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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*

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Abstract

Background—Research has shown involvement of hormones of the hypothalamic pituitary adrenal (HPA) axis and hypothalamic pituitary gonadal (HPG) axis in the regulation of behaviors that also contribute to SUD risk and its intergenerational transmission. Neighborhood environment has also been shown to relate to hormones of these two neuroendocrine systems and behaviors associated with SUD liability. Accordingly, it was hypothesized that 1) parental SUD severity and neighborhood quality correlate with activity of the HPG axis (testosterone level) and HPA axis (cortisol stability), and 2) transmissible risk during childhood mediates these hormone variables on development of SUD measured in adulthood.

Method—Transmissible risk for SUD measured by the Transmissible Liability Index (TLI; Vanyukov et al., 2009) along with saliva cortisol and plasma testosterone were prospectively measured in boys at ages 10–12 and 16. Neighborhood quality was measured using a composite score encompassing indicators of residential instability and economic disadvantage. SUD was assessed at age 22.

Results—Neither hormone variable cross-sectionally correlated with transmissible risk measured at ages 10–12 and 16. However, the TLI at age 10–12 predicted testosterone level and cortisol stability at age 16. Moreover, testosterone level, correlated with cortisol stability at age 16, predicted SUD at age 22.

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Conclusion—HPA and HPG axes activity do not underlie variation in TLI, however, high transmissible risk in childhood predicts neuroendocrine system activity presaging development of SUD.

Keywords

addiction; vulnerability; genetics; androgen; stress hormone; etiology

1. Introduction

Up to 100% of genetic variance and 80% of phenotypic variance are congenerous to the risk for developing all categories of substance use disorder (SUD; Kendler et al., 2003; Tsuang, et al., 1998; Vanyukov et al., 2003b). Whereas SUD risk is phenotypically and genetically correlated with disinhibitory and externalizing behavior in childhood (Elkins et al., 2007; Fu et al., 2002; Grove et al., 1990; Krueger et al., 2002; Iacono et al., 2008), many additional psychological characteristics are also related to risk for SUD (Bechara, 2005; Elkins et al., 2006; Giancola et al., 1996; Spear, 2000) of which a subset are indicators of a continuous latent trait representing the component of SUD liability that is transmissible across generations (Vanyukov et al., 2003a,b, 2009). Termed the *transmissible liability index (TLI)*, the score on this trait is highly heritable (Hicks et al., 2012; Vanyukov et al., 2009) and predicts all SUD categories in adults (Ridenour et al., 2011) as well as SUD outcome between childhood and adulthood (Kirisci et al., 2009). Notably, the interval between first cannabis experience and cannabis use disorder diagnosis negatively covaries with severity of transmissible risk. Not only is the TLI superior to parental SUD as a predictor of SUD in offspring (Ridenour et al., 2011) it bypasses the need for intrusive questioning in children about substance use and its adverse consequences in their parents. To date, five age-specific computer adaptive versions of the TLI have been validated for children, adolescents and young adults using the Web platform (Kirisci et al., 2012) thereby enabling quantitative measurement of this component of SUD risk in less than ten minutes.

The biological mechanisms underlying variation in transmissible risk remain, however, to be elucidated. One fruitful line of research pertains to neuroendocrine mechanisms based on findings that cortisol level regulated by the HPA axis is related to behaviors that are widely recognized to amplify risk for SUD. Negative results obtained in one study notwithstanding (Sondeijker et al., 2007), the evidence accrued to date points to an association between attenuated cortisol level and externalizing disorder in youths (Fairchild et al., 2008; Kobak et al., 2009; McBurnett et al., 2000; Moss et al., 1995; Shoal et al., 2003; Snock et al., 2004; Van Goozen, 2005; Virkkunen, 1985). Moreover, aggressive behavior, the most ubiquitous feature of externalizing disorder, is also associated with attenuated cortisol level in boys whose fathers have early age onset SUD (Vanyukov et al., 1993); this SUD variant has high genetic loading evinced at a young age by disinhibited and antisocial behavior (Cloninger et al., 1981). Notably, frequency of marijuana consumption during adolescence also covaries negatively with cortisol level (Huizink et al., 2006; Moss et al., 1999). In view of these results showing that externalizing behavior is a salient characteristic of transmissible risk for SUD and marijuana use is related to low cortisol level, it is hypothesized that HPA axis activity is among the mechanisms underlying variation in severity of transmissible risk.

