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The dopamine D2 receptor gene DRD2 and the nicotinic acetylcholine receptor gene CHRNA4 interact on striatal gray matter volume: Evidence from a genetic imaging study

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ABSTRACT

Dopaminergic activity is modulated by acetylcholine with relevance for cognitive functioning, as shown by pharmacological work in a rodent model. In humans, the two transmitter systems' joint effort on cognition has been described on the molecular genetic level: DRD2 rs6277, a single nucleotide polymorphism (SNP) on the dopamine D2 receptor gene and CHRNA4 rs1044396, a SNP on the nicotinic acetylcholine receptor gene interact on visuo-spatial and phonological working memory. The present study uses structural MRI and voxel based morphometry to extend this behavioral work to an intermediate phenotype on the neural level. We found significantly reduced gray matter volume in the right putamen in carriers of the DRD2 C/C and CHRNA4 T/T groups. This genotype combination has previously proven to be beneficial for working memory capacity. Results are in line with the idea that the two genes jointly influence the gating signals from subcortical structures to the prefrontal cortex.

Introduction

Dopaminergic activity in the midbrain is modulated by acetylcholine (ACh), particularly through its nicotinic receptors (nAChR). Especially in the striatum, nAChr can be found on dopaminergic neurons and vice versa (Exley and Cragg, 2008; Zhou et al., 2003). The joint effect of these two neurotransmitter systems on cognitive functioning is well documented in rodents. Radial maze performance, a behavioral measure for working memory in rats, is improved after the application of a nAChR agonist (Levin and Rose, 1991) but impaired by an antagonist (Levin et al., 1987). Importantly, the adverse effect of the nAChR antagonist can be reversed by a dopamine D2 receptor agonist (Levin et al., 1989) while a co-administration of a D2- and a nAChR antagonist leads to an even stronger impairment compared with the effect of each pharmacological agent alone (McGurk et al., 1989). The interactive effect is constrained to dopamine D2 receptors as dopamine D1 agonists are not capable of neutralizing the detrimental effect of nAChR antagonists (Levin et al., 1989).

In humans, the influence of both neurotransmitter systems on working memory has been studied in isolation (Levin et al., 1998; Luciana et al., 1992, 1998). Evidence for an interaction, however, is scarce. Cognitive functioning, including general cognitive ability, executive control and working memory capacity, are highly heritable phenotypes (Friedman et al., 2008; Kremen et al., 2007; Plomin et al., 1994). Having this in mind, we conducted two studies that addressed the interaction between the two transmitter systems on a molecular genetic level (Markett et al., 2010, 2011a). We focussed our analysis on the single nucleotide polymorphisms (SNPs) rs6277 (also known as DRD2 C957T) on the dopamine D2 receptor gene DRD2 and rs1044396 on the CHRNA4 gene which codes for the alpha4 subunit of nicotinic acetylcholine receptors. The DRD2 gene is located on the q22–23 strand of chromosome 11, the CHNRA4 gene on q13.3 of chromosome 20.

DRD2 rs6277 is a SNP that – even though synonymous – alters the folding of the messenger RNA (mRNA). Consequently, the mRNA is less stable which leads to markedly reduced protein synthesis rates (Duan et al., 2003). This in vitro work is complemented by positron emission tomography (PET) studies that demonstrate the functional significance of the SNP for D2 receptor functioning in vivo: Carriers of the C-allele show a reduced density and affinity of D2 receptors in the striatum (Hirvonen et al., 2004, 2009). On a behavioral level, rs6277 has been associated with performance on the Wisconsin Card Sorting Test (Rodriguez-Jimenez et al., 2006), working memory (Xu et al., 2007), impulsivity (Colzato et al., 2010) and individual differences in responding to a nicotinic pharmacological challenge (Jacobsen et al., 2006).

