FULL-LENGTH REPORT

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A Preliminary Study of *DBH* (Encoding Dopamine Beta-Hydroxylase) Genetic Variation and Neural Correlates of Emotional and Motivational Processing in Individuals With and Without Pathological Gambling

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Background and aims: Corticostriatal-limbic neurocircuitry, emotional and motivational processing, dopaminergic and noradrenergic systems and genetic factors have all been implicated in pathological gambling (PG). However, allelic variants of genes influencing dopaminergic and noradrenergic neurotransmitters have not been investigated with respect to the neural correlates of emotional and motivational states in PG. Dopamine beta-hydroxylase (DBH) converts dopamine to norepinephrine; the T allele of a functional single-nucleotide polymorphism rs1611115 (C-1021T) in the *DBH* gene is associated with less DBH activity and has been linked to emotional processes and addiction. Here, we investigate the influence of rs1611115 on the neural correlates of emotional and motivational processing in PG and healthy comparison (HC) participants. *Methods:* While undergoing functional magnetic resonance imaging, 18 PG and 25 HC participants, all European Americans, viewed gambling-, sad-, and cocaine-related videotapes. Analyses focused on brain activation differences related to *DBH* genotype (CC/T-carrier [i.e., CT and TT]) and condition (sad/gambling/cocaine). *Results:* CC participants demonstrated greater recruitment of corticostriatal-limbic regions, relative to T-carriers. *DBH* variants were also associated with altered corticostriatal-limbic activations across the different videotape conditions, and this association appeared to be driven by greater activation in CC participants relative to T-carriers during the sad condition. CC relative to T-carrier subjects also reported greater subjective sadness to the sad videotapes. *Conclusions:* Individual differences in genetic composition linked to aminergic function contribute significantly to emotional regulation across diagnostic groups and warrant further investigation in PG.

Keywords: *DBH* gene, functional magnetic resonance imaging, addiction, mood, cue responsivity

INTRODUCTION

Pathological gambling (PG; gambling disorder in DSM-5) has an estimated heritability of 50%–66% (Xian, Giddens, Scherrer, Eisen, & Potenza, 2014). Most reported genetic studies of PG are limited to very small-scale (in terms of a genetic association design for complex traits) candidate-gene approaches ($N = 68$ to 186 PGs) for candidate genes involving mainly in dopaminergic and serotonergic systems with weak or inconsistent effects (Gyollai et al., 2014). The first large-scale genome-wide association study (GWAS) on a PG-related trait (Lind et al., 2013) identified no gene variants reaching genome-wide significance. However, larger effect sizes may be observed in regard to functional variants of enzyme-encoding genes; these may relate to brain activations and treatment outcome in PG (Grant et al., 2013).

Both dopamine and norepinephrine alterations have been implicated in PG (Boileau et al., 2014; Linnet et al., 2012). The *DBH* gene variant, which regulates the enzyme DBH's conversion of dopamine to norepinephrine, is linked with various addiction and impulsive phenotypes (Cubells et al., 2000; Hess et al., 2009). In European Americans (EAs), the functional promoter variant in *DBH*, single-nucleotide polymorphism (SNP) rs1611115, is associated with 35%–52% of the variance in plasma DBH activity; in a dosage-effect fashion, the TT genotype is associated with the lowest DBH plasma activity, followed by CT demonstrating intermediary activity, and CC showing the greatest levels of enzymatic function (Zabetian et al., 2001). These genotypes are further associated with alterations in emotional/motivational processing; for example, T-carriers demonstrate diminished empathic ability relative to CC genotypes (Gong, Liu, Li, & Zhou, 2014). The TT genotype (relative to CC or CT) is also

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associated with higher neuroticism and novelty seeking but lower conscientiousness, suggestive of an impulsive personality style (Hess et al., 2009). The TT genotype is also associated with increased severity of heroin use (Xie et al., 2013) and poorer cocaine treatment outcomes (Kosten et al., 2013). Moreover, drugs affecting DBH activity, such as disulfiram, demonstrate promise in treatment of substance and non-substance addictions (Carroll et al., 2004; Kosten et al., 2013; Muller, Banas, Heinz, & Hein, 2011; Skinner, Lahmek, Pham, & Aubin, 2014).

The current study used functional magnetic resonance imaging (fMRI) to investigate effects of *DBH* polymorphisms on emotional/motivational processing. Given the infrequency of TT homozygotes and prior studies highlighting the clinical relevance of T-carrier status, we investigated T-carriers and those with CC genotypes. Both PG and healthy comparison (HC) participants were scanned while watching videos designed to elicit sad feelings, gambling urges, or cocaine cravings (Kober et al., 2016; Potenza et al., 2003; Wexler et al., 2001). Sad, gambling, and cocaine videos were studied with the following rationale and goals. Experiential sadness has been linked to changes in dopaminergic and noradrenergic function (Harmer, Perrett, Cowen, & Goodwin, 2001; Peron, Grandjean, Drapier, & Verin, 2014), and the *DBH* polymorphism is associated with empathic responses (Gong et al., 2014). As some investigators have considered PG within an affective framework (Grant, 2004), PG has been associated with negative affective states and mood disorders (Potenza, Xian, Shah, Scherrer, & Eisen, 2005) and prior findings using the same task have identified alterations in the neural correlates of sad states in PG subjects (Kober et al., 2016; Potenza et al., 2003), sad videos were included in this study. Finally, the cocaine videos served as an active control condition to identify specificity of neural responses to the gambling (a main focus of the study) and sad videos; previous evidence demonstrates that the cocaine videos elicit emotional responses in people with and without cocaine dependence (CD; Wexler et al., 2001).

