testosterone and cortisol predicts distinct effects on response inhibition and risky decision-making in adolescents

Supplemental Material

Supplemental Method

Participants. Participants were recruited through local schools, community flyers, and Listservs. We only invited adolescents who did not use or take any psychoactive drugs (e.g., antidepressants, ADHD medication). Exclusion criteria were use of psychoactive medications and drugs or an age below 12 years or of/above 16 years. The sample was fairly diverse in terms of race/ethnicity: 61.8% Non-Hispanic White, 10.9% African American, 9.1% Hispanic/Latino, 5.5% Asian/Asian American, and 12.7% Mixed or Other. Our sample size of 55 participants provided 73% power to detect a moderate correlation (i.e., r = .30) in an expected direction and 99% power to detect a strong correlation (i.e., r = .50) in an expected direction.

Cognitive tasks. The following describes the parameters and procedures used for each cognitive task.

Response inhibition. Response inhibition was assessed using the stop-signal task (Verbruggen and Logan, 2009). During each trial, participants were presented with an arrow ("<" or ">") encompassed by a white circle. Participants were instructed to indicate the direction of the arrow (i.e., "go" response) with a button press unless the circle encompassing the arrow turned red (i.e., "stop" trials), in which case they had to withhold the button press. Go trials lasted for 750ms. On stop trials, arrows were presented for a determined amount of time (i.e., the stop-signal delay [SSD]; bounded at 50 to 350ms) within a white circle before the enclosing circle turned red, after which time the arrow remained on the screen for the duration of the 750ms trial. Between each trial was a random intertrial interval drawn from a gamma distribution centered at 1000ms. The SSD was initially set to 150ms and was subject to a standard staircase procedure, with successful stop trials increasing the SSD by 50ms and failed stop trials decreasing the SSD by 50ms. The task consisted of 186 trials, two-thirds of which were go trials and one-third stop trials. The primary index of response inhibition in this task is the stop-signal reaction time

(SSRT), which quantifies the time a participant requires to inhibit an activated response. We calculated SSRT using the recommended integration method (Verbruggen et al., 2013); greater SSRTs indicate poorer response inhibition.

Risky decision-making. Risky decision-making was assessed using a driving simulation known as the yellow light game (YLG), which is a basic decision-making task masked as a driving game (Op de Macks et al., 2018). The YLG has been used to appropriately and successfully assess risk taking in other adolescent samples (Op de Macks et al., 2018; Rogers et al., 2018; van Hoorn et al., 2018). In the YLG, participants complete a virtual driving course and are instructed to complete it as quickly as possible. At each intersection, participants must choose to either stop or go; decisions must be made quickly and failing to make a decision results in a penalty. A go decision is fastest if successful; however, if another car crosses the intersection (which occurs on 50% of the trials, although this is not made explicit to participants), a go decision results in a crash, causing a 5s delay and results in feedback of a honking car, crash sound, and broken windshield. A stop decision causes a 2.5s delay regardless of whether another car crosses the intersection. Participants were informed of the time delays resulting from the possible decisions. In this task, therefore, "go" decisions at intersections represent risky decision-making; greater "go" decisions indicate more risky decision-making. Participants completed 2 rounds of the YLG, each of which included 20 intersections. Participants made "stop" decisions on 43% of trials on average per round. In our sample, none of the participants were old enough to have driven before.

Hair sampling and assays. Three hair segments approximately 3mm in diameter were taken from the posterior vertex position of the scalp. Hormone assays were done by Dresden LabService GmbH (Germany) following procedures described previously (Gao et al., 2013). Based upon an average hair growth rate of 1cm/month, hormone concentrations in hair segments were assumed to represent 3-month hormone concentrations (Gao et al., 2013). Assays were performed using liquid chromatography-tandem mass spectrometry (LCMS). Inter- and intra-assay coefficients of variation were less than 12% for cortisol, and less than 15% for testosterone.

