Polymorphisms in dopamine system genes are associated with individual differences in attention in infancy

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Polymorphisms in Dopamine System Genes are Associated with Individual Differences in Attention in Infancy

Karla Holmboe
Birkbeck, University of London

Zsofia Nemoda
Semmelweis University

R. M. Pasco Fearon
University of Reading

Gergely Csibra
Birkbeck, University of London

Maria Sasvari-Szekely
Semmelweis University

Mark H. Johnson
Birkbeck, University of London

Abstract

Knowledge about the functional status of the frontal cortex in infancy is limited. This study investigated the effects of polymorphisms in four dopamine system genes on performance in a task developed to assess such functioning, the Freeze-Frame task, at 9 months of age. Polymorphisms in the catechol-*O*-methyltransferase (*COMT*) and the dopamine D4 receptor (*DRD4*) genes are likely to impact directly on the functioning of the frontal cortex, while polymorphisms in the dopamine D2 receptor (*DRD2*) and dopamine transporter (*DAT1*) genes might influence frontal cortex functioning indirectly via strong fronto-striatal connections. A significant effect of the *COMT* Val^{158}Met polymorphism was found. Infants with the Met/Met genotype were significantly less distractible than infants with the Val/Val genotype in Freeze-Frame trials presenting an engaging central stimulus. In addition, there was an interaction with the *DAT1* 3' VNTR polymorphism; the *COMT* effect was only present in infants who did not have two copies of the *DAT1* 10-repeat allele. These findings indicate that dopaminergic polymorphisms already affect selective aspects of attention in infancy, and further validate the Freeze-Frame task as a frontal cortex task.

Key words: Frontal cortex, Infancy, Dopamine genes, Attention, Frontal-subcortical circuits

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Introduction

The frontal cortex is associated with important cognitive functions such as working memory and various aspects of cognitive control (for review, see Fuster, 1997; Gazzaley & D'Esposito, 2007). Despite years of intensive study of this area in adults and non-human primates, relatively little is known about the functional status of the frontal cortex in infancy.

The frontal cortex has a more protracted development than other areas of the brain, with synaptogenesis continuing well into middle childhood (Glantz, Gilmore, Hamer, Lieberman, & Jarskog, 2007; Huttenlocher, 1990). Glucose metabolism and regional cerebral blood flow also peak later in the frontal cortex (Chugani & Phelps, 1986; Chugani, Phelps, & Mazziotta, 1987; Franceschini et al., 2007). Despite this protracted developmental course, infant neuroimaging studies have shown activation in the frontal cortex during language processing, processing of novel stimuli, and working memory (Baird et al., 2002; Bell, 2001; Bell & Fox, 1992, 1997; Dehaene-Lambertz, Dehaene, & Hertz-Pannier, 2002; Homae, Watanabe, Nakano, & Taga, 2007; Nakano, Watanabe, Homae, & Taga, 2008). Furthermore, Diamond and colleagues have shown that performance on a task which has been directly associated with the frontal cortex, the A-not-B task (Piaget, 1954), improves drastically during the second half of the first year of life (Diamond, 1985; Diamond & Goldman-Rakic, 1989; Diamond, Zola-Morgan, & Squire, 1989).

A previous report sought to validate a new infant frontal cortex task, the Freeze-Frame task, by investigating the relationship between this task and other infant and toddler frontal cortex tasks (Holmboe, Fearon, Csibra, Tucker, & Johnson, 2008). The Freeze-Frame task was developed to assess various aspects of inhibitory control in infancy using eye movements

as the dependent measure. In the task, infants are encouraged to stay fixated on an animated cartoon in the centre of a computer screen. On every trial a peripheral distractor (a white square) is presented. If the infant looks to this distractor, the animation is frozen for a brief period of time. Furthermore, the task involves two alternating trial types. In the *interesting* trials a dynamic and changeable animation is presented, whereas the *boring* trials present the same simple animation (a rotating orange star) every time.

In the study by Holmboe and colleagues (2008) it was found that 9-month-old infants stopped looking to the distractors during the course of the test session. Infants also looked less to the distractors in the interesting trials right from the beginning of the session. No evidence of an interaction between trial type and phase of the test session was found. Individual performance indices suggested that infants who looked less to the distractors in the interesting trials than the boring trials early in the Freeze-Frame session performed better on the A-not-B task at 9 months of age. Another index, which assessed infants' ability to selectively learn to inhibit looks to the distractors, was associated with significantly better performance on a frontal cortex task at 24 months of age, the Spatial Conflict task (Gerardi-Caulton, 2000; Rothbart, Ellis, Rueda, & Posner, 2003), suggesting that Freeze-Frame performance at 9 months is predictive of later frontal cortex functioning (Holmboe et al., 2008).

Even though these results indicate that performance on the Freeze-Frame task shares a significant proportion of its variance with performance on other infant and toddler frontal cortex tasks, this is still relatively indirect evidence that the task depends on the frontal cortex. More definitive evidence that the task is indeed associated with the functioning of the frontal cortex would involve establishing a direct relationship between performance on the task and biological markers of frontal cortex functioning. One way to address this issue is to investigate the potential effect of genetic variation. In the present study we therefore

investigated the relationship between performance on the Freeze-Frame task and well-established candidate polymorphisms in dopamine system genes.

The neurotransmitter dopamine plays a major role in the frontal cortex. For example, depletion of dopamine, but not noradrenaline or serotonin, in the dorsolateral prefrontal cortex causes delayed-response deficits similar to those seen after ablation of that area (Brozoski, Brown, Rosvold, & Goldman, 1979; Collins, Roberts, Dias, Everitt, & Robbins, 1998; Roberts et al., 1994). Furthermore, recordings from prefrontal dopamine-sensitive neurons in primates have shown these neurons to be active during the delay period in working memory tasks (Goldman-Rakic, Muly, & Williams, 2000; Sawaguchi & Goldman-Rakic, 1991; Vijayraghavan, Wang, Birnbaum, Williams, & Arnsten, 2007). Finally, Diamond and colleagues investigated children treated early and continuously for phenylketonuria (PKU) and found that estimated dopamine levels in the frontal cortex affected children's performance on frontal cortex tasks throughout infancy and early childhood (Diamond, Prevor, Callender, & Druin, 1997).

We investigated two dopamine system genes which have been demonstrated to impact on frontal cortex function in several studies: the catechol-*O*-methyltransferase (*COMT*) gene and the dopamine D4 receptor (*DRD4*) gene. However, the dopamine system is not restricted to the frontal cortex. It also plays an important role in subcortical areas such as the striatum. We therefore included two dopaminergic polymorphisms believed to affect neurotransmission primarily in the striatum: the TaqIA polymorphism in the dopamine D2 receptor (*DRD2*) gene and the 40-bp 3' VNTR polymorphism in the dopamine transporter (*DAT1, SLC6A3*) gene. These polymorphisms could potentially affect performance in the Freeze-Frame task via frontal-subcortical circuits linking the frontal cortex to distinct areas of the striatum (Alexander, DeLong, & Strick, 1986; Cummings, 1993; Cummings & Miller, 2007; Di Martino et al., 2008; Nieoullon, 2002).

