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Genetic moderation of cocaine subjective effects by variation in the *TPH1, TPH2,* and *SLC6A4* serotonin genes

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Abstract

Objective—This study investigated variants of *TPH1, TPH2*, and *SLC6A4* in the moderation of the subjective effects of cocaine.

Methods—Non-treatment seeking cocaine-dependent individuals ($N = 66$) were intravenously administered saline and cocaine (40 mg) in randomized order. Participants self-reported subjective effects of cocaine using a visual analog scale starting before administration of saline or cocaine (−15 min) to up to 20 min post-infusion. Self-report ratings on the visual analog scale ranged from 0 (no effect) to 100 (greatest effect). Participants were genotyped for the *TPH1* rs1799913, *TPH2* rs4290270, and *SLC6A4 5-HTTLPR* variants. Repeated measures analysis of covariance (ANCOVA) was used to examine change in subjective effect scores over time while controlling for population structure.

Results—Participants carrying the *TPH1* rs1799913 A allele reported greater subjective response to cocaine for 'stimulated' and 'access' relative to the CC genotype group. Those carrying the *TPH2* rs4290270 A allele reported higher 'good effect' and lower 'depressed' effect relative to the TT genotype group. Those carrying the *SLC6A4 5-HTTLPR* S′ allele reported greater 'desire' and 'access' compared to the L′L′ genotype group.

Conclusions—These findings indicate that *TPH1, TPH2*, and *SLC6A4* variants moderate the subjective effect of cocaine in non-treatment seeking cocaine-dependent participants.

Keywords

cocaine; tryptophan hydroxylase; genetics; serotonin; polymorphism; subjective; variant; substance use; TPH; transporter

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Introduction

In the United States, over 38 million individuals greater than 11 years old reported cocaine use in their lifetime (SAMHSA, 2014). Several psychosocial and biological risk factors predict the trajectory from cocaine use to addiction including high impulsivity (Molander *et al.*, 2011), changing method of drug administration (e.g., switching to smoking or intravenous injection; Gawin & Khalsa-Denison, 1996), and episodes of binging (Dackis & O'brien, 2001). Individuals with cocaine use disorder (CUD) are a heterogeneous population and up to 56% of the heritability and variance of their cocaine use may be due to genetic factors (Gelernter *et al.*, 2014, Kendler *et al.*, 2003, Tsuang *et al.*, 1999). For example, a recent genome-wide association studies (GWAS) supports the role of genetic factors in vulnerability to develop cocaine dependence (Gelernter *et al.*, 2014).

Our laboratory previously reported that the subjective effects of cocaine use are moderated, in part, by variants in the *ankyrin repeat and kinase domain-containing 1* (*ANKK1*) (Spellicy *et al.*, 2014), *dopamine transporter (DAT1*, *SLC6A4),* and *serotonin transporter (SLC6A4) 5-HTTLPR* (Liu *et al.*, 2015) genes. Herein, we continue to investigate the relation between genetic variants and subjective effects of cocaine by exploring the role of the serotonergic system in the subjective effects of cocaine use in non-treatment seeking cocaine-dependent individuals.

Serotonin (5-hydroxytryptamine; 5-HT) is a neurotransmitter associated with psychobiological reward processes (Russo & Nestler, 2013). We previously have reviewed the role of the 5-HT system in substance use disorders, including tryptophan hydroxylase 1 (*TPH1*; rs1799913), tryptophan hydroxylase 2 (*TPH2;* rs4290270), and the serotonin transporter promoter (5-*HTTLPR*) (Bauer *et al.*, 2015, Nielsen *et al.*, 2012, Nielsen *et al.*, 2008, Nielsen *et al.*, 2014, Yuferov *et al.*, 2010).

The biosynthesis of 5-HT relies on the conversion of tryptophan by TPH, the rate-limiting enzyme in the biosynthesis of serotonin (Cooper & Melcer, 1961). Specifically, after an individual consumes food containing tryptophan, tryptophan is absorbed by the intestine and transported by the blood to the brain, where it is transported across the blood-brain barrier and biotransformed into 5-HT. TPH is encoded by two separate genes, *TPH1*, located on chromosome 11p15.1, and *TPH2*, located on chromosome 12q21.1. Both TPH isozymes convert L-tryptophan via hydroxylation into 5-hydroxytryptophan (5-HTP), which is subsequently decarboxylated to 5-HT. *TPH2* is expressed primarily in the raphe nuclei of the brain (Patel *et al.*, 2004, Walther & Bader, 2003, Walther *et al.*, 2003, Zill *et al.*, 2004, Zill *et al.*, 2007) whereas *TPH1* is expressed mainly in the pineal gland, the raphe nuclei during late development, and the enterochromaffin cells of the gut (Nakamura *et al.*, 2006, Zill *et al.*, 2007).

The tightly linked variants (rs1799913 and rs1800532) in *TPH1* (originally designated *TPH* prior to the discovery of *TPH2*) have been associated with behaviors including suicidality (González-Castro *et al.*, 2014, Nielsen *et al.*, 1994), alcoholism (Nielsen *et al.*, 1998), impulsive behavior (Staner *et al.*, 2002), and with cerebrospinal fluid (CSF) 5-hydroxyindoleacetic acid (5-HIAA) levels (Jönsson *et al.*, 1997, Nielsen *et al.*, 1994).

