

Developmental Psychology

Dopamine, Working Memory, and Training Induced Plasticity: Implications for Developmental Research

Stina Söderqvist, Sissela Bergman Nutley, Myriam Peyrard-Janvid, Hans Matsson, Keith Humphreys, Juha Kere, and Torkel Klingberg

Online First Publication, November 21, 2011. doi: 10.1037/a0026179

CITATION

Söderqvist, S., Bergman Nutley, S., Peyrard-Janvid, M., Matsson, H., Humphreys, K., Kere, J., & Klingberg, T. (2011, November 21). Dopamine, Working Memory, and Training Induced Plasticity: Implications for Developmental Research. *Developmental Psychology*. Advance online publication. doi: 10.1037/a0026179

Dopamine, Working Memory, and Training Induced Plasticity: Implications for Developmental Research

Stina Söderqvist, Sissela Bergman Nutley,
Myriam Peyrard-Janvid, Hans Matsson,
and Keith Humphreys
Karolinska Institutet

Juha Kere
Karolinska Institutet and University of Helsinki

Torkel Klingberg
Karolinska Institutet

Cognitive deficits and particularly deficits in working memory (WM) capacity are common features in neuropsychiatric disorders. Understanding the underlying mechanisms through which WM capacity can be improved is therefore of great importance. Several lines of research indicate that dopamine plays an important role not only in WM function but also for improving WM capacity. For example, pharmacological interventions acting on the dopaminergic system, such as methylphenidate, improve WM performance. In addition, behavioral interventions for improving WM performance in the form of intensive computerized training have recently been associated with changes in dopamine receptor density. These two different means of improving WM performance—pharmacological and behavioral—are thus associated with similar biological mechanisms in the brain involving dopaminergic systems. This article reviews some of the evidence for the role of dopamine in WM functioning, in particular concerning the link to WM development and cognitive plasticity. Novel data are presented showing that variation in the dopamine transporter gene (*DAT1*) influences improvements in WM and fluid intelligence in preschool-age children following cognitive training. Our results emphasize the importance of the role of dopamine in determining cognitive plasticity.

Keywords: working memory, fluid intelligence, dopamine, plasticity, cognitive training

Working memory (WM) is the ability to manipulate and keep task relevant information in mind for a short period of time. This is important for reasoning, which typically involves several steps of planning and execution. WM deficits are commonly observed in several neuropsychiatric disorders occurring during development, such as attention-deficit/hyperactivity disorder (ADHD; Castellanos & Tannock, 2002; Martinussen, Hayden, Hogg-Johnson, & Tannock, 2005). WM capacity is also strongly associated with general intellectual ability and is a predictor for later academic

performance (Alloway, Gathercole, Kirkwood, & Elliott, 2009; Gathercole, Brown, & Pickering, 2003; Kane et al., 2004). These observations emphasize the importance of understanding the basis of WM function, its development, and plasticity.

Dopamine and Working Memory

Evidence From Primates

The link between WM and dopamine has been investigated at a cellular level by studying neurons exhibiting memory fields in the prefrontal cortex (PFC) of monkeys. These neurons are believed to be the cellular basis for visuospatial WM as they are specifically active in response to distinct spatial locations of a stimulus and are also active during the delay period between stimulus presentation and response. Dopamine D1 receptor antagonists enhance the response of these neurons (Williams & Goldman-Rakic, 1995). This effect seems restricted to the D1 receptor, as no effect of a D2 agonist was observed in the same study. Effects were also dose dependent and specific to the neurons displaying memory field properties. D1 receptor stimulation can also lead to long-term improvements in WM performance, as observed in rhesus monkeys with either age-related (Castner & Goldman-Rakic, 2004) or drug-induced (Castner, Williams, & Goldman-Rakic, 2000) cognitive impairments. The specific mechanism by which D1 receptor manipulation acts to enhance visuospatial WM performance has

Stina Söderqvist, Sissela Bergman Nutley, and Torkel Klingberg, Department of Neuroscience and Stockholm Brain Institute, Karolinska Institutet, Stockholm, Sweden; Myriam Peyrard-Janvid and Hans Matsson, Department of Biosciences and Nutrition at Novum, Karolinska Institutet, Huddinge, Sweden; Keith Humphreys, Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, Stockholm, Sweden; Juha Kere, Department of Biosciences and Nutrition at Novum, Karolinska Institutet, Huddinge, Sweden; Science for Life Laboratory Stockholm, Karolinska Institutet, Stockholm, Sweden; Department of Medical Genetics, Haartman Institute, University of Helsinki, Helsinki, Finland.

Torkel Klingberg and Sissela Bergman Nutley are both main inventors of the non-verbal reasoning training program that has been submitted in an international patent application, financed by Pearson Assessment.

Correspondence concerning this article should be addressed to Stina Söderqvist, Karolinska Institutet, Department of Neuroscience, Retzius väg 8, 171 77 Stockholm, Sweden. E-mail: stina.soderqvist@ki.se

been suggested to occur by spatial tuning of neurons through decreasing the neurons' response to nonpreferred directions in a spatial WM task (Vijayraghavan, Wang, Birnbaum, Williams, & Arnsten, 2007). In conclusion, research on primates highlights the importance of cortical dopamine D1 receptor activity for WM performance.

Human Pharmacological Evidence

Evidence for dopamine's involvement in WM functioning in humans stems from pharmacological research, where a distinction between effects of D1 and D2 receptors has also been reported. Whereas the D1/D2 receptor agonist pergolide improves performance on WM tasks, the D2 receptor agonist bromocriptine does not, suggesting a predominate effect of D1 over D2 receptors for WM performance (Müller, von Cramon, & Pollmann, 1998), consistent with the literature on primates. Further studies have demonstrated pergolide's effects to be dependent on baseline performance, with high-performing individuals benefiting more from the treatment than lower performers (Kimberg & D'Esposito, 2003). However, there is inconsistent evidence regarding the effect of D2 receptors and some suggestion that bromocriptine influences WM performance in participants with low baseline WM performance (Kimberg, D'Esposito, & Farah, 1997). The effect of D2 agonists on WM has also been suggested to be domain specific, improving performance only on a spatial WM task (Luciana & Collins, 1997).

Methylphenidate is a psychostimulant drug that is commonly used to alleviate symptoms of ADHD by blocking reuptake of dopamine and norepinephrine, thus increasing their availability in the synapse (Patrick, Caldwell, Ferris, & Breese, 1987; Solanto, 1998). In humans, methylphenidate improves WM performance, specifically in the visuospatial domain (Mehta et al., 2000). Furthermore, the drug effect also depends on baseline WM capacity, with greater benefits observed in participants with lower baseline performance (Mehta, Goodyer, & Sahakian, 2004; Mehta et al., 2000).

