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Neural response to alcohol taste cues in youth: effects of the *OPRM1* gene

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ABSTRACT

Genetic variations in the mu-opioid receptor (*OPRM1*) gene have been related to high sensitivity to rewarding effects of alcohol. The current study focuses on the neural circuitry underlying this phenomenon using an alcohol versus water taste-cue reactivity paradigm in a young sample at relatively early stages of alcohol use, thus limiting the confound of variations in duration of alcohol use. Drinkers (17–21 years old) were selected on genotype carrying the AA—($n = 20$) or the AG—($n = 16$) variant of the A118G single nucleotide polymorphism (SNP) of the *OPRM1* gene (rs1799971), and underwent functional magnetic resonance imaging (fMRI). Magnitude of the neural activity and frontostriatal functional connectivity in response to alcohol versus water were investigated. The AG-group demonstrated reduced activation in prefrontal and parietal regions, including the inferior and middle frontal gyrus, superior and inferior parietal lobule, compared with the AA-group. No activation differences were observed in the mesolimbic pathway. Connectivity from the ventral-striatum to frontal regions for alcohol > water trials was higher in the AG than the AA group. For the dorsal-striatum seed region, the AG group showed increased connectivity to non-PFC regions. These results indicate that adolescents carrying the G-allele may be more vulnerable for the alcohol to hijack the reward system in the absence of frontal control to regulate craving. This implies that findings of hyperactivation in the mesolimbic structures of G-allele carriers in earlier studies might result from both genetic susceptibility and heavy drinking.

Keywords Alcohol dependence, cue-reactivity, dorsal/ventral striatum, functional connectivity, imaging genetics, *OPRM1*.

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INTRODUCTION

Incentive sensitization towards drugs and drug-related stimuli develops due to neuroadaptations in the mesolimbic dopaminergic system controlling the incentive values assigned to drug stimuli (Berridge & Robinson 2003; Berridge *et al.* 2009). At early stages, drug use is goal-directed, and drug-taking behavior is promoted by the hedonic properties of drugs (associated with 'liking') in order to obtain pleasurable outcomes. In susceptible individuals, long-term drug use can produce changes in the brain, leading to incentive salience ('wanting'). Evidence from animal studies has shown that individual differences in the tendency to attribute incentive salience to drug-related stimuli is associated with vulnerability for the transition to compulsive drug seeking behavior (Flagel *et al.* 2009). In humans, genetic variants, which play a role in the brain reward circuitry, have been proposed as one factor contributing to the extent of incentive salience attribution (Blum *et al.* 2011).

A single-nucleotide polymorphism (SNP) located in the *OPRM1* gene of the mu opioid receptor (A118G) contributes to individual differences in sensitivity to the rewarding effects of alcohol. The A118G SNP is thought to increase receptor binding affinity for β-endorphin by threefold (Bond *et al.* 1998). Moreover, alcohol consumption induces opioid release (primarily β-endorphin) binding to the mu opioid receptors and leads to heightened dopamine levels in brain reward circuitry (Merrer 2009). Thus, G-allele carriers with higher binding affinity experience higher reinforcement from acute administration of alcohol. In experimental studies, heavy drinking G-allele carriers of the *OPRM1* gene demonstrated relatively strong automatic approach action-tendencies (Wiers *et al.* 2009), attentional bias towards alcohol-related stimuli (Pieters *et al.* 2011), alcohol craving (Van den Wildenberg *et al.* 2007) and stronger subjective feelings of intoxication, stimulation, sedation after alcohol as compared with participants homozygous for the A-allele (Ray & Hutchison 2004). This polymorphism is also associated with more potent striatal dopamine response to alcohol (Ramchandani *et al.* 2011) and stronger neural activity in the mesocorticolimbic pathway [i.e., ventral striatum, ventromedial prefrontal cortex (PFC) and orbitofrontal cortex (OFC)] to alcohol taste cues (Filbey *et al.* 2008b). Furthermore, activations in these regions were correlated with state measures of alcohol craving and with measures of drinking behavior and problems. Therefore, the A118G polymorphism may be associated with increased sensitivity towards the rewarding effects of alcohol, which in return is consistent with the role of the opioidergic system in the hedonic properties of alcohol as well as natural rewards (Robinson & Berridge 1993).

While incentive sensitization to alcohol-related cues strengthens due to acute rewarding properties of drugs on mesolimbic structures, control over drug use could also fail as a result of a weak frontal regulatory mechanism, either pre-dating or preceding chronic alcohol use (Gladwin *et al.* 2011; Volkow *et al.* 2004; Wiers *et al.* 2007). The current literature suggests the involvement of two interacting systems (limbic and frontal) in addiction and craving. Therefore, the role of dysregulation of frontostriatal circuitry in sustained drug-seeking behavior is a topic of increased interest (e.g., Feil *et al.* 2010). An additional mechanism involves the shift from ventral to dorsal striatal (VS/DS) activation to drug cues, which co-occurs with increasing habitual responses to alcohol (Everitt *et al.* 2008) and with the transition from alcohol use to abuse. A recent study in alcohol dependent adults reported *OPRM1* genotype involvement in the regulation of frontostriatal functional connectivity during an alcohol-taste cue paradigm (Ray *et al.* 2014). In this study, G-allele carriers showed stronger negative correlations between the VS/DS and the frontal regions interpreted as greater demand for cortical control over both ventral and dorsal striatal regions during processing of alcohol taste cues. Furthermore, in an alcohol dependent sample, disrupted frontostriatal connectivity predicted maladaptive drug-related behaviors and impairments in learning (Park *et al.* 2010). These studies show that frontal regulation of striatal activation towards rewarding effects of drugs and alcohol could play an important role in addiction.

In dependent and/or adult samples, alcohol use history (duration) and patterns (frequency and dose) vary drastically, potentially confounding the results and making it difficult to distinguish pre-existing neural predispositions from neural dysregulations induced by chronic use (Fernandez- Serrano *et al.* 2011). Therefore, studying young people enables us to compare responses towards alcohol cues at early stages of alcohol use without the confound of duration of use. Yet most of the cue reactivity studies on the *OPRM1* gene have been conducted in adult samples with a wide age-range (Courtney & Ray 2014 and Ray *et al.* 2014, age-range 21–51). Furthermore, these studies exclusively selected individuals based on their alcohol consumption severity (Filbey *et al.* 2008a; Van den Wildenberg *et al.* 2007; Wiers *et al.* 2009;) or dependence status (Courtney & Ray 2014; Ray *et al.* 2014). To our knowledge, studies in younger samples with limited age-range (permitting a more homogeneous group in terms of alcohol use profile) are largely lacking. To overcome this limitation, here we studied the neural circuitry involved in the processing of alcohol-taste-cues in a young sample at early stages of alcohol use, while comparing two groups with different genetic vulnerability for the acute reinforcing effects of alcohol.