The striatum used to be regarded as a subcortical relay of information from diverse cortical areas, especially in relation to movement control (reviewed in Alexander et al., 1986). However, Alexander and colleagues (1986) proposed a model whereby distinct basal ganglia-thalamo-cortical circuits process information relevant to different functional domains. Two of these circuits involve parts of the prefrontal cortex (the dorsolateral prefrontal and the lateral orbitofrontal circuits), and one involves the anterior cingulate. In support of this model, work on experimental animals as well as neuropsychological studies of human patients have shown deficits in the functions associated with specific frontal areas (e.g., working memory function associated with the dorsolateral prefrontal cortex) after lesion of other nodes in the relevant frontal-subcortical circuit (Cummings, 1993; Divac, Rosvold, & Szwarcbart, 1967; Stuss et al., 1998; Yehene, Meiran, & Soroker, 2008). Furthermore, the existence of strong functional connections between the striatum and different parts of the frontal cortex has been confirmed in an analysis of human functional magnetic resonance imaging (fMRI) data (Di Martino et al., 2008). Given this extensive evidence for frontal-subcortical networks, it seemed important to investigate not just dopamine genes likely to affect processing in the frontal cortex, but also dopamine genes acting at the subcortical level.

Looking at the individual genes in more detail, the COMT enzyme metabolizes catecholamines such as dopamine and noradrenaline (Chen et al., 2004; Männistö & Kaakkola, 1999; Tunbridge, Harrison, & Weinberger, 2006). The role of COMT in catabolizing dopamine in the frontal cortex is particularly important due to the relative lack of dopamine transporters and the positioning of these transporters at a distance from synaptic release sites (Sesack, Hawrylak, Matus, Guido, & Levey, 1998). Thus, COMT accounts for approximately 50-60% of the metabolic degradation of dopamine in the frontal cortex (Karoum, Chrapusta, & Egan, 1994; Yavich, Forsberg, Karayiorgou, Gogos, & Männistö, 2007). In contrast, COMT catabolism only plays a minor role in the striatum where the

dopamine transporter is abundant and better situated for dopamine reuptake (Karoum et al., 1994; Yavich et al., 2007; for review, see Tunbridge et al., 2006). Consistent with this, studies of COMT-deficient mice have demonstrated increased dopamine availability in the frontal cortex, but not the striatum (Gogos et al., 1998; Yavich et al., 2007). The important role of COMT in the cortex compared to the striatum has also recently been shown *in vivo* in the human brain using positron emission tomography (PET) (Slifstein et al., 2008).

The Val^{158}Met polymorphism in the *COMT* gene affects the activity level of the COMT enzyme. The polymorphism is an evolutionarily recent G (guanine) to A (adenine) missense mutation at codon 158, resulting in a substitution of methionine (Met) for valine (Val) in the COMT enzyme (Chen et al., 2004; Lachman et al., 1996; Tunbridge et al., 2006; Tunbridge et al., 2007). The Val and Met alleles are almost equally frequent in populations of European descent (Met-allele frequency = .47; heterozygosity = .48), whereas the Val-allele is more common in other parts of the world (Met-allele frequency = .16-.34; heterozygosity = .27-.45) (Palmatier, Kang, & Kidd, 1999).

The Met variant of the enzyme is less stable at body temperature (Chen et al., 2004; Lotta et al., 1995), resulting in 3 to 4 times less COMT enzyme activity in the human liver and red blood cells (Männistö & Kaakkola, 1999). In the human brain this difference is smaller, but still considerable, with Met/Met homozygotes having approximately 40% less COMT activity than Val/Val homozygotes in the prefrontal cortex (Chen et al., 2004). The alleles are codominant, resulting in Val/Met heterozygotes having an intermediate level of COMT activity (Egan et al., 2001; Männistö & Kaakkola, 1999; Tunbridge et al., 2006). This evidence strongly suggests that Met/Met homozygotes have the *highest* baseline level of dopamine available in the prefrontal cortex (because less dopamine is catabolized) with Val/Met heterozygotes having an intermediate level, and Val/Val homozygotes having the lowest level of prefrontal dopamine (Tunbridge et al., 2006; Tunbridge et al., 2007).

Several studies have demonstrated a relationship between the *COMT* Val^{158}Met polymorphism and performance on tasks associated with the frontal cortex. For example, Egan et al. (2001) found that the *COMT* Val^{158}Met polymorphism affected performance on the Wisconsin Card Sorting Test (WCST). Val/Val homozygotes performed significantly worse than Met/Met homozygotes and heterozygotes. Furthermore, the number of Met-alleles (0-2) that an individual had significantly predicted neural efficiency in the frontal cortex during an fMRI task, the N-back task (Egan et al., 2001). In this task all genotype groups performed at the same level, but Val/Val homozygotes showed significantly greater activation (indicating lower neural efficiency) in the frontal cortex than heterozygotes, and heterozygotes showed significantly greater activation than Met/Met homozygotes. Recent meta-analyses have been inconsistent in terms of the relationship between performance on the WCST and the *COMT* Val^{158}Met polymorphism (Barnett, Jones, Robbins, & Müller, 2007; Barnett, Scoriels, & Munafò, 2008). However, the evidence for an effect on neural efficiency as well as on a range of frontal cortex tasks has been replicated in several studies (Bertolino et al., 2006; Blasi et al., 2005; Caldú et al., 2007; Diaz-Asper et al., 2008; Krämer et al., 2007; Mattay et al., 2003; Meyer-Lindenberg et al., 2006; Sheldrick et al., 2008; Stefanis et al., 2005), and has recently been extended to a mouse model of the Val^{158}Met polymorphism (Papaleo et al., 2008). Finally, a study by Diamond and colleagues (2004) demonstrated an effect of the *COMT* Val^{158}Met polymorphism on school-age children's performance on a task hypothesized to depend on dopamine in the prefrontal cortex. This finding demonstrates the potential effect of variation in COMT activity at younger ages, and opens up the possibility that the *COMT* Val^{158}Met polymorphism might have an effect on frontal cortex functioning already in infancy.

