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Striatal Dopamine Release and Genetic Variation of the Serotonin 2C Receptor in Humans

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Mesoaccumbal and nigrostriatal projections are sensitive to stress, and heightened stress sensitivity is thought to confer risk for neuropsychiatric disorders. Serotonin 2C (5-HT_{2C}) receptors mediate the inhibitory effects of serotonin on dopaminergic circuitry in experimental animals, and preclinical findings have implicated 5-HT_{2C} receptors in motivated behaviors and psychotropic drug mechanisms. In humans, a common missense single-nucleotide change (rs6318, Cys23Ser) in the 5-HT_{2C} receptor gene (*HTR2C*) has been associated with altered activity *in vitro* and with clinical mood disorders. We hypothesized that dopaminergic circuitry would be more sensitive to stress in humans carrying the Ser23 variant. To test this hypothesis, we studied 54 healthy humans using positron emission tomography and the displaceable D_2/D_3 receptor radiotracer [^{11}C]raclopride. Binding potential (BP_{ND}) was quantified before and after a standardized stress challenge consisting of 20 min of moderate deep muscular pain, and reduction in BP_{ND} served as an index of dopamine release. The Cys23Ser variant was genotyped on a custom array, and ancestry informative markers were used to control for population stratification. We found greater dopamine release in the nucleus accumbens, caudate nucleus, and putamen among Ser23 carriers, after controlling for sex, age, and ancestry. Genotype accounted for 12% of the variance in dopamine release in the nucleus accumbens. There was no association of Cys23Ser with baseline BP_{ND}. These findings indicate that a putatively functional *HTR2C* variant (Ser23) is associated with greater striatal dopamine release during pain in healthy humans. Mesoaccumbal stress sensitivity may mediate the effects of *HTR2C* variation on risk of neuropsychiatric disorders.

Introduction

Disturbances of dopaminergic projections from midbrain to striatum have been implicated in mood disorders, psychotic disorders, and addictions. Furthermore, dopaminergic circuitry is sensitive to stress in experimental animals (Berton et al., 2006; Cao et al., 2010; Ungless et al., 2010; Cabib and Puglisi-Allegra, 2012) and humans (Scott et al., 2006; Borsook et al., 2010; Admon et al., 2012), suggesting that this system may mediate the effects of stress on psychiatric disease in vulnerable individuals. Given the heritability of these diseases, a better understanding of their origins may come from identifying genetic variants that influence dopaminergic circuitry and disease vulnerability.

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Serotonin 2C (5-HT_{2C}) receptors regulate dopaminergic circuitry in experimental animals. Serotonin activates 5-HT_{2C} receptors on midbrain interneurons and dopamine neurons, suppressing local dopaminergic cell activity (Eberle-Wang et al., 1997; Di Giovanni et al., 2001; Berg et al., 2008). Activation of 5-HT_{2C} receptors reduces striatal dopamine release, while antagonists of 5-HT_{2C} receptors increase striatal dopamine (Berg et al., 2008; Di Matteo et al., 2008; Egerton et al., 2008). Pharmacologic or genetic manipulations of 5-HT_{2C} receptors alter locomotor responses, the reinforcing value of psychostimulants, food intake and obesity, and the behavioral effects of antidepressants and antipsychotics (Tecott et al., 1995; Rocha et al., 2002; Cannon et al., 2004; Giorgetti and Tecott, 2004; Abdallah et al., 2009).

5-HT_{2C} receptors have been implicated in stress, anxiety, and pain. Manipulation of 5-HT_{2C} receptors in experimental animals causes anxiety and alters behavioral and neuroendocrine responses to various forms of stress (Kahn and Wetzler, 1991; Bagdy et al., 2001; Burghardt et al., 2007; Heisler et al., 2007; Hawkins et al., 2008; Christianson et al., 2010; Strong et al., 2011). Several reports have implicated 5-HT_{2C} receptors in pain-stress and spinal mechanisms of neuropathic pain (Jeong et al., 2004; Obata et al., 2004; Hawkins et al., 2008; Nakae et al., 2008; Liu et al., 2010; Brasch-Andersen et al., 2011). How these receptors might be involved in the response to pain in central stress-sensitive circuitry such as the mesolimbic pathway remains unclear.

