Interactive Effects of the Serotonin Transporter 5-HTTLPR Polymorphism and Stressful Life Events on College Student Drinking and Drug Use

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**Background:** A common functional polymorphism, 5-HTTLPR, in the serotonin transporter gene has been associated with heavy drinking in college students. We examined this polymorphism as it interacted with negative life events to predict drinking and drug use in college students.

**Methods:** Daily reports of drinking and drug use obtained using a daily web-based survey were combined with self-reports of past-year negative life events and 5-HTTLPR genotypes in a regression analysis of alcohol and nonprescribed drug use in a sample of 295 college students.

**Results:** Genotype and negative life events significantly interacted in relation to drinking and drug use outcomes. Individuals homozygous for the short (s) allele who experienced multiple negative life events in the prior year reported more frequent drinking and heavier drinking, stronger intentions to drink, and greater nonprescribed drug use. In individuals homozygous for the long (l) allele, drinking and drug use were unaffected by past-year negative life events. Heterozygous subjects showed drinking outcomes that were intermediate to the two homozygous groups.

**Conclusions:** The 5-HTTLPR s-allele is associated with increased drinking and drug use among college students who have experienced multiple negative life events. The s-allele carriers may be at risk for a variety of adverse behavioral outcomes in response to stress.

**Key Words:** Gene-environment interaction, heavy drinking, alcohol, SLC6A4, 5-HTTLPR, daily diary

Heavy drinking among college students is widespread and has been the recent focus of considerable public and scientific attention (Goldman et al. 2002). College is normatively a time of heavy drinking (Chen and Kandel 1995), which has been associated with negative consequences, including academic, interpersonal, and legal problems (Bucholz 1990; Engs and Hanson 1988; Syre et al. 1999; Wechsler et al. 1994). Further, longitudinal studies suggest that whereas most students “mature out” of heavy drinking, some students continue to drink heavily following college graduation (Gotham et al. 1997; Schulenberg et al. 1996; Zucker et al. 1995), putting them at risk for the development or persistence of alcohol use disorders. A better understanding of the determinants of heavy drinking among college students has important public health implications.

Serotonin (5-HT), a monoamine neurotransmitter with multiple sites of action, affects mood, sensory processing, cognition, and sleep. Animal and human studies suggest that central 5-HT activity may influence alcohol consumption (George et al. 1997; Heinz et al. 2003; Higley and Linnoila 1997; Virkkunen and Linnoila 1997) and is moderated by acute and chronic effects of alcohol (Heinz et al. 2000; Smith and Weiss 1999; Yoshimoto et al. 1992, 2000). The 5-HT transporter (5-HTT) plays a central role in the serotonergic system by regulating the reuptake of 5-HT following synaptic release. A common functional polymorphism, the short (s) versus long (l) allele in the 5-HTT linked promoter region (5-HTTLPR) of SLC6A4, the gene encoding 5-HTT, involves a variable number of tandem repeats (14- vs. 16-repeat elements) upstream of the transcription start site and modulates transcriptional activity of SLC6A4 and levels of 5-HTT messenger RNA (mRNA) (Bradley et al. 2005; Greenberg et al. 1999; Heils et al. 1996; Hranilovic et al. 2004; Lesch et al. 1996; Mortensen et al. 1999).

Recent studies highlight the potential importance of this functional variation for behavioral responses in young adults, including alcohol consumption. A higher frequency of the 5-HTTLPR s-allele has been observed in young adults with high alcohol tolerance (Turker et al. 1998) and college students homozygous for the s-allele report more episodes of binge drinking and drinking to “get drunk” (Herman et al. 2003). Additionally, the effects of the s-allele have been shown to interact with exposure to life stressors to increase the risk of depression among college-age adults (Casp et al. 2003). Using a prospective study design, Caspi et al. (2003) found that, while the 5-HTTLPR genotype did not directly predict the onset of depression by age 26, exposure to stressful life events in combination with the s-allele doubled the likelihood of developing depression. Subsequently, three studies (Eley et al. 2004; Kaufman et al. 2004; Kendler et al. 2005) have replicated the 5-HTTLPR genotype x environment interaction on risk for depressive symptoms, while two studies of middle-aged and older adults failed to replicate the finding (Gillespie et al. 2005; Surtees et al. 2006).

