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ORIGINAL INVESTIGATION

# *DRD2* genotype predicts prefrontal activity during working memory after stimulation of D2 receptors with bromocriptine

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**Abstract**
*Rationale* Pharmacological stimulation of D2 receptors modulates prefrontal neural activity associated with working memory (WM) processing. The T allele of a functional single-nucleotide polymorphism (SNP) within *DRD2* (rs1076560 G > T) predicts reduced relative expression of the D2S receptor isoform and less efficient neural cortical responses during WM tasks.
*Objective* We used functional MRI to test the hypothesis that *DRD2* rs1076560 genotype interacts with pharmacological stimulation of D2 receptors with bromocriptine on prefrontal responses during different loads of a spatial WM task (*N*-Back).
*Methods* Fifty-three healthy subjects (38 GG and 15 GT) underwent two 3-T functional MRI scans while performing the 1-, 2- and 3-Back versions of the *N*-Back WM task. Before the imaging sessions, either bromocriptine or placebo was administered to all subjects in a counterbalanced order. A factorial repeated-measures ANOVA within SPM8 ($p<0.05$, family-wise error corrected) was used.
*Results* On bromocriptine, GG subjects had reduced prefrontal activity at 3-Back together with a significant decrement in performance, compared with placebo. On the other hand, GT subjects had lower activity for the same level of performance at 1-Back but a trend for reduced behavioral performance in the face of unchanged activity at 2-Back.
*Conclusions* These results indicate that bromocriptine stimulation modulates prefrontal activity in terms of disengagement or of efficiency depending on *DRD2* genotype and working memory load.

**Keywords** Dopamine · Prefrontal cortex · Working memory · Bromocriptine · *DRD2* rs1076560 · fMRI

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## Introduction

Working memory (WM) refers to the ability to maintain, manipulate and access mental representations as needed to support complex cognition (Baddeley 1992). Single-cell recording studies in nonhuman primates and functional neuroimaging studies in humans support the critical involvement of the prefrontal cortex (PFC) in WM (Goldman-Rakic 1999; Callicott et al. 1999; Barbas 2000; Passingham and Sakai 2004). Prefrontal neuronal activity during performance of WM tasks is modulated by dopamine (Bertolino et al. 2006; Tan et al. 2007). More specifically, dopamine directly regulates firing of pyramidal neurons and of their GABA inhibitory surround within PFC to focus neuronal resources to the task at hand (Seamans and Yang 2004). The relationship between dopamine levels and neuronal activity describes a nonlinear function (inverted-U) (Seamans and Yang 2004). In other words, there is a critical range of dopamine stimulation within which task-related activity is more focused while task-unrelated activity is attenuated, i.e. more efficient neuronal activity for better behavioral performance (Mattay et al. 2003). Above or below this critical range of dopamine stimulation, behavioral performance is deteriorated and the balance between task-related and task-unrelated neuronal activity varies engendering globally attenuated or excessive PFC activity (Mattay et al. 2003). Several studies in nonhuman primates and in humans have demonstrated that this relationship is strongly modulated by dopamine D1 receptors (Sawaguchi and Goldman-Rakic 1991; Seamans and Yang 2004; Takahashi et al. 2008). However, other single-cell recording studies in nonhuman primates have also demonstrated that dopamine D2 receptors selectively modulate prefrontal neural activity associated with memory-guided responses during spatial WM (Wang et al. 2004). Further behavioral experiments in humans have indicated that systemic administration of D2 receptor agonists like bromocriptine or antagonists like haloperidol and sulpiride is respectively associated with relative improvement or deterioration of spatial WM performance (Luciana et al. 1992; Luciana and Collins 1997; Kimberg et al. 2001; Mehta et al. 2003, 2004; Fischer et al. 2010). Several studies have also reported that the drug-induced effects on cognitive tests are correlated with individual differences in WM capacity, often measured with the listening span task (Daneman and Carpenter 1980; Kimberg et al. 1997; Mehta and Riedel 2006; Frank and O'Reilly 2006). Previous results indicate that performance during cognitive tasks is improved by D2 agonists in subjects with lower WM capacity and impaired in subjects with higher WM capacity (Kimberg et al. 1997). These findings have been accounted for by the inverted-U-shaped dose–response curve model, i.e. D2 agonists might stimulate the dopamine system to 'optimal' or to overdosed levels in individuals with low vs high baseline dopamine system functioning (Meyer-Lindenberg and Weinberger 2006). Consistent with the behavioral experiments, several studies with functional imaging have reported that bromocriptine administration is beneficial for prefrontal activity in subjects with lower capacity, whereas it is detrimental in subjects with higher WM capacity (Gibbs and D'Esposito 2005a, b). All these results together suggest that improvement or deterioration of behavioral performance and cortical activity from manipulation of D2 signaling is associated with the individual, possibly genetic, makeup of individuals. This hypothesis has been supported by several recent studies investigating the relationship between genetic variation within *DRD2* and pharmacological D2 receptor stimulation in different cognitive domains (Kirsch et al. 2006; Cohen et al. 2007).

