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Initial Evidence that OPRM1 Genotype Moderates Ventral and Dorsal Striatum Functional Connectivity During Alcohol Cues

Lara A. Ray, Kelly E. Courtney, Kent E. Hutchison, James MacKillop, Adriana Galvan, and Dara G. Ghahremani

Background: Endogenous opioids and striatal dopamine have been implicated in cue-induced alcohol craving and have been hypothesized to play a role in goal-directed, as opposed to habitual, alcohol use. This initial study examines dorsal and ventral striatal functional connectivity during alcohol-cue processing as a function of the A118G single-nucleotide polymorphism of the mu-opioid receptor (OPRM1) gene.

Methods: Seventeen individuals with alcohol dependence (6 females; 90% Caucasian; mean age = 29.4) underwent blood oxygen level-dependent functional magnetic resonance imaging, while performing an alcohol taste-cues task. Psychophysiological interaction analyses investigated associations of the OPRM1 genotype with ventral and dorsal striatum functional connectivity, using the ventral striatum and the caudate as the seed region, respectively.

Results: Compared to A-allele homozygotes, G-allele carriers of the OPRM1 gene showed (i) greater activation of the insula and orbitofrontal cortex and (ii) stronger negative fronto-striatal functional connectivity for both ventral and dorsal striatal seed regions during processing of alcohol versus water cues.

Conclusions: These preliminary findings suggest that, relative to A-allele homozygotes, G-allele carriers show unstable frontal regulation over reward and/or habit-driven inputs from the striatum resulting from greater reward sensitivity combined with limited self-control resources.

Key Words: OPRM1, Functional Magnetic Resonance Imaging, Cue Reactivity, Functional Connectivity, Dorsal Striatum, Ventral Striatum.

CRAVING IS INHERENTLY a subjective experience best described as a state of desire or wanting (Monti et al., 2004). The neural basis of craving has been highlighted in the most prominent neurobiological theories of addiction (Kalivas and Volkow, 2005; Koob and Le Moal, 2008; Robinson and Berridge, 1993). A number of neuroimaging studies have examined brain activation in response to alcohol cues thought to provoke craving. A functional magnetic resonance imaging (fMRI) study by George and colleagues (2001) found greater activation of the anterior thalamus and left dorsal lateral prefrontal cortex (dlPFC) in response to alcohol versus control cues, when comparing alcohol-dependent patients to controls. Myrick and colleagues (2004) found that alcohol cues elicited activation of the left orbitofrontal cortex (OFC), nucleus accumbens (NAc), and anterior cingulate (ACC), with the magnitude of activation in these regions found to correlate with alcohol craving in alcohol-dependent patients, but not in social drinkers. Other studies have supported the positive association between activation of the dorsal striatum and cue-induced craving in alcohol-dependent patients (Wrase et al., 2002) and implicated the magnitude of activation in these brain regions with the risk of relapse (Grusser et al., 2004). While these studies have demonstrated that alcohol versus control cues elicit differential brain activation in alcohol-dependent patients as compared to controls (Schacht et al., 2013), less is known about individual variation within alcohol-dependent individuals.

Neural circuitry related to reward sensitivity is reliably involved in alcohol craving, yet how craving influences functional connectivity between brain regions remains unclear. The ventral striatum receives synaptic inputs from the OFC, dlPFC, and limbic structures, such as the amygdala and hippocampus (Groenewegen et al., 1999), and preclinical data suggests functional activity in the striatum is directly influenced by input from the cortex (Brown et al., 1998). Thus, the fronto-striatal pathway is likely affected in alcohol

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dependence as abnormal fronto-striatal functional connectivity has been associated with impairments in learning from prediction errors as well as with the magnitude of alcohol craving (Park et al., 2010). These findings are taken as evidence that fronto-striatal coupling is related to alcohol-dependent patients' ability to control their craving for alcohol, even though the expression of reward prediction errors in the ventral striatum was intact in these patients (Park et al., 2010). A recent study of tobacco and food craving found that decreases in craving correlated with decreases in ventral striatum activation and increases in dlPFC activation, with ventral striatal activation fully mediating the relationship between lateral prefrontal cortex and self-reported craving (Kober et al., 2010). This suggests that craving can be controlled cognitively via the effects of prefrontal control systems on the ventral striatum and implies a top-down control process. Further, it has been postulated that processing of alcohol cues shifts from ventral to dorsal striatum during the transition from goal-directed (reward driven, "wanting") to habitual and compulsive alcohol use (Vollstadt-Klein et al., 2010). This is consistent with the incentive–sensitization model of addiction, whereby compulsive alcohol use is under control of the dorsal striatum (Everitt and Robbins, 2005). Based on these findings, the present study seeks to test ventral and dorsal striatal functional connectivity during alcohol-cue processing.

Positron emission tomography (PET) imaging findings suggest that in alcohol-dependent individuals, D_2-dopamine receptor availability in the ventral striatum is negatively associated with alcohol craving (Heinz et al., 2005b). Moreover, lower availability of D_2-like receptors in the ventral striatum was associated with greater alcohol craving and greater cue-induced activation of the mPFC and ACC during an fMRI task in alcohol-dependent patients (Heinz et al., 2004). PET imaging has implicated the mu-opioid receptors in alcohol craving, such that higher availability of mu-opioid receptors in the ventral striatum and mPFC was positively associated with the degree of craving in alcohol-dependent patients (Heinz et al., 2005a). Furthermore, drinking alcohol induces opioid release in the NAc and OFC (Mitchell et al., 2012), and a genetic polymorphism of the mu-opioid receptor (OPRM1) gene is associated with stronger dopamine release in the ventral striatum following alcohol consumption (Ramchandani et al., 2011). These studies implicate both endogenous opioids and dopamine in the ventral striatum with the expression of alcohol craving and alcohol "high," which are thought to subserve goal-directed drinking. Thus, variation in striatal opioid levels may moderate the efficiency of fronto-striatal control over craving and genetic markers subserving opioidergic activity warrant examination.

The present study examines genetic variation in the A118G single-nucleotide polymorphism (SNP; rs17799971) of the OPRM1 gene as a moderator of neural responses to alcohol cues and of dorsal and ventral striatal functional connectivity during an alcohol taste-cues task. This SNP is thought to increase receptor binding affinity for β-endorphin by 3-fold (Bond et al., 1998). Further, the G-allele is associated with deleterious effects on both mRNA and OPRM1 protein yield (Zhang et al., 2005). Experimental studies found that G-allele carriers report greater subjective reinforcement from alcohol in the laboratory (Ray and Hutchison, 2004) and in the natural environment (Ray et al., 2010). Among heavy drinkers, the G-allele was associated with greater hemodynamic response to alcohol cues in mesocorticolimbic areas (Filbey et al., 2008b) and greater striatal dopamine release following acute alcohol administration (Ramchandani et al., 2011). This study extends the literature to alcohol-dependent individuals. The present study also differs from Filbey and colleagues (2008b) by evaluating functional connectivity during an alcohol-cue-exposure task.

