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Modulation of orbitofrontal-striatal reward activity by dopaminergic functional polymorphisms contributes to a predisposition to alcohol misuse in early adolescence

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Abstract

Background: Abnormalities in reward circuit function are considered a core feature of addiction. Yet, it is still largely unknown whether these abnormalities stem from chronic drug use, a genetic predisposition, or both. **Methods**: In the present study, we investigated this issue using a large sample of adolescent children by applying structural equation modeling to examine the effects of several dopaminergic polymorphisms of the D1 and D2 receptor type on the reward function of the ventral striatum and orbital frontal cortex, and whether this relationship predicted the propensity to engage in early alcohol misuse behaviours at 14 years of age and again at 16 years of age. **Results**: The results demonstrated a regional specificity with which the functional polymorphism rs686 of the DRD1 gene and Taq1A of the ANKK1 gene influenced medial and lateral orbital frontal cortex activation during reward anticipation, respectively. Importantly, our path model revealed a significant indirect relationship between the rs686 of the DRD1 gene and early onset of alcohol misuse through a medial orbital frontal cortex and the ventral striatum interaction. **Conclusions**: These findings highlight the role of D1 and D2 in adjusting reward-related activations within the mesocorticolimbic circuitry, as well as in the susceptibility to early onset of alcohol misuse.

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Keywords: adolescence; dopamine D1/D2 receptor; ventral striatum; orbital frontal cortex; reward; addiction;

Introduction

More than a decade of neuroimaging studies point towards functional abnormalities of the mesocorticolimbic reward system in substance use disorders (Redish et al., 2008, Volkow et al., 2009). Overall, the data imply that chronic drug use can lead to increased neuronal activation in response to drug-associated cues, and reduced response to natural rewards, a maladaptive process thought to facilitate the progression towards excessive drug choice (Volkow et al., 2009). While much attention in the field has focused on identifying addiction-related endophenotypes contributing to pre-existing abnormalities in reward circuit function, the search has been plagued by questions of causality: Do the observed reward-related abnormalities stem from chronic drug use itself, or from factors related to genetics that facilitate a progression towards excessive drug use, or some combination of both? Of importance, the longitudinal path from a potential genetic vulnerability to substance misuse outcomes later in life have not been investigated from such a neurodevelopmental perspective, due, in part, to the lack of sufficiently powered longitudinal genetic-neuroimaging studies (Conrod and Nikolaou, 2016).

Using a uniquely large genetic-neuroimaging dataset (IMAGEN study (Schumann et al., 2010), we addressed this unsolved issue by applying structural equation modeling [SEM]) to examine whether the selective modulation of key components of the reward circuitry –ventral striatum (VS) and orbital frontal cortex (OFC) – by dopaminergic functional polymorphisms contribute to the degree of perilous alcohol use behaviour observed at 14 years of age and again at 16 years of age. In particular, functional magnetic resonance imaging (fMRI) data collected from 14 year old adolescence participants performing the monetary incentive delay (MID) task were used to quantify the blood-oxygen-level dependent (BOLD) response of the VS and OFC during the anticipation of large and small rewards. The MID task has been used extensively to

investigate changes in neural activity in response to the processing of different stages of reward processing (e.g. reward prediction, anticipation of obtaining rewards of different magnitude or avoiding punishment, outcome processing) in typical and atypical populations, with findings that converge with both animal and human studies emphasizing the essential role of the VS and OFC in processing reward-related information (for review, see (Lutz & Widmer, 2014; Balodis & Potenza, 2015; Knutson & Heinz, 2015). However, both hypo-responsiveness and hyper-responsiveness of reward-related brain regions (e.g., VS) have been reported during anticipation of reward in the MID task in substance dependent adults (for review, see (Balodis and Potenza, 2015), so it remains uncertain what functional state (hyper vs hypo) of the reward system may actually precipitate a substance use disorder.

Nevertheless, the relevant reward signal (i.e. positive and negative reward prediction error signals) critical to the functioning of the VS and OFC are thought to originate in the midbrain dopamine system (Schultz, 2001; Schultz et al., 2000). These reward signals are conveyed to the neural targets of the dopamine system where their impact reorganizes synaptic connectivity in a way that drives learning and motivation (Schultz, 2011, Schultz, 2001). For this reason, we focused on functional polymorphisms that would appear to alter dopaminergic signaling in the VS and OFC during reward valuation and prediction. To be specific, we selected the 7-SNP haplotype of the PPP1R1B gene − mRNA expression highest for G alleles of the rs87694 SNP (Meyer-Lindenberg et al., 2007) – because of its critical function in integrating dopaminergic and glutaminergic signaling (Svenningsson et al., 2004), and its association with reward learning (Frank et al., 2007) and cognitive performance (Meyer-Lindenberg et al., 2007). The rs686 SNP of the DRD1−the G allele linked to increases in DRD1 expression (Huang and Li, 2009) – selected because of the role D1 has in reward signalling (Ikemoto et al., 1997; Suhara

