INTRODUCTION
Cocaine dependence remains a challenging public health problem with relapse cited as a major determinant in its chronicity and severity. Environmental contexts and stimuli become reliably associated with its use leading to durable conditioned responses (‘cue reactivity’) that can predict relapse as well as treatment success. Individual variation in the magnitude and influence of cue reactivity over behavior in humans and animals suggest that cue-reactive individuals may be at greater risk for the progression to addiction and/or relapse. In the present translational study, we investigated the contribution of variation in the serotonin (5-HT) 5-HT2C receptor (5-HT2C-R) system in individual differences in cocaine cue reactivity in humans and rodents. We found that cocaine-dependent subjects carrying a single nucleotide polymorphism (SNP) in the HTR2C gene that encodes for the conversion of cysteine to serine at codon 23 (Ser23 variant) exhibited significantly higher attentional bias to cocaine cues in the cocaine-word Stroop task than those carrying the Cys23 variant. In a model of individual differences in cocaine cue reactivity in rats, we identified that high cocaine cue reactivity measured as appetitive approach behavior (lever presses reinforced by the discrete cue complex) correlated with lower 5-HT2C-R protein expression in the medial prefrontal cortex and blunted sensitivity to the suppressive effects of the selective 5-HT2C-R agonist WAY163909. Our translational findings suggest that the functional status of the 5-HT2C-R system is a mechanistic factor in the generation of vulnerability to cocaine-associated cues, an observation that opens new avenues for future development of biomarker and therapeutic approaches to suppress relapse in cocaine dependence.

Variation within the serotonin (5-HT) 5-HT2C receptor system aligns with vulnerability to cocaine cue reactivity
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Cocaine dependence remains a challenging public health problem with relapse cited as a major determinant in its chronicity and severity. With a history of cocaine use, environmental contexts and stimuli (for example, paraphernalia) become reliably associated with its use leading to durable conditioned responses (‘cue reactivity’) that can predict relapse as well as treatment success. Individual variation in the magnitude and influence of cue reactivity over behavior in humans and animals suggest that cue-reactive individuals may be at greater risk for the progression to addiction and/or relapse.1 With a history of cocaine use, environmental contexts and stimuli (for example, paraphernalia) become reliably associated with its use leading to durable conditioned responses (‘cue reactivity’) that can predict relapse as well as treatment success. Individual variation in the magnitude and influence of cue reactivity over behavior in humans and animals suggest that cue-reactive individuals may be at greater risk for the progression to addiction and/or relapse.1 With a history of cocaine use, environmental contexts and stimuli (for example, paraphernalia) become reliably associated with its use leading to durable conditioned responses (‘cue reactivity’) that can predict relapse as well as treatment success. Individual variation in the magnitude and influence of cue reactivity over behavior in humans and animals suggest that cue-reactive individuals may be at greater risk for the progression to addiction and/or relapse.

Drug cue reactivity is the attentional orientation toward drug-associated cues that are measurable as conditioned physiological effects (for example, heart rate), subjective properties (for example, craving), appetitive approach behaviors (for example, drug-seeking), and activation of specific corticostriatal subcircuits.1,2,3 Individual variation in the magnitude and influence of cue reactivity over behavior in humans7,8 and animals9,10 suggest that cue-reactive individuals may be at greater risk for the progression to addiction and/or relapse.8,11,12 A greater understanding of the neural underpinnings of cocaine cue reactivity promises to shed light on therapeutic approaches to effectively intervene in cocaine dependence and improve recovery outcomes.

