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Developmental Pathways from Genetic, Prenatal, Parenting and Emotional/Behavioral Risk to Cortisol Reactivity and Adolescent Substance Use: A TRAILS Study

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Abstract

Cortisol reactivity is a frequently studied biomarker of substance use, though infrequently examined in adolescence. However, past research provides evidence that multiple developmental influences, including genetics and both prenatal and postnatal environmental influences, contribute both to cortisol reactivity and adolescent substance use. The aim of this study was to assess the impact of these earlier developmental influences on cortisol reactivity to a social stress challenge and adolescent substance use (smoking, alcohol, and marijuana use frequency assessed at age 16 years), using data from the TRacking Adolescents' Individual Lives Survey (TRAILS; $N = 2,230$ adolescents, 51% female). Developmental pathways included polygenic risk, prenatal stress, warm parenting (age 11), and internalizing and externalizing problems (intercepts and change from 11–16 years). Cortisol reactivity was associated with smoking but not alcohol or marijuana use. Externalizing problems were the stronger predictor of adolescent substance use, but internalizing problems also had an important role. Prenatal stress and middle childhood parenting operated via

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Authors Contributions

KM conceived of the study aims, analyzed the data, and drafted the manuscript; VSK aided in the conceptualization of study aims, interpretation, and edited the manuscript; LB aided in the data analysis and edited the manuscript; SAR was instrumental in data collection and provided access to the data, aided in the conceptualization of key variables and interpretation, and edited the manuscript. All authors read and approved the final manuscript.

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Conflicts of Interest: The authors declare that they have no conflict of interest.

Data Sharing Declaration

The data that support the findings of this study are available from the TRAILS consortium. Some restrictions apply to the availability of these data, which were used under license for the current study, and so are not publicly available. However, data are available from the authors upon reasonable request and with permission of the TRAILS consortium.

Ethical Approval

All procedures involving human participants were in accordance with the APA Ethical Standards in the treatment of the participants and approved by the Dutch Central Committee on Research Involving Human Subjects and by the Institutional Review Board at Purdue University.

Informed Consent

Informed consent was obtained from all individual participants included in the study.

middle childhood externalizing problems; trajectories of growth of externalizing problems were uniquely important in predicting adolescent substance use outcomes. Further, there were protective effects of internalizing problems for alcohol and marijuana use in the context of a more comprehensive model. These developmental influences did not attenuate the association of cortisol reactivity and smoking. These findings suggest a need to understand the broader developmental context regarding the impact of internalizing pathways to substance use, and that it is unlikely that cortisol reactivity and smoking are associated solely because of common developmental influences.

Keywords

prenatal stress; warm parenting; behavior problems; cortisol reactivity; adolescent substance use

Introduction

Substance use and addictions have a well-established neurobiological component (Chassin, Colder, Hussong, & Sher, 2016; Goodman, 2008). The stress-response system is theoretically implicated in the development of substance use (Goodman, 2008; Stephens & Wand, 2012), although associations are infrequently tested in adolescence, despite it being a key developmental period for both phenotypes. Blunted cortisol reactivity earlier in adolescence has been associated with increased involvement in substance use later in adolescence when considering the risky use of multiple substances (Evans et al., 2016) or regular monthly smoking and marijuana (but not alcohol) use (Moss, Vanyukov, Yao, & Kirillova, 1999). However, these associations are not always found (e.g., Lovallo et al., 2019). Theoretically, these associations could be explained by fearlessness and sensation seeking (Marceau, Ruttle, Shirtcliff, Essex, & Susman, 2015), which posit that cortisol reactivity is a biomarker that reflects low fear and/or low arousal. Youth with low arousal may seek to use substances in order to attain higher levels of arousal, and youth with low fear are not dissuaded from use because of potential negative consequences. Notably, this is a causal interpretation whereby cortisol reactivity either marks or contributes to risk for substance use.

However, many of the same developmental influences have been found to contribute to associations of cortisol reactivity and substance use, including genetic vulnerability and both prenatal and postnatal environmental influences (e.g., Dodge et al., 2009; Zucker, Hicks, & Heitzig, 2016). Thus, these influences may lead to both cortisol reactivity and substance use through a process of multi-finality (e.g., where the same cause leads to different outcomes; Cicchetti & Rogosch, 1999). If this is the case, then 1) the same key developmental influences would be expected to predict both outcomes, and 2) any observed association of cortisol reactivity and substance use would be expected to attenuate when including key developmental influences of both phenotypes in the model. The literature testing multiple influences on substance use or cortisol reactivity in a comprehensive model is sparse, and their co-development from the same early influences in a process of multi-finality has not been tested. The present study tests developmental pathways including several key influences highlighted by the literature: genetic risk, prenatal stress, less warm parenting,

and internalizing and externalizing problems cortisol reactivity and substance use during adolescence, and tests whether earlier developmental influences explain (e.g., confound, or attenuate) any observed associations of cortisol reactivity and substance use during adolescence.

Genetic, Prenatal, and Postnatal Influences on Cortisol Reactivity and Substance Use

Familial factors, including genetic influences, have been associated with both cortisol reactivity and substance use. Associations of fathers' substance use with boys' lower cortisol reactivity and associations of blunted cortisol reactivity with substance use among youth at familial risk of substance use (Andersen & Teicher, 2009; Tarter et al., 2013) may reflect a stressful family environment contributing to alterations in cortisol function (Keller et al., 2015). Or, these associations may be indicative of common genetic underpinnings that contribute both to cortisol function and substance use since both phenotypes are heritable (e.g., Kendler, Maes, Sundquist, Ohlsson, & Sundquist, 2014; Steptoe, van Jaarsveld, Semmler, Plomin, & Wardle, 2009). Few studies, however, have tested whether genetic or environmental mechanisms (endogenous or exogenous) underlie cortisol-substance use associations. Recently, 28 genes have been identified in the literature as having been associated both with cortisol and substance use phenotypes (Marceau & Abel, 2018). Variations in these genes may be important for the development of both cortisol and substance use.

Prenatal stress.—Multiple types of prenatal exposures have been linked both to cortisol responses to stress (though findings are mixed with regard to direction) and to substance use. Maternal reports of prenatal stress (e.g., Glover, O'Connor, & O'Donnell, 2010), symptoms of anxiety and depression (e.g., Barker, Jaffee, Uher, & Maughan, 2011; Laurent et al., 2012; Oberlander et al., 2008), and exposure to use of several substances (e.g., alcohol, tobacco, marijuana, cocaine) during pregnancy (e.g., Irner, 2012; Zhang, Sliwowska, & Weinberg, 2005) have each been associated both with cortisol function and with adolescent substance use. Given that these exposures rarely occur in isolation, it has been hypothesized that these and other prenatal risks operate through a cumulative stress mechanism that organizes the development of the child's stress response (Marceau, Ram, et al., 2013) and also has longer-term behavioral consequences (Marceau, Laurent, et al., 2015).

Parenting.—Parental warmth (e.g., positivity, sensitivity) during childhood is a key parenting influence for adolescent substance use (Donaldson, Handren, & Crano, 2016). Warm parenting may facilitate monitoring (knowledge of adolescents' whereabouts and activities), because youth who experience warm parenting tend to be more compliant to parents' rule-setting behavior and may be more likely to disclose information about their whereabouts and activities (Grusec, Goodnow, & Kuczynski, 2000). Parental warmth earlier in childhood is also expected to be related to less adolescent substance use indirectly through child characteristics (including fewer emotional and behavioral difficulties) as peers become more influential as primary or direct influencers (Lee, Padilla-Walker, & Memmott-Elison, 2017).

Warm and supportive parenting during childhood (as opposed to adolescence) is important for the social buffering of the cortisol response to stress (Hostinar, Johnson, & Gunnar, 2015) and is related to more regulated cortisol function during adolescence (Lippold, Davis, McHale, Buxton, & Almeida, 2016). Children experiencing warm parenting feel more secure and lean on parents to help regulate their response to stress, rather than mounting a hormonal stress response. This is adaptive, given the detrimental downstream effects of high levels of cortisol across the lifespan (Lupien, McEwen, Gunnar, & Heim, 2009).

Emotional/behavioral risk.—Higher levels of internalizing and especially externalizing problems are among the strongest risk factors for adolescent substance use, although internalizing problems are sometimes a protective factor against substance use (e.g., Colder et al., 2013; Hussong, Jones, Stein, Baucom, & Boeding, 2011). Further, *trajectories of growth* in internalizing and externalizing problems may be particularly important for the development of substance use during adolescence (Lynne-Landsman, Graber, Nichols, & Botvin, 2011; McCarty et al., 2013). It is important to note that internalizing and externalizing problems are comorbid during childhood and adolescence (Zahn-Waxler, Shirtcliff, & Marceau, 2008). A higher-order latent factor underlying both problem types has been described, providing further evidence for their shared variance (Caspi et al., 2014). The generalist gene and specialist environments hypothesis suggests that genetic influences explain correlations of internalizing and externalizing problems whereas environmental influences explain their divergence. This idea has been empirically supported (Rhee, Lahey, and Waldman, 2015). Thus, it may be the case that externalizing problems are the main risk and internalizing problems are only associated with substance use through shared etiology. Or, internalizing problems may confer a different or additional risk for substance use.

