The Association between Dopamine DRD2 Polymorphisms and Working Memory Capacity Is Modulated by a Functional Polymorphism on the Nicotinic Receptor Gene CHRNA4

Sebastian A. Markett, Christian Montag, and Martin Reuter

Abstract

Working memory capacity is extremely limited and individual differences are heritable to a considerable extent. In the search for a better understanding of the exact genetic underpinnings of working memory, most research has focused on functional gene variants involved in the metabolism of the neurotransmitter dopamine. Recently, there has been investigation of genes related to other neurotransmitter systems such as acetylcholine. The potential relevance of a polymorphism located in the gene coding for the alpha4 subunit of the nicotinic acetylcholine receptor (rs#1044396) has been discussed with respect to working memory, but empirical investigations have provided mixed results. However, pharmacological studies in both rodents and humans have shown that the effect of nicotinic agonists on cognitive functions is mediated by dopamine. We therefore hypothesized that such an interaction can be found on a molecular genetic level as well. In order to test this hypothesis, we genotyped 101 healthy subjects for rs#1044396 and three functional polymorphisms on the dopamine d2 receptor gene (rs#1800497, rs#6277, rs#2283265). These subjects performed a visuospatial working memory task in which memory load was systematically varied. We found a significant interaction between rs#1044396 and a haplotype block covering all three dopaminergic polymorphisms on working memory capacity. This effect only became apparent on higher levels of working memory load. This is the first evidence from a molecular genetic perspective that these two neurotransmitter systems interact on cognitive functioning. The results are discussed with regard to their implication for working memory theories and their clinical relevance for treatment of substance abuse and schizophrenia.

INTRODUCTION

Working memory is the memory system that allows the maintenance of information in a highly accessible state over a brief period (Cowan, 1999). Working memory has both storage and processing functions and is involved in the goal-directed execution of a vast range of cognitive tasks (D’Esposito, 2007; Baddeley, 1986). Despite the centrality of the working memory construct for cognition, its capacity is surprisingly limited (Miller, 1956). Estimates of working memory capacity suggest that merely four items can be retained in the absence of external stimulation (Owen, McMillan, Laird, & Bullmore, 2005; Cowan, 2000). Despite this limitation, there is, nevertheless, a considerable amount of variation in working memory capacity between individuals, and differences in working memory capacity have proven to be excellent predictors of performance in many cognitive domains including general intelligence (Ackermann, Beyer, & Boyle, 2005; Conway, Kane, & Engle, 2003; Daneman & Merikle, 1996).

Individual differences in working memory capacity seem to be under genetic influence. In a twin study, heritability estimates ranged from 0.27 to 0.51 (Kremen et al., 2007). In the population, working memory capacity is a normally distributed phenotype, and thus, it is obvious that no single gene locus but many loci on several genes, so-called quantitative trait loci, form its genetic foundation (Plomin et al., 1994). The probing question is which particular genes determine working memory capacity. A more detailed understanding of the molecular genetics of working memory would not only add valuable insight into working memory theories but could also benefit the diagnosis and treatment of disorders which are associated with impairments in working memory functioning, such as attention deficit hyperactivity disorder (Martinussen, Hayden, Hogg-Johnson, & Tannock, 2005) or schizophrenia (Barch & Smith, 2008).

The neurotransmitter dopamine plays a pivotal role in working memory processes (Aalto, Brück, Laine, Någren, & Rinne, 2005; Luciana, Depue, Arbisi, & Leon, 1992; Brozoski, Brown, Rosvold, & Goldman, 1979), and individual differences in dopaminergic neurotransmission strongly covariate with working memory capacity (Cools, Gibbs, Miyakawa, Jagust, & D’Esposito, 2008). This evidence renders dopamine-related genes as candidate genes for genetic association studies. An association between COMT
rs#4680 (COMT val158met), a single nucleotide polymorphism (SNP) on the gene coding for the dopamine degrading enzyme catechol-O-methyltransferase (COMT) and working memory has been consistently reported (Mattay et al., 2003; Egan et al., 2001; for a review, see Savitz, Solms, & Ramesar, 2006). The COMT rs#4860 SNP affects the availability of dopamine, but neurotransmitter functioning does not only depend on the availability of a transmitter but also on the existence of sufficient binding sites. Although both dopamine d1 and d2 receptors are important for working memory functioning, molecular genetic research in this area has so far almost exclusively focused on the DRD2 gene which codes for the D2 receptor. The DRD2 rs#1800497 polymorphism (DRD2/ANKK1 Taq-Ia), which is associated with striatal d2 receptor density (Pohjalainen et al., 1998), demonstrates an interaction with COMT rs#4860 on working memory (Gosso et al., 2008). Such an interaction, a so-called epistasis effect, provides direct evidence that more than one gene affects working memory capacity and that their influence is not necessarily additive.

The dominant paradigm in the literature which reports COMT rs#4860 effects on working memory is the n-back task, in which participants view a stream of stimuli in succession and are instructed to respond with a button press each time the nth stimulus repeats. Working memory load can be manipulated by varying the parameter n. The n-back task was also used by Zhang et al. (2007), who demonstrated that an intrinsic SNP on DRD2 (DRD2 rs#2283265), which is linked to individual differences in DRD2 mRNA expression, affects working memory. The n-back task is a global working memory task which taxes maintenance, updating, and manipulation processes (Cowan, 2000). Although the generality of the task has made it an excellent means of establishing the behavioral and neural relevance of the COMT and the DRD2 gene, it remains unclear which exact process is modulated by the genes. In a similar way, composite working memory measures, as used in the Gosso et al. (2008) study on the DRD2 by COMT epistasis, are problematic for the identification of the modulated task components. Recently, Stelzel, Basten, Montag, Reuter, and Fiebach (2009) used structural equation modeling to identify latent variables that account for performance in various working memory tasks. They found that the DRD2 by COMT epistasis selectively affected a latent variable that was interpreted as the executive component of working memory, which involves the manipulation and updating of information.