An influence of the CHNRA4 gene on dopaminergic functioning in the midbrain has been demonstrated in a knock-out model. Mice

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lacking a 750 bp fragment in exon 5 exhibit higher striatal dopamine levels and altered morphology of dopaminergic neurons in the midbrain (Marubio et al., 2003; Parish et al., 2005). CHRNA4 rs1044396 is a synonymous SNP located on this exonic region. Allelic variants of this SNP have repeatedly been associated with various phenotypes including smoking status and nicotine addiction (Chu et al., 2011; Feng et al., 2004), attentional task performance (Greenwood et al., 2005; Parasuraman et al., 2005; Reinvang et al., 2009), neural activation differences during attentional tasks (Espeseth et al., 2007; Winterer et al., 2007), negative emotionality (Markett et al., 2011b), internet addiction (Montag et al., in press) and depression and loneliness in elderly adults (Tsai et al., 2012). The associations with nicotine addiction, attention and affective traits have all been replicated in independent samples, indicating that rs1044396 is of phenotypic relevance, even though the precise molecular mechanisms remain undiscovered.

In a previous study we were able to confirm an interaction between DRD2 rs6277 and CHNRA4 rs1044396 on working memory performance: The combination of the C/C genotype at the DRD2 rs6277 gene locus and the T/T variant at CHRNA4 rs1044396 is beneficial for visuospatial working memory capacity in a delayed-match-to-sample task (Markett et al., 2010) and for phonological working memory in the Wechsler forward and backward digit span task (Markett et al., 2011a). In both studies, the interaction affected variables related to the encoding of contextual information into working memory while retrieval of information from working memory was not related to the two genes. While behavioral assays in the study of genetics can reveal the importance of the given gene loci for a behavioral or cognitive phenotype, they are not capable of elucidating the biological pathway that leads from genetic variation to individual differences in behavior. Imaging genetics, however, provides a tool for the study of intermediate phenotypes on the neural level (Hariri et al., 2006). In the present study, we seek to investigate the neurostructural impact of the DRD2 and the CHRNA4 gene and extend the evidence on their role in normal cognition to a further biological variable.

The tripartite working memory model proposed by Baddeley (Baddeley, 1986; Baddeley and Hitch, 1974) describes two storage buffers for visuo-spatial and phonological materials that are entirely independent from each other with the exception that they are both controlled by the central executive. One of the functions designated to the central executive is the selection of behaviorally relevant information. As the observed interaction between DRD2 and CHRNA4 is independent from the stimulus modality on the one hand and related to the encoding of new information in both modalities on the other, we hypothesized that DRD2 and CHRNA4 affect jointly the functioning of the central executive. The prime neural correlate of psychological functions subsumed under the concept of the central executive is the prefrontal cortex (Baddeley, 2003; Goldman-Rakic, 1996; Miller and Cohen, 2001). Ascending dopaminergic pathways that project from the midbrain's ventral tegmental area via the basal ganglia to the prefrontal cortex are thought to gate new, behaviorally relevant, information into working memory (Hazy et al., 2007; Miller and Cohen, 2001). This dopaminergic signaling is putatively modulated by ACh (Dehaene et al., 1998). An explanation for the interaction between DRD2 and CHRNA4 on working memory is a joint effect of the two genes on this gating mechanism. Another line of indirect evidence for this claim comes from functional imaging studies demonstrating that the encoding efficiency of new information in the face of distraction is a powerful predictor for working memory capacity in the paradigm used in our study on visual working memory (Vogel et al., 2005). The main neural correlates of this encoding efficiency are the basal ganglia (McNab and Klingberg, 2008) indicating that this neural structure plays a crucial role in working memory.

Taking these considerations together, there is plenty of reason to hypothesize that the two genetic variants on DRD2 and CHRNA4 affect the basal ganglia. This, however, has not been tested empirically. With the present study we seek to fill this gap using structural genetic imaging applying voxel-based morphometry. In our previous work, we identified better working memory capacity in carriers of the DRD2 C/C and CHRNA4 T/T genotypes compared to the other eight possible genotype combinations (Markett et al., 2011b), supporting our findings presented in Markett et al. (2010). In the present study's scope, we expect to find structural differences in the DRD2 C/C and CHRNA4 T/T groups as well. Therefore, and to increase statistical power, we choose to test the genetic interaction by grouping participants in four genotype groups according to the presence or absence of the DRD2 C/C (vs. DRD2 C/T or T/T) and CHRNA4 T/T (vs. C/C or CT) genotypes.

