Published in final edited form as:

Cortex. 2013 March ; 49(3): 877–890. doi:10.1016/j.cortex.2012.01.010.

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

Marta Peciña¹, Brian J. Mickey^{1,3}, Tiffany Love¹, Heng Wang¹, Scott A. Langenecker^{1,3}, Colin Hodgkinson², Pei-Hong Shen², Sandra Villafuerte¹, David Hsu¹, Sara L. Weisenbach¹, Christian S. Stohler⁵, David Goldman², and Jon-Kar Zubieta^{1,3,3,*}

¹Molecular and Behavioral Neuroscience Institute, University of Michigan, Ann Arbor, Michigan.

²Laboratory of Neurogenetics, National Institute on Alcohol Abuse and Alcoholism, National Institutes of Health, Rockville, Maryland. ³Departments of Psychiatry and Radiology, University of Michigan, Ann Arbor, Michigan. ⁴Departments of Psychiatry and Radiology, University of Michigan, Ann Arbor, Michigan. ⁵University of Maryland, School of Dentistry

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 interindividual 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 (fMRI) 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 D_{2/3} receptor mediated neurotransmission confirmed a gene effect on the functional connectivity of the DLPFC during reward anticipation and subcortical stress induced dopamine 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.

Keywords

imaging; dopamine; DLPFC; reward; emotion

* To whom correspondence should be addressed Jon-Kar Zubieta, MD, PhD University of Michigan 205 Zina Pitcher Place Ann Arbor, MI 48109-0720 Phone: 734-763-6843, Fax: 734-647-4130 zubieta@umich.edu.

Conflicts of Interest: None

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 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.

METHODS

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 hours 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.

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, Yuan 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 ancestry informative markers. 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 BOLD signal change or NEO scores, respectively. Thus, no confounding was present due to ethnic differences. Moreover, genotype and allele frequencies were similar for both Caucasians and no-Caucasians groups.

fMRI Tasks

For each task stimuli were projected onto a screen at the rear of the magnet using a 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). Subjects responded to cues via a MRI compatible response pad (Psychology Software Tools Inc., Pittsburgh PA).

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-s trials and each subject completed 2 runs. A trial consisted of a cue representing a monetary value ($\pm\$0.20$, $\pm\$1.00$, and $\pm\$5.00$, $\$0$) 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 $45 \pm 24\%$ of the gain trials, and $44 \pm 24\%$ of the loss response trials. Their average reaction time on gain trials was (165 ± 41.34) and on loss trials was (165 ± 43.10).

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 each displayed for 3 s each, separated by a fixation cross displayed for 1 s. 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-second epochs of positive, negative, or neutral words (six words of a single valence per block), separated by 18-second resting epochs. Subjects were instructed to relax and look at the crosshair during the rest periods. Each fMRI run included 6 emotional word blocks (each followed by a resting block), counterbalanced 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.

fMRI data acquisition

The blood oxygenation level dependent (BOLD) signal was measured using a GE Signa 3-Tesla scanner (General Electric, Milwaukee WI) with standard RF coil, using a T2* weighted pulse sequence (single-shot combined spiral in/out, gradient echo; repetition time = 2 s; echo time = 30 ms; 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 (Acqirre 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 ms, echo time= 3.4 ms, flip-angle= 25 deg, 124 contiguous images, 1.5-mm thickness). To allow comparisons between individuals, subject's MRI and functional images were co-registered 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 (full width at half maximum 6-mm) to reduce residual inter-individual variability. Smoothed functional images were band pass-filtered with a 128 second high pass filter to eliminate low frequency signals.

fMRI data analysis

The BOLD responses were modeled with SPM2 software (Department of Cognitive Neurology, Wellcome Trust Centre for Neuroimaging, London, England) using a general linear model and canonical hemodynamic response function. Statistical analysis proceeded in 2 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 placed 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 tests were also applied to 2 primary contrast

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 2 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.

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. 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 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 regions 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 $p < 0.05$ FDR, two-sided. ROI-based connectivity maps were generated by CONN.

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 the MID, would show increases in DA release during a pain challenge in those regions (Ko et al., 2008).

A single PET study with [^{11}C]raclopride, a DA $\text{D}_{2/3}$ 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 [^{11}C] raclopride administered to each subject was 15.0 ± 2.2 mCi. Fifty percent of the [^{11}C] 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 minutes with an increasing duration (30 seconds up to 10 minutes) 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 (K_1 ratio), and (b) a receptor-related measure at equilibrium (binding potential at equilibrium, BPeq), the latter using data obtained from 35–45 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 ($B_{\max}/K_d$) (Carson et al., 1997). For each scan, two measures were calculated, baseline DA $D_{2/3}$ 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 Montreal Neurological Institute (MNI) atlas template. To compensate for small residual anatomic variations across subjects and to improve signal to noise ratios, a three-dimensional Gaussian filter (Full width at half maximum: FWHM 6 mm) was applied to each image.

PET data analysis

A mixed model was conducted in SPM5 (SPM5; Wellcome Department of Cognitive Neurology, University College, London, England), where each genotype represented the between subjects factor and the 2 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 > 0.2) was used.

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.

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 $p < 0.01$ 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 SPM (Friston et al., 1994) where the resulting voxel wise maps ($2 \times 2 \times 2$ mm) were thresholded with 2-sided uncorrected $P < .001$ and extent $k < 61$ voxels (488mm³), which protected against overall type I error at $P < .01$ according to Monte Carlo simulations with AlphaSim (Ward, 2010) as previously described (Mickey et al., 2011).