The second candidate gene in our study was the *DRD4* gene. Knowledge about the distribution of the D_4 receptor in the human brain is limited due to the lack of appropriate

radioligands (Hurd & Hall, 2005; Oak, Oldenhof, & Van Tol, 2000). However, existing evidence suggests that D_4 receptors are most abundant in the retina, followed by the prefrontal cortex (Oak et al., 2000). Hurd and Hall (2005) suggest that transmission via D_4 receptors is predominantly inhibitory in nature, resulting in disinhibition of excitatory transmission when these receptors are blocked (Hurd & Hall, 2005). Thus, a lack of or less efficient D_4 receptors may lead to deficits in frontal cortex functioning.

The most widely studied polymorphism of the *DRD4* gene is located in the third exon and contains a 48 base pair variable number of tandem repeats (48-bp VNTR). Nine alleles of the *DRD4* 48-bp VNTR have been identified world-wide, with the number of repeats ranging between 2 and 10. The 4- and 7-repeat alleles are the most common globally, though the 2-repeat allele is prevalent in South and East Asia. In a population of mixed European ancestry, allele frequencies are .57, .21 and .12 for the 4-, 7- and 2-repeat alleles respectively (Chang, Kidd, Livak, Pakstis, & Kidd, 1996).

The number of 48-bp repeats has been hypothesized to affect the transmitted signal in the postsynaptic neuron. However, findings from *in vitro* studies have shown that the *DRD4* 48-bp VNTR does not significantly alter D_4 receptor activity (Oak et al., 2000). A more recent study suggests that the different repeat sequences may affect gene expression differentially, i.e., the density of D_4 receptors in the brain. This study found that the 7-repeat allele had reduced expression compared to the 2-repeat and 4-repeat alleles (Schoots & Van Tol, 2003).

The *DRD4* 48-bp VNTR has been extensively studied in relation to Attention Deficit Hyperactivity Disorder (ADHD) (Li, Sham, Owen, & He, 2006). ADHD has been linked to performance deficits on tasks assessing frontal cortex functions such as response inhibition, selective attention and set shifting (for review, see Cornish et al., 2005). The 7-repeat allele has been consistently associated with ADHD in recent meta-analyses (Faraone et al., 2005; Li et al., 2006). Furthermore, the *DRD4* 48-bp VNTR has been shown to affect prefrontal grey

matter volume in a sample of boys diagnosed with ADHD, their siblings and controls (Durston et al., 2005). Recently, the 7-repeat allele has also been found to be associated with impulsivity and lower levels of response inhibition in healthy adults, both on its own (Congdon, Lesch, & Canli, 2008) and in combination with other polymorphisms in dopamine system genes (Congdon et al., 2008; Eisenberg et al., 2007). Finally, the 7-repeat allele has been linked to faster habituation in infancy and increased novelty seeking in adolescence (Laucht, Becker, & Schmidt, 2006), and to sensation seeking in toddlers when combined with poor parenting (Sheese, Voelker, Rothbart, & Posner, 2007). Therefore, the *DRD4* 48-bp VNTR can be considered a candidate polymorphism for frontal cortex functioning in infancy.

Turning to the genes most likely to act at the subcortical level, the D_2 receptor is considerably less prevalent in the cerebral cortex than in the striatum (Ito, Okubo, Halldin, & Farde, 1999; Lidow, Goldman-Rakic, Rakic, & Innis, 1989). The *DRD2* TaqIA polymorphism is located in the 3' untranslated region, 10 kb downstream from the *DRD2* gene, actually in the adjacent gene *ANKK1* (Neville, Johnstone, & Walton, 2004). A1 is the minor allele. The A1-present (A1+) genotype has a prevalence of approximately 31% in Caucasian individuals (Noble, 2000). The presence of this allele has been associated with lower D_2 receptor density in the human brain using PET, especially in the striatum (Jönsson et al., 1999; Pohjalainen et al., 1998; Ritchie & Noble, 2003; Thompson et al., 1997).

In contrast to the *DRD4* 48-bp VNTR, the *DRD2* TaqIA polymorphism is not associated with ADHD (Faraone et al., 2005). However, the A1 allele has been associated with various addictions (Munafò, Matheson, & Flint, 2007; Young, Lawford, Nutting, & Noble, 2004) and a more impulsive response style in a monetary reward task in healthy adults (Eisenberg et al., 2007). Little evidence exists for a role of the *DRD2* TaqIA polymorphism in frontal cortex functioning. However, Reuter and colleagues (2005) showed a significant interaction between the *DRD2* TaqIA polymorphism and the *COMT* Val^{158}Met polymorphism on a Stroop-like

task where participants had to respond to the written form of color words written in incongruent colors as quickly as possible. The interaction effect accounted for 13% of the variance in performance on this task. This result opens up the possibility that the *DRD2* gene (and perhaps other subcortical dopaminergic genes) impacts indirectly on frontal cortex functioning via interactions with genes affecting dopaminergic neurotransmission directly in the frontal cortex (e.g., *COMT* and *DRD4*).

Finally, we investigated the potential effect of a well-known polymorphism of the dopamine transporter (*DAT1*) gene. The dopamine transporter is primarily expressed in the mesencephalon (a subcortical area with strong dopaminergic projections to the striatum and frontal cortex), with the highest density in the basal ganglia (Hurd & Hall, 2005). The *DAT1* gene contains a 40-bp VNTR in the 3' untranslated region. Alleles range from 3 to 13 repeats, but the most common are the 9-repeat and 10-repeat alleles (Cornish et al., 2005). In populations of European ancestry the frequencies of the 9- and 10-repeat alleles vary, but most studies report frequencies of approximately .30 for the 9-repeat allele and .70 for the 10-repeat allele (Kang, Palmatier, & Kidd, 1999). Although analyses of mRNA levels in brain regions resulted in contradictory findings (Mill, Asherson, Browes, D'Souza, & Craig, 2002; Wonodi et al., 2009), two independent large-scale *in vivo* single photon emission computed tomography (SPECT) studies have shown that healthy individuals with at least one copy of the 9-repeat allele (9/9 and 9/10 genotypes) had higher transporter density, and therefore presumably more effective dopamine removal at the synapse, than the 10/10 genotype (van de Giessen et al., 2008; van Dyck et al., 2005).

In terms of phenotypes, the *DAT1* gene has been studied extensively in relation to ADHD because stimulant medication used in its treatment acts by blocking the dopamine transporter. Evidence suggests that 10/10 homozygosity is associated with a slightly increased risk of ADHD (Faraone et al., 2005). Furthermore, Cornish and colleagues (2005) reported an

association between the 10/10 genotype and ADHD symptoms in a general population sample. This group also found an independent association between the 10/10 genotype and poorer performance on measures of selective attention and response inhibition in their selected high- and low-risk sample. A similar trend was found by Congdon and colleagues (2008) in a sample of healthy adults. Despite these findings, recent neuroimaging studies in adults have indicated a more efficient neural response in the prefrontal cortex of 10/10 homozygotes during a working memory task (Bertolino et al., 2006; Caldú et al., 2007), a pattern similar to that which is seen in subjects with the *COMT* Met/Met genotype. One recent study also found higher levels of impulsivity in healthy adults with at least one 9-repeat allele (Forbes et al., 2007), contradicting other behavioral results. The behavioral effects of the *DAT1* 3' VNTR polymorphism may depend on the population studied.