Cortisol reactivity has been associated with adolescent internalizing and externalizing problems as well. Concurrently, higher cortisol reactivity is often associated with internalizing whereas lower cortisol reactivity is often associated with externalizing problems (Marceau, Ruttle, et al., 2015). However, the findings are mixed with regard to the direction of association, and not always found (particularly for externalizing problems; Alink et al., 2008). Longitudinal research is relatively rare in this area (Salis, Bernard, Black, Dougherty, & Klein, 2016), but may indicate that increased cortisol reactivity precedes the development of internalizing problems (Burke, Davis, Otte, & Mohr, 2005; Stroud, Chen, Doane, & Granger, 2018). Despite evidence that internalizing and externalizing problems are associated with cortisol reactivity and adolescent substance use, it is unclear whether cortisol reactivity is independently associated with substance use apart from emotional/behavioral risk.

Developmental Pathways that include Genetic, Prenatal, Parenting, and Emotional/Behavioral Risk

Based on the literature suggesting that there are multiple common early influences on cortisol reactivity and substance use, associations of cortisol reactivity and substance use (if or when they are observed) may arise only because cortisol reactivity and substance use are separate consequences of similar developmental processes. Thus, findings reported in the literature may be completely confounded by the developmental influences highlighted herein

(e.g., genetic, prenatal, parenting, and earlier emotional/behavioral problems). Cortisol reactivity, in particular, is typically conceptualized as a mechanism for the development of substance use (Horn, Roos, Berkman & Fisher 2018). As such, several studies have assessed whether cortisol reactivity could be a mediating mechanism implicated in developmental pathways to substance use or related phenotypes (e.g., externalizing problems). During adolescence, cortisol reactivity mediated the effect of prenatal drug exposure on aggression and a composite measure of drug experimentation (Buckingham-Howes, Mazza, Wang, Granger, & Black, 2016), and a similar developmental pathway including prenatal polysubstance exposure, early adversity, and blunted cortisol reactivity predicted externalizing problems (Conradt et al., 2014). However, other studies have found that adolescent cortisol reactivity did not mediate associations of prenatal stress or parenting influences with externalizing problems (Marsman, Rosmalen, Oldehinkel, Ormel, & Buitelaar, 2009; Ryan, Schechter, & Brennan, 2012). One study found that developmental pathways including genetic, prenatal, parenting, and cortisol influences distinguished which youth developed internalizing vs. externalizing problems: Lower morning cortisol mediated pathways including a) genetic risk and prenatal stress, and b) over-reactive parenting on child internalizing but not externalizing problems (Marceau, Laurent, et al., 2015). Further, blunted cortisol reactivity (at age 7 years) also partially mediated associations of earlier (age 5 years) parental warmth on lower internalizing problems at age 11 years (Kuhlman, Olson, & Lopez-Duran, 2014). Together, these studies suggest that developmental pathways including genetic, prenatal, parenting, and cortisol may be more important for substance use that develops via internalizing rather than via externalizing pathways.

There is also evidence to suggest that developmental pathways to substance use via externalizing problems may be more general, whereas developmental pathways to substance use via internalizing problems may be more substance specific. A pair of recent studies found that externalizing behaviors mediated genetic risk for substance use similarly for alcohol, cigarette, and marijuana use (Trucco et al., 2016), but that pathways from genetic risk through depression to substance use varied for alcohol, cigarette, and marijuana use (Trucco, Villafuerte, Hussong, Burmeister, & Zucker, 2018). This pattern of findings lends credence to the hypothesis that internalizing problems may confer a different or additional risk for substance use than externalizing problems despite their comorbidity. Although these studies did not include measures of cortisol reactivity, Trucco et al. (2018) found that depression mediated the association of a specific gene variant (the *BDNF* Met allele) that has been previously related to increased cortisol reactivity with alcohol, but not cigarette or marijuana use.

Present Study

Current theories highlight dynamic relations between the child and multiple contextual influences (parents, peers, etc.) and biological and environmental influences (Chassin et al., 2016; Dodge et al., 2009; Trucco et al., 2018). However, relatively fewer studies integrate across multiple biological and environmental influences. Examining few variables at a time is vital for in-depth understanding of specific mechanisms in controlled settings, but this approach can mask the relative magnitudes of multiple influences and provide potentially misleading findings due to a failure to account for other factors. The goal of this study was

to test a more comprehensive developmental model of cortisol reactivity and substance use that incorporates several biological and environmental influences in a unified model. It is noted, however, that several potentially relevant factors (i.e., peer and sibling influences, cognitive development) could not be considered given the complexity of these models.

Specifically, path model tested whether a polygenic score of genes related to cortisol, in conjunction with indicators of prenatal stress and parenting at age 11 years, predicted trajectories of internalizing and externalizing problems from age 11–16, and whether these trajectories predicted cortisol reactivity to a social stress challenge at age 16 years, and smoking, drinking, and marijuana use at 16 years. There were three main hypotheses: First, 1) that lower cortisol reactivity would be associated with higher smoking, drinking, and marijuana use frequency at age 16 years. Second, 2) that developmental pathways including genetic, prenatal, and parenting influences would influence substance use via internalizing and externalizing trajectories, as expected based on prior findings in childhood (Marceau, Laurent, et al., 2015) and adolescence (Trucco et al., 2016; Trucco et al., 2018). Based on the prior work on developmental pathways, it was also expected that mediation by prenatal stress may be important for pathways to substance use involving internalizing but not externalizing problems. Thus the final hypothesis 3) was that the association of cortisol reactivity with substance use (if found) would partially attenuate after accounting for genetic, prenatal, parenting, and emotional/behavioral influences, consistent with a multifinality explanation (Lovallo et al., 2019).

Method

Participants were from TRAILS (TRacking Adolescents' Individual Lives Survey). TRAILS is a longitudinal Dutch study of 2,230 adolescents (51% female, 85% European ancestry, representative of the Northern part of the Netherlands) followed prospectively beginning at age 11 years ($M = 11.11$, $SD = 0.56$, range = 10–13 years), designed to track the development of mental and physical health from preadolescence into adulthood. In March, 2001 in the Netherlands, the names and addresses of inhabitants born within a 1-year period were requested from five municipalities (spanning 1989–1991). Primary schools within those municipalities were requested to participate (90% agreed). Parents of children in those schools were informed of the study and recruited ($N = 3,145$). Exclusion criteria included mental retardation or physical illness preventing participation, and unavailability of a Dutch-speaking parent or guardian. Of the eligible children, 76% ($N = 2,230$) were enrolled. The study was approved by the Dutch Central Committee on Research Involving Human Subjects and by Purdue University "BioBehavioral Developmental Origins of Adolescent Substance Use" #1702018756. Please see Oldehinkel et al., (2015) and Ormel et al., (2012) for further detail on TRAILS. The current study used data from the first three assessment waves, at (average) ages 11, 13, and 16 years.

Measures

Genetic data.—In total, 1,491 children were genotyped for 295,173 genome-wide single nucleotide polymorphisms (SNPs; autosomes + the X chromosome) using the Illumina Cyto SNP12 v2 array. Missing data was imputed to the 1000G using the Phase 1 global reference

set (March 2012). The goal was to generate a SNP-based polygenic risk score for each individual based on aggregate information from markers relevant to cortisol activity. To derive meaningful scores based on relevant markers, risk was weighted based on SNPs effect sizes that were generated from a large, published, independent genome-wide association study (GWAS). The GWAS catalog was searched (MacArthur et al., 2017) using the keyword “cortisol”, and found that Neumann et al., 2017 was the most recent and largest GWAS of (basal) cortisol as measured via saliva. This study yielded 10 SNPs significant at a p-value of $< 5e-06$ (rs6768297, rs1170109, rs11899245, rs10244501, rs7248779, rs11557092, rs2983496, rs6849009, rs6069930, rs5927) with associated beta-weights and effect alleles that were used in the current study to derive the polygenic score. Cortisol-related genes, specifically, were selected for this analysis because cortisol is theoretically a mechanism of the development of substance use, although this study cannot separate that direction of effect. In brief, TRAILS data were coded in terms of the number of copies of the effect allele (cortisol-increasing alleles according to Neumann et al., 2017) each individual had for each of the 10 SNPs. Then, the number of effect alleles was multiplied by the beta-weight presented in Neumann et al., (2017) for each SNP for each individual, and the resulting 10 values per-person were summed (across SNPs), yielding a single PGS for each individual. Thus, the PGS reflects individuals’ genetic predisposition to higher cortisol levels. Of additional importance to the current study, Marceau and Abel (2018) identified a list of 28 genes that were related both to cortisol and substance use; this list was used in an alternative analytic strategy and conceptualization of polygenic risk (see Appendix 1, part 2). To be circumspect, a similar PGS comprised only of substance use-related GWAS hits was also calculated and investigated; see sensitivity analyses discussed in Appendix 1.