Next, in exploratory analyses, we examined the individual SNPs (rs4274224 and rs451480) within the significant haplotype block in the EWT and MID using the same statistical threshold. Based on these findings, we then performed further exploratory analyses on the two SNPs where significant effects on reward and/or emotional processing were found (rs4274224 and rs12364283) using functional connectivity, PET and dopamine related personality traits. Functional connectivity statistical analyses have been described in detail above. For the PET data, due to the *a priori* hypothesis concerning the striatum, the summary statistical maps were thresholded at $p < 0.005$ uncorrected for multiple comparisons (Friston, 1997), with a voxel extent greater than 10 voxels. Planned correlations between imaging data and Openness to Experience and Extraversion personality traits were conducted with Pearson correlations at $p < 0.05$.

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).

RESULTS

Main Effects of fMRI Task

Monetary Incentive Delay Task (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 Table1).

Emotional Word Task (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 Table1).

DRD2 effect on fMRI Task

Haplotype-based analysis—86 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 (Figure 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.

Diploypes 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. Diploypes groups with less than 5 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 4 haplotype analysis and one *a priori* SNP (rs12364283) analysis a $p < 0.01$ 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/488mm³), 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 4 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 dorsolateral prefrontal cortex (DLPFC) for both gain minus null contrast [(x, y, z, MNI coordinates, cluster size mm³, Z); (-50, 42, 12, 1512 mm³, Z=4.09)] and loss minus null contrast (-48, 40, 6, 2936 mm³, 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/GC; AC/GT; GT/GT) (See supplementary Figure 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 mm³, Z=3.66). PSC in the subgenual ACC was extracted for *post hoc* analysis, revealing a greater activation of the sgACC in the AC/AC diplotype group (AC/AC>AC/GC, AC/GT, GC/GT) (See supplementary Figure 1).

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 block 2, but the magnitude was larger for rs4274224. No significant effect was observed for the other SNP in the 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 3 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 mm³, Z=4.35); loss-null: -48, 42, 6, 4304 mm³, 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 (Figure 2). Linear effects were also observed in the temporal cortex during gain minus null contrast (-52, 10, -16, P<0.001, cluster=544 mm³) and loss minus null contrast (-54, 6, -14, P<0.001, cluster=696 mm³) (GG>AG>AA).

EWT: Similarly to its haplotype block, the ANCOVA model showed a significant main effect of rs4274224 in the left pregenual and subgenual ACC (Brodmann areas 32/25) for negative *minus* neutral words contrasts (-2, 16, -4, 496 mm³, 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 (Figure 2, Table 2). Linear effects were also observed in the dorsal ACC (-12, -70, 24, P<0.001, cluster=976 mm³) and occipital cortex (-2, -40, 50, P<0.001, cluster=1472 mm³) (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 rs12364283 in the right frontal cortex (BA 10) and (42,

40, 10, 616 mm³, $Z=3.81$) and as a trend in the left ACC (BA 32) (-6, 46, 4, 448 mm³, $Z=4$) during gain minus null contrast (T/T>T/C) (Figure 2). No significant effects were found for the inverse contrast (T/C >T/T) or during emotional processing.

Gene effect of rs4274224 on DLPFC 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 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: $\beta = -0.01$, $t = -0.36$; left: $\beta = 0.01$, $t = 0.43$]; GG [right: $\beta = -0.01$, $t = -0.20$; left: $\beta = -0.02$, $t = -0.41$], all p 's > 0.05). 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: $\beta = -0.11$, $t = -2.24$, $p < .09$, 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) ($r = -.41$, $p = .004$) and losses ($r = -.46$, $p = .002$) as observed in AG subjects and explained 20% of the variance of the activation signal.

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: $r = -.24$, $p < .001$; right: $r = .27$, $p < .001$), although no significant differences were observed among genotype groups.

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 3 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 differences with respect to sex or ethnicity among the 2 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 mm³= 1360, $z=3.19$, $p < 0.001$; left: x, y, z , -17, 26, 10, cluster= 912 mm³, $z=3.47$, $p < 0.001$) and the left anterior putamen (right, x, y, z coordinates (MNI) -20, 16, 3, cluster size in mm³= 1360, $z=3.19$, $p = 0.003$), compared to AA and GG subjects (Figure 3). Although not *a priori* hypothesized, these differences were also observed in the right putamen (right: x, y, z , 33, 1, 9, cluster= 1864 mm³, $z=3.33$, $p < 0.001$) and the nucleus accumbens bilaterally (right, x, y, z , 14, 15, -10, $z=2.48$, $p < 0.007$; left, x, y, z , -7, 16, -7,

$z=2.51$, $p<0.006$). No genotype effects were observed for baseline binding measures. No genotype effects on dopamine release or baseline were found for rs12364283.

The presence of extensive corticostriatal 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 ($r=0.46$, $p<0.001$) and at trend levels with left nucleus accumbens ($r=0.27$, $p=0.07$). DLPFC activation during gain and loss anticipation was on the other hand negatively correlated with DA release in the left caudate (gain: $r=-0.37$, $p=0.013$; loss: $r=-0.42$, $p=0.004$), the right caudate/putamen (gain: $r=-0.46$, $p=0.001$; loss: $r=-0.47$, $p=0.001$), and with trends in the same direction in the nucleus accumbens bilaterally (gain: left: $r=-0.28$, $p=0.06$; loss: right: $r=-0.28$, $p=0.06$).