The present research examined whether an interaction of the 5-HTTLPR polymorphism and negative life events is related to other potentially problematic behaviors in college students (i.e., alcohol or drug use). We employed a longitudinal observational study of college student drinking that incorporated month-long assessments of drinking and drug use using a web-based diary during each academic year. In this article, we report the results for the first 2 years of data collection.
Methods and Materials

Subjects

College students taking part in a multiyear longitudinal study of daily experience and health-related behaviors, including alcohol and other drug use, were invited via e-mail during their second year of data collection to participate in a genetic study by providing a mouthwash oral rinse for collection of DNA. Among the 535 eligible participants whose participation was solicited for the genetic study, 345 (64.5%) provided consent to collect genetic data, 17 (3.2%) declined, and 173 (32.3%) did not respond. Study participants who provided DNA samples did not differ from those who did not on nearly all variables, including age, in college, gender, race, negative life events, and psychological and alcohol/drug behavioral measures. One exception was that individuals who provided DNA samples reported higher levels of heavy drinking in the second year of data collection ($p < .05$) compared with those not providing DNA.

Students enrolled in our multiyear study were very similar to the introductory psychology subject pool from which our subjects were recruited, as well as the university student population in general. Our sample was 86% Caucasian versus 85% for the subject pool and 83% for the university in general. The mean math/verbal Scholastic Assessment Test (SAT) scores for our sample was 1167 versus 1168 for the university-wide incoming class of 2003. Based on surveys completed by introductory psychology students ($n = 2414$), our participants were also similar to the general subject pool in terms of age, $t = .34, p = .7$; hours worked per week, $t = -0.019, p = .9$; and number of times they reported drinking in the past month, $t = 1.5, p = .13$.

To avoid potential confounds due to racial/ethnic differences in allele frequencies and drinking behavior, we limited our analysis to non-Hispanic Caucasian subjects ($n = 302$) who provided at least 1 year of usable data ($n = 295$). Of the 295 subjects who constituted our sample, 54% were women, and at study entry, 59% percent were freshmen, 33% were sophomores, and 8% were upper classmen. The average age was 18.7 ± 0.8 years. Most subjects (89%) lived in coeducational campus dormitories and all were unmarried. All subjects gave written informed consent to participate in the protocol, which was conducted under guidelines approved by the University of Connecticut Institutional Review Board.

Psychological and Behavioral Measures

In each of the 2 academic years, participants were asked to complete an annual baseline assessment 1 to 2 months following the start of the fall or spring semester by logging onto a secured website using a unique password. Annual baseline assessment measures included a 36-item checklist, the Life Events Scale for Students (LESS) (Clements and Turpin 1996; Linden 1984), an empirically derived inventory of stressful life adapted from the Social Readjustment Rating Scale (Holmes and Rahe 1967) for use with college students. In this checklist, students indicated which stressful life events happened to them over the past year (e.g., broke up with boy/girlfriend, failed a course, family health problems, financial problems). Of the 36 events included in the checklist, only the 25 unambiguously negative events were retained for analysis to be comparable with Caspi et al (2003). Eleven events that were independently identified by three of the investigators (HT, SA, and TSC) to have either a positive or ambiguous affective valence (e.g., getting your own car, finding a part-time job, family get-togethers) were excluded. The 25 items were summed such that a score of 0 (to 25) indicated that none (to all) of the stated negative events occurred in the past year. The range on this measure was 0 to 15 ($M = 4.25; SD = 2.90$) for year 1 and 0 to 14 ($M = 3.67; SD = 2.73$) for year 2.

In addition, several personality questionnaires were administered in the initial annual assessment to obtain measures of sensation seeking and neuroticism for use as covariates. For a measure of sensation seeking, participants rated the eight items of the excitement seeking subscale of the NEO Personality Inventory-Revised (Costa and McCrae 1992). Responses were made using seven-point Likert-type scales (1 = extremely disagree to 7 = extremely agree); items were averaged together ($\alpha = .59$) and scores ranged 2.63 to 6.88 ($M = 5.26, SD = .80$). Using the same response scale, participants also rated 12 items of the neuroticism subscale of the 60-item NEO Five-Factor Inventory (Costa and McCrae 1992). Items were averaged together ($\alpha = .87$) and ranged from 1.08 to 6.58 ($M = 3.57; SD = 1.04$). Lastly, we administered the 13-item short form of the Beck Depression Inventory (BDI) (Beck and Beck 1972) each year as a potential mediator or moderator of genetic effects on drinking. Beck Depression Inventory scores were summed (year 1, $M = .83$; year 2, $M = .84$) and were generally low (year 1, range = 0–22, $M = 4.47$, $SD = 4.14$; year 2, range = 0–24, $M = 3.66$, $SD = 4.05$), comparable with other U.S. college student samples (Anderson 1999). Evidence for the psychometric properties of these scales can be found in their source articles.