D2 receptors exist in two alternatively spliced isoforms, the D2 long (D2L) which is mainly post-synaptic and the D2 short (D2S) which has predominant presynaptic autoreceptor functions (Khan et al. 1998; Usiello et al. 2000). Earlier studies suggest association between an intronic polymorphism (rs1076560, G > T) and relative expression of these two isoforms: subjects carrying the minor (T) allele have reduced relative prefrontal and striatal expression of D2S (Zhang et al. 2007) and less efficient neural cortical and subcortical responses during performance of the 2-Back WM task compared with GG subjects (Zhang et al. 2007; Bertolino et al. 2009a, b, 2010). More specifically, the differences between the two genotypes emerged at the 2-Back working memory condition, whereas no such difference was found at 1-Back. These results suggested that the 2-Back condition was within the working memory capacity range of GG subjects but exceeded that of GT subjects (Zhang et al. 2007).

In the present double-blind, crossover, randomized, placebo-controlled study in healthy subjects, we examined whether *DRD2* rs1076560 predicts prefrontal responses to pharmacological stimulation of D2 receptors with bromocriptine during a spatial WM task. Based on our earlier studies, we increased the range of working memory load by also using a 3-Back working memory condition so as to evaluate the interaction between drug, genotype and working memory load presumably both within and beyond working memory capacity for both genotype groups. Based on our earlier studies, we hypothesized that the effects of bromocriptine on prefrontal behavior and activity would be genotype- and load-dependent.

## Materials and methods

### Subjects

Fifty-three healthy subjects (24 males, mean age ± SD 27.2± 4.4 years) entered the study. All subjects were Caucasians from the region of Puglia in Italy. Inclusion criteria were absence of any psychiatric disorder, as evaluated with the

Structured Clinical Interview for Diagnostic and Statistical Manual of Mental Disorders IV; of any significant neurological or medical condition revealed by clinical and magnetic resonance imaging evaluation; of history of head trauma with loss of consciousness; and of pharmacological treatment or drug abuse in the past year. The Wechsler Adult Intelligence Scale—Revised was used to evaluate intelligence quotient (IQ), the Hollingshead Scale (Hollingshead and Redlich 1958) to calculate the socioeconomical status and the Edinburgh Inventory (Oldfield 1971) to measure handedness.

The present experimental protocol was approved by the local institutional review board. After complete description of the study to the subjects, written informed consent was obtained.

## Experimental procedure

Each subject underwent a very well-established protocol which makes use of systemic administration of bromocriptine to stimulate D2 receptors (Luciana et al. 1998; Mehta et al. 2001; Gibbs and D'Esposito 2005a, b; Kirsch et al. 2006; Cools et al. 2007, 2009; van Holstein et al. 2011). Each subject was tested twice, once after administration of 1.25 mg bromocriptine and once after placebo (lactose), in a randomized, double-blind, crossover design counterbalanced for the order of drug administration. Both bromocriptine and placebo were administered orally in identical capsules together with 10 mg domperidone, a peripheral dopaminergic antagonist which does not pass the blood–brain barrier, to reduce possible side effects, like nausea, vomiting and dizziness known to be induced by bromocriptine intake. The dose of 1.25 mg was carefully chosen as it has previously been reported to modulate spatial short-term memory without producing appreciable adverse effects, unlike the higher dose of 2.5 mg (Luciana et al. 1998; Mehta et al. 2001; Gibbs and D'Esposito 2005a, b; Kirsch et al. 2006; Cools et al. 2007, 2009; van Holstein et al. 2011). The participants were not able to differentiate bromocriptine from placebo. The two functional MRI (fMRI) sessions, separated by 2 weeks, occurred approximately 150 min after administration of bromocriptine or of placebo, since the range of peak effectiveness for bromocriptine was estimated from previous studies to occur between 90 and 180 min after pill ingestion (Luciana et al. 1992; Luciana and Collins 1997; Kimberg et al. 1997, 2001; Mehta et al. 2001; Gibbs and D'Esposito 2005a, b; Kirsch et al. 2006; Cools et al. 2007). A blood sample was obtained immediately before and at the end of the fMRI session to test blood prolactin levels. This procedure was performed with the objective of evaluating in each individual that bromocriptine had caused the known reduction of prolactin over and beyond those correlated with circadian rhythm and the stress induced by the fMRI procedure.