Recently, we reported on data from this sample (Kober et al., 2016). In this study, we restricted the sample to EA subjects given the genetic focus and thus focused on PG and HC to have sufficient samples for each group. We also focused on sustained emotional/motivational responses as we have done previously (Potenza, 2008). We hypothesized observing main effects of *DBH* genotype on corticolimbic activations and interactions with condition and *DBH* genotype. For *DBH*-genotype-by-condition interaction effects, we hypothesized that T-carriers would report less sadness to sad videos, in line with reports of reduced empathic responses in these individuals (Gong et al., 2014); further, we hypothesized that the neural expression of this effect would be reflected in diminished corticostriatal recruitment in T-carriers relative to the CC group during sad videos. Based on prior findings (Potenza, 2008; Potenza et al., 2003), we also expected group-by-condition interaction effects involving diminished ventromedial prefrontal cortex (vmPFC) and ventral striatum (VS) activity in the PG group during gambling videos.

METHODS AND MATERIALS

Participants

EA subjects, who provided fMRI and genetic data, were included from a larger sample of multi-ethnic individuals, whose data are reported elsewhere (Kober et al., 2016). To avoid potential genetic population stratification, only EA individuals were included: 18 individuals meeting PG criteria and 25 HC subjects. All subjects were native English speakers with no history of neurologic disorders or injury. A structured clinical interview (First, 1997) determined that PG subjects met DSM-IV criteria for PG as the primary diagnosis and that HC subjects had no axis-I disorders except possible nicotine dependence (ND; one ND subject in the HC group). Detailed participant characteristics including PG comorbidity with other psychiatric disorders are described in Supplementary Material I, with demographic information provided in Table 1 (including information on use of the Barratt impulsiveness scale (BIS-11) and South Oaks Gambling Screen (SOGS) and Supplementary Material II).

Genotyping

DNA was extracted from whole blood. The SNP rs1611115 was genotyped for all subjects by a fluorogenic 5' nuclease assay of the TaqMan method using the ABI PRISM 7900 Sequence Detection System (ABI, Foster City, CA). No discrepancy was detected between the duplicates for one PG subject, one HC subject, and one control Centre d'Etude du Polymorphisme Humain (representative of the EA population) in the quality control of genotyping procedures. Genotypes were in Hardy–Weinberg equilibrium for the PG, HC, and combined PG and HC groups (all $p > .05$).

Experimental task

Participants viewed six videos in random and counterbalanced order (see Supplementary Material III), including two videos each related to gambling, sad and cocaine scenarios in which actors depict each specific scenario. The detailed scenarios are previously described (Kober et al., 2016; Potenza et al., 2003; Wexler et al., 2001), with further descriptions in Supplementary Material IV. Subjective responses to videos were recorded on a scale of 0–10 and involved rating emotional intensity, gambling urges, and drug cravings (Potenza et al., 2003; Wexler et al., 2001).

Image acquisition

Images were obtained using a 3-T Siemens Trio MRI system equipped with a standard quadrature head coil, using T2*-sensitive gradient-recalled single-shot echo-planar pulse sequence. Subjects were positioned in the coil and head movements were restrained using foam pillows. Anatomical images at the functional slice locations were next obtained with spin-echo imaging in the axial plane parallel to the anterior-commissure/posterior-commissure (AC–PC) line with repetition time (TR) = 300 ms, echo time (TE) = 2.47 ms, bandwidth = 300 Hz/pixel, flip angle (FA) = 60°,

Table 1. Subject characteristics

(A)	PG	HC	*p*-value
Sample size	18	25	
Sex, male (%)	13 (72.2%)	15 (60.0%)	.613
Age (mean ± SD)	36.6 ± 12.0	30.0 ± 11.4	.079
Education years	13.8 ± 1.8	14.6 ± 2.2	.242
FTND	1.7 ± 2.8	.1 ± .6	.038*
South Oaks Gambling Screen Score	12.5 ± 3.5	.08 ± .3	2.1×10^{-11}*
BIS-11 total score	71.7 ± 13.6	54.6 ± 8.4	7.5×10^{-5}*

	CC	T-carrier		
(B) *DBH* genotype	Count (%)	Count (%)	Total	*p*-value
PG	9 (50.0)	9 (50.0)	18	.937
HC	11 (44.0)	14 (56.0)	25	
Male	13 (46.4)	15 (53.6)	28	~1
Female	7 (46.7)	8 (53.3)	15	
BIS-11 total score	58.3 ± 9.6	65.3 ± 16.2		.093
Sad video–Emotional intensity	6.88 ± 1.7	5.52 ± 2.3		.035*

Note. BIS-11, Barratt impulsiveness scale, version 11; FTND, Fagerstrom test for nicotine dependence; PG, pathological gambling; HC, healthy comparison.
*$p < .05$.

field of view = 220 × 220 mm, matrix = 256 × 256, 25 slices with slice thickness = 5 mm and no gap. Functional blood-oxygen-level-dependent (BOLD) signals were then acquired with a single-shot gradient echo-planar-imaging sequence. Twenty-five axial slices parallel to the AC–PC line covering the whole brain were acquired with TR = 1500 ms, TE = 27 ms, bandwidth = 2520 Hz/pixel, FA = 60°, field of view = 220 × 220 mm, matrix = 64 × 64, 25 slices with slice thickness = 5 mm and no gap. Six fMRI runs (two per condition) were acquired. Following functional imaging, high-resolution 3-D Magnetization-Prepared Rapid-Gradient Echo (MPRAGE) data (TR = 2530 ms; TE = 3.34 ms; bandwidth = 180 Hz/pixel; FA = 7°; slice thickness = 1 mm; field of view = 256 × 256 mm; matrix = 256 × 256) were acquired for multi-subject registration.