In light of the research implicating endogenous opioids (Heinz et al., 2005a; Ramchandani et al., 2011) and striatal dopamine (Heinz et al., 2004, 2005b) in cue-induced alcohol craving, this study examines dorsal and ventral striatal functional connectivity during alcohol-cue processing as a function of OPRM1 genotype. To the extent to which the ventral striatal pathway subserves reward-driven drinking (Everitt and Robbins, 2005) and based on the literature suggesting this polymorphism is implicated in reward drinking (Ray et al., 2012), we predicted differences in ventral striatal functional connectivity as a function of OPRM1 genotype. These differences would have the potential to explain variation in reward-driven drinking between the allelic groups based on fronto-striatal connectivity. First, we hypothesized that these differences are driven by weakened frontal control over ventral striatal reward signals. This would be predicted by weaker fronto-striatal connectivity in G-allele carriers. Alternatively, stronger connectivity in G-allele carriers would suggest a greater need for frontal regulation of disproportionately greater reward sensitivity in this group. No differences in dorsal striatal connectivity were hypothesized, as this polymorphism has not been implicated in habitual drinking. Given the hypothesized contribution of alcoholism severity to the shift from ventral to dorsal striatum control of alcohol-cues processing (Vollstadt-Klein et al., 2010), analyses control for alcoholism severity.

MATERIALS AND METHODS

Sample Characteristics

Nontreatment seeking individuals reporting alcohol problems (n = 295) were recruited through community flyers and online advertisements. The protocol was approved by the University of California, Los Angeles Institutional Review Board. Upon providing written informed consent, participants were screened for alcohol dependence and prospectively genotyped resulting in a sample of 43 individuals who completed the alcohol administration study (Ray et al., 2013). Of the 43 eligible alcohol-dependent individuals prospectively matched on OPRM1 genotype, 20 were selected for the neuroimaging study described herein. These individuals were selected to ensure equal numbers of male and female participants with and without the minor allele (G) of the OPRM1 gene (AA, n = 10; AG, n = 10). Of those, 3 were excluded due to excessive motion, resulting in a sample of 17 completers (AA, n = 9; AG, n = 8). Ethnicity was

matched across groups to account for population stratification at the OPRM1 locus. Inclusion criteria for the study were as follows: (i) ages between 21 and 55, (ii) met DSM-IV criteria for current alcohol dependence, (iii) no serious medical illness, use of psychotropic medications, history of psychotic disorders, bipolar disorder, or suicidal ideation, (iv) no current drug use (other than tobacco and marijuana), verified by a toxicology screen, and (v) no DSM-IV drug abuse or dependence in the past 12 months. Participants had a blood alcohol concentration of 0.00 g/dl prior to scanning.

Individual Difference Measures

Demographic information is presented in Table 1. There were no significant differences between the genotype groups on demographic variables ($ps > 0.10$). Alcohol use was assessed using the 30-day timeline follow back (Sobell and Sobell, 1980). Alcohol dependence and the exclusionary diagnoses were assessed using the Structured Clinical Interview for DSM-IV (First et al., 1995). All participants completed the Clinical Institute Withdrawal Assessment for Alcohol (CIWA-Ar; Sullivan et al., 1989), Alcohol Dependence Scale (ADS; Skinner and Allen, 1982), Drinkers Inventory of Consequences (DrInC-2R) questionnaire (Miller et al., 1995), and the Penn Alcohol Craving Scale (PACS; Flannery et al., 1999).

To model the shared variance between the alcohol problem indices (ADS, PACS, Symptom Count, DrInC-2R, and CIWA-Ar), principal components analyses were conducted on the full sample of problem drinkers ($n = 295$) to derive factor scores capturing alcohol problem severity, as described in detail elsewhere (Ray et al., 2013). Participants' score on the single factor, labeled alcoholism severity, was used in subsequent analyses.

Alcohol-Cues Task

While in the scanner, participants underwent an alcohol taste-cue paradigm, previously reported to elicit blood oxygen level-dependent response in mesocorticolimbic areas (Filbey et al., 2008a,b). Alcohol and water taste stimuli were delivered via Teflon tubing using a computer-controlled delivery system (Infinity Controller; J-KEM Scientific, Inc., St. Louis, MO) as described by Filbey and colleagues (2008a). The paradigm consisted of 12 taste-cue trials (6 alcohol and 6 water trials) in which 1 ml of liquid was delivered. Each trial consisted of a 24-second taste delivery period, followed by a 6-second rest period, a 12-second urge rating period, and a 2-second delay before the initiation of the next trial (Fig. 1). The words "Alcohol Taste" or "Control Taste" were visually presented during cue delivery; although it was previously reported that the presentation of explicit, versus nonexplicit, taste instructions did not alter responses to alcohol (Filbey et al., 2008a). Participants rated their urge to drink alcohol using a scale of 1 (no urge at all) to 4 (very high urge) using a 4-button response box placed in their right hand. Sauvignon Blanc wine was used for the alcohol taste cue and distilled water for the control cue. Self-reported urge to drink ratings during alcohol and water cues are presented in Fig. 1. The presentation of visual stimuli and response collection were programmed using E-Prime (Psychology Software Tools, Inc., Sharpsburg, PA). Visual and auditory stimuli were presented using MRI compatible goggles and headphones (Resonance Technologies, Van Nuys, CA).

Table 1. Sample Demographics by OPRM1 Genotype

	Frequency or mean (SD)	
Variable	AA ($n = 10$)	AG/GG ($n = 10$)
Age	32.1 (11.0)	26.7 (5.8)
Sex—Male/Female	7/3	7/3
Ethnicity		
Caucasian	9	9
African American	1	1
Drinks per drinking day	6.9 (1.9)	5.9 (2.6)
Percent drinking days (past 30 days)	65.3 (0.6)	58.3 (0.2)
Withdrawal symptoms (Total CIWA-Ar Score)	2.5 (1.5)	2.0 (1.8)
Symptom count	6.70 (2.26)	5.70 (1.94)
ADS Total score	18.80 (5.55)	15.40 (4.65)
DrInC-2R Total score	103.20 (22.17)	93.80 (15.39)
Dependence severity factor	0.4957 (0.9378)	−0.0327 (0.6415)
Education (years)	15.7 (2.5)	14.3 (1.9)
Shipley IQ (standard score)	113.3 (16.5) ($n = 9$)	106.7 (22.2)
Working memory (digit span scaled score)	12.3 (1.9) ($n = 7$)	11.0 (3.3) ($n = 8$)
Marijuana use—none/moderate	7/3	7/3
Cigarettes per day		
0	3	3
1 ≤ 10	6	6
>10	1	1

DrInC-2R, Drinkers Inventory of Consequences; ADS, Alcohol Dependence Scale.

Except for the trend-level group difference on alcohol dependence severity ($p = 0.09$), no significant group differences were found ($ps > 0.10$).