In summary, the present study investigated whether performance on the Freeze-Frame task at 9 months of age was associated with genetic polymorphisms affecting important aspects of dopamine function in the brain. Since dopamine plays an important role in both the frontal cortex and the striatum, direct effects of the *COMT* Val^{158}Met and *DRD4* 48-bp VNTR were hypothesized, with potential interacting or indirect effects of the *DRD2* TaqIA and the *DAT1* 3' VNTR polymorphisms.

Methods

Sample

Infants were recruited from the greater London area. Data from two independent cohorts of infants were combined in the present study. Cohort 1 consisted of a small group of infants (N = 24). Behavioral results from this cohort have been reported previously (Holmboe et al., 2008). Cohort 2 consisted of a considerably larger group of infants (N = 104) who took part in a longitudinal study of frontal cortex functioning during the first year of life. Ninety-four

infants from the original cohort of 104 infants (recruited at 4 months) participated in the study at 9 months. Data from this cohort have not been reported previously.

Data on parental education and household income were only collected in Cohort 2, but generally represent families recruited for studies at our laboratory. Parents were in their mid-thirties (mothers: $M = 34.43$, $SD = 4.90$; fathers: $M = 36.45$, $SD = 6.61$) and primarily, but not exclusively, of middle or upper-middle class socio-economic status (maternal years of education: $M = 17.80$, $SD = 3.55$; household income in £: $M = 65{,}076$, $SD = 61{,}854$[1]). Seventy-nine percent of the infants tested (Cohorts 1 and 2 combined) had a White/Caucasian ethnic background (approx. ¾ of these infants were of British or Irish descent), and 21% had other or mixed ethnic background. Of the infants with other than Caucasian ethnic background ($N = 26$), 8% of infants had an Asian ethnic background, 15% had a Black ethnic background, and 77% had a mixed ethnic background (e.g., mother Asian and father Caucasian). Ethical permission for the study was obtained from the School of Psychology ethics board at Birkbeck, University of London.

The Freeze-Frame task

A detailed description of the Freeze-Frame task can be found in Holmboe et al. (2008). In short, infants were presented with animations in the centre of a 19-inch color monitor. Infants were seated in their parent's lap at a 60-cm distance from the monitor. On every trial a white square was flashed on the right or left side of the screen (the distractor). If the infant looked to the distractor, the animation was stopped for 3000 ms. If the infant did not look to the distractor, the animation continued after distractor presentation for the duration of the trial. Distractor duration was calibrated individually for each infant by increasing it by 40 ms on

[1] Approximate US$ equivalent: $M = 120{,}511$, $SD = 114{,}544$, based on the average GB£ per US$ exchange rate of 0.54 in 2006 (NationMaster.com, 2009) when the majority of the data was collected.

every trial where the infant did not look to the distractor. When the infant had looked to the distractor on two consecutive trials, distractor duration was fixed at the current duration for the rest of the test session. The even-numbered trials presented dynamic and colorful animations changing every 2 s (interesting trials), whereas the odd-numbered trials always presented the same uninteresting rotating orange star (boring trials). Infants were encouraged to complete 60 trials.

A few minor adjustments were made to the task used in Cohort 2. Most importantly, the animations were slightly smaller and a different set of animations was used for the interesting trials. The procedure used in Cohort 2 was the same as the procedure used in Cohort 1. In the new version distractor duration did not increase beyond 1200 ms. Infants were encouraged to complete 80 trials. The data were analyzed as described in Holmboe et al. (2008). That is, the session was divided into phases (from two trials before the calibration trial), invalid trials were excluded, and the proportion of looks to the distractors was calculated separately for boring and interesting trials in each phase. However, the additional data collection allowed an extra phase in the analyses. Thus, there were 4 phases of the experiment, each containing 16 trials (8 boring and 8 interesting).

Video recordings of each infant's behavior were coded offline. The coding procedure in Cohort 2 was similar to the procedure reported in Holmboe et al. (2008). The trial was considered invalid if the infant was not looking at the central stimulus at distractor onset. The trial was also considered invalid if the infant blinked (i.e., the pupils were fully covered) during distractor presentation. In addition, the trial was considered invalid if these behaviors occurred during the 1000 ms following distractor presentation. This criterion was added because in trials where the infant looks away immediately following distractor presentation, it is impossible to know whether the infant *would have* looked to the distractor if they had not looked away from the screen. On rare occasions, a trial was excluded because the infant's

eyes were out of view (e.g., if the infant's hand was in front of his or her eyes); such trials were considered invalid if the eyes were out of view for more than 2 frames (80 ms) during distractor presentation or within the 1000 ms following distractor presentation. Finally, trials where a saccade to the distractor was initiated earlier than 3 frames (120 ms) after distractor onset were also considered invalid; such saccades were most likely anticipatory or random. Inter-coder reliability in Cohort 2 was satisfactory for both looking behavior ($\kappa = .94$) and trial validity ($\kappa = .86$), based on data from 10 participants. (Inter-coder reliability in Cohort 1 was similar; see Holmboe et al., 2008.)

Collection of buccal swabs and DNA extraction

Buccal (cheek) swabs were collected at 3.5 years of age in Cohort 1 as part of a follow-up study, and at 4 months in Cohort 2. The buccal swab was collected by the parent in the lab (by rubbing the cotton bud on the inside of the child's cheeks for approximately 5-10 seconds), and then put in a sample tube by the experimenter. Two swabs per DNA sample tube were collected, and two independent samples per infant were shipped and isolated separately using a DNA-purification kit obtained from Gentra (Minneapolis, US), yielding total of 2-10 μg DNA per sample.

Genotyping

Genotyping procedures were carried out using published protocols (*DRD2* TaqIA: Grandy, Zhang, & Civelli, 1993; *DRD4* 48-bp VNTR: Ronai et al., 2000; *COMT* $Val^{158}Met$: Tarnok et al., 2007; *DAT1* 3' VNTR: Vandenbergh et al., 1992). Both DNA samples from each infant were genotyped for all the investigated polymorphisms. In order to ensure successful genotyping, the following precautions were taken: In case of unsuccessful amplification (~10%) at the *DRD4* and *DAT1* VNTR genotyping, the PCR reaction was repeated, hence the genotyping success rate was 100%. In addition, independent

amplification reactions were carried out for 50% of the samples at the DRD4 VNTR, because of the problematic amplification of the longer alleles (Ronai et al., 2000), this quality checkup yielded the same genotypes as the ones originally obtained. At the DRD2 TaqI restriction enzyme digestion genotyping was repeated in case of unsuccessful amplification (~5%) or non-identical results for the two samples (~8%). The *COMT* Val^{158}Met SNP (rs4680) was also genotyped by an alternative method using a pre-designed TaqMan kit (C_25746809_50, Applied BioSystem, Foster City, USA) on a 7300 Real-Time PCR System; the genotypes were in accordance with the original ones.