Approximately 2 weeks after completion of the annual baseline assessment, participants used a secure website to complete a 5-minute daily survey between 3:30 pm and 7:00 pm for 30 days. Each survey contained questions about events from the past day, including questions about drinking and drug use. Specifically, students were asked to report the number of alcoholic drinks they consumed the previous night (one drink equaled one 12-oz can or bottle of beer, one 5-oz glass of wine, one 12-oz wine cooler, or a 1.5-oz measure of liquor straight or in a mixed drink), whether they intended to drink alcohol later that night (1 = not at all; 7 = definitely), and whether they “used any non-prescribed drugs other than alcohol last night (e.g., marijuana, ecstasy, amphetamines).” From these data, we computed four aggregate daily variables for each person: drinking frequency, to assess the regularity with which students drank one or more drinks; heavy drinking frequency, to assess the regularity with which students drank one or more alcoholic drinks they consumed the previous night (one drink equaled one 12-oz can or bottle of beer, one 5-oz glass of wine, one 12-oz wine cooler, or a 1.5-oz measure of liquor straight or in a mixed drink), whether they intended to drink alcohol later that night (1 = not at all; 7 = definitely), and whether they “used any non-prescribed drugs other than alcohol last night (e.g., marijuana, ecstasy, amphetamines).” From these data, we computed four aggregate daily variables for each person: drinking frequency, to assess the regularity with which students drank one or more drinks; heavy drinking frequency, to assess the regularity of “binge drinking” (days on which five or more drinks were consumed by men and four or more were consumed for women); mean drinking intentions, to assess psychological awareness of their plans to drink alcohol; and drug use frequency, to assess the frequency of nonprescribed drug use. Frequency variables reflected proportion scores (i.e., number of days on which each behavior occurred divided by total days surveyed) to take into account within-person differences in the number of daily surveys completed, which ranged from 15 (required minimum) to 30 (maximum) across both years (year 1, $M = 25.7, SD = 3.5$; year 2, $M = 25.7, SD = 3.7$).

The completion rate for the study measures was good. Of the 302 non-Hispanic Caucasian subjects eligible for analysis, 267 (88%) successfully completed all questionnaires and diary measures to criterion (15 of 30 usable records) across both years; 28 additional subjects (9%) successfully completed all measures for one but not both years ($n = 15$, year 1; $n = 13$, year 2). Seven individuals (2%) had poor quality data across both years and were omitted entirely from analyses, leaving a sample of 295 with usable data for at least in 1 year. The 5-HTTLPR allele frequencies for the seven subjects excluded entirely and the other subjects omitted selectively across years due to insufficient responding.

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did not differ from the included subjects (\( \chi^2 (2) = 1.23, p = .54 \)). To retain data and maximize statistical power, analyses were conducted on subsets of the 295 subjects with complete and usable data in year 1 \((N = 282)\) and year 2 \((N = 280)\).

**Genotyping Procedure**

Genomic DNA was extracted from Scope (Procter and Gamble, Cincinnati, Ohio) mouthwash-stabilized saliva samples using a commercial DNA isolation protocol (PureGene, Gentra Systems, Minneapolis, Minnesota). The DNA yield was measured using a SYBR Green (Invitrogen, Carlsbad, California) fluorescence quantification method. Average yield per subject was 3.7 ± 2.4 μg (range, 2 to 10.9 μg). The 5-HTTLPR polymorphism was genotyped using a TaqMan 5’ nucleic acid assay modified from that originally described by Hu et al (2005). The 25-μL polymerase chain reactions (PCRs) contained 200 nM each of forward and reverse primers (GCAACCTCCAGCAACTCTCTGTA and GAG-GTGCAGGGGGATGCTGGGAA), 120 nM of an l-allele specific FAM-labeled probe, and 60 nM of a VIC-labeled internal control probe whose target is present in the PCR amplicon for both l-alleles and s-alleles (6FAM-TGCAGCCCCCCCCAGCATCTCC-MGB and VIC-TCCCCCTTCTCACCTGCGGCGCATC-MGB), (ABI-Applied Biosystems Inc., Foster City, California); 1 mol/L Betaine (Sigma-Aldrich, St. Louis, Missouri); 1X ABI TaqMan Universal master mix (ABI-Applied Biosystems Inc.); and 25 ng genomic DNA. Samples were heated to 95°C for 10 minutes to activate Taq DNA polymerase, followed by 40 thermal cycles of 98°C for 15 seconds, followed by 62.5°C for 90 seconds. The number of l-alleles (0, 1, or 2) for each subject was identified by examination of scatter plots of end point FAM versus VIC fluorescence levels captured using an ABI 7500 Sequence Detection System. Fifteen percent of samples were repeated with no discrepancy in genotype between assays.

We previously validated this closed-tube fluorescent assay of 5-HTTLPR alleles by comparing results obtained for 300 DNA samples using the 5’-nuclease TaqMan assay with those from a traditional 5-HTTLPR agarose gel-based PCR fragment length assay, which yielded 100% agreement between methods. Samples were also genotyped at the Duffy antigen FY+/− (−365 T/C) polymorphism to identify the degree of African chromosome admixture (Covault et al 2004; Lautenberger et al 2000) for the sample of self-identified Caucasian subjects used for our analysis. The self-reported racial status of participants was consistent with FY genotype results: .6% of Caucasian chromosomes were FY−.