Previous structural genetic imaging studies which looked at the relationship between variants in the vicinity of the DRD2 gene (DRD2/ANKK1 TaqIa) and gray matter volume have reported a volume reduction in carriers of variants associated with reduced d2 receptor density (Cerasa et al., 2009; Montag et al., in press). As the C/C genotype at DRD2 rs6277 is also associated with reduced binding potential of striatal D2 receptors, we hypothesized a reduction of gray matter volume in this group. Even though this may sound counterintuitive at first glance, a reduction in gray matter volume is not at odds with the enhancement of cognitive functioning: A recent study by Takeuchi et al. (2011) was able to demonstrate reductions in regional gray matter volume after working memory training that led to improved cognitive performance. A possible explanation for this relationship is the neurocognitive model proposed by Hazy et al. (2007): The striatum is thought to be part of functional loops that connect the thalamus with the prefrontal cortex (Alexander et al., 1986). Within these loops, the striatum provides constant inhibitory input to the globus pallidus and the substantia nigra. Only when this inhibition is released, the substantia nigra can activate the thalamus, which in turn sends signals to the prefrontal cortex. Thus, a reduction in striatal tissue might lower the tonic inhibition of the globus pallidus and the substantia nigra thereby enhancing the updating signals of the thalamus to the prefrontal cortex.

Methods

Participants

A total of N = 142 healthy Caucasian participants (n = 114 females, n = 28 males; mean age M = 23.09, SD = 4.25) underwent structural MRI scanning and provided buccal swabs for the genetic analyses after giving informed written consent. Most participants were enrolled in the University of Bonn's psychology program on the undergraduate or graduate level, leading to a relatively uniform sample with respect to educational background, intelligence, age and gender. Exclusion criteria were past or present neurological and psychiatric conditions, which were assessed by a screening questionnaire and contraindications to MRI. N = 29 participants reported to be regular smokers. Smoking was no exclusion criterion, as previous work of our group did not find an association between smoking and CHRNA4 or an influence of smoking status on the interaction between CHRNA4 and DRD2 on working memory. No participant reported illicit drug use or alcohol use that exceeded the maximum daily allowance issued by the World Health Organization (three beverages for males, two for females). The study protocol was in accordance with the declaration of Helsinki and approved by the ethics committee of the University Hospital in Bonn.

Genotyping

DNA was extracted from buccal cells. Automated purification of genomic DNA was conducted by means of the MagNA Pure(R) LC system using a commercial extraction kit (MagNA Pure LC DNA

isolation kit; Roche Diagnostics, Mannheim, Germany). Genotyping was performed by real time polymerase chain reaction (RT-PCR) using melting curve detection analysis on a Light Cycler System (Roche Diagnostics, Mannheim, Germany). The protocols (TIB MOLBIOL, Berlin, Germany) for the RT-PCR were as follows:

DRD2 c957t:
Forward primer: 5′-GAACTTGTCCGGCTTTACC-3′
Backward primer: 5′-CAATCTTGGGGTGGTCTTT-3′
Anchor hybridization probe: 5′-LCRed640-CCCCGCCAAACCAG AGAAGAAT-phosphate-3′
Sensor hybridization probe: 5′-TCCACAGCACTCCCGACA-fluorescein-3′
CHRNA4 rs1044396:
Forward primer: 5′-TCTCGCAACACCCACTC-3′
Backward primer: 5′-GTCTGTGTCTTCGGCCTTCA-3′
Anchor hybridization probe: 5′-LCRed640-CACCGAAGAGGGCT CCTTCTTGCAT-phosphate-3′
Sensor hybridization probe: 5′-TCTTGACCGTGGCACTCGGG-fluorescein-3′.

Image acquisition

Three dimensional high-resolution T1-weighted images were acquired from all participants using a Magnetization Prepared RApid Gradient Echo (MP-RAGE) sequence with 160 sagittal slices on a 1.5 T MRI scanner (Avanto, Siemens, Erlangen) with 1 mm slice thickness, a field of view of 256 mm × 265 mm and a matrix size of 256 × 256 which yielded an isotropic resolution of 1 mm^3 voxels.