Data analysis

Data were converted from Digital Imaging and Communication in Medicine (DICOM) format to ANALYZE format using XMedCon (Nolf, 2003). The first six volumes of each functional series were discarded to enable signal to achieve steady-state equilibrium. Functional images were first slice-time-corrected and then realigned (motion corrected) with the Statistical Parametric Mapping 5 algorithm (www.fil.ion.ucl.ac.uk/spm/software/spm5) for three translational directions (*x*, *y*, or *z*) and three possible rotations (pitch, yaw, or roll). Trials with linear motion that had a displacement in excess of 2 mm or rotation in excess of 3° were rejected. Corrected images were spatially smoothed by using a Gaussian filter with a full-width-at-half-maximum of 6.876 mm. Analysis-of-covariance (ANCOVA) approaches as described below controlled for covariates and analyzed signal change during the entire period of video-viewing relative to the combined pre- and post-baseline (see Supplementary Material X). Post hoc *t*-test comparisons were implemented to dissect what drived the effects identified in the ANCOVAs and examined the nature of the effects. For subjective-response data, analyses of variance (ANOVAs) tested emotional/motivational ratings between the PG and HC groups. ANOVAs tested *DBH* genotype differences for the sad condition.

Analysis-of-covariance

Data were converted to AFNI format (http://afni.nimh.nih.gov) for ANCOVAs. We applied a 2 × 2 × 3 ANCOVA with age adjustment involving two *DBH* genotypic groups (CC/T-carrier), two diagnostic groups (PG/HC), and three conditions (gambling, sad, and cocaine), in which subject was treated as a random factor using the GroupAna program from the AFNI MATLAB library (http://afni.nimh.nih.gov/afni/matlab/). Results were masked, converted back to ANALYZE format and viewed in Yale BioImage Suite (http://wwww.bioimagesuite.org). Data were thresholded at a voxel level of $p < .05$, corrected for multiple comparisons by spatial extent of contiguous suprathresholded individual voxels at a family-wise-error-corrected (FWE corrected) threshold of $p < .05$. In a Monte-Carlo simulation using AFNI (applying a smoothing kernel of 6.876 mm and a connection radius of 6.97 mm on 3.44 mm × 3.44 mm × 5 mm voxels), an activation volume of 311 voxels (8402 μl) satisfied the FWE-corrected $p < .05$ threshold.

Ethics

The investigation was carried out in accordance with the latest version of the Declaration of Helsinki; the protocol was approved by the Yale Human Investigation Committee. All subjects were recruited through advertisements and provided written informed consent after the nature of the procedures had been fully explained.

RESULTS

Diagnostic group differences

The PG group (versus HC group) was more impulsive ($p = 7.5 \times 10^{-5}$) and demonstrated greater problem-gambling severity on the SOGS ($p = 2.1 \times 10^{-11}$).

Genotype distribution

No between-group differences in genotypic distributions of SNP rs1611115 were observed between PG and HC subjects ($p > .05$) or between male and female subjects ($p > .05$) (Table 1B). Modestly higher impulsivity scores in T-carriers relative to CC individuals did not reach significance ($p = .093$).

Brain activations elicited by videos

Significant two-way interaction effects emerged for *DBH*-genotype-by-condition (Figure 1A) and group-by-condition (Figure 1C) analyses (see Supplementary Material VI for additional activation maps). No activations in group-by-*DBH*-genotype and group-by-*DBH*-genotype-by-condition interactions survived whole-brain correction. Main effects of *DBH* genotype and condition on brain activation were identified; main effects of group did not survive whole-brain correction. Extracted BOLD-signal changes from the identified regions were used to understand the nature of the significant effects, as described below.

DBH-genotype-by-condition interaction

A *DBH*-genotype-by-condition interaction identified one cluster involving the bilateral thalamus and putamen extending to the left insula, hippocampus, caudate, dorsolateral prefrontal cortex (dlPFC), and anterior cingulate cortex (ACC) (Figure 1A and B; Cluster-a). Another cluster involved the right dlPFC and posterior cingulate cortex (Figure 1A and B; Cluster-b; Table 2C). For both clusters, significant differences appear driven by greater activation in the *DBH* CC participants relative to T-carriers during the sad