In summary, human pharmacological studies provide further evidence for the importance of the dopamine neurotransmitter system in WM function. However, the specific roles of task modality, receptor subtypes, and their interaction with subjects' baseline WM capacity need further investigation to provide clarity to the somewhat conflicting evidence.

Dopamine During Development

The dopamine system is believed to undergo numerous changes during development. In rhesus monkeys, dopamine concentration changes during development, with patterns of change being region specific (Goldman-Rakic & Brown, 1981, 1982). Lower dopamine concentrations are observed in somatosensory and posterior parietal cortices than in the frontal lobe. In these regions, a more rapid decrease in density is also observed, reaching adult levels of concentration already at 5 months of age. In contrast, dopamine levels in the prefrontal areas follow a nonlinear developmental pattern. At birth, levels of dopamine in prefrontal areas are high and similar to adult levels. These levels then decrease significantly during the first 6 months of life before once again increasing to reach adult levels (Goldman-Rakic & Brown, 1982). A recent human positron emission tomography (PET) study investigated

dopamine D1 receptor binding potential in humans 10 to 30 years of age (Jucaite, Forssberg, Karlsson, Halldin, & Farde, 2010). Interestingly, no age effects were observed in the posterior parietal cortex for the age range studied, possibly reflecting earlier developmental changes in this region as suggested by Goldman-Rakic and Brown (1982). For other areas, including the frontal, anterior cingulate, and occipital cortices, an average decrease of 26% in D1 binding potential was observed during adolescence. This level of decrease is comparable with that observed throughout the entire adult life span.

Dopamine and Development of WM Capacity

The effect of the gene coding for the catechol-O-methyltransferase (COMT) enzyme has been extensively studied in the context of both typical and atypical cognitive development. The COMT enzyme is important for the degradation of *catecholamines*, such as dopamine, especially within the neocortex (Matsumoto et al., 2003) and is hypothesized to be of particular importance for tasks relying on PFC functioning. The Val¹⁵⁸Met polymorphism of the *COMT* gene has been associated with WM function in both adults and children, independently and in interaction with the *DRD2* gene, coding for the dopamine D2 receptor (Stelzel, Basten, Montag, Reuter, & Fiebach, 2009; Xu et al., 2007). Two studies (Barnett, Heron, Goldman, Jones, & Xu, 2009; Wahlstrom et al., 2007) have observed associations between WM performance and *COMT* polymorphisms in children and adolescents. The effects were found to be curvilinear, with an optimal level of expression being beneficial, but either too much (Val/Val genotype) or too little (Met/Met genotype) enzymatic activity having negative effects on performance. Furthermore, a longitudinal study of typically developing children and adolescents demonstrated a developmental dependency of the *COMT* genotype effect on WM performance (Dumontheil et al., 2011). It was shown that, whereas the Val-allele tended to be associated with superior performance on a visuospatial WM task in younger ages (6–10 years), the Met allele was beneficial after the age of 10. This is in line with the tendency for adult Met carriers to show better WM performance than Val carriers, although evidence is currently inconsistent (for a review, see Dickinson & Elyevag, 2009). These differences in the effect of *COMT* observed across development are consistent with the developmental changes reported in dopamine concentrations discussed earlier, with changes in basal levels of dopamine affecting optimal levels of dopamine degradation (Wahlstrom et al., 2007).

Another genetic variant that has been associated with typically developing WM function is a variable number tandem repeat (VNTR) polymorphism located in the 3-untranslated region of the dopamine transporter (*DAT1*) gene. This polymorphism is believed to be involved in the expression of the gene, with higher expression being associated with the 10-repeat allele in vitro (Fuke et al., 2001; Mill, Asherson, Browes, D'Souza, & Craig, 2002). In children ages 7 to 12 years, 9/10-repeat heterozygosity has been associated with better WM performance than 10-repeat homozygosity (Stollstorff et al., 2010).

A VNTR polymorphism located in exon 3 of the dopamine receptor 4 gene, *DRD4*, has been associated with WM performance in young children (Froehlich et al., 2007), with carriers of the 7-repeat allele showing lower performance on a spatial WM

task. Furthermore the polymorphism was found to interact with the deteriorating effects of lead levels, such that children not carrying the 7-repeat allele were most severely affected by lead levels and showed worse cognitive performance. Also, activity in the PFC during performance on an *N*-back WM task has been associated with the 7-repeat allele, with young adults carrying the 7-repeat allele showing a larger difference in brain activation as a result of task difficulty (Herrmann et al., 2007). In summary, the 7-repeat is associated with poorer WM performance and what can be interpreted as more ineffective brain activity. This genetic evidence suggests that several genes controlling dopamine levels and signaling have an influential effect on WM during development. These effects on WM are likely to be complicated by Gene \times Gene interactions and factors affecting basal dopamine levels, such as age.

Dopamine and Plasticity

Dopamine has been suggested to be important for plasticity by enhancing neural sprouting and synaptogenesis (Stroemer, Kent, & Hulsebosch, 1998). In stroke patients, treatment with stimulant medication, which increases dopamine concentrations in the synapse, enhances motor recovery resulting from physiotherapy (Scheidtmann, Fries, Muller, & Koenig, 2001; Walker-Batson, Smith, Curtis, Unwin, & Greenlee, 1995). These effects are long lasting, with significant improvements remaining up to 12 months after treatment.

The effects of behavioral parenting interventions have also been linked to dopaminergic function, with outcomes associated with variants of a VNTR in the *DRD4* gene (Bakermans-Kranenburg, Van, Pijlman, Mesman, & Juffer, 2008). This evidence is of particular interest as it suggests an interaction between the dopaminergic system and environmental influences on behavioral changes.