The selective role of the DRD2 gene on updating and manipulating fits into the framework outlined by Winterer and Weinberger (2004), who suggest that the flexible updating of representations is mediated by D2 receptors, whereas D1 receptors are involved in the maintenance of information. However, Xu et al. (2007) showed that the SNP DRD2 rs#6277 (DRD2 C957T), which is also associated with reduced striatal receptor density, interacts with COMT on working memory in a serial position test. The serial position test is a working memory task which emphasizes maintenance-related processes. During task performance, however, no steps were taken to prevent the subjects from using memory-aiding strategies such as chunking. Thus, the question remains whether the observed gene effect indeed influences maintenance or reflects other executive processes associated with meta-memory. At present, it is not entirely clear which working memory components are targeted by the investigated polymorphisms. It is advisable that future studies focus on the clear isolation of component processes, either by identifying latent variables or by using measures that are regarded as pure estimates of the component under investigation.

The executive component of working memory is related to attention. Baddeley (1986) adopted the supervisory attentional system (Norman & Shallice, 1980) as a blueprint for the central executive, and a more recent model postulates a capacity-limited attentional focus that amplifies the active representations of context information in working memory in the service of ongoing tasks (Cowan, 1999). Because attentional control itself is an integral part of working memory, gene variants which are associated with attentional network functioning might be of relevance for working memory processes as well. Pharmacological studies point toward acetylcholine, especially its receptors of the nicotinic subtype, as a crucial transmitter system for attention (Cools & Robbins, 2004; Mancuso, Andres, Anseau, & Tirelli, 1999; Levin et al., 1998; Provost & Woodward, 1991). The SNP rs1044396 in the gene coding for the alpha subunit of the nicotinic acetylcholine receptor (CHRNA4) was intensively studied with respect to its role in attention, and there is abundant evidence that this SNP is associated with attentional network functioning (Reinvang, Lundervold, Rootwelt, Wehling, & Espeseth, 2009; Espeseth, Endestad, Rootwelt, & Reinvang, 2007; Winterer et al., 2007; Greenwood, Fossella, & Parasuraman, 2006; Parasuraman, Greenwood, Kumar, & Fossella, 2005). However, its role is less clear with regard to working memory. Parasuraman et al. (2005) could not relate the CHRNA4 SNP to working memory in a visuospatial delayed-match-to-sample task. Greenwood et al. (2008) addressed the genetic role of CHRNA4 in the interaction between attention and working memory. They used a delayed-match-to-sample task together with a visual cueing task. Working memory performance declined with an increase in task difficulty, however, the CHRNA4 predicted whether beneficial visual cues could diminish this effect. This provides evidence that the CHRNA4 SNP modulates the overlapping processes between attention and working memory.

Although there is abundant evidence for the role of dopaminergic gene loci in working memory functioning, the results regarding cholinergic genes are ambiguous. Inconsistent findings regarding a specific gene locus might be partially due to an interaction with other gene loci that conceal the individual impact of a given SNP when examined in isolation. In the present study, we seek to examine the hypothesis that CHRNA4 rs1044396 affects working memory in interaction with dopamine-related gene loci in
a working memory task without additional requirements on attentional network functioning. We ground this hypothesis on three clusters of evidence. First, it was suggested that cholinergic and dopaminergic neurotransmission in mesocortical pathways subserve the dynamic goal-directed attentional amplification of representations in working memory (Dehaene, Kerszberg, & Changeux, 1998). There is a strong overlap between the cholinergic and the dopaminergic system in mesocortical pathways. Especially in the striatum, dopaminergic neurons share nicotinic acetylcholine receptors and dopamine receptors can be found on cholinergic interneurons (Esley & Cragg, 2008; Zhou, Wilson, & Dani, 2003). Functionally, the striatum acts as a funnel for corticothalamical loops through the basal ganglia in service of cognitive functions, among other things (Alexander, DeLong, & Strick, 1986). Given the overlap of the dopaminergic and nicotinic systems, there is reason to assume that dopamine and acetylcholine do not affect attentional amplification in working memory independently.

Second, evidence from pharmacological studies in rodents indicate that nicotinic acetylcholine receptor functioning affects working memory and that this influence is least partially mediated by dopamine. In the rat model, nicotine receptor agonists improve radial maze performance (Levin & Rose, 1991), whereas antagonists lead to impairment (Levin, Castonguay, & Ellison, 1987). The administration of a dopamine D2 agonist, however, can reverse the impairments induced by nicotinic antagonists (Levin, McGurk, Rose, & Butcher, 1989), whereas the coadministration of both dopamine and nicotine antagonists leads to additional impairment when compared to the individual impact of each substance (McGurk, Levin, & Butcher, 1989). Remarkably, reversal of the impairment induced by the nicotinic antagonist cannot be accomplished by a dopamine D1 agonist. This indicates a selective role of dopamine D2 receptors in the observed interaction between dopaminergic neurotransmission and nicotinic acetylcholine receptors during working memory tasks (Levin et al., 1989).