Prenatal stress.—The measures of prenatal stress used here have been previously used and described (e.g., Jaspers, de Meer, Verhulst, Ormel, & Reijneveld, 2010; Nederhof, Bouma, Oldehinkel, & Ormel, 2010). In brief, the TRAILS Family History Interview, was administered to mothers when children were age 11 years and the following variables were constructed. Data on low/high birth weight, smoking during pregnancy, alcohol use during pregnancy, preterm, postterm, complicated deliveries, and hospitalization of the mother and/or child were summed to create a composite prenatal stress score, and winsorized so that 4 was the maximum in order to correct the distribution following previous studies using this measure (see Table 1 for descriptive statistics). Each of the risk factors were coded in terms of what constitutes potential risk to the fetus based on prior studies, and cutoffs are described in Jaspers et al., (2010), Nederhof, et al., (2010) and Appendix 1 (part 1). As a sensitivity check, a prenatal stress count variable was also constructed that included only smoking during pregnancy, alcohol use during pregnancy, and maternal physical, social or psychological problems experienced during pregnancy which excludes indicators measured postnatally that may be further removed from the intended construct of *prenatal* stress. This variable was slightly zero-inflated (45.5%) and skewed (1.07), with an observed range of 0–5. Results did not differ except in one case (noted as a footnote in the results and Figure note).

Warm parenting.—Warm parenting at age 11 years was a multi-rater, multi-measure composite (Ellis, Oldehinkel, and Nederhof, 2016). Separately for mothers and fathers,

children completed the rejection (hostility, punishment, derogation, blaming child; $\alpha = .85 - .88$, mother-father $r = .68$), and emotional warmth (giving special attention, praise, unconditional love, support, affection; α 's = .91, mother-father $r = .71$) subscales of the EMBU (a Swedish acronym for My Memories of Upbringing; Markus, Lindhout, Boer, Hoogendijk, & Arrindell, 2003) for Children. Rejection and warmth composites were moderately correlated ($r = -.35$, $p < .001$). After reverse-coding rejection scores, child reports on warmth and rejection were averaged in order to arrive at a single score that reflects parent-child relationship quality, with higher scores indicating more warmth/support.

Internalizing and externalizing problems.—Internalizing and externalizing problems were measured using the Child Behavior Checklist (Achenbach, 1991a) and Youth Self Report (Achenbach, 1991b) at 11, 13, and 16 years of age. These measures have been shown to be internally consistent and valid (e.g., Ormel et al., 2012). Parent and youth report were correlated at each assessment (internalizing: $r = .27 - .43$; externalizing: $r = .31 - .40$, p 's < .001), and thus averaged to create multi-rater indices of internalizing and externalizing problems. Linear mixed effects models were fit using SAS PROC MIXED in order to estimate individuals' intercepts (e.g., estimated middle childhood levels) and slopes (e.g., rates of change from the 11 to 16 year assessments) of internalizing and externalizing problems across adolescence, using participants' exact age (centered on the youngest observed age at the first assessment, 10.01 years) as the metric of time (results presented in Appendix 1, part 1). The Bayes Empirical Estimates from each model were saved as measures of middle childhood levels (intercept) and rates of change (slope) in internalizing and externalizing problems. Middle childhood levels and rates of change across adolescence were not correlated within each problem type (internalizing: $r = -.01$, $p = .79$; externalizing: $r = .02$, $p = .33$), but middle childhood levels and rates of change were correlated across problem types (intercept: $r = .50$, $p < .0001$; slope: $r = .47$, $p < .0001$). The original intent was to include internalizing and externalizing in a single model, formally modeling this covariance, but multicollinearity was an issue, and the patterns of findings were quite different than expected based on the correlation matrix, prior findings in the literature, theory, and models including one phenotype at a time (which generally matched the aforementioned expectations more closely). Thus, separate hypothesis testing models were run including internalizing and then externalizing problems to avoid these issues.

Cortisol reactivity.—Cortisol reactivity was assessed in response to the Groningen Social Stress Task (GSST; Bouma, Riese, Ormel, Verhulst, & Oldehinkel, 2009), the Dutch version of the Trier Social Stress Test, which elicits a cortisol response through an uncontrollable social evaluative stressor (see Appendix 1 for task procedures). Salivary cortisol was collected with the Salivette sampling device (Sarstedt, Numbrecht, Germany; see Bouma et al., 2009 for details on collection, storage, and assays). Cortisol values for 42 participants were set to missing due to medical exclusions (corticosteroids, SSRIs, pain medications, and smoking prior to the GSST). Relevant to the current study, cortisol levels were assessed just before the start of the GSST as well as 20 minutes later, immediately after the GSST ended (40 minutes after baseline). As in prior reports (e.g., Nederhof, Marceau, Shirtcliff, Hastings, & Oldehinkel, 2015), the post-GSST sample was regressed on the pre-task sample, and the

standardized residuals saved as the measure of cortisol reactivity. Higher values indicate higher cortisol reactivity.

Substance use outcomes.—Adolescent self-report of past-month frequency of alcohol (number of times alcoholic drinks were consumed), tobacco (number of cigarettes consumed), and marijuana (number of times used) use were asked by individual questions at the age 16 assessment. Alcohol and marijuana use were reported on the following scale: 0 = no use, 1 = 1 time, 2 = 2 times, 3 = 3 times, 4 = 4 times, 5 = 5 times, 6 = 6 times, 7 = 7 times, 8 = 8 times, 9 = 9 times, 10 = 10 times, 11 = 11–19 times, 12 = 20–39 times, 13 = 40 or more times. Tobacco use was assessed on a scale of 0 = none, 1 = less than 1 cigarette per week, 2 = less than 1 cigarette per day, 3 = 1–5 cigarettes per day, 4 = 6–10 cigarettes per day, 5 = 11–20 cigarettes per day, and 7 = more than 20 cigarettes per day.

Analytic Strategy

To test the *first hypothesis*, that lower cortisol reactivity would be concurrently associated with higher smoking, drinking, and marijuana use frequency at age 16 years zero-order associations among these variables were examined. The subsequent hypotheses were tested using path models. To test the *second* and *third hypotheses*, hypothesis-testing path analyses were conducted in R(lavaan) (Rosseel, 2012), using the `se="bootstrap"` command to accommodate the skewed nature of the outcome data and to obtain unbiased *p*-values for indirect effects, and Full Information Maximum Likelihood (FIML) to accommodate missing data. The models are based on all 2,230 adolescents who provided data on at least one construct included in the model (see Table 1 for construct-specific *ns*). Specifically, paths were included allowing the PGS and prenatal stress to correlate and to predict warm parenting at age 11 years, middle childhood levels and rates of change in internalizing or externalizing problems from 11 to 16 years, and cortisol reactivity, alcohol, smoking, and marijuana use at age 16 years. Paths allowing warm parenting to predict middle childhood levels and rates of change in internalizing or externalizing problems, and cortisol reactivity, alcohol, smoking, and marijuana use at age 16 years were also included, as were paths allowing middle childhood levels and rates of change in internalizing or externalizing problems to predict cortisol reactivity, alcohol, smoking, and marijuana use at age 16 years. Finally, cortisol reactivity, alcohol, smoking, and marijuana use at age 16 years were allowed to correlate. Prior to data analysis, all study variables were standardized ($M = 0$, $SD = 1$).

Within this model, the *second hypothesis* (that there would be developmental pathways by which genetic, prenatal, and parenting influences would influence substance use via internalizing and to a lesser extent externalizing trajectories – particularly considering prenatal stress effects), was tested via indirect effects and would be confirmed by finding significant indirect effects in the model including prenatal stress and internalizing but not externalizing behaviors.

The *third hypothesis* (that the association of cortisol reactivity with substance use would somewhat, but not completely attenuate when considering genetic, prenatal, parenting, and behavioral influences) was tested by the significance and strength of the association of

cortisol reactivity and each substance use phenotype that was significant in the test of hypothesis 1 in the full model.

Results

34.3% of adolescents reported any amount of smoking, 77.2% of adolescents reported any amount of drinking, and 13% of adolescents reported any amount of marijuana use in the past 4 weeks; see Table 1 for descriptive statistics.

Associations of Cortisol Reactivity and Substance Use (Hypothesis 1)

Correlations (Table 2) showed that lower cortisol reactivity was associated with a higher smoking (but not use of alcohol or marijuana) frequency at 16 years, partially confirming the first hypothesis (e.g., only for smoking). Frequencies of use of all three substances at age 16 were moderately and positively correlated. Therefore, although the path models were run for all substances (hypothesis 2), hypothesis 3 could only be tested for smoking.