Cognitive Training as a Model for Human Cognitive Plasticity

During the past decade, there has been increasing interest in improving cognitive functions through targeted training. Many training programs have shifted focus from explicit training, teaching strategies to improve performance, to implicit training that involves repetition practice, feedback, and gradually increasing the cognitive load required to solve the training tasks (Klingberg, 2010). Implicit training of WM has been shown to improve performance on nontrained WM tasks, reflecting a true increase in WM capacity (Klingberg et al., 2005; Klingberg, Forssberg, & Westerberg, 2002). Some studies report improvements in non-trained cognitive functions associated with WM, such as attention, reading comprehension, mathematical ability, and fluid reasoning (K. Dahlin, 2011; Holmes, Gathercole, & Dunning, 2009; Jaeggi, Buschkuhl, Jonides, & Perrig, 2008; Klingberg et al., 2005; Klingberg et al., 2002). WM training can also have positive effects on symptoms of ADHD and cognitive performance after stroke (Klingberg et al., 2005; Klingberg et al., 2002; Westerberg et al., 2007). This has obvious potential benefits for other clinical populations as well, and this area of research is currently growing rapidly. WM training is also related to changes in brain activity. For example, improvements in WM observed after training have

been related to changes in activity in the caudate nucleus and prefrontal and parietal cortices (E. Dahlin, Neely, Larsson, Backman, & Nyberg, 2008; Olesen, Westerberg, & Klingberg, 2004). For a review and discussion, see Klingberg (2010).

Dopamine and Cognitive Training

One study has used PET to investigate the association between cognitive training and dopamine D1 and D2 receptor density (McNab et al., 2009). Participants, who were all men in early adulthood, underwent a 5-week WM training scheme, training for 35 min 5 days a week and were scanned using functional and structural magnetic resonance imaging and PET before and after training. The functional magnetic resonance imaging was used to identify regions showing WM-related activity. These regions guided PET analyses, investigating changes of dopamine D1 receptor binding potential in cortical regions and D2 receptor binding potential in subcortical regions. There were no significant associations between D1 or D2 receptor binding potential and WM performance at baseline, although a trend was observed for cortical D1. Notably, improvements in WM capacity observed after training were significantly related to changes in D1 receptor binding potential. Furthermore, fitting a quadratic model significantly increased the variance explained, suggesting an inverted-U-shaped relationship, in line with previous primate and human research findings discussed earlier. No relation between improvements in WM capacity and D2 receptor binding potential was found. The results provide further evidence for a greater importance of the D1 receptor compared with the D2 receptor not only for WM functioning but also for cognitive plasticity. Note, however, the recent findings by Bäckman et al. (2011), demonstrating changes in D2 receptor binding potential in the striatum after 5 weeks of updating training. Whether the differences between these two studies result from differences in types of training (purely updating or tasks not involving updating) needs further investigation. Differences might also arise from methodological differences. As McNab et al. (2009) performed PET scanning during rest, changes observed in this study reflect task-independent changes. On the other hand, Bäckman et al. (2011) used PET scanning during task performance and a ligand sensitive to endogenous DA release. The results might therefore also reflect changes that are task specific, such as differences in behavior during scanning.

Brehmer et al. (2009) found additional evidence for involvement of the dopaminergic system in training induced plasticity from genetic analyses in young adults who completed a WM training program similar to the program used by McNab et al. (2009). A sample of 29 young adults were genotyped for the *DAT1* VNTR and were grouped according to 10-repeat homozygosity or 9-repeat carriership (heterozygotes or homozygotes for the 9-repeat allele) while controlling for the *COMT* Val¹⁵⁸Met polymorphism. No significant effects of the *DAT1* genotype on baseline performance on tasks measuring WM, attention, and fluid intelligence (*Gf*) were observed. An initial superior (but nonsignificant) performance on the visuospatial WM tasks used in training was observed in 9/10-repeat carriers compared with 10-repeat carriers. There was a trend for this difference to increase throughout the training period with 10-repeat carriers increasing their performance more steeply than noncarriers throughout the training period. No training related effects were observed for the *COMT*

genotype, nor were there any significant effects of either genotype on the verbal WM tasks being trained. In summary, the effect of the *DAT1* genotype was apparent across the training period, although not seen at baseline. This pattern of results suggests that the genotype has an effect on susceptibility to training induced improvements per se (E. Dahlin et al., 2008). It is important to note that this study was underpowered for analyzing genetic effects that tend to be particularly small for complex traits, such as cognitive functions, and because the results were nonsignificant, a replication is needed.

In the present study, we investigated the effects of polymorphisms in five genes involved in the dopaminergic system (Table 1) on the effectiveness of WM and nonverbal reasoning (NVR) training in preschool children. A training program consisting of NVR tasks was designed to assess the feasibility of improving fluid intelligence (Gf) (Bergman Nutley et al., 2011). Gf is referred to as the ability to identify patterns and relations and infer rules for novel problems (Horn & Cattell, 1966). Gf is independent from previously learned knowledge, is highly correlated with WM capacity, and similar to WM, is a predictor of academic performance (Alloway & Alloway, 2010; Lynn, Meisenberg, Mikk, & Williams, 2007). Deficits in Gf, in combination with deficits in other types of intelligence, are core symptoms of mental retardation, a particularly common mental disorder with a prevalence of around 3% (Roeleveld, Zielhuis, & Gabreels, 1997). It would therefore be of great potential benefit if this function could be improved with training similar to that used for WM. Bergman Nutley et al. (2011) recently showed that Gf could be enhanced in 4-year-old children. Each child was randomly assigned to train WM, to train NVR, or to train a combination (CB) of the two. For comparison, a placebo group was also included. Training of WM (in both WM and CB groups) resulted in significantly improved performance on WM transfer tasks (i.e., nontrained tasks) compared with the placebo group. Training of NVR led to significantly improved performance on transfer tasks of Gf, and this group also showed a trend toward transfer between constructs with improvements on a measure of visuospatial WM. On the basis of the evidence so far, we investigated here whether these improvements were associated to polymorphisms in some genes related to the dopaminergic system.

Methods

Participants

DNA was available from 96 children ages 4.0 to 4.5 years (56 boys and 40 girls; mean age = 51.2 months, *SD* = 3.0) who had completed a 5-week training scheme. Participants were recruited with flyers distributed at preschools in the local Stockholm area and by advertisements in the local newspaper and on the lab website. The children received a small reward (a toy) after completion of each set of 5 days of training. After completion of the whole training period (a minimum of 20 completed training days), participants received an additional monetary reward. The study was approved by the local ethics committee, and informed consent was collected from the guardians of all participants (for further details, see Bergman Nutley et al., 2011).

Training Program and Procedure

Participants were pseudorandomly assigned (stratifying for sex) to one of four different training programs: WM training, NVR training, a CB training of WM and NVR, and a placebo training designed as the CB training but with task difficulty kept constant at the lowest levels throughout the training period. The WM group trained with a program developed by Cogmed Systems containing seven different versions of visuospatial WM tasks. Training tasks used for the NVR training program were based on three tests from the Leiter Battery that load highly on Gf: Repeated Patterns, Sequential Order, and Classification (Roid & Miller, 1997). Task difficulty was carefully assessed to allow for an automatic generation of multiple items of varying difficulty. The WM, NVR, and CB training programs were adaptive, with level of difficulty automatically adjusted according to each child's performance. Training took place in the home and lasted approximately 15 min per day 5 days per week until 25 sessions had been performed (Bergman Nutley et al., 2011).