Third, in a recent pharmacogenetic imaging study in healthy subjects, it was shown that nicotine affects working memory differently depending on the individual’s genotype (Jacobsen, Pugh, Mencl, & Gelernter, 2006). Using fMRI, it was shown that transdermally administered nicotine led to a stronger BOLD response in the left anterior insula during a demanding n-back task. Furthermore, error rates were elevated. These effects were restricted to individuals carrying at least one T-allele of the DRD2 rs#6277 polymorphism. This allele variant is associated with an elevated receptor density in the striatum (Hirvonen et al., 2006). This finding points directly toward the question of whether functional alternations in nicotinic receptor functioning have a different impact on working memory depending on dopaminergic gene variants.

As outlined above, it is not entirely clear which working memory processes are targeted by dopaminergic genes. Working memory capacity appears to be an ideal dependent measure for the present purpose because the idea that individual differences in working memory are best reflected in working memory capacity has a long tradition in working memory research (Daneman & Merikle, 1996) and genetic influence on working memory capacity has been demonstrated in twin studies. Cowan (2001) outlined boundary conditions which allow for observation of a pure estimate of working memory capacity. The brief visuospatial array task represents a pure capacity estimate in the visuospatial domain (Luck & Vogel, 1997). In such tasks, an array consisting of a variable number of stimuli is flashed briefly. The short presentation duration discourages eye movements and prevents participants from grouping individual stimuli into chunks. Thus, one can assume that each stimulus in the array is encoded as one chunk, which allows for inference on an individual’s capacity. It was shown that a critical predictor of working memory capacity in brief visuospatial array tasks is the efficiency of the selective encoding of relevant information in combined selective attention/working memory tasks (McNab & Klingberg, 2008; Vogel, McCollough, & Machizawa, 2005). The selection of task-relevant information to the exclusion of irrelevant information has been associated with executive control (Posner & Dehaene, 1994). Based on first evidence that dopaminergic gene loci are involved in the executive component of working memory, we assume that the genes under investigation affect working memory capacity by influencing the updating of information into working memory.

The interaction between dopamine and nicotine is mainly mediated by dopamine d2 receptors. Thus, we focused on functional SNPs on the DRD2 gene coding for this receptor subtype. We chose three SNPs which had previously been shown to be functional and related to working memory, namely, DRD2 rs#1000497 (Goss et al., 2008; Pohjalainen et al., 1998), DRD2 rs#6277 (Xu et al., 2007; Hirvonen et al., 2006), and DRD2 rs#2283265 (Zhang et al., 2007). Instead of testing every DRD2 SNP on interaction with CHNRA4 rs#1033496 in isolation, we opted for a different strategy: We made use of the fact that adjacent alleles on a chromosome are not inherited independently but in so-called haplotype blocks with a strong recombinatory variation between and little or no recombination within these blocks (Gabriel et al., 2002). We constructed haplotype blocks from the genotype data by means of statistical linkage analysis and then tested the haplotype blocks on the DRD2/ANKK1 gene for interaction with the nicotinic SNP. By doing so, we increased the power of our test and reduced the risk of false-positive results due to multiple testing (The International HapMap Consortium, 2003).

METHODS

Participants

A total of 101 white subjects (n = 83 women, n = 18 men, mean age M = 24.76 years, SD = 6.45) gave their informed written consent to participate in the study. Participants...
were recruited from the gene data bank of the Bonn Gene Brain Behavior Project (BGBBP) and had previously delivered DNA samples together with their approval to use the genetic information in experimental studies. All subjects were enrolled in the psychology program at the University of Bonn and were compensated with course credit for their efforts. The high proportion of female participants reflects the gender distribution in German psychology classes. Before testing, subjects were screened for neurological and psychiatric disorders and questioned about their smoking habits. The study was carried out in accordance with the ethical principals of the Declaration of Helsinki of the World Medical Association and was approved by the ethics committee of the German Psychologist Association.

Genotyping
DNA was extracted from buccal cells. Automated purification of genomic DNA was conducted by means of the MagNA Pure LC system using a commercial extraction kit (MagNA Pure LC DNA isolation kit; Roche Diagnostics, Mannheim, Germany). Genotyping of the four SNPs was performed by real-time polymerase chain reaction (RT-PCR) using fluorescence melting curve detection analysis by means of the Light Cycler System (Roche Diagnostics). The protocols for the PCR were as follows:

**DRD2 rs#1800497:**
Forward primer: \(5'\text{CGGCTGGCCAAGTTGTCTAA}3'\)
Backward primer: \(5'\text{AGCACCTTCCTGAGTGTCATCA}3'\)
Anchor hybridization probe: \(5'\text{LCRed640-TGAGGATGGC-TGTGTTG-CCCTT-phosphate}3'\)
Sensor hybridization probe: \(5'\text{CTGCCTCGACCAGCACT-fluorescein}3'\)

**DRD2 rs#6277:**
Forward primer: \(5'\text{GAACCTTGTCCGGCTTTACC}3'\)
Backward primer: \(5'\text{CAATCTTGGGGTGGTCTTT}3'\)
Anchor hybridization probe: \(5'\text{LCRed640-CCCCGCCAAAC-CAGAGAAGAAT-phosphate}3'\)
Sensor hybridization probe: \(5'\text{TCCACAGCACTCCCGA-CA-fluorescein}3'\)

**DRD2 rs#2283265:**
Forward primer: \(5'\text{TCTTGCGCTAGACGCAT}3'\)
Backward primer: \(5'\text{GTGGAATCCTCAAGACCACC}3'\)
Anchor hybridization probe: \(5'\text{LCRed640-CCTGTTTCC-TCATCTGTTAAATGGGAAT-phosphate}3'\)
Sensor hybridization probe: \(5'\text{TCTTGACCGTGACCTCCCA-fluorescein}3'\)