We expected that G-allele carriers would be more sensitive to alcohol taste-cues than non-carriers. We studied both activation and connectivity measures. First, we studied regional activations in the reward circuitry, expecting increased responses in G-allele carriers. Second, we studied frontostriatal functional connectivity in processing alcohol taste cues in both groups. Functional connectivity analysis focused on *a priori* selected seed regions of NAc and dorsal caudate (VS/DS). We expected that the mu-opioid system would be uniquely involved in the brain circuitry associated with hedonic responses to drugs (NAc), thus hypothesizing that G-alleles would show an increased ventral-to-frontal connectivity. Finally, we investigated the relationship between in-scanner 'pleasantness/urge' ratings (reflecting 'liking/wanting' aspects of drug use) with neural responses during the alcohol taste-cue exposure in relation to the *OPRM1* gene.

MATERIALS AND METHODS

Participants

Thirty-six participants (17–21 year olds) were selected from a larger group of adolescents ($n = 145$), who participated in a study in which they were genotyped (information on the parent study can be found in *supplementary materials, and in* Korucuoglu *et al.* 2015). In the larger sample, only one participant was GG carrier and not included in this study. Groups were created in such a way to have AA and AG groups well-matched on demographics and drinking patterns so that the observed differences could be attributed to genetic variance alone. Because of technical problems with liquid administration, taste cue-reactivity task failed with five participants, these participants were replaced based on their demographics from the same pool. In the final sample, 20 participants were homozygous for the A-allele of the A118G SNP of the *OPRM1* gene (rs1799971), while 16 participants had the AG genotype. At the time of the fMRI study, our participants had 3–4 years of experience with alcohol, were in secondary education, scored an average of 7.5 on the AUDIT and had fairly stable drinking pattern for the last 2 years (Table 1), therefore they could be considered as being at an early stage of alcohol use. Participants were instructed to abstain from any alcohol for at least 24 hours and any legal or illegal drugs for at least 1 week (for exclusion criteria, see supplementary materials). The study was approved by the Ethics Committee of the Faculty of Social and Behavioral Sciences of the University of Amsterdam. For participants under the age of 18, parental consent was mandatory to take part in the study. A written informed consent was obtained from all participants prior to the experiment. Participants received financial compensation (€35) for their participation.

Genotyping

Saliva samples were collected using Oragene saliva collection kit (DNA Genotek, Inc., Ottawa, Ontario, Canada) for DNA analysis. Genotyping was performed with a Taqman assay (Life Technologies) on a LC480 lightcycler (Roche) at the Genetics core facility of the Academic Medical Center, the Netherlands. Sanger sequencing of five samples with the different genotypes was perform to confirm the genotypes of the Taqman assays. Duplicate genotyping was performed for five samples as a quality control, which showed 100% consistency. The allele frequencies did not violate Hardy-Weinberg Equilibrium ($X^2(1)_{HW} = 0.233$, $P = 0.63$).

Table 1 Demographic information, drug and alcohol use and urge-pleasantness ratings for the AA and AG groups of the *OPRM1* genotype.

Variable	*AA (n = 20)*	*AG (n = 16)*	*AA vs AG*
Age (mean, SD) (T4)	19.2(1.82)	18.81(1.72)	ns.
Sex (M/F)	10/10	13/3	–
Ethnicity (Caucasian/other)	20/0	13/3	–
DAQ (mean, SD)	36.5(11.58)	38.38(8.46)	ns.
PANAS—Positive affect(mean, SD)	28.2(7.35)	26.7(5.02)	ns.
PANAS—Negative affect(mean, SD)	12.5(2.21)	13.31(2.24)	ns.
AUDIT T1(last 90 days) (mean, SD)* ($n = 18,14$)	7.06(4.24)	7.5 (5.52)	ns.
AUDIT T2(last 90 days) (mean, SD)* ($n = 19,14$)	7.37(4.98)	6.93 (4.43)	ns.
AUDIT T3(last 90 days) (mean, SD)* ($n = 19,14$)	7.42(4.34)	6.62 (4.01)	ns.
AUDIT T4(last 90 days)—fMRI session(mean, SD)* ($n = 20,16$)	7.65(4.85)	7.56(4.11)	ns.
Age of first full drink (mean, SD)	15.1(1.62)	14.94(1.34)	ns.
Smoking? (Yes/No, frequency)	9/11, 11–20 times	9/7, 21–30 times	–
Drug use (last 90 days)			–
Marijuana (Yes/No, frequency)	6/14, < 10 times	9/7, 11–20 times	
Ecstasy (Yes/No, frequency)	2/18, < 10 times	4/12, < 10 times	
Volatile substances (Yes/No, frequency)	2/18, < 10 times	0/16	
In-scanner urge and pleasantness ratings			
Alcohol taste pleasantness	4.58(1.8)	4.97(1.55)	ns.
Water taste pleasantness	5.34(1.92)	4.9(1.94)	ns.
Alcohol taste urge	5(1.68)	5.5(1.69)	ns.
Water taste urge	5.36(1.78)	4.91(1.7)	ns.

*In this study participants were selected from a pool of subjects ($n = 145$), who took part in a larger study in which they were genotyped (Time 1, T1). Participants filled out AUDIT questionnaire once again, 3, and 6 months after the inclusion to the study (T2 and T3, respectively). The fMRI session (T4) took place approximately 1 to 2 years after T1. AUDIT = Alcohol Use Disorder Identification Test; DAQ = Desire for alcohol Questionnaire; F = female; M = male; PANAS = Positive and Negative Affect Scale; SD = standard error.

Procedure

Upon arrival, participants filled out questionnaires (see *supplementary information*). Participants first completed a behavioral testing session, where they completed an Electromyogram (EMG) measurement and performed two unrelated tasks, followed by an fMRI session. A minority of participants performed their behavioral session last due to scheduling related problems (six participants performed one of the behavioral tasks last and two participants both behavioral tasks). In the scanner participants performed two tasks, of which the second one was the taste cue-reactivity task. Before and after the scanning session, participants received a sample of the experimental taste stimuli (alcohol and water) and rated the pleasantness on a 10-point scale.