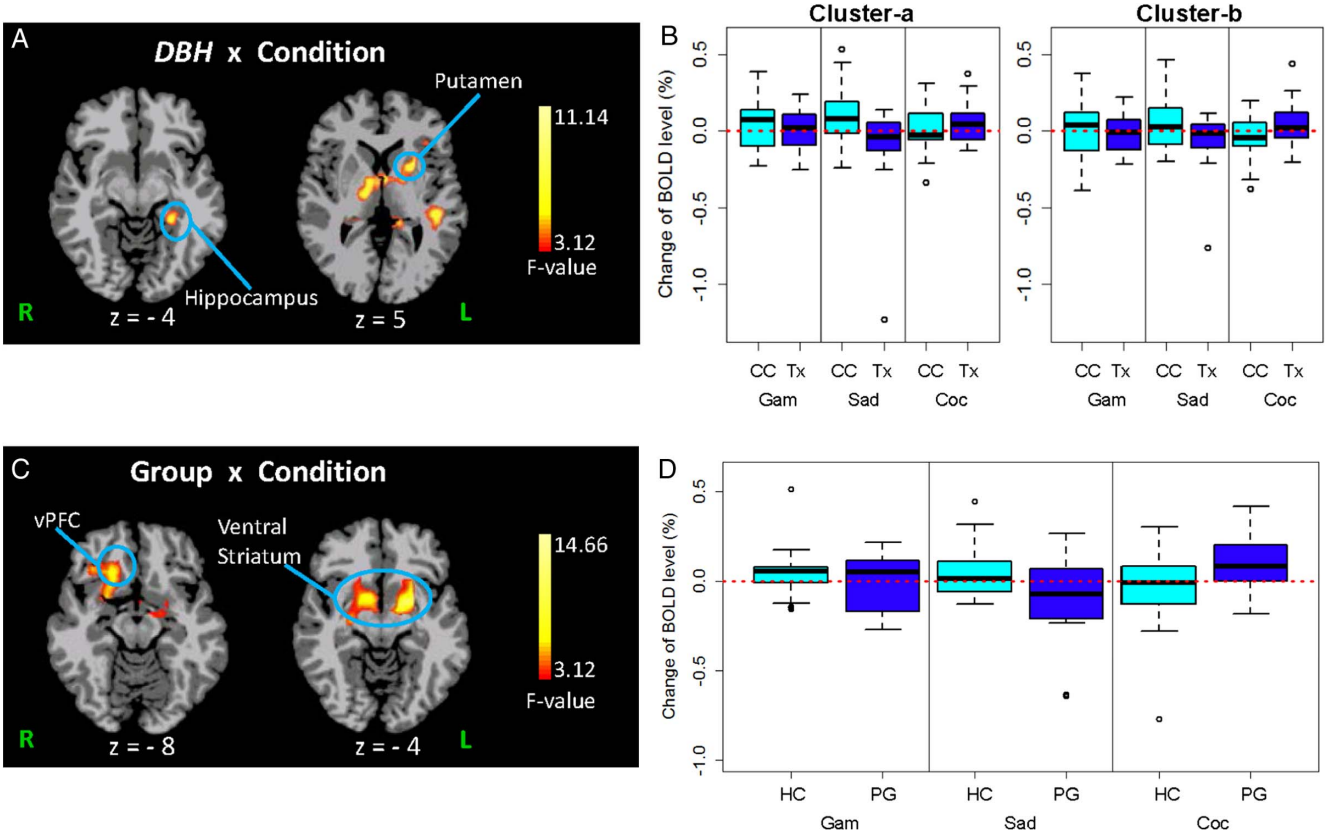

Figure 1. Activation maps displaying interactive effects of *DBH* genotype, condition and diagnostic group. The axial brain sections highlighting regions showing significant two-way interactions adjusted by age involving two genotypic groups (CC, T-carrier [shown as Tx]), two diagnostic groups (pathological gambling, healthy comparison, shortened as PG and HC, respectively) and three cue conditions (gambling, sad, and cocaine, shortened as Gam, Sad, and Coc, respectively) are shown. The maps are thresholded at $p < .05$, with a family-wise-error correction. Talairach *z* levels are indicated (A, C). The bar on the right side of the Figures A and C indicates the strengths of identified interactions. Right side of the brain is shown on the left. Indicated on the right (B and D) are extracted values of percentage signal changes according to respective interactions for genotype (CC, T-carrier [shown as Tx]), diagnostic group (PG, HC) or condition (gam, sad, and coc). Outliers of BOLD-signal are marked by circles; outlier evaluation by exclusion and retest; all the effects in the retests remained similar. mOFC = medial orbitofrontal cortex; vPFC = ventral prefrontal cortex. Cluster-a: Thalamus/putamen/caudate/insula/dorsal lateralprefrontal cortex/hippocampus/anterior cingulate/pars triangularis/superior temporal gyrus/primary sensory/primary motor. Cluster-b: Frontal eye fields/dorsolateral prefrontal cortex/posterior cingulate

Table 2. Age-adjusted main and interactive effects of *DBH* genotype, condition and diagnostic group on regional brain activations

	Left/ Right	Volume mm^3 (voxels)	Talairach CenterMass (*x, y, z*)	*F*-values Mean	*F*-values Stdev
(A) Main effect *DBH* genotype					
Cerebellum_parahippocampus_hippocampus_fusiform		35375 (1310)	−9, −56, −22	5.85	1.56
Ventral striatum_orbital frontal cortex_anterior cingulate_rostrolateral prefrontal					
Cortex_I nferior frontal gyrus_amygdala		19055 (706)	−8, 20, −6	5.96	1.72
Subclusters: Ventral striatum	L	955 (35)	−13, 0, −5	6.45	2.21
Anterior cingulate	L/R	2301 (85)	3, 32, 0	5.09	.88
Amygdala	L	500 (19)	−17, −7, −10	6.04	1.48
Caudate	L	774 (29)	−12, 14, 0	5.63	1.22
Putamen	L	525 (19)	−21, 7, −4	5.67	1.65
vmPFC	L/R	5146 (191)	−8, 13, −15	6.72	2.19
(B) Main effect condition					
Occipital cortex_temporal gyrus_angular gyrus_fusiform gyrus_temporopolar area_thalamus_posteror cingulate_cerebellum		249810 (9252)	−1, −52, 4	9.00	6.52
Subclusters: Thalamus	L	2089 (77)	−11, −23, 3	4.18	.83
Thalamus	R	563 (21)	7, −23, 2	3.76	.48
Posterior cingulate	L/R	4181 (155)	−8, −55, 33	4.82	1.37
Anterior cingulate cortex_anterior prefrontal cortex_orbitofrontal gyri_I nsula		12983 (481)	−9, 42, −6	4.39	1.18
Subclusters: Insula	L	232 (9)	−26, 20, 0	3.66	.40
vmPFC	L/R	4151 (154)	−8, 37, −20	4.18	1.03
Anterior cingulate	L/R	1402 (52)	−4, 39, 8	4.58	1.29
Medial frontal gyrus	L/R	5907 (219)	−8, 48, −4	4.57	1.27
(C) *DBH* genotype X condition					
Thalamus_putamen_caudate_I nsula_dlPFC_ Hippocampus_anterior cingulate_pars triangularis_superior temporal gyrus_primary		56708 (2100)	−24, −1, 19	4.30	1.00
Subclusters: Thalamus	R	2938 (109)	11, −15, 7	4.21	.77
Thalamus	L	2213 (82)	−10, −16, 10	4.11	.84
Putamen	R	280 (10)	25, −1, 0	3.68	.41
Putamen	L	1475 (55)	−22, 2, 6	4.28	.87
Insula	L	544 (20)	−26, 16, 9	4.12	.65
Hippocampus	L	905 (34)	−28, −34, 0	4.42	.94
Caudate	L	1181 (44)	−14, 15, 13	5.31	1.71
dlPFC	L	13771 (510)	−30, 27, 20	4.36	1.00
Anterior Cingulate	L/R	794 (29)	−7, 21, 22	3.76	.54
Frontal eye fields_dlPFC_posterior cingulate	R	8789 (326)	17, 15, 28	4.23	1.00
Subcluster: Posterior cingulate	R	409 (15)	4, −18, 33	4.17	.82
(D) Group X condition					
Putamen_caudate_thalamus_orbital frontal cortex_ventral striatum_anterior cingulate_insula_hypothalamus		26068 (965)	−1, 1, 3	4.63	1.50
Subclusters: Putamen	L	2918 (108)	−24, 0, 4	5.17	1.90
Caudate		1364 (51)	14, 4, 13	4.04	.78
Caudate	L	1449 (54)	−13, 9, 7	4.45	1.04
Thalamus		1592 (59)	9, −15, 9	4.01	.70
Thalamus	L	717 (27)	−8, −17, 6	3.61	.42
Ventral Striatum		2004 (74)	10, 3, −2	5.43	1.93
Ventral Striatum	L	1159 (43)	−12, 1, −2	5.55	2.31
Medial Orbital Frontal Cortex	L/R	474 (18)	10, 11, −14	4.37	.98
Lateral Orbital Frontal Cortex	L/R	2299 (85)	24, 16, −10	4.43	1.04
Insula	L	1208 (45)	−33, −4, 13	4.45	.94