5-HT_{2C} receptors have been linked to neuropsychiatric disorders (Drago and Serretti, 2009). The single nucleotide variant

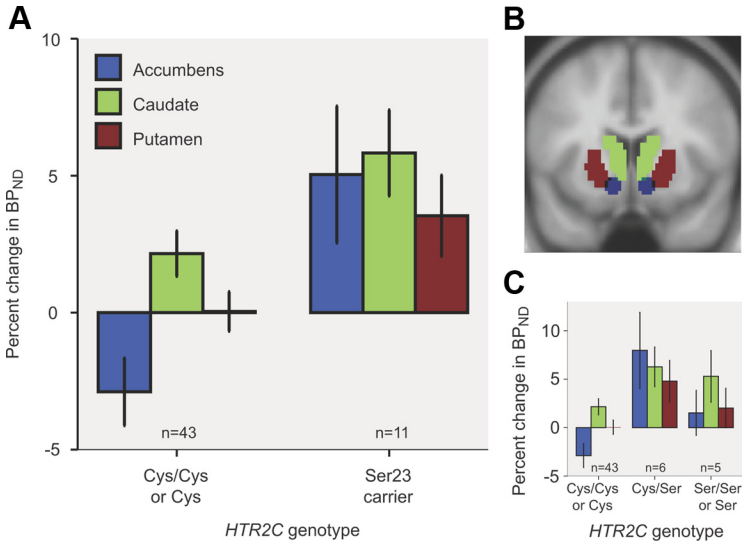

Figure 1. Effects of pain-stress on striatal dopamine release as a function of *HTR2C* genotype. ***A***, Stress-induced dopamine release, expressed as percentage change in D_2/D_3 binding potential (BP_{ND}) relative to baseline, in three striatal regions of interest. Positive change represents pain-induced decrease in BP_{ND}, which reflects dopamine release. Error bars indicate SEM. ***B***, Nucleus accumbens, caudate nucleus, and putamen regions of interest are shown over a coronal MRI section in standardized space ($y = 10$). ***C***, Stress-induced dopamine release versus *HTR2C* genotype for the 3-group classification.

Cys23Ser (rs6318) in the human 5-HT_{2C} receptor gene (*HTR2C*) has been a focus of attention because a serine is substituted for a cysteine in the extracellular N terminus of the receptor, potentially altering the protein's structure or stability by eliminating a disulfide bond (Lappalainen et al., 1995). The Ser23 variant has been associated with greater constitutive activity, lower affinity, and altered resensitization in some assay systems (Lappalainen et al., 1995; Okada et al., 2004; Fentress et al., 2005; Walstab et al., 2011). A genetic association study linked Ser23 with recurrent major depression and bipolar disorder (Lerer et al., 2001).

We hypothesized that stress-sensitivity of the dopamine system would be greater among individuals carrying the *HTR2C* Ser23 variant. To test this possibility, we genotyped healthy humans who participated in a pain challenge—a controlled physical and emotional stressor that is valid across species—during positron emission tomography (PET) with the D_2/D_3 receptor tracer [^{11}C]raclopride.

Materials and Methods

Subjects. Fifty-four healthy, right-handed adults (60% female; mean age, 27 years; SD, 5 years; range, 19–40 years) completed PET and provided blood for genetic analyses. PET data from 17 of these subjects have been reported previously (Scott et al., 2006) and are reanalyzed here. Participants had no personal history of major medical illness or psychiatric disorder, including substance use disorders. They were not taking medications with CNS activity (including birth control pills and other exogenous hormones) and they were instructed to abstain from all psychoactive substances for 24 h before the study. Women were studied in the follicular phase of the menstrual cycle (days 4–10). Written informed consent was obtained and all procedures were approved by the Institutional Review Board and Radioactive Drug Research Committee at the University of Michigan.