Cue reactivity task with tastes

A blocked-design taste-cue paradigm was adapted from Filbey *et al.* (2008a, 2008b). The task consisted of 16 mini blocks during which either an alcohol-containing beverage or a control taste was delivered (eight alcohol and eight control blocks). Each block comprised of two taste-delivery periods of 10 seconds, in which 1 ml liquid was administered, each followed by a swallowing period of 2 seconds. During the taste and swallowing periods, participants were presented with visual instructions of 'Taste' and 'Swallow' (Fig. 1). Vodka-apple pre-mixed spirit (Smirnoff, commercial ready-to-drink alcohol beverage with a 6.4% Vol) was used as alcoholic taste and distilled water was used as control taste. Note that, contrary to some of the previous alcohol-taste cue studies (Filbey *et al.* 2008a, 2008b), in which subjects received their preferred alcoholic beverage, in the current study a standard alcoholic beverage was administered to all participants. At the time of the experiment, participants under the age of 18 were allowed to consume only alcohol containing drinks with a relatively low percentage of alcohol, including pre-mixed spirits (alcopops), but not spirits straight or in a personal mix. Given that the administration of their preferred beverage would not be possible for all participants, a standard beverage type was used. Taste stimuli were delivered via a plastic tube attached to an electronic syringe pump positioned in the scanner control room, using a computer-controlled delivery system running under E-prime2 (Psychology Software Tools, Inc., Sharpsburg, PA). Each taste was equally presented across blocks and randomized with the restriction that two consecutive blocks would be of the same type. The block was completed with a rest period of 16 seconds followed by taste ratings for *pleasantness* and *urge* in that order (with a maximum duration of 5 seconds). Participants rated the tastes on a 1–10 Likert scale (1: no urge, 10; very high urge) via an MRI compatible optic response device (fORP) with a four-button paddle. For the taste ratings, a two-step response procedure was used: two buttons were assigned to move the centered anchor to the left or the right, a separate button was assigned for confirming the choice when the anchor is on the desired point of the scale. The start of the next block was informed via a 'Ready?' warning on the screen (2 seconds).

Image acquisition

Functional and anatomical images were acquired on a Philips 3 Tesla Achieva TX MRI scanner with a 32-channel SENSE head coil, at the Spinoza Center, Amsterdam, the Netherlands. A structural T1-weighted echo planar image was acquired with the following parameters: voxel size of $1 \times 1 \times 1$ mm, FOV = 240×188, TR = 8.17 ms, TE = 3.8 ms, flip angle = 8°, slice thickness = 1 mm, 0 mm gap, matrix = 240×240, and 220 slices per volume, with a total scan duration of ~6 minutes. Functional T2*-weighted images were acquired with a single-shot gradient echo EPI sequence. The following parameters were used for the functional scans: FOV = 240×240, voxel size of $3 \times 3 \times 3$ mm, 420 volumes, TR = 2000 ms, TE = 27.63 ms, matrix size = 80×80, flip angle of 76.1°, 37 slices per volume, slice gap 0.3 mm, and slice thickness = 3 mm, sensitivity encoding factor of 2. Stimuli were projected on a projection screen, which the participants viewed through a tilted mirror attached to the head coil.

Image processing and statistical analyses

Magnetic resonance imaging data were analyzed using statistical parametric mapping (SPM8, Wellcome

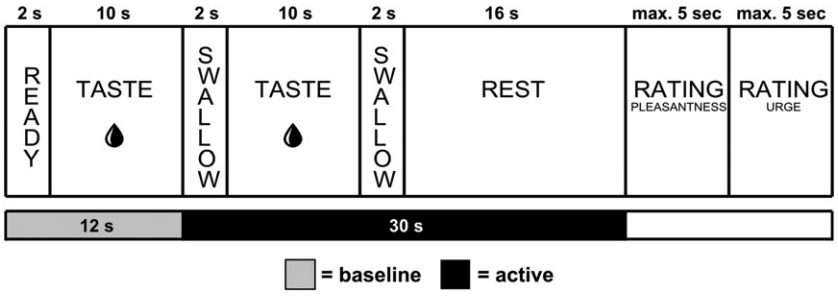

Figure 1 Schematic representation of the alcohol-taste cue reactivity task

Department of Cognitive Neurology, London, UK) implemented in Matlab 7.11. Preprocessing steps included motion correction using rigid body transformations, coregistration to the anatomical scans, spatial normalization to a T1 template based on Montreal Neurological Institute (MNI) stereotaxic space, spatial smoothing (8 mm full width—half maximum) and high pass filtering with a cutoff period of 128 s.

Specifics of the fMRI analysis (the events modeled, the contrast selected etc.) were based on previous studies (Filbey *et al.* 2008a; Ray *et al.* 2014) and were as follows: For the first-level analysis, hemodynamic response function was convolved with the time course of the blocked design. Realignment parameters were used as model regressors. Alcohol-cue and water taste-cue exposure were modeled as separate event types. Each event included the period starting from the instructions for the first swallow until the end of the rest, depicted as active period in Fig. 1. Fixation, first taste delivery, urge and pleasantness rating periods were not modeled (Filbey *et al.* 2008a). To verify main effects of the task, a whole brain analysis was conducted on the contrast of Alcohol > Water taste delivery for all participants with a threshold of $P = 0.05$ (FWE), 10 voxels. Group analysis was conducted on Alcohol > Control contrast image as well. Given that the influence of a single SNP on brain responses is usually modest, the statistical threshold for group comparison contrasts were set to $P < 0.005$, with a minimum cluster size of 20. This threshold produces a desirable balance between Type-I and Type-II errors (Lieberman & Cunningham 2009). A separate regression analysis was conducted to investigate genotype effects on the relationship between in-scanner *pleasantness* and *urge* ratings within the limbic clusters identified with a whole-brain analysis on the full sample. First, for the behavioral data, a contrast score was calculated by subtracting the mean rating for the water from the mean rating for the alcohol taste for in-scanner pleasantness and urge ratings, separately (i.e., Contrast score for urge rating = Urge rating alcohol—Urge rating water). Following that, contrast scores for *pleasantness* and *urge* ratings were centered by subtracting the overall mean score from each participant's rating score. Inspection of contrast scores for *urge* and *pleasantness* ratings revealed a strong correlation ($r = 0.95$, $P < 0.001$). Note that there was a significant positive correlation between *pleasantness* and *urge* ratings for each liquid type as well ($r_{\text{urge-pleasantness for water}} = 0.95$, $P < 0.001$ and $r_{\text{urge-pleasantness for alcohol}} = 0.93$, $P < 0.001$). Given that the contrast scores for the *pleasantness* and *urge* ratings were highly correlated, first a principle component analysis (PCA) method was applied in order to reduce two correlated variables into one factor. Subsequently, the PCA factor was used in the regression model to predict neural pattern of activation commonly relating to both scales.