Data analysis. Data were analyzed in R, version 3.5.1. As mentioned in the main text, some participants did not have enough hair to provide the standard sample, so all hormone analyses (except for those stated as using the alternative adjustment for unequal hair length) controlled for hair length; correlations with hormones given are therefore partial correlations controlling for hair length. All primary analyses were regressions. Moderated regression analyses examined interactions between hormones, and between sex and each hormone, in predicting outcomes. In some analyses, we controlled for the other hormone in the model (stated within the Results section for the corresponding models). This was done to examine whether an association between a hormone and the outcome is not due to a general pattern of greater or lower hormone secretion, and that the associations hold even when controlling for the potential influence of the other hormone on the outcome. We examined all variables for outliers, and found that after log transformation there were no outliers (i.e., values > 3 SDs ± the mean) in either of the hormones.

Supplemental Results

We conducted a series of analyses to better explore our data after conducting our *a priori* analyses. The results of these analyses are presented below.

Effects of Sex on Cognitive Task Performance

We also examined whether male and female adolescents differed in cognitive task performance. We found no sex differences in either stop-signal reaction time (M_{male} = 343.32, SE_{male} = 9.36; M_{female} = 336.80, SE_{female} = 7.85), $t(53) = 0.54$, $p = .593$, $d = 0.15$, or "go" decisions (M_{male} = 10.65, SE_{male} = 0.43; M_{female} = 9.64, SE_{female} = 0.69), $t(53) = 1.22$, $p = .227$, $d = 0.33$. It should be noted, though, that these nonsignificant effects may have been due to a lack of power; the effect size for sex differences in "go" decisions was small-to-moderate, and boys made approximately 1 more risky decision than girls (i.e., 5% more risky decisions per round of the YLG).

Examining Associations with Age

Age was not associated with any of the primary variables of interest in this study. In particular, age was not associated with hair testosterone, $r = -.028$, $p = .858$, hair cortisol, $r = .009$, $p = .953$, stop-signal reaction time, $r = -.085$, $p = .539$, or "go" decisions in the yellow light game, $r = .117$, $p = .395$.

Age was also unassociated with hair testosterone and hair cortisol using the alternative adjustment for unequal hair length across participants (described below); in these analyses, age was not associated with hair testosterone, r = -.033, p = .829, or hair cortisol, r = .005, p = .977.

Alternative Adjustment for Unequal Hair Length

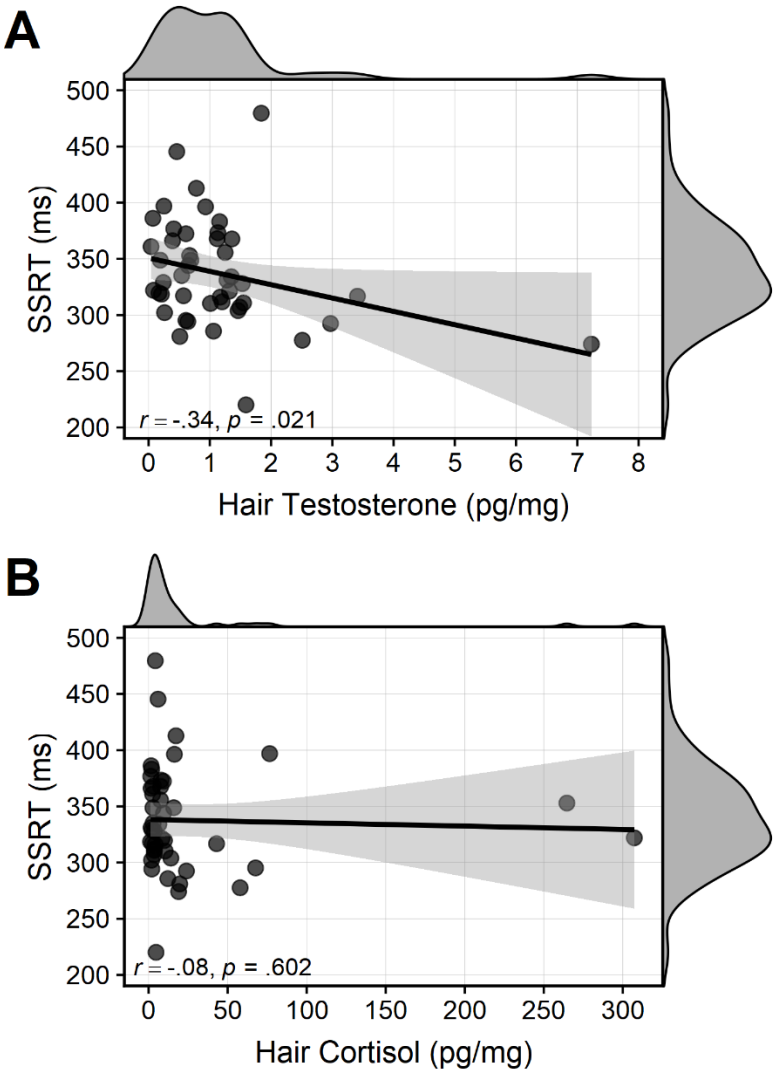
Because 7 out of 55 participants (12.7%) did not have long enough hair for a full 3cm sample, all of our primary analyses controlled for hair length in the interest of retaining the largest sample possible. However, an alternative approach to adjusting for unequal hair length between participants is to multiply each participant's observed hormone values by the ratio of the standard sample length in centimeters (i.e., 3 cm) to their hair sample in centimeters, such that a participant with a 2 cm sample would have their hormone values multiplied by 3/2, and a participant with a 3 cm sample would have their hormone values multiplied by 3/3 (i.e., 1). This approach therefore provides an estimate of a participant's 3 cm (i.e., three-month) hormone concentration. We present the analyses using this approach below; in brief, all results were virtually identical to those obtained in our primary analyses. Note that these models do not control for hair length.