Data analyses

Behavioral data were analyzed using repeated measures analysis of variance (ANOVA). For the genotype analyses, data were analyzed using a Linear Mixed Model analysis (LMM) assuming a diagonal covariance structure. Phase and Trial Type were entered as repeated measures and proportion of looks to the distractors was entered as the dependent measure. The advantage of LMM is that data from participants with missing data points, in this case missing data from one or more phases of the experiment, can be included in the analysis (Garson, 2008). Missing data points are inevitable in infant studies, and, given the fact that the genotype effects we were interested in were likely to be modest in magnitude, we wished to include as much of the data in the analyses as possible.

Due to the risk of population stratification in ethnically mixed samples (Hutchison, Stallings, McGeary, & Bryan, 2004), genotype analyses were carried out on both the entire sample and on the subsample of infants of Caucasian ethnic origin. Significant main effects and interactions were followed up by posthoc tests and checked against a False Discovery Rate (FDR) adjusted *p*-value based on the total number of posthoc tests carried out across all genotype analyses in both the total sample and the Caucasian subsample (33 posthoc tests in total). The FDR was controlled at $p < .05$ using the method described by Benjamini et al.

(2001). Only posthoc comparisons that remained significant after controlling the FDR are reported.

The Hardy-Weinberg (HW) equilibrium test was calculated using Knud Christensen's program (Christensen, 1999); for the *DAT1* 3' VNTR the three common genotypes from two frequent alleles (9- and 10-repeat) were included in the analysis, and for the *DRD4* 48-bp VNTR, genotypes from 4 common alleles (2-, 3-, 4-, 7-repeat) were analyzed. For the *COMT* Val^{158}Met and *DRD2* TaqIA polymorphisms there were only 3 genotypes, and therefore all infants could be included in the HW test.

In the analyses investigating potential genotype effects on Freeze-Frame performance, the most frequent 10/10 genotype of the *DAT1* 3' VNTR was compared to all other genotypes (9/9, 9/10 and other types of heterozygotes, i.e., 3/10, 7/10, 10/11). The latter group is referred to as the non-10/10 group. Genotype grouping for the *DRD4* 48-bp VNTR polymorphism was based on the presence or absence of the 7-repeat allele (the 7+ group and the 7- group, respectively). One infant with the genotype 4/8 was included in the 7+ group.

Results

Genotype and allele distribution

Genotype data were available for 19 out of the 24 infants in Cohort 1. Seventeen of these infants were of Caucasian ethnic origin. In Cohort 2 genotype data were available for all 94 infants (71 Caucasian) tested at 9 months of age. When the two cohorts were pooled, genotype data were available for 113 infants (88 Caucasian). One hundred and two of these infants calibrated in the task (see below) and could be included in the analyses. Genotype frequencies for each of the four polymorphisms are presented in Table 1, and allele frequencies are presented in the Supplementary Table. Alleles and genotypes were in Hardy-Weinberg equilibrium for all polymorphisms, with the exception of the *DRD4* 48-bp VNTR

polymorphism in the total sample (see note to Table 1). When the Hardy-Weinberg analysis of the *DRD4* 48-bp VNTR was restricted to the Caucasian subsample, the *p*-value increased to .45. In order to ensure a genetically homogenous population, every genetic analysis was carried out in the Caucasian subsample as well. Allele and genotype frequencies were generally in agreement with the frequencies reported for a mixed European population (see Introduction), and were very similar in the total sample and the Caucasian subsample.

Freeze-Frame behavioral results

One hundred and two infants out of the 113 infants with genotype data available calibrated in the Freeze-Frame task (79 in the Caucasian subsample), i.e. they looked to the distractor on two consecutive trials (6 infants did not calibrate, and 5 infants were incorrectly calibrated by the experimenter; these infants could not be included in the analyses). Distractor duration was on average calibrated in 5.53 trials (*SD* = 8.13, ranging from 2 to 64), and the mean calibrated distractor duration was 324 ms (*SD* = 181, ranging from 200 to 1200). The average proportion of valid trials was .82 (*SD* = .10). Infants in Cohort 2 had a slightly lower proportion of valid trials than infants in Cohort 1 (.81 vs. .90), probably due to the session being a few minutes longer in the former cohort, but the groups did not differ significantly in terms of calibration data (data not shown).

The proportion of looks to the distractors in each phase and trial type is presented in Table 2. Freeze-Frame results from Cohort 1 have been reported previously (Holmboe et al., 2008). In the previous study a repeated measures ANOVA indicated that there were significant main effects of Phase and Trial Type, but no interaction. Results were unchanged in the sample of infants from Cohort 1 for whom genotype data were available (data not shown). These results were also replicated in Cohort 2 (Trial Type: $F(1,68) = 79.29$, $p < .001$, $\eta_p^2 = .54$; Phase: $F(2,136) = 99.63$, $p < .001$, $\eta_p^2 = .59$; Phase × Trial Type: $F(2,136) = 0.63$, p

= .53), and in the total sample (Trial Type: $F(1,81) = 105.99$, $p < .001$, $\eta_p^2 = .57$; Phase: $F(2,162) = 117.42$, $p < .001$, $\eta_p^2 = .59$; Phase × Trial Type: $F(2,162) = 0.59$, $p = .55$). The same significant effects were found when 4 phases were included in the ANOVA of data from Cohort 2 (data not shown). These results indicate that there is a clear main effect of Trial Type on looks to the distractors such that infants look less to the distractors in the interesting trials than in the boring trials. Infants also show a decrease in looks to the distractors during the test session, and this decrease is similar in the two trial types, i.e., no interaction (Table 2).

For the genotype analyses we wished to combine the data from the two cohorts to increase power. In order to combine all the available data, it was important to establish that infants in the two cohorts performed the task in the same way. A few minor parameters of the Freeze-Frame task differed between the two cohorts (see Methods). Therefore, the repeated measures ANOVA was repeated with Cohort as a between-subjects factor. This analysis clearly replicated the main effects and lack of interaction (data not shown). Importantly, there was no significant main effect of, or interactions involving, Cohort (all *p*s > .30). Given this lack of significant differences between the two cohorts, it was deemed appropriate to pool the data for the genotype analyses.