The distributed corticostriatal circuitry that controls the incentive-motivational properties of drug-associated cues involves a key modulatory role for dopamine neurotransmission.13 Serotonin (5-HT) innervation of these interlooping pathways is also prominent14,15 and evidence suggests a modulatory role for 5-HT neurotransmission in cue reactivity processes (for review15). The 5-HT2C receptor (5-HT2C-R) is one of fourteen 5-HT-receptive proteins in brain and is prominently localized to corticostriatal subregions including the medial prefrontal cortex (mPFC) in rodents,16 a homolog of the orbitofrontal cortex in humans.18 This cortical region is a critical component within the circuit responsive to cocaine-associated cues in humans19 and animals.20,21 Stimulation of the 5-HT2C-R localized to the mPFC suppressed cocaine-seeking in rats,22 an observation that recapitulates the efficacy of systemic administration of selective 5-HT2C-R agonists (RO 60-0175, WAY163909) to consistently reduce cue- and cocaine-primed drug-seeking.23–27 This 5-HT2C-R agonist-induced functional antagonism of cocaine cue reactivity is reversed by the selective 5-HT2C-R antagonist SB242084 and occurs at doses of the 5-HT2C-R agonists that do not significantly alter general behaviors (for example, locomotor activity).21–27 Consistent with this behavioral profile, SB242084 also increased cocaine-seeking although inter-individual variability in its efficacy was observed.25,28–30 Finally, we recently demonstrated that cocaine cue reactivity was significantly elevated in rats following virally mediated loss of the 5-HT2C-R in mPFC31 establishing that reduced mPFC 5-HT2C-R function is a neurobiological mediator of cocaine cue reactivity.
Natural variation within the 5-HT2CR system through single nucleotide polymorphisms (SNPs) could contribute to individual differences in sensitivity to reward-associated cues in humans. The single nucleotide variant Cys235Ser (rs6318) in the human 5-HT2CR gene (HTR2C) results in the substitution of a serine for a cysteine in the extracellular N-terminus of the receptor. This SNP is predicted to alter protein structure and/or stability, which would be expected to alter the ability of a ligand to bind to the receptor and initiate downstream signal transduction.

In support of this concept, there is evidence that the Ser23 variant has been associated with lower sensitivity to the effects of 5-HT2CR agonists in human studies. As a putative reduced-function SNP in the HTR2C in humans, we tested the hypothesis that the Ser23 variant may associate with higher cocaine cue reactivity, measured as attentional bias (attentional orienting response in a computerized cocaine-word Stroop task). Alignment of the human and rat 5-HT2CR gene shows no sequence homology at the rs6318 position. However, given our recent finding that knockdown of the 5-HT2CR in the mPFC resulted in vulnerability to the expression of cocaine cue reactivity in rats, we tested the hypothesis that individual differences in cocaine cue reactivity as measured by the Cocaine-word Stroop task were analyzed with a paired Student t-test. Differences in age, sex, race, years of cocaine use, percent treatment-seekers, percent positive urine cocaine screens, percent alcohol abuse and percent cannabis abuse among subjects with different HTR2C genotypes were analyzed using one-way analysis of variance (ANOVA). The obtained values were similar to those calculated without correction for these covariates. The alpha level for all analyses was set at P=0.05.

Assessment of the 5-HT2CR system and associated cocaine cue reactivity in rodents.

Animals. Experimentally naive, male, Sprague-Dawley rats (n=105) weighing 225-250g at arrival were housed two per cage under a 12-h light-dark cycle at constant temperature (21–23 °C) and humidity (40–50%). Food and water were available ad libitum. All experiments were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (2011) and with the approval of the Institutional Animal Care and Use Committee at the University of Texas Medical Branch.

Drugs. Self-administration and cue reactivity assessments. Implantations of intravenous catheters with back mounts were performed under anesthesia with a cocktail containing xylazine (8.6 mg kg⁻¹), acropromazine (1.5 mg kg⁻¹) and ketamine (43 mg kg⁻¹) in bacteriostatic saline. Self-administration took place in standard operant chambers equipped with two retractable levers, a stimulus light above each lever, and a houselight housed within vented and sound-attenuating chambers (Med-
Associates, St Albans, VT, USA). Cocaine infusions were delivered by a syringe attached to an infusion pump located outside the chamber. Daily flushes with a solution of bacteriostatic saline containing heparin sodium (10 U ml⁻¹), streptokinase (0.67 mg ml⁻¹) and ticarcillin disodium (66.67 mg ml⁻¹) were performed to maintain catheter patency.