Cortisol production, however, correlates negatively with testosterone level (Blanchard et al., 1993; Plymate et al., 1989). Research into SUD etiology has to date not taken into account this synergy between the HPA and HPG neuroendocrine systems (Viau, 2002) even though psychological characteristics frequently reported to be associated with SUD risk (e.g., impulsivity, aggressiveness, fearlessness) are similarly manifest by individuals having low cortisol and high testosterone concentration (see Terberg et al., 2009 for review). Whereas

not all studies have reported an association between testosterone level and aggressivity (Constantino et al., 1993), research conducted on a subset of the present sample has shown that disruptive behavior in childhood mediates the association between testosterone level and SUD in adulthood (Reynolds et al., 2007). Furthermore, social dominance striving in concert with indifference to societal norms in mid-adolescence mediates the association between testosterone level in early adolescence and SUD in adulthood (Tarter et al., 2009). Moreover, elevated testosterone concentration has been reported in aggressive youths (Scerbo and Kolko, 1994) and men qualifying for Type 2 (antisocial) alcoholism (Dabbs et al., 1995). Indeed, it has been shown that testosterone level mediates the relation between SUD in parents and sensation seeking motivation in their adolescent sons (Kirillova et al., 2001). Importantly, parental SUD load (number of affected parents) is related to sexual maturation rate in boys that in turn predicts deviant peer affiliations, disruptive behavior disorders and subsequently SUD (Kirillova et al., 2008). Accordingly, the hypothesis is advanced that high testosterone concentration also underlies transmissible risk for SUD.

Regulation of the HPA axis (Bartels et al., 2003) and puberty onset (Silventoinen et al., 2008) are significantly heritable. Socioeconomic disadvantage is also related to HPA (Evans and English, 2002; Lupie et al., 2001) and HPG axis activity (Schaal et al., 1996; Tarter et al., 2009) as well as risk for SUD (Compton et al., 2007; Lopez-Quintero et al., 2011). Notably, genetic factors account for most variance on the TLI presaging SUD (Hicks et al., 2012; Vanyukov et al., 2009), and severe scores on this index cluster among youths domiciling in disadvantaged neighborhoods (Ridenour et al., under review). Accordingly, as shown in Figure 1, the theoretical model guiding this investigation, it was hypothesized that parental SUD correlated with neighborhood quality predicts hypothalamic pituitary adrenal (HPA) axis and hypothalamic pituitary gonadal (HPG) axis activity in children underlying transmissible risk predisposing to SUD.

2. Methods

2.1 Participants

The sample was comprised of boys enrolled in the Center for Education and Drug Abuse Research (CEDAR), a longitudinal family/high-risk study of SUD etiology (Tarter and Vanyukov, 2001). The boys (N=451) were ascertained at age 10-12 through proband fathers who either qualified for a DSM-III-R lifetime diagnosis of SUD concomitant to consumption of illegal drugs or had no axis 1 psychiatric disorder. DSM-III-R criteria were utilized because this longitudinal investigation was initiated before publication of the DSM-IV. Boys who completed the baseline study were tracked for follow-up study when they attained 16 and 22 years of age. Current SUD diagnoses were determined at age 22. The presence of any of the following characteristics disqualified potential subjects from participation: i) WISC-III-R IQ below 80, ii) inability to comprehend English, iii) medical history of neurological injury requiring hospitalization, iv) chronic life threatening medical illness, and v) psychosis. From the baseline sample (N=451), 138 participants either refused to participate or could not be located for the outcome evaluation at age 22. Thirty-seven subjects had not yet attained age 22 and thus were not included in the analyses. Accordingly, the analyses were conducted on 276 subjects (451 baseline minus 138 attrition minus 37 not old enough to participate).