Transfer Tests

Gf was measured with the Repeated Patterns, Sequential Orders, and Classifications subtests from the Leiter Battery (Roid &

Table 1
Eleven SNPs From Five Genes Involved in Dopamine-Related Pathways

SNP	Alleles ^a	MAFs ^b	Chromosome ^c	Position (bp) ^d	Location in the gene	Gene symbol (alternative)	Gene name (alternative name)
rs13140817	G/A	0.37 (0.38)	4p16.1	9372073	20 kb from 5'	<i>DRD5</i>	Dopamine receptor 5
rs3863145	A/G	0.29 (0.24)	5p15.3	1445711	10 kb after 3'	<i>SLC6A3 (DAT1)</i>	Solute carrier family 6 member 3 (dopamine transporter)
rs27072	T/C	0.14 (0.18)		1447522	in 3' UTR	<i>SLC6A3 (DAT1)</i>	
rs40184	T/C	0.49 (0.46)		1448077	intron 14 (of 14)	<i>SLC6A3 (DAT1)</i>	
rs1541332	A/G	0.43 (0.45)	9q34	135501337	intron 5 (of 11)	<i>DBH</i>	Dopamine beta-hydroxylase
rs2797853	T/C	0.37 (0.35)		135502336	intron 5 (of 11)	<i>DBH</i>	
rs7124601	A/C	0.48 (NA)	11p15.5	629273	intron 1 (of 3)	<i>DRD4</i>	Dopamine receptor 4
rs11246226	A/C	0.48 (0.51)		631191	500 bp after 3'	<i>DRD4</i>	
rs936465	G/C	0.49 (0.42)		633568	3 kb after 3'	<i>DRD4</i>	
rs740601	G/T	0.43 (0.42)	22q11.2	18330763	intron 3 (of 5)	<i>COMT</i>	Catechol-O-methyltransferase
rs4680	A/G	0.47 (0.48)		18331271	exon 4 (of 6)	<i>COMT</i>	

^a Alleles of the corresponding single nucleotide polymorphism (SNP) with the minor allele first. ^b Minor allele frequency (MAF) in our sample set and corresponding allele frequency in the HapMap CEU data set in parenthesis (NA = no frequency available). ^c Chromosomal location of the gene based on ideogram with chromosome number and band. ^d SNP position, in base pair (bp), on the respective chromosome (Genome Assembly Build 36.3).

Miller, 1997), Raven's Coloured Progressive Matrices (Raven, 1998), and Block Design from the Wechsler Preschool and Primary Scale of Intelligence—Third Edition (Wechsler, 2004). To assess WM capacity, we used a visuospatial grid task (Bergman Nutley, Söderqvist, Bryde, Humphreys, & Klingberg, 2009; Westerberg, Hirvikoski, Forssberg, & Klingberg, 2004), the Odd One Out task from the Automated Working Memory Assessment (Alloway, 2007), and the Word Span test, a verbal WM test similar to the Digit Span Forward subtest from the Wechsler Intelligence Scale for Children—Third Edition (Wechsler, 1991) but with unrelated nouns instead of numbers (Thorell & Wählstedt, 2006). For the measures of WM and Gf, respectively, the three measures were modeled as independent linear functions of a continuous latent variable.

Genes and SNPs Studied

Genetic data for the current sample were available as a subset of a previous larger study (see Söderqvist et al., 2010). Genotypes from single nucleotide polymorphisms (SNPs) in five genes belonging to the dopamine pathway were available and were here tested for association with training performance. The genes were dopamine receptors D4 (*DRD4*) and D5 (*DRD5*), solute carrier family 6 member 3 (*SLC6A3*), also known as dopamine transporter (*DAT1*), dopamine beta-hydroxylase (*DBH*), and *COMT*. Table 1 summarizes the SNPs genotyped in these genes, their chromosomal location and base pair positions according to Genome Build 36.3. We selected SNP markers available from the HapMap Genome Browser to analyze genotype–phenotype association using the genes described here. For the dopamine receptors *DRD4* and *DRD5*, SNPs were selected so that they tagged the complete gene regions. The SNPs genotyped are in strong linkage disequilibrium with the previously reported VNTR and Taq1 restriction site polymorphism for the *SLC6A3/DAT1* and *DBH* genes, respectively. The VNTR markers reported in previous studies were not used for genotyping as the SNP genotyping technology available allowed for multiplexing of a large number of SNPs in one reaction, which was the method of choice for screening a large number of genes. All samples had a genotyping success rate of greater than 95% and a genotype call rate of greater than 80%. The methods for blood and saliva sampling, genomic DNA extraction, and SNP genotyping are described in (Söderqvist et al., 2010).

Results

Genetic Effect on Transfer

The 11 SNPs were included (separately) as fixed effects in mixed effects models. As the dependent variable we used either the subject loading on the latent variable for WM (Grid task, Odd One Out task, and Word Span test) or the subject loading on the latent variable for Gf (Leiter Battery, Raven's Coloured Progressive Matrices, and Block Design tests). Because of the small number of participants, all active groups (WM, NVR, and CB) were collapsed into one training group for these analyses. The independent fixed factors were time (Time 1, before training, or Time 2, after training) to account for change in performance related to baseline, additive effect of genotype (0, 1, or 2 copies of a specific allele of each SNP) to assess main effect of genotype, training factor

(training or no training) to assess main effect of training, and an interaction term of genotype and training factor to assess the effect of genotype on the influence of training. Person ability was entered as a random effect to account for within-person correlation. The strongest association was found for the Gf latent variable and one SNP (rs27072, T/C) from the *DAT1* gene significantly interacting with the training factor, $F(4, 91) = 6.971, p = .01$. The T-allele of rs27072 seemed to be advantageous, as carriers showed a larger training gain than noncarriers on the Gf factor (see Figure 1). Two additional SNPs from the *DAT1* gene also showed significant associations. One, rs40184, was found to associate with the Gf latent factor, $F(6, 93) = 3.445, p = .036$, whereas another, rs3863145, showed significant association with the WM latent factor, $F(6, 96) = 3.545, p = .032$. No other polymorphisms showed significant training interaction effects (Table 2), and no genotype effects were observed on baseline performance. All p values are uncorrected for multiple comparisons of the 11 SNPs tested. Thus, results should be considered preliminary and in need of replication in a larger sample set.