Note. All the main and interactive effects were detected with an uncorrected threshold of $p < .05$ and familywise error (FWE)-corrected with $c = 311$ except the main effect of condition with an uncorrected threshold of $p < .005$ and $c = 311$ FWE-corrected. Brain regions starting with "R" or "L" indicate right and left, respectively.

condition ($p = .0087$ and .041, respectively) but not during the other two conditions (Figure 1B).

Group-by-condition interaction

A group-by-condition interaction occurred in a ventral striatal cluster extending to the medial and lateral orbitofrontal cortex (OFC), thalamus, caudate, left insula, and left putamen (Figure 1C and Table 2D). Relative to the HC group, the PG group demonstrated significantly greater recruitment during the cocaine condition ($p = .0098$), less activation during the sad condition ($p = .031$), and no difference during the gambling condition ($p = .24$; Figure 1D).

Main effects of DBH genotype

A main *DBH*-genotype effect demonstrated greater CC group activity in the left VS extending to the OFC, ACC, amygdala, caudate, putamen, and vmPFC (Table 2A and Figure 2A), relative to the T-carriers (Figure 2B, Cluster-a). Another cluster extended from the cerebellum to the hippocampus and also displayed relatively greater activity in the CC versus T-carrier groups (Figure 2B, Cluster-b).

Main effects of condition

A main condition effect recruited the bilateral thalamus extending to the posterior cingulate and appeared to be driven by greater activation to the gambling versus cocaine ($p = 1.62 \times 10^{-5}$) and gambling versus sad ($p = .0067$) conditions, with no difference in the cocaine versus sad condition ($p = .10$) (Figure 2D, Cluster-c). Another cluster extending from the vmPFC to the ACC and medial frontal gyrus (Table 2B and Figure 2C) appeared to be driven by decreased activation in the cocaine versus gambling ($p = .0021$) and cocaine versus sad ($p = .00094$) conditions, with no difference in the gambling versus sad conditions ($p = .57$) (Figure 2D, Cluster-d).

Potential effects of outliers of BOLD-signal changes, as depicted in Figures 1 and 2, were evaluated by exclusion and retest; all the effects in the retests remained similar. Main effects of *DBH* genotype and condition remained strong. Effects of *DBH*-genotype-by-condition kept the striatal cluster ($p = .012$) and the cluster with the posterior cingulate cortex was in marginal significance ($p = .074$), and both appeared to be driven by the sad condition. Effects of group-by-condition for the PG group relative to the HC group demonstrated similar results, which reflected greater