MRI analysis

All data analyses were carried out in SPM8 (http://www.fil.ion.ucl.ac.uk/spm/) implemented in Matlab 7.8.0 (The Mathworks) running on a 2.53 GHz Intel MacBook Pro. The structural T1-weighted images were preprocessed with the VBM toolbox by Christian Gaser (VBM8, version 369; http://dbm.neuro.uni-jena.de/vbm/). Preprocessing included the segmentation of the images into gray matter, white matter and cerebrospinal fluid. During segmentation, a spatial adaptive non-local means (SANLM) filter with internally estimated optimal weighting and a Markov random field (MRF) filter with a medium weighting of 0.15 were used to denoise the data. Images were normalized on a standard MNI template using the high dimensional DARTEL algorithm. The normalization procedure comprised a modulation step only for non-linear transformations, resulting in voxels that include information about volume. Finally, the images were smoothed with an 8 mm Gaussian kernel. A quality check of the preprocessed images was performed with the respective modules as recommended in the VBM8 manual: First, one slice per normalized and bias-corrected image was visually inspected to identify artifacts or wrongly-oriented images. This step was complemented by the analysis of the covariance matrix between the normalized gray matter segments modulated for the non-linear components. No statistical outlier could be identified and the images of all participants were submitted to the individual differences analysis.

For this analysis, a two-way analysis of variance (ANOVA) model with the two genes as independent factors and age and gender as covariates was fitted to the smoothed and normalized gray matter images. We first computed a whole brain analysis with a statistical threshold of $p<.001$ and an extend threshold of $k>100$. Because of our hypothesis that the two genes interact on the basal ganglia, we further ran a region-of-interest (ROI) analysis in this structure. For this purpose, an anatomical mask including the bilateral putamen, caudate nuclei and the globus pallidus was constructed using the WFU PickAtlas toolbox (www.ansir.wfubmc.edu). Tests in this search volume (9264 voxels) were thresholded at $p<.05$, corrected for the family wise error (FWE).

Results

Genotyping

The genotype frequencies for both SNPs did not deviate from those expected according to the Hardy–Weinberg-equilibrium: DRD2 rs6277: C/C n = 37, C/T n = 65, T/T n = 40 (CHI^2 (1) = 1.004, ns) and CHRNA4: C/C n = 31, C/T n = 60, T/T n = 51 (CHI^2 (1) = 2.697, ns). Based on previous work and our hypothesis we tested on the allelic level and grouped our sample into DRD2 T+ (C/T and T/T) and T− (C/C) and into CHRNA4 C+ (C/C and C/T) and C− (T/T) carriers. The resulting allelic distribution can be seen in Table 1.

Whole brain analysis

The whole brain analysis revealed an interaction of DRD2 and CHRNA4 on gray matter volume in the putamen, the lingual gyrus of the occipital lobe, the precuneus, the posterior cingulate, the inferior frontal gyrus and the superior temporal gyrus (see Fig. 1 for a glass brain projection and Table 2 for details on the localization of the clusters). No significant clusters showed up when the contrast was reversed in its direction.

ROI analysis

The results of the ROI analysis are depicted in Fig. 2. We found a significant interaction of DRD2 and CHRNA4 in the right putamen (peak voxel x = 32, y = 5, z = 1, cluster size K = 181, t(136) = 3.71, p = .046, FWE-corrected). Fig. 3 shows the mean parameter estimates from the cluster for the four allelic groups. Post-hoc analyses with the LSD procedure confirmed that the interaction was driven by significantly reduced gray matter volume in participants carrying both the DRD2 C/C and CHRNA4 T/T variants compared to carriers of all other allelic groups (all $p<.05$). All other three groups did not differ except for the DRD2 C/C and CHRNA4 C+ groups which showed a larger volume than the DRD2 T+/CHRNA4 C+ group ($p=.01$).