& Miyoshi, 2007) and addiction (Batel et al., 2008; Comings et al., 1997; Zhu et al., 2013; Huang et al., 2008). To date, the rs686 SNP of the DRD1 has yet to be investigated in the context of human reward-related learning or behaviour. Further, we selected the promoter rs12364283 SNP of the DRD2 gene – the C allele has been shown to confer higher transcriptional activity (Zhang et al., 2007)− because of the association D2 has with reward signalling (Assadi et al., 2009; Suhara & Miyoshi, 2007), reinforcement learning (Baker et al., 2013; Frank & Hutchison, 2009), and addiction (Noble, 1994; Noble, 2000). Likewise, the Taq1A polymorphism (rs1800497) of the ANKK1 gene was also selected because of its association with striatal D2 receptor function (Thompson et al., 1997) but see (Laruelle et al., 1998), altered activation of OFC (Cohen et al., 2005) and VS (Nymberg et al., 2014), impaired reinforcement learning (Klein et al., 2007), and addiction (Munafo et al., 2007; Noble et al., 1994; Abi-Dargham, 2004; Noble, 1998; Noble, 2003; Noble, 2000).

Taken together, we hypothesized that these specific dopaminergic functional polymorphisms − $DRD1^{rs686}$, $DRD2^{rs12364283}$, $ANKK1^{rs1800497}$, and $PPP1R1B^{rs87694}$ – may selectively modulate the VS and OFC BOLD signal (hyper vs hypo) during reward anticipation. In turn, we predicted that the relationship between these SNPs and alcohol related behaviour at 14 years and 16 years of age would be indirect and be mediated by their effect on the reward response in these selected brain regions. Although less explored, because both the VS and OFC have been proposed to play an important role in reward learning (Frank & Claus, 2006), adolescent risk-taking behaviours (Galvan et al., 2006; Conrod & Nikolaou, 2016) and the development of addiction (Pujara & Koenigs, 2014), we used an interaction term to investigate the influence of the balance of activity between these two regions during reward anticipation as a

variable of interest in our SEM. Our proposed imaging genetics approach constitutes a natural application of SEM, which provides a means for modeling such complex interrelationships.

Methods

Participants and Procedure

A community-based sample of young adolescents (N=2463) was recruited for the IMAGEN study (for details on the IMAGEN project, see Schumann et al. 2010). Individuals who provided assent, and whose parents provided informed written consent, completed an extensive battery of neuropsychological, clinical, personality and drug use assessments online and at the testing centers. Participants were excluded if, among other criteria, they had contra-indications for MRI (for example, metal implants, claustrophobia). After data quality control, complete and reliable data sets were available for 1840 participants at Time 1 (1666 participants at Time 2). Of these volunteers at Time 2, 1639 had complete neuroimaging data. The demographic information of the participants at time 1 was: mean age = 14.55 ± 0.447 years, 51.7% female, 88.80% right-handed, verbal IQ = 110.67 ± 14.85, performance IQ = 107.57 ± 14.77.

Alcohol Use Disorders Identification Test

Problematic alcohol use behaviors were assessed twice, at 14 and 16 years of age, using the total score of the Alcohol Use Disorders Identification Test (AUDIT) (Bohn et al. 1995) via the online computer Psytools ® (Delosis Ltd, London, UK) platforms at the participant's home. Of the 1840 adolescents in Time 1 (AUDIT mean = 1.56 ± 0.06), 877 scored 0 on the AUDIT and thus had never used alcohol, whereas 963 adolescents reported the use of alcohol at some degree (score >0) (Table 1). Of the 1666 adolescents in Time 2 (AUDIT mean = 3.7 ± 0.08), 288 scored 0 on the AUDIT and thus had never used alcohol, whereas 1378 adolescents reported the

use of alcohol (score >0) (for an overview of these data, see Table 1). To note, participants AUDIT score were significantly larger at Time 2 compared to Time 1, $t(1461) = -25.8$, $p < .001$.

Table1

fMRI task, acquisition and analysis

Monetary incentive delay task In order to assess reward processing during fMRI in an adolescence population, a modified version of the MID task was used (Figure 1). In brief, each trial consisted of anticipation, response, and feedback related cues. Before the anticipation phase, a cue signaled the position of the target as well as the type of reward that could be attained by a correct response. Different cues distinguished between large reward (10 points), small reward (2 points) and neutral (zero points) conditions. After a random anticipation interval of 4000–4500ms length, the target appeared. Participants were instructed to respond to the target as quickly as possible via button press and informed that the points they earned would be converted into chocolate treats after scanning (i.e. 1 candy [M&Ms] for every 5 points scored). The duration of the target was continuously adapted to the performance of the subject, ensuring a successful performance on approximately 66% of all the trials. Immediately following the response, feedback indicated the number of points attained in the recent trial as well as the total points earned during the task. The inter-trial interval varied so that each trial took approximately 10,000 ms (Figure 1). Large, small, and neutral conditions were randomized throughout the task (22 trials each, summing up to 66 trials in total). Task presentation and recording of the behavioral responses were performed using Visual Basic 2005 and NET Framework Version 2.0, as well as the visual and response grip system from Nordic Neuro Lab (NordicNeuroLab AS, Bergen, Norway).