Discussion

Previous literature suggests that dopamine might not be important only for performance on WM tasks and other cognitive tasks relying on prefrontal and parietal function but also for its plasticity. Computerized cognitive training, such as WM training (Klingberg et al., 2002, 2005) and reasoning training (Bergman Nutley et al., 2011), could be useful not only for rehabilitation, but also as a method of studying cognitive plasticity in humans, as highlighted by our study. We investigated the role of genetic polymorphisms on the change in cognitive performance resulting from training and found that polymorphisms of the *DAT1* gene are associated with training effects. As cognitive training increases in popularity, a

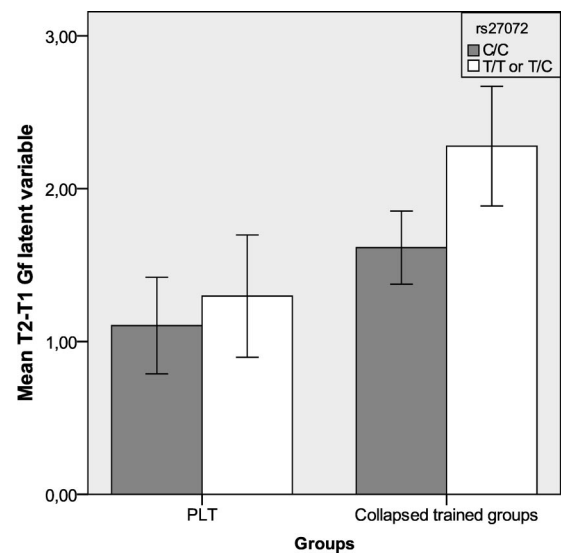


Figure 1. Mean fluid intelligence (Gf) gains per group and genotype. Bars show mean gains on the Gf latent variable for the collapsed trained groups and placebo training (PLT) group and sorted by genotype on the *DAT1* (rs27072) with standard error of the mean. T1 = Time 1; T2 = Time 2.

Table 2
Significance Levels (*p* Values) From Mixed Model Analyses for All SNPs Tested for Main Effect of Genotype on Gf and WM Performance as Well as Training \times Genotype Interactions

SNP (gene)	Main effect on Gf	Interaction effect on Gf	Main effect on WM	Interaction effect on WM
rs13140817 (<i>DRD5</i>)	.580	.436	.768	.622
rs3863145 (<i>DATI</i>)	.151	.570	.006	.032
rs27072 (<i>DATI</i>)	.511	.010	.060	.161
rs40184 (<i>DATI</i>)	.644	.036	.073	.132
rs1541332 (<i>DBH</i>)	.360	.229	.293	.089
rs2797853 (<i>DBH</i>)	.357	.082	.494	.778
rs7124601 (<i>DRD4</i>)	.253	.964	.779	.774
rs11246226 (<i>DRD4</i>)	.615	.850	.909	.916
rs936465 (<i>DRD4</i>)	.332	.664	.987	.495
rs740601 (<i>COMT</i>)	.247	.825	.248	.655
rs4680 (<i>COMT</i>)	.767	.191	.195	.972

Note. Significant interactions indicate that training effects were associated with genotype. Bold indicates $p < .05$ (uncorrected for multiple comparisons). SNP = single nucleotide polymorphism; Gf = fluid intelligence; WM = working memory.

better understanding of the mechanisms underlying its effects is of great value.

The SNP with strongest association in our sample (rs27072) is located in the 3' untranslated region of the gene and has previously been implicated in genetic studies of ADHD, with the carriers of the C/G allele exhibiting higher risk for ADHD (for a review, see Galili-Weisstub & Segman, 2003; Gizer, Ficks, & Waldman, 2009). The same allele (T) found to be advantageous in terms of training gains in the present study has also been shown to have a protective effect for ADHD (Brookes et al., 2006; Feng et al., 2005). Furthermore, for rs27072, allelic imbalance of *DATI* mRNA expression has been observed, with the minor allele (T) accounting for increased expression in both in vitro and in vivo studies (Pinsonneault et al., 2011).

Dopamine transporter protein removes dopamine from the synaptic cleft. Consequently, concentration of dopamine transporter protein could influence the activity of both D1 and D2 receptors.

The sample size of the current study is considered small for analyses of genetic effects, and the effects observed do not remain significant after correcting for multiple comparisons. Thus, the findings need further replication in larger independent samples. The low power is also a reason for caution when interpreting the lack of effects for other genotypes analyzed. It might, for example, seem surprising that no effect was observed for the *COMT* Val¹⁵⁸Met polymorphism considering its extensively observed effects on WM and other cognitive functions and its importance for degradation of dopamine in the PFC, one of the areas that show training related changes in activity. It is possible that analyzing a *COMT* haplotype would have provided stronger effects, as has been previously suggested in studies of pain perception and attention (Diatchenko et al., 2005) and attention alone in 2-year-old children (Voelker, Sheese, Rothbart, & Posner, 2009). Currently available data do not allow for such analyses, and we suggest that haplotype analysis of genetic markers in *COMT* can be addressed in future studies. Furthermore, effects of dopaminergic genes are likely to follow complicated patterns across development depend-

ing on interactions with other genes and background factors, such as age and baseline performance. Future studies should include larger samples to allow such interactions to be analyzed. It would also be of interest to investigate the importance of domain in which the cognitive training is performed. As discussed earlier, there have been suggestions that dopamine function is of particular importance for visuospatial WM function. In the current study, training included only visuospatial WM tasks. Thus, such a distinction is not possible. Understanding domain-specific interactions with dopamine function would provide a good foundation for better understanding how cognitive training can be individualized to best suit people with different baseline capacities and perhaps with different genetic makeup.

It has been suggested that some neurodevelopmental disorders can be understood as disorders of learning, rather than a fixed cognitive deficit (Karmiloff-Smith, 1998). The capacity of the brain to adapt and learn in response to environmental influence is considered crucial for the development of cognitive functions. Thus, a general learning impairment can lead to more specific disruptions later in life. The data presented here suggest that dopamine is important not only for cognitive performance but perhaps, in particular, for plasticity. The same genotype associated with lower plasticity in the present study is also associated with ADHD (Gizer et al., 2009). Considering the importance of dopamine-related genotypes in many neurodevelopmental disorders, it will be interesting to further investigate to what extent these effects can be explained through an influence on learning and plasticity rather than on permanent and fixed functions.