## Genotype determination

Subjects were genotyped for *DRD2* rs1076560. This single-nucleotide polymorphism (SNP) was analysed in our laboratory with allele-specific PCR primers as described previously (Zhang et al. 2007; Bertolino et al. 2009a, b). Thirty-eight subjects were GG and 15 were GT. None of the subjects were homozygous for the T allele, consistent with previous studies (Zhang et al. 2007). Allelic distribution of this genetic variant in our sample was in Handy–Weinberg equilibrium ($\chi^2=1.44$, $p=0.23$), and it was consistent with the known distribution of these two alleles in the Caucasian population (http://www.ncbi.nlm.nih.gov/projects/SNP/snp_ref.cgi?rs=1076560), indicating that our distribution of genotypes is representative of the general population.

## *N*-Back working memory paradigm

During fMRI, all subjects completed a blocked paradigm of the *N*-Back task. Briefly, '*N*-back' refers to how far back in the sequence of stimuli the subject had to recall. The stimuli consisted of numbers (1–4) shown in a random sequence and displayed at the points of a diamond-shaped box, and in WM conditions, the task required recollection of a stimulus seen one, two or three stimuli previously (1-, 2- or 3- Back, respectively) while subjects continued to encode additionally incoming stimuli. There was also a non-memory-guided control condition (0-Back) that simply required subjects to identify the stimulus currently seen. Three different task runs were used, each alternating four 30-s blocks of a 0-Back condition with four 30-s blocks of a WM condition (1-, 2- or 3-Back, respectively). Each run lasted 4 min and 8 s. Stimuli were presented via a back-projection system, and behavioral responses were recorded through a fiber optic response box which allowed measurement of accuracy and reaction time for each trial. All subjects were trained on the task before the fMRI session.

## Demographic, clinical and behavioral data analysis

ANOVAs and $\chi^2$ were used to assess potential differences between the two *DRD2* genotype groups for all demographic variables and to assess the effect of bromocriptine vs placebo on blood levels of prolactin. Behavioral data (accuracy and reaction time at 1-, 2- and 3-Back) were compared using repeated-measures factorial ANOVA with genotype as between-subjects factor and load and drug as repeated-measures factors. A dependent-sample *t* test was used for post hoc analysis on behavioral data within each genotype group.



$\chi$

### fMRI data acquisition

Blood oxygen level-dependent (BOLD) fMRI was performed on a GE Signa 3-T scanner (General Electric, Milwaukee, WI), equipped with a standard quadrature head coil. A gradient-echo planar imaging sequence (repetition time, 2,000 ms; echo time, 28 ms; 20 interleaved axial slices; thickness, 4 mm; gap, 1 mm; voxel size, 3.75×3.75×5; flip angle, 90°; field of view, 24 cm; matrix, 64×64) was used to acquire 120 volumes for each task run. The first four scans were discarded to allow for a T1 equilibration effect.

### fMRI data analysis

Analysis of the fMRI data was completed using Statistical Parametric Mapping (SPM8, http://www.fil.ion.ucl.ac.uk/spm). Images, for each subject, were realigned to the first volume in the time series, and movement parameters were extracted to exclude subjects with excessive head motion (>2 mm of translation, >2° rotation). Images were then resampled to a 3.75-mm isotropic voxel size, spatially normalized into a standard stereotactic space (Montreal Neurological Institute, MNI, template) and smoothed using a 10-mm full-width half-maximum isotropic Gaussian kernel to minimize noise and to account for residual inter-subject differences. A box car model convolved with the haemodynamic response function (HRF) at each voxel was modeled. Subject-specific movement parameters, obtained from the realignment procedure, were included in the general linear model (GLM) as covariates, taking into account the effect of subject motion. In the first-level analysis, linear contrasts were computed producing a *t* statistical map at each voxel for the 1-, 2- and 3-Back conditions, assuming the 0-Back condition as a baseline. All individual contrast images were entered in a second-level random-effects analysis. A factorial ANOVA was then performed, with load and drug as repeated-measures factors and *DRD2* rs1076560 genotype as the between-subjects factor. Furthermore, *t* test for dependent or independent samples as appropriate outside of SPM was used for post hoc comparisons. We used a statistical threshold of $p<0.005$ (minimum cluster size [*k*]=3), with further family-wise error correction at $p<0.05$, using as volume of interest the Wake Forest University PickAtlas (http://fmri.wfubmc.edu/cms/software#PickAtlas) Brodmann's area 9 (BA9). This region of interest (ROI) included 193 voxels. This region was chosen a priori based on our earlier studies (Zhang et al. 2007; Bertolino et al. 2009a, b; Fazio et al. 2011) and because of our strong hypothesis on D2 modulation of dorsolateral prefrontal cortical activity. Outside the ROI, no activation encompasses the threshold of family-wise error (FWE) $p<0.05$ whole-brain correction.

BOLD responses were extracted from significant clusters using MarsBaR (http://marsbar.sourceforge.net/). Finally, to explore the relationship between behavior and BOLD responses in the PFC region showing a genotype by WM load by drug interaction (see 'Results'), we used linear regression analyses within SPM entering the single-subject contrasts and accuracy as variables. This analysis was performed separately for the two genotype groups and for all conditions (i.e. WM loads and drug). Specific T contrasts were built in order to investigate correlations between accuracy and prefrontal activity. These analyses were corrected with the same algorithm described for the factorial ANOVA and using the same prefrontal ROI (see above).