MRI Data Acquisition

Neuroimaging was conducted using a 3 Tesla Siemens Trio MRI scanner (Siemens Medical Solutions USA, Inc., Malvern, PA), at the UCLA Ahmanson–Lovelace Brain Mapping Center. The protocol began with initial structural scans followed by a series of 4 functional runs, including the alcohol-cue-exposure task, a stop-signal task, a delay-discounting task, and a risky decision-making task (results from the latter 3 tasks will be reported elsewhere). A T2-weighted, high-resolution, matched bandwidth, anatomical scan (MBW) and a magnetization-prepared rapid-acquisition gradient echo (MPRAGE) were acquired for each subject to enable registration (TR, 1.9 seconds; TE, 2.26 ms; FOV, 250 mm; matrix, 256 × 256; sagittal plane; slice thickness, 1 mm; 176 slices). The orientation for MBW and echoplanar image (EPI)

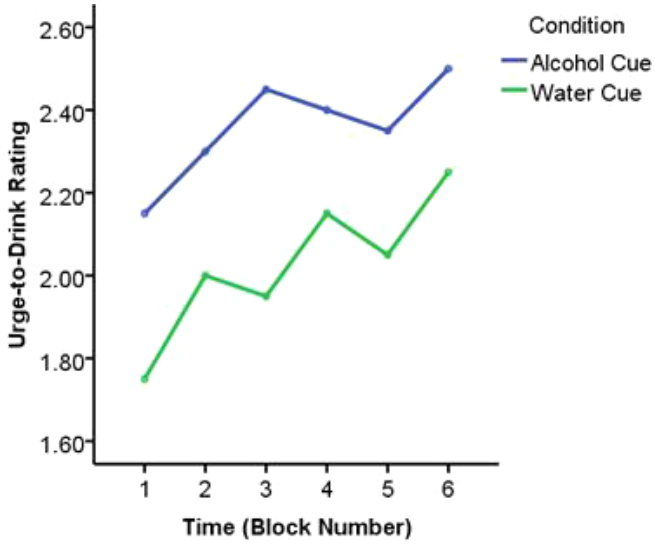

Fig. 1. Urge to drink ratings across taste delivery blocks for alcohol and water cues. There was a significant main effect of condition (alcohol vs. water; $p < 0.01$) and a main effect of time, such that urge ratings increased across trial ($p < 0.01$). However, there was no significant Condition × Time interaction ($p = 0.65$).

scans was oblique axial to maximize brain coverage. The alcohol taste-cues scan included 184 functional T2*-weighted EPIs (TR, 2 seconds; TE, 30 ms; flip angle, 90°; FOV, 192 mm; matrix, 64 × 64; voxel size, 3 × 3 × 4 mm^3; slice thickness, 4 mm; 34 slices). The first 6 volumes collected were discarded to allow for T1 equilibrium effects.

Imaging Preprocessing and Registration

FSL 4.1 (FMRIB's Software Library, www.fmrib.ox.ac.uk/fsl; Analysis Group, FMRIB, Oxford, UK) was used for the imaging analyses. Motion correction was carried out using FSL's Motion Correction Linear Image Registration Tool (McFLIRT, Version 5.0; Analysis Group)) with the estimated motion parameters entered as covariates in the general linear model. Nonbrain tissue/skull removal was conducted with the Brain Extraction Tool. The images were smoothed using a FWHM Gaussian kernel (5 mm) and high-pass filtered (100-second cutoff) in the temporal domain using a Gaussian weighted straight line using FSL's FMRI Expert Analysis Tool (FEAT, Version 5.63; Analysis Group)). The EPIs were first registered to the MBW, then to the MPRAGE using affine linear transformations, and finally into standard (Montreal Neurological Institute [MNI] avg152 template) space for between subject analyses refined by FSL's FNIRT nonlinear registration (Andersson et al., 2007). Three subjects (2 G-allele carriers and 1 A-allele homozygote) were excluded from further analyses due to excessive motion (exceeding 3 mm of translation).

Genotyping

Saliva samples were collected using Oragene saliva collection kits (Kanata, Ontario, Canada) and sent to the UCLA Genotyping and Sequencing Core for genotyping. Polymerase chain reaction was performed on Applied Biosystems (Carlsbad, CA) dual block PCR thermal cyclers. SNPs were run on an AB 7900HT Fast Real-Time PCR System and analyzed using the Sequence Detection Systems software version 2.3 (Applied Biosystems, Grand Island, NY). Genotypes were automatically scored by the allele calling software, and each genotype was verified by visual inspection.

Statistical Analyses

Whole-brain statistical analysis was performed using a multistage approach to implement a mixed-effects model treating participants as a random-effects variable. Explanatory variables for the alcohol taste-cues task were created by convolving delta functions representing the onset of the taste period for each trial type (Fig. 2) with a double-gamma hemodynamic response function (HRF) in FEAT. The event period was chosen to stay consistent with previous applications of this task (Filbey et al., 2008a,b). Alcohol- and water-cue exposure were modeled as separate event types. The onset for each event was set at the first instruction to swallow (10 seconds after the initial taste cue was presented) with duration of 20 seconds plus the response time for the urge to drink rating. The delay in onset was chosen to capture the period in which participants are actively "experiencing" the taste of alcohol or water. We excluded the first taste period of each trial because this includes the time for the gustometer to deliver the liquid, which could be slightly startling to the subjects, and is likely too soon for the subjects to be "experiencing" the taste. The second taste period of each trial was included; however, because participants are still "experiencing" the taste of the first delivery of liquid during this time. These procedures are consistent with Filbey and colleagues (2008a,b).

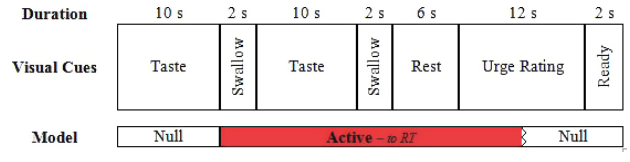

Fig. 2. Schematic of a single taste-cue trial and model. A rest period immediately followed the taste delivery period for 6 seconds, wherein "Rest" was presented visually. A single urge question ("What is your urge to drink alcohol?") was presented for a total of 12 seconds at the end of the rest period, followed by "Ready?" for 2 seconds.

Temporal derivatives were included as covariates of no interest to improve statistical sensitivity. Null events, consisting of the postresponse rating period, rest period, and first cue delivery, were not explicitly modeled and therefore constituted an implicit baseline. The following contrasts were computed: (i) water versus alcohol and (ii) alcohol versus water, which was the primary contrast of interest.

Group analyses were conducted on contrast images transformed into standard space. *Z*-statistic images were thresholded with cluster-based corrections for multiple comparisons based on the theory of Gaussian Random Fields with a cluster-forming threshold of $Z > 1.96$, 2.3, or 3.7, depending on the analysis (see each Table and Figure for analysis-specific details), and a probability threshold of $p < 0.05$ (Worsley, 2001). In particular, the more stringent thresholds were used to refine the localization of the clusters of activation thus informing the interpretation of the findings. Specifically, the voxel height threshold (i.e., "cluster-forming threshold") of the *Z* map (Gaussianized *t*-map) was first specified followed by a corrected cluster significance threshold of $p = 0.05$. Alcoholism severity factor scores were modeled as the explanatory variable on the whole-brain contrast maps. Anatomical localization within each cluster (maximum *Z*-statistics and MNI coordinates) was obtained by searching within maximum-likelihood regions from the FSL Harvard–Oxford probabilistic atlas. To test the primary aim, OPRM1 genotype (i.e., AA and AG/GG) was entered as a predictor variable and examined in relation to the computed contrasts using a whole-brain approach.