As in our primary analyses, we found that hair testosterone significantly predicted inhibitory control, such that *greater* hair testosterone predicted *lower* stop signal reaction time (i.e., better response inhibition), β=-.334, p=.036, and hair testosterone remained a significant predictor of better inhibitory control when controlling for hair cortisol, β=-.323, p=.043. In contrast to hair testosterone, hair cortisol was unassociated with inhibitory control, p=.296. Hair cortisol and hair testosterone did not interact to predict inhibitory control, p=.399. As in our primary analyses, hair testosterone was unassociated with risky decision-making, p=.420. However, hair cortisol predicted fewer risky decisions, β=-.366, p=.016, and hair cortisol remained a significant predictor of less risky decision-making when controlling for hair testosterone, β=-.377, p=.013. Hair cortisol and hair testosterone did not interact to predict risky decision-making, p=.535.

Using Untransformed Variables, and Models without Covariates

Because hair hormone values were skewed, and because age and sex can dramatically affect hormone values, we log transformed hair hormone values and controlled for age and sex in all of our primary analyses. In the interest of full data transparency, however, we present analyses including the raw hair hormone values—both with and without controlling for age and sex—as well as analyses including log-transformed hair hormone values without controlling for age and sex here in this supplemental material. Because some participants were unable to provide the full 3cm hair sample for analysis, we controlled for hair sample length in all the following analyses.