We also found significant main effects on the allele level of both genes. DRD2 C/C carriers showed reduced gray matter volume in the left caudate nucleus compared to carriers of the T+ variant (peak voxel x = −12 y = 14, z = −11, cluster size K = 88, t(136) = 4.03, p = .015, FWE-corrected). Furthermore, gray matter volume in the left putamen was reduced in carriers of the CHRNA4 T/T variant compared to CHRNA4 C+ carriers (peak voxel x = −32, y = 3, z = −5, cluster size K = 50, t(136) = 3.8, p = .031, FWE-corrected).

The absence of an interaction effect in the basal ganglia of the left hemisphere despite two significant main effects might be due to the conservative statistical threshold. We therefore lowered the threshold to $p<.005$, uncorrected. Using such a liberal criterion, we found similar main effects and a similar interaction in both hemispheres (see Table 3). The two large clusters (1006 voxels in the right putamen and 499 voxels in the left) extend from the putamen into the globus pallidus. Thus, the differences in lateralization observed on the initial statistical threshold is attributable to weaker effects in the respective contralateral hemispheres which, in consequence, did not show up in the initial conservative test.

Table 1
Sample distribution depending on allelic variants on the DRD2 and CHRNA4 genes.

		DRD2	
		CC	CT and TT
CHRNA4	CC and CT	70	21
	TT	16	35

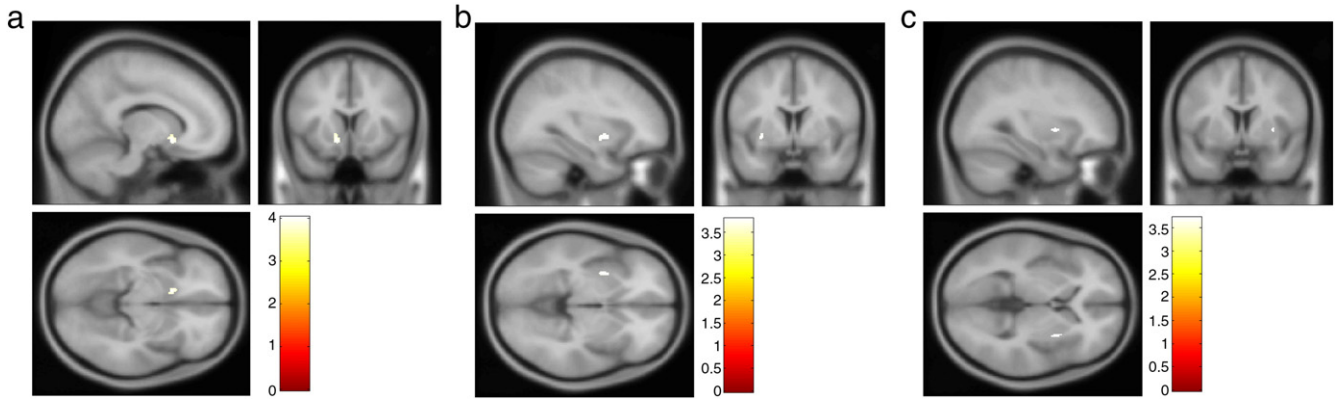

Fig. 1. Statistical parametric maps for the DRD2 main effect (a), the CHRNA4 main effect (b) and the interaction (c). All contrasts were computed with p<.05, FWE corrected.

Main effects on the genotype level

In the present study, we decided to test on the allele and not on the genotype level with a clear hypothesis in mind that was derived from previous work. As the literature reports main effects of both SNPs under investigation on various phenotypes on the genotype level while structural MRI studies on both SNPs are lacking, we also tested for main effects on the genotype level. For that purpose, we fitted separate one-way ANOVA models with the three different genotypes as factor levels for both genes. For both analyses, an omnibus F-test did not reveal any significant clusters at the whole brain level (double thresholded at p<.001, k>100) or at the ROI level (p<.05, FWE-corrected).

Discussion

The present study's purpose was to test for an interaction effect between variants on the dopamine D2 receptor gene DRD2 and the nicotinic acetylcholine receptor gene CHRNA4 on the structure of the basal ganglia. We found a statistically robust reduction of gray matter volume in the right putamen in participants carrying both the DRD2 C/C and the CHRNA4 T/T variant. With a more liberal statistical threshold, the interaction effect was also visible in the left putamen and the globus pallidus.