Functional connectivity was assessed using psychophysiological interaction (PPI) analysis (Friston et al., 1997; Gitelman et al., 2003), which measures coupling of brain regions during specific task conditions. As described by O'Reilly and colleagues (2012), PPI analysis seeks to identify task-specific changes in the relationship between brain areas (i.e., functional connectivity). As such, if 2 areas interact more in the context of a certain psychological task (e.g., alcohol-cue exposure), activity in one area should regress more strongly on activity in the other area during task blocks compared with during control blocks (O'Reilly et al., 2012). To examine fronto-striatal functional connectivity during cue exposure, we separately examined the coupling of (i) the right ventral striatum (including the NAc) and (ii) the dorsal striatum (caudate) and the rest of the brain during alcohol- versus water-cue presentation. The right ventral striatum as well as the caudate (ROIs) were anatomically defined using the high-resolution MPRAGE anatomical images, segmented on a subject-specific basis in native space using FMRIB's Integrated Registration and Segmentation Tool in FSL. The average time course of the right ventral striatum and the caudate was extracted from motion-corrected, high-pass-filtered image data (same preprocessing steps as outlined above). PPI analyses were conducted using components of SPM and FSL's FEAT. The models were identical to the first-level model described above with the inclusion of 3 additional regressors: "psychological," "physiological," and "psychophysiological interaction." These regressors were generated separately by computing 3 vectors using the PPI algorithms implemented in SPM5 (http://www.fil.ion.ucl.ac.uk/spm/software/spm5). The psychological vector was specified by a delta function with alcohol-cue events represented by 1 and water-cue events represented by −1 (zero centered), the physiological vector estimated "neural" activation of the right ventral striatum via

Table 2. Means and Standard Deviations (SD) for the Task-Related Variables by OPRM1 Genotype

Alcohol-cues task variable	AA		AG/GG	
	Mean	SD	Mean	SD
Urge rating to alcohol cue	2.20	0.88	2.52	0.87
Urge rating to water cue	1.90	0.57	2.15	0.90
Rating response time to alcohol cue (ms)	3,317.62	1,173.17	3,045.73	1,308.17
Rating response time to water cue (ms)	2,997.78	1,387.21	2,553.42	1,263.50

No significant group differences observed (ps > 0.10).

hemodynamic deconvolution of the average preprocessed time course, and the PPI vector was the product of the two. The 3 vectors were convolved with an HRF (using SPM functions), prior to inclusion in FSL's FEAT model. A whole-brain contrast image for the PPI was computed from this model and submitted for group analyses described above using a cluster-forming threshold of $Z > 1.96$ and a probability threshold of $p < 0.05$.

RESULTS

Urge ratings and response times during the task are presented in Table 2. The mean length of abstinence from alcohol prior to scanning was 1.63 days (SD = 0.83). No group differences were observed across OPRM1 genotype in subjective urge ratings during the task or response times as analyzed by independent t-tests (ps > 0.10). Across all subjects, urge to drink alcohol and rating response time following cue presentation were unrelated to alcohol dependence indices (ps > 0.10). There was a trend toward greater alcoholism severity among A-allele homozygotes, $t(15) = -1.763$, $p = 0.09$, and alcoholism severity was controlled for in subsequent analyses.

Whole-Brain Alcohol-Cue Contrasts

The main contrast of interest (alcohol vs. water) activated a broad set of regions including mesocorticolimbic areas such as the ventral striatum and ventrolateral frontal areas.

Table 3. Locations of Significant Activation from the Alcohol- Versus Water-Cue Contrast Across All Subjects (Whole-Brain Cluster-Corrected at $Z > 3.7$, $p < 0.05$), and Regions Significantly Moderated by OPRM1 Genotype Within this Contrast (Whole-Brain Cluster-Corrected at $Z > 2.3$, $p < 0.05$)

Clusters/Brain regions	Hemisphere	Cluster voxels	Max Z	X	Y	Z
Alcohol versus Water cue						
Postcentral gyrus	L	38,161	6.06	−54	6	−2
Middle temporal gyrus	L		6.04	−48	−12	10
Insular cortex	R/L		5.87/4.55	36/−38	−10/10	4/2
Superior parietal lobule	R/L		4.42/5.66	30/−24	−58/−32	50/54
Caudal anterior cingulate gyrus	R		4.98	2	18	38
Supplementary motor cortex	R		4.53	2	−2	58
Thalamus	R/L		4.51/4.93	16/−14	−14/−16	14/6
Putamen	L		4.72	16	12	−6
Cerebellum	L		4.81	−12	−58	−28
Occipital pole	R/L		4.47/4.03	30/−28	−90/−88	10/8
Middle frontal gyrus	R/L	339/332	4.86/4.80	38/−30	48/50	24/28
Posterior cingulate/Precuneus	R/L	80/279	4.40/4.99	10/−8	−40/−52	20/16
Caudate/Putamen (ventral)	R	257	5.37	14	16	−4
Rostral anterior cingulate/Medial prefrontal cortex	R/L	190/91	4.57/4.08	6/−6	4/40	−42/20
Lateral occipital cortex	L	47	4.40	−54	−70	28
Alcohol versus Water cue moderated by OPRM1 genotype (AG/GG vs. AA)						
Posterior supramarginal gyrus	L	801	3.24	−54	−44	28
Anterior supramarginal gyrus	L		2.94	−56	−36	30
Parietal operculum cortex	L		2.92	−54	−38	20
Cerebellum	R/L	748	3.12	14	−62	−20
Lingual gyrus	R		2.96	20	−60	−14
Cuneal cortex	R	725	2.92	8	−88	24
Supracalcarine cortex	R		2.88	2	−84	12
Occipital pole	L		2.73	−8	−94	10
Intracalcarine cortex	R		2.70	8	−80	12
Precuneus cortex	L	719	3.01	−6	−50	52
Superior parietal lobule	R		2.84	32	−40	54
Insular cortex	R	511	2.92	36	12	−6
Temporal pole	R		2.85	62	12	−4
Central opercular cortex	R		2.73	40	10	4
Orbitofrontal cortex	R		2.65	44	20	−8
Frontal opercular cortex	R		2.61	40	20	2
Angular gyrus	R	374	2.92	58	−50	30
Posterior supramarginal gyrus	R		2.92	60	−42	20
Anterior supramarginal gyrus	R		2.83	64	−30	30

X, Y, and Z MNI coordinates indicate the location of peak voxel activation (or local maxima for subregions) within each cluster. R, right, L, left.

Genotype moderated results are from the G-allele carrier versus A-allele homozygote contrast. No significant activation differences were found for the AA versus AG/GG contrast. MNI, Montreal Neurological Institute.

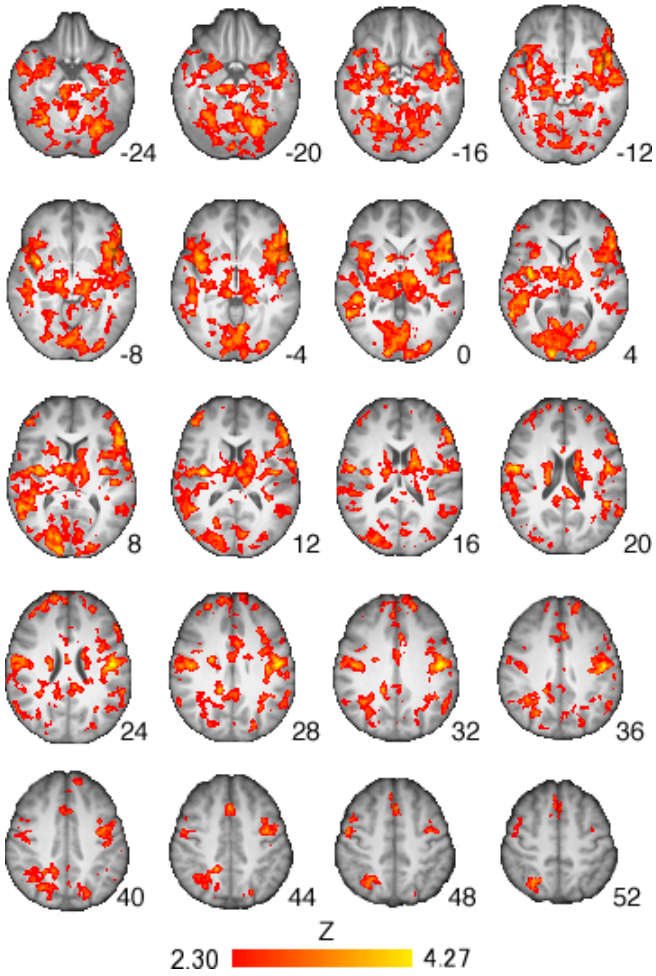

Fig. 3. Brain activation within the alcohol- versus water-cue contrast (see Table 3 for full list of regions). *Z*-statistic maps are whole-brain cluster-corrected, $Z > 2.3$, $p = 0.05$. The threshold for the statistical map was decreased for visualization purposes. Coordinates are in MNI space, and the brain is displayed in radiological convention (left = right). MNI, Montreal Neurological Institute.