***** Figure 1. Here*****

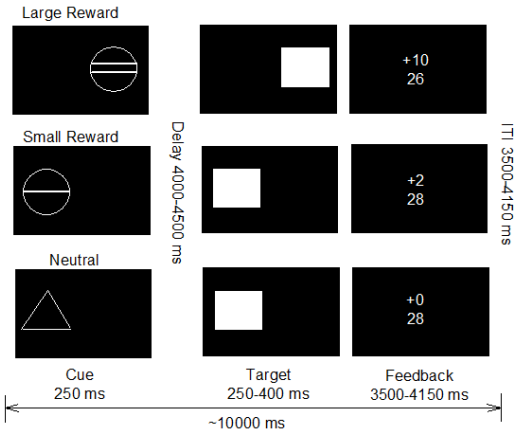

Figure 1. Monetary Incentive Delay (MID) task, adapted from Knutson et al (2001).

Imaging parameters All scanning was performed with a 3T whole body MRI system made by several manufacturers (Siemens, Philips, General Electric, Bruker) at the eight IMAGEN assessment sites (London, Nottingham, Dublin, Mannheim, Dresden, Berlin, Hamburg, and Paris). To ensure a comparison of MRI data acquired on these different scanners, we implemented image-acquisition techniques using a set of parameters compatible with all scanners that were held constant across sites (cf., Schumann et al, 2010). We acquired 40 slices in descending order (2.4 mm, 1 mm gap) using a gradient-echo T2*-weighted sequence (EPI) with the following image parameters: TR=2200 ms, TE=30 ms, and an in-plane matrix size of 64 × 64 pixels. We used a plane of acquisition tilted to the anterior–posterior commissure line (rostral>caudal). For anatomical reference, a 3D magnetization prepared gradient-echo sequence (MPRAGE) based on the ADNI protocol (http://www.loni.ucla.edu/ADNI/Cores/index.shtml) with TR=6.8 ms and TE=3.2 ms over the whole brain was carried out.

Functional preprocessing and analysis The fMRI data were analyzed with Statistical Parametric Mapping (SPM8, Wellcome Department of Imaging Neuroscience, University

College London, London, UK). All individual data were slice time corrected using the first slice as reference, then spatially realigned to correct for head movement, and non-linearly warped on the MNI space using custom EPI template based on an average of mean images of 400 adolescents. This custom template image (53 × 63 × 46 voxels) was subsequently applied to all functional T2* data and voxels were resampled at a resolution of 3 × 3 × 3 mm. The functional data were smoothed using an isotropic Gaussian kernel for group analysis (5 mm full-width at half-maximum). First level statistics were performed by modeling reward anticipation and reward feedback as predictor variables within the context of the GLM on a voxel-by-voxel basis, with AR noise model against a design matrix. Estimated movement was added to the design matrix in the form of 18 additional columns (3 translational, 3 rotations, 3 quadratic and 3 cubic translations, 3 translations shifted 1 TR before, and 3 translations shifted 1 TR later). A movement threshold of 2 mm was employed. Furthermore, each individual fMRI time series underwent an automatic spike detection method.

For anticipation cues of neutral, small reward, and large reward, as well as information on feedback (hit [response within the correct time window] vs missed [response outside the correct time window]) trials, were entered in a parametric design, and study center was included as a covariate. The regressors modeling the experimental conditions (e.g. cues predicting large reward, small reward, and neutral reward trials) were convolved using SPM's default hemodynamic response function. The individual contrast images were entered in a second-level random-effects analysis (full flexible procedure of SPM8), and a non-sphericity correction was performed. A one-sample t-test was conducted, testing activity on large reward trials (and separately on small reward trials) against the implicit baseline of the neutral condition, removing variance associated with the other

regressors in the design matrix. A significance level of $p<0.05$ was selected (FWE-corrected), with a minimum cluster size of 10 voxels.

Based on previous IMAGEN studies, (cf., Nees et al, 2012, Whelan et al. 2012), the analyses focused on weighted mean BOLD signal change of the designated ROIs (OFC and VS) over both hemispheres for anticipation of large reward vs neutral (large reward condition) and anticipation of small reward vs neutral (small reward conditions). Furthermore, we analyzed two distinct regions in OFC (medial OFC and lateral OFC) based on evidence suggesting dissociable functions in reward processing (Elliott et al., 2000; Elliott et al., 2008; Frank & Claus, 2006) (O'Doherty et al., 2001; Diekhof et al., 2012). The ROI masks were taken from the Wake Forest University Pick-Atlas (Maldjian et al. 2003) using various atlases (medial OFC [aal atlas], lateral OFC [Broadman's area 47], ventral striatum [nucleus accumbens]), and the mean contrast value for each ROI was calculated for each subject for both large reward and small reward contrasts[1]. To note, only trials that subjects made a successful response were included in this analysis and our analysis focused on the reward anticipation period of the task.