Several methods were employed to accrue the sample. Public service announcements in the electronic media, random telephone calls, posters, and a market research firm were the primary methods used to identify potential participants in metropolitan Pittsburgh. Approximately 25% of the sample had fathers who recently completed treatment for SUD. It is noteworthy that this sample of youths is not atypical. Cannabis, the most frequently consumed illegal drug, had a lifetime prevalence of 34.4% in this sample at age 16

compared to 34.5% of tenth grade students in the 2011 Monitoring the Future survey (Johnston et al., 2012).

Table 1 presents the characteristics of the sample at baseline. As can be seen, grade in school, neighborhood quality, severity of transmissible liability index (TLI), severity of SUD in parents, ethnicity, and the neuroendocrine variables are not different between the retained and attrited segments of the sample. Full scale IQ is lower in the subjects who attrited ($F = 18.91$, $p < .05$); however, intelligence level of both retained and attrited subjects is in the normal range.

A test of equivalence (Rowe, 2007) of retained and attrited subjects was also conducted to evaluate attrition. To demonstrate equivalence, we computed the probability of containing the whole of the 95% confidence interval of difference between two groups within the limits of the “equivalence zone.” The equivalence zone reveals how much the variable needs to change before there is a realistic possibility of practical consequences. In this study, we set limits of the equivalence zone to $\pm \frac{1}{3} \sigma$ for the variables. The results, shown in Table 1, indicate that there is no difference large enough to have practical significance. Based on these analyses, it is concluded that attrition did not bias the results.

2.2 Instrumentation

2.2.1 SUD severity in fathers and mothers—The parents were administered an expanded version of the Structured Clinical Interview for Diagnosis (SCID; Spitzer et al., 1990). The SCID was expanded in our research to more comprehensively characterize antisocial behavior and legal history. The diagnostic formulation was performed by a committee consisting of a psychiatrist certified in addiction psychiatry, another psychiatrist or a psychologist, and master-level clinical associates who conducted the interviews. Following documentation of current and lifetime SUDs, item response theory (IRT) methodology was employed using established procedures (Kirisici et al., 2006) to derive a continuous scale that quantified SUD severity. The SUD scores of the mother and father were summed to document severity of parental SUD.

2.2.2 Neighborhood quality—Several indicators were recorded from the most recent census to assess neighborhood quality. Five variables (total income below poverty level, proportion of households headed by a single female, proportion of households receiving public assistance, proportion of households having an unemployed parent, number of residents below 18 years of age) were aggregated into a composite scale measuring *economic disadvantage*. Two variables (proportion of households on the block having new occupants since the last census and proportion of vacant residential units) were combined to document *residential instability*. The composite scores were summed to index neighborhood quality. A high score has previously been shown to predict cannabis use disorder (Ridenour et al., 2009).

2.2.3 Testosterone level (ages 10-12 and 16)—Blood was drawn between 8-9 a.m. Serum was analyzed in triplicate by radioimmunoassay using DSL-4000 Active kits (Diagnostic Systems Laboratories). The intra-assay coefficients of variation are 9.6%, 8.1% and 7.8 % at 0.94, 7.01 and 19.71 ng/ml of testosterone. The inter-assay coefficients are 8.6%, 9.1% and 8.4% at 0.7, 5.95 and 16.06 ng/ml of testosterone.

2.2.4 Cortisol level (age 10-12 and 16)—Cortisol concentration in saliva was measured at two timepoints. The first sample was taken between 9-10 a.m. while placing scalp electrodes in preparation for an event related potentials study. The second sample was

obtained 50-60 minutes later upon completion of this study. The difference score reflecting stability of cortisol level was used in the analyses.

The methods used to acquire and analyze the saliva samples have been previously described (Pajer et al., 2001; Yao et al., 1998). First, a mouth rinse was performed by the boys to remove debris. Next, they chewed a wax plug to stimulate saliva flow. Between 2-5 ml of saliva were then expectorated into a plastic tube and the sample was stored at -80°C. The assay was conducted in batches. Saliva samples were defrosted and purified from the debris by centrifugation (1,500 g) at room temperature. Purified saliva (0.5 ml) was lyophilized and then reconstituted with 100 µl of cortisol-free serum (DELFI diluent). Using a commercial fluoro-immunoassay (DELFI: Wallac Oy), the reconstituted samples (25 µl) were processed in triplicate in streptavidin coated 96-well microplates. The intra-assay coefficients of variation were 7.0%, 4.5%, and 5.0% at concentrations of 3.3, 8.3, and 30 µg/dl. The inter-assay coefficients were 5.0%, 3.5% and 4%.

