Clinical neuroanatomy

**DRD2 polymorphisms modulate reward and emotion processing, dopamine neurotransmission and openness to experience**

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**ABSTRACT**

Dopamine (DA) neurotransmission through D2 receptors (DRD2) has been implicated in the regulation of reward processing, cognition and the effects of drugs of abuse, and also has significant effects in responses to stressors and salient aversive stimuli. An examination of the influence of genetic variation across multiple psychophysical measures therefore appears critical to understand the neurobiology of DA-modulated complex personality traits and psychiatric illnesses. To examine inter-individual variation in the function of DRD2 modulated mechanisms in healthy humans, we used a haplotype-based and single nucleotide polymorphism (SNP) investigation. Their effects were interrogated with functional magnetic resonance imaging during reward and emotional processing. We found that a haplotype block composed by two SNPs, rs4274224 and rs4581480, affected the hemodynamic responses of the dorsolateral prefrontal cortex (DLPFC) during reward expectation and the subgenual anterior cingulate cortices (sgACC) during implicit emotional processing. Exploratory analysis within the significant haplotype block revealed the same functional effects only for the SNP rs4274224. Further analysis on rs4274224 using functional connectivity and positron emission tomography (PET) measures of DA D2/3 receptor mediated neurotransmission confirmed a gene effect on the functional connectivity of the DLPFC during reward anticipation and subcortical stress induced DA release. At a phenotypic trait level, significant effects of genotype were obtained for the NEO PI-R "Openness to Experience" and further correlated with neuroimaging data. Overall, these results show significant neurobiological effects of genotype variation in DRD2 on multiple functional domains, such as emotional, stress and reward processing. As such, it contributes to normal variation and potentially to vulnerability to psychopathology associated with those functions, such as risk for mood and substance use disorders.

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1. Introduction

Evidence from animal studies (Berridge and Robinson, 1998; Hamidovic et al., 2009; Ikemoto and Panksepp, 1999) and growing evidence from human in vivo imaging studies (Badgaiyan, 2010; Schott et al., 2008; Kobiella et al., 2010) suggest that dopamine (DA) neurotransmission is critically involved in incentive-motivational mechanisms (Schultz, 2006; Wise, 2004) but also in responses to salient aversive stimuli (Horvitz, 2000; Thierry et al., 1976), in addition to its more traditional roles in cognitive and motor function. Recent literature has linked variation in genes related to DA function (COMT, DAT and D4) with reward (Aarts et al., 2010; Camara et al., 2010; Dreher et al., 2009; Forbes et al., 2009; Hahn et al., 2010; Krugel et al., 2009; Schmack et al., 2008; Yacubian et al., 2007) and emotional processing phenotypes (Aleman et al., 2008; Herrmann et al., 2009; Rasch et al., 2010; Smolka et al., 2005; Swart et al., 2011; Williams et al., 2010). However, understanding how genetic variation might provide a linkage between these processes seems critical to understand the neurobiology of DA-mediated complex personality traits (Ebstein, 2006) and psychiatric illnesses (Noble, 2003).

A key gene that might contribute to variation in reward and emotional processing is the D2 receptor (DRD2). Located on chromosome 11 (q22-q23), this receptor is a member of the D2-like family (Civelli et al., 1993), and is highly expressed in the striatum and prefrontal cortex. In mice, the D2 long isoform plays a role in regulating emotional responses (Hranilovic et al., 2008) and the acquisition of context-stimulus associations to rewards (Smith et al., 2002). In humans, D2 antagonists disrupt the attribution of incentive-motivational value (Danna and Elmer, 2010), and induced blunting of self-reported affective responses (Mizrahi et al., 2007).

Several DRD2 single nucleotide polymorphisms (SNPs) potentially affecting transcription have been described (−141 C Ins/Del, Ser311Cys, Taq1A ANKK1, Taq1B, C957T, rs12364283, rs2283265 and rs1076560) (Zhang et al., 2007), however there has been minimal exploration of their potential effects on reward (Forbes et al., 2009; Kirsch et al., 2006) or emotion processing in humans (Blasi et al., 2009). Moreover, the functional significance and clinical relevance of these SNPs remains controversial (Finckh et al., 1996; Frank and Hutchison, 2009; Lucht et al., 2007). For this reason, it has been suggested that haplotype-based analyses, as a means of exploring a larger number of SNPs and their relationship with functional endophenotypes, may provide additional relevant information about the effects of DRD2 variation on brain function.

Here we investigated the role of a previously reported SNP with putative functional effects on transcription (rs12364283) (Zhang et al., 2007) along with 13 other SNPs using a haplotype-based approach during the processing of emotional lexical content and monetary reward expectation. Further exploratory analyses on the significant haplotype block and functional SNP were conducted using a SNP-based analyses, which included functional connectivity during reward anticipation and direct measures of baseline D2 receptor availability in vivo and of DA release during a standardized stress challenge using positron emission tomography (PET). Further, we explored both, potential linear and non-linear effects, since both have been described in the context of DA function in humans (Gjedde et al., 2010; Monte-Silva et al., 2009). In addition, we examined the relationship between DRD2 variation, brain function and two personality traits previously related to DA function, Extraversion (Depue and Collins, 1999) and Openness to Experience (DeYoung et al., 2005). We hypothesized that DRD2 variation would affect reward and emotion processing in the striatum and prefrontal cortex, D2 neurotransmission measures in the striatum, and the way that these processes interact with each other, and explain some of the variability in DA-linked personality traits.