## Results

### Demographic data

ANOVAs and $\chi^2$ tests indicated that there were no significant differences between genotype groups in any demographic variable (all $p>0.1$) (Table 1).

### Prolactin reduction

ANOVA in the whole sample of subjects demonstrated that bromocriptine decreased plasma levels of prolactin relative to placebo ($F_{(1,51)}=28.8$, $p<0.001$). There was no main effect of genotype or any interaction between genotype and drug (all $p>0.3$).

### Behavioral data

A repeated-measures ANOVA of accuracy data indicated a main effect of load ($F_{(2,102)}=41.58$, $p=0.0001$; increasing WM load was associated with declining accuracy), no main effect of *DRD2* genotype ($F_{(1,51)}=0.23$, $p=0.63$), no main effect of drug ($F_{(1,51)}=0.60$, $p=0.43$), no significant drug by genotype interaction ($F_{(1,51)}=0.005$, $p=0.94$) and a significant interaction between WM load, *DRD2* genotype and drug ($F_{(2,102)}=8.2$, $p=0.0004$) (Fig. 1). Post hoc analysis

**Table 1** Demographics of the subjects included in the study

| | *DRD2* (total N) | | *p value* |
|---|---|---|---|
| | GG (38) | GT (15) | |
| Gender (males) | 15 | 9 | 0.17 |
| Age (years) | 27.5 (±5) | 26.4 (±2.5) | 0.44 |
| IQ (WAIS-R) | 111.5 (±13.7) | 110.7 (±15.2) | 0.87 |
| Socioeconomic status (Hollingshead Scale) | 44.9 (±14.8) | 48.1 (±15.8) | 0.5 |
| Handedness (Edinburgh Inventory) | 0.7 (±0.3) | 0.6 (±0.5) | 0.41 |

*IQ* intelligence quotient, *WAIS-R* Wechsler Adult Intelligence Scale—Revised

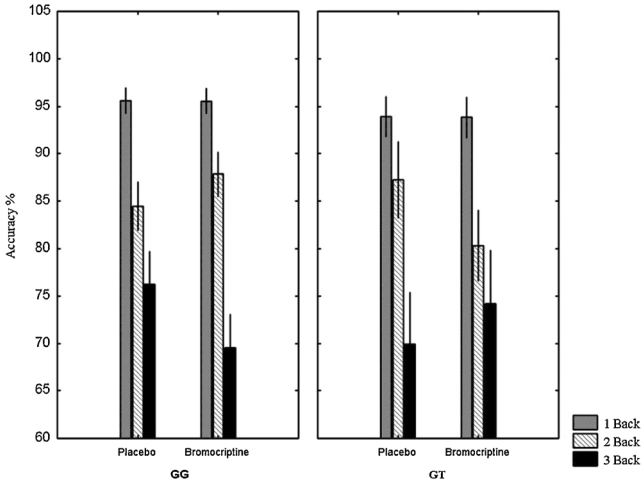


**Fig. 1** Behavioral data. Bar graph (mean ± SE) of working memory accuracy. An interaction between WM load, *DRD2* genotype and drug was found (GG subjects have a significant decrement of accuracy at 3-Back on bromocriptine; GT subjects have a trend for reduced performance at 2-Back on bromocriptine; see text for statistics)

demonstrated that after bromocriptine administration, GG subjects had reduced WM performance at 3-Back compared with placebo ($p$=0.002), while GT subjects tended to have reduced performance at 2-Back compared with placebo ($p$=0.07). All other post hoc comparisons were not statistically significant (all $p$>0.1). Thus, in line with a previous study (Markett et al. 2009), individual differences in WM capacity were only present at a greater WM load.

No statistically significant main effects or interactions were present on reaction time data (all $p$>0.5).