Specifically, male *TPH1* A allele carriers have been shown to have lower levels of serotonin production as measured by CSF 5-HIAA levels (Jönsson *et al.*, 1997) and decreased risk for suicide attempt (González-Castro *et al.*, 2014, Nielsen *et al.*, 1994). *TPH2* rs4290270 has been shown to have differential allelic expression in the cortex, hypothalamus, thalamus, hippocampus, amygdala, and cerebellum (Lim *et al.*, 2007). The T allele is expressed at two times the level of the A allele in heterozygous subjects. The TT genotype may be related to the production of more serotonin compared to that in A allele carriers. *TPH1* and *TPH2* are expressed in several regions associated with reward processes (e.g., hippocampus, amygdala, frontal cortex; Russo & Nestler, 2013, Zill *et al.*, 2004). Genetic variation in *TPH1* and *TPH2* may be related to altered serotonergic function and thus may impact reward processes (e.g., in the hippocampus, amygdala, frontal cortex) associated with the serotonergic system including effects of cocaine. Particularly, the low serotonin production associated with *TPH1* A-allele and *TPH2* A-allele carriers may increase an individual's vulnerability to the subjective effects of cocaine.

A promoter variant in the promoter region of the *serotonin transporter* (*5-HTT*) gene (*SLC6A4*), *5-HTTLPR*, alters the transcription and availability of 5-HT in humans (Lesch *et al.*, 1996, Murphy *et al.*, 2004). This polymorphism contains 16 or 14 repeats characterized as either the long (L) or short (S) form, respectively. The L allele typically has higher transcription activity than the S allele (Lesch *et al.*, 1996), however, an A to G transition (rs25531) occurs in the L allele of *5-HTTLPR*. The L allele containing the G transition codes for an allele (L_G) with low transcriptional activity similar to that of the S allele (Hu *et al.*, 2006, Praschak-Rieder *et al.*, 2007). These two low transcription alleles (L_G and S) are referred to as S′, while the L_A allele, which has higher transcriptional activity, is referred to as L′. Recent findings suggest that individuals with the S′ allele may be more sensitive to the environment than those with the L′ L′ genotype (Fox *et al.*, 2011, Graham *et al.*, 2013). Specifically, S′ allele carriers appear more easily biased by environmental stimuli and more attuned to their perceived limitations.

We hypothesized that individuals who are carriers of alleles associated with lower serotonergic production (*TPH1* A and *TPH2* A alleles) or lower levels of serotonin transporter (5-*HTTLPR* S′ allele) would demonstrate more sensitivity to the positive and negative effects of cocaine (see *Subjective Effects* section below for description). Specifically: 1) participants who were carriers of the *TPH1* rs1799913 A allele would have higher positive and lower negative subjective ratings to cocaine administration relative to participants with the CC genotype, 2) carriers of the *TPH2* rs4290270 A allele would have higher positive and lower negative subjective ratings to cocaine administration compared to participants with the TT genotype, and 3) carriers of the S′ allele of *5-HTTLPR* would have higher positive and lower negative subjective ratings to cocaine. Non-treatment seeking cocaine-dependent individuals enrolled in the present study were genotyped and completed self-report measures on the subjective effects of cocaine versus saline. *TPH1*, *TPH2*, and *5-HTTLPR* genotype differences were examined on the self-reported subjective effects. Additionally, cardiovascular effects were investigated to examine differences between subjective and objective effects of cocaine.

Experimental Methods

Participants

Sixty-six participants between the ages of 18 and 55 were recruited from March 2010–July 2012 through an ongoing research trial at Baylor College of Medicine (see cohort details in prior publication; Brewer *et al.*, 2015). Briefly, inclusion criteria for the study were that subjects: (1) gave informed consent; (2) had a negative pregnancy test for the women; (3) were administered the Mini-International Neuropsychiatric Interview (M.I.N.I.) (Sheehan *et al.*, 1998) and met Diagnostic and Statistical Manual of Mental Disorders 4th Edition (DSM-IV-TR; Association, 2000) criteria for cocaine-dependence; and (4) were non-treatment seeking. Exclusion criteria included: (1) a history of head trauma, epilepsy, heart disease, acquired immunodeficiency syndrome (AIDS), asthma, or other serious medical conditions, (2) dependence on drugs other than cocaine or nicotine, (3) inability to sense the effects of cocaine, (4) presence of any other axis I psychiatric disorder, or (5) use of psychotropic medications or medications affecting blood pressure. All participants This study was approved by the Institutional Review Board of Baylor College of Medicine and the Research and Development committee of the Michael E. DeBakey Veteran Affairs Medical Center.

Subjective & Objective Effects

Congruent with previously published studies from our laboratory (Brewer *et al.*, 2015, Spellicy *et al.*, 2014), a double-blind, placebo-controlled, within-subjects experimental design was used. Participants were randomized and intravenously administered 0 mg (saline) or 40 mg of cocaine in the morning or afternoon. Each participant received either: 1) one morning dose (administered at approximately 9 AM or 10 AM) of saline and one afternoon dose of cocaine (administered at approximately 1 PM or 2 PM) or 2) one morning dose cocaine (at approximately 9 AM or 10 AM) and one afternoon dose of saline (at approximately 1 PM or 2 PM). Four hours separated the doses (i.e., patients received doses at 9 AM and 1 PM or 10 AM and 2 PM) to minimize any carry over effects. Participants rated baseline subjective effects fifteen minutes prior to receiving an infusion. Subjective effect ratings also were collected at 5, 10, 15, and 20 minutes post-infusion (see Figure 1). Participants rated subjective effects on a visual analog scale that ranged from 0 (“no effect”) to 100 (“most effect”). Positive subjective effects were: ‘high’ (“How high are you right now?”), ‘any drug effect’ (“Do you feel any drug effect right now?”), ‘stimulated’ (“How stimulated do you feel right now?”), ‘good effect’ (“Does the drug have any good effects right now?”), ‘desire’ (“How much do you desire the drug right now?”), ‘access’ (“If you had access to the drug right now how likely would you be to use it right now?”), and ‘like’ (“How much do you like the drug right now?”). Negative effects were: ‘bad effect’ (“Does the drug have any bad effects right now?”), ‘anxious’ (“How anxious do you feel right now?”), and ‘depressed’ (“How depressed do you feel right now?”). Heart rate and systolic and diastolic blood pressure were measured throughout using standard hospital equipment, GE Dash 3000 (GE Medical Systems, Milwaukee, WI). Cardiovascular effects were investigated to highlight differences between subjective and objective effects.