**CHRNA4 rs#1044396:**
Forward primer: \(5'\text{TCTCGCAACACCACACTC-3'}\)
Backward primer: \(5'\text{GTCTGTGTCTTCTTGGCCTTCA-3'}\)
Anchor hybridization probe: \(5'\text{LCRed640-CACCGAAGAG-GGCTCCTTCTTGCAT-phosphate}3'\)
Sensor hybridization probe: \(5'\text{TCTTGACCGTGACCTCCCA-fluorescein}3'\)

Haplotype Analysis
Linkage analyses between SNPs and construction of haplotype blocks were conducted by means of Haplovew 3.32 (www.broad.mit.edu/mpg/haplovew/index.php). Individual haplotypes were calculated with PHASE, version 2.1. PHASE implements a Bayesian statistical method for reconstructing haplotypes from population genotype data (Stephens, Smith, & Donnelly, 2001). The construction of haplotype blocks was conducted via the Solid Spine of LD method.

Behavioral Task and Stimuli
We applied a version of a brief visual array task (Luck & Vogel, 1997) as it was used in McNab and Klingberg (2008). The procedure is depicted in Figure 1. Following a 1000-msec fixation, a grid comprising 16 positions was presented to the subject for 1000 msec. In this grid, red squares were arranged at a variable number of positions, and participants were instructed to memorize the exact location of the squares. After a retention interval of 2000 msec, the grid was presented again with one position cued. Participants indicated with different button presses on a response pad (Cedrus, San Pedro, CA) whether a red square had stood on the cued position or not. Subjects performed on a total of 150 trials in five blocks. Working memory load was increased blockwise in ascending order from two to six items (i.e., red squares). Participants

![Figure 1](http://www.mitpressjournals.org/doi/pdfplus/10.1162/jocn.2009.21354)
were instructed to respond as fast as possible while avoiding errors.

**Measures**

We computed Cowan’s K as an estimate of working memory capacity, which is the measure most widely used in research on visual working memory (McNab & Klingberg, 2008; Song & Jiang, 2006; Vogel et al., 2005). K is defined as \( K = (H + CR - 1) \times n \), where \( H \) and \( CR \) are the observed hit rate and correct rejection rate, respectively, and \( n \) is the number of items in the array (Cowan, 2001). The K measure uses the correct rejection rate to correct for guessing and assumes that a person who can hold \( K \) items in working memory will answer correctly in \( K \) out of \( n \) trials if an item has actually stood at the cued position. For each working memory load (\( n = 2, 3, \ldots, 6 \) items), a separate \( K \) value was computed. Following McNab and Klingberg (2008), \( K_{\text{max}} \), the highest individual \( K \) value irrespective of working memory load, served as a global index of working memory capacity.

We further analyzed participants’ reaction times (RTs). It is widely apprehended that the slope in RT with increasing working memory load reflects the efficiency of mental search in working memory, which is regarded as an important working memory process (Sternberg, 1969). The rationale here is that an efficient search in working memory allows a memory-guided correct response before the memory trace decays. Hence, individuals with a steeper slope are thought to search less efficiently in working memory and, therefore, show a reduced performance in working tasks. Prior to analysis, RTs were submitted to an exploratory data analysis to identify outliers according to Tukey’s (1977) criterion. Outlier analysis was computed separately for each participant and working memory load, and led to the exclusion of 7.1 trials per participant on average.

**RESULTS**

**Genetic Analyses**

The genotype frequencies were as follows: DRD2 rs#1800497—A1A1, \( n = 5 \); A1A2, \( n = 31 \); A2A2, \( n = 61 \) [test for Hardy–Weinberg equilibrium \( \chi^2(1) = 0.17, \text{ ns} \); DRD2 rs#6277—C/C, \( n = 30 \); C/T, \( n = 44 \); T/T, \( n = 25 \) [test for Hardy–Weinberg equilibrium \( \chi^2(1) = 1.17, \text{ ns} \); DRD2 rs#2283265—G/G, \( n = 69 \); G/T, \( n = 27 \); T/T, \( n = 2 \) [test for Hardy–Weinberg equilibrium \( \chi^2(1) = 0.12, \text{ ns} \); CHRNA4 rs#1044396—C/C, \( n = 20 \); C/T, \( n = 48 \); T/T, \( n = 31 \) [test for Hardy–Weinberg equilibrium \( \chi^2(1) = 0.03, \text{ ns} \). Due to technical reasons, some samples could not be genotyped for some SNPs: DRD2 rs#1800497, \( n = 4 \); DRD2 rs#6277, \( n = 2 \); DRD2 rs#2283265, \( n = 3 \); and CHRNA4 rs#1044396, \( n = 2 \). Genotype data for at least one DRD2 SNP was available for each subject, which is sufficient for the haplotype analysis. The two subjects whose samples could not be genotyped for CHRNA4 were excluded from further analysis.

The three DRD2 SNPs built a haplotype block spanning 15.5 kb on the DRD2/ANKK1 gene (see Figure 2). Five different haplotypes could be identified (see Table 1). We grouped the sample into TCT-haplotype carriers and noncarriers, as this haplotype covers all three alleles associated with reduced or altered dopamine D2 receptor density. As is common in the literature (Reinvang et al., 2009; Parasuraman et al., 2005), we further divided the sample into CHRNA4 C-allele carriers (C/C and C/T genotypes) and into T/T carriers. The resulting distribution of the sample according to haplotype and CHRNA4 allele is depicted in a \( 2 \times 2 \) format in Table 2. Gender frequencies were equally distributed across cells (Fisher’s exact test, \( p = .57 \)).