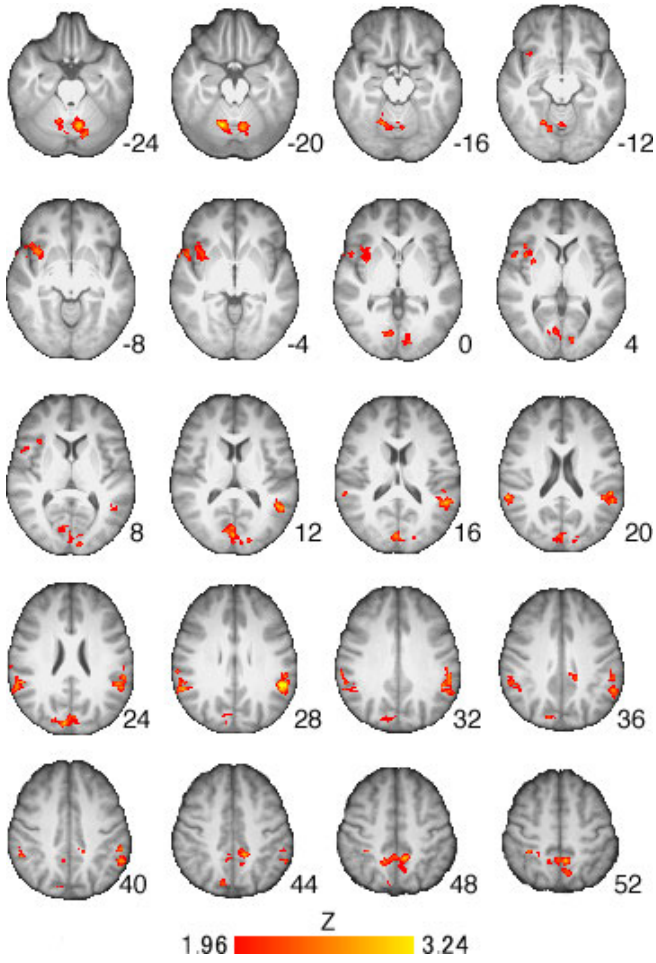

Fig. 4. Brain activation moderated by OPRM1 genotype (AG/GG vs. AA) within the alcohol- versus water-cue contrast. The left insula was a primary area of activation that found to be greater for G-allele carriers (see Table 3 for full list of regions). *Z*-statistic maps are whole-brain cluster-corrected, $Z > 1.96$, $p = 0.05$. The threshold for the statistical map was decreased for visualization purposes. Coordinates are in MNI space, and the brain is displayed in radiological convention (left = right). MNI, Montreal Neurological Institute.

Additional areas of activity were found in limbic cortex (insula, anterior and posterior cingulate gyrus/precuneus), superior parietal areas, medial frontal gyrus, medial prefrontal cortex, the thalamus, and occipital areas (Table 3, Fig. 3). No significant activation was found for the complementary contrast (water vs. alcohol). OPRM1 genotype (AG/GG vs. AA) was found to moderate the activation within the alcohol versus water contrast such that G-allele carriers displayed greater activation within regions including the right insula, bilateral supramarginal gyri, left precuneus, right superior parietal lobule, right OFC, and right angular gyrus associated with the presentation of the alcohol cue (Table 3, Fig. 4).

PPI Analyses

To determine fronto-striatal functional connectivity during alcohol-cue presentation, separate whole-brain PPI analyses from the alcohol versus water contrast describing strength of connectivity between the right ventral striatum (including the accumbens), and the rest of the brain and the right primarily dorsal striatum (caudate) and the rest of the brain were performed. These analyses carefully followed published procedures for PPI implementation and interpretation (O'Reilly et al., 2012). No regions of significant positive activation clusters were found across all subjects for either seed region in the PPI analysis; however, the negative PPI results revealed weaker correlations (decreased functional connectivity) between striatal seed regions and mostly posterior brain regions during the presentation of the alcohol versus water cues (Table 4, Figs 5 and 6). Furthermore, functional connectivity with each seed region was found to differ between OPRM1 genotypes in the G-allele carriers versus A-allele homozygotes contrast (parameter estimates for the cluster: G carriers = −0.271, A homozygotes = 0.012;

Table 4. Negative Main Effect Fronto-Striatal Functional Connectivity Results Within the Alcohol- Versus Water-Cue Contrast Across All Subjects (Cluster-Corrected at $Z > 1.96$, $p < 0.05$)

Brain region	Hemisphere	Cluster voxels	Max *Z*	*X*	*Y*	*Z*
(A) Ventral striatum seed negative PPI for alcohol versus water cue						
Superior lateral occipital cortex	R	92,319	4.61	16	−82	36
Cuneal cortex	R		4.49	2	−74	24
Occipital pole	R		4.48	2	−94	8
(B) Dorsal striatum (Caudate) seed negative PPI for alcohol versus water cue						
Precuneus cortex	L	53,827	4.31	−4	−64	64
Superior lateral occipital cortex	L		4.30	−34	−60	54
Intracalcarine cortex	R		4.27	10	−88	4
Precentral gyrus	L		4.26	−4	−30	70
Occipital fusiform gyrus	R		4.25	38	−72	−22

X, *Y*, and *Z* MNI coordinates indicate the location of peak voxel activation (or local maxima for subregions) within each cluster. R, right, L, left.

Results are from a psychophysiological interaction (PPI) analysis using the right ventral striatum (A) and right dorsal striatum (caudate) (B) as anatomically defined regions of interest determined for each participant individually (see Materials and Methods). MNI, Montreal Neurological Institute.

for PPI activation maps for each genotype group see Figs S1 and S2). Specifically, G-allele carriers exhibited stronger negative correlations between the right ventral striatum and the insula, frontal medial cortex, superior parietal lobule, thalamus, putamen, and paracingulate gyrus as compared to A-allele homozygotes (Table 5, Fig. 7). G-allele carriers were also found to have stronger negative correlations between the right dorsal striatum (caudate) seed region and the insula, OFC, paracingulate gyrus, and subcallosal cortex (Table 5, Fig. 8). Analyses using the left ventral striatum as the seed revealed the same pattern of results, with the exception of the temporal and lateral occipital areas.

DISCUSSION

This study examined fronto-striatal functional connectivity during an alcohol-cue-exposure task in a sample of alcohol-dependent individuals. Inputs from the prefrontal cortex to the striatum regulate the expression of reward-driven decision making (Haber et al., 2006). Shifts from ventral to dorsal striatal control of alcohol-cues processing are thought to mark the transition from goal-directed to habitual drinking (Everitt and Robbins, 2005). Thus, we examined 2 distinct pathways of fronto-striatal connectivity, namely ventral and dorsal pathways, and tested whether the A118G locus, putatively involved in alcohol reinforcement, moderates functional connectivity patterns during alcohol-cue exposure.