2. Methods

2.1. Subjects

Eighty-six healthy subjects (43 males, 43 females), aged 19–54 years (mean 28 ± 8) were recruited via advertisement. Participants were right handed, non-smokers and fluent English speakers, with no personal history of major medical illnesses, psychiatric, or substance use disorders. Volunteers were not taking psychotropic medications or exogenous hormones, including hormonal birth control, and they were instructed to abstain from all psychoactive substances (e.g., alcohol) for 24 h prior to the study. Urine illicit drug screens were used prior to scanning session. Written informed consent was obtained and all procedures were approved by the Institutional Review Board and the Radioactive Drug Research Committee at the University of Michigan.

2.2. Genotyping

Genotyping of 14 SNPs of the DRD2 gene was performed in all subjects using the Illumina. Golden Gate platform, employing the Addictions Array content of 130 genes (1350 SNPs) and 186 Ancestry Informative Markers (AIMs), described elsewhere (Hodgkinson et al., 2008). Clustering and genotype calling for each locus was manually verified and loci with call rates <90% were excluded. AIMs scores were calculated by comparison to similarly derived genotype data from the CEPH Diversity panel using a minimum of 160 markers. Genotyping accuracy was confirmed by replicate genotyping of 10% of the total sample. Completion rates for the 14 DRD2 SNPs was >93% (mean 99.4%, median 100%) and replicates showed no errors at these loci.

Population stratification was evaluated as a potential confounder using the same AIMs. Factor analysis resulted in a 7-factor solution that yielded ethnic factor scores for each individual. To test for population stratification in the neuroimaging and personality traits data, we performed Spearman correlations between ethnic factor scores and percentage of blood oxygenation level dependent (BOLD) signal change or NEO PI-R scores, respectively. Thus, no confounding was present due to ethnic differences. Moreover, genotype and allele frequencies were similar for both Caucasians and non-Caucasians groups.
2.3. **Functional magnetic resonance imaging (fMRI) tasks**

For each task stimuli were projected onto a screen at the rear of the magnet using an LCD projector. Subjects viewed words/pictures by way of a mirror mounted on the head coil. Stimuli were displayed and synchronized with image acquisition using E-Prime software (Version 1.1, Psychology Software Tools Inc., Pittsburgh PA, USA). Subjects responded to cues via an MRI compatible response pad (Psychology Software Tools Inc., Pittsburgh PA, USA).

2.3.1. **Monetary incentive delay task (MID)**

Forty-five subjects (19 males, 26 females), aged 19–54 years (mean 27 ± 5) also completed a modified version of the MID (Knutson et al., 2000). Each session consisted of 72 6-sec trials and each subject completed two runs. A trial consisted of a cue representing a monetary value (±$2.00, ±$1.00, and ±$5.00, $0) followed by an anticipation phase and a neutral target requiring button press with their right thumb. Subjects were then informed of their success on the preceding trial, where they either gained or avoided losing the cued amount of money for monetary gain or loss trials respectively. In the null trials, subjects experienced no monetary gain or loss but were still instructed to respond to the target. Subjects successfully hit the target an average of 45 ± 24% of the gain trials, and 44 ± 24% of the loss response trials. Their average reaction time on gain trials was (165 ± 41.34) and on loss trials was (165 ± 43.10).

2.3.2. **Emotion word stimulus task (EWT)**

Eighty-two subjects (43 males, 39 females), aged 19–54 years (mean 28 ± 8) completed an affective word task during which they silently read emotionally valenced words, as described previously (Heitzeg et al., 2008). Words were selected from entries of the Affective Norms for English Words list, which provides normative emotional ratings for valence and arousal (Bradley and Lang, 1999). On the nine-point scale, we used negative words with valence ratings less than 3, neutral words with valence ratings between 4.5 and 5.5, and positive words with valence ratings greater than 7. Words were displayed for 3 sec each, separated by a fixation cross displayed for 1 sec. Participants were asked to press a button using their right index finger after silently reading each word to acknowledge that they understood the meaning of the presented word. We confirmed that response rates were greater than 90% for all participants. The blocked design incorporated 24-sec epochs of positive, negative, or neutral words (six words of a single valence per block), separated by 18-sec resting epochs. Subjects were instructed to relax and look at the crosshair during the rest periods. Each fMRI run included six emotional word blocks (each followed by a resting block), counter-balanced by emotional valence using a Latin squares design, and each subject completed three runs. Subjects with usable data from at least three runs were included in analyses.