Self-administration consisted of 14 days of 180-min sessions, during which rats were trained to lever press to obtain a cocaine infusion (0.75 mg kg⁻¹ per 0.1 ml infusion) on a fixed ratio (FR) 1 schedule before progressing to an FR5.23,27,31.49 Schedule completions on the active lever resulted in delivery of cocaine over a 6-s period along with simultaneous illumination of the house and stimulus lights and activation of the infusion pump (discrete cue complex paired with delivery of cocaine); responses on the inactive lever were recorded but had no scheduled consequences. After cocaine delivery, the pump and stimulus light were inactivated simultaneously. The house light remained illuminated for a 20-s timeout period, during which lever presses had no scheduled consequences. Following stable self-administration on an FRS (seven infusions per hour for at least three sessions with < 10% variation in the number of infusions received for three consecutive sessions), cocaine-trained rats were subjected to a probe trial on self-administration day 12 to stratify individual rats as high cue reactive (HCR) or low cue reactive (LCR). During this 60-min probe trial, responses on the active lever were reinforced by presentation of the discrete cue complex (stimulus light, pump) previously associated with cocaine delivery. Self-administration was reinstated immediately following the end of the probe trial followed by an additional two self-administration sessions. The number of previously active lever presses during the probe trial was used to stratify rats within the HCR or LCR phenotype; a median split was used. The probe session did not interfere with the stability of self-administration as performance on the post-probe sessions was identical to the stable baseline established before the probe trial (data not shown).

Rats were returned to their home cage after 14 days of cocaine self-administration. In Experiment 1, rats (n = 12 rats per phenotype) were reintroduced to the self-administration chambers 24 h later and assayed in a test session comprised of two sequential components. The first component evaluated whether HCR and LCR rats would exhibit differential levels of lever presses when placed in the context in the absence of the discrete cue complex. To this end, responses on both levers on an FR1 schedule were recorded but no discrete cues (for example, stimulus light, pump) were present nor delivered during the initial 10 min of the session. The second component was signaled by a single, non-response contingent delivery of the discrete cue complex presented at the termination of the first 10-min component. To assess cocaine cue reactivity, presses on the previously-active lever in the 60-min (second component) were reinforced by the discrete cue complex on an FR1; inactive lever presses were recorded but produced no scheduled consequences.31

In Experiment 2, rats were stratified as HCR or LCR on the basis of their performance on the probe trial (above) and returned to the self-administration chambers at 24 h of withdrawal. To assess cocaine cue reactivity, presses on the previously-active lever were reinforced by the discrete cue complex on an FR1 during a 60-min session;31 inactive lever presses were recorded but produced no scheduled consequences. For ex vivo neurochemical studies, rats were sacrificed immediately after the cue reactivity test session (HCR (n = 5), LCR (n = 6)) or upon removal from their home cage at the expected time of that test session without re-exposure to the self-administration chambers (HCR (n = 6), LCR (n = 6)); this second group of rats served as control for the behavioral experience during the cue reactivity session. For pharmacological analyses, a cohort of HCR (n = 16 per treatment) and LCR rats (n = 16 per treatment) were administered vehicle (saline, 1 ml kg⁻¹, intraperitoneal) or WAY1663909 (0.5 mg kg⁻¹, intraperitoneal) 15 min before the start of the cue reactivity test session.

**Immunoblotting.** The HCR or LCR rats stratified on the probe test in Experiment 2 were evaluated for cue reactivity at 24 h of withdrawal and sacrificed immediately following the cue reactivity test session or remained in their home cages and sacrificed at 24 h of withdrawal. Rats were anesthetized [chloral hydrate solution (400 mg kg⁻¹)] and decapitated; the midbrain was microdissected and flash frozen in liquid nitrogen and stored at −80 °C for subsequent crude synaptosomal protein extraction and immunoblotting.50,51 Equal amounts of protein were separated by SDS–PAGE using 4–12% Bis-Tris gels and transferred to a PVDF membrane for immunoblotting with 5-HT2CR antibody (D-12, sc-17797, Santa Cruz, Dallas, TX, USA; 1:100) or pan-cadherin antibody (AB6528, Abcam, Cambridge, MA, USA; 1:1000). Membranes were incubated with mouse IgG IRDye (1:10 000) for detection by Odyssey Imaging System (LI-COR Biosciences, Lincoln, NE, USA). The integrated intensity of each band (arbitrary units) was analyzed with the Odyssey Software. The ratio of the intensity of the 5-HT2CR-immunoreactive band to the cadherin-immunoreactive band was determined for normalization.

**Statistical analyses.** A one-way ANOVA (SAS 9.3) for repeated measures was used to analyze the dependent measures of the total number of active and inactive lever presses per session over the last three sessions of the self-administration phase. Student's t-test was employed to compare HCR and LCR rats on the total number of responses (previously active and inactive levers) and the latency to respond on the previously-active lever during the probe trial, the context-associated and cue reactivity test sessions as well as the density of 5-HT2CR protein expression. For pharmacological analyses, a two-way ANOVA for the factors of phenotype and treatment was conducted; a priori comparisons between the total number of responses on the previously-active and inactive levers as well as the latency to respond on the previously active lever during the test session were made using Student's t-test. The experimentwise alpha level was set at P = 0.05.