References

- Alloway, T. P. (2007). *Automated Working Memory Assessment*. Oxford, United Kingdom: Harcourt.
- Alloway, T. P., & Alloway, R. G. (2010). Investigating the predictive roles of working memory and IQ in academic attainment. *Journal of Experimental Child Psychology*, *106*, 20–29. doi:10.1016/j.jecp.2009.11.003
- Alloway, T. P., Gathercole, S. E., Kirkwood, H., & Elliott, J. (2009). The cognitive and behavioral characteristics of children with low working memory. *Child Development*, *80*, 606–621. doi:10.1111/j.1467-8624.2009.01282.x
- Bäckman, L., Nyberg, L., Soveri, A., Johansson, J., Andersson, M., Dahlin, E., . . . Rinne, J. O. (2011, August 5). Effects of working-memory training on striatal dopamine release. *Science*, *333*, 718. doi:10.1126/science.1204978
- Bakermans-Kranenburg, M. J., Van, I. M. H., Pijlman, F. T., Mesman, J., & Juffer, F. (2008). Experimental evidence for differential susceptibility: Dopamine D4 receptor polymorphism (*DRD4* VNTR) moderates intervention effects on toddlers' externalizing behavior in a randomized controlled trial. *Developmental Psychology*, *44*, 293–300. doi:10.1037/0012-1649.44.1.293
- Barnett, J. H., Heron, J., Goldman, D., Jones, P. B., & Xu, K. (2009). Effects of catechol-O-methyltransferase on normal variation in the cognitive function of children. *American Journal of Psychiatry*, *166*, 909–916. doi:10.1176/appi.ajp.2009.08081251
- Bergman Nutley, S., Söderqvist, S., Bryde, S., Humphreys, K., & Klingberg, T. (2009). Measuring working memory capacity with greater precision in the lower capacity ranges. *Developmental Neuropsychology*, *35*, 81–95. doi:10.1080/87565640903325741
- Bergman Nutley, S., Söderqvist, S., Bryde, S., Thorell, L. B., Humphreys, K., & Klingberg, T. (2011). Gains in fluid intelligence after training non-verbal reasoning in 4-year-old children: A controlled, randomized