2.4. **fMRI data acquisition**

The BOLD signal was measured using a GE Signa 3-Tesla scanner (General Electric, Milwaukee, WI, USA) with standard RF coil, using a T2* weighted pulse sequence (single-shot combined spiral in/out, gradient echo; repetition time = 2 sec; echo time = 30 msec; flip-angle = 90 deg; field-of-view = 20 or 24 cm; 64-by-64 image matrix; slice thickness = 3 or 4 mm; 30 oblique-axial slices) (Glover and Law, 2001). This imaging protocol was selected to minimize signal loss due to magnetic susceptibility effects (Noll, 2002).

Data were reconstructed off-line, slice-time corrected to the middle slice (Acquire and D’Sposito, 1999), and realigned to the first volume of each run to correct for intrascan movement using Statistical Parametric Mapping (SPM) -based algorithms (Friston et al., 1995). Each session was visually inspected for artifacts and screened for excessive head movement. High resolution anatomical MRI studies were also acquired using an axial spoiled gradient recall T1-weighted sequence (repetition time = 10.5 msec, echo time = 3.4 msec, flip-angle = 25 deg, 124 contiguous images, 1.5-mm thickness). To allow comparisons between individuals, subject’s MRI and functional images were coregistered and anatomically normalized by warping the anatomical T1-weighted image to a standard stereotactic space (Montreal Neurological Institute, MNI) using SPM2 (Wellcome Department of Cognitive Neurology, University College, London, UK). Finally, functional images were smoothed with a Gaussian kernel of [full width at half maximum (FWHM) 6-mm] to reduce residual inter-individual variability. Smoothed functional images were band pass-filtered with a 128 sec high pass filter to eliminate low frequency signals.

2.5. **fMRI data analysis**

The BOLD responses were modeled with SPM2 software (Department of Cognitive Neurology, Welcome Trust Centre for Neuroimaging, London, UK) using a general linear model and canonical hemodynamic response function. Statistical analysis proceeded in two stages. At the first level, activation maps were derived for individual subjects, including task-related covariates of interest and nuisance covariates (head translation and rotation). At the second level, contrast images were place into MNI space using the transformation matrix derived from the linear and non-linear warping transformation matrices (described above) and random-effects analysis was used to determine group effects, resulting in statistical parametric (t or F ) maps. For the MID, statistical test were also applied to two primary contrasts of interest, combined gain (small, medium and large) minus neutral and combined loss (small, medium and large) minus neutral conditions. For the EWT, statistical tests were applied to the two primary contrasts of interest, negative minus neutral words and positive minus neutral words. A mask excluded the cerebellum and brainstem below the midbrain because these regions were not well represented.

2.6. **fMRI connectivity analysis**

A functional connectivity analysis was conducted in the 45 subjects who performed the MID, using the functional Connectivity (CONN) toolbox (http://web.mit.edu/swg/software.htm). The CONN toolbox performs seed-based analysis by computing the temporal correlation between the
BOLD signals from a given voxel to all other voxels in the brain. CONN also allows for ROI-based analysis, by grouping voxels into ROIs based upon Brodmann’s Areas (BA). Given our a priori interest in specific regions related to reward and emotional processing this last strategy was used for our analysis. White matter, cerebrospinal fluid (CSF) and physiological noise source reduction were taken as confounders, following the implemented CompCor strategy (Behzadi et al., 2007). Specifically, motion artifact in the scanner and task effects were taken as confounders. Bi-variate correlations were calculated between each pair of ROIs as reflections of connections. All Brodmann areas were imported as possible connections for our selected ROI source. Z-score standardizing was introduced to validate the multiple comparisons, and the significance tests were based on the Z-scores.

Based on the second level results of our fMRI analysis, we choose the dorsolateral prefrontal cortex (DLPFC) as the primary seed for the analysis, with specific interest in its connectivity with the sgACC and the nucleus accumbens. A DLPFC ROI, MNi-based, was generated using WFU PickAtlas toolbox (Maldjian et al., 2003) (Department of Radiology, Wake Forest University School of Medicine, Winston-Salem, NC, USA) based on the significant effect of rs4274224 on this region during the MID (combined gains-null contrast). Differences in functional connectivity within the three rs4274224 alleles were evaluated separately. For the DLPFC, CONN determined the inter-ROI connectivity with each ROI in the BA atlas according to the correlation analysis. The threshold was set at p < .05 FDR, two-sided. ROI-based connectivity maps were generated by CONN.

2.7. Stress challenge and PET data acquisition

Since cortical gene effects were observed during both, reward and emotional processing, we hypothesized that DRD2 rs4274224 would likely influence DA release during a stress challenge through cortical modulation. For this purpose, PET analysis was conducted in a sample of 52 subjects (22 males, 30 females), aged 20—40 years (mean 26.8 ± 4.9). Burst stimulation-induced inhibition of the left DLPFC function has been associated with greater DA release in the bilateral caudate and ipsilateral putamen, thus we hypothesized that the genotype with a lower BOLD response in the DLPFC during caudate and ipsilateral putamen, thus we hypothesized that been associated with greater DA release in the bilateral stimulation-induced inhibition of the left DLPFC function has been introduced to validate the multiple comparisons, and the significance tests were based on the Z-scores.