## BOLD response

We evaluated associations of *DRD2* genotype and D2 stimulation with brain activity during WM using a parametric *N*-back working memory task involving increasing cognitive load (1-, 2- and 3-Back). ANOVA demonstrated a strong main effect of load in the whole WM network, including the left inferior frontal gyrus (BA9 $x$=−48, $y$=16, $z$=25, $F_{(2,306)}$=18.65, $k$=87, $Z$=5.47, FWE $p$<0.001) where BOLD responses increased linearly from 1- to 2- and 3-Back (3-Back >1-Back: $p$<0.0001, 3-Back >2-Back: $p$=0.008, 2-Back >1-Back: $p$=0.04) (Fig. 2). Consistent with previous studies (Zhang et al. 2007; Bertolino et al. 2009a, b; Fazio et al. 2011), the results also demonstrated a main effect of genotype in the left superior frontal gyrus (BA9 $x$=−33, $y$=49, $z$=40, $F_{(1,306)}$=18.28, $k$=15, $Z$=4.05, FWE $p$=0.003) where GT subjects have a significantly greater activity compared with GG subjects (Fig. 3). No interactions of genotype by WM load, of genotype by drug and of WM load by drug were found (all $p$>0.05). Finally, ANOVA demonstrated an interaction between WM load, *DRD2* genotype and drug in the left superior frontal gyrus (BA9 $x$=−33, $y$=46, $z$=32, $F_{(2,306)}$=6.54, $k$=14, $Z$=2.94, FWE $p$=0.04) (Table 2). BOLD responses extracted from this cluster revealed the following: GG subjects on placebo increase prefrontal activity from 1- or 2- to 3-Back (3-Back >1-Back: $p$=0.0008, 3-Back > 2-Back: $p$=0.003, 2-Back vs 1-Back: $p$=0.49), whereas on bromocriptine, this pattern was attenuated, especially from 2- to 3-Back (3-Back >1-Back: $p$=0.07, 3-Back vs 2-Back: $p$=0.98, 2-Back > 1-Back: $p$=0.08). Moreover, GG subjects after bromocriptine have decreased BOLD responses at 3-Back compared with placebo ($p$=0.02). On the other hand, the pattern of BOLD responses in GT subjects was different across the two sessions. When on placebo, there was no significant difference between the three WM loads (all $p$>0.35). After bromocriptine, GT subjects have a more physiological pattern of activity which tended to increase linearly from 1- to 2- and 3-Back (3-Back > 1-Back: $p$=0.01, 3-Back >2-Back: $p$=0.02, 2-Back >1-Back: 0.09). Moreover, in GT subjects, bromocriptine is associated with decreased BOLD responses at 1-Back compared with placebo ($p$=0.009) (Fig. 4a, b).

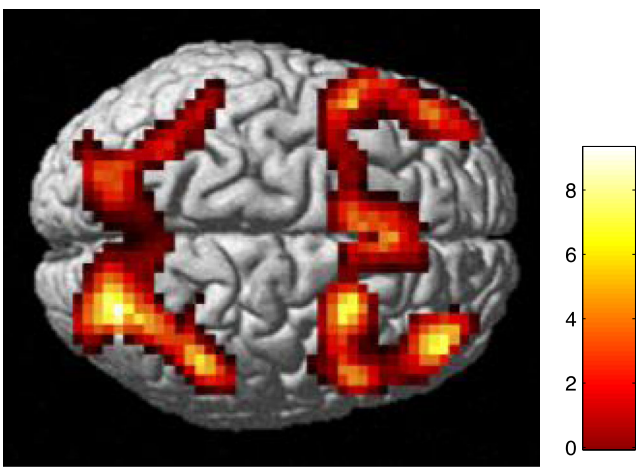


**Fig. 2** Rendered image of the brain showing the network activated during *N*-Back task. The *color bar* represents *t* values

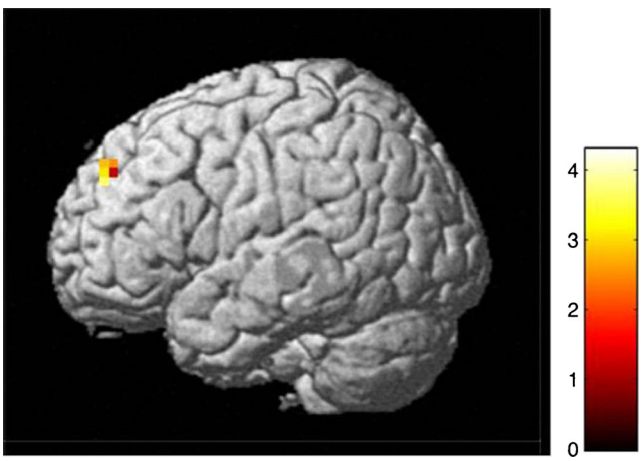


**Fig. 3** Rendered image of the brain showing the cluster in the left superior frontal gyrus where a main effect of *DRD2* genotype was found (GT > GG, see text for statistics). The *color bar* represents *t* values

**Table 2** Statistics and Montreal Neurological Institute coordinates for the effects of load and *DRD2* genotype as well as for interaction between drug, genotype and load on brain activity during *N*-Back task

| | Brain region | BA | MNI coordinates | | | *F* | *k* | *Z* | FWE *p* |
|---|---|---|---|---|---|---|---|---|---|
| | | | *x* | *y* | *z* | | | | |
| Main effect of WM load | Left inferior frontal gyrus | BA9 | −48 | 16 | 25 | 18.65 | 87 | 5.47 | 0.0001 |
| Main effect of *DRD2* | Left superior frontal gyrus | BA9 | −33 | 49 | 40 | 18.28 | 15 | 4.05 | 0.003 |
| Drug by genotype by WM load interaction | Left superior frontal gyrus | BA9 | −33 | 46 | 32 | 6.54 | 14 | 2.94 | 0.04 |