Positron emission tomography. Striatal D_2/D_3 receptor availability was quantified with PET and the displaceable radiotracer [^{11}C]raclopride, as described previously (Scott et al., 2006). In brief, [^{11}C]raclopride was synthesized at high specific activity (>2000 Ci/mmol) by reaction of *O*-desmethyl-raclopride with [^{11}C]methyl-triflate. Fifty percent of the radiotracer dose was administered as a bolus and the remainder as a continuous infusion to more rapidly achieve constant tracer levels (total mean ± SD administered, 15.0 ± 2.2 mCi). Under these conditions, equilibrium across kinetic compartments is achieved after ~35 min (Carson et al., 1997). PET scans were acquired with a Siemens HR+ scanner in 3-D mode, with a reconstructed full-width-at-half-maximum resolution of 5.5 mm in-plane and 5.0 mm axially. Twenty-eight image frames of increasing duration were acquired over 90 min.

The stress challenge consisted of moderate, sustained, muscular pain, as described previously (Scott et al., 2006). A fine-gauge needle was inserted into the left masseter muscle before the scan. During the baseline period (0–45 min after radiotracer administration) isotonic saline (0.9%) was infused, which in all cases caused no pain. Participants were not told at what point during the scan pain would begin, so an expectation of pain was created during the baseline period. Beginning at 45 min, infusion of hypertonic saline (5%) maintained a steady level of pain for 20 min. Pain intensity was rated every 15 s from 0 (no pain) to 100 (most intense pain imaginable) using a visual analog scale, and a computer controller adjusted the infusion to maintain the pain near a target of 40 visual analog units. Pain sensitivity was calculated as the average visual analog intensity rating divided by the total volume of infused hypertonic saline. Immediately after the pain challenge, participants completed the McGill Pain Questionnaire (MPQ) (Melzack and Torgerson, 1971), which reflects an individual's overall subjective experience, as measured by weighted pain descriptors. Subjective emotional state was assessed before the challenge (baseline) and after the challenge using the Positive and Negative Affect Schedule (PANAS) (Watson and Clark, 1994).

Dynamic image data were transformed voxelwise into two sets of images: a tracer transport measure (K_1 ratio) and a receptor-related measure, the nondisplaceable binding potential (BP_{ND}) relative to a cerebellar reference region (Carson et al., 1997; Watabe et al., 2000; Love et al., 2012). BP_{ND} measures were calculated for the period before pain (35–45 min after tracer infusion) and during and immediately after pain (60–80 min). Reduction in BP_{ND} during the pain-stress challenge is thought to reflect release of dopamine and competition between radiotracer and endogenous ligand (Laruelle, 2000), but changes in dopamine receptor concentration or affinity cannot be ruled out with this method. Images were spatially normalized to standardized space (Montreal Neurological Institute, MNI). Mean BP_{ND} values were extracted from regions of interest (shown in Fig. 1*B*) using the MarsBaR toolbox (Brett et al., 2002). Nucleus accumbens regions were defined by two spheres of 5 mm radius centered at MNI coordinates ±10, 10, −10. Caudate and putamen regions were based on the Talairach atlas as implemented in the Wake Forest University PickAtlas toolbox (Lancaster et al., 2000; Maldjian et al., 2003).

Genetics. Approximately 6 ml of whole blood was collected during the PET scan. DNA was extracted and genotyped on an Illumina GoldenGate platform as previously described (Hodgkinson et al., 2008). The *HTR2C* single nucleotide polymorphism Cys23Ser (rs6318) was selected because this variant substitutes a serine for a cysteine in the putative extracellular N terminus of the receptor, increasing the likelihood of functional effects (Lappalainen et al., 1995). Because *HTR2C* is on the X chromosome, males are hemizygous (Cys or Ser), and females may be homozygous or heterozygous (Cys/Cys, Cys/Ser, or Ser/Ser). The *HTR2C* gene is subject to X inactivation in females. Genotype frequencies were in Hardy-Weinberg equilibrium.