**Statistical Analysis**

We used ordinary least squares multiple regression procedures to test the effect of genotype and its interaction with negative life events in relation to drinking and drug use outcomes. Analyses were run separately for the year 1 and year 2 data. For all models, a dummy coding procedure was used to compare the genotype groups, specifying the l/l group as the reference group. This yielded two separate comparisons: 1) l/l versus l/s, and 2) l/l versus s/s. This strategy was more conservative than treating genotype either as a single quantitative predictor with equal intervals (which assumes a linear or additive relationship between genotype and outcomes) or as a dichotomous variable, i.e., comparing l/l versus l/s and s/s (which assumes identical effects for s carriers). By using the dummy codes, we could examine whether any observed genotype effects were consistent with recessive, additive, or dominant genetic models. To test the genotype x life events interaction, we created a set of interaction terms by multiplying each dummy code by the negative life events variable, which was mean-centered prior to creating the composites. Centering allowed for a meaningful interpretation of the dummy code (genotype) main effects in the final models including the interaction terms (i.e., they reflected the main effect of genotype group on the outcomes, at average levels of negative life events controlling for the other predictors). For all analyses, we report the significance (\( \alpha = .05 \)) of the main effect and interaction dummy coefficients. For significant effects, we report the effect size suitable for regression analyses—the squared part correlation (\( \Delta r^2 \)) or the change in \( r^2 \) due to inclusion of the predictor.

We also included several theoretically relevant covariates in the regression analyses. First, we controlled for sex, because men tend to drink more than women (Dawson et al 1995). We also controlled for excitement seeking based on prior findings showing that college students high on this personality dimension tend to drink more than those low on this dimension (McCabe 2002). Finally, we controlled for neuroticism because of its role in the stress-reactivity process (Bolger and Schilling 1991) and its possible relation to the 5-HTTLPR polymorphism (Munafo et al 2005; Schinka et al 2004; Sen et al 2004).

**Results**

**Descriptive Statistics**

The distribution of 5-HTTLPR genotypes was in Hardy-Weinberg equilibrium (\( \chi^2 = .05, p = .83 \)). Ninety-one subjects were l-allele homozygotes, 144 were heterozygotes, and 60 were s-allele homozygotes. The observed s-allele frequency (.45) is typical of non-Hispanic European American samples (Gelernter et al 1997). Genotype distribution did not differ by sex (\( \chi^2 = 1.23, p = .54 \)), by race (\( \chi^2 = 1.23, p = .54 \)), or current depressive symptoms either in year 1 (BDI means, l/l = 3.56; l/s = 3.83; s/s = 4.09) or year 2 (BDI means, l/l = 3.50; l/s = 3.71; s/s = 3.77) and neuroticism (\( \chi^2 = 1.23, p = .54 \)). Genotype was unrelated to excitement seeking (means, l/l = 5.32; l/s = 5.21; s/s = 5.28; significance tests, l/l vs. l/s, \( b = -.111, p = .305 \); l/l vs. s/s, \( b = -.044, p = .744 \)), neuroticism (means, l/l = 3.61; l/s = 3.60; s/s = 3.48; significance tests, l/l vs. l/s, \( b = .032, p = .875 \); l/l vs. s/s, \( b = -.369, p = .595 \)) or year to year change in neuroticism (\( \Delta r^2 = .05, p = .053 \)).

**Genetic Analyses**

Table 1 presents the descriptive statistics for the drinking and drug use outcomes for the sample overall and separately for each genotype. As seen in the first column, overall in year 1, students reported drinking on average 20.3% of the days sampled and they reported heavy drinking on an average 13.3% of days. As seen in the fifth column, in year 2, the drinking percentages were significantly higher, with drinking occurring on 25.1% of days \( [t(266) = -5.77, p < .01] \) and heavy drinking occurring on 15.9% of days \( [t(266) = -4.37, p < .01] \). These data are consistent with other work showing that drinking tends to increase across the early college years (Jackson et al 2005; Schulenberg and Maggs 2002). Scores on the intention to drink variable showed that people had rather low intentions to drink alcohol that increased from year 1 to year 2 \( [t(266) = -4.15, p < .01] \). Nonprescribed...
drug use was less common than alcohol use. In year 1, participants reported using drugs on an average of 6.2% of the days sampled. In year 2, this percentage increased only slightly to 7.1% (t(266) = 1.66, p < .10). Of the participants who reported at least some drug use (n = 83 in year 1; n = 89 in year 2), drugs were used on an average of 21.0% of the days sampled in year 1 and 22.3% of the days sampled in year 2. All of the drug use variables showed strong associations across time (r = .33 to .42). Within each year, the three drinking variables were strongly associated with each other (r = .69, .72, .70, .74, respectively). Within each year, the three drinking variables were strongly associated with each other (r = .76 to .88) and less so with drug use (r = .33 to .42).

