RICE UNIVERSITY

Is Response Time Variability on an Exogenous Visual Orienting Task Associated with Specific Genetic Markers?

by

Rebecca A. Lundwall

A THESIS SUBMITTED IN PARTIAL FULFILLMENT
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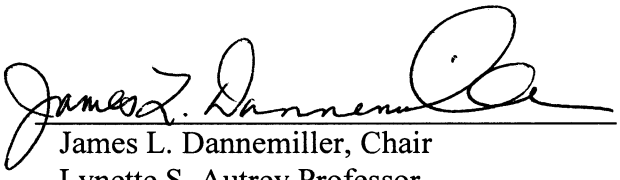
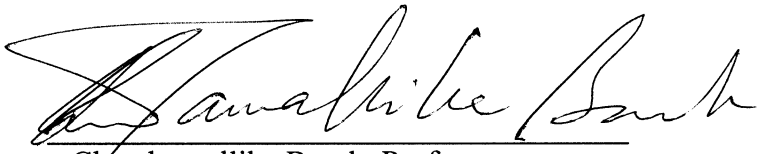


James L. Dannemiller, Chair
Lynette S. Autrey Professor
Psychology

Fred Oswald, Professor
Psychology



Chandramallika Basak, Professor
Psychology

HOUSTON, TEXAS

JULY 2011

ABSTRACT

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Attention is a vital component of everyday functioning, and deficits in attention feature in many psychological disorders. Improved understanding of attention may eventually be critical to early identification and treatment of attentional deficits. One step in that direction is to acquire a better understanding of genetic associations with performance on a measure of reflexive visual attention. We have previously studied the relationship between selected genes and mean RT. This thesis reports on a new analysis of the same data which were used to examine mean differences but now examines the contributions of genetic markers to RT variability. I consider the relationship between mean RT and RT variability and account for other potential predictors of RT variability such as age, ethnicity, and sleepiness. I ask, after accounting for other reasons that RT variability might be increased in some subjects, does increased RT variability depend also on genotype?

Acknowledgements

I am indebted to a great number of people for their support to me as I worked on this thesis. First and most of all I would like to express my deep appreciation to Jim Dannemiller, my advisor, for his support. I think it quite likely he is the best possible mentor for me. I especially appreciate his willingness to treat me as an intellectual equal. I would also like to thank Fred Oswald and Chandramallika Basak, both professors at Rice University. I sought Fred's advice on statistics many times and he was always ready (and quick!) to help. Chandramallika was equally welcoming when I sought her advice and I appreciate her cheerfulness. I am also grateful to all my other colleagues (student and professor alike) at Rice University. I can't think of a better place to grow intellectually.

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Introduction

Attentional measure/gene association studies are one method for finding genetic influences on attention, which is important in many inherited psychological disorders as well as in the normal population. Attention is a broad concept that has often been distinguished in the literature into at least two types: 1) reflexive and 2) sustained. Reflexive attention refers to a stimulus-driven re-orienting of the brain's resources, often to an external object that newly appears, has a relatively salient color, or involves motion. The cued-orienting task (Posner, 1980) that we (Jim Dannemiller and I) used in our first study is an example of a reflexive task. Stimuli flash briefly on the computer display and subjects automatically move their attention. It is taken as evidence that attention was captured if subjects were faster at responding to a target that was preceded by the presentation of a brief pre-cue even though the stimuli presentation is too brief to depend on eye movement. Sustained attention is measured with an effortful task requiring vigilance over time. An example would be a task that presents a stream of stimuli, some of which require a response and others that require that a response be withheld. The Continuous Performance Test (CPT; Conners, 1992) and the Sustained Attention to Response Task (SART; Bellgrove, Hawi, Kirley, Gill, & Robertson, 2005) are two examples. Both missed targets and responses to non-targets can be examined. Performance on any type of behavioral task can be examined for correlation with specific markers[1] in the genetic code of an individual. These are referred to as genetic association studies.

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[1] A genetic marker is a DNA sequence or single location (base pair) associated with a particular gene.

Genetic association studies using these types of tasks provide information on genetic influences on behavior. They are valuable in cognitive neuroscience because they offer a way to establish connections between the brain and behavior. Establishing this link usually involves determining the influences of the gene on the availability of a neurotransmitter. That is, the biological link between the brain and behavior often includes neurotransmitters whose availability impacts certain cognitive behaviors (such as attending, deciding, or planning), making these behaviors more or less efficient.

Below I provide the rationale for performing a RT variability analysis, discuss relevant literature, and outline the research questions to be investigated. I then describe behavioral, genetic, and statistical methodologies. Results are then presented, followed by discussion and conclusions, including contributions of this study and implications for future research.

Genetics Background

An individual's genetic code is made up of long strands of nucleotide bases; the nucleotide bases and their abbreviations are cytosine (C), guanine (G), adenine (A), and thymine (T). The 'double helix' of deoxyribo-nucleic acid (DNA) is created from two strands of nucleotide bases linked by their complementary base pairs: the base A always forms a base pair with T, and the base G forms a base pair with C. These base pairs are organized into genes (that have coding and non-coding regions) and non-genetic DNA sequences (that is, sequences outside of a gene) which may have functions which are still uncertain (Svensson, Arvestad, & Lagergren, 2006). A gene usually produces a protein, such as a neurotransmitter or an enzyme that degrades it. It does this by assembling a string of amino acids, each specified by a set of three sequential base pairs called codons.