2.2.5 Transmissible liability index (TLI) (ages 10-12 and 16)—The TLI was derived when the boys attained ages 10-12 and 16 using the method described by Vanyukov et al. (2009). Psychometric analyses have shown that the TLI has construct, discriminative, incremental, concurrent, and predictive validity (Kirisci et al., 2009; Ridenour et al., 2011). Thus, this measure is an accurate indicator of transmissible risk. Indeed, one study revealed that the TLI (age 10-12) has an odds ratio of 2.27 for predicting cannabis use disorder at age 22 (Kirisci et al., 2009).

Development of the TLI (Vanyukov et al., 2003a,b; 2009) began with selection of items from a large pool of questionnaires and interviews. The items were assigned to constructs implicated in the empirical literature to be associated with SUD risk. This procedure was implemented by a faculty panel at CEDAR. The provisional constructs were subsequently verified using exploratory factor analysis (EFA) followed by confirmatory factor analysis (CFA). Sons of SUD+ and SUD- men were then compared on each construct. The constructs that were significantly different were retained and their items submitted to EFA and CFA to ensure unidimensionality (a prerequisite for item response theory modeling) and to further reduce the item set. Finally, the items were submitted to item response theory analysis for calibration. The resulting item set is shown in Supplementary Materials¹.

2.2.6 Substance use disorder (SUD) in the boys (age 22)—The clinical committee performed the diagnostic formulation blind to SUD status of the parents. Current diagnosis of SUD (abuse or dependence) other than nicotine dependence was present in 37.3% of the sample. This high SUD rate is expected considering that half the sample had one or more parents with SUD.

2.3 Procedure

Written informed consent using the procedure approved by the University of Pittsburgh IRB was obtained from the biological parents prior to administering the research protocols when the boys were 10-12 and 16 years of age. Informed consent was provided by the boys prior to initiating the age 22 evaluation. Before commencing the evaluation an alcohol breathalyzer test and urine drug screen were conducted to ensure that the results were not confounded by substance-induced altered physiological state. A positive result required rescheduling the participant. After completing the research protocol, the subjects were debriefed in a private room. Lastly, they were compensated for their time at the rate of \$10/hr. before discharge from the laboratory.

¹Supplementary material can be found by accessing the online version of this paper at <http://dx.doi.org> and by entering doi: ...

2.4 Statistical Analysis

Path analysis with dichotomous outcome was conducted. Model parameters were estimated using Mplus (Muthen and Muthen, 2001). Mplus uses the weighted least square parameter estimation method with diagonal weight matrix with robust standard errors. Four indices of model fit were used: 1) χ^2 goodness-of-fit index, 2) root mean square error of approximation (RMSEA), 3) comparative fit index (CFI), and, 4) Tucker-Lewis index. A nonsignificant χ^2 value ($p > .05$) indicates that the data are consistent with the model. Mediated paths were tested using the method described by Sobel (1982) using the following formula:

$$z = \frac{b_1 b_2}{\sqrt{b_2^2 \sigma_{b_1}^2 + b_1^2 \sigma_{b_2}^2}},$$

where b_1 is the regression coefficient between predictor and mediator, b_2 is the regression coefficient between mediator and dependent variable, and σ^2 is the square of the estimate of the standard error of the corresponding regression coefficient. All of the variables used in the path analysis were manifest variables.

3. Results

The results of model testing are shown in Figure 2. Excellent fit was obtained between the model and data ($X^2 = 17.36$, $df = 16$, $p = .38$, $RMSEA = < .013$, $Tucker-Lewis Index = .99$, $CFI = .99$). The pattern of associations among the variables reflects three pathways to SUD.

Pathway a (shown in red) traces from neighborhood quality (correlated with and severity of parental SUD) to TLI at ages 10-12 and 16 years of age culminating in SUD at age 22.