Functional connectivity was assessed using psychophysiological interactions (PPI) analysis (Friston *et al.* 1997). The aim of a PPI analysis is to detect regions whose activity is coupled with the activity of a seed region over the time course of the alcohol taste blocks, but not during the water blocks. The regions of interest for the PPI analysis were based on previous research (Ray *et al.* 2014) and included the following regions: (1) the right NAc and (2) the right dorsal caudate, to investigate connectivity between the ventral/dorsal striatum (VS/DS) and the PFC. A mask image for the right NAc and caudate were acquired from the IBASPM 71 anatomical atlas toolbox (Alemán-Gómez *et al.* 2006). The tail of the caudate mask (ventral part) was excluded using an in-house package programmed in Matlab (for masks, see Fig. S1, supplementary materials). The mean deconvolved time courses in these seed regions were extracted from the preprocessed individual images. Regressors were created by multiplying extracted time courses of ROIs with condition specific regressors. The PPI analysis was conducted for each individual separately and then entered into a random-effects analysis using a one sample *t*-test. Between-group analysis was conducted using a two-sample *t*-test with the thresholds described earlier. Anatomical labeling was based on the AAL atlas (Tzourio-Mazoyer *et al.* 2002) with the SPM probabilistic toolbox (Eickhoff *et al.* (2005) and the Hiro software (Gladwin & Vink 2008). When the effect of task condition on the activity of the seed region increases, increases and decreases in activity in the other regions represent the positive and negative connectivity, respectively.

RESULTS

Participant characteristics

Allele groups were not different in any of the demographical or substance use characteristics ($P > 0.1$, see Table 1).

In-scanner urge and pleasantness ratings

In-scanner urge and pleasantness ratings and response times are shown in Fig. 2. Urge and pleasantness rating scores and reaction times during fMRI scanning were subjected to a repeated-measures ANOVA (RM-ANOVA) with *Taste* (alcohol and water) as within-subjects factors and *Group* (AA and AG) as between-subjects factor. Analysis of ratings scores and reaction times revealed no main or interaction effects for Group.

Pleasantness rating pre-scanning and post-scanning

Pleasantness ratings before/after the scanning session were analyzed with a RM-ANOVA, with *Time*

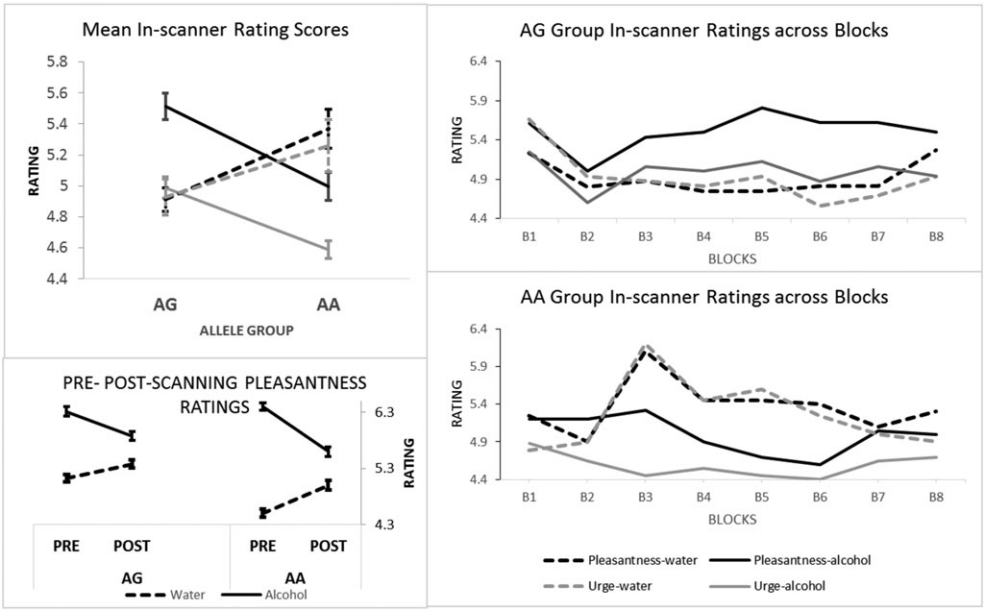

Figure 2 Mean scores for the in-scanner pleasantness and urge ratings (upper-left) and pre-scanning post-scanning pleasantness ratings (lower-left) for the alcohol and control tastes and for the AG and AA alleles of the *OPRM1* gene. In-scanner pleasantness and urge ratings across blocks of the taste-cue-reactivity task are also presented separately for the allele group (right side)

(pre-scanning and post-scanning) and *Taste* (alcohol and water) as within-subject variables and *Group* (AA and AG) as between-subject variable. No group differences were observed. Overall, participants liked alcohol more than water (a main effect of *Taste*; $F(1, 34) = 5.733$, $P = 0.022$, $\eta_p^2 = 0.14$). An interaction effect of *Time* by *Taste* was observed ($F(1, 34) = 5.49$, $P = 0.025$, $\eta_p^2 = 0.14$). This two-way interaction was inspected by separately examining the effect of *Time* on each *Taste*. Results revealed that compared with pre-scanning, participants rated alcohol less pleasant during post-scanning ($F(1, 34) = 5.31$, $P = 0.027$, $\eta_p^2 = 0.14$). Pre-scanning and post-scanning ratings for water were the same. Lastly, during pre-scans, participants rated alcohol as more pleasurable than water ($F(1, 34) = 12.41$, $P = 0.001$, $\eta_p^2 = 0.27$).

Whole-brain analysis alcohol > water contrast

The whole brain analysis in the full sample revealed activation in several regions over the frontal, parietal, temporal and limbic regions. The alcohol-taste versus water-taste cues elicited activation in the thalamus, inferior frontal gyrus (IFG), superior temporal gyrus and caudate (Fig. 3a and Table 2). No deactivations were observed. Comparison between genotypes (AA > AG) revealed that AA-carriers showed higher activation over the frontal and parietal areas; including middle and IFG, angular gyrus, superior and IFG; compared with G-allele carriers (Fig. 3b and Table 3). The G-allele carriers (AG > AA) revealed higher activation in the hippocampus.

Functional connectivity

For the VS seed region, relative to the AA group G-allele carriers exhibited stronger alcohol-taste cue related connectivity with middle and superior frontal gyrus, parahippocampal and motor cortex, as well as caudate and insula (Fig. 4a and Table 4). For the DS seed region, the G-allele carriers revealed stronger connectivity with hippocampal, thalamic, precuneus and occipital regions, however, no connectivity was observed with frontal regions (Fig. 4b and Table 4). There was no significant increased connectivity across the brain in the AA group versus the AG group.

Neural correlates of pleasantness and urge ratings

Given that the difference scores for the *urge* and *pleasantness* ratings correlated significantly, we reported the neural activity across genotypes during the alcohol > water contrast that has been predicted by the combined urge and pleasantness PCA factor. The strength of the connectivity from the VS to the frontal regions (inferior and superior frontal regions) positively correlated with the PCA factor of urge/pleasantness ratings in G-carriers compared with A-carriers. Moreover, G-carriers compared with A-carriers also demonstrated a positive correlation with the level of DS-to-frontal connectivity (to inferior frontal cortex) and the PCA factor (Table 5 for the full list).