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A single PET study with [11C]raclopride, a DA D2/3 receptor radiotracer, that included a baseline period and a stressor, moderate levels of muscular pain [hypertonic saline solution (5%) into the left masseter muscle] applied over 20 min, was conducted as previously described (Scott et al., 2006). We assessed the internal emotional state of the volunteers with the Positive and Negative Affective Scale (PANAS) before radiotracer administration, after the baseline control and after completion of the pain challenge (Watson et al., 1988).

The total activity of [11C]raclopride administered to each subject was 15.0 ± 2.2 mCi. Fifty percent of the [11C]raclopride dose was administered as a bolus with the remainder delivered as a continuous infusion by a computer-controlled automated pump to more rapidly achieve steady-state tracer levels. Under these conditions, equilibrium conditions across kinetic compartments are achieved approximately 35 min after tracer administration (Carson et al., 1997). Twenty-eight image frames were acquired over 90 min with an increasing duration (30 sec up to 10 min) and were coregistered to each other (Minoshima et al., 1993). Dynamic image data for each of the receptor scans were transformed, on a voxel-by-voxel basis, into two sets of images, coregistered to each other: (a) a tracer transport measure (K1 ratio), and (b) a receptor-related measure at equilibrium (binding potential at equilibrium, BPeq), the latter using data obtained from 35 to 45 min (baseline) or 45—90 min (pain stress) after tracer administration and using the cerebellum as non-specific region. BPeq is proportional to the concentration of receptors, divided by their affinity (Bmax/Kd) (Carson et al., 1997). For each scan, two measures were calculated, baseline DA D2/D3 BPeq, and the reduction in this measure with the pain-stress challenge. The latter reflects the release of DA and the competition between the endogenous ligand and the radiotracer (Innis et al., 1992).

T1-weighted MR and PET images of each subject were then coregistered to each other (Meyer et al., 1997). The accuracy of coregistration and warping algorithms were confirmed for each subject individually by comparing the transformed MRI and PET images to each other and the MNI atlas template. To compensate for small residual anatomic variations across subjects and to improve signal to noise ratios, a three-dimensional Gaussian filter (FWHM 6 mm) was applied to each image.

2.8. PET data analysis

A mixed model was conducted in SPM5 (Wellcome Department of Cognitive Neurology, University College, London, UK), where each genotype represented the between subjects factor and the two conditions (baseline and pain) represented the within subjects factors. No global normalization was applied to the data, and therefore the calculations are based on absolute BPeq values. A mask that included only regions with specific DAD2/D3 receptor binding potential (BPeq > .2) was used.

2.9. Personality measures

Revised NEO Personality Inventory (NEO PI-R) data were available for 72 subjects (41 males, 31 females), aged 19—54 years (mean 25 ± 4). We explored two of the five main dimensions for a gene effect for its reported association with the DA system: Extraversion (Depue and Collins, 1999) and Openness to Experience (DeYoung et al., 2005) using sex corrected standardized scores.

2.10. Statistical analyses

First, we tested our primary hypothesis of a DRD2 effect in the EWT and MID tasks on whole brain ANCOVA models for each task separately controlling for age, sex and ethnicity using the European AIM score for each individual. A p < .01 was used considering that four haplotype blocks and one a priori hypothesized SNP (rs12364283) were included in the analysis. A cluster extend-based multiple correction was conducted in
Extraversion personality traits were conducted with Pearson correlation with a voxel extent greater than 10 voxels. Planned correlational analyses were found in the striatum, the summary statistical maps were thresholded at p < .005 uncorrected for multiple comparisons (Friston, 1997), with a voxel extent greater that 10 voxels. Planned correlations between imaging data and Openness to Experience and Extraversion personality traits were conducted using Pearson correlations at p < .05.

All reported p- and z-values were two-sided, and coordinates were in MNI space. BOLD response data was extracted for the second level significant peaks for the SPM Beta images and averaged them using Matlab. For the PET images raw data was extracted using MarsBar (Brett et al., 2002).

3. Results

3.1. Main effects of fMRI task

3.1.1. MID

Consistent with previous reports (Knutson et al., 2000), anticipation of monetary gain was associated with activation in the bilateral nucleus accumbens, caudate, thalamus, anterior cingulate, cuneus, lingual and fusiform gyri, the supplementary motor area and the precentral gyrus. Anticipation of loss induced regional activation in the nucleus accumbens, bilaterally, albeit of lesser magnitude than for reward processing, as well as in the caudate, thalamus, cuneus, the lingual, fusiform and temporal gyri (Supplementary Table 1).

3.1.2. EWT

As previously reported (Mickey et al., 2011) a main task effect of reading words with emotional valence was found in the medial prefrontal cortex for the negative minus neutral word contrast and in the cuneus for the positive minus neutral contrast (Supplementary Table 1).

3.2. DRD2 effect on fMRI task

3.2.1. Haplotype-based analysis

Eighty-six healthy subjects were genotyped for the 14 DRD2 SNPs in the Addiction array. We examined linkage disequilibrium (LD) among the 14 DRD2 SNPs, to help narrow the location of a potential susceptibility locus. We observed a total of four blocks in LD (Fig. 1). Haplotype Block 1 contained SNPs rs2242592, rs2587548, rs1076563, rs1079596, rs1125394, rs2471857, rs7103679 and rs4648318; Haplotype Block 2 contained rs4274224 and rs451480; Haplotype Block 3 contained rs4648317 and rs4350392 and Haplotype Block 4 contained rs1799978 and rs12364283.