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 ($F=2.86$, $p=0.079$) was observed, with largest increases in AA subjects. No gene effects ($F=1.08$, $p=0.35$) were found for changes in positive affect.

Trait Personality Measures

Finally, we tested for effects of rs4274224 and rs12346283 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, $df_{2,72}$, $F=4.02$, $p=0.023$). 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. $=-0.87$, s.e. $=0.32$, $p=0.026$) and as a trend, the GG volunteers (mean dif. $=-0.81$, s.e. $=0.36$, $p=0.089$). 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 Openness to Experience domain ($r=-0.25$, $p=0.05$), and activation of the left DLPFC during anticipation of gain showed a trend towards a positive correlation with this measure ($r=0.26$, $p=0.08$).

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 3 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 (Aleman 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 (Blasi et al., 2009). None of these studies have reported a specific effect in the sgACC. However, the role of the sgACC in processing negative affective stimuli is well described (Phan et al., 2002). 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 interindividual 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 D_{2/3} 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 DLPFC 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 DLPFC 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 DLPFC and sgACC during motivated behavior for AA homozygote 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 feedback is primarily exerted through glutamatergic efferents from the PFC (Christie et al., 1985). In humans, disruption of glutamatergic neurotransmission with ketamine leads to increased amphetamine-induced DA release in the striatum (Kegeles et al., 2000) and reductions of frontal excitatory input to mesolimbic and mesocortical DA neurons are associated with an excessive mesolimbic response to exogenous stimuli such as stressors (Laruelle et al., 2003; Moore et al., 1999; Meyer-Lindenberg et al., 2002). Importantly, in the rodent (Carr and Sesack, 2000), PFC afferents to midbrain neurons projecting to the striatum are selectively located on GABAergic (inhibitory) neurons, suggesting a direct anatomical mechanism by which a pathological decrease in PFC excitatory output would lead to disinhibition of striatal dopaminergic function. This anatomical account is also consistent with the proposal that prefrontal cortical glutamatergic projections are a ‘brake’ on the striatal DA system (Carlsson et al., 2001). In fact, changes in stress-induced DA release in these regions were negatively correlated with left DLPFC activation during reward anticipation. Similarly, the positive relationship between the sgACC activity during negative emotional processing and ventral striatal DA release during stress is not surprising given the dense unreciprocated glutamatergic projections from the sgACC to the ventral striatum (Hamani et al., 2011), and recent findings suggesting that sadness-induced DA release takes place in dorsal areas of the caudate and putamen (Badgaiyan, 2010).

Although the mechanism through which this particular SNP (rs4274224) might affect receptor function is presently unknown, other *DRD2* intronic SNPs have shown to affect transcription in cortical areas (Zhang et al., 2007; Bertolino et al., 2009; Blasi et al., 2009) by affecting alternative splicing of exon 6 to yield *DRD2L* (long, primarily postsynaptic) and *DRD2S* (short, primarily presynaptic) isoforms (Khan et al., 1998; Bertolino et al., 2009). Studies in mice have found that mice expressing only the short isoform display reduced exploratory behavior and increased latency to escape harmful situations (Hranilovic et al., 2008), suggesting a more anxious phenotype. Differences in the D2S/D2L ratio within the prefrontal cortex might explain why AA individuals show greater responses in the sgACC to negative lexical stimuli, greater stress-induced DA release and lower levels of Openness to Experience, reflecting a more “internalizing” and potentially vulnerable phenotype. AG individuals, on the other hand, that demonstrate greater responses in the DLPFC in a motivational context as well as higher levels of Openness to Experience may represent a more resilient phenotype. Future *in vivo* studies, however, will have to confirm the possibility that this SNP will influence the formation of these two isoforms.

It should also be noted that both behavioral trait and imaging data have shown molecular heterosis, which might occur 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 too 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 DLPFC activation during the expectation of monetary gains and losses.

In summary, our results implicate the rs4274224 SNP in interindividual 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.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Work was supported by R01 DA 016423, R01 DA 022520 (JKZ), a career development award, K01 MH 074459 (SAL), the Phil F. Jenkins Foundation and the Alicia Koplowitz Foundation. We would also like to acknowledge the contribution of the technologists of the functional MRI laboratory and the PET Center at the University of Michigan.

REFERENCES

- Aarts E, Roelofs A, Franke B, Rijpkema M, Fernandez G, Helmich RC, Cools R. Striatal dopamine mediates the interface between motivational and cognitive control in humans: evidence from genetic imaging. *Neuropsychopharmacology*. 2010; 35(9):1943–1951. [PubMed: 20463658]
- Acquiere, GK.; D'Sposito, M. *Medical Radiology: Functional MRI*. Springer-Verlag; Heidelberg: 1999. Experimental Design for Brain fMRI.; p. 369-380.
- Aleman A, Swart M, van Rijn S. Brain imaging, genetics and emotion. *Biological Psychology*. 2008; 79(1):58–69. [PubMed: 18329779]
- Apkarian AV, Hashmi JA, Baliki MN. Pain and the brain: specificity and plasticity of the brain in clinical chronic pain. *Pain*. 2010; 152(3 Suppl):S49–64. [PubMed: 21146929]
- Badgaiyan RD. Dopamine is released in the striatum during human emotional processing. *Neuroreport*. 2010; 21(18):1172–1176. [PubMed: 21057339]