For hypothesis testing, participants were classified as Ser23 carriers and non-carriers (Ser23-dominant model, Table 1) in accord with a previous association study (Lerer et al., 2001). Other classifications were explored *post hoc*. Gene effects were examined in females and males separately, excluding the single Ser/Ser participant. Because male hem-

Table 1. *HTR2C* Cys23Ser genotypes of the study sample

Genotype group	*N*
All	54
Cys/Cys female	24
Cys/Ser female	6
Ser/Ser female	1
Cys male	19
Ser male	4
Cys/Cys and Cys	43
Ser carrier	11

izygotes are in theory functionally equivalent to female homozygotes, we also explored a 3-group classification: Cys/Cys or Cys versus Cys/Ser versus Ser/Ser or Ser.

To control for population stratification, samples were genotyped for 186 ancestry informative markers (AIMs) using an Illumina GoldenGate assay as described previously (Hodgkinson et al., 2008). Factor analysis resulted in a seven-factor solution which yielded ethnic factor scores for each individual. In our sample, the mean (median) ancestry factor scores were as follows: Europe, 0.67 (0.94); Africa, 0.14 (0.001); Asia, 0.10 (0.02); Middle East, 0.06 (0.02); East Asia, 0.02 (0.003); America, 0.008 (0.003); Oceania, 0.004 (0.002). Because factor scores were correlated and the sample was predominantly Caucasian, the Europe factor score was included as a covariate in all analyses to account for ancestral variability in allele frequency.

Statistical analysis. Initial power analysis indicated that, with group sizes of 11 and 43, we would have ~80% power to detect a standardized effect size of ~1.0 with a 5% type I error rate (Kraemer and Thiemann, 1987). To test our primary hypothesis that Cys23Ser would be associated with dopaminergic neurotransmission, we used a multivariate repeated-measures general linear model (PASW Statistics 18.0, Chicago, IL). The dependent variables were BP_{ND} in each of the three regions of interest (nucleus accumbens, caudate nucleus, and putamen) before and after pain. Left and right were averaged because pain activates the striatum bilaterally (Scott et al., 2006), and we had no basis for hypothesizing lateralized gene effects. The Hotelling multivariate test accounted for testing of three brain regions. Pain condition was the within-subject factor. Ser23 carrier classification (Table 1) was the between-subjects factor. Sex, age, and AIMs Europe factor score were included as covariates in all statistical models because Cys23Ser distribution varies with ancestry (Lerer et al., 2001; Drago and Serretti, 2009), because *HTR2C* is X-linked, and because of previous reports that D_2/D_3 receptor availability and dopamine release vary with sex and age (Pohjalainen et al., 1998; Munro et al., 2006). Furthermore, our own data showed that age and sex were associated with BP_{ND} (see Results). Where statistically significant effects were found with the general linear model, to aid interpretation, we computed the percentage change in BP_{ND} as an index of dopamine release.

Psychophysical measures of pain and emotion were also analyzed with a general linear model. As for the PET analysis, Ser23 carrier classification was the between-subjects factor, and sex, age, and AIMs Europe factor score were included as covariates. Highly skewed measures (pain sensitivity, MPQ Affect subscale, PANAS Negative subscale) were transformed to normal scores before analysis (Blom proportion estimation, PASW Statistics 18.0).

Results

Fifty-four individuals completed PET and the pain-stress challenge. Participants were classified as carriers or non-carriers of the *HTR2C* Ser23 allele (Table 1). Pain intensity ratings during the challenge were not associated with Ser23 carrier status (Table 2), demonstrating that the challenge paradigm resulted in comparable levels of subjective pain for the two groups. A trend toward greater pain sensitivity (ratio of pain intensity rating to volume of saline administered) was found among Ser23 carriers (Table 2). Cys23Ser genotype was not associated with emotion ratings on the Positive and Negative Affect Schedule prechallenge or postchallenge (Table 2). Scores on the McGill Pain Questionnaire, which reflected an individual's overall qualitative experience of pain immediately after the challenge, were greater among Ser23 carriers (Table 2).