**RESULTS**

Assessment of 5-HT2CR genotype and cue reactivity in cocaine-dependent subjects

The demographics of the study population are presented in Table 1. Cocaine-dependent subjects were stratified into three groups, those homozygous (CC; females) and hemizygous (C; males) for the C allele which encodes for the Ser23 variant, those homozygous (GG; females) and hemizygous (G; males) for the G allele or heterozygous (CG; females only) which encodes for the Cys variant. Genotype was not associated with the distribution of age (F2,111 = 0.78; NS) or race (Fisher’s exact test, NS), but was associated with sex (Fisher’s exact test, P < 0.01) (Table 1). Genotype was not associated with years of cocaine use (F2,111 = 0.15, NS), percent positive cocaine urine screens (Fisher’s exact test, NS), percent alcohol abuse (Fisher’s exact test, NS), percent cannabis abuse (Fisher’s exact test, NS), or percent treatment-seekers (Fisher’s exact test, NS) (Table 1).

The response to presentation of cocaine-associated cues (‘cue reactivity’) was measured as attentional bias in the cocaine-word Stroop task in cocaine-dependent subjects. Several studies have reported that cocaine-dependent subjects show attentional bias to cocaine-related words whereas healthy control subjects do not.24,44 Here, cocaine-dependent subjects had significantly longer reaction times to indicate the word color in trials with cocaine-related words than in trials with neutral words (Figure 1a; t = 6.96;
both sexes with the C or CC genotype was significantly greater than that for African-American subjects with the G/GG genotype. Mean reaction times (msec ± s.e.m.) for African-American subjects with the C/CC genotype which encodes for the Ser23 protein variant were presented as mean (± s.e.m.) attentional bias in msec calculated for each subject and averaged across subjects. *P < 0.05 vs G (males only). *P < 0.05 vs G/GG (both males and females).

C/CC genotype was significantly higher than AA subjects with the G/GG genotype (Figure 2; P < 0.05).

Assessment of cocaine cue reactivity in rodents
We tested the hypothesis that individual differences in HCR vs LCR rats would be observable within the context (self-administration chambers) or in the levels of cocaine cue reactivity (lever presses reinforced by the discrete cue complex). Rats in Experiment 1 readily acquired cocaine self-administration to stability; across the last three sessions (data not shown), there was no main effect of session for the number of active lever presses (F2,29 = 1.41; NS), inactive lever presses (F2,29 = 1.07; NS) or the number of infusions received (F2,29 = 0.05; NS). Rats were stratified (median split) as HCR or LCR on the basis of the number of lever presses for cocaine-associated cues during the probe session (data not shown; see Methods). Total cocaine intake did not differ between HCR and LCR rats (373.9 ± 18.3 mg kg⁻¹) and LCR rats (395.4 ± 16.6 mg kg⁻¹; NS). There was a positive correlation between previously active lever presses on the probe session with that seen on the cue reactivity test session for individual animals (r = 0.304; P < 0.05).

Table 2. Genotype, sex and attentional bias in cocaine-dependent subjects

| Genotype | Male* | Female | Both |
|----------|-------|--------|------|
| C        | 75.88 ± 14.13 | 152.83 (1) | 78.73 ± 13.89 |
| CG       | 26.03 ± 11.04 | 26.03 ± 11.04 | 41.65 ± 8.64 |
| G        | 37.80 ± 9.02  | 68.95 ± 27.94 | 41.65 ± 8.64 |

The numbers in the parenthesis indicate the subject number. *Data are presented as mean (± s.e.m.) attentional bias in msec calculated for each subject and averaged across subjects. *P < 0.05 vs G (males only). *P < 0.05 vs G/GG (both males and females).
The levels of operant behavior within the cocaine-taking context only or reinforced by the discrete cue complex were assessed in HCR vs LCR rats at 24 h of withdrawal. Previously active lever presses did not differ between HCR and LCR rats upon exposure to the cocaine-taking context in the absence of the discrete cue complex (Figure 3, left; t = 0.77, NS). HCR rats displayed significantly higher previously active lever presses that were reinforced by the discrete cue complex vs LCR rats (Figure 3, right; t = 2.81; P < 0.05). Inactive lever presses did not differ between HCR and LCR rats during the context only component (HCR = 2.5 ± 0.4; LCR = 2 ± 0.9; t = 0.77; NS) or the cue reactivity component (HCR = 8.4 ± 1.5; LCR = 6 ± 1.3; t = 1.17; NS). These data suggest that HCR and LCR rats exhibit distinct appetitive approach behavior when provided with the opportunity to deliver the discrete cue complex. The propensity to engage in appetitive behavior to deliver the discrete cue complex may represent a useful construct within which to investigate individual differences in cocaine cue reactivity.