- Barbas H, Pandya DN. Architecture and intrinsic connections of the prefrontal cortex in the rhesus monkey. *The Journal of Comparative Neurology*. 1989; 286(3):353–375. [PubMed: 2768563]
- Behzadi Y, Restom K, Liao J, Liu TT. A component based noise correction method (CompCor) for BOLD and perfusion based fMRI. *Neuroimage*. 2007; 37(1):90–101. [PubMed: 17560126]
- Berridge KC, Robinson TE. What is the role of dopamine in reward: hedonic impact, reward learning, or incentive salience? *Brain Research Reviews*. 1998; 28(3):309–369. [PubMed: 9858756]
- Bertolino A, Fazio L, Caforio G, Blasi G, Rampino A, Romano R, Di Giorgio A, Taurisano P, Papp A, Pinsonneault J, Wang D, Nardini M, Popolizio T, Sadee W. Functional variants of the dopamine receptor D2 gene modulate prefronto-striatal phenotypes in schizophrenia. *Brain*. 2009; 132(Pt 2): 417–425. [PubMed: 18829695]
- Blasi G, Lo Bianco L, Taurisano P, Gelao B, Romano R, Fazio L, Papazacharias A, Di Giorgio A, Caforio G, Rampino A, Masellis R, Papp A, Ursini G, Sinibaldi L, Popolizio T, Sadee W, Bertolino A. Functional variation of the dopamine D2 receptor gene is associated with emotional control as well as brain activity and connectivity during emotion processing in humans. *The Journal of Neuroscience*. 2009; 29(47):14812–14819. [PubMed: 19940176]
- Bradley, M.; Lang, P. Technical report C-1. The Center for Research in Psychophysiology, University of Florida; Gainesville, FL: 1999. Affective norms for English words (ANEW): Stimuli, instruction manual and affective ratings..
- Brett, M.; Anton, J-L.; Romain Valabregue, J-BP. Region of interest analysis using an SPM toolbox [abstract].. Presented at the 8th International Conference on Functional Mapping of the Human Brain; Sendai, Japan. June 2-6; 2002. CD-ROM in *NeuroImage*
- Camara E, Kramer UM, Cunillera T, Marco-Pallares J, Cucurell D, Nager W, Mestres-Misse A, Bauer P, Schule R, Schols L, Tempelmann C, Rodriguez-Fornells A, Munte TF. The effects of COMT (Val108/158Met) and DRD4 (SNP -521) dopamine genotypes on brain activations related to valence and magnitude of rewards. *Cerebral Cortex*. 2010; 20(8):1985–1996. [PubMed: 20038544]
- Carlsson A, Waters N, Holm-Waters S, Tedroff J, Nilsson M, Carlsson ML. Interactions between monoamines, glutamate, and GABA in schizophrenia: new evidence. *Annual Review of Pharmacology and Toxicology*. 2001; 41:237–260.
- Carr DB, Sesack SR. Projections from the rat prefrontal cortex to the ventral tegmental area: target specificity in the synaptic associations with mesoaccumbens and mesocortical neurons. *The Journal of Neuroscience*. 2000; 20(10):3864–3873. [PubMed: 10804226]
- Carson RE, Breier A, de Bartolomeis A, Saunders RC, Su TP, Schmall B, Der MG, Pickar D, Eckelman WC. Quantification of amphetamine-induced changes in [¹¹C]raclopride binding with continuous infusion. *Journal of Cerebral Blood Flow & Metabolism*. 1997; 17(4):437–447. [PubMed: 9143226]
- Christie MJ, Bridge S, James LB, Beart PM. Excitotoxin lesions suggest an aspartatergic projection from rat medial prefrontal cortex to ventral tegmental area. *Brain Research*. 1985; 333(1):169–172. [PubMed: 2859910]
- Civelli O, Bunzow JR, Grandy DK. Molecular diversity of the dopamine receptors. *Annual Review of Pharmacology and Toxicology*. 1993; 33:281–307.
- Cohen MX, Young J, Baek JM, Kessler C, Ranganath C. Individual differences in extraversion and dopamine genetics predict neural reward responses. *Cognitive Brain Research*. 2005; 25(3):851–861. [PubMed: 16289773]
- Comings DE, MacMurray JP. Molecular heterosis: a review. *Molecular Genetics and Metabolism*. 2000; 71(1-2):19–31. [PubMed: 11001792]
- Costa P, McRae R. Normal personality assessment in clinical practice: the NEO Personality Inventory. *Psychological Assessment*. 1992; 4(1):5–13.
- Danna CL, Elmer GI. Disruption of conditioned reward association by typical and atypical antipsychotics. *Pharmacology Biochemistry and Behavior*. 2010; 96(1):40–47.
- Depue RA, Collins PF. Neurobiology of the structure of personality: dopamine, facilitation of incentive motivation, and extraversion. *The Behavioral and Brain Science*. 1999; 22(3):491–517. discussion 518-469.