Table 2. *HTR2C* Ser23 carrier status and psychophysical measures during the pain-stress challenge

	Mean (SD)			
	Cys23 only $n = 43$	Ser23 carrier $n = 11$	p^a	$F_{(1,49)}$
Pain intensity (visual analog scale)	32 (12)	39 (11)	0.12	2.49
Saline infusion volume (ml)	2.9 (1.2)	2.2 (1.3)	0.12	2.57
Pain sensitivity (intensity / saline volume)	1.6 (1.5)	3.1 (3.3)	0.07	3.52
PANAS Positive subscale, before pain	14.5 (6.9)	16.5 (7.2)	0.41[b]	0.69[b]
PANAS Positive subscale, after pain	9.9 (7.0)	11.0 (7.0)	0.64[c]	0.22[c]
PANAS Negative subscale, before pain	3.2 (3.6)	8.1 (12.5)	0.22[b]	1.53[b]
PANAS Negative subscale, after pain	3.3 (5.4)	5.8 (5.9)	0.72[c]	0.13[c]
MPQ Total	23.1 (9.4)	31.9 (14.3)	**0.023**	**5.48**
Sensory subscale	15.1 (6.2)	20.1 (8.5)	**0.040**	**4.45**
Affect subscale	1.6 (2.5)	2.6 (3.1)	0.21	1.61
Evaluative subscale	1.8 (1.3)	2.6 (1.3)	0.09	2.97
Miscellaneous subscale	4.7 (2.2)	6.6 (3.6)	**0.039**	**4.51**
MPQ Present Pain Intensity subscale	2.3 (0.7)	2.6 (0.7)	0.40	0.71

PANAS, Positive and Negative Affect Schedule; MPQ, McGill Pain Questionnaire. Bold indicates $p < 0.05$.

[a]*p* values represent the effect of Ser23 carrier status on each psychophysical variable, separately evaluated using a general linear model, adjusted for sex, age, and European ancestry factor score.

[b]Ser23 main effect, repeated-measures model.

[c]Pain × Ser23 interaction, repeated-measures model.

Striatal D_2/D_3 receptor BP_{ND} was quantified before and after pain, and genetic association with BP_{ND} was tested using a repeated-measures general linear model. Sex, age, and ancestry were included as covariates for the following reasons. In agreement with previous reports (Pohjalainen et al., 1998; Munro et al., 2006), we found that BP_{ND} was associated with sex (main effect, $p = 0.009$, $F_{(3,50)} = 4.35$; sex-by-pain interaction, $p = 0.013$, $F_{(3,50)} = 3.96$; multivariate test) and age (main effect, $p = 0.005$, $F_{(3,50)} = 4.78$; age-by-pain interaction, $p = 0.58$, $F_{(3,50)} = 0.66$; multivariate test). Furthermore, previous work showed that *HTR2C* allelic distribution varies with ancestry (Lerer et al., 2001; Drago and Serretti, 2009). The repeated-measures general linear model demonstrated a significant interaction between Ser23 carrier status and pain condition, and no main effect of Ser23 (Table 3).

To interpret these findings, we calculated the fractional change in BP_{ND} as an index of pain-induced dopamine release, and found that release was greater among Ser23 carriers for all three striatal regions of interest (Fig. 1*A*,*B*). Genotype accounted for 12%, 5%, and 7% of the variance in dopamine release in the nucleus accumbens, caudate, and putamen, respectively (standardized effect size, 0.7–1.0). Dopamine release in the nucleus accumbens actually decreased post-pain in the Cys23 group, on average (Fig. 1*A*), indicating greater synaptic dopamine release during anticipation of pain than during the subsequent experience of pain. Ser23 was not associated with baseline BP_{ND} ($p = 0.30$, multivariate general linear model, controlling for sex, age, and ancestry).