Developmental Pathways (Hypothesis 2)

Internalizing pathways.—The model fit was acceptable: $\chi^2(1)=0.27$, $p=.60$, CFI = 1.0, TLI = 1.03, RMSEA = 0.000, SRMR = 0.001. The variance explained (R^2) was as follows: smoking frequency (5%), drinking frequency (1%), marijuana use frequency (1%), cortisol reactivity (2%) and initial internalizing (9%). Because the main study hypotheses were tested via indirect effects, only findings from the indirect effects are presented here (for full results see Figure 1; Supplemental Table S1). There were significant indirect effects from warm parenting through lower levels of initial levels of internalizing problems to more frequent drinking ($\beta = .022$, SE = .008, $p = .005$), marijuana use ($\beta = .021$, SE = .009, $p = .019$), and cortisol reactivity ($\beta = .032$, SE = .014, $p = .026$). There was also a significant indirect effect from warm parenting through greater increasing internalizing problems to more frequent smoking ($\beta = .008$, SE = .003, $p = .030$). Finally, there was a significant indirect effect from prenatal stress through lower levels of initial levels of internalizing problems to more frequent drinking ($\beta = -.005$, SE = .002, $p = .032$).¹

Externalizing pathways.—The model fit was adequate: $\chi^2(1)=5.11$, $p=.02$, CFI = .998, TLI = .895, RMSEA = .043, SRMR = .007. The variance explained (R^2) was as follows: smoking frequency (28%), drinking frequency (6%), marijuana use frequency (11%), cortisol reactivity (2%) initial externalizing (17%), change in externalizing (1%). Again, as the main study hypotheses were tested via indirect effects, only indirect effects are presented here (for full results see Figure 2; Supplemental Table S2). There were significant indirect effects from prenatal stress through higher levels of initial levels of externalizing problems to more frequent smoking ($\beta = .035$, SE = .007, $p < .001$), drinking ($\beta = .018$, SE = .005, $p = .009$), and marijuana use ($\beta = .023$, SE = .006, $p < .001$). There were also significant indirect effects from warm parenting through lower levels of initial levels of externalizing problems to less frequent smoking ($\beta = -.112$, SE = .013, $p < .001$), drinking ($\beta = -.056$, SE

¹This effect barely did not meet the threshold for significance when using the more focused prenatal stress variable: $\beta = -.0046$, SE = .002, $p = .054$, however the findings are judged to be consistent.

= .012, $p < .001$), and marijuana use ($\beta = -.074$, $SE = .016$, $p < .001$). Finally, significant indirect effects also indicated a path from warm parenting through growth in externalizing problems to more frequent smoking ($\beta = .029$, $SE = .011$, $p = .006$), drinking ($\beta = .015$, $SE = .006$, $p = .009$), and marijuana use ($\beta = .018$, $SE = .007$, $p = .0109$).

Attenuation of Cortisol Reactivity – Substance Use Associations (Hypothesis 3)

Given the null correlations between cortisol reactivity and alcohol and marijuana use (hypothesis 1), the hypothesis that the association of cortisol reactivity and substance use (if present) would be attenuated by developmental influences could only be assessed for smoking. These analyses for smoking showed that associations were not substantially attenuated in models of internalizing pathways; compared to a zero-order correlation (Table 2) of $-.16$, the standardized coefficient for the focal association was $-.15$ in the model including internalizing but very slightly attenuated ($-.09$) in the model including externalizing. This hypothesis was thus generally not confirmed.

Discussion

Although a strong neurobiological component has been described for the development of substance use (Stephens & Wand, 2012), the literature on associations of cortisol reactivity and substance use in adolescence is sparse and mixed. Nonetheless, several theories conceptualize cortisol reactivity as a biomarker of risk for the development of substance use: a causal hypothesis (Marceau, Ruttle et al., 2015). The present study builds on prior literature investigating earlier developmental influences on cortisol reactivity and substance use to investigate whether these phenotypes are related in adolescence, co-develop (e.g., show a pattern of multi-finality), and whether associations, if observed, hold after accounting for common developmental influences. Specifically, developmental pathways that included a polygenic score of genes related to cortisol, as well as indicators of prenatal stress, parenting, and trajectories of internalizing and externalizing problems predicted cortisol reactivity, smoking, alcohol, and marijuana use at age 16 years were tested. The initial hypothesis, that lower cortisol reactivity would be associated with higher smoking, drinking, and marijuana use frequency at age 16 years, was only confirmed for smoking. In testing the second hypothesis, findings revealed pathways by which 1) prenatal stress and colder parenting influenced substance use (but not cortisol reactivity) via middle childhood externalizing levels, 2) warmer parenting influenced substance use and cortisol reactivity via externalizing trajectories, and 3) colder parenting influenced marijuana use, alcohol use, and cortisol reactivity via middle childhood internalizing levels but warmer parenting influenced smoking via internalizing trajectories. Findings from the third hypothesis provided minimal evidence that the association of cortisol reactivity with smoking was attenuated after considering genetic, prenatal, parenting, and behavioral influences. Thus, findings generally support the notion that cortisol reactivity and substance use co-develop with some overlapping developmental influences (particularly pathways including parenting, and middle childhood levels of internalizing problems or changes in externalizing problems) in line with a multi-finality model of the development of cortisol reactivity and substance use. There was less evidence for the potential for cortisol reactivity to be a key mechanism of substance use development during adolescence.

Cortisol and Substance Use

This is the second study seeking to place cortisol reactivity in a model of pathways from genetic and earlier life influences to substance use. The first study (Lovallo et al., 2019) examined substance use in young adults, a developmental period marked by higher rates of substance use than mid-adolescence, but still found little evidence of links between cortisol reactivity with alcohol use frequency and the number of illicit drugs tried. They concluded that the earlier risk factors that predict substance use also predict cortisol reactivity. In other words, multi-finality of common developmental risk factors are likely to explain how associations arise, if they do. The present findings partially support this supposition, insofar as there were some common developmental profiles leading to both phenotypes. However, there were also some differences in developmental pathways to substance use and cortisol phenotypes. Further, there was little evidence that the cortisol reactivity-smoking association diminished in the full models. This finding could suggest that cortisol reactivity is a biomarker that plays a relatively small role in the development of smoking, or that much of the association was explained by unmeasured common developmental influences.

When critically reviewing published cortisol reactivity-substance use associations, one might conclude that the “riskier” phenotypes – more frequent and recent use of less frequently used substances (e.g., smoking, cannabis, other drugs) tend to be the phenotypes associated with cortisol reactivity, and alcohol use (a more normative substance use outcome at this developmental period) is unrelated to cortisol reactivity. The null correlation for alcohol use was confirmed in the present study. It should be noted, however, that van Leeuwen and colleagues (2011) found an association in the TRAILS data of blunted cortisol reactivity and lifetime cannabis use, whereas in the present analysis the association with past 4-weeks use was not significant, so it may not be frequent use that is the key feature for observing associations. In sum, the small literature assessing cortisol reactivity-adolescent substance use associations is quite mixed, and further experimentation is needed in order to decipher when or if the association is present, prior to further explorations of the mechanisms by which they should theoretically be associated.

Internalizing and Externalizing Pathways

Consistent with prior literature, findings showed that externalizing problems were more robustly associated with adolescent substance use than internalizing problems; however, the current findings indicated pathways including both externalizing and internalizing problems (Hussong et al., 2011). Similar to previous work by Trucco and colleagues (2016, 2018), pathways including internalizing problems varied for smoking, drinking, and marijuana use, whereas pathways including externalizing problems were quite consistent across substances. It may be that externalizing pathways to substance use operate via mechanisms related to sensation seeking or desire to engage in delinquent behaviors (Quinn & Harden, 2013). Initial correlations suggested that changes in externalizing problems were more strongly associated with substance use than were middle childhood levels of externalizing problems. This could be explained by more proximal externalizing behaviors simply being more highly correlated with concurrent substance use. However, middle childhood levels of externalizing problems were more important in developmental pathways from prenatal stress to substance use, illustrated by the indirect effects that involved middle childhood levels but not changes

in externalizing across adolescence. These findings may suggest that the timing of externalizing problems matters for progression to substance use; particularly, higher levels earlier in childhood compared to adolescence are more indicative of developmental pathways beginning at birth. Steeper increases in externalizing problems across adolescence are indeed a strong predictor of adolescent substance use outcomes, but likely via different mechanisms, not including prenatal risk.