- study. *Developmental Science*, *14*, 591–601. doi:10.1111/j.1467-7687.2010.01022.x
- Brehmer, Y., Westerberg, H., Bellander, M., Furth, D., Karlsson, S., & Backman, L. (2009). Working memory plasticity modulated by dopamine transporter genotype. *Neuroscience Letters*, *467*, 117–120. doi:10.1016/j.neulet.2009.10.018
- Brookes, K., Xu, X., Chen, W., Zhou, K., Neale, B., Lowe, N., . . . Johansson, L. (2006). The analysis of 51 genes in *DSM-IV* combined type attention deficit hyperactivity disorder: Association signals in DRD4, DAT1 and 16 other genes. *Molecular Psychiatry*, *11*, 934–953. doi:10.1038/sj.mp.4001869
- Castellanos, F. X., & Tannock, R. (2002). Neuroscience of attention-deficit/hyperactivity disorder: The search for endophenotypes. *Nature Reviews Neuroscience*, *3*, 617–628. doi:10.1038/nrn896nrm896
- Castner, S. A., & Goldman-Rakic, P. S. (2004). Enhancement of working memory in aged monkeys by a sensitizing regimen of dopamine D1 receptor stimulation. *Journal of Neuroscience*, *24*, 1446–1450. doi:10.1523/JNEUROSCI.3987-03.200424/6/1446
- Castner, S. A., Williams, G. V., & Goldman-Rakic, P. S. (2000, March 17). Reversal of antipsychotic-induced working memory deficits by short-term dopamine D1 receptor stimulation. *Science*, *287*, 2020–2022. doi:10.1126/science.287.5460.2020
- Dahlin, E., Neely, A. S., Larsson, A., Backman, L., & Nyberg, L. (2008, June 13). Transfer of learning after updating training mediated by the striatum. *Science*, *320*, 1510–1512. doi:10.1126/science.1155466
- Dahlin, K. (2011). Effects of working memory training on reading in children with special needs. *Reading and Writing*, *24*, 479–491. doi:10.1007/s11145-010-9238-y
- Diatchenko, L., Slade, G. D., Nackley, A. G., Bhalang, K., Sigurdsson, A., Belfer, I., . . . Maixner, W. (2005). Genetic basis for individual variations in pain perception and the development of a chronic pain condition. *Human Molecular Genetics*, *14*, 135–143. doi:10.1093/hmg/ddi013
- Dickinson, D., & Elvevag, B. (2009). Genes, cognition and brain through a COMT lens. *Neuroscience*, *164*, 72–87. doi:10.1016/j.neuroscience.2009.05.014
- Dumontheil, I., Roggeman, C., Ziermans, T., Peyrard-Janvid, M., Matsson, H., Kere, J., & Klingberg, T. (2011). Influence of the COMT genotype on working memory and brain activity changes during development. *Biological Psychiatry*, *70*, 222–229. doi:10.1016/j.biopsych.2011.02.027
- Feng, Y., Wigg, K. G., Makkar, R., Ickowicz, A., Pathare, T., Tannock, R., . . . Barr, C. L. (2005). Sequence variation in the 3'-untranslated region of the dopamine transporter gene and attention-deficit hyperactivity disorder (ADHD). *American Journal of Medical Genetics: Neuropsychiatric Genetics*, *139B*, 1–6. doi:10.1002/ajmg.b.30190
- Froehlich, T. E., Lanphear, B. P., Dietrich, K. N., Cory-Slechta, D. A., Wang, N., & Kahn, R. S. (2007). Interactive effects of a DRD4 polymorphism, lead, and sex on executive functions in children. *Biological Psychiatry*, *62*, 243–249. doi:10.1016/j.biopsych.2006.09.039
- Fuke, S., Suo, S., Takahashi, N., Koike, H., Sasagawa, N., & Ishiura, S. (2001). The VNTR polymorphism of the human dopamine transporter (DAT1) gene affects gene expression. *Pharmacogenomics Journal*, *1*, 152–156.
- Galili-Weisstub, E., & Segman, R. H. (2003). Attention deficit and hyperactivity disorder: Review of genetic association studies. *Israel Journal of Psychiatry and Related Sciences*, *40*, 57–66.
- Gathercole, S. E., Brown, L., & Pickering, S. J. (2003). Working memory assessments at school entry as longitudinal predictors of National Curriculum attainment levels. *Educational and Child Psychology*, *20*, 109–122.
- Gizer, I. R., Ficks, C., & Waldman, I. D. (2009). Candidate gene studies of ADHD: A meta-analytic review. *Human Genetics*, *126*, 51–90. doi:10.1007/s00439-009-0694-x
- Goldman-Rakic, P. S., & Brown, R. M. (1981). Regional changes of monoamines in cerebral cortex and subcortical structures of aging rhesus monkeys. *Neuroscience*, *6*, 177–187. doi:10.1016/0306-4522(81)90053-1
- Goldman-Rakic, P. S., & Brown, R. M. (1982). Postnatal development of monoamine content and synthesis in the cerebral cortex of rhesus monkeys. *Developmental Brain Research*, *256*, 339–349.
- Herrmann, M. J., Walter, A., Schreppe, T., Ehlis, A. C., Pauli, P., Lesch, K. P., & Fallgatter, A. J. (2007). D4 receptor gene variation modulates activation of prefrontal cortex during working memory. *European Journal of Neuroscience*, *26*, 2713–2718. doi:10.1111/j.1460-9568.2007.05921.x
- Holmes, J., Gathercole, S. E., & Dunning, D. L. (2009). Adaptive training leads to sustained enhancement of poor working memory in children. *Developmental Science*, *12*, F9–F15. doi:10.1111/j.1467-7687.2009.00848.x
- Horn, J. L., & Cattell, R. B. (1966). Refinement and test of the theory of fluid and crystallized general intelligences. *Journal of Educational Psychology*, *57*, 253–270.
- Jaeggi, S. M., Buschkuhl, M., Jonides, J., & Perrig, W. J. (2008). Improving fluid intelligence with training on working memory. *Proceedings of the National Academy of Sciences of the United States of America*, *105*, 6829–6833. doi:10.1073/pnas.0801268105
- Jucaite, A., Forsberg, H., Karlsson, P., Halldin, C., & Farde, L. (2010). Age-related reduction in dopamine D1 receptors in the human brain: From late childhood to adulthood, a positron emission tomography study. *Neuroscience*, *167*, 104–110. doi:10.1016/j.neuroscience.2010.01.034
- Kane, M. J., Hambrick, D. Z., Tuholski, S. W., Wilhelm, O., Payne, T. W., & Engle, R. W. (2004). The generality of working memory capacity: A latent-variable approach to verbal and visuospatial memory span and reasoning. *Journal of Experimental Psychology: General*, *133*, 189–217. doi:10.1037/0096-3445.133.2.189
- Karmiloff-Smith, A. (1998). Development itself is the key to understanding developmental disorders. *Trends in Cognitive Sciences*, *2*, 389–398. doi:10.1016/S1364-6613(98)01230-3
- Kimberg, D. Y., & D'Esposito, M. (2003). Cognitive effects of the dopamine receptor agonist pergolide. *Neuropsychologia*, *41*, 1020–1027. doi:10.1016/S0028-3932(02)00317-2
- Kimberg, D. Y., D'Esposito, M., & Farah, M. J. (1997). Effects of bromocriptine on human subjects depend on working memory capacity. *NeuroReport*, *8*, 3581–3585. doi:10.1097/00001756-199711100-00032
- Klingberg, T. (2010). Training and plasticity of working memory. *Trends in Cognitive Sciences*, *14*, 317–324. doi:10.1016/j.tics.2010.05.002
- Klingberg, T., Fernell, E., Olesen, P. J., Johnson, M., Gustafsson, P., Dahlstrom, K., . . . Westerberg, H. (2005). Computerized training of working memory in children with ADHD—A randomized, controlled trial. *Journal of the American Academy of Child and Adolescent Psychiatry*, *44*, 177–186. doi:10.1097/00004583-200502000-00010
- Klingberg, T., Forsberg, H., & Westerberg, H. (2002). Training of working memory in children with ADHD. *Journal of Clinical and Experimental Neuropsychology*, *24*, 781–791. doi:10.1076/jcen.24.6.781.8395
- Luciana, M., & Collins, P. F. (1997). Dopaminergic modulation of working memory for spatial but not object cues in normal humans. *Journal of Cognitive Neuroscience*, *9*, 330–347. doi:10.1162/jocn.1997.9.3.330
- Lynn, R., Meisenberg, G., Mikk, J., & Williams, A. (2007). National IQs predict differences in scholastic achievement in 67 countries. *Journal of Biosocial Science*, *39*, 861–874. doi:10.1017/S0021932007001964
- Martinussen, R., Hayden, J., Hogg-Johnson, S., & Tannock, R. (2005). A meta-analysis of working memory impairments in children with attention-deficit/hyperactivity disorder. *Journal of the American Academy of Child and Adolescent Psychiatry*, *44*, 377–384. doi:10.1097/01.chi.0000153228.72591.73
- Matsumoto, M., Weickert, C. S., Akil, M., Lipska, B. K., Hyde, T. M., Herman, M. M., . . . Weinberger, D. R. (2003). Catechol O-methyltransferase mRNA expression in human and rat brain: Evi-