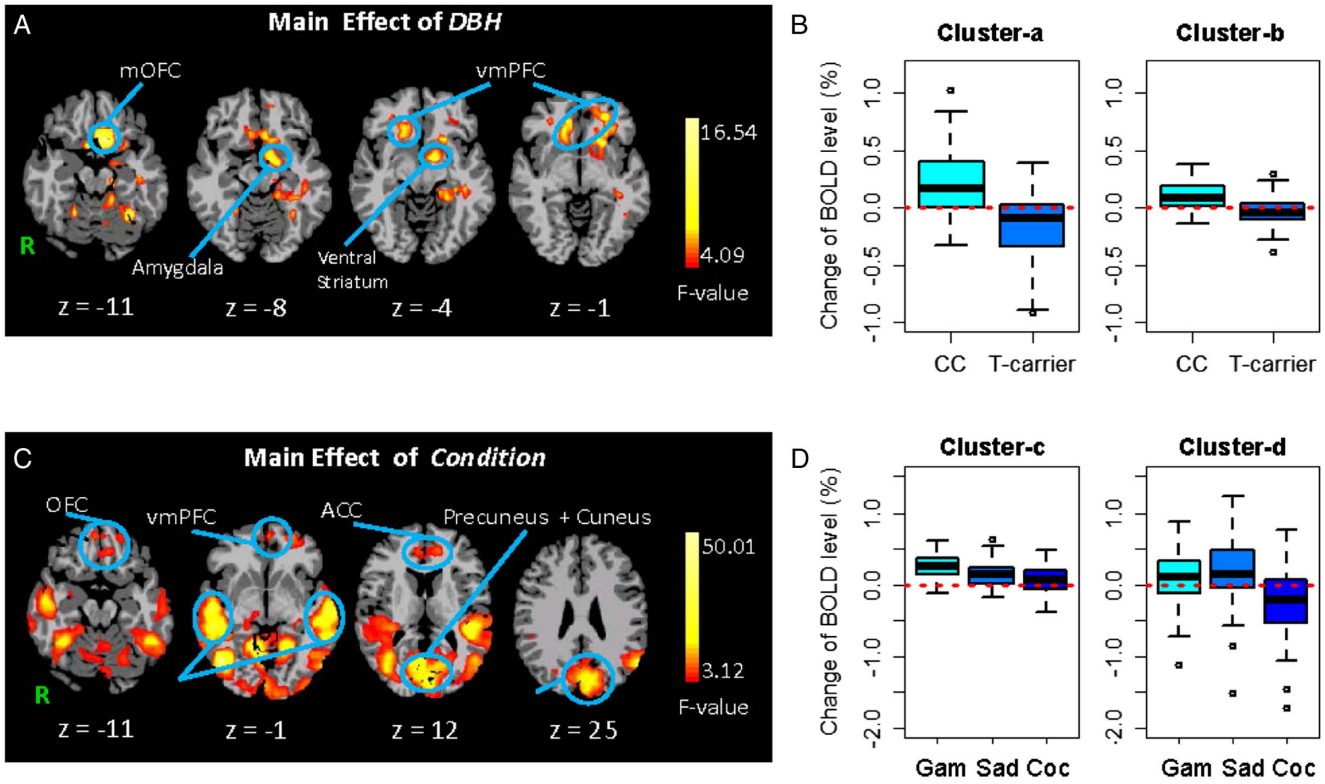

Figure 2. Main effects of *DBH* genotype and condition. Axial brain sections highlighting regions showing significant main effects of *DBH* genotype (A) and condition (C) with age adjustment are shown. The maps are thresholded at $p < .05$, with a family-wise-error correction. Talairach *z* of slices levels are indicated. The bar on the right side of the Figures A and C indicates the strengths of effects. Right side of the brain is shown on the left. Indicated on the right are extracted values of percentage signal change according to (B) genotype (CC, T-carrier) or (D) condition (gambling, sad, and cocaine). Outliers of BOLD-signal are marked by circles; outlier evaluation by exclusion and retest; all the effects in the retests remained similar. Cluster-a: ventral striatum/orbital frontal cortex/anterior cingulate/rostrolateral prefrontal cortex/inferior frontal gyrus/amygdala. Cluster-b: Cerebellum/parahippocampus/hippocampus/Fusiform. Cluster-c: Occipital cortex/temporal gyrus/angular gyrus/fusiform gyrus/temporopolar area/thalamus/posteror cingulate/cerebellum. Cluster-d: Anterior cingulate cortex/anterior prefrontal cortex/orbitofrontal gyri/insula

Table 3. Subjective responses to the tape viewing

Videotape condition	Subjective responses	PG mean [*SD*]	HC mean [*SD*]	PG versus HC *p*-values
Gambling	Emotional intensity	7.89 [1.27]	4.78 [2.17]	<.00001*
Gambling	Gamble urge	7.53 [1.97]	1.16 [.40]	<.00001*
Gambling	Drugs urge	1.28 [.55]	1 [0]	.046
Sad	Emotional intensity	7.25 [2.01]	5.36 [1.93]	.0038*
Sad	Gamble urge	3.33 [2.66]	1 [0]	.0017*
Sad	Drugs urge	1.11 [.32]	1 [0]	.163
Cocaine	Emotional intensity	6.97 [2.38]	4.74 [2.18]	.0034*
Cocaine	Gamble urge	3.28 [2.71]	1.04 [.20]	.0028*
Cocaine	Drugs urge	1.94 [1.85]	1.08 [.28]	.066

Note. Multiple comparison was adjusted by Bonferroni correction. The significance threshold was set at .05/9 = .00556 by taking into the account that three subjective responses were tested for each of the three scenarios for each subject. *SD* = 0 reflected the same rating for all of the responses in the subgroup analyzed.
*$p < .00556$.

recruitment during the cocaine condition ($p = .016$), less recruitment during the sad condition ($p = .09$), and no difference during the gambling condition ($p > .4$).

Subjective responses

The subjective response ratings to the videos, involving ratings of emotional intensity, gambling urges, and drug cravings, are summarized by comparisons of the diagnostic groups (Table 3) and the genotype groups for the sad condition (Table 1B). The T-carriers responded to the sad videos with less subjective sadness ($p = .035$, Table 1B). More detailed descriptions regarding the subjective responses of the PG versus HC groups are presented in Supplementary Material VII. In comparison to the T-carriers, CC participants demonstrated stronger correlations between brain activations and subjective emotional intensity in response to the sad videos in a cluster extending from the parietal cortex (including inferior parietal lobule) to the inferior occipital gyrus (whole-brain correction $p < .05$; Supplementary Material VII, Figure S3).

DISCUSSION

This first study investigating *DBH* genetic influences on brain activity found that the functional T allele in *DBH* SNP rs1611115, which reduces the conversion of dopamine to norepinephrine, corresponds with different BOLD-signal changes in responses to gambling, drug or sad cues in individuals with and without PG. A main effect of genotype was observed with respect to responses to the videotapes in the amygdala, vmPFC, and striatal areas, and a *DBH*-genotype-by-condition, as well as group-by-condition interactions, implicated corticostriatal-limbic regions. *DBH* genotype was also linked to subjective responses to the sad videotapes. The finding of *DBH* effects across diagnostic groupings is consistent with transdiagnostic research efforts (e.g., research domain criteria). Finally, cue-condition differences were found in occipital and temporal cortices and ventral prefrontal cortical regions. These findings, particularly the novel *DBH*-related findings, suggest an important role for dopamine and norepinephrine in subjective sadness and brain correlates of emotional regulation, impulse control, and motivational processes.