Genotyping

DNA was extracted from participant's blood using the Gentra Puregene blood kit (Qiagen, Germantown, MD) per manufacturer protocol. A researcher, who was blind to participant clinical status, determined the *TPH1* rs1799913 and *TPH2* rs4290270 genotypes in duplicate using 5′-fluorogenic exonuclease assays (TaqManÒ, Applied Biosystems, Foster City, CA). The TaqManÒ primer-probe sets ID C__2645661_10 was used to genotype *TPH1* rs1799913 and the primer probe set C__26385365_10 for the *TPH2* rs4290270 using the PlatinumÒ quantitative PCR SuperMix-UDG (Invitrogen, Carlsbad, CA) on a ViiA 7 (Applied Biosystems) in duplicate. Data analysis was conducted with ViiA 7 Software v1.1. The *TPH1* rs1799913 variant is an A to C transversion with the A allele being the ancestral allele and the C allele the derived allele. The *TPH2* rs4290270 variant is an A to T transition with the A allele being the ancestral allele and the T allele the derived allele. The *5-HTTLPR* is a repeat of either 14 (short) or 16 (long) copies of a 22 base pair repeat in the promoter region of the *SLC6A4* gene (Lesch *et al.*, 1996). In the 14 copy repeat is rs25531, an T to C transition, when examined in the same orientation as the *SLC6A4* gene. Both repeats are derived from a longer repeat region of 18 or 20 repeats found great apes, including chimpanzee, gorilla, and orangutan (Lesch *et al.*, 1997). Negative controls were empty wells containing no DNA and positive controls were wells containing DNA from control samples that are standardized across our studies.

The s*erotonin transporter 5-HTTLPR* was determined as described in Nielsen *et al.*, 2012 (Nielsen *et al.*, 2012). Briefly, the classic long "L" and short "S" alleles (rs4795541) (Lesch *et al.*, 1996) were determined by PCR amplification. The L allele yields a 181 base pair (bp) fragment and the S allele a 138 bp fragment The internal variant rs25531 in the L allele was genotyped by digestion of the amplified DNA with HpaII (New England Biolabs, Ipswich, MA) (Hu *et al.*, 2006). The "LG" (Stein *et al.*, 2006) G containing allele is into 96 and 85 bp fragments, while the "LA" A containing allele remains undigested. The functionally similar LG and S alleles are designated as S′ and the higher transcriptional rate LA allele as L′.

Sex was confirmed by genotyping SRY (Kosten *et al.*, 2013). Population structure was calculated by genotyping ten ancestry informative markers (AIMs). Data from the current participant sample were compared to the Centre d'Etude du Polymorphisme Humain–Human Genome Diversity Panel (CEPH-HGDP) samples (1,035 subjects of 51 populations) as described (Kosten *et al.*, 2013). Previously, it has been demonstrated that 94.6% of the maximum informativity value is obtained using these ten AIMs (Lao *et al.*, 2006).

Statistical analyses

R version 2.9.1 (R_Development_Core_Team, 2009) was used to conduct all statistical analyses. Participant's subjective effect values were calculated by 1) subtracting baseline (−15 min) cocaine or saline values from all post-administration subjective effect values (normalization) and then 2) subtracting the normalized saline subjective effect values from the normalized cocaine subjective effect values. A dominant model was used for all statistical analysis. A repeated measures analysis of covariance (ANCOVA) was used to examine the change in subjective effects scores over time while controlling for population structure. Repeated measures ANCOVA was used to examine genotype differences between

groups: *TPH1* rs1799913 genotype (0 = CC genotype vs. 1 = AA/AC), *TPH2* rs4290270 genotype (0 = TT genotype vs. 1 = AA/AT), *5-HTTLPR* minor S′ allele (0 = L′L′ vs. 1 = L′S′/S′S′), and by genotype pattern (0 = AA/AC, AA/AT, L′S′/S′S′ vs. 1 = others). Between group (i.e., genotype) differences in demographic variables were analyzed using analysis of variance (ANOVA) and Fisher's exact tests. Effect size was calculated as a partial eta-squared statistic using condition or polymorphism variance over residual variance and were compared to the established scale of small (η^2 = .01), medium (η^2 = .06), and large (η^2 = .014) effects (Cohen, 1988). Note: although there were 52 statistical tests performed, the subjective and objective effect outcomes were highly correlated and dependent (*r* between .224–.924); therefore, correction for multiple hypothesis testing (e.g., Bonferroni) was not completed since these corrections are only valid when outcome variables are independent and uncorrelated (Blakesley *et al.*, 2009).

Outside of the SRY assay and the ten AIMs, 27 variants have been examined for pharmacogenetic moderation of cocaine subjective effect with this cohort. As such, corrections for multiple testing were performed to evaluate experiment-wise significance (P <.05/27 = 0.0019) by applying a Bonferroni correction. Results presented below are the moderation analyses that demonstrated findings at $P < 0.0019$.

Results

Demographics

Between group (CC vs. AC/AA) differences were found for ethnicity ($P = 0.029$) and race ($P = 0.006$) for *TPH1*. An experiment-wise difference remained for race after using the Bonferroni correction. There was a point-wise difference with more Hispanic individuals having the *TPH1* AA/AC, *TPH2* AA/AT, 5-HTTLPR L′S′/S′S′ genotype pattern ($P = 0.032$) when compared to all other genotype groups, but this did not remain significant after Bonferroni correction. No point- or experiment-wise differences were found between *TPH2* or *5-HTTLPR* genotype groups for any of the demographic variables.