Assessment of the 5-HT₂CR system and associated cue reactivity in rodents

We tested the hypothesis in Experiment 2 that individual differences in levels of cue reactivity would correlate with the expression of 5-HT₂CR ex vivo. HCR rats displayed significantly higher previously active lever presses for the discrete cue complex vs LCR rats (Figure 4a; t = 3.65; P < 0.01). Inactive lever presses (Figure 4a; t = 0.5; NS) and the latency to the first lever press (data not shown; t = 0.97; NS) were not different between HCR and LCR rats. A two-way, repeated measures ANOVA on the last three sessions of stable self-administration indicated no main effect of phenotype (F(1,29) = 0.02; NS), day (F(2,29) = 1.43; NS), and no phenotype × day interaction (F(2,29) = 1.02; NS) for active lever presses, indicating that individual differences in cue reactivity in rats are unrelated to previous cocaine-taking history.

Figure 4b depicts representative immunoblots for mPFC synaptosomal protein from HCR and LCR rats sacrificed immediately following the cue reactivity test session. HCR rats displayed significantly lower 5-HT₂CR synaptosomal protein levels in the mPFC vs LCR rats (Figure 4c; t = −3.75; P < 0.01); an inverse correlation was observed between mPFC 5-HT₂CR synaptosomal protein and responses on the previously-active lever for the discrete cue complex in individual rats (Figure 4d; r = 0.69; P < 0.05). Because these rats underwent the cue reactivity session, such exposure could account in part for the observed changes in mPFC 5-HT₂CR protein levels. Thus, 5-HT₂CR protein levels were assessed in a cohort of cocaine-trained rats that were retained in their home cage and sacrificed 24 h after termination of cocaine self-administration sessions (that is, not tested for cue reactivity). The differential protein expression observed in HCR (0.051 ± 0.002 arbitrary units) and LCR (0.076 ± 0.008 arbitrary units) (Figure 4) rats (stratified on the probe session) is not related to the cue reactivity test itself as comparable 5-HT₂CR mPFC protein levels were observed in HCR (0.049 ± 0.01 arbitrary units) and LCR rats (0.087 ± 0.02 arbitrary units) that were not exposed to the cue reactivity test session. These data suggest that high levels of cue reactivity are associated with lower 5-HT₂CR expression in the mPFC supporting our hypothesis that differential 5-HT₂CR neurobiology may contribute to individual differences in cocaine cue reactivity.

We then tested the hypothesis that HCR and LCR rats during early withdrawal would exhibit differential pharmacological sensitivity to the suppressive effects of the selective 5-HT₂CR agonist WAY163909 over cocaine cue reactivity (Figure 5). A main effect of phenotype (F(1,41) = 30.93; P < 0.0001), treatment (F(1,41) = 11.34; P < 0.01), and a phenotype × treatment interaction (F(1,41) = 4.23; P < 0.05) for previously active lever presses was observed. Saline-treated HCR rats displayed higher previously-active lever presses vs saline-treated LCR rats (Figure 5; P < 0.05). LCR rats treated with WAY163909 exhibited lower previously-active lever presses vs saline-treated LCR rats (Figure 5; P < 0.05); WAY163909 did not significantly alter previously-active lever presses vs saline in HCR rats (Figure 5; NS). WAY163909 (0.5 mg kg⁻¹) suppressed previously-active lever presses ~48% in LCR rats and ~12% in HCR rats. No main effect of phenotype (F(1,41) = 1.1;
DISCUSSION