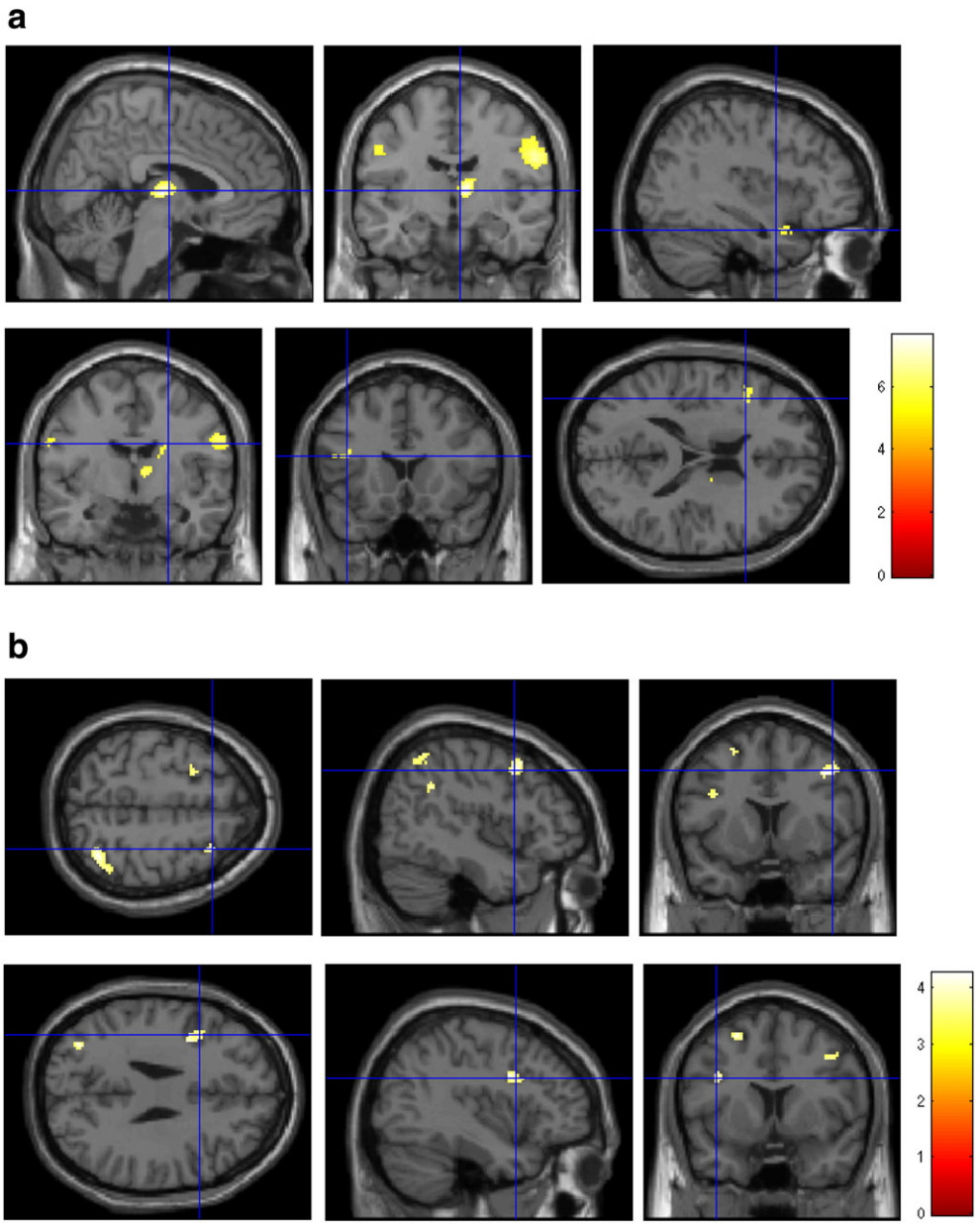

Figure 3 (a) Significant areas of activation for the Alcohol > Control Taste contrast (FWE, $P < 0.05$, $k \geq 10$ voxels); top-row left to right; thalamus (sagittal and coronal view), and temporal pole; bottom-row left to right; caudate and inferior frontal gyrus (coronal and transverse). (b) Regions showing greater activation for the AA carriers of the *OPRM1* genotype compared with AG carriers (AA > AG contrast, $P < 0.005$, uncorrected, $k \geq 20$ voxels); top-row; middle frontal gyrus (transverse, sagittal and coronal view); bottom-row; inferior frontal gyrus (transverse, sagittal and coronal view)

DISCUSSION

The main aim of this study was to assess differences in neural activity and frontostriatal functional connectivity during an alcohol-taste cue paradigm between the *OPRM1* AG-genotypes and AA-genotypes in a sample of young individuals (17–21 year olds) at the early stage of their drinking career. Main findings can be summarized as follows: concerning brain activations across genetic groups, G-allele carriers of the *OPRM1* gene demonstrated reduced activation by alcohol in the prefrontal and parietal regions, including the inferior and middle frontal gyrus, superior and inferior parietal lobule, compared with A-allele homozygotes. Contrary to our expectations, no activation differences were observed in the mesolimbic reward pathway between the A-allele homozygotes and G-allele carriers. Concerning connectivity, we observed that the coupling from the VS seed region to the frontal regions (middle—including dorsolateral prefrontal cortex (DLPFC)-and superior frontal gyrus) after alcohol tasting

Table 2 Significant areas of activation for the Alcohol > Control Taste contrast (Whole-brain analysis, FWE, $P < 0.05$, k ≥ 10 voxels).

Region	*Hemisphere*	*Cluster size (in voxels)*	*Peak value*	*MNI coordinates x,y,z*
Thalamus	R	298	7.63	8,–10, 6*
			6.64	14,–20, 4*
Superior temporal gyrus	L	60	6.46	−48,–26, 8*
Inferior frontal gyrus	L	26	6.13	−40, 18, 18*
Caudate	R	16	6.08	22,–4, 24^
Temporal pole	R	23	5.99	38, 12,–22*
Postcentral gyrus	R	407	6.75	60,–8, 30*
			6.45	66,–14, 34*
			6.22	48,–16, 38*
	L	85	6.07	−44,–16, 34*
			5.9	−52,–12, 36*
			5.53	−44,–20, 42*
	L	17	5.84	−58,–4, 28*

*(Eickhoff *et al.* (2005) and the Hiro software^ (Gladwin & Vink 2008). L = left; MNI = Montreal Neurological Institute; R = right. Anatomical labeling was based on the AAL atlas (Tzourio-Mazoyer *et al.* 2002) with the SPM probabilistic toolbox.

Table 3 Whole-brain group comparison by *OPRM1* polymorphism genotype for the alcohol > control taste contrast ($P < 0.005$, uncorrected, k ≥ 20 voxels).