Cocaine use

Daily cocaine use was compared between those with the AA/AC, AA/AT, L′S′/S′S′ genotype pattern and those without this genotype pattern (Table 1). Greater cocaine daily use reported in the combined AA/AC, AA/AT, L′S′/ S′S′ genotype pattern group ($P = 0.039$; 3.3 grams ± 3.6 s.d.) compared to the other participants (1.9 grams ± 1.3 s.d.).

Subjective Effects

The role of *TPH1* rs1799913 variant in moderating participants' subjective effects to cocaine was evaluated. For the subjective score of 'stimulated' (for description of subjective effect measures please see *Subjective Effects* section in the methods above) there was a main effect of genotype group ($F = 13.67$; df = 1, 255; $P < 0.001$, with an effect size of 0.054) and time ($F = 15.26$; df = 1, 255; $P < 0.001$, with an effect size of 0.060) (Figure 2A). The AA/AC genotypes group (carriers of the A allele) demonstrated greater 'stimulated' across time. The largest difference between the genotype groups was at 5 minutes for 'stimulated,' where the

AA/AC genotypes group had values of 69.1 ± 10.8 (s.e.m.) and the CC genotype group had values of 50.3 ± 6.2.

Regarding the subjective report of 'access', a main effect of genotype group ($F = 17.57$; df = 1, 255; $P < 0.001$ with an effect size of 0.069) was found (Figure 2B). The AA/AC genotypes group reported higher 'access' relative to the CC genotype group. The greatest difference between the genotype groups for 'access' was at 15 minutes where the AA/AC genotype group had values of 48.1 ± 12.4 and the CC genotype group had values of 18.3 ± 7.0.

The *TPH2* rs4290270 variant also was examined for its moderation of the subjective effects of cocaine. For the subjective score of 'good effect' there was a significant main effect of genotype group ($F = 12.3$; df = 1, 255; $P = 0.001$, with an effect size of 0.048) (Figure 3A). The AA/AT genotype group reported greater 'good effect' than the TT genotype. The largest difference between the genotype groups was at 5 minutes for 'good effect' where the AA/AT genotypes group had values of 59.2 ± 6.0 and the TT genotype group had values of 36.7 ± 7.8.

For the subjective scores of 'depressed' there was a main effect of genotype group ($F = 10.40$; df = 1, 255; $P = 0.001$, with an effect size of 0.041) (Figure 3B). Participants with the TT genotype reported a greater 'depressed' subjective effect than those carrying an A allele. The largest difference between the genotype groups for 'depressed' was at 15 minutes, where the AA/AT genotypes group had values of 4.7 ± 2.1 and the TT genotype group had values of 13.3 ± 5.2.

The subjective effects of cocaine were also examined for *5-HTTLPR*. For the subjective scores of 'desire', there was a main effect of genotype ($F = 17.98$; df = 1, 255; $P < 0.001$, with an effect size of 0.070) (Figure 4A). The L′S′/S′S′ genotypes group reported higher 'desire' than the L′L′ genotype group. The difference between the genotype groups was the largest for 'desire' at 5 minutes into the trial where the L′S′/S′S′ genotypes group had values of 41.4 ± 6.4 and the L′L′ genotype group had values of 11.8 ± 4.4.

Subjective effect scores of 'access' showed a main effect for genotype group (F = 14.62; df = 1, 255; $P < 0.001$, with an effect size of 0.060) (Figure 4B). The L′S′/S′S′ genotypes group reported higher 'access' than the L′L′ genotype group. Genotype groups reported the greatest difference for 'access' at 15 minutes where the L′S′/S′S′ genotypes group had values of 37.3 ± 7.0 and the L′L′ genotype group had values of 9.8 ± 4.2.

The subjective effects of cocaine were compared between the genotype pattern of those participants carrying a *TPH1* A-, *TPH2* A-, and *5-HTTLPR* S′-alleles to those who were not *TPH1* A, *TPH2* A, and *5-HTTLPR* S′ allele carriers (had either a *TPH1* CC, *TPH2* TT, or *5-HTTLPR* L′L′ genotype). There was a main effect for the genotype pattern group for 'access' ($F = 24.09$; df = 1, 253; $P < 0.001$, with an effect size of 0.100) (Figure 5A), with the AA/AC, AA/AT, L′S′/S′S′ group self-reporting significantly higher 'access.' The largest difference between genotype groups was at 10 minutes where the genotype pattern AA/AC, AA/AT, L′S′/S′S′ group had values of 57.9 ± 17.5 compared to the other participants who had values of 19.3 ± 6.5.

Objective Effects

A genotype group main effect was found ($F = 13.17$; df = 1, 255; $P < 0.001$ with an effect size of 0.051) for heart rate (Figure 2C). The CC genotypes group had higher heart rate relative to the AA/AC genotype group. Heart rate had the greatest group difference at 5 minutes where the AA/AC genotype group had values of 33.4 ± 5.2 and the CC genotype group had values of 23.9 ± 2.3.

A main effect of genotype (F = 4.47; df = 1, 255; $P < 0.05$, with an effect size of 0.020) was found for heart rate (Figure 4C). The L′S′/S′S′ genotype group had higher heart rate relative to the L′L′ group. Heart rate demonstrated the greatest difference between the genotype groups at 5 minutes where the L′S′/S′S′ genotypes group had values of 30.0 ± 3.2 and the L′L′ genotype group had values of 21.0 ± 3.2.