This study used an alcohol taste-cues task previously found to activate both ventral and dorsal striatal regions in heavy drinkers (Filbey et al., 2008a,b). Whole-brain analyses of the alcohol versus water contrast revealed greater activation of the insula, OFC, precuneus, and bilateral supramarginal gyrus (BA40) among G-allele carriers, compared with A-allele homozygotes. These results differ from those of Filbey and colleagues (2008b) who used the same task in a sample of heavy drinkers. Filbey and colleagues (2008a) found greater activation of multiple mesocorticolimbic areas among G-allele carriers in the alcohol versus control contrast, whereas in the present study, only a few areas of activation differentiated the 2 genotype groups. These differences may be the result of comparing cue reactivity in heavy drinkers versus alcohol-dependent individuals. As noted above, differences in the neural correlates of cue reactivity have been reported as a function of alcohol use (Vollstadt-Klein et al., 2010). Conversely, the different pattern of mesolimbic activation found in previous studies by Filbey and colleagues (2008a,b) may reflect differences in statistical power as well as the statistical approach. The studies by Filbey and colleagues (2008a,b) primarily focused on ROI analyses as compared to whole-brain analyses, which are more statistically stringent due, in part, to the multiple comparisons correction applied. Further, we note that the exploratory whole-brain analysis reported in Filbey and colleagues (2008a) did not find significant mesolimbic activation for the alcohol versus control taste contrast. These methodological differences are likely to account, at least in part, for the discrepancy in the findings while also suggesting that the results may be more congruent at the whole-brain level of analysis.

The present findings extend previous research with heavy drinking samples and suggest that cue-induced activation of opioid receptor-rich neural regions (Cross et al., 1987; Zubieta et al., 2001), such as the insula and OFC, is moderated by OPRM1 genotype. It is plausible that individuals (G-allele carriers) expressing the more potent mu-opioid receptors experience enhanced neural response to alcohol reward-signaling cues and that this pattern of activation provides a neural signature to the enhanced alcohol reward observed in behavioral pharmacology paradigms (Heilig et al., 2011; Ray et al., 2012). Placebo-controlled studies combining fMRI with in vivo alcohol exposure are needed to more directly test this hypothesis. Nevertheless, these results are generally consistent with the hypothesized role of this genetic marker in alcoholism phenotypes (Ray et al., 2012).

Results of PPI analyses in this sample showed patterns of activation that were consistent with the known neuroanatomical pathways connecting these brain regions such that dorsal striatum (caudate) revealed greater connectivity with the prefrontal cortex and inferior temporal gyrus, whereas the ventral striatum showed greater connectivity with the insula and putamen (Leh et al., 2007; all in the negative direction). Testing the OPRM1 gene as a moderator of functional connectivity with the right ventral striatum as the seed revealed stronger negative correlations with the insula, putamen, frontomedial cortex, thalamus, and paracingulate gyrus among G-allele carriers.

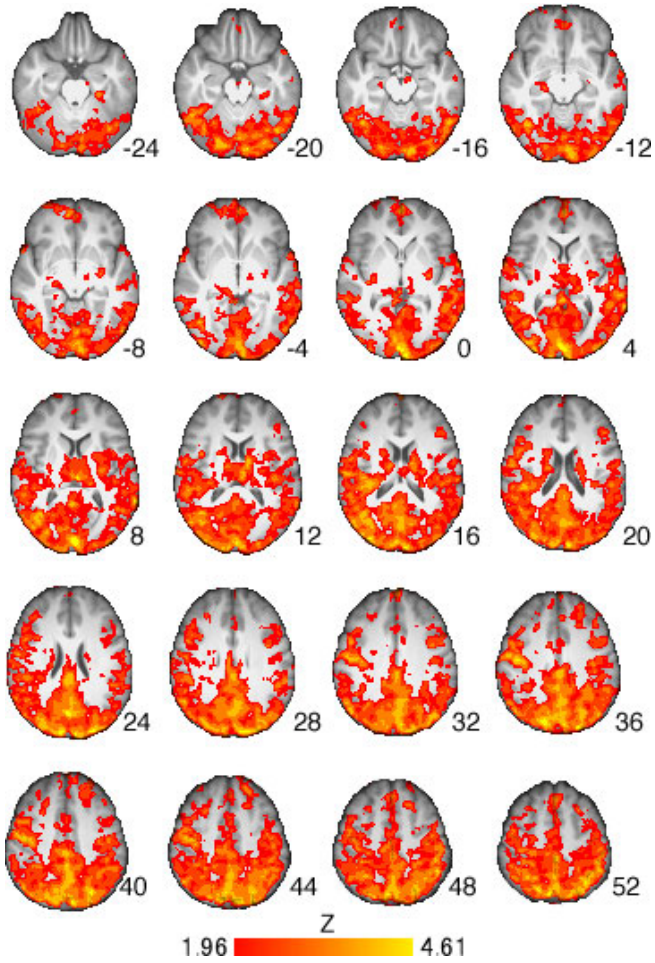

Fig. 5. Results of the negative main effect functional connectivity analysis indicating areas of decreased functional connectivity to the ventral striatum during the presentation of alcohol versus water cues (whole-brain cluster-corrected at $Z > 1.96$, $p < 0.05$). Brain regions showing decreased functional connectivity were largely posterior and included the occipital and cuneal cortices. Results are from a psychophysiological interaction analysis using the right ventral striatum (including the nucleus accumbens) as an anatomically defined region of interest determined for each participant individually (see Materials and Methods). Coordinates are in MNI space, and the brain is displayed in radiological convention (left = right). MNI, Montreal Neurological Institute.