For internalizing, findings suggested small initial correlations which suggest that changes in internalizing problems (e.g., increasing problems) were more strongly associated with smoking frequency than were initial levels. However, middle childhood levels of internalizing problems and changes in internalizing problems were similarly associated with less frequent alcohol use. After accounting for earlier influences, the effect of childhood internalizing problems on smoking frequency was completely attenuated, and a protective effect of childhood internalizing problems for marijuana use frequency emerged. These patterns were clearly substance-specific, in contrast to the findings including externalizing problems. It is unclear why internalizing pathways may be substance-specific. Trucco and colleagues (2018) examined different early risks (behavioral control and resiliency) in developmental pathways including depression, specifically. They found that pathways including specific genes, behavioral control and resiliency, and depression were important for higher levels of alcohol use, but not smoking or marijuana use. In contrast, in the current study, internalizing symptoms were predictive of more frequent smoking (i.e., changes in internalizing symptoms), but less frequent alcohol and marijuana use (i.e., middle childhood levels of internalizing symptoms). Consistent with the present findings, a recent review suggested that internalizing symptoms were more often protective against, rather than risks for, alcohol and marijuana use, although most studies found null effects (Hussong, Ennett, Cox, & Haroon, 2017). However, for tobacco, internalizing symptoms were more often predictive of more use, though again most studies found null results (Hussong et al., 2017). Speculatively, anxiety-related behaviors (rather than depression) may lead to less involvement with deviant and substance-using peers which then serve to decrease risk for substance use later in adolescence (Colder et al., 2013). Further, the present findings suggest that the risk and protective effects of internalizing behavior may only be observable in the context of (e.g., after accounting for) earlier influences on the development of substance use. Because emotional and behavioral risk is a part of a rich developmental context for each individual, the present findings suggest that the lack of contextualization of transitions to substance use may partly explain mixed findings regarding internalizing risk in the literature.

The current findings showed stronger effects of increases in internalizing problems across adolescence than middle childhood levels of internalizing problems on smoking frequency. Thus, it may be that adolescents who experience more dramatic increases in internalizing problems, which are quite different from the normative decrease found in this sample, may be particularly at risk for engaging in cigarette use as a means to self-medicate. Alternatively, when this cohort of children were approximately age 16 years (around 2007), policies toward adolescent cigarette use in the Netherlands were quite a bit more permissive than they are now (and compared to the United States). Thus, youth with internalizing problems may have chosen to use cigarettes in particular as a means of self-medication because they were particularly easy to access, or because at the time cigarette use was

common at any time of day whereas alcohol and marijuana use were more typically limited to evening leisure-time.

Pathways to Cortisol Reactivity

Interestingly, findings revealed an indirect pathway such that colder parenting predicted more internalizing problems and subsequently blunted cortisol reactivity several years later. This suggests that earlier parenting continues to have lasting effects on the development of mental health problems, even beyond concurrent benefits of social buffering for reducing internalizing problems. A different pathway including externalizing trajectories was significant, suggesting that warmer parenting predicted greater increases in externalizing problems and subsequently lower cortisol reactivity. It was interesting that changes in externalizing problems across middle childhood and adolescence were associated with blunted cortisol reactivity, rather than middle childhood levels of externalizing problems. Given the mixed findings in the literature regarding associations of cortisol reactivity and externalizing problems (Alink et al., 2008), the present findings provide intriguing initial evidence that considering the trajectories of increasing externalizing problems may be particularly salient for understanding the links between cortisol reactivity and childhood and adolescent externalizing. This effect must be replicated; studies examining developmental trajectories of behavior and cortisol are needed to understand the implications of this finding. Further, it is important to keep in mind that the present findings suffer the same limitation as much of the longitudinal literature on cortisol reactivity and behavior problems: the present study was unable to test both directions, and thus the findings may reflect bidirectional or concurrent associations rather than directional associations.

Parenting and Prenatal Influences

Interestingly, despite some zero-order correlations, only the direct effect of colder parenting on more frequent smoking and marijuana use persisted in the context of the full model and only in the models including the internalizing pathway. In other words, the majority of the influence of prenatal stress and parenting operated indirectly through internalizing and especially externalizing problems. The current finding of no direct effects of prenatal stress or parenting but significant indirect effects in the model including externalizing problems highlights the important organizing role of putatively environmental influences on substance use specifically through their influence on earlier behavior problems and subsequent transitions from behavior problems to substance use. These findings have meaningful implications for intervention studies, particularly for those at risk based on prenatal stress and colder parenting. Based on these findings, interventions to reduce prenatal stress and increase warm parenting may help to directly reduce middle childhood internalizing and externalizing problems, and have longer-term indirect effects serving to reduce the frequency of use across multiple substances during adolescence. However, these interventions could not be expected to completely attenuate adolescent substance use, as there were independent pathways to substance use that were marked by increasing internalizing and externalizing problems during adolescence that would not be expected to be affected by such interventions. Future work exploring trajectories of externalizing problems in pathways that include other developmental risk and protective factors (e.g., peer

and sibling influences) may yield other intervention targets that play a more important role later in development.

Genetic Influences

Contrary to hypotheses, the polygenic scores did not predict cortisol reactivity, substance use, or any other study variables. It may be that the 10 markers included in the polygenic score simply do not exert a strong enough influence on cortisol reactivity or substance use to be detected in samples of this size. Another explanation may be that these markers are related to different phenotypes (e.g., morning or diurnal cortisol) that are less relevant to reactivity. The main limitations of using the Neumann et al., (2017) study to construct the polygenic score are as follows. First, there is limited coverage of the cortisol reactivity system because only 10 SNPs were considered top hits in the GWAS. Second, this discovery sample, though the largest available for salivary cortisol, only includes 7,708 individuals which is considered small for a discovery sample and likely yielded unstable weights. Given that many complex traits are polygenic in nature, it is possible that the inclusion of more markers (e.g., all of the SNPs reported in the study, even if they do not pass genome-wide significance) may result in the aggregation of numerous very small effects, thereby yielding a more powerful risk score. However, in this case, the estimated heritability of cortisol was quite low (6%; Neumann et al., 2017) and it is unlikely that incremental increases in variance by including more non-significant SNPs (particularly that have potentially unstable weights because of the small discovery sample size) would meaningfully increase prediction of cortisol reactivity or substance use. Of note, four distinct methods of creating a polygenic score were attempted (detailed in Appendix 1), and while each has its own limitations, none produced significant results. Therefore, if a single polygenic risk factor does influence both cortisol reactivity and substance use in adolescence, the magnitude and clinical meaningfulness of such a measure is likely to be quite small, and pale in comparison to the other developmental influences assessed here. However, future work using a polygenic score conditioned on a larger discovery sample is critical to adequately test the role of shared genes on cortisol and substance use in developmental pathways to adolescent substance use.

Limitations

There are several study limitations important to consider when interpreting findings. First, substance use frequency was assessed at age 16 years, which occurs prior to the peak of substance use frequency. This is particularly clear for smoking and marijuana use, which had lower frequency in the present data. Thus, the smoking and marijuana phenotypes here may represent earlier onset (and thus potentially more detrimental) of frequent use; conversely, the analysis would not pick up on many youths who will go on to use in the following couple of years, and effects (including potential associations with cortisol reactivity) may thus be underestimated here.

Second, girls who used contraceptives were not excluded from the analytic sample in order to avoid further reducing the sample size for the polygenic score. There is some evidence that these girls did not exhibit the expected cortisol reactivity in TRAILS (Bouma et al., 2009), which may increase error or bias in the polygenic score and associations with other study variables. Similarly, although most frequently used in the literature, difference scores

20 minutes post-stressor may not fully capture cortisol reactivity. A final data limitation is the limited validity of retrospective recall of prenatal stress a decade after birth (Jaspers et al., 2010).

In terms of design limitations, the original study did not use a genetically informed design that is capable of disentangling genetic from environmental influences (e.g., adoption, twin/family). Thus the ‘environmental’ influences of prenatal stress and warm parenting may actually represent correlated effects of genotypes and environments. Further, it is not possible to interpret the direction of effects in the current study, like most studies on developmental pathways to substance use. Temporal ordering was presumed by virtue of the timing of assessment of the study variables. In the future, cross-lagged panel designs (particularly including random intercepts) may provide a better test of the direction of pathways of development.

The racial/Ethnic composition of this sample was such that Ethnicity could not be considered in the current analysis. Whereas this is a strength ensuring homogeneity for genetic influences, the racial/Ethnic composition limits generalizability, especially in the context of polygenic scores (Duncan et al., 2019). Notably, in the literature, developmental pathways including cortisol reactivity for adolescent externalizing problems and substance use were found in African American (Buckingham-Howes et al., 2016; Conratt et al., 2014) but not White populations (Marsman et al., 2009; Ryan et al., 2012). As such, the results should only be generalized to White populations.