Subjective responses

Subjective responses to the videos provide a validity check (i.e., specificity of responsiveness as designed) and are consistent with previous studies reporting stronger gambling-urge responses during the gambling-cue condition in the PG group (Potenza et al., 2003) and for cue-specificity as in the larger sample (Kober et al., 2016). Interestingly, the PG group also reported stronger gambling urges to the sad and cocaine-cue conditions, suggesting that other states (dysphoric, drug-cue-related) may trigger gambling urges; alternatively, persistent baseline gambling urges may endure across conditions in the PG group.

Main effect of DBH genotype

Collapsed across diagnostic groups and video conditions, the increased recruitment by the CC relative to the T-carrier subjects in dopaminergic projection sites suggests that greater conversion of dopamine to norepinephrine may relate to increased corticostriatal-limbic activation in regions implicated in emotional regulation, motivation, executive and cognitive functions, and impulse control, among other processes (Goldstein & Volkow, 2011; Li & Sinha, 2008; Satterthwaite et al., 2007). Given the links between DBH activity and addiction characteristics, manipulating the balance between dopamine and norepinephrine could prove an effective treatment strategy for multiple neuropsychiatric disorders (Marecos, Ng, & Kurian, 2014). For example, the drug disulfiram is effective in the treatment of alcohol and CD, possibly through effects on craving (Carroll et al., 2004; Skinner et al., 2014). DBH inhibition by disulfiram (Kosten et al., 2013) may reduce craving via increasing dopamine levels and decreasing norepinephrine levels (Carroll et al., 2004). Accordingly, clinical case reports suggest that disulfiram may reduce gambling urges in PG (Muller et al., 2011). These reports provide a proof-of-principle for PG treatment via DBH inhibition to regulate the balance between dopamine and norepinephrine, which have both been implicated to varying degrees in PG (Leeman & Potenza, 2012).

Subjective reports of greater sadness to sad videos were reported in the CC group, relative to the T-carriers (Table 1B), a finding that is consistent with reports of lowered empathic characteristics in the T-carrier group (Gong et al., 2014). Our results with fMRI extend these findings to demonstrate diminished recruitment of frontostriatal circuitry in T-carriers, specifically during the sad condition. These data link the *DBH* polymorphism with alterations in frontostriatal responses while listening to the personal distress of another person. Specifically, the observed *DBH*-genotype-by-condition differences implicate the dlPFC, midbrain, hippocampus, insula, caudate, putamen, and thalamus. These brain regions are associated with dopaminergic network differences in multiple addictive behaviors and neuropsychiatric disorders (Leeman & Potenza, 2012). The dlPFC is sensitive to levels of norepinephrine and dopamine, in which imbalances may impair prefrontal cortex function (Arnsten, 2011) and influence emotional regulation (Perlstein, Elbert, & Stenger, 2002; Urry et al., 2006). As the T allele is associated with impulsivity, aggressive hostility, addiction vulnerability, and altered noradrenergic function (Cubells et al., 2000; Hess et al., 2009; Kalayasiri et al., 2007; Kohnke et al., 2002), the diminished recruitment of corticostriatal areas by T-carriers suggests possible neural substrates of emotion dysregulation that may be conferred by the "risk" T allele.

The identified brain areas, including left insula, caudate, and putamen, are implicated in effort-based decision-making (Treadway et al., 2012), preferences for rewarding substances (Szczypka et al., 2001), and, arguably most relevant to the current task, emotional regulation (Martinot et al., 2001). Although small, the current sample demonstrated numerically but not statistically higher impulsivity in the T-carrier group ($p < .10$, Table 1B), consistent with reports of a more impulsive personality style associated with this genotype (Hess et al., 2009). Higher impulsivity has been related to lower D_2/D_3 dopamine receptor availability in the midbrain (Buckholtz et al., 2010), with greater amphetamine-related dopamine release related to mibrain (substantia nigra) levels of D_3 dopamine receptors in PG subjects (Boileau et al., 2014). In PG, a disorder characterized by increased impulsivity, striatal dopamine D_2/D_3 receptor binding negatively correlates with mood-related impulsivity (Clark et al., 2012). Future studies with larger samples could examine potential interactions between impulsivity, mood, and dopaminergic function in PG as compared to non-PG samples.

Group effects across conditions

Group differences occurred in ventral PFC and ventral striatal areas. Specifically, during the sad condition, the PG group demonstrated diminished frontostriatal activity and greater subjective sadness relative to the HC group (Table 3). A previous study using the same task but an independent sample demonstrated correlations between sadness ratings and medial prefrontal regions in PG but not HC subjects, suggesting differential sensitivities and alterations in affective processing in PG (Balodis, Lacadie, & Potenza, 2012). As impairments in emotional clarity and awareness are noted in PG (Williams, Grisham, Erskine, & Cassedy, 2012), further investigations could examine how these activations relate to emotional dysregulation in PG.

The finding of relatively increased activity within this cluster in the PG group to the cocaine-cue condition was unanticipated and persisted after exclusion of the three subjects with comorbid cocaine abuse/dependence. Further research should examine the extent to which PG subjects' responses to cocaine cues reflect predilections toward or histories of cocaine use, particularly given the elevated co-occurrence between PG and CD and genetic contributions to their co-occurrence (Xian et al., 2014).