Systolic blood pressure also had a main effect of genotype (F = 15.45; df = 1, 255; $P < 0.001$, with an effect size of 0.060) (Figure 4D). The L′S′/S′S′ genotype group had higher systolic blood pressure relative to the L′L′ group. The largest difference between the genotype groups was systolic blood pressure at 20 minutes where the L′S′/S′S′ genotypes group had values of 18.1 ± 2.1 and the L′L′ genotype group had values of 7.3 ± 3.2.

There was a main effect for genotype group for heart rate ($F = 16.39$; df = 1, 253; $P < 0.001$, with an effect size of 0.060) (Figure 5B), with the AA/AC, AA/AT, L′S′/S′S′ genotype pattern group having higher heart rate. Heart rate differences were greatest between genotype groups at 5 minutes where the AA/AC, AA/AT, L′S′/ S′S′ group had values of 36.9 ± 6.4 and the other participants had values of 24.2 ± 2.4.

All other subjective and objective effect variables did not significantly differ by the aforementioned genotype groups and are not reported.

Discussion

The present study investigated the role of *TPH1, TPH2*, and *SLC6A4* variants in the moderation of the subjective effect of cocaine in non-treatment seeking cocaine-dependent individuals. Our findings indicate that alleles associated with low serotonin production (*TPH1* A, *TPH2* A) or low serotonin transporter levels (*5-HTTLPR* S allele) are related to more positive self-reported subjective effects of cocaine post-cocaine administration.

The *TPH1* AA/AC genotypes group reported greater subjective response to cocaine for ‘stimulated’ and ‘access’ relative to the CC genotype group, the *TPH2* AA/AT genotypes group reported significantly more ‘good effect’ and less ‘depressed’ effect relative to the TT group, and the *5-HTTLPR* L′S′/S′S′ genotypes group reported greater ‘desire’ and ‘access’ compared to the L′L′ genotype group. Further, when these alleles associated with low serotonin production or transporter levels were combined into one genotype pattern group (i.e., *TPH1* AA/AC, *TPH2* AA/AT, *5-HTTLPR* L′S′/S′S′) and compared to those without these genotype patterns, the *TPH1* A, *TPH2* A, and *5-HTTLPR* S′ allele carrier genotype pattern group demonstrated more positive subjective effects to cocaine regarding “access” than those without this genotype pattern. The positive subjective effects of the low serotonin

genotype pattern group (AA/AC, AA/AT, L′S′/S′S′) was also associated with greater daily cocaine use. Physiological measurement of heart rate and blood pressure during the study indicated that the *TPH1* AA/AC genotype groups had higher heart rate post-cocaine administration compared to the CC genotype group, the *5-HTTLPR* L′S′/S′S′ genotype groups had higher heart rate and systolic blood pressure relative to the L′L′ genotype group, and the AA/AC, AA/AT, L′S′/S′S′ genotype pattern group had higher heart rate compared to the other participants.

Neurotransmitter and brain mechanisms related to reward processes help an individual make quick judgments regarding the aversive or rewarding aspects of a stimulus. The outcome of this cognitive processing (i.e., aversive or rewarding judgment) informs an individual's future response to stimuli (for review see Schultz, 2011). Therefore it is not surprising that alterations of the serotonergic system have been associated with psychiatric diagnosis and symptomatology, including mood disorders, suicidality, impulsivity, and substance-related disorders (Mann, 1999). Cocaine has a particularly disruptive effect by inhibiting serotonin uptake (Han & Gu, 2006) and after long term exposure results in reduced 5-HT levels that increase cocaine-seeking and may maintain addictive behaviors (e.g., Kirby *et al.*, 2011, Pelloux *et al.*, 2012).

The lower serotonin levels associated with the *TPH1* A and *TPH2* A alleles may indicate an altered vulnerability to cocaine addiction due to their association with greater positive subjective effects of cocaine and increased daily cocaine use. In contrast, individuals without these alleles and thus higher basal serotonin levels may experience a "saturation" of serotonin. Further, serotonin levels are not able to increase upon using cocaine as much as in participants with lower baseline serotonin levels (i.e., carriers of the *TPH1* A and *TPH2* A); therefore, leading to less positive subjective effects of cocaine. Moreover, it is interesting that the participants with the AA/AC, AA/AT, L′S′/S′S′ genotype pattern group experienced more positive and less negative effects of cocaine, greater physiological response, and more daily cocaine use. These findings support prior literature that demonstrates that lower serotonin production associated with *TPH1* A and *TPH2* A is related to suicidality, alcoholism, and impulsive behavior (González-Castro *et al.*, 2014, Nielsen *et al.*, 1994, Nielsen *et al.*, 1998, Slof-Op't Landt *et al.*, 2013, Staner *et al.*, 2002). This indicates a potential genetic marker of vulnerability to cocaine addiction. It is possible that these genotypes, which are related to lower serotonin levels, and lower levels of the serotonin transporter drive increased cocaine-seeking and cocaine addiction (e.g., Kirby *et al.*, 2011, Pelloux *et al.*, 2012). Specifically, in the current study, these lower levels of serotonin and serotonin transporter are related to an increased likelihood that an individual would continue to use cocaine if they had access to it and to a greater physiological response (i.e., higher heart rate). Both this increased likelihood of continuing to use and being in the physiologically mobilized state (i.e., higher heart rate) may be mechanisms by which cocaine seeking and addiction are drive and contribute to this group's higher daily use of cocaine. Participants with lower levels of serotonin and serotonin transporter may require less cocaine than individuals with higher levels of basal serotonin in order to feel similar subjective and objective (physiological) effects to cocaine use. Notably, the S′ allele of 5-*HTTLPR* also has been associated with response to treatment for cocaine dependence in prior studies (Nielsen *et al.*, 2012). Additionally, it is possible that low serotonin and

serotonin transporter levels reflect an increased sensitivity to impulsivity and to the positive subjective effects of cocaine (as well as other substances, like alcohol) and place an individual at greater risk for addiction.