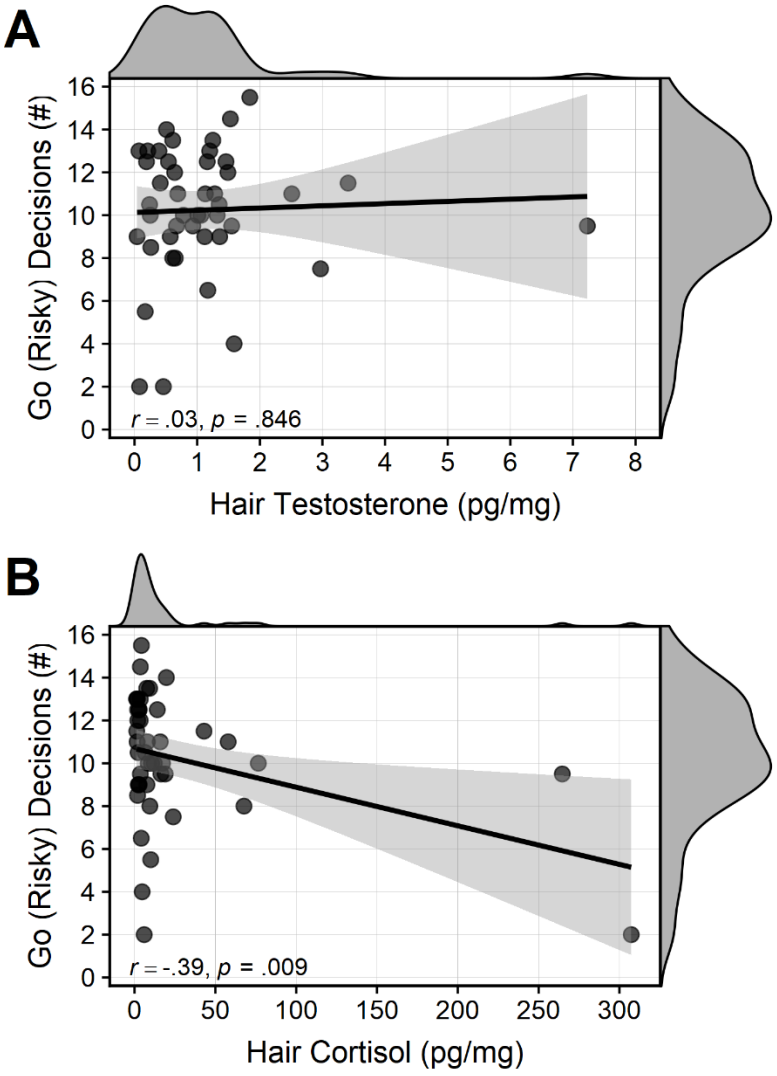
Like the results presented in the main text, greater hair testosterone (untransformed) was associated with smaller stop signal reaction times (i.e., better response inhibition), β=-.343, *p*=.022 (Supplemental Figure 1a), whereas hair cortisol (untransformed) was not, β=-.080, *p*=.606 (Supplemental Figure 1b). Controlling for age and sex did not alter these results; controlling for age and sex, greater hair testosterone (untransformed) remained a significant predictor of smaller stop signal reaction times, β=-.376, *p*=.019, and hair cortisol (untransformed) remained unassociated with stop signal reaction times, β=-.080, *p*=.634. Log transforming the hair hormone values (not including age and sex as covariates; see the main text for those results) did not alter these results; greater log-transformed hair testosterone remained a significant predictor of smaller stop signal reaction times, β=-.319, *p*=.039, and log-transformed hair cortisol remained unassociated with stop signal reaction times, β=-.194, *p*=.224.



Supplemental Figure 1. Untransformed hair testosterone was significantly associated with better response inhibition (smaller stop signal reaction times; panel A), whereas untransformed hair cortisol was not associated with response inhibition (panel B).

Again like the results presented in the main text, hair testosterone (untransformed) was not associated with risky decision-making (i.e., "Go" decisions in the Yellow Light Game), β=.030, *p*=.848 (Supplemental Figure 2a), whereas hair cortisol (untransformed) was a significant predictor of less risky decision-making, β=-.394, *p*=.009 (Supplemental Figure 2b). Controlling for age and sex did not alter these results; controlling for age and sex, hair testosterone remained unassociated with risky decision-making, β=.014, *p*=.933, and hair cortisol (untransformed) remained a significant predictor of less risky

decision-making, β=-.399, *p*=.011. Log transforming the hair hormone values (not including age and sex as covariates; see the main text for those results) did not alter these results; greater log-transformed hair testosterone remained unassociated with risky decision-making, β=.151, *p*=.347, and log-transformed hair cortisol remained a significant predictor of less risky decision-making, β=-.395, *p*=.012.



Supplemental Figure 2. Untransformed hair testosterone was not associated with risky decision-making ("Go" decisions in the Yellow Light Game; panel A), whereas untransformed hair cortisol was a significant predictor of less risky decision-making (panel B).

In brief, results of analyses using untransformed variables and results of analyses without including covariates were all in agreement with the results presented in the main text.

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