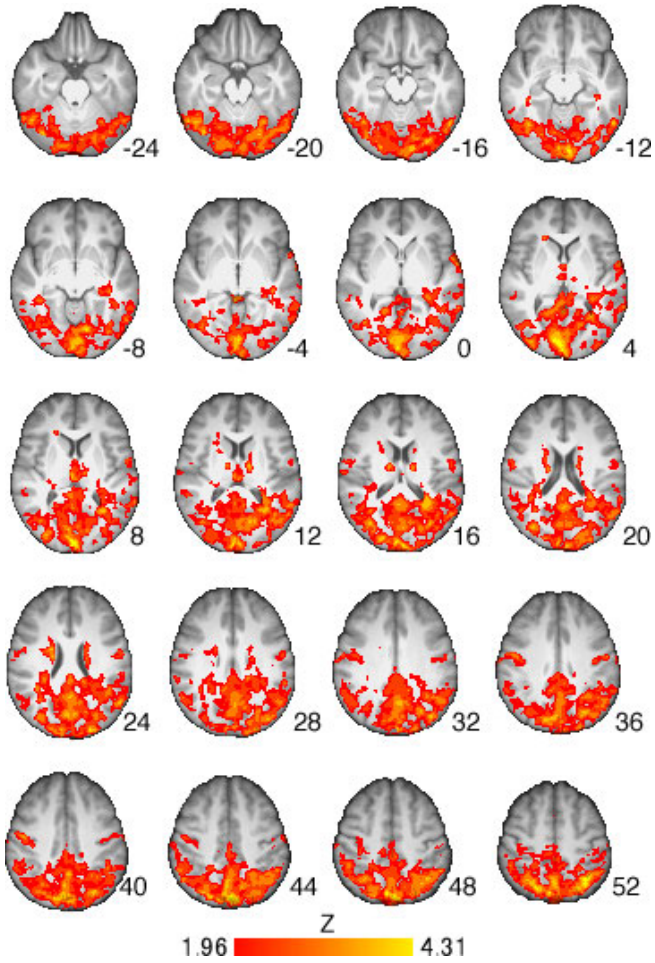

Fig. 6. Results of the negative main effect functional connectivity analysis indicating areas of decreased functional connectivity to the dorsal striatum (caudate) during the presentation of alcohol versus water cues (whole-brain cluster-corrected at $Z > 1.96$, $p < 0.05$). Brain regions showing decreased functional connectivity were largely posterior and included the precuneus and occipital cortices. Results are from a psychophysiological interaction analysis using the right dorsal striatum (caudate) as an anatomically defined region of interest determined for each participant individually (see Materials and Methods). Coordinates are in MNI space, and the brain is displayed in radiological convention (left = right). MNI, Montreal Neurological Institute.

Two hypotheses concerning fronto-striatal functional connectivity were considered based on alternative explanations for greater reward-driven behavior in G-allele carriers versus A-allele homozygote groups. First, we hypothesized weaker connectivity in the G-allele carriers suggesting dysregulation of ventral striatal reward signals by frontal regions; however, this was not confirmed as we found stronger connectivity between the 2 regions. Our alternative hypothesis was consistent with this finding as it assumed that fronto-striatal connectivity reflects greater demand for frontal regulation over subcortical signals reflecting heightened reward sensitivity (Heatherton and Wagner, 2011). The negative direction of functional connectivity (negative correlation between signals across regions) suggests the possibility that G-allele carriers require greater frontal recruitment for self-regulation. Given studies indicating self-control as a limited resource (Baumeister et al., 1998; Hagger et al., 2010), the negative connectivity observed in this study may reflect a cycle of increased frontal control of reward-related striatal signals (i.e., increased frontal and decreased striatal activation) followed by depletion of frontal control processes resulting in unregulated reward signals (i.e., decreased frontal and decreased striatal activation). Such unstable control processes may underlie the differences in reward-driven drinking behavior observed across allelic groups. Such behavioral differences are indicated by literature implicating this polymorphism in alcohol-induced reward (Ramchandani et al., 2011; Ray and Hutchison, 2004; Ray et al., 2010) and craving (Wiers et al., 2009; van den Wildenberg et al., 2007) and with the notion that reward-driven drinking is subserved by ventral, but not

Table 5. OPRM1 Moderated (AG/GG vs. AA) Negative Fronto-Striatal Functional Connectivity Results Within the Alcohol- Versus Water-Cue Contrast (Cluster-Corrected at $Z > 1.96$, $p < 0.05$)

Brain region	Hemisphere	Cluster voxels	Max *Z*	*X*	*Y*	*Z*
(A) Ventral striatum seed negative PPI for alcohol versus water cue moderated by OPRM1 genotype (AG/GG vs. AA)						
Postcentral gyrus	L	16,672	3.74	−40	−18	28
Thalamus	L		3.60	−6	−16	12
Precuneus/Posterior cingulate	R		3.57	2	−52	8
Occipital fusiform gyrus	R		3.55	38	−70	−22
Insula	R	1,778	3.21	32	−30	10
Central opercular cortex	R		3.10	58	−2	14
Putamen	R		3.09	26	−16	10
Postcentral gyrus	R		3.06	60	−8	38
Superior parietal lobule	R	1,016	3.23	36	−40	52
Angular gyrus	R		2.96	58	−50	46
Superior lateral occipital cortex	R		2.86	36	−60	50
Paracingulate gyrus	R/L	840	3.31	4	40	−12
Subcallosal cortex	R/L		3.20/3.02	2/−2	22/28	−14/−14
Frontal medial cortex	R		2.99	6	46	−12
(B) Dorsal striatum (Caudate) seed negative PPI for alcohol versus water cue moderated by OPRM1 genotype (AG/GG vs. AA)						
Insula/Orbitofrontal cortex	R	6,282	4.01	34	22	−6
Paracingulate gyrus	L		3.37	−4	46	−6
Subcallosal cortex	R/L		3.26	0	26	−10

X, *Y*, and *Z* MNI coordinates indicate the location of peak voxel activation (or local maxima for subregions) within each cluster. R, right, L, left.
Results are from a psychophysiological interaction (PPI) analysis using the right ventral striatum (A) and right dorsal striatum (caudate) (B) as anatomically defined regions of interest determined for each participant individually (see Materials and Methods). MNI, Montreal Neurological Institute.

dorsal, striatal pathways (Everitt and Robbins, 2005; Vollstadt-Klein et al., 2010).

Functional connectivity analysis with the dorsal striatum (caudate) as the seed region revealed a similar pattern, whereby G-allele carriers showed stronger negative connectivity with the insula, OFC, paracingulate gyrus, and subcallosal cortex. These findings are contrary to the initial hypothesis that differences in fronto-striatal functional connectivity patterns across groups would be unique to the ventral striatum (signaling reward-driven drinking), and not present for the dorsal striatum (signaling habit-driven drinking). In fact, these results suggest that this candidate gene contributes to negative correlations in both ventral and dorsal fronto-striatal functional connectivity during alcohol-cues processing. In other words, there was no evidence for dissociation of the functional connectivity patterns across striatal subregions moderated by this polymorphism.

While a recent study found greater ventral striatum activation to alcohol cues in social drinkers and greater dorsal striatum activation to alcohol cues in heavy drinkers (Vollstadt-Klein et al., 2010), alcoholism severity was controlled for in the present study. Controlling for severity allowed us to more effectively test the effects of genotype at matched levels of clinical impairment. As such, the present findings do not address the role of clinical severity on ventral versus dorsal striatum connectivity and instead focus on the role of the A118G SNP to these putative risk pathways for alcoholism. To that end, whole-brain analyses suggest that the G-allele of the OPRM1 gene is associated with (i) greater activation of opioid receptor-rich areas of the brain (insula, OFC) and (ii) negative fronto-striatal functional connectivity in both ventral and dorsal striatum during processing of alcohol cues. These findings suggest that inhibition of reward and/or habit-driven inputs from the striatum by the prefrontal control circuitry may be unstable among G-allele carriers. Importantly, the G-allele could show similar instability in fronto-striatal connectivity to other cues with incentive salience. Moreover, these results suggest the possibility that strengthening self-control in this group may result in more stable frontal control. Future research elucidating the specificity of this effect seems warranted.