- DeYoung CG, Peterson JB, Higgins DM. Sources of openness/intellect: cognitive and neuropsychological correlates of the fifth factor of personality. *Journal of Personality*. 2005; 73(4): 825–858. [PubMed: 15958136]
- Dreher JC, Kohn P, Kolachana B, Weinberger DR, Berman KF. Variation in dopamine genes influences responsivity of the human reward system. *Proceedings of the National Academy of Science of the United States of America*. 2009; 106(2):617–622.
- Drevets WC, Savitz J, Trimble M. The subgenual anterior cingulate cortex in mood disorders. *CNS Spectrums*. 2008; 13(8):663–681. [PubMed: 18704022]
- Dunlop BW, Nemeroff CB. The role of dopamine in the pathophysiology of depression. *Archives of General Psychiatry*. 2007; 64(3):327–337. [PubMed: 17339521]
- Ebstein RP. The molecular genetic architecture of human personality: beyond self-report questionnaires. *Molecular Psychiatry*. 2006; 11(5):427–445. [PubMed: 16534505]
- Finckh U, von Widdern O, Giraldo-Velasquez M, Podschus J, Dufeu P, Sander T, Harms H, Schmidt LG, Rommelspacher H, Rolfs A. No association of the structural dopamine D2 receptor (DRD2) variant 311Cys with alcoholism. *Alcoholism: Clinical and Experimental Research*. 1996; 20(3): 528–532.
- Forbes EE, Brown SM, Kimak M, Ferrell RE, Manuck SB, Hariri AR. Genetic variation in components of dopamine neurotransmission impacts ventral striatal reactivity associated with impulsivity. *Molecular Psychiatry*. 2009; 14(1):60–70. [PubMed: 17893706]
- Frank MJ, Hutchison K. Genetic contributions to avoidance-based decisions: striatal D2 receptor polymorphisms. *Neuroscience*. 2009; 164(1):131–140. [PubMed: 19393722]
- Friston KJ. Imaging cognitive anatomy. *Trends in Cognitive Science*. 1997; 1(1):21–27.
- Friston KJ, Ashburner J, Frith CD, Poline JB, Heather JD, Frackowiak RSJ. Spatial registration and normalization of images. *Human Brain Mapping*. 3(165-89):1995.
- Friston KJ, Worsley KJ, Frackowiak RSJ, Mazziotta JC, Evans AC. Assessing the significance of focal activations using their spatial extent. *Human Brain Mapping*. 1994; 1:210–220.
- Gilbert AM, Fiez JA. Integrating rewards and cognition in the frontal cortex. *Cognitive Affective, & Behavioral Neuroscience*. 2004; 4(4):540–552.
- Gjedde A, Kumakura Y, Cumming P, Linnet J, Moller A. Inverted-U-shaped correlation between dopamine receptor availability in striatum and sensation seeking. *Proceedings of the National Academy of Science of the United States of America*. 2010; 107(8):3870–3875.
- Glover GH, Law CS. Spiral-in/out BOLD fMRI for increased SNR and reduced susceptibility artifacts. *Magnetic Resonance in Medicine*. 2001; 46(3):515–522. [PubMed: 11550244]
- Goldman-Rakic PS. The cortical dopamine system: role in memory and cognition. *Advances in Pharmacology*. 1998; 42:707–711. [PubMed: 9327997]
- Haber SN, Knutson B. The reward circuit: linking primate anatomy and human imaging. *Neuropsychopharmacology*. 2010; 35(1):4–26. [PubMed: 19812543]
- Hahn T, Heinzel S, Dresler T, Plichta MM, Renner TJ, Markulin F, Jakob PM, Lesch KP, Fallgatter AJ. Association between reward-related activation in the ventral striatum and trait reward sensitivity is moderated by dopamine transporter genotype. *Human Brain Mapping*. 2010
- Hamani C, Mayberg H, Stone S, Laxton A, Haber S, Lozano AM. The subcallosal cingulate gyrus in the context of major depression. *Biological Psychiatry*. 2011; 69(4):301–308. [PubMed: 21145043]
- Hamidovic A, Dlugos A, Skol A, Palmer AA, de Wit H. Evaluation of genetic variability in the dopamine receptor D2 in relation to behavioral inhibition and impulsivity/sensation seeking: an exploratory study with d-amphetamine in healthy participants. *Experimental and Clinical Psychopharmacology*. 2009; 17(6):374–383. [PubMed: 19968402]
- Heitzeg MM, Nigg JT, Yau WY, Zubieta JK, Zucker RA. Affective circuitry and risk for alcoholism in late adolescence: differences in frontostriatal responses between vulnerable and resilient children of alcoholic parents. *Alcoholism: Clinical and Experimental Research*. 2008; 32(3):414–426.
- Herrmann MJ, Wurflein H, Schreppe T, Koehler S, Muhlberger A, Reif A, Canli T, Romanos M, Jacob CP, Lesch KP, Fallgatter AJ. Catechol-O-methyltransferase Val158Met genotype affects neural correlates of aversive stimuli processing. *Cognitive, Affective, & Behavioral Neuroscience*. 2009; 9(2):168–172.