The present study demonstrated that cocaine-dependent subjects who carry the less-common Ser23 variant of the HTR2C exhibit significantly higher cocaine cue reactivity than did those who carry the Cys23 variant, adding the HTR2C to handful of genes potentially identified as candidates involved in cocaine cue reactivity.53,54 Likewise, in a model of individual differences in cocaine cue reactivity in rats, we identified that high cocaine cue reactivity correlated with lower levels of mPFC 5-HT2CR protein expression and a blunted sensitivity to the suppressive effects of the selective 5-HT2CR agonist WAY163909. Interestingly, we discovered that individual differences in drug-seeking were evident when rats were given the opportunity to deliver the discrete cue complex but not when given the opportunity to simply press levers in the cocaine-taking context, supporting the incentive-motivational value of the discrete cue complex as a key defining characteristic in provoking cocaine-seeking.55 Together with our previous observation that knockdown of the mPFC 5-HT2CR resulted in vulnerability to the expression of cocaine cue reactivity in rats,31 we propose that the functional status of the 5-HT2CR system is a mechanistic driver in the generation of vulnerability to cocaine-associated cues.

Our new finding that the Cys23Ser SNP aligns with cue reactivity in cocaine-dependent subjects supports the concept that inherent variability in 5-HT2CR neurobiology may contribute to the liability of individuals to cocaine cues and cue-related relapse phenomena. The manner in which the Ser23 variant impacts baseline 5-HT2CR function is not yet fully defined. The replacement of the cysteine in the extracellular N-terminus of the 5-HT2CR encoded by the Ser23 variant localized predominantly to the cell surface in HEK293 cells and was aligned with faster binding and downstream signaling responsivity. In COS-7 cells, the Ser23 variant exhibited lower high-affinity binding and downstream signaling responsivity. In COS-7 cells, the Ser23 variant exhibited lower high-affinity binding, but not low-affinity binding, to the 5-HT2CR and the agonist response in these cells was more markedly desensitized relative to the Cys23 variant.34 The 5-HT2CR encoded by the Ser23 variant localized predominantly to the cell surface in HEK293 cells and was aligned with faster recovery of 5-HT-evoked cellular signaling following prolonged

Figure 4. High cue reactive (HCR) rats exhibit lower 5-HT2CR protein expression in medial prefrontal cortex (mPFC) relative to low cue reactive (LCR) rats. (a) Mean total lever presses (± s.e.m.) on the previously-active and inactive levers are presented for the cue reactivity test session. Each previously-active lever press resulted in the presentation of the discrete cue complex in the absence of cocaine delivery on an FR1. Rats identified as HCR (n = 5) displayed significantly higher lever presses for cocaine-associated cues vs LCR rats (n = 6; *P < 0.01). Inactive lever presses did not differ between HCR and LCR rats. (b) Qualitative and (c) quantitative data demonstrate phenotypic differences in mPFC 5-HT2CR synaptosomal protein expression. HCR rats displayed lower cortical synaptosomal 5-HT2CR protein levels relative to LCR rats (*P < 0.05). (d) An inverse correlation was observed between mPFC 5-HT2CR synaptosomal protein and responses on the previously-active lever for cocaine-associated cues in individual rats (r = 0.815; P < 0.01). The differential protein expression observed in HCR (0.051 ± 0.002 arbitrary units) and LCR (0.076 ± 0.008 arbitrary units) rats was not related to the cue reactivity test itself as comparable 5-HT2CR mPFC protein levels were observed in HCR (n = 6; 0.049 ± 0.01 arbitrary units) and LCR rats (n = 6; 0.087 ± 0.02 arbitrary units) that remained in their home cage until sacrifice.
exposure to an inverse agonist. It is possible that aberrant 5-HT2CR-mediated functions in Ser23 carriers may exhibit differential responsivity to stress or pharmacological triggers, including 5-HT2CR agonists. However, there have been no experimental evaluations in animal models in vivo which would be valuable to tease apart the mechanisms by which the Cys23Ser SNP may drive 5-HT2CR neurobiology and its impact on cocaine cue reactivity. Such studies are as a recent publication found that the Ser23 and Cys23 variants behaved indistinguishably in HEK293 and NIH-3T3 cells. Thus, although there is in vitro evidence that the Ser23 variant leads to altered cellular responses to stimuli, definitive information remains to be collected to best understand the association reported here between expression of the Ser23 variant and enhanced cocaine cue reactivity, as well as in the clinical course of some psychiatric disorders.