Conclusion

There is gap in the literature between the complexity of theories of the development of cortisol reactivity and substance use and the relatively narrow models used to test these theories. The present study addressed this gap by testing more comprehensive developmental models that included polygenic, prenatal, parenting, and emotional/behavioral risk on both cortisol reactivity and substance use outcomes in adolescence. Findings generally showed little evidence for the potential for cortisol reactivity to be a key mechanism of substance use development during adolescence, instead supporting a multi-finality model of the development of cortisol reactivity and substance use with some overlapping developmental influences (e.g., including parenting, and middle childhood levels of internalizing problems or changes in externalizing problems). There was little evidence of cortisol reactivity-substance use associations during adolescence despite being commonly assumed in theory. Key findings included first that the only association found between cortisol reactivity and smoking frequency was somewhat but not greatly attenuated by the inclusion of growth of externalizing problems. Therefore, changes in externalizing problems across early to mid-adolescence could play a small but important role for how the stress response system develops during adolescence and how it integrates with substance use phenotypes later in life. Second, the present findings highlight the importance of earlier influences, particularly prenatal stress and parenting, for understanding the magnitude of developmental associations of internalizing and externalizing problems with substance use. Third, consistent with a growing body of literature, the present findings provide further support for the notion that despite externalizing problems being stronger predictors of substance use,

there is still an important role for internalizing problems in the development of substance use. And the current findings lend credence to the relatively new idea that the development of substance use via externalizing pathways are general, whereas the development of adolescent substance use via internalizing pathways are substance-specific. There was some evidence of protective direct effects of internalizing problems for marijuana and alcohol use that emerged in the context of a fuller model including prenatal stress and parenting influences, both levels and changes in internalizing problems, and associations among use of multiple substances. These findings may help to explain the mixed effects reported in the literature (Hussong et al., 2017), as they suggest a critical need for understanding a broader developmental context for uncovering meaningful internalizing pathways to adolescent substance use. However, future work with better-powered samples and more robust associations of cortisol reactivity and substance use (perhaps later in development) are needed to fully explore the potential role of shared genes in developmental pathways of cortisol and substance use.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

- Achenbach TM (1991a). Manual for the Child Behavior Checklist/4–18 and 1991 Profile. Burlington, VT: Department of Psychiatry.
- Achenbach TM (1991b). Manual for the youth self-report and 1991 profile. Burlington: University of Vermont: Department of Psychiatry.
- Alink LRA, van Ijzendoorn MH, Bakermans-Kranenburg MJ, Mesman J, Juffer F, & Koot HM (2008). Cortisol and externalizing behavior in children and adolescents: Mixed meta-analytic evidence for the inverse relation of basal cortisol and cortisol reactivity with externalizing behavior. *Developmental Psychobiology*, 50, 427–450. doi:10.1002/dev.20300 [PubMed: 18551461]
- Andersen SL, & Teicher MH (2009). Desperately driven and no brakes: Developmental stress exposure and subsequent risk for substance abuse. *Neuroscience & Biobehavioral Reviews*, 33, 516–524. doi: 10.1016/j.neubiorev.2008.09.009 [PubMed: 18938197]
- Barker ED, Jaffee SR, Uher R, & Maughan B (2011). The contribution of prenatal and postnatal maternal anxiety and depression to child maladjustment. *Depression and Anxiety*, 28, 696–702. [PubMed: 21769997]
- Bouma EMC, Riese H, Ormel J, Verhulst FC, & Oldehinkel AJ (2009). Adolescents' cortisol responses to awakening and social stress; Effects of gender, menstrual phase and oral contraceptives. The TRAILS study. *Psychoneuroendocrinology*, 34, 884–893. [PubMed: 19195792]
- Buckingham-Howes S, Mazza D, Wang Y, Granger DA, & Black MM (2016). Prenatal Drug Exposure and Adolescent Cortisol Reactivity: Association with Behavioral Concerns. *Journal of Developmental and Behavioral Pediatrics* : JDBP, 37, 565–572. [PubMed: 27541579]
- Burke HM, Davis MC, Otte C, & Mohr DC (2005). Depression and cortisol responses to psychological stress: A meta-analysis. *Psychoneuroendocrinology*, 30(9), 846–856. doi:10.1016/j.psyneuen.2005.02.010 [PubMed: 15961250]
- Caspi A, Houts RM, Belsky DW, Goldman-Mellor SJ, Harrington H, Israel S, ... & Moffitt TE (2014). The p factor: one general psychopathology factor in the structure of psychiatric disorders?. *Clinical Psychological Science*, 2, 119–137. [PubMed: 25360393]
- Chassin L, Colder CR, Hussong A, & Sher KJ (2016). Substance use and substance use disorders. *Developmental psychopathology: Maladaptation and psychopathology*, Vol. 3, 3rd ed. (pp. 833–897). Hoboken, NJ, US: John Wiley & Sons Inc.

- Cicchetti D, & Rogosch FA (1999). Psychopathology as risk for adolescent substance use disorders: A developmental psychopathology perspective. *Journal of Clinical Child Psychology*, 28, 355–365. [PubMed: 10446685]
- Colder CR, Scalco M, Trucco EM, Read JP, Lengua LJ, Wieczorek WF, & Hawk LW Jr. (2013). Prospective associations of internalizing and externalizing problems and their co-occurrence with early adolescent substance use. *Journal of Abnormal Child Psychology*, 41, 667–677. [PubMed: 23242624]
- Conradt E, Abar B, Lester BM, LaGasse LL, Shankaran S, Bada H, ... Hammond JA (2014). Cortisol Reactivity to Social Stress as a Mediator of Early Adversity on Risk and Adaptive Outcomes. *Child Development*, 85, 2279–2298. doi:10.1111/cdev.12316 [PubMed: 25376131]
- Dodge KA, Malone PS, Lansford JE, Miller S, Pettit GS, & Bates JE (2009). A dynamic cascade model of the development of substance-use onset. *Monographs of the Society for Research on Child Development*, 74, vii–119. doi:10.1111/j.1540-5834.2009.00528.x
- Donaldson CD, Handren LM, & Crano WD (2016). The Enduring Impact of Parents' Monitoring, Warmth, Expectancies, and Alcohol Use on Their Children's Future Binge Drinking and Arrests: a Longitudinal Analysis. *Prevention Science*, 17, 606–614. doi:10.1007/s11121-016-0656-1 [PubMed: 27178008]
- Dudbridge F (2013). Power and predictive accuracy of polygenic risk scores. *PLoS Genet*, 9, e1003348. [PubMed: 23555274]
- Duncan L, Shen H, Meijse J, Ressler K, Feldman,., & Domingue B (2019). Analysis of polygenic risk score usage and performance in diverse human populations. *Nature Communications*, 10, 3328.
- Ellis BJ, Oldehinkel AJ, & Nederhof E (2016). The adaptive calibration model of stress reactivity: An empirical test in the Tracking Adolescents' Individual Lives Survey study. *Development and Psychopathology*, 29, 1001–1021. doi:10.1017/S0954579416000985 [PubMed: 27772536]
- Evans BE, Greaves-Lord K, Euser AS, Thissen S, Tulen JH, Franken IH, & Huizink AC (2016). Stress reactivity as a prospective predictor of risky substance use during adolescence. *Journal for Studies on Alcohol and Drugs*, 77, 208–219.
- Geer LY, Marchler-Bauer A, Geer RC, Han L, He J, He S, ... Bryant SH (2010). The NCBI BioSystems database. *Nucleic Acids Research*, 38, D492–496. doi:10.1093/nar/gkp858 [PubMed: 19854944]
- Glover V, O'Connor TG, & O'Donnell K (2010). Prenatal stress and the programming of the HPA axis. *Neuroscience & Biobehavioral Reviews*, 35, 17–22. doi:10.1016/j.neubiorev.2009.11.008 [PubMed: 19914282]
- Goodman A (2008). Neurobiology of addiction: An integrative review. *Biochemical Pharmacology*, 75, 266–322. [PubMed: 17764663]
- Grusec JE, Goodnow JJ, & Kuczynski L (2000). New directions in analyses of parenting contributions to children's acquisition of values. *Child Development*, 71, 205–211. [PubMed: 10836575]
- Horn SR, Roos LE, Berkman ET, & Fisher PA (2018). Neuroendocrine and immune pathways from pre- and perinatal stress to substance abuse. *Neurobiology of Stress*, 9, 140–150. [PubMed: 30450380]
- Hostinar CE, Johnson AE, & Gunnar MR (2015). Parent support is less effective in buffering cortisol stress reactivity for adolescents compared to children. *Developmental Science*, 18, 281–297. doi:10.1111/desc.12195 [PubMed: 24942038]
- Hussong AM, Ennett ST, Cox MJ, & Haroon M (2017). A systematic review of the unique prospective association of negative affect symptoms and adolescent substance use controlling for externalizing symptoms. *Psychology of Addictive Behaviors : Journal of the Society of Psychologists in Addictive Behaviors*, 31, 137–147. doi:10.1037/adb0000247 [PubMed: 28134539]
- Hussong AM, Jones DJ, Stein GL, Baucom DH, & Boeding S (2011). An internalizing pathway to alcohol and substance use disorders. *Psychology of Addictive Behaviors : Journal of the Society of Psychologists in Addictive Behaviors*, 25, 390–404. doi:10.1037/a0024519 [PubMed: 21823762]
- Irner TB (2012). Substance exposure in utero and developmental consequences in adolescence: a systematic review. *Child Neuropsychology*, 18, 521–549. doi:10.1080/09297049.2011.628309 [PubMed: 22114955]