Main effects of condition

The main effects of condition on brain activations, collapsed across the *DBH* genotypes and the diagnostic groups, implicated the OFC, vmPFC, anterior and posterior cingulate, thalamus, caudate, left insula, and medial frontal gyrus. Greater activation of occipital and temporal cortices, i.e., audio-visual-processing regions, was observed during the gambling condition. These findings complement those that have implicated in PG patient's dorsal visual processing stream involving the cuneus in motivational states underlying gambling urges (Crockford, Goodyear, Edwards, Quickfall, & el-Guebaly, 2005), although the findings may also reflect qualities of the gambling tape given the finding across diagnostic groups. Future studies with larger samples may dissect effects of cue conditions in individuals with different characteristics.

Main effect of diagnostic group

In contrast to our previous studies (Kober et al., 2016; Potenza et al., 2003) using this task, the current study did not detect a main effect of group on BOLD signal changes. Sample characteristics and analytic technique differences may underlie the discrepant findings. Our previous study (Potenza et al., 2003) focused on male-only PG versus HC, while the recent study (Kober et al., 2016) aimed at comparing neurobiological similarities and differences between cocaine craving and gambling urges using both males and females of multi-ethnic backgrounds in three diagnostic groups of PG, CD, and HC individuals. These two previous studies evaluated the first and the last viewing periods, and investigated between-group differences in neural activities during viewing of videos of gambling, happy, or sad content (Potenza et al., 2003), or with gambling, sad, and cocaine content (Kober et al., 2016). The study (Potenza et al., 2003) found that the most pronounced between-group differences (PG versus HC) in neural activities were observed during the initial period of viewing of the gambling conditions (Potenza et al., 2003). In that study, PG subjects displayed relatively decreased activity in frontal and OFC, caudate/basal ganglia, and thalamus compared with controls. In our recent study (Kober et al., 2016), a diagnostic group by video interaction identified mPFC, which was activated mainly to cocaine videos in CD participants during the initial viewing period, and a more dorsal mPFC region that was most strongly activated for cocaine videos in CD participants, gambling videos in PG participants, and sad videos in HC during the last viewing period. Our current

study includes both men and women of EA-only PG versus HC, and investigates signal changes during the entire period of tape-viewing (a different analytic approach used in [Potenza, 2008]). We have reanalyzed the current study data separated by sex using the previous approaches, partially replicating our previous findings in an independent sample (Potenza et al., 2003) with relatively decreased activities in VS ($p = .033$) of PG versus HC for the male-only sample, but not for the vmPFC (see Supplementary Material X).

Strengths, limitations and future directions

Together, the findings suggest important roles for dopamine and norepinephrine in emotion regulation and impulse control and provide a context for the novel *DBH*-related findings reported here. Strengths of this initial study lie in the whole-brain analytic approach to assess neural activity and sizable samples of CC and T-carrier subjects. While some effects between cue conditions may linger, the counterbalancing approach was designed to mitigate this possibility. Responses to sad-scenario content (parental divorce, a relative's death) may differ based on individual subjects' experiences; information regarding parental divorce or death was not collected and limited us from evaluation of its impact on the responses to sad cues. Nonetheless, the qualitative and emotional intensity ratings by participants provide support that the scenarios were successful in generating the intended emotional responses. Additionally, findings in this study substantiate previous reports of diminished empathy in T-carriers and extend these findings to demonstrate the neural substrates underlying this effect. Together with subjective reports, these findings therefore provide proof-of-concept of the video task.

Small sample sizes when classifying by both diagnostic group and *DBH* genotypes may have precluded identification of some significant findings, such as the two-way and three-way interaction effects of group-by-*DBH*-genotype and group-by-*DBH*-genotype-by-condition, respectively. Nonetheless, the sample sizes for main effects of *DBH* genotype ($N\text{-}_{CC} = 20/N\text{-}_{T\text{-carrier}} = 23$) and condition ($N = 43$) and for two-way interactions of group-by-condition ($N\text{-}_{PG} = 18/N\text{-}_{HC} = 25$) and *DBH*-genotype-by-condition ($N\text{-}_{CC} = 20/N\text{-}_{T\text{-carrier}} = 23$) comply with recommended guidelines for power and sample size consideration for clinical fMRI studies (Carter, Heckers, Nichols, Pine, & Strother, 2008). Additionally, some p values may not withstand correction for multiple comparisons (e.g., with respect to allelic variation and subjective responses to sad videos).

Imbalances in co-occurring psychiatric disorders existed between PG and HC groups; however, the PG pattern of comorbidity is reflective of this clinical population (Barnes, Welte, Tidwell, & Hoffman, 2015). After controlling for comorbid conditions (i.e., cocaine and alcohol dependence), results remained similar (see Supplementary Material VIII). Further analyses contrasting smokers versus non-smokers within the PG group found no difference between PG smokers and PG non-smokers for the gambling and cocaine scenarios but discovered some differences in the sad scenario (see Supplementary Material IX). However, the brain regions in response to the sad condition differ from the brain regions, implicating corticostriatal-limbic neurocircuitry we identified across the three conditions. Therefore, the differences between PG versus HC groups for the sad condition do not appear attributable to smoking status.

Despite limitations, the current study identified *DBH*-related differences in brain function in the processing of affective and motivational cues in PG and HC groups. Given DBH's role in dopamine/norepinephrine balance, future studies should investigate the extent to which targeting dopamine and/or norepinephrine function in corticostriatal-limbic brain regions might be helpful for PG treatment, particularly in individuals with specific *DBH* allelic identities.

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Authors' contribution: B-ZY analyzed the data, wrote the draft of the manuscript and revised the manuscript and addressed the reviewers' comments. IMB helped revise the manuscript and provided guidance of the revision. CML analyzed the imaging data. JX offered interpretation of the results and discussion. MP designed and guided the study, proposed the analyses, and revised the manuscript. All authors read and approved the manuscript.

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