There are reports of altered 5-HT2CR responsivity after cocaine exposure in humans and experiment-delivered cocaine in animals. Our observations that mPFC 5-HT2CR expression and pharmacological sensitivity to a selective 5-HT2CR agonist associate with individual variations in levels of cue reactivity in rodents are consistent with the possibility that reduced mPFC 5-HT2CR expression observed here translates directly to differential functional output of the receptor to manifest cue reactivity, however, high cue reactive rats were less sensitive to the suppressive effects of WAY163909. The composition of the cellular microenvironment (that is, protein-binding partners) also contributes to 5-HT2CR-mediated signaling and agonist responsiveness. It is currently unknown whether the difference in cortical 5-HT2CR expression observed here translates directly to differential functional output of the receptor to manifest cue reactivity. However, high cue reactive rats were less sensitive to the suppressive effects of WAY163909. The composition of the cellular microenvironment (that is, protein-binding partners) also contributes to 5-HT2CR-mediated signaling and agonist responsiveness.

We have reported that the 5-HT2CR is localized to the postsynaptic density in PFC and thus positioned to directly modulate synaptic plasticity in cortical neurons; the 5-HT2CR agonist MK212 is reported to enhance long-term potentiation in forebrain. Taken together, these biochemical and behavioral data suggest that high cocaine cue reactivity (but not sucrose cue reactivity) may be governed by a blunted response capacity of the 5-HT2CR. The discovery that individual differences in cue reactivity coexist concomitantly with distinct 5-HT2CR expression patterns in the synaptosomal compartment indicates that balance in the cortical 5-HT2CR functional status may be the key to shaping the neural state that contributes to cocaine-associated cue reactivity during abstinence.

Some limitations of this study should be noted. With the small number of female subjects in the human data set in this study and the exclusion of females in the rodent data set, the findings of this study cannot be extrapolated to women. As the HTR2C is X-linked, future studies should investigate the role of 5-HT2CR neurotransmission in sex differences observed in cocaine cue reactivity as sex may be a factor that contributes to cocaine cue-related neurobiology. The direct translatability of the studies presented herein is somewhat limited as there are key discrepancies in cocaine exposure patterns and cocaine use history between humans and rodents. The human data set included subjects with extensive cocaine histories, whereas the rodent data set included animals with shorter exposures to cocaine self-administration. Further, the Cys23Ser SNP has not been identified in rodents nor has the Cys23Ser SNP been tied directly to frontocortical activation patterns in response to drug-associated cues or the cortical 5-HT2CR functional status in cocaine-dependent subjects. Nonetheless, the inclusion of the rodent study allowed for the experimental test of the hypothesis that individual differences in cocaine cue reactivity during early abstinence are associated with differential measures of cortical 5-HT2CR neural integrity.

Our translational findings cumulatively suggest that susceptibility to cocaine cue reactivity may be related to inter-individual variation within the 5-HT2CR system. Although other studies have examined the association of genotype with cue reactivity in cocaine users our study employed the largest sample size to date, and we are the first to have examined the association of the HTR2C genotype in experimentally measured cue reactivity. The rodent studies suggest that a differential 5-HT2CR functional status, marked by lower cortical 5-HT2CR synaptosomal protein expression and reduced pharmacological sensitivity, associates with greater reactivity to cocaine-associated cues. Future studies are required to expand on our observations to consider the 5-HT2CR system as a risk factor or predictor of cocaine cue reactivity, and perhaps explore as a biological marker of propensity toward craving and relapse in cocaine dependence.
CONFLICT OF INTEREST
Dr Moeller is a consultant for Boehringer-Ingelheim. Dr Cunningham is a consultant for Arena Pharmaceuticals and an editor of Neuropsychopharmacology Reviews for which she receives compensation from the American College of Neuropsychopharmacology. The remaining authors declare no conflict of interest.

ACKNOWLEDGMENTS
We thank Dr David Goldman (Laboratory of Neurogenetics, National Institute on Alcohol Abuse and Alcoholism) for thoughtful discussions and comments on the manuscript. We thank Ms Sonja J Litstutz (UTMB) for assistance with rat self-administration surgeries and procedures. This work was supported by the National Institute on Drug Abuse grants K99 DA033734 (NCA), P20 DA042157 (KAC), K05 DA200887 (KAC), K02 DA000403 (FGM), P50 DA09262 (FGM), MD Anderson’s Cancer Center Support Grant DA026120 (SL) and the Center for Addiction Research at the University of Texas Medical Branch. The work was also supported in part by the Toomin Family Fund (DMN). This material is the result of work supported in part by the use of resources and the use of facilities at the Michael E DeBakey VA Medical Center, Houston, TX, USA.

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