- Jaspers M, de Meer G, Verhulst FC, Ormel J, & Reijneveld SA (2010). Limited validity of parental recall on pregnancy, birth, and early childhood at child age 10 years. *Journal of Clinical Epidemiology*, 63, 185–191. doi:10.1016/j.jclinepi.2009.05.003 [PubMed: 19682856]
- Keller PS, Granger DA, Tyler J, Gilbert LR, Haak EA, & Bi S (2015). Parental problem drinking is associated with children's adrenocortical reactivity to stress. *Journal of Child and Family Studies*, 24(10), 3145–3153. doi:10.1007/s10826-015-0118-6
- Kendler KS, Maes HH, Sundquist K, Ohlsson H, & Sundquist J (2014). Genetic and family and community environmental effects on drug abuse in adolescence: a Swedish national twin and sibling study. *American Journal of Psychiatry*, 171, 209–217. doi:10.1176/appi.ajp.2013.12101300 [PubMed: 24077613]
- Kent WJ, Sugnet CW, Furey TS, Roskin KM, Pringle TH, Zahler AM, & Haussler D (2002). The human genome browser at UCSC. *Genome research*, 12, 996–1006. [PubMed: 12045153]
- Kuhlman KR, Olson SL, & Lopez-Duran NL (2014). Predicting developmental changes in internalizing symptoms: Examining the interplay between parenting and neuroendocrine stress reactivity. *Developmental Psychobiology*, 56, 908–923. doi:10.1002/dev.21166 [PubMed: 24009085]
- Laurent HK, Leve LD, Neiderhiser JM, Natsuaki MN, Shaw DS, Harold GT, & Reiss D (2013). Effects of prenatal and postnatal parent depressive symptoms on adopted child HPA regulation: Independent and moderated influences. *Developmental Psychology*, 49, 876–886. [PubMed: 22686176]
- Lee CT, Padilla-Walker LM, & Memmott-Elison MK (2017). The role of parents and peers on adolescents' prosocial behavior and substance use. *Journal of Social and Personal Relationships*, 34, 1053–1069. doi:10.1177/0265407516665928
- Li C-Y, Mao X, & Wei L (2008). Genes and (common) pathways underlying drug addiction. *PLoS computational biology*, 4, e2. [PubMed: 18179280]
- Lippold MA, Davis KD, McHale SM, Buxton OM, & Almeida DM (2016). Daily stressor reactivity during adolescence: The buffering role of parental warmth. *Health Psychology*, 35(9), 1027–1035. doi:10.1037/hea0000352 [PubMed: 27175577]
- Lovallo WR, Cohoon AJ, Sorocco KH, Vincent AS, Acheson A, Hodgkinson CA, & Goldman D (2019). Early-life adversity and blunted stress reactivity as predictors of alcohol and drug use in persons with COMT (rs4680) Val158Met genotypes. *Alcoholism: Clinical and Experimental Research*.
- Lupien SJ, McEwen BS, Gunnar MR, & Heim C (2009). Effects of stress throughout the lifespan on the brain, behaviour and cognition. *Nature Reviews Neuroscience*, 10, 434. [PubMed: 19401723]
- Lynne-Landsman SD, Graber JA, Nichols TR, & Botvin GJ (2011). Trajectories of aggression, delinquency, and substance use across middle school among urban, minority adolescents. *Aggressive Behavior*, 37, 161–176. doi:10.1002/ab.20382 [PubMed: 21274853]
- MacArthur J, Bowler E, Cerezo M, Gil L, Hall P, Hastings E, ... Parkinson H (2017). The new NHGRI/EBI Catalog of published genome-wide association studies (GWAS Catalog). *Nucleic Acids Research*, 45, D896–D901. doi:10.1093/nar/gkw1133 [PubMed: 27899670]
- Marceau K, & Abel E (2018). Mechanisms of cortisol – substance use associations across the substance use progression: A theoretical synthesis and hypothesis generation through gene enrichment. *Neuroscience & Biobehavioral Reviews*, 92, 128–193. [PubMed: 29802855]
- Marceau K, Laurent HK, Neiderhiser JM, Reiss D, Shaw DS, Natsuaki MN, ... Leve LD (2015). Combined influences of genes, prenatal environment, cortisol, and parenting on the development of children's internalizing versus externalizing problems. *Behavior Genetics*, 45, 268–282. [PubMed: 25355319]
- Marceau K, Ram N, Neiderhiser JM, Laurent HK, Shaw DS, Fisher P, ... Leve LD (2013). Disentangling the effects of genetic, prenatal and parenting influences on children's cortisol variability. *Stress*, 16, 607–615. doi:10.3109/10253890.2013.825766 [PubMed: 23947477]
- Marceau K, Ruttle PL, Shirtcliff EA, Essex MJ, & Susman EJ (2015). Developmental and contextual considerations for adrenal and gonadal hormone functioning during adolescence: Implications for adolescent mental health. *Developmental Psychobiology*, 57, 742–768. [PubMed: 24729154]

- Marees AT, de Kluiver H, Stringer S, Vorspan F, Curis E, Marie-Claire C, & Derks EM (2018). A tutorial on conducting genome-wide association studies: Quality control and statistical analysis. *International journal of methods in psychiatric research*, 27, e1608. [PubMed: 29484742]
- Markus MT, Lindhout IE, Boer F, Hoogendijk THG, & Arrindell WA (2003). Factors of perceived parental rearing styles: the EMBU-C examined in a sample of Dutch primary school children. *Personality and Individual Differences*, 34, 503–519.
- Marsman R, Rosmalen JM, Oldehinkel A, Ormel J, & Buitelaar J (2009). Does HPA-axis activity mediate the relationship between obstetric complications and externalizing behavior problems? The TRAILS study. *European Child & Adolescent Psychiatry*, 18, 565–573. [PubMed: 19353232]
- McCarty CA, Wymbs BT, Mason WA, King KM, McCauley E, Baer J, & Vander Stoep A (2013). Early adolescent growth in depression and conduct problem symptoms as predictors of later substance use impairment. *Journal of Abnormal Child Psychology*, 41, 1041–1051. [PubMed: 23624771]
- Moss HB, Vanyukov M, Yao JK, & Kirillova GP (1999). Salivary cortisol responses in prepubertal boys: the effects of parental substance abuse and association with drug use behavior during adolescence. *Biological Psychiatry*, 45, 1293–1299. doi:10.1016/s0006-3223(98)00216-9 [PubMed: 10349035]
- Nederhof E, Bouma EMC, Oldehinkel AJ, & Ormel J (2010). Interaction Between Childhood Adversity, Brain-Derived Neurotrophic Factor val/met and Serotonin Transporter Promoter Polymorphism on Depression: The TRAILS Study. *Biological Psychiatry*, 68, 209–212. [PubMed: 20553751]
- Nederhof E, Marceau K, Shirtcliff EA, Hastings PD, & Oldehinkel AJ (2015). Autonomic and adrenocortical interactions predict mental health in late adolescence: The TRAILS Study. *Journal of Abnormal Child Psychology*, 43, 847–861. [PubMed: 25421943]
- Neumann A, Direk N, Crawford AA, Mirza S, Adams H, Bolton J, ... & Milaneschi Y (2017). The low single nucleotide polymorphism heritability of plasma and saliva cortisol levels. *Psychoneuroendocrinology*, 85, 88–95. [PubMed: 28843169]
- Oberlander TF, Weinberg J, Papsdorf M, Grunau R, Misri S, & Devlin AM (2008). Prenatal exposure to maternal depression, neonatal methylation of human glucocorticoid receptor gene (NR3C1) and infant cortisol stress responses. *Epigenetics*, 3, 97–106. [PubMed: 18536531]
- Oldehinkel AJ, Rosmalen JGM, Buitelaar JK, Hoek HW, Ormel J, Raven D, ... Hartman CA (2015). Cohort profile update: The TRacking Adolescents' Individual Lives Survey (TRAILS). *International Journal of Epidemiology*, 44, 76–76n. doi:10.1093/ije/dyu225 [PubMed: 25431468]
- Ormel J, Oldehinkel AJ, Sijtsma J, van Oort F, Raven D, Veenstra R, ... Verhulst FC (2012). The TRacking Adolescents' Individual Lives Survey (TRAILS): design, current status, and selected findings. *Journal of the American Academy of Child and Adolescent Psychiatry*, 51, 1020–1036. [PubMed: 23021478]
- Quinn PD, & Harden KP (2013). Differential changes in impulsivity and sensation seeking and the escalation of substance use from adolescence to early adulthood. *Development and Psychopathology*, 25, 223–239. [PubMed: 22824055]
- Rhee SH, Lahey BB, & Waldman ID (2015). Comorbidity among dimensions of childhood psychopathology: Converging evidence from behavior genetics. *Child Development Perspectives*, 9, 26–31. [PubMed: 26019716]
- Rossee Y (2012). *lavaan: An R package for structural equation modeling*. 2012, 48, 36.
- Ryan S, Schechter J, & Brennan P (2012). Perinatal factors, parenting behavior, and reactive aggression: Does cortisol reactivity mediate this developmental risk process? *Journal of Abnormal Child Psychology*, 40, 1211–1222. doi:10.1007/s10802-012-9649-0 [PubMed: 22644427]
- Salis KL, Bernard K, Black SR, Dougherty LR, & Klein D (2016). Examining the concurrent and longitudinal relationship between diurnal cortisol rhythms and conduct problems during childhood. *Psychoneuroendocrinology*, 71, 147–154. doi:10.1016/j.psyneuen.2016.05.021 [PubMed: 27266968]
- Stephens MA, & Wand G (2012). Stress and the HPA axis: role of glucocorticoids in alcohol dependence. *Alcohol Research*, 34, 468–483. [PubMed: 23584113]