*WM* working memory, *BA* Brodmann's area, *MNI* Montreal Neurological Institute, *FWE* family-wise error

In order to explore the potential for changes in BOLD response identified in the interaction to correlate with measurable behavioral differences, we performed a linear regression in SPM. This analysis indicated in GG subjects on bromocriptine a statistical trend for a positive correlation between BOLD responses in the left superior frontal gyrus (BA9) and accuracy at 3-Back ($x$=−44, $y$=8, z=40, $t_{(1,36)}$ = 3.07, $k$=4, FWE $p$= 0.08). On the other hand, in GT subjects on bromocriptine, we found a negative correlation between BOLD responses in BA9 and accuracy at 2-Back ($x$=−48, $y$=16, $z$=40, $t_{(1,13)}$= 4.97, $k$=16, FWE $p$=0.006) (Fig. 5a, b).

## Discussion

The present results demonstrate an interaction between bromocriptine stimulation, *DRD2* rs1076560 genotype and WM load on prefrontal behavior and activity. This study adds to a growing body of research demonstrating that the effects of dopamine drugs on brain activity and performance are modulated by genetic variables that might also index baseline dopamine levels (Mattay et al. 2003; Frank and O'Reilly 2006; Kirsch et al. 2006; Meyer-Lindenberg and Weinberger 2006; Cools et al. 2007). In particular, the present results reveal that bromocriptine stimulation interacts with *DRD2* genotype in modulating load-related prefrontal activity during WM processing. More specifically, bromocriptine stimulation in GG subjects attenuates a load-related linear increase of prefrontal activity which is present when they are on placebo. Moreover, on bromocriptine, prefrontal BOLD signal of GG subjects decreased at the higher WM load together with a significant decrement in performance (accuracy). A possible interpretation of these results is that these subjects reduce engagement of prefrontal resources to the task, because their capacity of WM is exceeded at 3-Back. Consistent with this interpretation, regression analysis revealed a statistical trend for a positive relationship between prefrontal activity and accuracy at 3-Back on bromocriptine (lower activity for worse performance). In GT subjects, we found a reduction in BOLD signal at 1-Back after bromocriptine compared with placebo for the same level of performance, suggesting that they had increased efficiency. However, in this genotype configuration, stimulation with bromocriptine is associated with a linear increase in prefrontal activity as the task becomes more

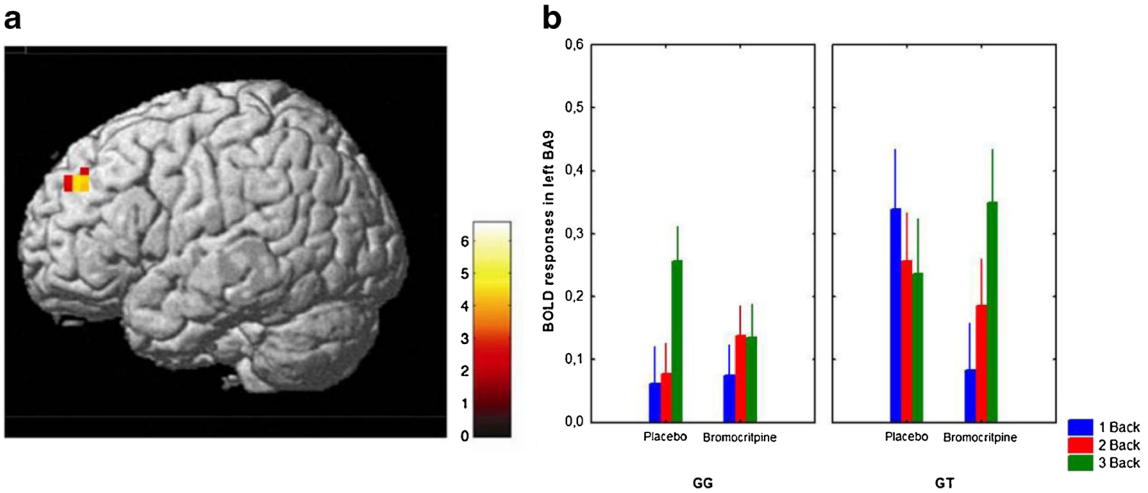


**Fig. 4** **a** Rendered image showing the cluster in the left superior frontal gyrus where a three-way interaction between WM load, *DRD2* genotype and treatment was found. The *color bar* represents *F* values. **b** Bar graph (mean ± SE) of BOLD responses extracted from the cluster depicted in **a**. GG subjects have a significant reduction of activity at 3-Back on bromocriptine, while GT subjects have reduced activity at 1-Back on bromocriptine. See text for statistics