Genetic data

***** Table 2. *****

After quality control, genome-wide data were available for N=1,839 of the participants. Details of quality control procedures are available in the supplementary online material. We investigated 4 SNPs, which were selected from each member of the full set of autosomal catecholamine genes; namely, those that that have empirical support for variation in the

[1] The ROIs are available from the corresponding author upon request [TEB]

degradation and receptor signalling of dopamine D1 and D2 receptors. In brief, we focused on two functional polymorphisms related to D1 receptors (DRD1rs686, PPP1R1Brs87694), and two genetic polymorphisms that affect D2 expression ($DRD2^{rs12364283}$, $ANKK1^{rs1800497}$).

Statistical analysis strategy

We performed two main sets of analyses using SPSS 17.0.1 and MPlus version 6.12 (Muthen & Muthen 2011). First, a simple regression analysis was performed to identify unique relationships between genetic data ($DRD1^{rs686}$, $DRD2^{rs12364283}$, $ANKK1^{rs1800497}$, and $PPP1R1B^{rs87694}$) and neuroimaging data (medial/lateral OFC and VS), and between neuroimaging data and alcohol misuse at 14 and 16 years. In addition, interactions terms (medial OFC*VS and lateral OFC * VS) were derived from the product of the medial/lateral OFC and VS standardized scores in order to examine whether the interaction between the two reward regions contribute to the prediction of alcohol misuse scores. Type 1 errors were statistically controlled following Benjamin and Hochberg (1995) with a corrected significance level of $\alpha = 0.05$. Sex, age, and imaging site (8 sites) were included in each regression model as nuisance variables using a stepwise approach.

Second, a SEM path model in Mplus was conducted, in which: 1) the robustness of these gene-brain associations could be tested once all associations were entered simultaneously in one model, and the effect of sex, age, and imaging site (as a cluster variable) was controlled for; and 2) indirect effects from genes to substance use behaviours could be tested using the product of coefficients method (Tofighi & MacKinnon, 2011). Full information maximum likelihood (FIML) was used to account for missing data. The SEM model was fit using a complex random effects design to control for sex, age, and site, and robust maximum likelihood estimation

(MLR), which is robust to non-normality. Model fit was assessed with the Chi-square and Comparative Fit Indices (CFI), the Standardized Root Mean Square Residual (SRMR) and the Root Mean Square Error of Approximation (RMSEA). Hu and Bentler (1999) suggest the following guidelines for interpreting Goodness-of-Fit Indices: SRMR and values close to or below .08, RMSEA values close to or below .06 and CFI close to or above .90 indicate acceptable model fit. To help interpret the interaction effects, these were plotted based on procedures by Aiken and West (1991), Dawson (2013) and Dawson and Richter (2006).

Results

Univariate results

Gene-Brain Associations SNP ($DRD1^{rs686}$, $DRD2^{rs12364283}$, $ANKK1^{rs1800497}$, and $PPP1R1B^{rs87694}$), and ROI (VS, medial and lateral OFC) associations were assessed using univariate regression models, while controlling for Sex, Age, and imaging site (corrected for multiple comparisons, B-H, $p<.0125$). All regression results are presented in Table S2. This analysis yielded two significant associations. First, $DRD1^{rs686}$ reliably predicted medial OFC BOLD signal (Beta = -.08, t = -2.7, p = .008) to the large reward anticipation cue ($F_{(10, 1230)} = 6.5$, $p < .001$, $r^2 = .05$,), indicating that increasing the number of G allele was associated with a stronger medial OFC BOLD response to the large reward anticipation cue (see Figure 2, middle panel). It is also worth noting that $DRD1^{rs686}$ also predicted medial OFC BOLD response to small reward anticipation, (Beta = -.07, t = -2.4, p = .014), but this relationship did not survive our correction for multiple-comparisons. Second, $ANKK1^{rs1800497}$ significantly predicted lateral OFC BOLD, (Beta = -.09, t = -3.1, p = .002) response to the large reward anticipation cue ($F_{(10, 1227)} = 2.9$, $p < .001$, $r^2 = .03$), indicating that increasing the number of A2 alleles was associated with a larger decreases in lateral OFC BOLD signaling during large reward anticipation (see

Figure 2, bottom panel). It is also worth noting that these SNP→ROI relationships remained significant (ANKK1^{rs1800497} [Beta = -.11, t = -3.1, p = .002]; DRD1^{rs686} [Beta = -.08, t = -2.3, p = .01]) when AUDIT Zone 0 participants (i.e. reported never using alcohol) were the only participants included in the regression analysis, suggesting that this genetic influence on reward activity precedes alcohol use at age 14.