Region	*Hemisphere*	*Cluster size (in voxels)*	*Peak value*	*MNI coordinates x,y,z*
		AG > AA		
Hippocampus/Heschl	R	36	3.08	28–40 16^*
		AA > AG		
Middle occipital gyrus	L	67	3.49	−32,–74, 32*
Middle frontal gyrus	R	132	3.46	44, 10, 44*
	L	35	3.32	−24, 8, 60*
	R	35	3.26	30, 20, 56*
Superior parietal lobule	R	260	3.47	36,–60, 56*
Inferior parietal lobule	R		3.04	44,–54, 52*
Angular gyrus	R		3.46	38,–64, 48*
Angular gyrus	R	23	3.14	46,–48, 32*
Precentral gyrus	L	45	3.35	−38, 4, 30*
Inferior frontal gyrus	L		2.99	−40, 12, 28*

*(Eickhoff *et al.* (2005) and the Hiro software^ (Gladwin & Vink 2008). L = left, MNI = Montreal Neurological Institute; R = right. Anatomical labeling was based on the AAL atlas (Tzourio-Mazoyer *et al.* 2002) with the SPM probabilistic toolbox.

(compared with water) was higher in G-allele carriers than in AA carriers. For the DS seed region, the AG group showed increased connectivity to non-PFC regions.

Our results did not confirm allele differences in the mesolimbic reward pathway across A and G alleles of the *OPRM1* and deviate from the findings of Filbey and colleagues study (Filbey *et al.* 2008b). The discrepancies between the previous and the current study should be considered in the context of sample and methodological differences. Filbey and colleagues tested the neural responses to alcohol taste cues in more senior heavy drinking A and G-carriers by using subjects' preferred drink. Associations have been reported between neural response to preferred alcohol taste cues and factors like years of alcohol exposure and severity of alcohol use, especially for the DLPFC, NAc and OFC activity (Claus *et al.* 2011). Therefore, earlier findings of increased reward-related neural response to alcohol taste cues could be explained by greater level of conditioning to a particular drink and associated neuroadaptations as a result of chronic alcohol use in older heavier drinking individuals. In line with this interpretation, in studies using other physiological measures of cue reactivity, a gradual reduction in the magnitude of response to alcohol cues has been observed for beverages more different than the preferred alcoholic drink and subjects elicited the largest response to the most commonly consumed beverage (Staiger & White 1991). Also imaging studies showed that reward-related brain responses scale with behavioral preference (O'Doherty *et al.*, 2006), and with the

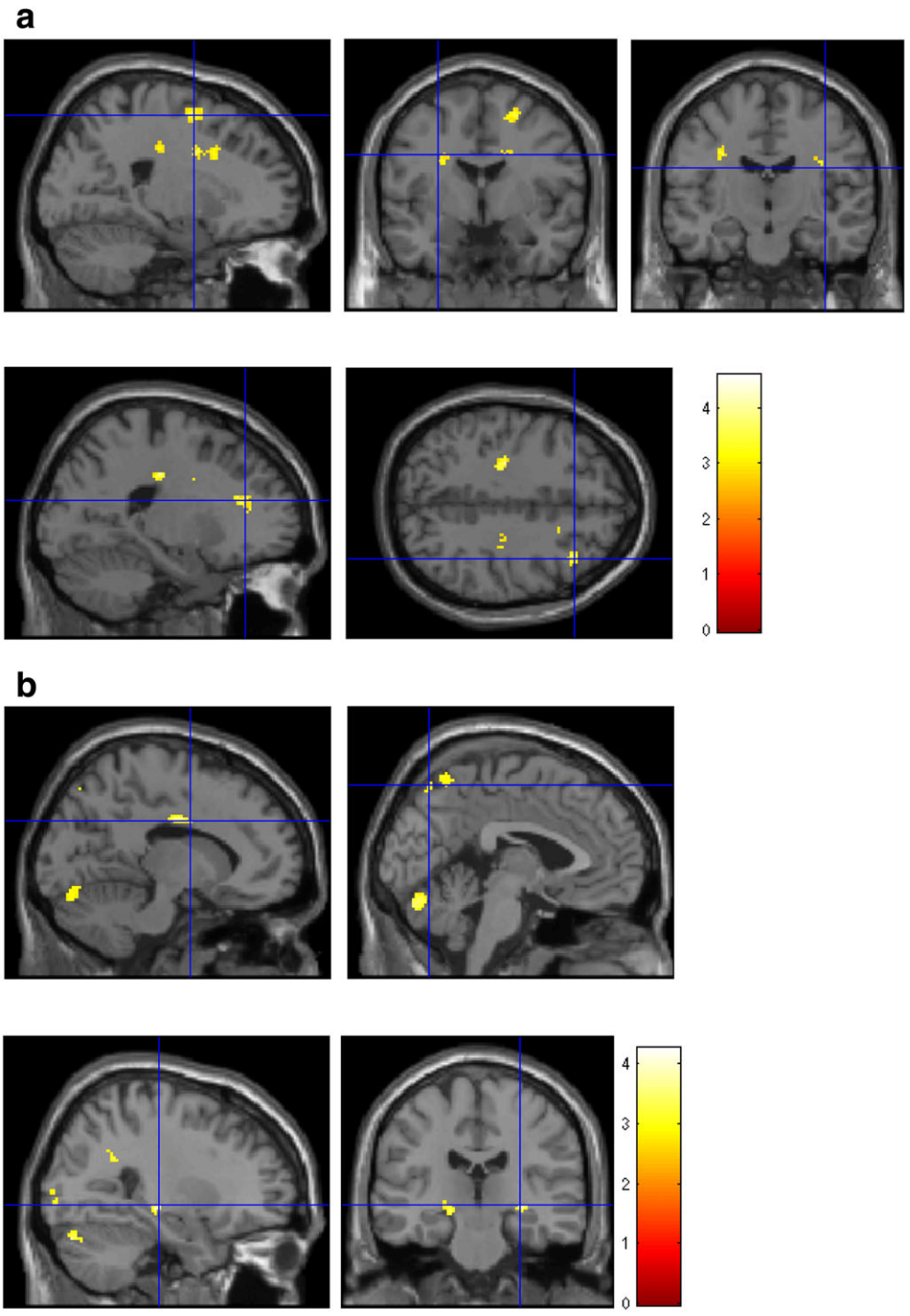

Figure 4 Regions showing greater positive functional connectivity for the AG > AA carriers of the *OPRM1* polymorphism with seed regions (a) the ventral (NAc) (top-row: superior frontal gyrus, caudate and insula; bottom-row: middle frontal gyrus) and (b) the dorsal striatum (caudate); (top-row: middle cingulate, precuneus; bottom-row: thalamus/hippocampus) ($P < 0.005$, uncorrected, $k \geq 20$ voxels)

degree to which subjects finds the stimuli pleasing and rewarding (Aharon *et al.* 2001; Knutson *et al.* 2001). However, some research findings contradict with this interpretation, for instance Berns *et al.* (2001) found that predictability but not preference effects the recruitment of NAc activity, yet in this study non-alcoholic beverages were administered. Two other studies (Courtney & Ray 2014; Ray *et al.* 2014), which administered a standard alcoholic beverage (white wine) instead of subjects' preferred one, also failed to replicate some of the activation differences in the mesolimbic pathway across alleles reported in the Filbey's study. All in all, recruitment of reward-related brain responses in alcohol cue reactivity paradigms might involve long-term

Table 4 Regions showing greater positive functional connectivity with the ventral and dorsal striatum for the AG > AA carriers of the *OPRM1* polymorphism genotype for the alcohol > control taste contrast ($P < 0.005$, uncorrected, k ≥ 20 voxels).