- Horvitz JC. Mesolimbocortical and nigrostriatal dopamine responses to salient non-reward events. *Neuroscience*. 2000; 96(4):651–656. [PubMed: 10727783]
- Hranilovic D, Bucan M, Wang Y. Emotional response in dopamine D2L receptor-deficient mice. *Behavioral Brain Research*. 2008; 195(2):246–250.
- Ikemoto S, Panksepp J. The role of nucleus accumbens dopamine in motivated behavior: a unifying interpretation with special reference to reward-seeking. *Brain Research Reviews*. 1999; 31(1):6–41. [PubMed: 10611493]
- Innis RB, Malison RT, al-Tikriti M, Hoffer PB, Sybirska EH, Seibyl JP, Zoghbi SS, Baldwin RM, Laruelle M, Smith EO, et al. Amphetamine-stimulated dopamine release competes in vivo for [123I]IBZM binding to the D2 receptor in nonhuman primates. *Synapse*. 1992; 10(3):177–184. [PubMed: 1532675]
- Kegeles LS, Abi-Dargham A, Zea-Ponce Y, Rodenhiser-Hill J, Mann JJ, Van Heertum RL, Cooper TB, Carlsson A, Laruelle M. Modulation of amphetamine-induced striatal dopamine release by ketamine in humans: implications for schizophrenia. *Biological Psychiatry*. 2000; 48(7):627–640. [PubMed: 11032974]
- Khan ZU, Mrzljak L, Gutierrez A, de la Calle A, Goldman-Rakic PS. Prominence of the dopamine D2 short isoform in dopaminergic pathways. *Proceedings of the National Academy of Science of the United States of America*. 1998; 95(13):7731–7736.
- Kirsch P, Reuter M, Mier D, Lonsdorf T, Stark R, Gallhofer B, Vaitl D, Hennig J. Imaging gene-substance interactions: the effect of the DRD2 Taq1A polymorphism and the dopamine agonist bromocriptine on the brain activation during the anticipation of reward. *Neuroscience Letter*. 2006; 405(3):196–201.
- Knutson B, Westdorp A, Kaiser E, Hommer D. fMRI visualization of brain activity during a monetary incentive delay task. *Neuroimage*. 2000; 12(1):20–27. [PubMed: 10875899]
- Ko JH, Monchi O, Pito A, Bloomfield P, Houle S, Strafella AP. Theta burst stimulation-induced inhibition of dorsolateral prefrontal cortex reveals hemispheric asymmetry in striatal dopamine release during a set-shifting task: a TMS-[(11)C]raclopride PET study. *European Journal of Neuroscience*. 2008; 28(10):2147–2155. [PubMed: 19046396]
- Kobiella A, Vollstadt-Klein S, Buhler M, Graf C, Buchholz HG, Bernow N, Yakushev IY, Landvogt C, Schreckenberger M, Grunder G, Bartenstein P, Fehr C, Smolka MN. Human dopamine receptor D2/D3 availability predicts amygdala reactivity to unpleasant stimuli. *Human Brain Mapping*. 2010; 31(5):716–726. [PubMed: 19904802]
- Krugel LK, Biele G, Mohr PN, Li SC, Heekeren HR. Genetic variation in dopaminergic neuromodulation influences the ability to rapidly and flexibly adapt decisions. *Proc Natl Acad Sci U S A*. 2009; 106(42):17951–17956. [PubMed: 19822738]
- Laruelle M, Kegeles LS, Abi-Dargham A. Glutamate, dopamine, and schizophrenia: from pathophysiology to treatment. *Annals of the New York Academy of Science*. 2003; 1003:138–158.
- Lawrence AD, Goerendt IK, Brooks DJ. Impaired recognition of facial expressions of anger in Parkinson's disease patients acutely withdrawn from dopamine replacement therapy. *Neuropsychologia*. 2007; 45(1):65–74. [PubMed: 16780901]
- Lelli-Chiesa G, Kempton MJ, Jogia J, Tatarelli R, Girardi P, Powell J, Collier DA, Frangou S. The impact of the Val158Met catechol- O-methyltransferase genotype on neural correlates of sad facial affect processing in patients with bipolar disorder and their relatives. *Psychological Medicine*. 2010; 1–10.
- Lozano AM, Mayberg HS, Giacobbe P, Hamani C, Craddock RC, Kennedy SH. Subcallosal cingulate gyrus deep brain stimulation for treatment-resistant depression. *Biological Psychiatry*. 2008; 64(6):461–467. [PubMed: 18639234]
- Lucht M, Barnow S, Schroeder W, Grabe HJ, Roskopf D, Brummer C, John U, Freyberger HJ, Herrmann FH. Alcohol consumption is associated with an interaction between DRD2 exon 8 A/A genotype and self-directedness in males. *Neuropsychobiology*. 2007; 56(1):24–31. [PubMed: 17943029]
- Maldjian JA, Laurienti PJ, Kraft RA, Burdette JH. An automated method for neuroanatomic and cytoarchitectonic atlas-based interrogation of fMRI data sets. *Neuroimage*. 2003; 19(3):1233–1239. [PubMed: 12880848]