Figure 2 here

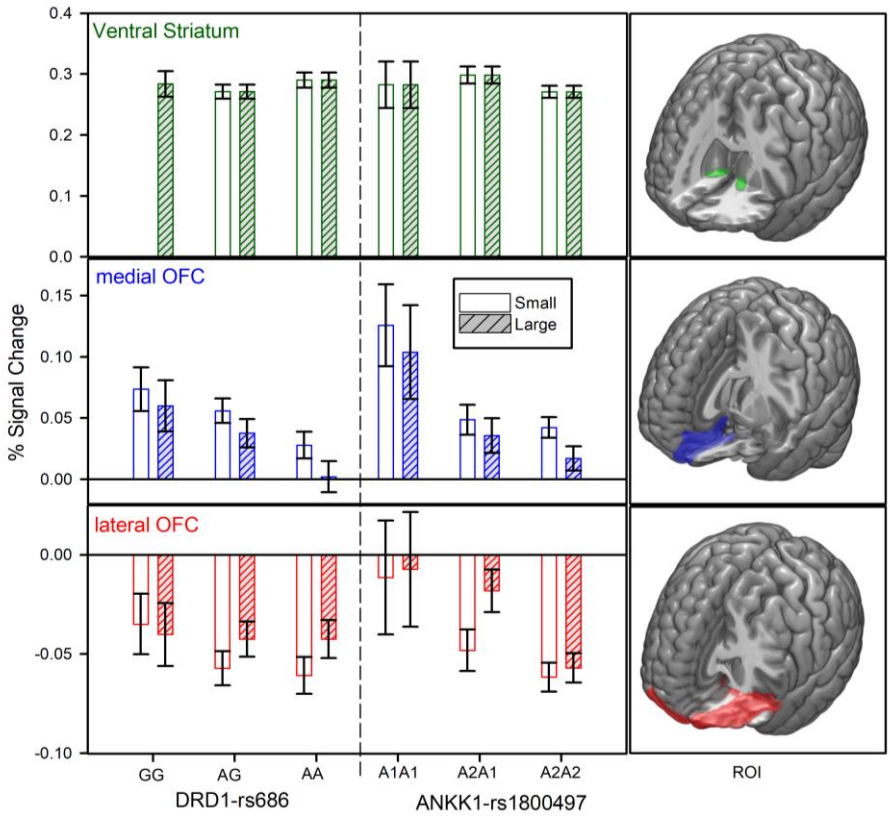

Figure 2. Gene-dose effects. DRD1 (left panel) and ANKK1 (right panel) gene-dose effects on small (clear columns) and large (dashed columns) reward anticipation cues for ventral striatum (top panel, green bars), medial OFC (middle panel, blue bars), and lateral OFC (bottom, red bars). Associated ROIs are displayed in right box. Error bars indicate standard errors of the means. OFC-orbital frontal cortex

Brain-AUDIT Associations The relationship between reward anticipation (large and small) and AUDIT scores (Time 1 and Time 2) were assessed using univariate regression models (corrected for multiple comparisons, B-H, p<.0125). All regression results are presented in Table S3. While the ROIs did not uniquely predict AUDIT scores at either time point, this analysis demonstrated that the interaction between medial OFC and VS (Beta = .09, t = 2.98, p < .005; $F_{(10, 1079)}$ = 5.3, p < .001, r^2= .05) and lateral OFC and VS (Beta = .08, t = 2.6, p < .01; $F_{(10, 1079)}$ = 5.3, p < .001, r^2= .05) during high reward anticipation uniquely predicted alcohol misuse at 14 years of age. No other associations were observed (p>.1). The finding suggests that when both the medial OFC and VS are highly active or inactive (i.e., synergistic), individuals displayed higher levels of Audit scores at 14 years of age (Figure 3C). It is interesting to note that the rs686 SNP of the DRD1 gene reliably predicted both medial OFC and VS interaction (Beta = -.10, t = -3.4, p < .001) for the large reward anticipation condition ($F_{(10, 1230)}$ = 4.3, p < .001, r2= .03)[2], and AUDIT scores (Beta = .07, t = 2.7, p = .008) at Time 2, ($F_{(10, 1385)}$ = 4.3, p < .001, r2= .03).

SEM results

Figure 3 here

[2] As a check, we tested all other SNP and OFC*VS interaction associations (Table S4). No associations were detected between the SNPs and the interaction between medial OFC and VS, p>.1, as well as the interaction between lateral OFC and VS, p>.1.