Table 1 also shows the raw drinking and drug use data separately for each of the three genotypes, the statistical analysis of which is presented in Table 2. In examining these means descriptively, it is apparent that s/s individuals reported the highest levels of drinking and drug use, whereas l/l individuals reported the lowest levels. To determine whether these genotype differences were statistically significant after correction for planned covariates and whether the differences were potentially moderated by negative life events, we examined several multiple regression models.

**Multiple Regression Models Related to Drinking Outcomes and Drug Use**

Table 2 presents the coefficients from the multiple regression models, which incorporated correction for covariates (i.e., sex, excitement seeking, and neuroticism). As seen in the left columns, significant main effects of genotype on the drinking and drug use outcomes were found in the year 2 data only. Carriers of the short allele (both l/s and s/s) reported significantly more frequent drinking occasions compared with l/l individuals (l/s Δr² = .013; s/s Δr² = .023), but only individuals homozygous for the s-allele (s/s) also reported significantly more frequent heavy drinking (Δr² = .016), stronger drinking intentions (Δr² = .017), and marginally more frequent drug use (Δr² = .010). The effect sizes (Δr²) for these genotype main effects in year 2 were relatively small, accounting for 1.0% to 2.3% of the unaccounted variance in the drinking and drug use outcomes.

Gene-environment interactive effects on the drinking and drug use outcomes were consistent across both years. As seen in the right columns of Table 2, the multiplicative term involving negative life events and the l/l versus s/s genotype was significant in seven of the eight models tested, and for the l/l versus l/s genotype, it was significant in two of eight models tested. For ease of interpretation, these interactions are graphed in Figures 1 and 2 for years 1 and 2, respectively. As can be seen across both figures, s/s individuals exhibited the strongest association between negative life events and the drinking and drug use outcomes, whereas l/l individuals exhibited the weakest associations; l/s individuals were mostly indistinguishable from l/l participants (year 1) or had outcomes intermediate between these two groups (year 2).

**Table 1. Means (and Standard Deviations) of Daily Drinking and Drug Use Outcomes for the Sample and Separately for Each 5-HTTLPR Genotype Group**

<table>
<thead>
<tr>
<th>Drinking and Drug Outcomes</th>
<th>Sample M (Range; SD)</th>
<th>l/l</th>
<th>l/s</th>
<th>s/s</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Year 1 (n = 282)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Drinking Frequency</td>
<td>.203 (.0885; .154)</td>
<td>.201 (.152)</td>
<td>.191 (.142)</td>
<td>.236 (.181)</td>
</tr>
<tr>
<td>Heavy Drinking Frequency</td>
<td>.133 (.0536; .123)</td>
<td>.130 (.120)</td>
<td>.127 (.116)</td>
<td>.151 (.137)</td>
</tr>
<tr>
<td>Drinking Intentions</td>
<td>2.32 (1.5–8.86)</td>
<td>2.25 (.89)</td>
<td>2.32 (8.85)</td>
<td>2.40 (95)</td>
</tr>
<tr>
<td>Drug Use Frequency</td>
<td>.062 (.0963; .160)</td>
<td>.053 (.143)</td>
<td>.056 (.138)</td>
<td>.109 (.139)</td>
</tr>
</tbody>
</table>

| **Year 2 (n = 280)**       |                      |     |     |     |
| Drinking Frequency         | .251 (.0941; .179)   | .211 (.156) | .265 (.177) | .280 (.203) |
| Heavy Drinking Frequency   | .159 (.0566; .141)   | .135 (.138) | .165 (.137) | .181 (.152) |
| Drinking Intentions        | 2.51 (10–5.82; 96)   | 2.35 (95) | 2.57 (90) | 2.62 (106) |
| Drug Use Frequency         | .071 (0.01; .186)    | .072 (.203) | .056 (.139) | .102 (.242) |

5-HTTLPR, serotonin transporter linked promoter region; l, long allele; s, short allele.

*Frequency outcomes reflect the proportion of the 30 days sampled on which people drank, drank heavily, or used unprescribed drugs.

Scale for drinking intentions: 1 = no intention to drink; 2 = most likely not; 3 = probably not; 4 = maybe; 5 = probably will; 6 = most likely; 7 = definitely.