This interaction effect between DRD2 and CHRNA4 was first described on visuo-spatial working memory (Markett et al., 2010) and later replicated on phonological working memory as well (Markett et al., 2011a). Therefore, the present study provides a second replication of the effect, this time, however, on a biological variable. The evidence that the two genes affect the neurostructural properties of the basal ganglia adds additional momentum to the successful associations with the cognitive variables in previous work and indicates that the interaction effect of both genes is a robust one.

The basal ganglia and especially the dopaminergic activity in the striatum are of major importance for human working memory functioning (Cools et al., 2007, 2008; Hazy et al., 2007; Miller and Cohen, 2001). D2 receptor-mediated gating signals provide the prefrontal cortex with information on what part of the presently available contextual information needs to be encoded in working memory (Bilder et al., 2004; Grace, 1991). There is evidence that the efficiency of this signaling is predictive for an individual's working memory capacity (McNab and Klingberg, 2008).

Taking all three genetic association studies on the interaction between DRD2 and CHRNA4 together, the following picture starts to evolve: Variants of the DRD2 gene which are associated with a reduced striatal dopamine binding potential at D2 receptors (DRD2 C/C) enhance – together with variants on the CHRNA4 gene which affect the neuromodulatory properties of cholinergic neurotransmission on dopaminergic signaling (CHRNA4 T/T) – the efficiency of the gating signals. This is reflected in the reduced gray matter volume in striatal tissue. These altered properties of the striatum affect the central executive of working memory and enable individuals to encode more information in working memory, irrespective of the stimulus modality. The negative relation between cognitive performance and regional gray matter volume depending on DRD2 and CHRNA4 variants is in line with the assumed role of the striatum within functional fronto-striatal-thalamic loops. Both the globus pallidus and the substantia nigra receive permanent inhibitory input from the striatum. The thalamus can only send gating signals to the prefrontal cortex upon a release of this inhibition. The reduction in striatal tissue might enhance the updating signals of the thalamus by reducing the tonic inhibition of the substantia nigra and the globus pallidus.

We are aware that this theoretical framework contains a good deal of speculation. However, it allows deducting hypotheses, which are testable by a combination of functional neuroimaging and molecular genetics. With the repeated replication of the interaction effect

Table 2
Clusters with an interaction between the DRD2 and CHRNA4 genes in the whole brain analysis.

Region	Cluster size	t(136)	MNI coordinates
Right lingual gyrus	292	4.21	14, −82, −6
Left posterior cingulate	166	4.06	5, −52, 7
Right inferior frontal gyrus	147	3.87	−18, 20, −23
Right cuneus	239	3.79	−6, −90, 28
Right superior temporal gyrus	277	3.70	−65, −9, 1
Left putamen	289	3.58	32, 5, 1

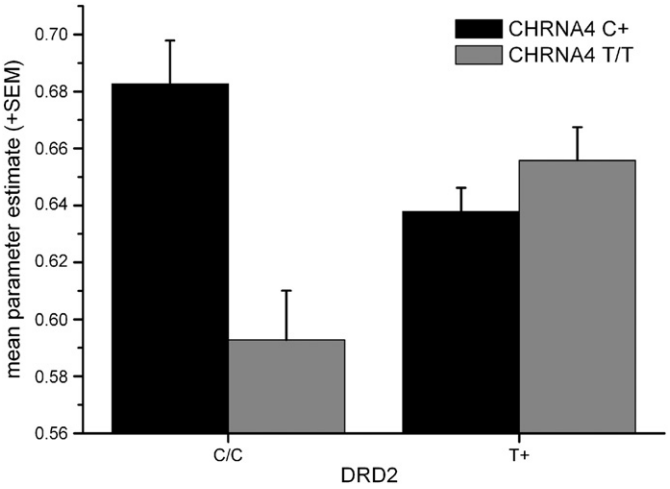

Fig. 2. Mean parameter estimates (+SEM) for the interaction effect within the significant cluster in the right putamen.