- Mayberg HS. Modulating dysfunctional limbic-cortical circuits in depression: towards development of brain-based algorithms for diagnosis and optimised treatment. *British Medical Bulletin*. 2003; 65:193–207. [PubMed: 12697626]
- Mayberg HS, Liotti M, Brannan SK, McGinnis S, Mahurin RK, Jerabek PA, Silva JA, Tekell JL, Martin CC, Lancaster JL, Fox PT. Reciprocal limbic-cortical function and negative mood: converging PET findings in depression and normal sadness. *American Journal of Psychiatry*. 1999; 156(5):675–682. [PubMed: 10327898]
- Mayberg HS, Lozano AM, Voon V, McNeely HE, Seminowicz D, Hamani C, Schwalb JM, Kennedy SH. Deep brain stimulation for treatment-resistant depression. *Neuron*. 2005; 45(5):651–660. [PubMed: 15748841]
- Meyer-Lindenberg A, Miletich RS, Kohn PD, Esposito G, Carson RE, Quarantelli M, Weinberger DR, Berman KF. Reduced prefrontal activity predicts exaggerated striatal dopaminergic function in schizophrenia. *Nature Neuroscience*. 2002; 5(3):267–271.
- Meyer CR, Boes JL, Kim B, Bland PH, Zasadny KR, Kison PV, Koral K, Frey KA, Wahl RL. Demonstration of accuracy and clinical versatility of mutual information for automatic multimodality image fusion using affine and thin-plate spline warped geometric deformations. *Medical Image Analysis*. 1997; 1(3):195–206. [PubMed: 9873906]
- Mickey BJ, Zhou Z, Heitzeg MM, Heinz E, Hodgkinson CA, Hsu DT, Langenecker SA, Love TM, Pecina M, Shafir T, Stohler CS, Goldman D, Zubieta JK. Emotion processing, major depression, and functional genetic variation of neuropeptide y. *Archives of General Psychiatry*. 2011; 68(2): 158–166. [PubMed: 21300944]
- Minoshima S, Koeppe RA, Mintun MA, Berger KL, Taylor SF, Frey KA, Kuhl DE. Automated detection of the intercommissural line for stereotactic localization of functional brain images. *Journal of Nuclear Medicine*. 1993; 34(2):322–329. [PubMed: 8429356]
- Mizrahi R, Rusjan P, Agid O, Graff A, Mamo DC, Zipursky RB, Kapur S. Adverse subjective experience with antipsychotics and its relationship to striatal and extrastriatal D2 receptors: a PET study in schizophrenia. *American Journal of Psychiatry*. 2007; 164(4):630–637. [PubMed: 17403977]
- Monte-Silva K, Kuo MF, Thirugnanasambandam N, Liebetanz D, Paulus W, Nitsche MA. Dose-dependent inverted U-shaped effect of dopamine (D2-like) receptor activation on focal and nonfocal plasticity in humans. *Journal of Neuroscience*. 2009; 29(19):6124–6131. [PubMed: 19439590]
- Moore H, West AR, Grace AA. The regulation of forebrain dopamine transmission: relevance to the pathophysiology and psychopathology of schizophrenia. *Biological Psychiatry*. 1999; 46(1):40–55. [PubMed: 10394473]
- Noble EP. D2 dopamine receptor gene in psychiatric and neurologic disorders and its phenotypes. *American Journal of Medical Genetics Part B: Neuropsychiatry Genetics*. 2003; 116B(1):103–125.
- Noll, DC. IEEE International Symposium on Biomedical Imaging. Washington, DC.: 2002. Rapid MR image acquisition in the presence of background gradients..
- Ongur D, Drevets WC, Price JL. Glial reduction in the subgenual prefrontal cortex in mood disorders. *Proceedings of the National Academy of Science of the United States of America*. 1998; 95(22): 13290–13295.
- Petrides M, Pandya DN. Dorsolateral prefrontal cortex: comparative cytoarchitectonic analysis in the human and the macaque brain and corticocortical connection patterns. *Eur J Neurosci*. 1999; 11(3):1011–1036. [PubMed: 10103094]
- Phan KL, Wager T, Taylor SF, Liberzon I. Functional neuroanatomy of emotion: a meta-analysis of emotion activation studies in PET and fMRI. *Neuroimage*. 2002; 16(2):331–348. [PubMed: 12030820]
- Pochon JB, Levy R, Fossati P, Lehericy S, Poline JB, Pillon B, Le Bihan D, Dubois B. The neural system that bridges reward and cognition in humans: an fMRI study. *Proceedings of the National Academy of Science of the United States of America*. 2002; 99(8):5669–5674.
- Rasch B, Spalek K, Buholzer S, Luechinger R, Boesiger P, de Quervain DJ, Papassotiropoulos A. Aversive stimuli lead to differential amygdala activation and connectivity patterns depending on