To explore whether the association of Cys23Ser with dopamine release could be accounted for by differences in sensitivity to sustained pain or overall pain experience, we added pain sensitivity and total McGill pain score as covariates in the repeated-measures model. Including these covariates weakened the Pain × Ser23 interaction in caudate and putamen, but the effect in the nucleus accumbens persisted (Table 3). We also explored an al-

Table 3. *HTR2C* genotype and D_2/D_3 binding potential during the pain-stress challenge

	Accumbens	Caudate	Putamen	Multivariate
Primary analysis: Ser23 dominant model (Ser23 carrier versus non-carrier)[a]				
Main effect of Ser23	0.31 (1.06, 0.021)	0.61 (0.27, 0.005)	0.22 (1.57, 0.031)	0.62 (0.60, 0.037)
Pain × Ser23	**0.003 (10.05, 0.170)**	**0.037 (4.62, 0.086)**	**0.034 (4.76, 0.089)**	**0.028 (3.30, 0.174)**
df	1, 49	1, 49	1, 49	3, 47
Exploratory analyses				
Ser23 dominant model with pain covariates[b]				
Main effect of Ser23	0.33 (0.98, 0.020)	0.85 (0.04, 0.001)	0.52 (0.43, 0.009)	0.81 (0.33, 0.021)
Pain × Ser23	**0.007 (8.07, 0.147)**	0.12 (2.51, 0.051)	0.12 (2.47, 0.050)	**0.040 (3.00, 0.167)**
df	1, 47	1, 47	1, 47	3, 45
Three-group model[c]				
Main effect of group	0.46 (0.79, 0.032)	0.21 (1.64, 0.064)	0.14 (2.09, 0.080)	0.56 (0.82, 0.052)
Pain × group	**0.005 (5.86, 0.196)**	0.08 (2.67, 0.100)	0.06 (2.99, 0.111)	0.09 (1.89, 0.112)
df	2, 48	2, 48	2, 48	6, 90
Females only[d]				
Main effect of group	0.15 (2.19, 0.078)	0.14 (2.31, 0.082)	**0.033 (5.08, 0.163)**	0.20 (1.69, 0.174)
Pain × group	**0.025 (5.69, 0.180)**	0.12 (2.54, 0.089)	0.09 (3.14, 0.108)	0.18 (1.76, 0.180)
df	1, 26	1, 26	1, 26	3, 24
Males only[e]				
Main effect of group	0.65 (0.22, 0.011)	0.31 (1.08, 0.054)	0.20 (1.79, 0.086)	0.61 (0.63, 0.100)
Pain × group	**0.022 (6.18, 0.245)**	0.16 (2.15, 0.102)	0.17 (2.05, 0.097)	0.16 (1.94, 0.255)
df	1, 19	1, 19	1, 19	3, 17

p values are shown for the repeated-measures multivariate general linear model. In parentheses are the *F* statistic and partial η^2 (a measure of effect size). *p* values for individual regions are uncorrected. The Hotelling multivariate test (right-hand column) accounts for multiple comparisons across the three regions. Dependent variables are binding potential in three regions of interest before and after the pain challenge. df, Degrees of freedom. Bold indicates $p < 0.05$.

[a]Primary model: between-subjects factor is Ser23 carrier status; covariates are sex, age, and European ancestry score.

[b]Between-subjects factor is Ser23 carrier status; covariates are sex, age, European ancestry factor score, pain sensitivity, and McGill Pain Questionnaire total score.

[c]Between-subjects factor is genotype group (Cys/Cys or Cys; Cys/Ser; Ser/Ser or Ser); covariates are sex, age, and European ancestry score.

[d]Between-subjects factor is genotype group (Cys/Cys versus Cys/Ser); covariates are age and European ancestry score; Ser/Ser participant was excluded.