Diplotypes assigned to each subject were used to study differences in activation during anticipation of monetary gain and loss and processing of positive, neutral and negative words. Diplotypes groups with less than five subjects were excluded from the analysis. Subject sample sizes for each block were, respectively for the larger (EWT) and the smaller (MID) sample, 66/36 (Block 1), 67/41 (Block 2), 85/43 (Block 3) and 79/35 (Block 4).

Considering four haplotype analysis and one a priori SNP (rs12364283) analysis a p < .01 was considered significant, using a cluster extend-based correction for multiple comparisons according to Monte Carlo simulations with AlphaSim (Ward, 2010) (p < .001 and extent k > 61 voxels/488 mm^3), which protected against overall type I error.

The only haplotype block with significant effects during reward and emotional processing was block 2 (rs4274224 and rs4581480). Here four diplotype groups (AC/AC; AC/GC; AC/GT and GT/GT) accounted for 97% of the total diplotypes included in the analysis. During the MID, a whole brain ANCOVA model showed a significant effect of the haplotype block 2 in the left DLPFC for both gain minus null contrast ([x, y, z, MNI coordinates, cluster size mm^3, Z]; (~50, 42, 12, 1512 mm^3, Z = 4.09)] and loss minus null contrast (~48, 40, 6, 2936 mm^3, Z = 4.87). Percentage signal change (PSC) in the DLPFC was also extracted for post hoc analysis, revealing a greater activation of the DLPFC in the G carriers (AC/GT; GT/GT) (See Supplementary Fig. 1). During the EWT, a whole brain ANCOVA model showed a close to significant effect of the haplotype block 2 (rs4274224 and rs4581480) in the left subgenual anterior cingulate (sgACC) for the negative minus neutral words contrast (0, 16, –6, 448 mm^3, Z = 3.66). PSC in the subgenual ACC was extracted for post hoc analysis, revealing a greater activation of the sgACC in the AC/GC diplotype group (AC/GC; GC/GC) (See Supplementary Fig. 1).

3.2.2. SNP-Based analyses

An exploratory SNP-based analysis was conducted within the significant haplotype block (rs4274224 and rs4581480). SNP rs4274224 showed qualitatively similar effects as its haplotype (rs4581480). Therefore, the remainder of this report will focus on rs4274224.

The DRD2 rs4274224 genotype distribution for the subjects that completed the EWT and MID tasks was, respectively, AA (23/13), AG (43/19) and GG (20/13), and A and G alleles were in Hardy–Weinberg equilibrium. There were no significant differences with respect to sex or ethnicity among the three genotype groups (Table 1).

MID: During the MID, a whole brain ANCOVA model showed significant non-linear effects of rs4274224 in the left DLPFC (gain-null: ~50, 42, 12, 2416 mm^3, Z = 4.35; loss-null: ~48, 42, 6, 4304 mm^3, Z = 5.44). In this case, PSC extracted data showed an inverted U-shaped pattern, where rs4274224 A/A exhibited a main effect of deactivation in the DLPFC, A/G...
was associated with activation of this region, and G/G showed neither activation nor deactivation of this region (Fig. 2).

Linear effects were also observed in the temporal cortex during gain minus null contrast ($t_{52, 10, \text{cluster} = 544 \text{ mm}^3}$) and loss minus null contrast ($t_{54, 6, \text{cluster} = 696 \text{ mm}^3}$) (GG > AG > AA).

**EWT**: Similarly to its haplotype block, the ANCOVA model showed a significant main effect of rs4274224 in the left

### Table 1 – Demographics, personality and behavioral data.

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pregenual and subgenual ACC (BA 32/25) for negative minus neutral words contrasts ($-2, 16, -4, 496 \text{ mm}^3, Z = 4$). PSC in the sgACC was extracted for post hoc analysis. A U-shaped effect was observed, where homozygosity (A/A and G/G) was associated with greater BOLD response in this region, while A/G was associated with deactivation (Fig. 2). Linear effects were also observed in the dorsal ACC ($-12, -70, 24, p < .001$, cluster $= 976 \text{ mm}^3$) and occipital cortex ($-2, -40, 50, p < .001$, cluster $= 1472 \text{ mm}^3$) (AA $>$ AG $>$ GG) for negative minus neutral words. No linear or non-linear significant gene effects were found for positive minus neutral words.