In all of the genotype analyses reported below the main effects of Phase and Trial Type remained highly significant with no interaction between Phase and Trial Type (data not shown). Furthermore, none of the polymorphisms was associated with basic task parameters such as the calibrated distractor duration or proportion of valid trials after controlling the FDR.

The COMT Val^{158}Met polymorphism and Freeze-Frame performance

All 4 phases of the Freeze-Frame task were included in the LMM since this analysis incorporates all available data. The LMM analysis indicated that there was a significant main effect of *COMT* Val^{158}Met Genotype on the proportion of looks to the distractors, $F(2,564.08) = 3.01$, $p < .050$. No interactions involving *COMT* Val^{158}Met Genotype reached significance in the total sample (all *p*s > .15). When the analysis was restricted to Caucasian infants this picture changed. The main effect of *COMT* Val^{158}Met Genotype was no longer significant, $F(2,418.32) = 2.20$, $p = .112$), but the *COMT* Val^{158}Met Genotype × Trial Type interaction was, $F(2,418.32) = 4.38$, $p = .013$, indicating that *COMT* Val^{158}Met Genotype affected performance in the two trial types differentially. No other interactions approached significance (all *p*s > .70).

Post hoc analyses on the main effect of *COMT* Val^{158}Met Genotype in the total sample indicated that none of the differences between genotype groups survived the FDR correction. Post hoc analyses of the *COMT* Val^{158}Met Genotype × Trial Type interaction observed in the Caucasian subsample indicated a significant difference in looks to the distractors in interesting trials both between the Met/Met and Val/Val group ($p < .0001$) and between the Met/Met and Val/Met group ($p < .01$). No other posthoc comparisons reached significance after controlling the FDR. The *COMT* Val^{158}Met genotype differences in the Caucasian subsample are illustrated in Figure 1a.

The DRD4 48-bp VNTR polymorphism and Freeze-Frame performance

The LMM analysis of the effect of the *DRD4* 48-bp VNTR on performance in the Freeze-Frame task showed no significant effects involving Genotype in either the total or the Caucasian subsample (all *p*s > .15). This indicates that, in the current sample, the 7+ group did not differ from the 7- group in terms of Freeze-Frame performance at 9 months of age.

The DRD2 TaqIA polymorphism and Freeze-Frame performance

The LMM showed no significant effects involving *DRD2* TaqIA Genotype (all *p*s > .70). This result was unchanged when the analysis was restricted to Caucasian infants (all *p*s > .20). The *DRD2* TaqIA polymorphism did not therefore have any significant effect on Freeze-Frame performance in the present sample.

The DAT1 3' VNTR polymorphism and Freeze-Frame performance

The LMM analysis of the *DAT1* 3' VNTR showed a significant main effect of Genotype in the total sample, $F(1,569.52) = 3.98$, $p = .047$. No interactions reached significance (all *p*s > .15). When the analysis was restricted to Caucasian infants, the main effect of *DAT1* 3' VNTR Genotype was only marginally significant, $F(1,427.74) = 2.92$, $p = .088$. There was also a marginally significant *DAT1* 3' VNTR Genotype × Phase interaction, $F(3,191.66) = 2.34$, $p = .075$. The main effect of *DAT1* 3' VNTR Genotype in the total sample was due to the 10/10 group looking less to the distractors overall than the non-10/10 group. This difference is illustrated in Figure 1b. No posthoc analyses were carried out since only the main effect of *DAT1* 3' VNTR Genotype was significant.

Analysis of the combined effect of the COMT Val^{158}Met and DAT1 3' VNTR polymorphisms on Freeze-Frame performance

The genotype distribution of the *COMT* Val^{158}Met and *DAT1* 3' VNTR, with genotype × genotype group sizes between 9 and 26 participants (see legend to Figure 1), allowed us to investigate the potential interaction between these two polymorphisms. (Genotype frequencies for the other polymorphisms investigated in the study resulted in group sizes that were too small to investigate interactions, with *n* for minor genotype × genotype groups being less than 5.) An LMM where both *DAT1* 3' VNTR Genotype and *COMT* Val^{158}Met Genotype were entered as independent variables showed a significant main effect of *COMT* Val^{158}Met Genotype, $F(2,528.98) = 3.41$, $p = .034$, and a marginally significant effect of

DAT1 3' VNTR Genotype, $F(1,529.47) = 3.30$, $p = .070$. In addition to these main effects, there was a significant *COMT* Val^{158}Met Genotype × Trial Type interaction, $F(2,528.98) = 3.19$, $p = .042$, and a significant *DAT1* 3' VNTR Genotype × *COMT* Val^{158}Met Genotype × Trial Type interaction, $F(2,528.98) = 4.09$, $p = .017$. The *DAT1* 3' VNTR Genotype × Phase interaction approached significance, $F(3,240.18) = 2.24$, $p = .084$, as did the *DAT1* 3' VNTR Genotype × *COMT* Val^{158}Met Genotype × Phase interaction, $F(6,241.10) = 1.91$, $p = .079$. No other interactions approached significance in the total sample (all *p*s > .35).

In the Caucasian subsample alone the results were slightly different. The main effect of *COMT* Val^{158}Met Genotype was marginally significant, $F(2,373.00) = 2.82$, $p = .061$. The same was the case for the *DAT1* 3' VNTR Genotype, $F(1,374.07) = 3.82$, $p = .051$. Again, the *COMT* Val^{158}Met Genotype × Trial Type interaction was significant, $F(2,373.00) = 4.13$, $p = .017$. Finally, the *DAT1* 3' VNTR Genotype × Phase interaction was significant in the Caucasian subsample, $F(3,170.63) = 2.98$, $p = .033$. No other interactions reached significance in the Caucasian subsample (all *p*s > .20).

Post hoc analyses were restricted to the novel interaction effects involving *COMT* Val^{158}Met and *DAT1* 3' VNTR because all significant and near-significant main effects were qualified by a significant interaction, and because other interactions, such as the *COMT* Val^{158}Met Genotype × Trial Type interaction, essentially indicated the same genotype effects as the analyses of the two polymorphisms separately. Posthoc analyses of the *DAT1* 3' VNTR Genotype × *COMT* Val^{158}Met Genotype × Trial Type interaction in the total sample indicated that within the *DAT1* non-10/10 group there was a significant difference in looks to the distractors in the interesting trials between the Met/Met and Val/Val groups ($p < .001$) and between the Met/Met and Val/Met groups ($p = .001$). In contrast, no *COMT* genotype differences reached significance in the *DAT1* 10/10 group after controlling the FDR. This pattern of results is illustrated in Figure 1c. Regarding performance in each *COMT* genotype

group across *DAT1* genotypes, infants with the Val/Met genotype who also had the *DAT1* 10/10 genotype looked significantly less to the distractors in the interesting trials than infants with the Val/Met genotype in the *DAT1* non-10/10 group ($p < .01$). The other *COMT* genotype groups did not differ significantly across *DAT1* genotype groups in the interesting trials (Figure 1c). None of the posthoc tests of the *DAT1* 3' VNTR Genotype × *COMT* Val^{158}Met Genotype × Trial Type interaction showed significant effects in the boring trials after controlling the FDR.