Pathway b (shown in blue) traces from parental SUD severity to TLI in childhood, to cortisol stability and testosterone level in mid-adolescence, and SUD in adulthood. Notably, TLI at age 10-12 mediates the association between parental SUD severity and testosterone level at age 16 ($b = .08$, $z = 2.04$; $p = < .042$) and testosterone (age 16) in turn mediates the relation between TLI (age 10-12) and SUD at age 22 ($b = .06$, $z = 2.98$, $p = .0001$).

Pathway c (shown in green) traces from poor neighborhood quality associated with elevated testosterone level at ages 10-12 and 16 resulting in SUD at age 22. As can be seen in Figure 2, testosterone level is correlated with cortisol stability at ages 10-12 and 16. Moreover, each variable predicts the other between ages 10-12 and 16.

4. Discussion

This study is a continuation of an ongoing research program directed at elucidating intergenerational transmission of liability to SUD (Vanyukov and Tarter, 2000; Vanyukov et al., 2003a,b; Kirillova et al., 2001, 2008; Kirisci et al., 2009; Vanyukov et al., 2009; Tarter et al., 2011). Previous research exploring the role of the HPG axis has shown that parental SUD is associated with faster rate of sexual maturation and higher testosterone level in male offspring that predispose to affiliation with deviant peers and behavior undercontrol leading to SUD (Kirillova et al., 2008). It has also been demonstrated that the HPA axis is involved in development of SUD. Specifically, attenuated cortisol reactivity is associated with antisocial and externalizing behaviors that are well-known to presage SUD (Vanyukov et al., 1993; Moss et al., 1995; Fairchild et al., 2008).

This study extends our prior research by demonstrating that the relationship between parental SUD severity and these hormonal variables in their children is traceable through the child's transmissible risk. In addition, it was shown in this study that neighborhood quality, correlated with parental SUD severity, is significantly related to child's testosterone level which is a predictor of SUD in adulthood. Whereas cortisol stability and testosterone level

are correlated and cross-predictive of each other between childhood and mid-adolescence, neither hormonal variable correlates with TLI cross-sectionally during childhood and adolescence. However, the TLI at age 10-12 predicts both variables at age 16 thereby indicating that they are involved in the system of biological mechanisms mediating behaviors relating transmissible risk and development of SUD. It is noteworthy in this regard that deviant socialization mediates the relationship between TLI in childhood and SUD in adulthood (Tarter et al., 2011). Moreover, the finding that high testosterone level potentiates aggressivity as a tactic to assert and maintain social status in youths residing in disadvantaged neighborhoods (Schaal et al., 1996) parallels results of our previous research on this sample showing that neighborhood quality mediates the relation between testosterone level and striving for social dominance presaging SUD (Tarter et al., 2009). Significantly, testosterone level also negatively covaries with the threshold for verbal and non-verbal aggression following provocation (Olwens et al., 1988). Thus, consistent with research on humans and other animals (see Archer, 2006 for review), the psychological characteristics comprising transmissible risk for SUD in context of adverse social environment is theorized to promote adaptation of the HPA and HPG neuroendocrine systems featured by low reactivity to stress and elevated androgen production.

Demonstrating that transmissible risk for SUD in children predicts neuroendocrine systems activity in adolescence has potentially important ramifications for elucidating the neurobiological mechanisms underlying the psychological predisposition to SUD. Romeo (2005) points out, for example, that cortisol and testosterone impact on neurogenesis, synaptogenesis and modulation of neuronal excitability. An intriguing body of findings from research conducted on animals and humans indicates that elevation of stress hormones and gonadotropins alter neurological processes during puberty and concomitantly regulation of emotion and behavior predisposing to substance use (Spear, 2000). Significantly, testosterone and cortisol bind to receptors in the amygdala, hippocampus, and prefrontal cortex (Herman and Cullinan, 1997). Results of neuroimaging studies reveal dysfunction in these regions among youth who are at high risk for SUD or are already engaging in substance use. For example, reduction in gray matter in prefrontal cortex has been recently reported in youths with conduct disorder (Fairchild et al., 2011) which commonly presages SUD. In effect, a heretofore unrecognized but potentially important etiologic pathway to SUD may involve hormone-induced injury of brain structures (resulting from combined influence of transmissible risk and adverse environment context) promoting a motivational propensity for substance use and subsequently SUD.