One additional SNP-based analysis was conducted for rs12364283, since the minor C allele has been reported to confer higher transcriptional activity compared to the major allele T (Zhang et al., 2007). The DRD2 rs12364283 genotype distribution of subjects in the EWT and MID samples were, respectively, T/T (60, 31 subjects), T/C (18, 10 subjects) and C/C (1, 1 subjects), and T and C alleles were in Hardy–Weinberg equilibrium. The one C/C subject was included in the heterozygote group for statistical analyses. During anticipation of reward we observed an effect of rs12346283 in the right frontal cortex (BA 10) and ($42, 40, 10, 616 \text{ mm}^3, Z = 3.81$) and as a trend in the left ACC (BA 32) ($-6, 46, 4, 448 \text{ mm}^3, Z = 4$) during gain minus null contrast (T/T $>$ T/C) (Fig. 2). No significant effects were found for the inverse contrast (T/C $>$ T/T) or during emotional processing.

3.3. **DRD2 effect on functional connectivity during the MID**

Connectivity within each allele group in rs4274224 was explored using the DLPFC as a seed region during the MID task. While positive functional connectivity in this region was extensive (data available upon request) for the three groups (AA/AG/GG), we concentrated on the relationship between the left DLPFC and the nucleus accumbens (NAC) and the sgACC, given the involvement of those regions in reward expectation and emotional processing. No effects of genotype on DLPFC
and nucleus accumbens connectivity were observed for each group separately [AA: right: $\beta = -0.05$, $t = -1.16$; left: $\beta = -0.03$, $t = -0.81$]; AG: right: $\beta = -0.01$, $t = -3.66$; left: $\beta = 0.01$, $t = 4.33$]; GG (right: $\beta = -0.01$, $t = -2.20$; left: $\beta = -0.02$, $t = -4.41$), all $p$'s < .05. However, during anticipation of gain, only the A/A group showed negative functional connectivity between the left DLPFC and the right sgACC (right: $\beta = -0.14$, $t = -3.06$, $p = .01$, FDR-corr.) that was not observed for the other two allelic variants. The same effect was observed, at least as a trend, during the anticipation of loss (right: $\beta = -1.11$, $t = -2.24$, $p < .09$, FDR-corr.).

Moreover, rs4274224 gene effects on DLPFC functional connectivity with the sgACC within task (MID), were also present across tasks (MID and EWT). Indeed, activation in the sgACC during the EWT as observed in the AA subjects was negatively correlated with the activation in left DLPFC during anticipation of gains (Pearson correlations) ($r = -0.41$, $p = .004$) and losses ($r = -0.46$, $p = .002$) as observed in AG subjects and explained 20% of the variance of the activation signal (Fig. 2).

Connectivity within each allele group in rs12364283 was explored using the right frontal cortex (BA 10) as a seed region during the MID task. Again, we concentrated on the relationship between the right frontal cortex and the nucleus accumbens and the sgACC. Whereas no functional connectivity between this region and the NAC was observed, a positive functional connectivity between the right frontal cortex and the sgACC was found (left: $r = -0.24$, $p < .001$; right: $r = 0.27$, $p < .001$), although no significant differences were observed among genotype groups.

3.4. **DRD2 effect on DA $D_{2/3}$ Receptor availability in vivo and stress-induced DA release**

We examined whether rs4274224 and rs12364283 would have a direct effect on DA $D_{2/3}$ receptor measures. The DRD2 rs4274224 genotype distribution for the subjects that completed the PET tasks was AA (12), AG (27) and GG (13), and genotypes were in Hardy–Weinberg equilibrium. There were no significant differences with respect to sex or ethnicity among the three genotype groups. The DRD2 rs12364283 genotype distribution for the subjects that completed the PET tasks was TT (31), CT (12), and there was no significant difference with respect to sex or ethnicity among the two genotype groups.

Again, a U-shaped pattern was observed for reductions in $D_{2/3}$ receptor availability during the stress challenge, where AG subjects showed a reduced dopaminergic response to the painful challenge in the caudate bilaterally [right: $x, y, z$ coordinates (MNI), 23, 0, 15, cluster size in $mm^3 = 1360$, $z = 3.19$, $p < .001$; left: $x, y, z = -17, 26, 10$, cluster = $912 mm^3$, $z = 3.47$, $p < .001$] and the left anterior putamen [right: $x, y, z$ coordinates (MNI) $-20, 16, 3$, cluster size in $mm^3 = 1360$, $z = 3.19$, $p = .003$], compared to AA and GG subjects (Fig. 3). Although not a priori hypothesized, these differences were also observed in the right putamen (right: $x, y, z$, 33, 1, 9, cluster = $1864 mm^3$, $z = 3.33$, $p < .001$) and the nucleus accumbens bilaterally (right: $x, y, z$, 14, 15, $-10$, $z = 2.48$, $p < .007$; left: $x, y, z$, $-7, 16, -7$, $z = 2.51$, $p < .006$). No genotype effects were observed for baseline binding measures. No genotype effects on DA release or baseline were found for rs12364283.

The presence of extensive cortico-striatal projections, thought to modulate DA-mediated responses to reward and other salient stimuli (Haber and Knutson, 2010) also opened the possibility that trait factors affecting cortical processing would be associated with differences in stress-induced DA release. Activation of the sgACC during the processing of negatively valenced words was indeed positively correlated with stress-induced DA release in the left caudate ($r = .46$, $p < .001$) and at trend levels with left nucleus accumbens ($r = .27$, $p = .07$). DLPFC activation during gain and loss anticipation was on the other hand negatively correlated with DA release in the left caudate (gain: $r = -0.37$, $p = .013$; loss: $r = -0.42$, $p = .004$), the right caudate/putamen (gain: $r = -0.46$, $p = .001$; loss: $r = -0.47$, $p = .001$), and with trends in the same direction in the nucleus accumbens bilaterally (gain: left: $r = -0.28$, $p = .06$; loss: right: $r = -0.28$, $p = .06$).