In participants with low serotonin transporter levels, cocaine would saturate a higher proportion of transporter sites than in those participant's with higher serotonin transporter levels. Hence, low amounts of cocaine would have a greater effect than in those individuals with low 5-HTT levels.

Limitations were present in this study. First, the combined AA/AC, AA/AT, L′S′/S′S′ genotype pattern group had a greater number of Hispanics then the group without the genotype pattern (although population structure was controlled for in all analyses). Secondly, we examined the subjective effects of only one dose of cocaine (40 mg). Thirdly, our sample size was small for a molecular genetic study; thus, replication of these findings will be needed to confirm these findings. Future studies could examine the subjective effects of several different doses a day (e.g., 0, 10, 20, and 40 mg; De La Garza *et al.*, 2015).

In summary, we demonstrated that genetic variation in the 5-HT system accounts for differences in the subjective and physiological effects of cocaine in non-treatment-seeking cocaine-dependent individuals. We also demonstrated that specific serotonergic alleles were associated with higher daily cocaine use. These results suggest that the variants associated with low serotonin and transporter production could potentially be used as markers for individuals who may have a greater propensity for relapse – similar to when individuals are not as sensitive to the subjective effects of alcohol, then they are more likely to become addicted to alcohol (Schuckit, 1984, Schuckit *et al.*, 2000). As such, these at-risk individuals may require intensive, personalized intervention in order to ensure maximal benefit.

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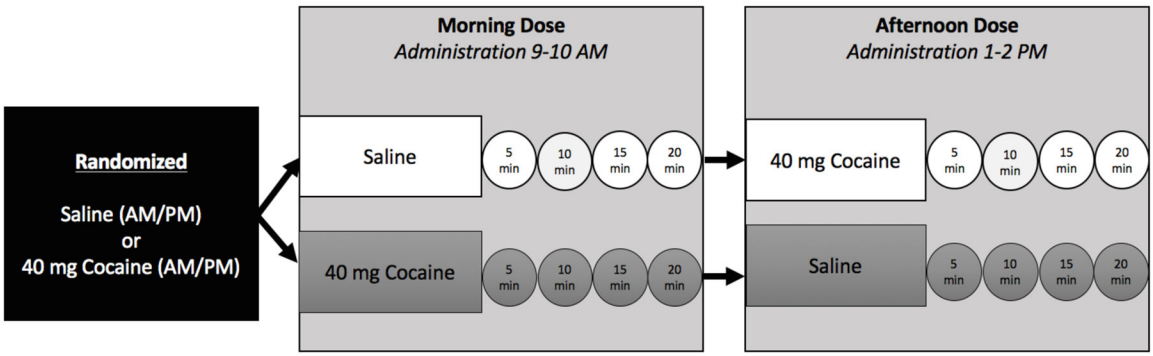

Figure 1.
Flow chart of challenge applied (squares) and timing of subjective effect ratings (circles).

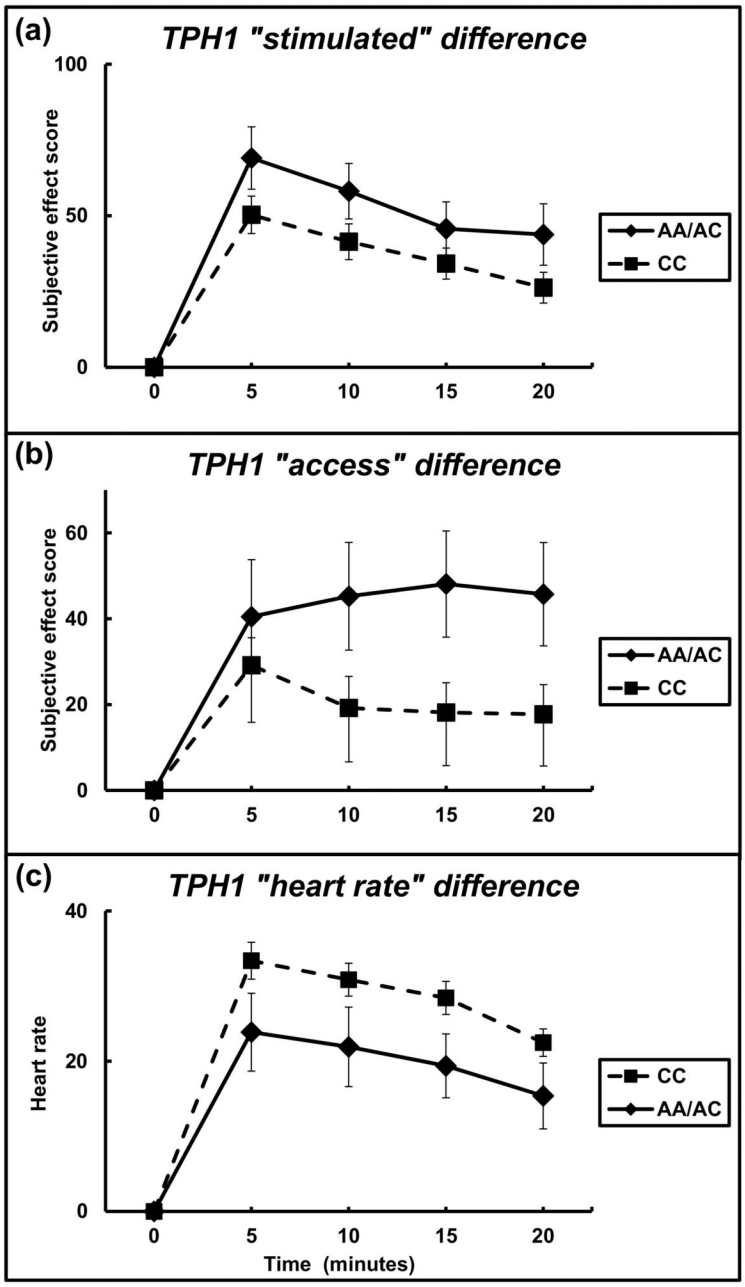

Figure 2.