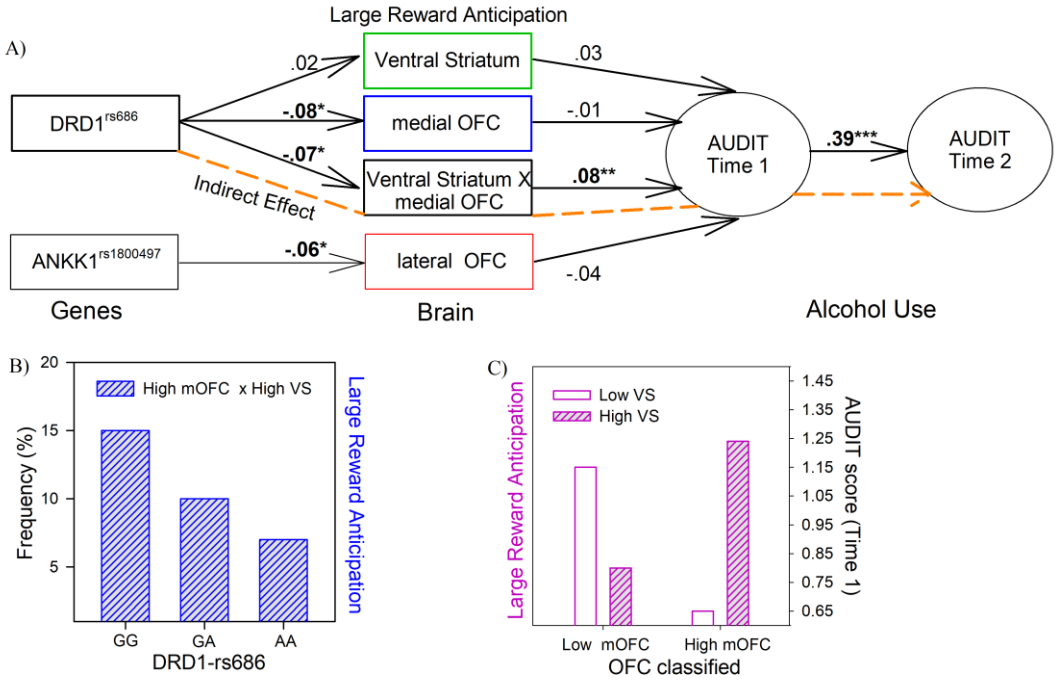

Figure 3. Results of the SEM A) Significant direct and indirect paths between gene, brain and alcohol misuse. Paths that are part of significant indirect effects are highlighted in orange, other direct effects are shown in black. * P<.05, **P<.005, ***p < .001 (two-tailed). B) DRD1 genotypes plotted by individuals classified as high medial OFC and high VS. C) Audit scores at 14 years plotted by groups classified as high and low medial OFC and VS activation during large reward anticipation. Note: VS-ventral striatum, OFC-orbital frontal cortex.

In the hypothesized model, all brain variables with genetic predictors were modeled to predict alcohol misuse at 14 years of age, which in turn predicted alcohol misuse at 16 years of age. Results from the SEM analysis showed that this model fit the data very well, $X^2_{(21, 2052)}$ = 29.69; CFI = .97; TLI = .95; RMSEA = .014 (90%CI= .00 - .025); SMRM = .018. The model indicated that the medial OFC*VS interaction term calculated for the large reward condition predicted alcohol use at 14 years of age (Beta = .08, t = 2.9, p<.01), which in turn significantly predicted alcohol use at 16 years (Beta = .39, t = 13.57, p <.001). In order to better understand the interaction effects, a Chi-Square Test of Independence was conducted and indicated that the $DRD1^{rs686}$ genotypes differ in the medial OFC*VS interaction, $X^2_{(4, 1396)}$ = 12.85, p =.012, namely, GG carriers, more than GA and AA carriers, were classified as high medial OFC and

high VS (15%, 10%, and 7% respectively) (Figure 3B). Furthermore, the interaction effect on alcohol use at 14 years was plotted (see Figure 3C), which indicated that when both the medial OFC and VS are highly active or inactive (i.e., synergistic), individuals displayed higher levels of Audit scores at 14 years of age. Finally, two significant indirect effects/paths from genes to alcohol misuse were identified: from rs686 SNP of the DRD1, through the medial OFC*VS interaction, to alcohol misuse at 14 years (ab= -.006, se=.003, 95% CI=-.013, -.01), and then on to alcohol misuse at 16 years (abc= -.002, se=.001, 95% CI= -.005, -.001) (Figure 3 A, orange path).

Discussion

Human neuroimaging studies confirm that the reward function of the mesocorticolimbic system is altered in substance use disorders (Volkow et al., 2011; Volkow et al., 2012). However, these data cannot distinguish whether the abnormalities observed in adults are induced by drug exposure or represent a pre-existing condition that predispose individuals to drug addiction, or a combination of both (Schoenbaum & Shaham, 2008; Schneider et al., 2012). In the present study, we attempted to resolve this issue by examining the relationship between dopaminergic functional polymorphisms, VS and OFC reward functioning, and alcohol use behaviour in early adolescence.