Table 3
Main effects and interaction of DRD2 and CHRNA4 on a liberal threshold (p<.005, uncorrected).

	Structure	k	x	y	z	t	df	p
DRD2:	Caudate nucleus left	756	−12	14	−11	4.03	136	<.001
	Caudate nucleus right	47	20	24	1	2.97	136	.002
	Caudate nucleus right	37	12	14	−11	3.08	136	.001
CHRNA4:	Putamen left	782	−32	3	−5	3.8	136	<.001
	Putamen right	438	30	3	−3	3.44	136	<.001
Interaction:	Putamen right	1006	32	5	1	3.71	136	<.001
	Putamen left	499	−30	9	−8	3.50	136	<.001
	Putamen left	49	−30	−16	−6	3.28	136	.001

between DRD2 and CHRNA4 on cognitive and biological phenotypes, such complex study designs are quite worthwhile.

Besides the results from the test of our hypothesis on the interaction between DRD2 and CHRNA4 on the structure of the basal ganglia, there were some unanticipated yet interesting additional results. The ROI analysis yielded reliable main effects for the allelic variants of both genes. Carriers of the DRD2 C/C genotype showed – compared to carriers of at least one DRD2 T-allele – a marked reduction of gray matter in the left caudate. Lowering the statistical threshold revealed a similar effect in the right caudate as well. For the CHRNA4 gene, the main effect showed a reduced volume of the left putamen in CHRNA4 T/T carriers, and this effect was also visible in the right putamen on a more liberal threshold. In contrast to the ROI analysis on the allele level, no main effect could be observed on the genotype level in a whole brain analysis. To our knowledge, the present study provides the first evidence for an association between CHRNA4 rs1044396 and structural differences in gray matter volume. The main effect of the DRD2 allelic variants fits into previous findings reporting an association of the DRD2 C/C genotype with reduced D2 receptor affinity in the striatum (Hirvonen et al., 2009). Previous structural MRI studies on the DRD2 gene have failed to report a DRD2 main effect on the basal ganglia (Cerasa et al., 2009; Montag et al., 2010). The focus of both studies, however, was on DRD2/ANKK1 rs1800497 (TaqIa), a SNP that resides more than 10,000 bp downstream from DRD2 rs6277. Previous linkage analyses have revealed that both SNPs are in strong but not perfect linkage disequilibrium which might explain the observed discrepancies across studies (Markett et al., 2010).

In the interaction analysis on the whole brain level, we found further clusters that showed a reduction in gray matter volume outside of the basal ganglia. As the analysis was not hypothesis driven and the threshold applied was rather liberal, the results should be interpreted with caution, if at all. The effect on the inferior frontal gyrus is of interest, as the basal ganglia and the prefrontal cortex are functionally linked (Alexander et al., 1986). Future studies should aim at a replication of this finding and examine if the structural and functional connectivity between the striatum and the prefrontal cortex is moderated by the DRD2 and CHRNA genes.

One drawback of the present study is the unequal gender distribution in our sample. The reason for this is the recruitment of the participants from psychology classes. As, however, the hypothesized results were obtained after statistically controlling for gender and also because gender did not affect the results in our previous work on the interaction between the DRD2 and CHRNA4 genes, we conclude that the unbalanced gender distribution does not bias the results.

The present results are of relevance for clinical conditions from the psychotic spectrum such as schizophrenia. Schizophrenia is a highly heritable disorder with heritability estimates of 81% (Sullivan et al., 2003). Knowledge of the molecular genetic basis of this high heritability is still scarce but several studies have reported an association between the DRD2 rs6277 SNP (under investigation in the present study) and the risk to develop schizophrenia (Betcheva et al., 2009; Hänninen et al., 2006; Hoenicka et al., 2006; Lawford et al., 2005; Monakhov et al., 2008). Understanding the effect of genetic variants that constitute risk factors for schizophrenia on intermediate phenotypes such as gray matter volume or cognitive variables in patients and healthy controls is necessary to derive a conclusive etiological model of the disorder.

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