**Working Memory Capacity**

One participant was excluded from the analysis because her performance was at below-chance level. There was no sex difference [multivariate \( F(6, 91) = 0.81, p = .562 \)

---

**Figure 2.** The haplotype block spanning three functional SNPs on the DRD2/ANKK1 gene. Values in the squares are \( d^2 \) values and represent the linkage disequilibrium between the SNPs. The T-allele of rs#1800497 and the C-allele of rs#6277 are both associated with reduced striatal receptor density (Hirvonen et al., 2006; Pohjalainen et al., 1998). The T-allele of rs#2283265 is associated with different mRNA expression for dopamine d2 receptors (Zhang et al., 2007).
nor was there a difference between smokers and non-smokers \[multivariate F(6, 91) = 0.37, p = .894\] on the \(K\) measures. Data were collapsed for further analysis. Figure 3 shows means and standard errors for Cowan’s \(K_{\text{max}}\) in the different genotype groups. A 2 × 2 between-subjects ANOVA revealed a significant main effect for the DRD2 haplotype \[F(1, 94) = 5.78, p = .018, \eta^2_{\text{est}} = .058\], and a significant interaction between DRD2 haplotype and CHRNA4 \[F(1, 94) = 9.23, p = .003, \eta^2_{\text{est}} = .089\]. Carriers of the TCT-haplotype outperformed their counterparts, but only when they were also homozygous for the CHRNA4 T-allele. No such difference could be found in subjects who carried at least one CHRNA4 C-allele.

Next, we examined working memory capacity in dependence of working memory load. Means and standard errors are depicted in Figure 4. Table 3 gives an overview of hit rates, correct rejection rates, and Cowan’s \(K\) values in dependence of the haplotype/genotype groups and working memory load. A 5 × 2 × 2 ANOVA on \(K\) with number of items in the array as within factor and the DRD2 haplotype and CHRNA4 genotype as between factors revealed significant main effects for working memory load \[F(4, 376) = 5.44, p < .0001, \eta^2_{\text{est}} = .089\]; gender, \(F(1, 91) = 11.516, p = .001, \eta^2_{\text{est}} = .112\]; and between DRD2 haplotype and CHRNA4 genotype as well. The combination of a DRD2 TCT-haplotype and a CHRNA4 T/T-genotype is beneficial for working memory capacity when the load on working memory increases. This was corroborated by exploratory \(t\) tests in the T/T carriers: The differences between the haplotype groups were significant on the four- \([t(26.165) = 2.46, p < .022]\) and on the five-item levels \([t(26.165) = 4.23, p < .0002]\). The latter contrast remained significant after adjusting for multiple testing.

In a last step, we assessed whether similar effects could be seen as a function of each DRD2 SNP separately, or whether stratification based on haplotype was necessary to reveal the effects. Table 4 shows the DRD2 main effects and DRD2 × CHRNA4 interactions for each dopaminergic SNP on \(K_{\text{max}}\). Each of the three SNPs interacts with the CHRNA4 SNP by itself. Upon examining the effect sizes, we can see that the haplotype analysis adds an increment in accounted variance as compared to the single SNP analyses.

### Reaction Time Analysis

Mean RTs under different working memory loads can be seen in Figure 5. Male subjects showed shorter response latencies, and sex was included as an additional factor in the model. A 5 × 2 × 2 × 2 ANOVA on RTs with number of items in the array as within factor and gender, the DRD2 haplotype, and CHRNA4 genotype as between-subject factors revealed significant main effects for number of items in the array \[F(4, 364) = 8.23, p = .005, \eta^2_{\text{est}} = .08\]. Most notably, the three-way interaction was significant \[F(4, 376) = 4.47, p = .002, \eta^2_{\text{est}} = .045\]. The TCT-haplotype affects working memory capacity differently depending on the number of items in the array and depending on the CHRNA4 genotype as well. The combination of a DRD2 TCT-haplotype and a CHRNA4 T/T-genotype is beneficial for working memory capacity when the load on working memory increases. This was corroborated by exploratory \(t\) tests in the T/T carriers: The differences between the haplotype groups were significant on the four- \((t(26.165) = 2.46, p < .022)\) and on the five-item levels \((t(26.165) = 4.23, p < .0002)\). The latter contrast remained significant after adjusting for multiple testing.

### Table 1. Observed and Expected Haplotypes as Reconstructed with PHASE

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>Observed</th>
<th>Expected</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 CCG</td>
<td>61</td>
<td>60.36</td>
</tr>
<tr>
<td>2 CTT</td>
<td>0</td>
<td>0.1</td>
</tr>
<tr>
<td>3 CTG</td>
<td>97</td>
<td>97.34</td>
</tr>
<tr>
<td>4 CTT</td>
<td>0</td>
<td>0.02</td>
</tr>
<tr>
<td>5 TCG</td>
<td>1</td>
<td>2.02</td>
</tr>
<tr>
<td>6 TCT</td>
<td>34</td>
<td>33.61</td>
</tr>
<tr>
<td>7 TTG</td>
<td>9</td>
<td>8.53</td>
</tr>
<tr>
<td>8 TTT</td>
<td>0</td>
<td>0.02</td>
</tr>
</tbody>
</table>

In the haplotype column, the first position refers to DRD2 rs#1800497, the second to DRD2 rs#6277, and the third to DRD2 rs#2283265.