It is also noteworthy that social cognition, frequently reported in conduct disordered and aggressive youths, and previously observed to mediate the relationship between behavior disinhibition in adolescence and SUD in adulthood (Kirisci et al., 2004), may also partly manifest from hormone-induced neurobiological dysfunction. Considering that high testosterone level is associated with weak functional integration of the cortex and subcortical structures (Schutter and Van Honk, 2004; Schutter and Van Honk, 2005), it is plausible to theorize that poor executive cognitive control of behavior (frequently reported in youths at high risk for SUD) partly results from injury caused by persistent elevation of androgen and stress hormones during child and adolescent development. It is also well-known that substance use typically begins during adolescence and first diagnosis of SUD usually occurs before the brain has fully matured; hence, it would appear important for future endocrinological research to take into account the possibility that environment, stress and individual vulnerability resulting in substance consumption additionally modifies HPA/HPG axes regulation that in turn exacerbates the psychological propensity for non-normative socialization leading to SUD.

Several limitations of this study are noted. The androgenic pathways to SUD observed in boys cannot be assumed to be present in girls. Also, testosterone level may vary due to heterogeneity in the onset of puberty. It should be noted, however, that our prior research has documented a relationship between rate of physiological maturation and SUD liability that is mediated by several facets of behavior self-regulation (Kirillova et al., 2001, 2008). Also, the outcome variable was any type of SUD diagnosis with the exception of nicotine dependence. Nicotine dependence was not included because consumption is not usually associated with behavioral or social maladjustment. Furthermore, attrition may have biased the results, even though the scores on the predictor variables at baseline did not distinguish retained and attrited subjects. Lastly, neighborhood quality was assessed only at baseline. Hence, it was not possible to determine whether relocating to a different neighborhood between baseline and outcome assessments impacted on the results. Lastly, it should be noted that the behavioral and hormone variables may have been influenced by substance consumption during adolescence presaging SUD. Clearly, future research needs to delineate the developmental pathways to SUD taking into account the possibility that substance use exacerbates liability, potentiating risk for SUD.

These limitations notwithstanding, the present study demonstrated that HPA and HPG axes hormones do not account for variation in psychological characteristics comprising transmissible risk. However, high transmissible risk in childhood predicts cortisol stability and elevated testosterone concentration in adolescence that in turn predicts SUD in young adulthood. Significantly, a poor-quality neighborhood is associated with elevated testosterone level that in turn predicts SUD. Whether these hormones influence neurological functioning, and accordingly impair cognitive control of behaviors and emotions predisposing to SUD, would appear to be an important topic of future research.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.
Theoretical model guiding this investigation.

Figure 2.

Results of path analysis depicting significant associations and standardized beta coefficients; see text for explanation of the coding of the paths. Mediated paths (not shown) are discussed in the text.

Table 1
Characteristics of participants at 10-12 years of age who completed the age 22 follow-up with participants who attrited

		Equivalence Test Between Groups									
		Retained (N=276)		Attrited (N=175)		Difference Score Between The Groups		95% CI for Group Difference		Probability of Equivalence	
		M	(SD)	M	(SD)						
Grade in school		4.6	1.1	4.5	1.0	.1		(-.10, .31)		.78	
Neighborhood quality		-.02	.97	.11	1.1	-.13		(-.33, .07)		.68	
Parental SUD severity		.14	.52	.18	.55	-.04		(-.15, .07)		.95	
Transmissible liability index		-.04	.99	.12	1.0	-.16		(-.36, .04)		.54	
Cortisol level (µg/dl) change (T ₁ -T ₂)		54.27	150.18	42.17	139.35	12.1		(-17.15, 41.35)		.81	
Testosterone concentration (ng/ml)		1.06	1.39	.98	1.23	.08		(-.19, .35)		.64	
Ethnicity		n	%	n	%	2.7		(-5.54, 10.94)		.93	
• European American		211	76.4	129	73.7						
• African American		57	20.7	38	21.7						
• Other		8	2.9	8	4.6						