As previously described (Scott et al., 2006; Zubieta et al., 2002), the pain stressor employed increases negative and reduces positive affect ratings. A trend towards significant gene effects on the change in negative affect, during the study ($F = 2.86$, $p = .079$) was observed, with largest increases in AA subjects. No gene effects ($F = 1.08$, $p = .35$) were found for changes in positive affect.

3.5. **DRD2 effect on trait personality measures**

Finally, we tested for effects of rs4274224 and rs12364283 on individual scores of the Extraversion and Openness to Experience domains of the NEO Personality Inventory (Costa and McRae, 1992). A significant effect of rs4274224 was obtained for the Openness to Experience domain (ANCOVA, $df = 272$, $F = 4.02$, $p = .023$). No effects were observed for the Extraversion domain. Post hoc analyses with Bonferroni correction for multiple comparisons showed that A/A subjects exhibited lower levels of Openness to Experience than A/G volunteers (mean dif. $= -0.87$, Standard Error $-SE = .32$, $p = .026$) and as a trend, the GG volunteers (mean dif. $= -0.81$, $SE = .36$, $p = .089$). No effect of rs12346283 was found for any of the traits examined.

Moreover, activation of the sgACC during negative words was negatively correlated with the NEO-PI-R Openness to Experience domain ($r = -0.25$, $p = .05$), and activation of the left DLPFC during anticipation of gain showed a trend towards a positive correlation with this measure ($r = 0.26$, $p = .08$).

4. **Discussion**

The data presented establishes a relationship between a previously unexplored haplotype and the SNP that was associated with its greatest effects, the intronic DRD2 gene polymorphism rs4274224, on the activation of the left DLPFC (AA<AG) during anticipation of reward and the sgACC (AA>AG) during processing emotional words. SNP effects were also detected in functional connectivity analyses between these two regions within the MID and between the MID and the EWT tasks. Moreover, genotype-related attenuation of stress-induced DA release was observed in the
bilateral caudate, putamen and accumbens of heterozygous individuals. Stress-induced DA release was positively correlated with sgACC activation during negative words and negatively correlated with left DLPFC activation during reward processing. This SNP also showed a significant effect on the Openness to Experience domain of the NEO-PI that was negatively correlated to activation of the sgACC during emotional processing and positively correlated, albeit at trend levels, to activation of the DLPFC during anticipation of reward. The reportedly functional SNP rs12364283 (Zhang et al., 2007), previously associated with avoidance-based decisions (Frank and Hutchison, 2009), working memory and negative symptoms of schizophrenia (TT > TC) (Bertolino et al., 2009), showed a single effect of greater BOLD responses in the right frontal cortex and ACC (BA32) during anticipation of gains. Finally, none of the other three haplotypes showed significant effects in the imaging tasks.

Among the studies where a DA-related gene (COMT, SLC6A3, DRD4 7-repeat, DRD4-521, DRD2-141C) has shown an effect on reward responses (Aarts et al., 2010; Camara et al., 2010; Dreher et al., 2009; Forbes et al., 2009; Hahn et al., 2010; Schmack et al., 2008; Yacubian et al., 2007), some have implicated the DLPFC (Dreher et al., 2009; Yacubian et al., 2007). DA neurotransmission in the DLPFC has important roles in higher-order cognitive control, especially in retaining and manipulating information within working memory (Goldman-Rakic, 1998). Additionally, a putative role for DLPFC in anticipatory responses to salient and rewarding events has been well described (Watanabe, 1998). Primate studies have shown that DLPFC neurons demonstrate reward expectancy related activity (Watanabe, 2007). In humans, increased activation of the DLPFC during anticipation of reward has been related to increased levels of reward and not to an increase in executive demands (Gilbert and Fiez, 2004; Pochon et al., 2002). In fact, it has been hypothesized that, because the DLPFC receives motivational information from the orbitofrontal cortex and cognitive information from posterior association cortices, the motivation and cognitive processing integration might occur in the DLPFC, and the integrated information might be used as a top-down signal for adaptive goal-directed behavior (Watanabe, 2007; Barbas and Pandya, 1989). For this network to be effective in a motivated cognitive context, activation in cognitive areas is necessary to maintain a high level of cognitive performance whereas affective areas, that might interfere with performance, need to be deactivated (Pochon et al., 2002).

Several imaging studies have described effects of the COMT val158met functional polymorphism on emotional processing, mainly in the prefrontal cortex and the amygdala (Alemán et al., 2008; Herrmann et al., 2009; Lelli-Chiesa et al., 2010; Rasch et al., 2010; Smolka et al., 2005; Swart et al., 2011; Williams et al., 2010; Blasi et al., 2009). One published study has examined a DRD2 intronic polymorphism (rs1076560) on emotional processing, with effects in these same regions.