These findings should be interpreted in the context of the study's strengths and limitations. Strengths include the well-validated alcohol-cue-exposure task and well-defined sample of alcohol-dependent patients prospectively genotyped to allow for meaningful comparisons across OPRM1 genotype. The use of PPI methods to extend upon whole-brain analyses of the alcohol- versus water-cues contrast represents a strength of the study. However, the correlative nature of PPI methods limit causal inferences about the directionality of connections between regions, precluding the ability to infer, for example, whether frontal activation occurred prior to striatal activation and vice versa. Study limitations include the relatively small sample size and the lack of a control group. The study sample size may only afford adequate statistical power to detect large effect sizes. Furthermore, the limited power afforded by the sample size precludes further analyses to examine the precise nature of the individual genotype group PPI effects, leaving open the potential for alternative interpretations. The a priori selection of alcohol-dependent patients as well as the prospective genotyping approach was consistent with the study aims of testing OPRM1 genotype on fronto-striatal functional connectivity during alcohol-cues processing. In addition, the use of a

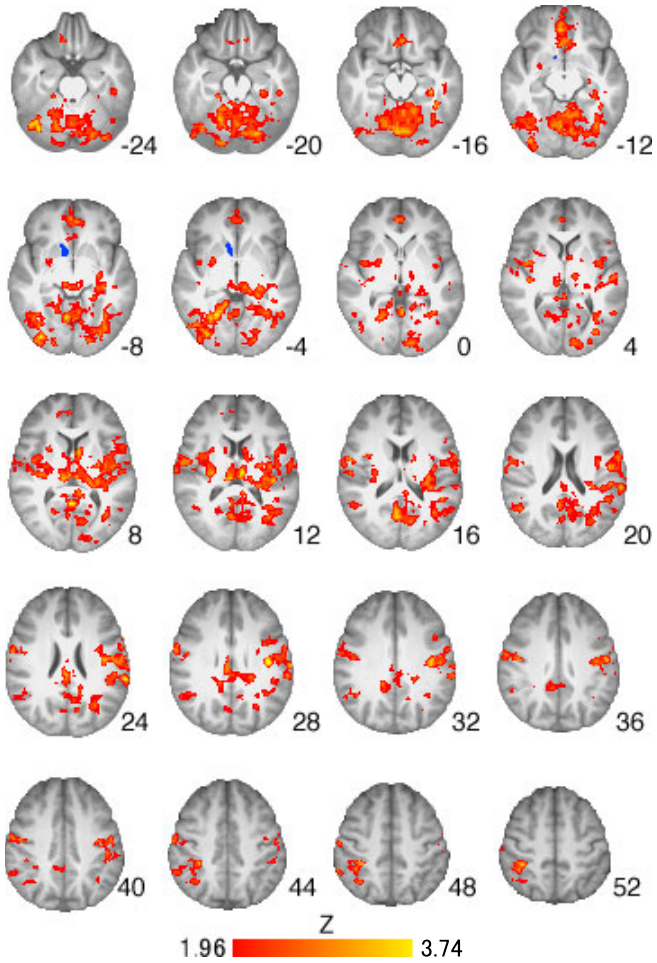

Fig. 7. Results of functional connectivity analysis revealed negative fronto-ventral striatal connectivity during the presentation of alcohol versus water cues was moderated by OPRM1 genotype (AG/GG vs. AA). The seed regions of interest is presented in blue. Brain regions whose negative connectivity with the right ventral striatum is greater for G-allele carriers as compared to A-allele homozygotes include the insula, frontal medial cortex, superior parietal lobule, thalamus, putamen, and paracingulate gyrus (whole-brain cluster-corrected at $Z > 1.96$, $p < 0.05$). Results are from a psychophysiological interaction analysis using the right ventral striatum (including the nucleus accumbens) as an anatomically defined region of interest determined for each participant individually (see Materials and Methods). No regions were found to be more positively functionally connected with the ventral striatum in the G-allele carriers as compared to the A-allele homozygotes. Coordinates are in MNI space, and the brain is displayed in radiological convention (left = right). MNI, Montreal Neurological Institute.

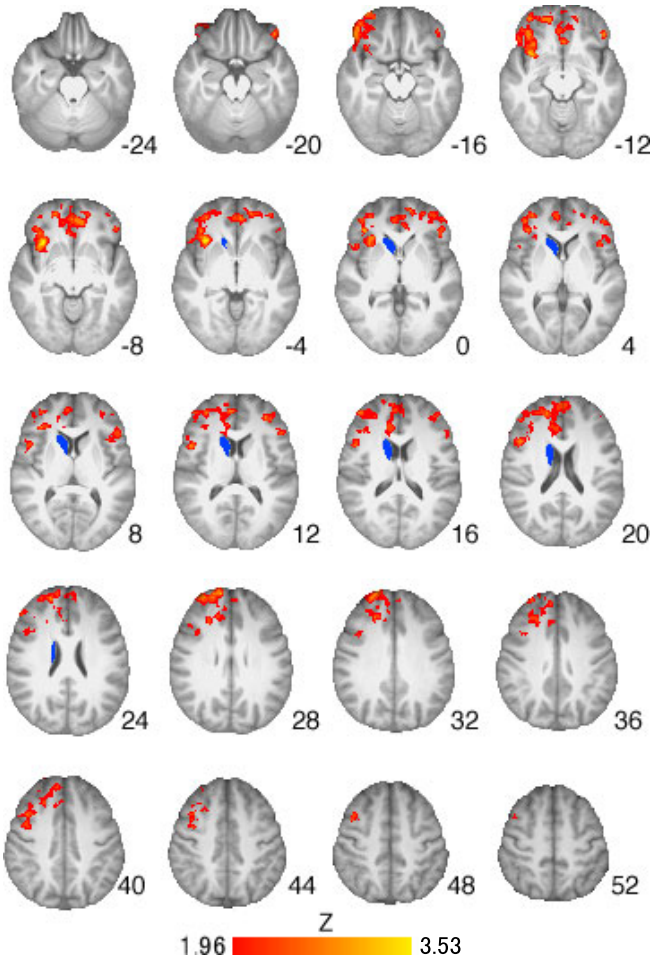

Fig. 8. Results of functional connectivity analysis revealed negative fronto–dorsal striatal connectivity during the presentation of alcohol versus water cues was moderated by OPRM1 genotype (AG/GG vs. AA). The seed regions of interest is presented in blue. Brain regions whose negative connectivity with the right dorsal striatum (caudate) is greater for G-allele carriers as compared to A-allele homozygotes include the insula, orbitofrontal cortex, paracingulate gyrus, and subcallosal cortex (whole-brain cluster-corrected at $Z > 1.96$, $p < 0.05$). Results are from a psychophysiological interaction analysis using the right dorsal striatum (caudate) as an anatomically defined region of interest determined for each participant individually (see Materials and Methods). No regions were found to be more positively functionally connected with the caudate in the G-allele carriers as compared to the A-allele homozygotes. Coordinates are in MNI space, and the brain is displayed in radiological convention (left = right). MNI, Montreal Neurological Institute.

common alcoholic beverage (white wine) as opposed to the participants' preferred (Filbey et al., 2008a), or most commonly consumed (Filbey et al., 2008b) alcoholic beverage represents a limitation. Likewise, the use of water as opposed to an active control (e.g., litchi juice) may limit the findings. In conclusion, this study provides initial evidence for differential fronto-striatal functional connectivity patterns during alcohol-cue-exposure among OPRM1 G-allele carriers versus A-allele homozygotes. Additional studies that can replicate these results and further delineate the clinical and treatment implications of these findings hold great promise for bridging the gap between preclinical and clinical studies of addiction neurobiology.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Fig. S1. Results of the negative functional connectivity analysis for A-allele homozygotes only, indicating areas of negative correlation with the ventral striatum during the presentation of alcohol versus water cues (whole-brain cluster-corrected at $Z > 1.96$, $p < 0.05$).

Fig. S2. Results of the negative functional connectivity analysis for G-allele carriers only, indicating areas of negative correlation with the ventral striatum during the presentation of alcohol versus water cues (whole-brain cluster-corrected at $Z > 1.96$, $p < 0.05$).