### Table 2. Distribution of the Sample According to DRD2 Haplotype and CHRNA4 Alleles

<table>
<thead>
<tr>
<th>CHRNA4 rs#1044396</th>
<th>CC and CT</th>
<th>TT</th>
</tr>
</thead>
<tbody>
<tr>
<td>DRD2 TCT+</td>
<td>21</td>
<td>10</td>
</tr>
<tr>
<td>DRD2 TCT−</td>
<td>47</td>
<td>21</td>
</tr>
</tbody>
</table>

TCT+ refers to carriers of the TCT haplotype and TCT− to participants without the TCT haplotype.

### Figure 3. Epistasis effect of the DRD2/ANKK1 haplotype and the CHRNA4 C-allele on an omnibus measure of working memory capacity (\(K_{\text{max}}\)).

Markett, Montag, and Reuter 1949
and CHRNA4, F(1, 91) = 6.215, p = .014, $\eta^2_{est} = .064$. No reliable interaction could be detected. RTs increased linearly with an increasing number of items in the array [linear trend, F(1, 91) = 14.28, p < .001, $\eta^2_{est} = .136] and CHRNA4 T/T-allele carriers showed uniformly higher RTs.

**DISCUSSION**

In the present study, we tested whether functional polymorphisms on the gene coding for the dopamine d2 receptor (DRD2/ANKK1) predict working memory capacity in a brief visuospatial array task in interaction with a functional polymorphism on CHRNA4, the gene coding for the nicotinic acetylcholine receptor $\alpha_4$ subunit. We were able to reconstruct individual haplotype blocks spanning 15.5 kb on the DRD2/ANKK1 gene from the genotype data. These haplotype blocks were then tested on an interaction with the CHRNA4 SNP. We found a main effect of the haplotype on an omnibus measure of working memory capacity (Cowan’s $K_{max}$): Carriers of the DRD2 TCT+ haplotype outperformed their counterparts by holding one additional item in working memory. The benefit of DRD2 TCT+, however, was restricted to carriers of the homozygous T/T variant of CHRNA4 (see Figure 3). This was reflected by a significant interaction effect of DRD2 and CHRNA4. No main effect for CHRNA4 rs#1044396 could be detected. The main effect for the haplotype and the null result for CHRNA4 are consistent with the literature (Xu et al., 2007; Zhang et al., 2007; Parasuraman et al., 2005).

The $K$ measure is thought to reflect the number of items stored in working memory but its computation is based on error rates. Thus, the gene effect on the global $K_{max}$ might not reflect differences in working memory capacity, but rather performance differences in accuracy irrespective of working memory load. A more detailed analysis revealed that the gene effects were only visible when the working memory load was high (5 items in the array). It was previously demonstrated that $K$ increases with set size until it reaches a plateau or even drops when the individual capacity limit is exceeded. Individual differences in working memory capacity only become apparent at higher working memory load: Although some individuals reach their plateau at five items or higher, the performance of others levels up at set sizes as little as three (Todd & Marois, 2005). The present significant three-way interaction between DRD2, CHRNA4, and working memory load renders it

![Figure 4](https://example.com/figure4.png)

**Figure 4.** Three-way interaction of the DRD2/ANKK1 haplotype, the CHRNA4 C-allele, and number of items in the array on working memory capacity. The graph on the left (A) shows the results of CHRNA4 T/T carriers, the graph on the right (B) those of C/C and C/T carriers.

<table>
<thead>
<tr>
<th>Items</th>
<th>DRD2 TCT+</th>
<th>DRD2 TCT+</th>
<th>DRD2 TCT+</th>
<th>DRD2 TCT+</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CHRNA4 C+</td>
<td>CHRNA4 C+</td>
<td>CHRNA4 C+</td>
<td>CHRNA4 C+</td>
</tr>
<tr>
<td></td>
<td>p(H)</td>
<td>p(CR)</td>
<td>K</td>
<td>p(H)</td>
</tr>
<tr>
<td>2</td>
<td>.83</td>
<td>.83</td>
<td>1.32</td>
<td>.87</td>
</tr>
<tr>
<td>3</td>
<td>.83</td>
<td>.80</td>
<td>1.85</td>
<td>.83</td>
</tr>
<tr>
<td>4</td>
<td>.74</td>
<td>.74</td>
<td>1.90</td>
<td>.75</td>
</tr>
<tr>
<td>5</td>
<td>.69</td>
<td>.61</td>
<td>1.51</td>
<td>.75</td>
</tr>
<tr>
<td>6</td>
<td>.64</td>
<td>.63</td>
<td>1.62</td>
<td>.66</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Items</th>
<th>DRD2 TCT+</th>
<th>DRD2 TCT+</th>
<th>DRD2 TCT+</th>
<th>DRD2 TCT+</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CHRNA4 C+</td>
<td>CHRNA4 C+</td>
<td>CHRNA4 C+</td>
<td>CHRNA4 C+</td>
</tr>
<tr>
<td></td>
<td>p(H)</td>
<td>p(CR)</td>
<td>K</td>
<td>p(H)</td>
</tr>
<tr>
<td>2</td>
<td>.85</td>
<td>.89</td>
<td>1.50</td>
<td>.86</td>
</tr>
<tr>
<td>3</td>
<td>.83</td>
<td>.82</td>
<td>1.95</td>
<td>.80</td>
</tr>
<tr>
<td>4</td>
<td>.73</td>
<td>.76</td>
<td>1.96</td>
<td>.70</td>
</tr>
<tr>
<td>5</td>
<td>.67</td>
<td>.69</td>
<td>1.94</td>
<td>.63</td>
</tr>
<tr>
<td>6</td>
<td>.56</td>
<td>.66</td>
<td>1.47</td>
<td>.56</td>
</tr>
</tbody>
</table>
in working memory capacity between the haplotype/DRD2 haplotype or with the CHRNA4 SNP was observed. (1969). However, with respect to RT, no interaction with the gene effect.