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**Fig. 3** – Effects of DRD2 rs4274224 on mean changes in binding potential (BPeq) and its SEs from baseline to the pain stress condition in the caudate, putamen and ventral striatum are displayed in blue. Positive values reflect increase release of DA in response to the challenge. AG subjects show a reduction in DA release from the baseline condition, potentially reflecting greater cortico-striatal regulation.
and fallypride, has been recently associated with greater depression (Kobiella et al., 2010). Previous brain imaging studies have also implicated the sgACC as a brain region implicated in the pathophysiology of Major Depression and persistent negative mood states (Mayberg et al., 1999; Drevets et al., 2008; Ongur et al., 1998). It has been additionally postulated that the sgACC may be a critical hub within distributed networks mediating depressive symptomatology and inter-individual variations in treatment response (Mayberg, 2003; Seminowicz et al., 2004). Consistent with an effect of DA on emotional regulation, Parkinson disease patients show impairment of emotional expression recognition (Lawrence et al., 2007). Moreover, prefrontal DA D2/D3 receptor availability, as measured with PET and [18F]fallypride, has been recently associated with greater amygdala responses to unpleasant visual stimuli during fMRI (Kobiella et al., 2010).

We confirmed an effect of DRD2 rs4274224 SNP on the relationship between the DLFCf function and that of the sgACC in the MID within task as well as between MID and EWT tasks. This is probably not surprising, since the DLPCf is heavily interconnected with the ACC (Mayberg et al., 2005; Petrides and Pandya, 1999). Within task, we observed the same pattern of negative functional connectivity between left DLPCf and sgACC during motivated behavior for AA homozygotes individuals. These same altered relationships between these two regions have been proposed for Major Depression (Mayberg et al., 1999) and their restoration has been linked remission from this illness (Ressler and Mayberg, 2007). In fact, the sgACC is one of the main targets for rTMS (repetitive transcranial magnetic stimulation) and DBS (deep brain stimulation) treatments in refractory depression (Lozano et al., 2008).

Consistent with the hypothesis that a gene effect on cortical regions would influence stress-induced DA release in striatal regions through cortico-striatal projections, reductions on striatal DA release were observed in the bilateral caudate, putamen and nucleus accumbens in heterozygous (AG) compared to homozygotes (AA or GG). Basic research strongly supports the concept that the activity of DA terminals in the striatum is under control of the PFC. The mechanism of regulation is complex, involving direct and indirect projections from the cortex to brainstem and striatum. This feed-back is primarily exerted through glutamatergic efferents from the cortex 49 (2013) 877–890 to little or too much gene expression is deleterious, with such as an inverted U-shaped response curve in which either optimal gene expression occurring in heterozygotes or occurs in up to 50% of all association analysis (Comings and MacMurray, 2000). This has been described for other DRD2 related processes, such as sensation seeking and neuronal plasticity (Monte-Silva et al., 2009; Gjedde et al., 2010). Several explanations for molecular heterosis have been proposed, such as an inverted U-shaped response curve in which either to little or too much gene expression is deleterious, with optimal gene expression occurring in heterozygotes or a broader range of gene expression in heterozygotes (Comings and MacMurray, 2000).

Finally, rs4274224 SNP explained some of the variance in the NEO-PI Openness to Experience domain, but not Extraversion, both personality traits associated with DA function (Depue and Collins, 1999; DeYoung et al., 2005; Smillie et al., 2010). This may not be entirely surprising, as Extraversion, with reward sensitivity as a core feature, may be more likely associated with DA projections to the nucleus accumbens, amygdala and the orbitofrontal cortex (Depue and Collins, 1999; Cohen et al., 2005) and other DRD2 functional polymorphisms like Taq1A/ANKK1 (Cohen et al., 2005; Smillie et al., 2010). On the other hand, Openness to Experience has been associated with DA projections to prefrontal cortex (DeYoung et al., 2005) in a motivated cognitive flexibility context. Consistent with this hypothesis, Openness to
Experience was, at least as a trend, positively correlated to the DLFPFC activation during the expectation of monetary gains and losses.

In summary, our results implicate the rs4274224 SNP in inter-individual variations in neural responses to expectations of reward and negative emotional processing, as well as stress-induced DA release, and the trait Openness to Experience. Further exploration of the functionality of this intronic gene variant both in vitro and in vivo appears warranted. One potential limitation of this study is the different sample sizes across tasks. However, it is unlikely that this would explain the results reported given that no significant demographic differences existed between samples. Overall, the studies presented demonstrate the influences of genetic variation in the DA D2 receptor gene that affect neurobiological responses to emotional, reward and stressful probes. These data are of direct relevance to the understanding of vulnerability and resiliency for the development of clinical conditions subserved by those mechanisms, which include the mood disorders (Dunlop and Nemeroff, 2007), addictions (Ungless et al., 2010) and even persistent pain conditions (Apkarian et al., 2010), where variations in DA function are thought to play a role.

Conflicts of interest

None.

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Supplementary material

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References