- catechol-O-methyltransferase Val158Met genotype. *Neuroimage*. 2010; 52(4):1712–1719. [PubMed: 20510373]
- Ressler KJ, Mayberg HS. Targeting abnormal neural circuits in mood and anxiety disorders: from the laboratory to the clinic. *Nature Neuroscience*. 2007; 10(9):1116–1124.
- Schmack K, Schlagenhauf F, Sterzer P, Wrase J, Beck A, Dembler T, Kalus P, Puls I, Sander T, Heinz A, Gallinat J. Catechol-O-methyltransferase val158met genotype influences neural processing of reward anticipation. *Neuroimage*. 2008; 42(4):1631–1638. [PubMed: 18634888]
- Schott BH, Minuzzi L, Krebs RM, Elmenhorst D, Lang M, Winz OH, Seidenbecher CI, Coenen HH, Heinze HJ, Zilles K, Duzel E, Bauer A. Mesolimbic functional magnetic resonance imaging activations during reward anticipation correlate with reward-related ventral striatal dopamine release. *Journal of Neuroscience*. 2008; 28(52):14311–14319. [PubMed: 19109512]
- Schultz W. Behavioral theories and the neurophysiology of reward. *Annual Review of Psychology*. 2006; 57:87–115.
- Scott DJ, Heitzeg MM, Koeppe RA, Stohler CS, Zubieta JK. Variations in the human pain stress experience mediated by ventral and dorsal basal ganglia dopamine activity. *Journal of Neuroscience*. 2006; 26(42):10789–10795. [PubMed: 17050717]
- Seminowicz DA, Mayberg HS, McIntosh AR, Goldapple K, Kennedy S, Segal Z, Rafi-Tari S. Limbic-frontal circuitry in major depression: a path modeling metanalysis. *Neuroimage*. 2004; 22(1):409–418. [PubMed: 15110034]
- Smillie LD, Cooper AJ, Proitsi P, Powell JF, Pickering AD. Variation in DRD2 dopamine gene predicts Extraverted personality. *Neuroscience Letters*. 2010; 468(3):234–237. [PubMed: 19897017]
- Smith JW, Fetsko LA, Xu R, Wang Y. Dopamine D2L receptor knockout mice display deficits in positive and negative reinforcing properties of morphine and in avoidance learning. *Neuroscience*. 2002; 113(4):755–765. [PubMed: 12182883]
- Smolka MN, Schumann G, Wrase J, Grusser SM, Flor H, Mann K, Braus DF, Goldman D, Buchel C, Heinz A. Catechol-O-methyltransferase val158met genotype affects processing of emotional stimuli in the amygdala and prefrontal cortex. *Journal of Neuroscience*. 2005; 25(4):836–842. [PubMed: 15673663]
- Swart M, Bruggeman R, Laroï F, Alizadeh BZ, Kema I, KorteKaas R, Wiersma D, Aleman A. COMT Val158Met polymorphism, verbalizing of emotion and activation of affective brain systems. *Neuroimage*. 2011; 55(1):338–344. [PubMed: 21156209]
- Thierry AM, Tassin JP, Blanc G, Glowinski J. Selective activation of mesocortical DA system by stress. *Nature*. 1976; 263(5574):242–244. [PubMed: 958479]
- Ungless MA, Argilli E, Bonci A. Effects of stress and aversion on dopamine neurons: implications for addiction. *Neuroscience & Biobehavioral Reviews*. 2010; 35(2):151–156. [PubMed: 20438754]
- Ward, BD. [January, 2010] <http://afni.nimh.nih.gov>. AlphaSim.
- Watanabe M. Cognitive and motivational operations in primate prefrontal neurons. *Reviews in the Neuroscience*. 1998; 9(4):225–241.
- Watanabe M. Role of anticipated reward in cognitive behavioral control. *Current Opinion in Neurobiology*. 2007; 17(2):213–219. [PubMed: 17336512]
- Watson D, Clark LA, Tellegen A. Development and validation of brief measures of positive and negative affect: the PANAS scales. *Journal of Personality and Social Psychology*. 1988; 54(6):1063–1070. [PubMed: 3397865]
- Williams LM, Gatt JM, Grieve SM, Dobson-Stone C, Paul RH, Gordon E, Schofield PR. COMT Val(108/158)Met polymorphism effects on emotional brain function and negativity bias. *Neuroimage*. 2010; 53(3):918–925. [PubMed: 20139013]
- Wise RA. Dopamine, learning and motivation. *Nature Reviews Neuroscience*. 2004; 5(6):483–494.
- Yacubian J, Sommer T, Schroeder K, Glascher J, Braus DF, Buchel C. Subregions of the ventral striatum show preferential coding of reward magnitude and probability. *Neuroimage*. 2007; 38(3):557–563. [PubMed: 17889562]
- Zhang Y, Bertolino A, Fazio L, Blasi G, Rampino A, Romano R, Lee ML, Xiao T, Papp A, Wang D, Sadee W. Polymorphisms in human dopamine D2 receptor gene affect gene expression, splicing,

- and neuronal activity during working memory. *Proceedings of the National Academy of Sciences of the United States of America*. 2007; 104(51):20552–20557. [PubMed: 18077373]
- Zubieta JK, Smith YR, Bueller JA, Xu Y, Kilbourn MR, Jewett DM, Meyer CR, Koeppe RA, Stohler CS. mu-opioid receptor-mediated antinociceptive responses differ in men and women. *Journal of Neuroscience*. 2002; 22(12):5100–5107. [PubMed: 12077205]

Figure 1. Linkage disequilibrium (LD) plot of the 14 *DRD2* loci included in the analysis. The haplotype of *DRD2* is similar to the haplotypes identified in the HapMap Project.

Figure 2.

The effect of *DRD2* rs4274224 in the left DLPFC (BA 46/9) during monetary gain/loss minus null condition are displayed in red (gain) and pink (loss). The bar graph shows the % signal change in the left DLPFC for gain/loss minus null condition for each genotype. The effect of *DRD2* rs4274224 on the bilateral subgenual and pregenual AC (BA25/32) during the condition Neg-Neu words is displayed in blue. The bar graph shows the % signal change in the subgenual while processing negative minus neutral words for each genotype. No effect of *DRD2* rs4274224 was found during the condition Pos-Neu words. The correlation between areas of activation showed that activation in the subgenual ACC was negatively correlated to the activation in the DLPFC and explained up to 21% of its variance.

Figure 3.

Effects of *DRD2* rs4274224 on mean changes in binding potential (BP) and its standard errors 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.

Table 1

Demographics, personality and behavioral data.

Demographics	AA (n=23)		AG (n=43)		GG (n=20)		ANOVA/ χ^2	
	Mean	SE	Mean	SE	Mean	SE	F	Sig
Age	32	2.31	27.28	1.023	25.7	0.711	4.273	0.017
Sex (M/F)	9/14		23/20		11/9		1.49	0.473
Ethnicity (Caucasian/Total)	17/23		32/43		15/20		5.031	0.540
Caucasian AIM	0.72	0.79	0.63	0.06	0.60	0.10	0.525	0.769
African American AIM	0.06	0.04	0.17	0.05	0.16	0.07	0.479	0.787
Asian AIM	0.09	0.04	0.06	0.02	0.08	0.04	2.64	0.26
NEO	AA (n=17)		AG (n=36)		GG (n=19)		ANOVA	
OPENNESS	115.88	4.746	129.5	3.227	128.84	4.101	4.014	0.023
Fantasy	18.35	1.422	19.97	0.94	22.84	0.09	3.955	0.024
Actions	15.12	1.039	18.97	0.622	16.58	0.846	5.618	0.006