Subjective effect scores by *TPH1* genotype. (A) Change over time (in minutes) of participant-reported subjective effect of "stimulated" by AA/AC genotypes ($n = 21$) vs. CC genotype ($n = 45$) groups. (B) Change over time (in minutes) of participant-reported subjective effect of "access" by AA/AC genotypes vs. CC genotype groups (*TPH1* rs1799913 minor T allele). Data reflect mean +/− S.E.M.

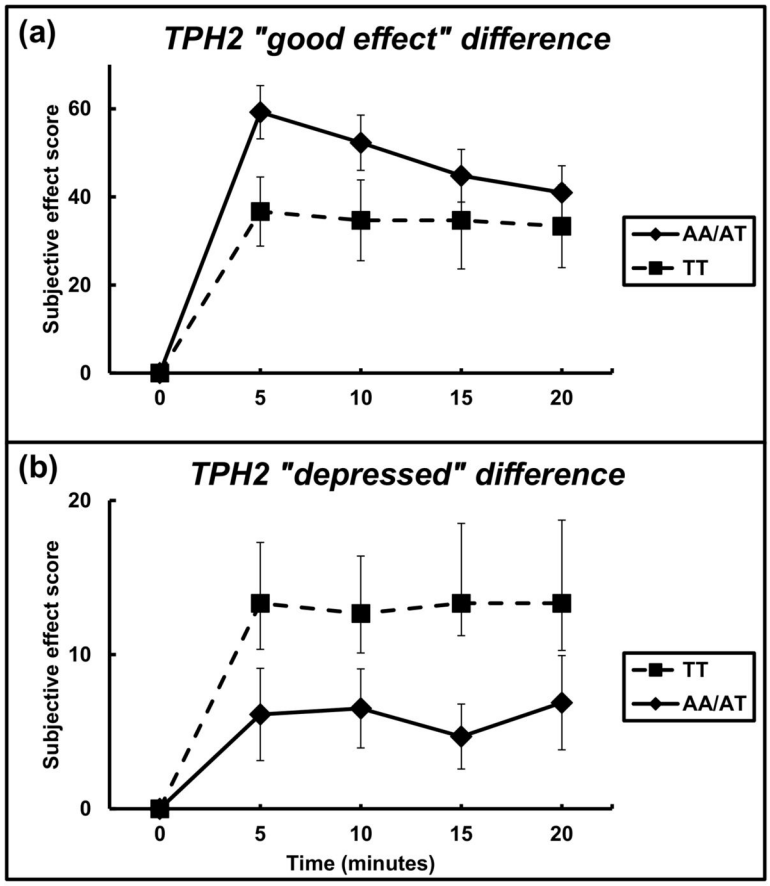

Figure 3.

Subjective effect scores by *TPH2* genotype. (A) Change over time (in minutes) of participant-reported subjective effect of "good effect" by TT genotype ($n = 15$) versus AA/AT genotype ($n = 51$) groups. (B) Change over time (in minutes) of participant-reported subjective effect of "depressed" by TT genotype versus AA plus AT genotype groups (*TPH2* rs4290270 minor A allele). Data reflect mean +/− S.E.M.

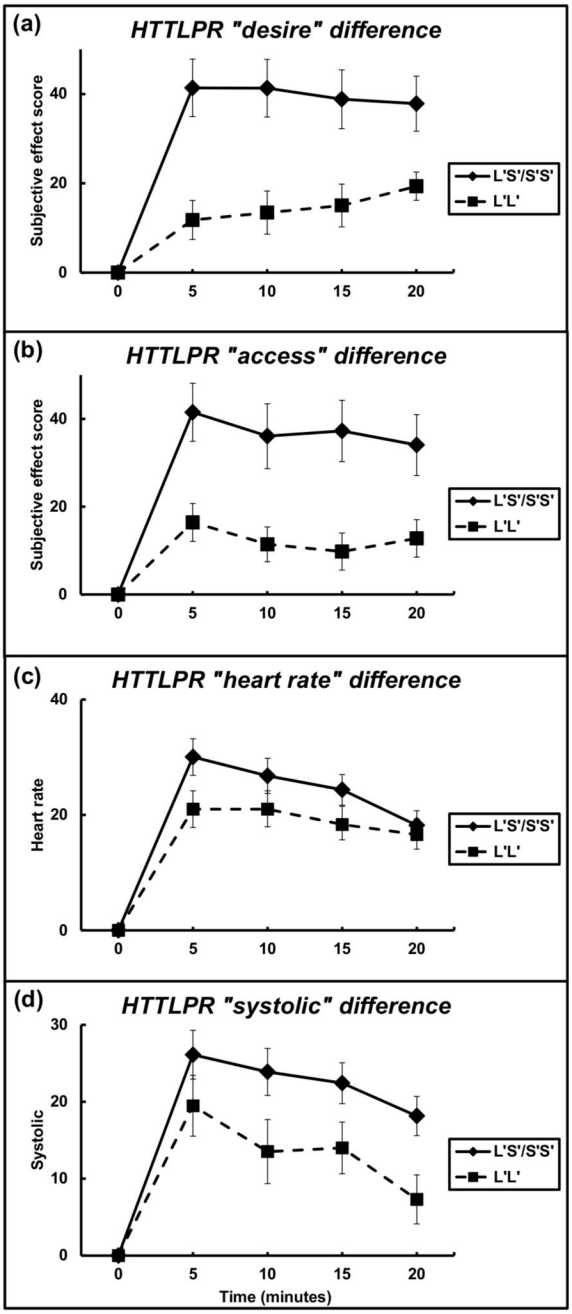

Figure 4.