Foremost, we found a novel association between the DRD1^{rs686} (Huang & Li, 2009) and medial OFC activation during reward anticipation: reducing DRD1 expression (increasing G alleles)(Huang & Li, 2009) predicted an increase in medial OFC response (but not lateral OFC or VS) to reward predicting cues. This finding appears consistent with a plethora of evidence highlighting the role of D1 receptors and medial OFC in reward-related learning (Cetin et al.,

2004; Durstewitz & Seamans, 2002; Hikosaka & Watanabe, 2000; Frank & Claus, 2006; Elliott et al., 2008; Elliott et al., 2000). Further, D1 density differs quantitatively between sub-compartments of the frontal cortex with the highest expression in the medial OFC (Hurd et al., 2001). Our findings suggest that a reduction in DRD1^{rs686} expression may allow a greater proportion of D1 housing medial OFC neurons to become stimulated by dopaminergic reward signals, thereby intensifying its hemodynamic response. Although suggestive, this idea aligns with the proposal that the intensity of a reward response depends on the absolute number of interactions between dopamine and its post-synaptic D1 (or D2) receptors (Cox et al., 2015)$^{pg.\ 99}$, and further, with evidence demonstrating that when D1 receptors are more highly activated in OFC, behaviours becomes more focused, and reward associations learned more rapidly (Garske et al., 2013). Taken together, this novel finding revealed that variation in expression DRD1^{rs686} can modulate the reward response of the medial OFC.

Perhaps more intriguing was the SEM findings, which point to a specific molecular pathway by which DRD1^{rs686} modulated the balance of activity between medial OFC and VS during reward anticipation, and this specific balance of activity predicted the level of problematic alcohol use behaviours early in adolescence. Consistent with anatomical, functional, and computational evidence highlighting the interplay between medial OFC and VS during learning (Pujara & Koenigs, 2014), a synergistic (hypo or hyper) response between medial OFC and VS during reward anticipation predicted elevated levels of problematic alcohol use behaviours. Such a synergistic relationship of activity between medial OFC and VS are interesting in light of known differential developmental trajectories for these regions in relation to reward processing and to increased risky behaviour during adolescents (Galvan et al., 2006). In particular, differential recruitment of frontostriatal regions are typically interpreted in terms of immature

prefrontal regions or an imbalance between prefrontal and subcortical regions (Galvan et al., 2006), a developmental pattern proposed to be exacerbated in those adolescents with a predisposition toward risk-taking (Galvan et al., 2006; Casey, 2015; Casey et al., 2008; Galvan et al., 2006). However, our results seem to suggest that a synergistic recruitment of medial OFC and VS during reward processing may facilitate a progression towards excessive drug use behaviours in adolescents.

Notably, the relationship between a synergistic medial OFC and VS reward response and problematic alcohol use may be explained in the context of a recent dual system model of decision making, which refers to the competition between an automatic and deliberative system during learning (McClure & Bickel, 2014). According to this model, behaviours reflected in VS and OFC circuitry (the automatic system) develop slowly through the regular co-occurrence of stimuli and reinforcers, a process facilitated by positive (increase in dopamine activity) or negative (decrease in dopamine activity) reward prediction error (RPE) signals (Schultz, 2010). With sufficient experience, this learning process is thought to give rise to stereotyped or habitual (automatic) behaviours (McClure & Bickel, 2014). By contrast, the role of the deliberative system, comprised of the dorsal lateral prefrontal/posterior parietal cortex, is to modulate behaviours by down regulating value-related responses in the automatic behavioural system (McClure & Bickel, 2014).

In line with this model, we propose that an automatic system with low DRD1 expression may function at a supraoptimal reward state during positive RPE signalling, allowing behaviours to become more focused, and associations learned more rapidly (for example, see Garske et al. 2013). Further, the dopamine-potentiation effects of addictive substances would compound this problem, resulting in an exaggerated reward response by the automatic system. Such a

maladaptive process may in turn prevent the deliberative system to sufficiently compete in the decision making process, failing to down regulate and implement control over high-valued drug-related stereotype, possibly explaining how early drug use can quickly spiral to problematic use. Alternatively, an automatic system with high DRD1 expression may function at a suboptimal reward state and antagonize positive RPE signalling. In turn, the automatic system may bias behaviours that are highly rewarding (e.g. following high risk behaviours, drug use) to compensate for a chronically low "reward" state (Blum et al., 2000; Comings & Blum, 2000). Furthermore, the deliberative system may fail to recognize the need to down regulate such high value-related responses by the automatic system since these reward responses may appear normalized. Although speculative, the association between a synergistic response between medial OFC and VS by DRD1^{rs686} (Huang & Li, 2009), and early onset of alcohol misuse behaviour may provide initial support for such possibilities.