- dence for a role in cortical neuronal function. *Neuroscience*, *116*, 127–137. doi:10.1016/S0306-4522(02)00556-0
- McNab, F., Varrone, A., Farde, L., Jucaite, A., Bystritsky, P., Forsberg, H., & Klingberg, T. (2009, February 6). Changes in cortical dopamine D1 receptor binding associated with cognitive training. *Science*, *323*, 800–802. doi:10.1126/science.1166102
- Mehta, M. A., Goodyer, I. M., & Sahakian, B. J. (2004). Methylphenidate improves working memory and set-shifting in AD/HD: Relationships to baseline memory capacity. *Journal of Child Psychology and Psychiatry and Allied Disciplines*, *45*, 293–305. doi:10.1111/j.1469-7610.2004.00221.x
- Mehta, M. A., Owen, A. M., Sahakian, B. J., Mavaddat, N., Pickard, J. D., & Robbins, T. W. (2000). Methylphenidate enhances working memory by modulating discrete frontal and parietal lobe regions in the human brain. *Journal of Neuroscience*, *20*, RC65.
- Mill, J., Asherson, P., Browes, C., D'Souza, U., & Craig, I. (2002). Expression of the dopamine transporter gene is regulated by the 3' UTR VNTR: Evidence from brain and lymphocytes using quantitative RT-PCR. *American Journal of Medical Genetics*, *114*, 975–979. doi:10.1002/ajmg.b.10948
- Müller, U., von Cramon, D. Y., & Pollmann, S. (1998). D1- versus D2-receptor modulation of visuospatial working memory in humans. *Journal of Neuroscience*, *18*, 2720–2728.
- Olesen, P. J., Westerberg, H., & Klingberg, T. (2004). Increased prefrontal and parietal activity after training of working memory. *Nature Neuroscience*, *7*, 75–79. doi:10.1038/nn1165
- Patrick, K. S., Caldwell, R. W., Ferris, R. M., & Breese, G. R. (1987). Pharmacology of the enantiomers of threo-methylphenidate. *Journal of Pharmacology and Experimental Therapeutics*, *241*, 152–158.
- Pinsonneault, J. K., Han, D. D., Burdick, K. E., Katakai, M., Bertolino, A., Malhotra, A. K., . . . Sadec, W. (2011). Dopamine transporter gene variant affecting expression in human brain is associated with bipolar disorder. *Neuropsychopharmacology*, *36*, 1644–1655. doi:10.1038/npp.2011.45
- Raven, J. C. (1998). *Manual for Raven's Progressive Matrices*. Oxford, United Kingdom: Oxford Psychologists Press.
- Roeleveld, N., Zielhuis, G. A., & Gabreels, F. (1997). The prevalence of mental retardation: A critical review of recent literature. *Developmental Medicine and Child Neurology*, *39*, 125–132. doi:10.1111/j.1469-8749.1997.tb07395.x
- Roid, G. H., & Miller, L. J. (1997). *Leiter International Performance Scale-Revised: Examiner's manual*. Wood Dale, IL: Stoelting.
- Scheidtmann, K., Fries, W., Muller, F., & Koenig, E. (2001). Effect of levodopa in combination with physiotherapy on functional motor recovery after stroke: A prospective, randomised, double-blind study. *Lancet*, *358*, 787–790. doi:10.1016/S0140-6736(01)05966-9
- Söderqvist, S., McNab, F., Peyrard-Janvid, M., Matsson, H., Humphreys, K., Kere, J., & Klingberg, T. (2010). The SNAP25 gene is linked to working memory capacity and maturation of the posterior cingulate cortex during childhood. *Biological Psychiatry*, *68*, 1120–1125. doi:10.1016/j.biopsych.2010.07.036
- Solanto, M. V. (1998). Neuropsychopharmacological mechanisms of stimulant drug action in attention-deficit hyperactivity disorder: A review and integration. *Behavioural Brain Research*, *94*, 127–152. doi:10.1016/S0166-4328(97)00175-7
- Stelzel, C., Basten, U., Montag, C., Reuter, M., & Fiebach, C. J. (2009). Effects of dopamine-related gene–gene interactions on working memory component processes. *European Journal of Neuroscience*, *29*, 1056–1063. doi:10.1111/j.1460-9568.2009.06647.x
- Stollstorff, M., Foss-Feig, J., Cook, E. H., Jr., Stein, M. A., Gaillard, W. D., & Vaidya, C. J. (2010). Neural response to working memory load varies by dopamine transporter genotype in children. *NeuroImage*, *53*, 970–977. doi:10.1016/j.neuroimage.2009.12.104
- Stroemer, R. P., Kent, T. A., & Hulsebosch, C. E. (1998). Enhanced neocortical neural sprouting, synaptogenesis, and behavioral recovery with D-amphetamine therapy after neocortical infarction in rats. *Stroke*, *29*, 2381–2393.
- Thorell, L. B., & Wåhlstedt, C. (2006). Executive functioning deficits in relation to symptoms of ADHD and/or ODD in preschool children. *Infant and Child Development*, *15*, 503–518. doi:10.1002/icd.475
- Vijayraghavan, S., Wang, M., Birnbaum, S. G., Williams, G. V., & Arnsten, A. F. (2007). Inverted-U dopamine D1 receptor actions on prefrontal neurons engaged in working memory. *Nature Neuroscience*, *10*, 376–384. doi:10.1038/nn1846
- Voelker, P., Sheese, B. E., Rothbart, M. K., & Posner, M. I. (2009). Variations in catechol-O-methyltransferase gene interact with parenting to influence attention in early development. *Neuroscience*, *164*, 121–130. doi:10.1016/j.neuroscience.2009.05.059
- Wahlstrom, D., White, T., Hooper, C. J., Vrshek-Schallhorn, S., Oetting, W. S., Brott, M. J., & Luciana, M. (2007). Variations in the catechol O-methyltransferase polymorphism and prefrontally guided behaviors in adolescents. *Biological Psychiatry*, *61*, 626–632. doi:10.1016/j.biopsych.2006.05.045
- Walker-Batson, D., Smith, P., Curtis, S., Unwin, H., & Greenlee, R. (1995). Amphetamine paired with physical therapy accelerates motor recovery after stroke. Further evidence. *Stroke*, *26*, 2254–2259. doi:10.1161/01.STR.26.12.2254
- Wechsler, D. (1991). *Wechsler Intelligence Scale for Children—Third Edition*. New York, NY: Psychological Corporation.
- Wechsler, D. (2004). *Wechsler Preschool and Primary Scale of Intelligence—Third Edition (WPPSI-III)*. New York, NY: Psychological Corporation.
- Westerberg, H., Hirvikoski, T., Forsberg, H., & Klingberg, T. (2004). Visuo-spatial working memory span: A sensitive measure of cognitive deficits in children with ADHD. *Child Neuropsychology*, *10*, 155–161. doi:10.1080/09297040490911014
- Westerberg, H., Jacobaeus, H., Hirvikoski, T., Clevberger, P., Ostensson, M. L., Bartfai, A., & Klingberg, T. (2007). Computerized working memory training after stroke—A pilot study. *Brain Injury*, *21*, 21–29. doi:10.1080/02699050601148726
- Williams, G. V., & Goldman-Rakic, P. S. (1995, August 17). Modulation of memory fields by dopamine D1 receptors in prefrontal cortex. *Nature*, *376*, 572–575. doi:10.1038/376572a0
- Xu, H., Kellendonk, C. B., Simpson, E. H., Keilp, J. G., Bruder, G. E., Polan, H. J., . . . Gilliam, T. C. (2007). DRD2 C957T polymorphism interacts with the COMT Val158Met polymorphism in human working memory ability. *Schizophrenia Research*, *90*, 104–107. doi:10.1016/j.schres.2006.10.001

Received February 15, 2011

Revision received August 30, 2011

Accepted September 27, 2011 ■