Furthermore, the epistasis effect of DRD2 by CHRNA4 was simply caused by differently affected error rates and was not only predictive for the amount of unnecessary storage of irrelevant information but also for individual working memory capacity which was measured outside the scanner in a task similar to the one used in the present study. The basal ganglia in general and the striatum in particular are thought to have a gating function for the access of context information into working memory (Cools, Sheridan, Jacobs, & D’Esposito, 2007; Braver & Cohen, 2000), presumably by means of the control of an attentional focus (McNab & Klingberg, 2008; Cowan, 1999; Dehaene et al., 1998). Dopamine D2 receptors are cardinaly expressed in the striatum (Camps, Cortes, Gueye, Probst, & Palacios, 1989), and nicotinic acetylcholine receptors with α4-subunits can be found on striatal dopaminergic neurons (Exley & Cragg, 2008). It was shown that the DRD2 SNPs under investigation influence striatal D2 receptor availability (Zhang et al., 2008). It was shown that the DRD2 SNPs under investigation influenced striatal D2 receptor availability (Zhang et al., 2008). It was shown that the DRD2 SNPs under investigation influenced striatal D2 receptor availability (Zhang et al., 2008). It was shown that the DRD2 SNPs under investigation influenced striatal D2 receptor availability (Zhang et al., 2008).

As expected, the RT analysis revealed a linear increase in RT depending on the number of items in the array. This is consistent with the widely replicated findings by Sternberg (1969). However, with respect to RT, no interaction with the DRD2 haplotype or with the CHRNA4 SNP was observed. Furthermore, the epistasis effect of DRD2 by CHRNA4 on working memory capacity could not be observed either. Thus, it seems unlikely that the observed differences in working memory capacity between the haplotype/genotype groups are caused by differently efficient search in working memory.

Working memory load, as measured with Cowan’s K, is associated with an increased negativity in the ERP across parietal cortex (Vogel & Machizawa, 2004) and an increase of the BOLD response in the left intraparietal and intracoccipital sulci (IPS/IOS; Todd & Marois, 2004). Both studies report similar characteristics for the increase in brain activity during the delay period of brief visuospatial array tasks. Individual differences in K_{\text{max}} have been related to individual differences in posterior brain areas, specifically the left intraparietal and intracoccipital sulci (Todd & Marois, 2005). It might be the case that these brain areas are modulated by the two genes under investigation. However, the brain activity was measured during the delay of the task and it is likely that it reflects maintenance-related processes which are not directly related to dopamine D2 receptors (Winterer & Weinberger, 2004).

Another neuromodulatory mechanism of the genetic interaction between CHRNA4 and DRD2 is conceivable. Vogel et al. (2005) demonstrated that the individual ability to exclude irrelevant information in a combined selective attention and working memory task predicted working memory capacity as measured with Cowan’s K in a separately administered brief visuospatial array task. Using fMRI, McNab and Klingberg (2008) were able to locate the effect: Activity in the basal ganglia, in preparation of the exclusion of irrelevant information, was not only predictive for the amount of unnecessary storage of irrelevant information but also for individual working memory capacity which was measured outside the scanner in a task similar to the one used in the present study. The basal ganglia in general and the striatum in particular are thought to have a gating function for the access of context information into working memory (Cools, Sheridan, Jacobs, & D’Esposito, 2007; Braver & Cohen, 2000), presumably by means of the control of an attentional focus (McNab & Klingberg, 2008; Cowan, 1999; Dehaene et al., 1998). Dopamine D2 receptors are cardinaly expressed in the striatum (Camps, Cortes, Gueye, Probst, & Palacios, 1989), and nicotinic acetylcholine receptors with α4-subunits can be found on striatal dopaminergic neurons (Exley & Cragg, 2008). It was shown that the DRD2 SNPs under investigation influence striatal D2 receptor availability (Zhang et al., 2008).

### Table 4. Separate ANOVAs with the DRD2 SNPs and CHRNA4 C-allele as Factors

<table>
<thead>
<tr>
<th>Effect</th>
<th>F</th>
<th>p</th>
<th>r^2_{est}</th>
</tr>
</thead>
<tbody>
<tr>
<td>DRD2 rs#1800497</td>
<td>4.79</td>
<td>.031</td>
<td>.05</td>
</tr>
<tr>
<td>DRD2 rs#1800497 × CHRNA4 C-allele</td>
<td>5.42</td>
<td>.024</td>
<td>.05</td>
</tr>
<tr>
<td>DRD2 rs#6277</td>
<td>2.127</td>
<td>.125</td>
<td>.04</td>
</tr>
<tr>
<td>DRD2 rs#6277 × CHRNA4 C-allele</td>
<td>4.80</td>
<td>.01</td>
<td>.09</td>
</tr>
<tr>
<td>DRD2 rs#2283265</td>
<td>5.07</td>
<td>.027</td>
<td>.05</td>
</tr>
<tr>
<td>DRD2 rs#2283265 × CHRNA4 C-allele</td>
<td>7.19</td>
<td>.009</td>
<td>.07</td>
</tr>
<tr>
<td>DRD2 haplotype</td>
<td>5.78</td>
<td>.018</td>
<td>.06</td>
</tr>
<tr>
<td>DRD2 haplotype × CHRNA4 C-allele</td>
<td>9.23</td>
<td>.003</td>
<td>.09</td>
</tr>
</tbody>
</table>

Due to the skewed distribution of the alleles of DRD2 rs#1800497 and DRD2 rs2283265, participants were grouped into A1+ (A1/A1 and A1/A2) and G+ (G/G and G/G) carriers, respectively. Effects for the haplotype/genotype analysis are also included.