Subjective effect scores by *SLC6A4* genotype. (A) Change over time (in minutes) of participant-reported subjective effect of "desire" by the L′S′/S′S′ (n = 43) and the L′L′ genotype (n = 23) groups. (B) Change over time (in minutes) of participant-reported subjective effect of "access" by the L′S′/S′S′ and the L′L′ genotype groups (*5-HTTLPR* minor S′ allele). Data reflect mean +/− S.E.M.

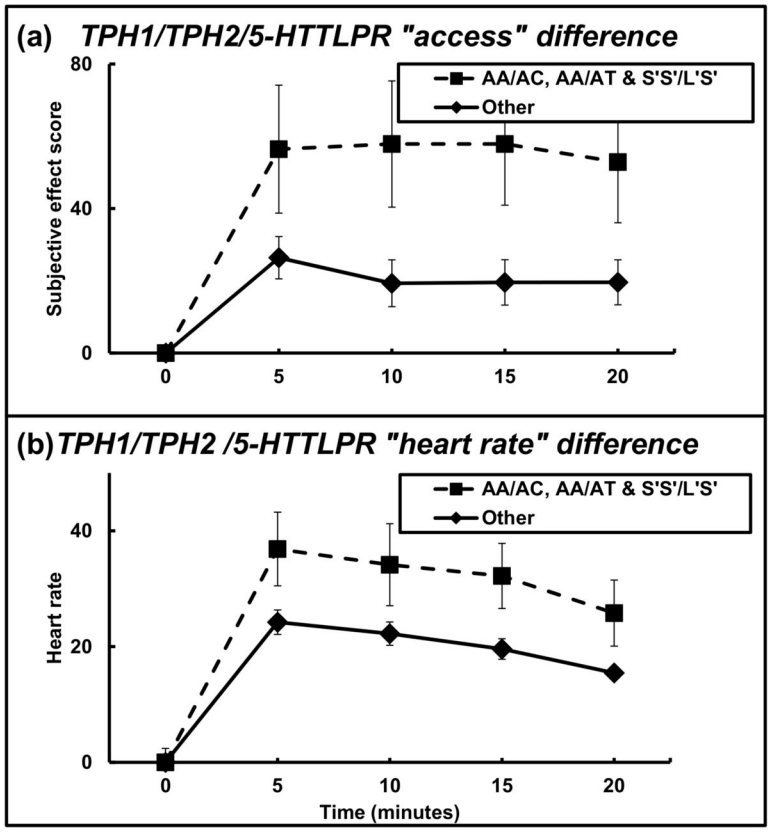

Figure 5.

Subjective effect scores by genotype pattern. (A) Change over time (in minutes) of participant-reported subjective effect of "access" by the AA/AC (*TPH1* rs1799913 minor T allele), AA/AT, and L′S′/S′S′ group ($n = 14$) and other participants ($n = 52$). (B) Change over time (in minutes) of participant-reported subjective effect of "heart rate" by the AA/AC, AA/AT, and L′S′/S′S′ group and "other" participants. Data reflect mean +/− S.E.M.

Table 1

Demographic comparison between genotype groups.

	TPH1		*TPH2*		*5-HTTLPR*		Genotype Pattern	
Genotype group	**CC**	**AA/AC**	**TT**	**AA/AT**	**L′L′**	**L′S′/S′S′**	**AA/AC AA/AT L′S′/S′S′**	**Other**
N	45	21	15	51	23	43	14	52
Male (%)	78.8	81.0	80.0	80.4	69.6	86.0	85.71	78.85
African American (%)	84.4	47.6	53.3	78.4	73.9	72.1	64.29	75
Caucasian (%)	11.1	42.9	40.0	15.7	21.7	20.9	28.57	19.23
Other (%)	4.4	9.5[a]	6.7	5.9	4.3	7.0	7.14	5.77
Hispanic (%)	4.4	23.8[b]	20.0	7.8	4.3	14.0	28.57	5.77 [c]
Education, years (SD)	12.7 (1.8)	12.5 (1.9)	12.3(1.8)	12.7 (1.8)	12.7 (1.9)	12.6 (1.8)	12.6 (2.0)	12.6 (1.8)
Age, years (SD)	43.8 (6.1)	40.7 (8.2)	43.9 (6.8)	42.5 (7)	43.0 (7.1)	42.7 (6.9)	40.3 (7.9)	43.5 (6.6)
Weight, lbs. (SD)	195.5 (35.4)	180.2 (37.7)	177.4 (30.7)	192.8 (37.4)	187.6 (34.6)	190.2 (37.7)	184.7 (42.4)	190.5 (35.0)
Nicotine use, years (SD)	21.7 (7.4)	20.5 (9.6)	20.8 (9.1)	21.4 (8)	19.8 (8.9)	22.1 (7.7)	20.4 (8.1)	21.5 (8.2)
Daily cocaine use, grams (SD) [d]	2.0 (1.4)	2.8 (3.0)	1.8 (1.4)	2.3 (2.2)	2.4 (1.5)	2.2 (2.3)	3.3 (3.6)	1.9 (1.3)
Years of cocaine use (SD)	17.8 (7.6)	15.2 (7.5)	16.4 (7.7)	17.2 (7.7)	17.0 (8.0)	17.0 (7.5)	15.4 (6.7)	17.5 (7.9)
Past 30 days cocaine use, % (SD)	19.1 (8.5)	16.1 (6.5)	19.5 (8.6)	17.8 (7.9)	20.2 (6.6)	17.1 (8.5)	17.6 (6.9)	18.4 (8.3)

Note.

[a] P = 0.029

[b] *P* = 0.006

[c] *P* = 0.032

[d] *P* = 0.039