A challenging question is why this pattern of activation between the medial OFC and VS directly predicted AUDIT scores at 14 years of age, and mediated the effect between the DRD1^{rs686} and AUDIT scores at 16 years of age. Presently we can only speculate about the answer to this riddle. In regards to the former, it is important to point out that the relationship between the pattern of activation between medial OFC and VS, and AUDIT scores at 14 years of age preceded early alcohol use (see results), providing an explanation of how dopamine-related genes may predispose individuals to alcohol misuse. In regards to the latter, given the critical developmental period that the frontal and striatal brain systems go through between 14 and 16 years of age, and taking into account the impact alcohol use may have during this time period, perhaps imaging data at 16 years of age may provide better predictions of AUDIT scores at Time 2, as well as other risky behaviors. Alternatively, these findings could also be interpreted in the

context of the many type I errors observed in candidate gene studies. Nevertheless, we hope that the results of this study will motivate future research on this issue.

The D2 dopamine receptor (DRD2) gene has received the most attention as a risk candidate for the genetic transmission of substance use disorders, yet, we did not observe such an association in this adolescent sample. Instead we observed that an increase in A2 alleles of the ANKK1^{1800497} gene (Thompson et al., 1997) was associated with an increase in lateral OFC deactivation or suppression during reward anticipation. To note, this association is complicated by the difficulty in determining whether suppression or deactivations reflect an active process such as inhibition, a passive consequence of the redistribution of blood as activity is orchestrated within a distributed network (i.e. due to increase medial OFC activation) or a product of the baseline (Frankenstein et al., 2002). Nevertheless, increasing A2 alleles, which have been associated with an increase in D2 density, may have strengthened the D2 inhibitory signal in lateral OFC, thereby reducing neuronal excitability for the purpose of suppressing competing behavioural responses maintained in working memory (Elliott et al., 2000; Elliott & Deakin, 2005). Based on these findings, perhaps D2's role in addiction is only observed in later stages of addiction (Blum et al., 1993; Noble et al., 1994; Munafo et al., 2007), which might be through impaired inhibitory control by lateral OFC. For instance, in a drug-using state (elevated dopamine levels), the lateral OFC should serve to inhibit the execution of competing behaviours to promote heighted drug-seeking behaviour. In an abstinent state (reduced dopamine levels), the lateral OFC may be unable to suppress drug-related behaviours that are not aligned with prosocial goals. Although speculative, how genetic variants related to D2 expression translate into a vulnerability to addiction warrants continued research.

Conclusion

Adolescence is thought to constitute a critical developmental period during which the frontal and striatal brain systems implicated in decision-making are particularly vulnerable to the addictive properties of drugs (Castellanos-Ryan et al., 2014; Conrod & Nikolaou, 2016). Our study provides a potential genetic link to this vulnerability, supporting the possibility that alterations in OFC and VS signalling by DRD1^{rs686} render youth susceptible to the early onset of substance misuse. Specifically, a genetic profile contributing to the presence of a suboptimal or supraoptimal balance between OFC and VS may present a primary risk factor of drug-seeking behaviour. Although speculative, it is possible that our findings may reflect a maladaptive U-shaped tuning of reciprocal projections between these brain regions during reward functioning (e.g., motivated behavior, working memory, and reward related learning) and dopamine signalling (e.g., dopamine concentration, dopamine receptor availability) (Cools & D'Esposito, 2011). By moving out of the optimum level of dopaminergic stimulation (trough) towards either peak by excessive or low levels of dopamine stimulation, the mesocorticolimbic system may become hyper or hypo sensitive to rewarding events, possibly biasing the adolescent's action toward drug-related behaviours. Lastly, our results point to a regional specificity in the relationship between functional polymorphisms associated with D1 and D2 receptors and reward-related activity in the medial and lateral OFC, respectively. By identifying such a dopamine-related genetic path in adolescence, our study points to targets for intervention at the genetic, neural, and cognitive level to help vulnerable youth prevent progression to heavy drinking.

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Author Contributions
M.F.-B., H.G. and T.W.R. carried out the functional neuroimaging. G.J.B., C.B., P.J.C., H.F., J.G., H.G., A.H., B.I., E.L., K.M., J.-L.M, F.N., M.N.S., T.P., M.R., R.S., D.S., T.W.R., M.S. and A.S. acquired the data. J.-B.P., R.W. and T.B. carried out neuroimaging data processing and analysis.. M.A.B., T.D.R.C., M.L., A.L. and G.S. carried out genotyping and genetic analysis. T.B. N.C-R, and P.J.C. designed and prepared the manuscript. P.J.C., and T.P edited the manuscript.

Competing financial Interests
Dr. Banaschewski has served as an adviser to or consultant for Eli Lilly, Hexal Pharma, Medice, Novartis, Otsuka, Oxford Outcomes, PCM Scientific, Shire, and Viforpharma and has been involved in clinical trials conducted by Eli Lilly, Shire, Viforpharma; he has received conference attendance support and conference support or received speaking fees from Eli Lilly, Medice, Novartis, and Shire. The other authors report no financial relationships with commercial interests.

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