![Figure 5](https://example.com/f5.png)

**Figure 5.** Results of the reaction time analysis dependent on the DRD2/ANKK1 haplotype, CHRNA rs#1044396 and working memory load. No reliable interaction could be detected.
encoded during the trial-wise updating of working memory contents. However, this view remains speculative until neuroimaging studies shed light into the neural underpinnings of the CHRNA4 by DRD2 interaction on working memory capacity.

The results from the haplotype analysis revealed a haplotype block which is especially interesting from a molecular genetics perspective, because the TCT-haplotype covers the allele variants of three functional SNPs on the DRD2/ANKK1 gene that are each associated with altered DRD2 density in the striatum. Carriers of at least one DRD2 rs#1800497 T-allele (also known as the A1 allele) show a 30–40% reduction of striatal dopamine d2 receptors compared to carriers of the C-allele (Pohjalainen et al., 1998), and striatal DRD2 availability decreases with each additional DRD2 rs#6277 C-allele (Hirvonen et al., 2006). The G → T transition of the DRD2 rs#2283265 SNP affects the splicing of the DRD2 gene after transcription. Carriers of the T-allele exhibit a reduced expression of mRNA for the presynaptic DRD2 short subtype in favor of the postsynaptic long version (Zhang et al., 2007). In the present study, we could demonstrate that a haplotype block, which covers all these three alleles (TCT) in conjunction, is of relevance for working memory capacity.

Unfortunately, the exact neurobiological consequence of the DRD2 TCT-haplotype has not yet been investigated. DRD2 rs#1800497 T-allele and DRD2 rs#6277 C-allele carriers exhibit a reduced DRD2 availability irrespective of the DRD2 subtype, whereas rs#2283265 T-allele carriers show an altered subtype proportion (pre- vs. postsynaptic) irrespective of general receptor availability. Hence, it might be the case that TCT+ carriers have a generally reduced receptor availability with an additionally shifted proportion of presynaptic to postsynaptic receptors. However, this consideration remains speculative until the exact interplay between the alleles on the TCT haplotype and the functional relation between haplotype block and striatal DRD2 availability is clarified. Despite the numerous successful association studies, the exact functional role of CHRNA4 rs#1033296 is unclear at the present time. The neurobiological mechanisms underlying the observed epistasis effect require further research. At the moment, we are under the assumption that the CHRNA4 polymorphism alters the affinity of presynaptic nAChRs on dopaminergic neurons. By this, it affects dopaminergic neurotransmission; taken together with the decreased D2 receptor density, this leads to an optimal saturation of D2 receptors.

There are remaining questions which should be addressed by future research. First, the neuronal correlate of the present effect should be investigated using neuroimaging methods; in particular, the aim should be to resolve the issue of whether parietal or striatal activity is modulated by the two genes. Another interesting endeavor would be an examination of a potential three-way interaction between SNPs on DRD2, CHRNA4, and the COMT gene, such as the COMT rs#4680 SNP that has been repeatedly related to working memory function. Such an investigation would require an even larger sample size than the present study. Future research may want to include more male participants. Due to the sampling strategy from psychology classes, we obtained a sample which was homogenous with respect to age and level of education. On the downside, however, the sample consisted of mainly female participants, reflecting the gender distribution in psychology classes. Gender was not significantly associated with any genotype/haplotype group in the factorial design, nor was it by any means related to the dependent variables under investigation. Thus, it seems very unlikely that an equal gender distribution across the sample would alter the results.

To our knowledge, the present finding is the first evidence on a molecular genetic level that the dopaminergic and the cholinergic system interact on cognitive functioning. This certainly adds momentum to the investigation of the exact molecular genetic underpinnings of working memory capacity and thereby also to its correlates such as general intelligence. Furthermore, the dopaminergic and nicotinic cholinergic epistasis provides a tentative ground for clinical and health-related investigations. Nicotine, as routinely self-administered by cigarette smokers, acts on nicotinic acetylcholine receptors and unfold its rewarding properties through the release of dopamine in mesostriatal pathways. Aside from being highly addictive, nicotine facilitates attentional processes and it was hypothesized that at least a subgroup of cigarette smokers self-administer nicotine to compensate for small attention deficits (Evans & Drobes, 2009). A similar self-medication hypothesis was put forward for schizophrenia. Patients suffering from schizophrenia exhibit elevated smoking prevalences compared to other clinical groups (Levin & Rezvani, 2007). It was suggested that these patients smoke to compensate for cognitive deficits associated with the negative symptoms (Weiser et al., 2004) or to reduce side effects from their neuroleptic medication (McEvoy, Freudenreich, Levin, & Rose, 1995). A better understanding of the dynamic interplay between nicotinic cholinergic and dopaminergic receptors, with respect to cognitive functions such as working memory, could benefit our understanding of addictive behavior and psychotic conditions and eventually lead to an appropriate treatment of cognitive deficits instead of hazardous cigarette smoking. We believe that a molecular genetics perspective appears to be a feasible means of investigation, as genetic variations are stable individual differences that can be easily assessed and could allow for a reliable allocation to an individual treatment in the future.

Acknowledgments
We thank Magdalena Jurkiewicz for editing the manuscript.
Reprint requests should be sent to Sebastian A. Markett, Department of Psychology, Kaiser Karl-Ring 9, 53111 Bonn, Germany, or via e-mail: markett@uni-bonn.de.