[e]Between-subjects factor is genotype group (Cys versus Ser); covariates are age and European ancestry score.

ternative 3-group genotype classification scheme, and examined gene effects in females and males separately. Similar effects of the Ser23 allele were observed in those analyses, especially in the nucleus accumbens (Table 3, Fig. 1*C*).

Discussion

Based on the pivotal role of 5-HT_{2C} receptors in the regulation of mesoaccumbal dopamine in experimental animals, we hypothesized that a putatively functional variant of the *HTR2C* gene would be associated with dopaminergic function in humans exposed to a salient, stressful stimulus—a standardized pain challenge. We found that carriers of the *HTR2C* Ser23 allele had greater release of dopamine in all striatal regions, but especially in the nucleus accumbens, where genotype accounted for 12% of the variance in dopamine release. To our knowledge, this is the first evidence for an effect of the Cys23Ser polymorphism on dopaminergic responses in humans.

We observed the association of Ser23 with dopamine release while controlling for sex, age, and ancestry. Adjusting for these variables is important for several reasons. First, genetic associations are vulnerable to the confounding effects of population stratification, and variation in Cys23Ser allele frequencies across ethnicities has been described (Lerer et al., 2001; Drago and Serretti, 2009). Second, *HTR2C* is on the X chromosome, so sex differences are possible in principle. Third, sex and age effects on D_2/D_3 receptor availability and dopamine release have been reported (Pohjalainen et al., 1998; Munro et al., 2006). For those reasons, we characterized each subject's genetic background by genotyping ancestry informative markers, and ancestry, sex, and age were included in all statistical models. We also detected the genetic association in males and females separately. That a genetic effect was observed regardless of sex, age, and ancestry strongly suggests that the association between Cys23Ser and dopamine release is not simply an artifact of population stratification or demographic factors. We cannot rule out the possibility that a genetic locus in strong linkage disequilibrium with Cys23Ser may be the actual causal factor, but there is currently no better candidate than Cys23Ser itself.

How the Cys23Ser polymorphism impacts 5-HT_{2C} receptor function is not altogether clear. The Ser23 variant lacks a cysteine residue in the putative extracellular N terminus of the receptor, which potentially eliminates a disulfide bond. *In vitro* functional studies have been discrepant, but the discrepancies might be accounted for by differences in assay systems. The Ser23 variant had lower high-affinity binding (but not low-affinity binding) and a steeper intracellular calcium response curve in COS-7 cells (Okada et al., 2004), greater constitutive activity in an Sf9 cell G-protein reconstitution system (Okada et al., 2004), and greater cell surface expression and faster resensitization following inverse agonist treatment in HEK293 cells (Walstab et al., 2011). On the other hand, in HEK293 and NIH-3T3 cells, no differences between Ser23 and Cys23 variants were found in cellular localization, high-affinity binding, phosphoinositide signaling, constitutive activity, or homodimerization (Fentress et al., 2005) and no differences were seen in frog oocytes under baseline conditions (Lappalainen et al., 1995). In addition to the usual difficulties in extrapolating *in vitro* findings to the intact nervous system, 5-HT_{2C} receptors are subject to an extraordinary degree of region-specific RNA editing (Niswender et al., 2001; Gurevich et al., 2002; Berg et al., 2008; Drago and Serretti, 2009; Iwamoto et al., 2009), so additional *in vivo* studies of Ser23 variant function are needed.