Region	*Hemisphere*	*Cluster size (in voxels)*	*Peak value*	*MNI coordinates x,y,z*
		Ventral Striatum seed region (NAc)		
Precentral	L	166	4.59	−28,–20, 34^
			3.5	−32,–12, 34^
	R	128	3.8	30,–22, 34^
Precentral/insula	R		3.22	34,–14, 28^
Postcentral	R	21	3.29	46,–34, 64*
	L	25	3.14	−40,–42, 66*
Middle frontal gyrus(BA9)	R	44	4.1	38, 26, 40*
	L	78	3.6	−20, 30, 22^
			3.24	−20, 38, 14^
			2.82	−28, 38, 20^
Superior frontal gyrus	R	77	3.54	22, 0, 58*
Mid cingulum	R	133	3.51	20, 12, 32^
			3.46	22, 4, 36^
			2.94	18, 16, 40^
Caudate	L	28	3.48	−24, 0, 26^
Parahippocampal	L	25	3.44	−34,–46,−2^
		Dorsal Striatum seed region (Dorsal Caudate)		
Hippocampus	R	39	4.26	28,−24,−6^
Hippocampus/thalamus	R		2.98	22,−24, 0*
Hippocampus/thalamus	L	51	3.49	−18,−24,−6*
Cerebellum	R	972	3.97	34,−78,−26*
			3.85	22,−80,−22*
			3.69	12,−82,−16*
Inferior orbital gyrus	R	36	3.73	38, 20,−20^
Mid cingulate cortex	L	79	3. 69	−12,−12, 34^
	L		2.92	−18,−16, 38^
	L		2.75	−8,−2, 30^
	R	29	3.47	18,−4, 36^
Inferior frontal tri	R	30	3.65	38, 22, 20^
Sup parietal lobule	R	201	3.56	24,−72, 54*
Precuneus	R		3.37	14,−74, 48*
	L		3.14	−2,−72, 56*
	R	163	3.42	4,−48, 64*
	R		3.1	2,−58, 60*
	R	25	3.14	28,−52, 26^
Angular gyrus/precuneus	R		2.95	38,−58, 26*^
Inferior occipital gyrus	L	74	3.29	−44,−76,−10*
	L		3.16	−42,−84,−8*
Fusiform gyrus	L		2.89	−42,−64,−16*
Fusiform gyrus	L	29	3.15	−42,−44,−22*
Middle occipital gyrus	R	29	3.21	34,−88, 12*
	R		2.99	40,−84, 8*

*(Eickhoff *et al.* (2005) and the Hiro software^ (Gladwin & Vink 2008). L = left, MNI = Montreal Neurological Institute; NAc = Nucleus Accumbens; R = right. Anatomical labeling was based on the AAL atlas (Tzourio-Mazoyer *et al.* 2002) with the SPM probabilistic toolbox.

neuroadaptations for preferred alcoholic beverages shaped by prior experience, which is consistent with the conditioning interpretation.

In the current study, activation differences across allele groups were more pronounced for the frontal structures; G-allele carriers showed reduced frontal activation compared with A-carriers. Both increases and decreases in the PFC activation have been implicated in the literature, albeit with distinct functional roles. Higher activation in OFC and DLPFC to alcohol cues has been observed in non-treatment seeking drug users but was lacking in treatment seeking drug users, which has been associated with context-dependent processing, for example, related to the actual availability of drugs (Wilson *et al.* 2004). Prefrontal activation could reflect the cue-evoked activation of expectancy of drug-related

Table 5 Regions correlated with the real-time pleasantness and urge ratings for the contrast AG > AA carriers of the *OPRM1* polymorphism genotype ($P < 0.005$, uncorrected, k ≥ 20 voxels).

Region	*Hemisphere*	*Cluster size (in voxels)*	*Peak value*	*MNI coordinates x,y,z*
PPI analysis—Ventral striatum seed region (NAc)				
Negative correlations for pleasantness and urge ratings (principle component)—AG > AA				
Superior occipital G	L	374	4.21	−8,−96,10*
Cuneus	L		4	−6,−92,20*
Superior occipital G	L		3.59	−20,−90,22*
Inferior frontal G. (BA47)	R	43	3.72	48,44,−10*
Inferior frontal G.	R		3.25	48,36,−12*
Superior orbital G.	R	33	3.52	34,60,−4*
Superior frontal G. (BA10)	R		2.92	30,66,2*
Insula	L	26	3.48	−26,−6,20^
Superior occipital G	R	153	3.40	22,−92,8*
Calcarine gyrus/cuneus	R		3.16	16,−88,12*^
Middle occipital G.	R		3.05	32,−84,8*
ACC/caudate	L	62	3.3	−8,14,18^
ACC	L		2.99	−10,22,20^
Frontal superior medial G. (BA8)	L	35	3.18	−2,28,46^
Positive correlations for pleasantness and urge ratings (principle component)—AG > AA				
Amygdala	R	23	3.32	22,2,−18*
Postcentral gyrus	R	36	3.29	30,−44,64*
	R		2.85	38,−42,64*
PPI analysis—dorsal striatum seed region (dorsal caudate)				
Negative correlations for pleasantness and urge ratings (principle component)—AG > AA				
Inferior frontal G.	L	33	3.36	−48,24,16*
Positive correlations for pleasantness and urge ratings (principle component)—AG > AA				
Amygdala	R	118	3.76	26, 0,−16*
Hippocampus	R		3.67	32,−8,−18*
Temporal pole	R		2.87	32, 8,−22*
Hippocampus	L	33	3.55	−12,−16,−12^
Putamen	R	26	3.3	20, 10, 6*

*(Eickhoff *et al.* (2005) and the Hiro software^ (Gladwin & Vink 2008). ACC = Anterior cingulate cortex; G = gyrus; L = left; MNI = Montreal Neurological Institute; NAc = Nucleus Accumbens; R = right. Anatomical labeling was based on the AAL atlas (Tzourio-Mazoyer *et al.* 2002) with the SPM probabilistic toolbox.

reinforcement and planning to acquire drugs (Wilson *et al.* 2004). Increased cue-induced activations in frontal regions have previously been found in emotion regulation areas (e.g., DLPFC) and has been associated with the regulation of craving and decreases in craving (Kober *et al.* 2010). Inferior and middle frontal cortex are part of the emotion network that has been documented in earlier reviews of emotion regulation (Quirk & Beer 2006). Thus, OFC and DLPFC may be associated with processes that are involved with problematic responses to drug cues as well as more healthy regulatory control, depending on other psychological factors. Given that G-allele carriers are more vulnerable to hazardous drinking, their reduced frontal activation may reflect a lack of regulatory responding. If this is the case, in the long run, G-allele carriers may be more vulnerable for the alcohol and drugs to hijack the reward system. Hence, G-allele carriers may be more prone to rapidly acquire incentive salience of alcohol cues with increasing alcohol use, also due to a decreased regulatory behavior to monitor or to control alcohol use.