- Author Manuscript
- Author Manuscript
- Author Manuscript
- Author Manuscript
- Step toe A, van Jaarsveld CH, Semmler C, Plomin R, & Wardle J (2009). Heritability of daytime cortisol levels and cortisol reactivity in children. *Psychoneuroendocrinology*, 34, 273–280. [PubMed: 18938040]
- Stroud CB, Chen FR, Doane LD, & Granger DA (2018). Early adversity and internalizing symptoms in adolescence: Mediation by individual differences in latent trait cortisol. *Development and Psychopathology*, 31, 509–524. doi:10.1017/S0954579418000044 [PubMed: 29559024]
- Tarter RE, Kirisci L, Kirillova G, Reynolds M, Gavalier J, Ridenour T, ... Vanyukov M (2013). Relation among HPA and HPG neuroendocrine systems, transmissible risk and neighborhood quality on development of substance use disorder: results of a 10-year prospective study. *Drug and Alcohol Dependence*, 127, 226–231. doi:10.1016/j.drugalcdep.2012.07.008 [PubMed: 22867990]
- Trucco EM, Hicks BM, Villafuerte S, Nigg J, Burmeister M, & Zucker RA (2016). Temperament and externalizing behavior as mediators of genetic risk on adolescent substance use. *Journal of Abnormal Psychology*, 125, 565–575. doi:10.1037/abn0000143 [PubMed: 26845260]
- Trucco EM, Villafuerte S, Hussong A, Burmeister M, & Zucker RA (2018). Biological underpinnings of an internalizing pathway to alcohol, cigarette, and marijuana use. *Journal of Abnormal Psychology*, 127, 79–91. doi:10.1037/abn0000310 [PubMed: 29172598]
- van Leeuwen AP, Creemers HE, Greaves-Lord K, Verhulst FC, Ormel J, & Huizink AC (2011). Hypothalamic–pituitary–adrenal axis reactivity to social stress and adolescent cannabis use: The TRAILS study. *Addiction*, 106, 1484–1492. [PubMed: 21631618]
- Zahn-Waxler C, Shirtcliff EA, & Marceau K (2008). Disorders of childhood and adolescence: gender and psychopathology. *Annual Review of Clinical Psychology*, 4, 275–303.
- Zhang X, Sliwowska JH, & Weinberg J (2005). Prenatal alcohol exposure and fetal programming: effects on neuroendocrine and immune function. *Experiments in Biological Medicine* (Maywood), 230, 376–388.
- Zucker RA, Hicks BM, & Heitzig MM (2016). Alcohol use and the alcohol use disorders over the life vcourse: A cross-level developmental review In Cicchetti D (Ed.), *Developmental Psychopathology*.

Figure 1.

Results from Structural Equation Models including Internalizing Trajectories

Only significant effects are depicted. Pathways including two (directional) bolded arrows indicate significant indirect effects. None of the longer indirect paths (e.g., including three arrows) were significant. *N* = 2,230 adolescents who provided data on at least one construct included in the model (see Table 1 for construct-specific *n*'s); missing data was accommodated using FIML. Unstandardized beta-weights are presented, with standard errors in parentheses.

* *p* < .05.

Figure 2.

Results from Structural Equation Models including Externalizing Trajectories

Only significant effects are depicted. Pathways including two (directional) bolded arrows indicate significant indirect effects. None of the longer indirect paths (e.g., including three arrows) were significant. N = 2,230 adolescents who provided data on at least one construct included in the model (see Table 1 for construct-specific n's); missing data was accommodated using FIML. Unstandardized beta-weights are presented, with standard errors in parentheses. The significant effect from prenatal stress to smoking was not significant in sensitivity analyses using a more focused measure of prenatal stress ($\beta = .006$, $SE = .03$, $p = .77$).

* $p < .05$

Table 1.

Sample Descriptive Statistics

	N	Mean	(SD)	Range
Prenatal stress	2119	1.47	(1.08)	0.00 – 4.00
Warm Parenting	2207	0.00	(0.81)	–5.03 – 1.56
Internalizing problems				
Age 11	2218	0.31	(0.18)	0.00 – 1.42
Age 13	2125	0.27	(0.19)	0.00 – 1.26
Age 16	1759	0.26	(0.20)	0.00 – 1.71
Externalizing problems				
Age 11	2223	0.26	(0.16)	0.00 – 1.18
Age 13	2127	0.24	(0.17)	0.00 – 1.34
Age 16	1764	0.26	(0.19)	0.00 – 1.20
Cortisol Reactivity	656	–0.03	(0.91)	–6.18 – 3.78
Smoking Frequency	1374	1.58	(2.38)	0.00 – 7.00
Drinking Frequency	1627	3.61	(3.58)	0.00 – 13.00
Marijuana Use Frequency	1632	0.68	(2.38)	0.00 – 13.00

Note. Smoking frequency = number of cigarettes consumed in the past month. Drinking frequency = number of times alcoholic drinks consumed in the past month. Marijuana use frequency = number of times used in the past month.

Table 2.

Zero-order correlations among study variables.

	PGS	Age 11			Age 11–16			Cortisol reactivity	Age 16 Smoking Frequency	Alcohol Frequency
		Prenatal stress	Warm Parenting	EXT intercept	EXT slope	INT intercept	INT slope			
Prenatal stress	–0.03 <i>1354</i>									
Warm Parenting	–0.01 <i>1347</i>	–0.02 <i>2207</i>								
EXT intercept	0.01 <i>1354</i>	0.07* <i>2230</i>	–0.40* <i>2207</i>							
EXT slope	0.01 <i>1354</i>	0.01 <i>2230</i>	0.07* <i>2207</i>	0.02 <i>2230</i>						
INT intercept	–0.03 <i>1354</i>	0.07* <i>2230</i>	–0.30* <i>2207</i>	0.50* <i>2230</i>	0.01 <i>2230</i>					
INT slope	0.01 <i>1354</i>	0.01 <i>2230</i>	0.06* <i>2207</i>	–0.05* <i>2230</i>	0.47* <i>2230</i>	–0.01 <i>2230</i>				
Cortisol reactivity	0.06 <i>556</i>	0.003 <i>656</i>	–0.02 <i>652</i>	–0.01 <i>656</i>	–0.12* <i>656</i>	–0.08* <i>656</i>	–0.04 <i>656</i>			
Smoking Frequency	0.02 <i>1086</i>	0.04 <i>1374</i>	–0.09* <i>1366</i>	0.29* <i>1374</i>	0.46* <i>1374</i>	0.07* <i>1374</i>	0.14* <i>1374</i>	–0.16* <i>536</i>		
Alcohol Frequency	0.02 <i>1285</i>	0.005 <i>1627</i>	–0.03 <i>1618</i>	0.14* <i>1627</i>	0.21* <i>1627</i>	–0.05* <i>1627</i>	–0.06* <i>1627</i>	–0.04 <i>628</i>	0.37* <i>1345</i>	
Marijuana Frequency	0.01 <i>1294</i>	–0.02 <i>1632</i>	–0.08* <i>1624</i>	0.20* <i>1632</i>	0.27* <i>1632</i>	–0.03 <i>1632</i>	–0.02 <i>1632</i>	–0.04 <i>634</i>	0.39* <i>1353</i>	0.24* <i>1609</i>

Note. *n*'s are provided in italics beneath each association.+ $p < .10$;* $p < .05$.