**Table 2. The Main and Interactive Effects of Past Year Negative Life Events and 5-HTTLPR Genotype on Drinking and Drug Use Outcomes (Controlling for Sex, Excitement Seeking, and Neuroticism)**

<table>
<thead>
<tr>
<th>Drinking and Drug Outcomes</th>
<th>Main Effects</th>
<th>Event x Gene Interactions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Events b (SE)</td>
<td>l/l vs. l/s b (SE)</td>
</tr>
<tr>
<td><strong>Year 1</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Drinking Frequency</td>
<td>-.003 (.005)</td>
<td>-.015 (.019)</td>
</tr>
<tr>
<td>Heavy Drinking Frequency</td>
<td>-.002 (.005)</td>
<td>-.005 (.016)</td>
</tr>
<tr>
<td>Drinking Intentions</td>
<td>-.004 (.030)</td>
<td>.053 (.112)</td>
</tr>
<tr>
<td>Drug Use Frequency</td>
<td>.004 (.006)</td>
<td>.001 (.021)</td>
</tr>
<tr>
<td><strong>Year 2</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Drinking Frequency</td>
<td>-.004 (.006)</td>
<td>.047 (.023)</td>
</tr>
<tr>
<td>Heavy Drinking Frequency</td>
<td>-.001 (.005)</td>
<td>.027 (.018)</td>
</tr>
<tr>
<td>Drinking Intentions</td>
<td>.026 (.032)</td>
<td>.201 (.123)</td>
</tr>
<tr>
<td>Drug Use Frequency</td>
<td>.002 (.006)</td>
<td>-.020 (.025)</td>
</tr>
</tbody>
</table>

Excitement seeking was a significant positive predictor in all models except for drug use. Sex was significant in all models (with men being higher than women) except for year 1 drug use. Neuroticism was a significant positive predictor of year 1 drinking frequency only.

5-HTTLPR, serotonin transporter linked promoter region; b, unstandardized regression coefficient; SE, standard error.

* p < .10.
* p < .05.
* p < .01.
* p < .001.
Individuals who were most at risk for alcohol or drug use were those with the s/s genotype who experienced multiple negative life events in the past year. These individuals, on average, drank 38% and 53% of days during the survey month in years 1 and 2, respectively (panel A, Figures 1 and 2), compared with the more normative 20% and 25% of days for the entire sample average. They were also more likely to drink heavily, especially in year 2, when they drank heavily on an average of 33% of the days (Figure 2, panel B) compared with the normative 16%. In year 1, they showed only a slight but nonsignificant elevation in reports of heavy drinking (Figure 1, panel B). As shown in panel C, Figures 1 and 2, s/s individuals who experienced more negative life events also reported the strongest intentions to drink alcohol, suggesting some psychological foresight of their drinking behavior. The gene-environment interaction was especially prominent for the drug use variable. As shown in panel D of Figures 1 and 2, s/s subjects reported taking drugs on 38% of the days sampled in year 1 and 44% of the days in year 2. Findings for drug use remained the same when the analyses controlled for drinking frequency.

Effect sizes (Δr²) for the gene environment effects were small but consistent, accounting for 1% to 4% of the variance in the drinking and drug use outcomes. For the significant or marginally significant interactions involving the L/L versus S/S contrast, the effect sizes were as follows: drinking frequency year 1 = .031, year 2 = .029; heavy drinking frequency year 1 = .011, year 2 = .016; drinking intentions year 1 = .025, year 2 = .033; proportion of drug use days year 1 = .041, year 2 = .029. The effect sizes for the significant or marginally significant interactions involving the L/L versus L/S contrast were as follows: drinking frequency year 2 = .017; drinking intentions year 1 = .012, year 2 = .024.

Because the drinking variables showed mild to moderate positive skew and the drug variables showed extreme skew, we examined whether the skewed outcomes produced nonnormally distributed residuals, a violation of linear regression assumptions. The residuals were normally distributed for the drinking outcomes but not for drug use. Because no transformation could normalize the drug use variables, we also analyzed drug use with a more appropriate nonlinear model—a multilevel logistic regression model for binary outcomes (Bernoulli sampling with logit link function; unit-specific model; Raudenbush and Bryk 2002). The findings were the same whether using the logistic model or the regular linear model. For ease of interpretation, we reported the results for the simpler linear model.

Figure 1. The relation between genotype, past-year negative life events, and outcomes from the daily diary survey in year 1. The average student reported 4 past-year negative life events; 10 events corresponded with approximately +2 standard deviations in the number of negative life events. Line symbols: solid, s/s subjects; dotted, L/S subjects; dashed, L/L subjects. Significant simple slopes are indicated. *p < .05; **p < .01; ***p < .001.