A recent study in alcohol-dependent adults reported *OPRM1* genotype involvement in the regulation of frontostriatal functional connectivity during an alcohol-taste cue paradigm (Ray *et al.* 2014). Specifically, this study in alcohol-dependent participants revealed negative frontostriatal connectivity in G-allele carriers, both for the ventral and the dorsal part of the striatum (Ray *et al.* 2014). Negative directionality of this connectivity suggests that heavy drinking G-allele carriers required inhibitory frontal control over both ventral and dorsal striatum during processing of alcohol taste cues. Contrary to earlier findings, in the current study with late adolescents and early adults, allele differences were specific to frontostriatal connectivity from the VS seed region only in G-allele carriers, but a greater connectivity of the DS with frontal structures was absent. The positive connectivity of the PPI analysis in the present study could

be due to the dominance of bottom-up feedback system in late adolescents in general (Gladwin *et al.* 2011). Previous findings of increased frontal to dorsal connectivity in heavy drinking adult samples might indicate increased need for frontal control of reward-related striatal signals because of neuroadaptations or cognitive impairments that took place in long term users (Ray *et al.* 2014). Alternatively, the negative connectivity observed in the previous study with an adult sample may be related to the recruitment procedure: individuals reporting alcohol problems, which might result in the context-dependent processing discussed earlier (Wilson *et al.* 2004).

Lastly, we tested whether observed differences between the genotypes in the activation and connectivity patterns would be related with observed differences in the real-time pleasantness and urge ratings. Regarding in-scanner urge and pleasantness ratings, two points are particularly worth noticing here. Earlier reviews stated that in the initial phases of drug use, wanting and liking are closely linked to each other. With repetitive use, liking behavior can either be stable or decrease, while 'wanting' increases with progression of alcohol and drug use (Berridge & Robinson 2003). In the current sample, in-scanner pleasantness and urge ratings were highly correlated, and therefore we looked at brain regions showing correlation with the variable accounting for the variance common to both rating scales. Interestingly, correlations of in-scanner pleasantness and urge ratings with connectivity from the striatum highlighted two frontal regions: inferior and superior frontal gyrus. Changes in coupling from the VS and DS to the IFG were correlated with both urge and pleasantness ratings. The IFG has been involved in successful inhibition and regulation of emotions (Shafritz *et al.* 2006). The correlation of urge and pleasantness with striatum connectivity to the superior frontal gyrus was specific to the VS seed region. The superior PFC has been associated with modulating craving reactivity in tobacco addiction (Rose *et al.* 2011). In sum, observed correlations between pleasure and urge ratings and frontostriatal connectivity patterns are in line with the idea of a conceptual and neural overlap between liking and wanting behavior in initial phases of drug use.

Some limitations of the current study need mentioning. It is important to note that although at pre-scanning participants rated the alcohol taste more pleasant than water. At post-scanning, there was a small decrease in pleasantness ratings of alcohol taste outside the scanner, this might have had an effect on the activation pattern. Moreover, the present study consists of a relatively small sample size. Future studies with a larger sample can aim to examine the association between brain responses to alcohol cues and measures of drinking history. However, we focused on a priori hypotheses based on earlier findings of imaging genetic studies and tested this in a sample with limited age range, which may (partly) compensate for this limitation. Another consideration is that the risk group in our study included only AG carriers which might have limited our power to detect small effects; stronger effects might have been observed with the inclusion of GG carriers. Despite these limitations, however, this is the first study testing the neural responses to taste cues in real-time in a genetically selected young group without excessive drinking histories.

In this imaging genetics study, we found that young individuals carrying the *OPRM1* G-allele genotype revealed lower activation in frontal regions compared with AA carriers in a taste cue paradigm. Functional connectivity analysis revealed that G-allele carriers had more dominant input from VS to frontal regions compared with A-allele homozygotes, which could be related to the observed lower PFC activity. Thereby, the present study provides various findings that may provide novel insights and new directions for the future studies. The role of *OPRM1* gene on the acquisition of alcohol addiction could be studied from a broader perspective, in different age groups and as a function of drinking profiles. In a recent review, it has been emphasized that besides its role in rewarding effects of alcohol, mu opioid receptors play a role in many other mechanisms; such as social reward, response inhibition and decision making processes (Lutz & Kieffer 2013). As a dysfunction in these processes contribute to the development of addiction, it may also be the case that such dysregulations might be attenuated in the G-carriers (Mitchell *et al.* 2007). If such causal links can be established, cognitive enhancers can be used in early stages of alcohol use for the vulnerable groups. Moreover, earlier studies showed that young adult carriers of the *OPRM1* G-allele have stronger approach tendencies towards alcohol-related cues (Wiers *et al.* 2009). Given that this approach bias for alcohol appears to be reversible through training (Wiers *et al.* 2011; C.E. Wiers *et al.* 2015), the *OPRM1* gene carriers could be a target group.

In conclusion, these results indicate that previous findings of hyperactivity in mesocorticolimbic structures observed in G-allele carriers of the *OPRM1* gene may result not only from genetic susceptibility but also from excessive alcohol use. In G-allele carrying adolescents without extensive alcohol use, the present study observed reduced prefrontal and parietal activations to alcohol taste-cues, together with increased VS to frontal coupling, which may constitute a mechanism of vulnerability that could be targeted in treatment.

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Authors Contribution

OK, TEG, and RWW were responsible for the study concept and the design of the study. OK performed the imaging data acquisition and data analysis. TEG assisted with the analysis of the imaging data, TEG and RWW contributed to the interpretation of the data and to the drafting of the manuscript. FB supervised genotyping of the data and contributed to parts of the manuscript. HGR, PFCG and RJTM contributed to the fMRI design. FB, HGR, PFCG and RJTM helped with the evaluation and the editing of the manuscript. RWW obtained funding for the study. All authors critically reviewed the content and approved the final version of the manuscript.

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