Posthoc analyses indicated that the *DAT1* 3' VNTR Genotype × Phase interaction found in the Caucasian subsample was due to a highly significant difference in proportion of looks to the distractors between the 10/10 group and the non-10/10 group in Phase 3 of the Freeze-Frame session ($p < .001$).

Discussion

The present study investigated whether performance in a novel task developed to assess frontal cortex functioning in infancy, the Freeze-Frame task (Holmboe et al., 2008), was associated with common polymorphisms in four dopamine system genes. Previous research has clearly shown that dopamine plays an important role in the frontal cortex (Brozoski et al., 1979; Collins et al., 1998; Diamond et al., 1997; Goldman-Rakic et al., 2000; Roberts et al., 1994; Sawaguchi & Goldman-Rakic, 1991; Vijayraghavan et al., 2007).

Behaviorally, we replicated previous findings on the Freeze-Frame task (Holmboe et al., 2008). In relation to the polymorphisms likely to impact directly on frontal cortex function, we found a significant association between Freeze-Frame performance and the *COMT* Val^{158}Met polymorphism. Given the extensive evidence for an association between the *COMT* Val^{158}Met polymorphism and performance on a range of frontal cortex tasks (Diamond et al., 2004; Diaz-Asper et al., 2008; Egan et al., 2001; Mattay et al., 2003; Sheldrick et al., 2008; Stefanis et al., 2005; see also Papaleo et al., 2008), as well as effects

on neural efficiency in the frontal cortex during performance of these tasks (Bertolino et al., 2006; Blasi et al., 2005; Caldú et al., 2007; Egan et al., 2001; Krämer et al., 2007; Mattay et al., 2003; Meyer-Lindenberg et al., 2006), it seems likely that *COMT* Val^{158}Met genotype affects dopamine levels in the frontal cortex and thereby Freeze-Frame task performance in our infant sample.

Furthermore, it is worth noting that this effect was specific to the interesting trials, at least in the Caucasian subsample (Figure 1a). This suggests that the *COMT* Val^{158}Met effect is not a general effect impacting on infants' distractibility level in any given situation. Rather, it seems to be the case that infants with the low-enzyme activity Met/Met genotype became particularly focused on the central stimulus compared to the high-enzyme activity Val/Val genotype when this stimulus was engaging. However, it should be noted that the interaction with trial type was significant in the Caucasian subsample only and therefore might not generalize to other populations.

We found little evidence that the *DRD4* 48-bp VNTR polymorphism affects performance on the Freeze-Frame task at 9 months of age, though the sample was too small to detect subtle effects. In terms of the polymorphisms which are likely to act in the striatum, we did not observe any effect of the *DRD2* TaqIA either. We did however observe an effect of the *DAT1* 3' VNTR polymorphism. In contrast to the effect of the *COMT* Val^{158}Met polymorphism, this effect did not appear to be specific to a particular trial type. Instead, we found evidence of an overall difference in the proportion of looks to the distractors with the 10/10 group looking less to the distractors than the non-10/10 group (Figure 1b). The results therefore suggest that the *DAT1* 3' VNTR polymorphism modulates overall distractibility in the Freeze-Frame task, though there was a tendency for this genotype effect to be stronger at the end of the test session. Given the fact that the dopamine transporter plays an important role in the striatum (Hurd & Hall, 2005; Karoum et al., 1994), this effect could be due to

modulation of general attentional mechanisms mediated by the subcortical dopamine system or frontal-subcortical connections (Alexander et al., 1986; Cummings, 1993).

Finally, we investigated the potential interaction between the *COMT* Val^{158}Met and *DAT1* 3' VNTR polymorphisms on Freeze-Frame performance. The results of these analyses broadly replicated the main effects and interactions found in the analysis of each polymorphism separately. However, the analyses also revealed a significant *DAT1* 3' VNTR Genotype × *COMT* Val^{158}Met Genotype × Trial Type interaction, suggesting that the *DAT1* 3' VNTR polymorphism modulated the effect of the *COMT* Val^{158}Met polymorphism on Freeze-Frame performance. Basically, the effect of the *COMT* Val^{158}Met polymorphism on the proportion of looks to the distractors in the interesting trials was strong in the *DAT1* non-10/10 group, with particularly large differences between the Met/Met group and the two other genotype groups (Figure 1c, right panel). In contrast, the equivalent effect in the *DAT1* 10/10 group virtually disappeared (Figure 1c, left panel).

Presuming that a lower level of distractibility in the interesting trials is an expression of a higher degree of selective inhibition, these results suggest that infants with the higher *COMT* enzyme activity alleles (Val/Val and Val/Met) actually benefit from having the *DAT1* 10/10 genotype, whereas this is not the case for infants with the low-activity enzyme (Met/Met). This was confirmed at least for the Val/Met genotype; this genotype showed a significant reduction in looks to the distractors in the interesting trials when combined with the 10/10 genotype rather than with the non-10/10 genotype (Figure 1c). Though preliminary given the sample size, these findings are particularly interesting because they suggest that the interaction between a predominantly frontal dopaminergic polymorphism (*COMT* Val^{158}Met) and a predominantly striatal dopaminergic polymorphism (*DAT1* 3' VNTR) results in large performance differences on the Freeze-Frame task already at 9 months.

It should be mentioned that it would have been ideal to investigate all possible interactions between the four polymorphisms in the study. However, only the *COMT* Val^{158}Met and the *DAT1* 3' VNTR polymorphisms had genotype frequencies providing enough power to investigate interaction effects (see Methods). For the *DRD4* 48-bp VNTR and the *DRD2* TaqIA polymorphisms the genotype frequencies involving the minor allele were too low to test meaningful interactions. Future studies should address the question of interactions between all four (and additional) polymorphisms in dopamine system genes in a larger infant cohort.

Despite the likely effect of both frontal and subcortical mechanisms in the reported results, it is not possible to establish the exact neural substrate of this interaction from the current data. Previous studies have found additive genetic effects of the *DAT1* 3' VNTR and *COMT* Val^{158}Met polymorphisms on neural efficiency in the frontal cortex (Bertolino et al., 2006; Caldú et al., 2007). However, an interaction between the two polymorphisms has not previously been reported (though see Prata et al., 2009, for a recent study which found an epistatic effect in the parietal cortex). Further research using neuroimaging data will help elucidate the potential role of the frontal cortex and the striatum in these genotype effects.