Figure 2. The relation between genotype, past-year negative life events, and outcomes from the daily diary survey in year 2. The average student reported 4 past-year negative life events; 10 events corresponded with approximately +2 standard deviations in the number of negative life events. Significant simple slopes are indicated. Line symbols: solid s/s subjects; dotted L/S subjects; dashed L/L subjects. *p < .05; **p < .01; ***p < .001.

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Supplemental Analyses of Gender and Depression

Given prior reports of sex effects with respect to the interaction of 5-HTTLPR genotype and stressful life events (Eley et al 2004; Grabe et al 2005), we also examined whether the findings differed for men and women. We created additional three-way (i.e., sex x negative life events x genotype) multiplicative terms and entered them into the regression models. Results indicated significant three-way interactions in only two of the eight models. There were significant effects for the sex x negative life events x 1/l versus s/s contrast in predicting year 1 heavy drinking frequency (b = .018, SE = .008, p = .021, Δr² = .016), as well as year 1 drinking intentions (b = .131, SE = .055, p = .017, Δr² = .017). The form of these effects indicated that for these two drinking outcomes, women, but not men, exhibited gene x environment effects. Specifically, women with the s/s genotype showed the strongest positive association between negative life events and reports of heavy drinking and the intention to drink, whereas women with the 1/l genotype showed no such association. For the remaining six outcomes, men and women showed similar patterns, suggesting only minimal evidence of sex differences.

Lastly, in view of reports of interactions of adverse life events and the 5-HTTLPR s-allele with clinical depression (Caspi et al 2003; Eley et al 2004; Grabe et al 2005; Kaufman et al 2004; Kendler et al 2005), we examined current depressive symptoms as a potential mediator (and moderator) of our genotype findings. To test for mediation, we ran our regression model substituting scores on the BDI as the primary outcome variable to determine whether genotype and its interaction with past year negative life events predicted current depressive symptoms, a statistical requirement for mediation. None of these interactions were significant in either year, regardless of whether the analysis included covariates (sex, excitement seeking, neuroticism). To test for moderation, we examined whether current depressive symptoms—rather than past year life events per se—was a more proximal moderator of genotype effects on alcohol and drug use outcomes. We ran our regression models substituting scores on the BDI for the negative life events variable and creating the appropriate interaction terms with genotype. Again, none of the interaction terms were significant. Thus, the results do not support a mediational or moderating role for current depressive symptoms on drinking or drug use outcomes.

Discussion

We found significant interactions between the 5-HTTLPR polymorphism and negative life events on drinking behavior across both study years. For individuals homozygous for the s-allele, increased negative life events during the past year were consistently associated with higher levels of alcohol consumption, whereas for individuals with the 1/l genotype, there was no relation between negative life events and alcohol use. In the second study year, when more drinking occurred for the entire sample, individuals with the 1/l genotype showed patterns intermediate to the s/s and 1/l subjects, consistent with a co-dominant effect of the s-allele for alcohol use outcomes.

A particularly robust effect was seen for the interaction of the genotype and negative life events on drug use, such that s/s subjects who had experienced 5 past-year life stressors used nonprescribed drugs on an average of 20% of days and subjects with 10 past-year life stressors used drugs on an average of 40% of days in both the years 1 and 2 sampling intervals. Nonprescribed drug use was infrequent and unrelated to life stressors for both 1/s and 1/l subjects. These patterns of interactive effects of life stress and 5-HTTLPR genotype on drug use are more suggestive of a recessive genetic effect, whereas those for alcohol use are more consistent with a co-dominant effect of the s-allele. Differences in the apparent genetic effect for drug versus alcohol use raise the possibility that different pathways may moderate the likelihood of drug versus alcohol use as a function of the interaction of the 5-HTTLPR s-allele and life stress (alternative explanations might include insufficient power to detect additive effects on drug use due to the lower baseline frequency of drug use compared with alcohol). Complex biological effects of functional polymorphisms may or may not mirror the mode of inheritance evident in proximal gene effects, and for the 5-HTTLPR polymorphism, it remains unclear whether the s-allele has a dominant (Hranić et al 2004) or additive effect (Bradley et al 2005) on mRNA levels. Further research to replicate these effects in relation to both alcohol intake and drug use and to address the hypothesis of a different genetic mechanism underlying the observed effects for alcohol versus drug use is warranted.