5-HT_{2C} receptors are thought to mediate the inhibitory effects of serotonin on mesoaccumbal function (Eberle-Wang et al.,

1997; Di Giovanni et al., 2001; Berg et al., 2008; Di Matteo et al., 2008; Egerton et al., 2008), so our finding of greater stress-induced dopamine release among Ser23 carriers would be consistent with weaker serotonergic modulation of mesoaccumbal projections in those individuals. There are several plausible mechanisms that might contribute. Release of serotonin in the ventral tegmental area (VTA) normally activates inhibitory interneurons via 5-HT_{2C} receptors, increasing local inhibition of VTA dopamine neurons, and reducing dopamine release in the striatum. This mechanism would be less efficient among Ser23 carriers if this receptor variant had lower affinity for endogenous serotonin (Okada et al., 2004), or if this variant had a smaller dynamic response to endogenous serotonin as a result of greater constitutive activity (Okada et al., 2004). 5-HT_{2C} receptors are also expressed by VTA dopamine neurons (Berg et al., 2008; Bubar et al., 2011), so a more direct influence of HTR2C polymorphism on dopamine release is also possible. Mechanisms outside the VTA could also be at play. For example, 5-HT_{2C} receptors likely mediate negative feedback in the raphe nuclei by activating interneurons which inhibit serotonin release (Di Matteo et al., 2008) so it is conceivable that the Ser23 variant alters negative feedback locally in the raphe, decreasing serotonin release in the VTA and elsewhere.

We are aware of one other neuroimaging study that investigated the Cys23Ser polymorphism (Kühn et al., 2004). Regional cerebral blood flow was quantified with and without a serotonergic challenge as a function of Cys23Ser genotype, and complex changes were described. As the authors point out, the pharmacologic challenge did not produce the expected changes in blood flow (Kühn et al., 2004) so the results are not easily interpreted. Furthermore, it is likely that the *HTR2C* gene effects reported would not survive correction for multiple comparisons. In our K1 images—which reflect baseline cerebral blood flow and tracer extraction—we found a whole-brain-corrected gene effect in only one small cluster in left occipital cortex (unpublished results) suggesting minimal effects of the Cys23Ser polymorphism on baseline blood flow.

If independently confirmed, our finding of greater stress-induced dopamine release among Ser23 carriers may have implications for neuropsychiatric disease. Although initial genetic studies found no association with mood disorders (Gutiérrez et al., 1996; Frisch et al., 1999), a larger subsequent study (total $n \approx 2000$) found a higher prevalence of Ser23 carriers among individuals with recurrent major depressive disorder or bipolar disorder, even after controlling for population stratification (Lerer et al., 2001). Ser23 has also been associated with lower reward dependence and persistence traits (Ebstein et al., 1997). In the context of convergent evidence that alterations in dopaminergic circuitry underlie depression and depressive behaviors (Nestler and Carlezon, 2006) our findings raise the possibility that excess risk of mood disorders among Ser23 carriers is mediated in part by greater mesoaccumbal reactivity to stress. Furthermore, the 5-HT_{2C} receptor has been suggested as a target for antidepressant drugs (Millan et al., 2005; Strong et al., 2009; Dekeyne et al., 2012). Our findings suggest that *HTR2C* genotype might be a useful predictor for an individual's clinical response to such antidepressants.

Our results also inform future studies of *HTR2C*. Most genetic association studies of Cys23Ser and other neuropsychiatric conditions (alcohol abuse, schizophrenia, eating disorders, suicide, medication effects) have been negative (recently reviewed by Drago and Serretti, 2009), but that may be due to small sample sizes and a focus on psychiatric diagnosis as the phenotype. Future human studies might usefully focus on dopamine-related cellular, circuit-level, and behavioral phenotypes (i.e., intermediate phenotypes) that transcend diagnostic categories. Given the close mechanistic links between 5-HT_{2C} receptors and the dopamine system, and potential interactions between *HTR2C* and dopaminergic gene variants (Ebstein et al., 1997), future studies with sufficiently large samples should also examine the effects of such gene interactions on intermediate phenotypes.

In conclusion, we found that a common, putatively functional variant of a key serotonergic gene was associated with greater stress-induced mesoaccumbal dopamine release—a plausible intermediate phenotype for mood disorders, addictions, and other stress-related illnesses. Future studies should explore other genetic and environmental factors that might contribute to stress responsiveness of mesoaccumbal dopaminergic circuitry in humans.

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