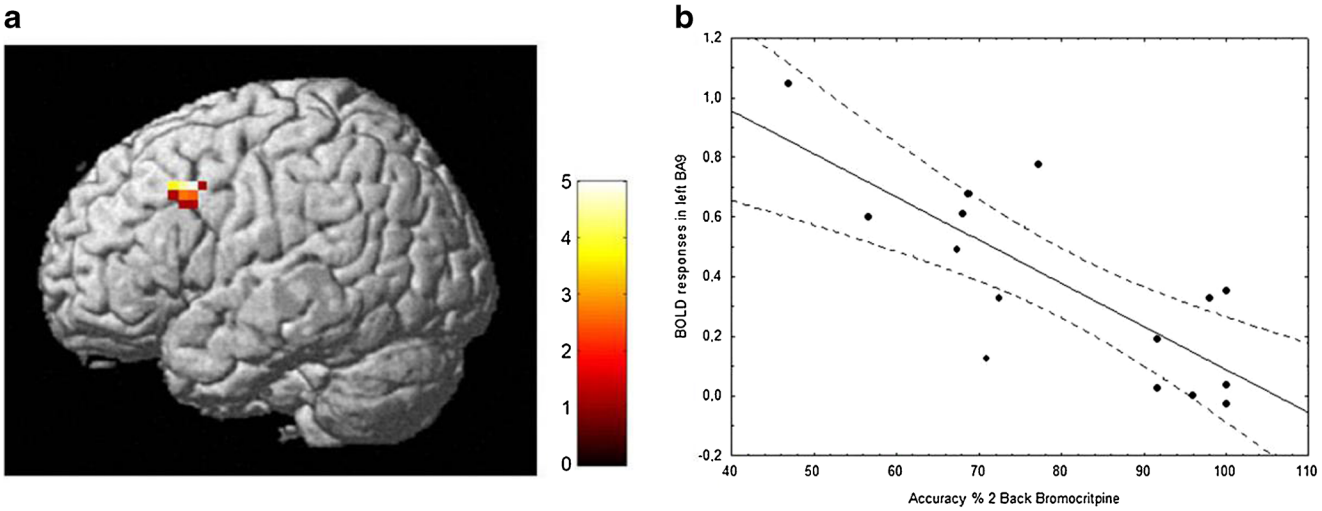


**Fig. 5** **a** 3-D rendering of the negative correlation between prefrontal activity and accuracy at 2-Back in GT subjects on bromocriptine with the relative scatterplot showing individual data points (**b**). The *color bar* represents *t* values. See text for statistics

difficult, with a trend for reduced behavioral performance already at 2-Back, suggesting greater effort for worse task performance. Consistently, we found a negative relationship between prefrontal activity of GT subjects and accuracy at 2-Back on bromocriptine. These results are consistent with other studies demonstrating that genetically determined D2 receptor levels are crucial to modulate cognition (Jocham et al. 2009; Markett et al. 2009).

A possible biological mechanism underlying the present results may be that the effect of bromocriptine on cortical activity is associated with its preferential presynaptic action and with the differential expression ratios of D2 isoforms in GG and GT subjects. Evidence from the animal literature suggests that low doses of D2 agonists actually exert their effects primarily via presynaptic mechanisms (Richfield et al. 1989; Usiello et al. 2000; Tamminga 2002; Tamminga and Carlsson 2002; Frank and O'Reilly 2006; Cools et al. 2009; Anzalone et al. 2012). Presynaptic D2 autoreceptors tightly control the level of dopamine release via an inhibitory feedback (Starke et al. 1989; Grace 1995; Schmitz et al. 2003). Thus, D2 agonists like bromocriptine stimulate presynaptic D2 autoreceptors and reduce dopamine release. Moreover, earlier studies have demonstrated that GG subjects have a relatively greater expression of D2S (Zhang et al. 2007) and are generally more efficient at 2-Back, presumably because their dopamine levels are within a hypothetical optimal range predicted by the inverted-U-shaped dose–response curve (Williams and Goldman-Rakic 1995). Consistent with this earlier literature, GG subjects reveal a reduction in terms of both accuracy and cortical activity only at the greater load (3-