Our results demonstrating the interaction of 5-HTTLPR genotype and negative life events on alcohol use parallel recent findings of 5-HTTLPR interactions with childhood maltreatment on alcohol use in children (Kaufman et al, in press). In that study, children who were carriers of the 5-HTTLPR s-allele and had experienced childhood maltreatment were at greatest risk for early use of alcohol and cigarettes. Our results for drinking as a main effect of the 5-HTTLPR s-allele were also consistent with the observations of Herman et al (2003) on college student drinking but appeared less robust. We observed statistically higher levels of drinking only for students with the s/s versus the 1/l genotype during the second year of data collection. Furthermore, unlike Herman et al (2003), we did not find that 5-HTTLPR had a greater association with heavy drinking episodes compared with any drinking episode. This limited replication regarding the main effects of genotype may be due, in part, to differences in environmental conditions across the two studies. Subjects in Herman et al (2003) lived in an urban setting (versus our rural setting), and so students may have had a higher baseline level of stressful life events, which could make genotype effects more apparent. Another difference is that we collected drinking and drug use data close to its real-time occurrence, relatively free of recall error and bias. Finally, limited replication is also consistent with the modest association of the 5-HTTLPR polymorphism with alcohol dependence, with several studies showing an association of alcohol dependence with the s-allele (Hallikainen et al 1999; Hammoumi et al 1999; Lichtermann et al 2000; Thompson et al 2000), while others do not (Edenberg et al 1998; Geletner et al 1997; Kranzler et al 2002). A meta-analysis of 17 reports, including some 5800 subjects, found a small increase in the odds of the s-allele (odds ratio [OR] = 1.18, 95% confidence interval [CI] = 1.03–1.35) among alcoholics (Feinn et al 2005).

We did not observe an interactive effect of the 5-HTTLPR genotype and past year negative life events on the Beck Depression Inventory. Earlier reports (Caspi et al 2003; Kaufman et al 2004; Kendler et al 2005) that found a higher incidence of clinical depression in the setting of stressful life events examined effects of the 5-HTTLPR s-allele and stressful events occurring over a 5-year period (Caspi et al 2003) or used measures of extreme life events (removal from the home due to parental maltreatment [Kaufman et al 2004], assault, robbery, divorce, serious illness, or serious housing problems [Kendler et al 2005]). Our results suggest that for less severe or a less prolonged series of negative life events, increased symptoms of depression may not be evident as a result of interac-
tion with the 5-HTTLPR s-allele, but young persons may be at risk for other potentially troublesome behavioral outcomes.

These findings must be viewed in the context of several limitations. First, our study population contained too few non-European American students to allow analysis of the effects of 5-HTTLPR in other ethnic groups, thereby limiting our capacity to generalize our findings beyond European Americans. Studies of environmental interaction with 5-HTTLPR in other ethnic/racial groups are needed. Similar studies of young adults not attending college would also be important to examine the generality of our findings. Second, our sample had insufficient power to fully examine the effect of sex on the interaction between the 5-HTTLPR polymorphism and stress. However, consistent with prior reports of gender differences in the interplay between 5-HTTLPR genotype and stressful life events (Eley et al. 2004; Grabe et al. 2005), our two significant gender findings suggested a larger effect may sometimes be present among women with respect to drinking behavior. Third, the substantial and variable time elapsed between past-year stressful life events and current alcohol/drug use behaviors limits interpretation of their direct causal relationship. Fourth, the presence of increased life stress in the past year may reflect a lifelong history of increased social stressors including those occurring during early childhood development. Such early life stressors have been reported to interact with the 5-HTTLPR s-allele to produce higher rates of depression (Kaufman et al. 2004) and earlier alcohol use (Kaufman et al., in press) in children, as well as greater levels of alcohol intake by nonhuman primates (Barr et al. 2004). Future studies will benefit from designs that collect information on early life adversity, as well as designs that allow a closer temporal analysis between change in life stress burden and drinking or drug use.

Our observation of an interaction between the 5-HTTLPR polymorphism and negative life events on drinking and drug use is the first evidence of this gene x environment interaction on behaviors other than those related to depression. Although this interaction accounted for a small amount of the total variance in alcohol and drug use in the entire sample (1% to 4%), for the subset of subjects who had experienced a high number of negative life events in the past year, the effect of genotype was large. Specifically, among individuals who had experienced 10 or more past-year life stressors, alcohol use was twice as frequent and drug use eight times as frequent for s/s versus l/l subjects. Other investigators have reported that young men with the l/l genotype are more likely to show reduced acute effects of alcohol as measured by body sway and the Subjective High Assessment Scale (Hu et al. 2005). We doubt that the interactive effects of life stress and 5-HTTLPR that we observed are driven by a direct biochemical effect of the 5-HTTLPR polymorphism on acute alcohol or drug effects. Rather, we suggest they may be due to biases in developmentally determined affective reactivity to life stressors related to the 5-HTTLPR genotype (such as shown for amygdala activation in healthy subjects; Hariri et al. 2002, 2005). In conclusion, our findings suggest that in the presence of multiple or repeated life stress, young adult carriers of the 5-HTTLPR s-allele may have a higher risk for a variety of adverse behavioral outcomes, including increased risk of alcohol and drug use disorders.

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