The current study constitutes a snapshot in time at 9 months of age. Future studies over a wider age range may help elucidate which patterns of Freeze-Frame performance are adaptive throughout infancy and early childhood, and how these patterns relate to polymorphisms in dopamine system genes. Some progress has already been made towards this at the behavioral level in the work by Holmboe and colleagues (2008) where performance indices on early frontal cortex tasks showed both positive and negative associations with later performance. Nevertheless, an important conclusion to be drawn from the results of the present study is that polymorphisms in dopamine system genes play an important role already in infancy. Previous studies have found effects of the *DRD4* 48-bp VNTR on temperament and relatively broad

aspects of attention in infancy (Auerbach et al., 1999; Auerbach, Benjamin, Faroy, Geller, & Ebstein, 2001; Auerbach, Faroy, Ebstein, Kahana, & Levine, 2001; Ebstein et al., 1998; Laucht et al., 2006; Sheese et al., 2007). The current study adds to this evidence by showing that the *COMT* Val^{158}Met polymorphism, which is thought to play an important role specifically in the frontal cortex, affects performance on a simple saccadic inhibition task in infancy.

In conclusion, the results of the present study further validate the Freeze-Frame task, and demonstrate that variation in dopamine neurotransmission in the frontal cortex and associated subcortical structures can have an impact on infant attention already at 9 months of age. The exact neural substrate and developmental course of these genotypic differences is a fruitful area for future research. This research holds the promise of deepening our understanding of the genetic underpinnings of individual differences in the important functions mediated by the frontal cortex from an early age.

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Table 1.

Genotype frequencies in the total sample and the Caucasian subsample (percentages in brackets).

Polymorphism	*Genotype*	*Total*	*Caucasian*	*Grouping*
DRD4 48-bp VNTR	2/3	2 (2.0)	2 (2.5)	7-
	2/4	10 (9.8)	9 (11.4)	7-
	2/7	8 (7.8)	4 (5.1)	7+
	3/4	8 (7.8)	6 (7.6)	7-
	3/7	2 (2.0)	2 (2.5)	7+
	4/4	48 (47.1)	36 (45.6)	7-
	4/5	2 (2.0)	0 (0.0)	7-
	4/7	21 (20.6)	19 (24.1)	7+
	4/8	1 (1.0)	1 (1.3)	7+
	7+	32 (31.4)	26 (32.9)	
COMT $Val^{158}Met$	Met/Met	28 (27.5)	19 (24.1)	Met/Met
	Val/Met	47 (46.1)	37 (46.8)	Val/Met
	Val/Val	27 (26.5)	23 (29.1)	Val/Val
DRD2 Taq1A	A1/A1	4 (3.9)	4 (5.1)	A1+
	A1/A2	29 (28.4)	20 (25.3)	A1+
	A2/A2	69 (67.6)	55 (69.6)	A1-
	A1+	33 (32.4)	24 (30.4)	
DAT1 3' VNTR	3/10	1 (1.0)	0 (0.0)	Non-10/10
	7/10	1 (1.0)	0 (0.0)	Non-10/10
	9/9	4 (3.9)	3 (3.8)	Non-10/10
	9/10	35 (34.3)	30 (38.0)	Non-10/10

	10/10	60 (58.8)	45 (57.0)	10/10
	10/11	1 (1.0)	1 (1.3)	Non-10/10
	Non-10/10	42 (41.2)	34 (43.0)	
Total *N*		102	79	

Note. Only data from infants who calibrated in the Freeze-Frame task are included in the table (data from infants who did not calibrate could not be used in the analyses). All polymorphisms except the *DRD4* 48-bp VNTR polymorphism conformed to Hardy-Weinberg equilibrium: *DRD4* 48-bp VNTR: $\chi^2 = 12.95$, $df = 6$, $p = .044$ (all participants); $\chi^2 = 5.80$, $df = 6$, $p = .45$ (Caucasians only). *COMT* Val^{158}Met: $\chi^2 = 0.63$, $df = 1$, $p = .43$ (all participants); $\chi^2 = 0.29$, $df = 1$, $p = .59$ (Caucasians only). *DRD2* Taq1A: $\chi^2 = 0.18$, $df = 1$, $p = .67$ (all participants); $\chi^2 = 1.37$, $df = 1$, $p = .24$ (Caucasians only). *DAT1* 3' VNTR: $\chi^2 = 0.16$, $df = 1$, $p = .69$ (all participants); $\chi^2 = 0.54$, $df = 1$, $p = .46$ (Caucasians only).

Table 2.

Descriptive statistics for the proportion of looks to the distractors across phases and trial types in the Freeze-Frame task.

	Mean	*SD*
Boring, Phase 1	.69	.22
Boring, Phase 2	.42	.28
Boring, Phase 3	.40	.24
Boring, Phase 4*	.39	.25
Boring, Total	.48	.17
Interesting, Phase 1	.45	.25
Interesting, Phase 2	.19	.19
Interesting, Phase 3	.13	.16
Interesting, Phase 4*	.11	.15
Interesting, Total	.22	.14

Note. *Only infants in Cohort 2 completed 4 phases.

Figure Caption

Figure 1. The effect of the *COMT* Val^{158}Met and *DAT1* 3' VNTR polymorphisms on Freeze-Frame performance. Error bars indicate the 95% confidence interval of the mean.

a, The mean proportion of looks to the distractors in the boring and interesting Freeze-Frame trials in the three *COMT* Val^{158}Met genotype groups in the Caucasian subsample (Met/Met, n = 19; Val/Met, n = 37; Val/Val, n = 23); the asterisk (*) indicates a significant difference from the Met/Met group at $p < .01$.

b, The mean proportion of looks to the distractors in the boring and interesting Freeze-Frame trials in the *DAT1* 10/10 and non-10/10 genotype groups (10/10, n = 60; non-10/10, n = 42); the overall difference between the two genotype groups (across trial types) was significant at $p < .05$.

c, The effect of the *COMT* Val^{158}Met polymorphism on the mean proportion of looks to the distractors in the interesting Freeze-Frame trials in the two *DAT1* genotype groups (10/10 + Met/Met, n = 19; 10/10 + Val/Met, n = 26; 10/10 + Val/Val, n = 15; non-10/10 + Met/Met, n = 9; non-10/10 + Val/Met, n = 21; non-10/10 + Val/Val, n = 12); the asterisk (*) indicates a significant difference from the Met/Met group at $p < .01$ within the non-10/10 group, and the triangle (▲) indicates a significant difference at $p < .01$ between the Val/Met group in the non-10/10 group compared to the Val/Met group in the 10/10 group.