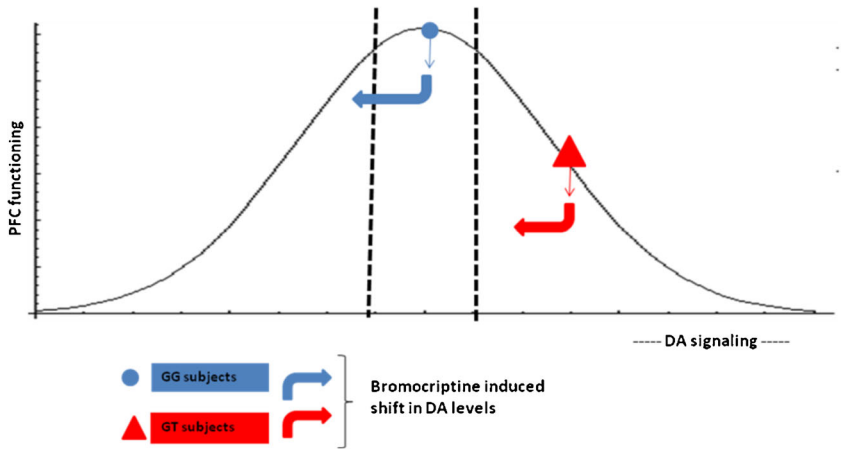


**Fig. 6** Hypothetical inverted-U model describing the effects of DRD2 genotype and bromocriptine on prefrontal dopamine signaling and function. During placebo, GG subjects are located near the peak of the D2-response curve, i.e. optimal signaling and prefrontal function. Instead, GT subjects have greater dopamine signaling and less optimal prefrontal function. Administration of bromocriptine in GG subjects may shift D2 signaling to suboptimal levels and lower prefrontal efficiency at high WM load. On the other hand, administration of bromocriptine in GT subjects may shift D2 signaling to more optimal levels, thus improving prefrontal function of GT individuals at low levels of working memory load (1-Back). However, this mechanism is not sufficient to increase prefrontal efficiency in GT subjects at greater levels of working memory loads (2-Back)

Back) after bromocriptine stimulation. This effect is presumably because their dopamine levels are shifted below the optimal range by bromocriptine. Conversely, in GT subjects who have relatively lower levels of D2S and who are physiologically less efficient to begin with (Zhang et al. 2007), bromocriptine stimulation of presynaptic D2 receptors reduces the supra-normal levels of dopamine (Bertolino et al. 2010), improving their efficiency at 1-Back, only. On the other hand, this mechanism would not be sufficient to improve their efficiency at 2-Back, which is actually reduced probably because of their more physiologically limited WM capacity (Zhang et al. 2007; Bertolino et al. 2009a, 2010) (Fig. 6). This hypothetical interpretation is supported by studies in humans, suggesting that the effects of dopamine D2 agonists and antagonists on cognition and prefrontal activity are correlated with the baseline levels of dopamine and with capacity limitations (Kimberg et al. 1997; Frank and O'Reilly 2006; Kirsch et al. 2006; Cohen et al. 2007, 2009; van Holstein et al. 2011). In particular, consistent with our interpretation, Frank and O'Reilly (2006) and Cools et al. (2009) demonstrated that low doses of D2 agonist impaired cognitive performance in subjects with high baseline levels of dopamine, presumably as a result of reduced dopamine bursts. Of note, our results are apparently at odds with earlier studies reporting improvement of cognitive performance in subjects with a lower WM capacity (Kimberg et al. 1997; Gibbs and D'Esposito 2005a, b). However, it is important to mention that our subjects had on average high WM performance (2-Back, mean ± SD 85.25± 15.48 %; 3-Back, mean ± SD 74.45±21.09 %) as compared with earlier studies with the same task (Mattay et al. 2003: 2-Back, mean ≈84 %; 3-Back, mean ≈65 %; Goldberg et al. 2003: 2-Back, mean ≈78 %), suggesting that there may be a ceiling effect. This interpretation also has another corollary. Earlier studies with this task have reported that activity in PFC is reduced at 3-Back compared with 2-Back, suggesting a nonlinear relationship between cognitive demand and prefrontal activity (Callicott et al. 1999). Here, we found that PFC activity is positively related with WM demands, such that activity increases linearly with greater memory loads. These results are consistent with the interpretation above detailed because it is possible that in our subjects, the cognitive demands elicited by the 3-Back did not breach average WM capacity, resulting in increased activity.

Some potential limitations of the present data have to be discussed. First, the inclusion of women may introduce additional variance in the data because the dopamine system is modulated by the menstrual cycle (Dreher et al. 2007). Although we did not collect menstrual cycle position information, it is likely that this variable would have been randomly distributed across all conditions and, therefore, would not have affected the results in any reliable way. Second, in this study, we used a block design paradigm in which the demand for executive processes involves information updating and temporal indexing increases concurrently with maintenance and retrieval demands. Earlier studies have demonstrated that the effects of bromocriptine are associated with specific phases of WM processing (Gibbs and D'Esposito 2005a, b; Cools and D'Esposito 2011). Since our task does not allow separation of updating and maintenance, we cannot disentangle potential effects of the drug or the gene on these WM components.

In conclusion, our study in healthy subjects indicates that genetic variation in *DRD2* modulating D2 presynaptic signaling is relevant to modulate specific aspects of physiology during cognitive processing. Additional studies not only with dopamine D2 agonist but also with antagonist are needed to elucidate the mechanisms of the effect of *DRD2* rs1076560 on WM processes.

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