A single nucleotide base change on a gene can alter the amino acid that is produced by a codon and therefore alter the functioning of the protein that is produced by the entire gene. Alternatively, a single base pair change can result in the same amino acid but slow production of the protein. If the change is related to the availability of a neurotransmitter, then it is often informative to associate these single nucleotide polymorphisms (SNPs) with performance on an appropriate behavioral measure. Polymorphic alleles exist when there are alternate forms that may occur at a given position in the genetic code. In a similar way, the number of identical copies of longer sections of base pairs (called variable number tandem repeats; VNTRs; see Figure 1) can also be associated with performance on behavioral measures. VNTRs are inherited from one's biological parents in the same way that SNPs are. An alteration in one of the SNPs or VNTRs on a gene will often impact the functioning of the entire gene. This altered functioning is not limited to those with a diagnosable disorder such as ADHD. Many polymorphisms, in fact, occur in the normal population at a frequency high enough that they can be used as a grouping factor. These polymorphisms are said to be risk alleles for the disorder. Disorders that are influenced by the risk alleles from many genes are said to be polygenetic.

CCT	CCA	CTC	AGG/	CCA	CTC	AGG/	CCA	CTC	AGG/	CTT
CCT	CCA	CTC	AGG/	CCA	CTC	AGG/				CTT

Figure 1. The top row represents a person with three repeats (copies) of a variable number tandem repeat (VNTR). The bottom row represents a person with two repeats. Slashes separate the repeats.

The markers that we have in the current data set include one VNTR (a 30 base pair unit on intron 8 of the DAT1 gene), three separate SNPs (on COMT, DBH and

DRD4), and an additional two SNPs (used to define APOE gene status). Because subjects receive one allele from each of their biological parents, there are three possible genotypes at each genetic marker. In the case of DAT1, it is the number of copies inherited from each parent (combinations of 5- and 6-repeat alleles: 5R/5R, 5R/6R, and 6R/6R). In the case of APOE, the three groups have been defined as ε2/ ε3, ε3/ ε3, and either ε3/ ε4 or ε4/ ε4 (see Hubacek, Lánská, Skodová, Adámková, & Poledne, 2008). These genes influence the availability of the neurotransmitters dopamine (in the case of DAT1, COMT, DBH, and DRD4) and acetylcholine (for APOE).

Association studies between attention tasks and certain genes generally use mean RT differences between those predicted to have more or less neurotransmitter available as determined by their genotype. There are many of these studies in the literature. On the other hand, there are relatively few studies that examine RT variability in association with genotype. This is despite the fact that greater RT variability has been found in certain populations and may reflect difficulties in regulating attentional arousal, such as the ability to maintain alertness or sustain attention. In addition, greater RT variability is predicted by certain theories.

Rationale

The first step in the rationale for this project is to note that there are theories which predict in whom we will see greater variability. For example, the Moderate Brain Arousal model predicts that those with low dopamine will have increased variability (Söderlund, 2007). This neurocomputational model hypothesizes that there is less general activation in the brains of those with less dopamine so that the effective signal to noise ratio is lower. It specifically predicts greater RT variability in those with less dopamine.

Lower dopamine is seen in those with ADHD but also in normal individuals who carry certain risk alleles.

The Dynamic Developmental Behavioral theory likewise predicts that failure of the dopamine pathways (mesolimbic, mesocortical, and nigrostriatal) to respond to signals from other neurotransmitter pathways (such as glutamate and GABA) will lead to attention deficits (Sagvolden, Johansen, Aase, & Russell, 2005). This highlights the role of dopamine as a neuromodulator that tunes the sensitivity of other neurotransmitters in their responsiveness to the environment.

The two theories just described specifically mention dopamine. On the other hand, the Barkley model (Barkley, 1997) only predicts that ADHD should be associated with secondary impairments and greater variability in many cognitive tasks that lead to more variable RTs. No genetic pathways are hypothesized. Similarly, Sergeant's Cognitive–Energetic Model (CEM) proposes that the overall efficiency of information processing at several levels determines deficits that increase variability in a wide range of behaviors (Sergeant, 2005). The levels include 1) lower cognitive processes such as motor organization (i.e., intention, or planning for a motor response), 2) "energetic pools" such as activation (influenced by stimulus intensity and novelty) and effort, and 3) executive functions (such as error detection and monitoring). Sergeant does not specifically mention RT variability, but all three levels may influence this aspect of task performance. Subjects with ADHD (and, by extension, perhaps those who carry risk alleles for ADHD) may be especially sensitive to task features that make their performance more variable over the course of the task. They may also be more variable as they attempt to monitor response errors.

In addition to theoretical work, empirical studies also contribute to the rationale for investigating genetic association with RT variability. For example, two different groups of researchers have used a warned four-choice task to compare ADHD boys and age-matched controls, including on RT variability. The 'warning' involves temporal information that a target will soon appear. The task involves responding with one of four fingers (two from each hand) to the location of a target, which is sometimes preceded by a spatially informative pre-cue. Leth-Steensen, Elbaz, and Douglas (2000) created ex-Gaussian distributions for boys with ADHD and control subjects. RTs are represented as composed of two components: a normally distributed and an exponentially distributed component, which together create a positively skewed RT distribution.[2] Leth-Steensen et al. (2000) found that the ex-Gaussian distributions of ADHD subjects differed from those of control subjects with respect to the size of the tail (indicating greater variability for the ADHD boys). For this task, time between cue and target (the preparation interval) varied from 2 to 8 seconds. Hurks et al. (2005) also found greater RT variability in children with ADHD across preparation intervals (from 100 ms to 1,000 ms) and pre-cue conditions. Although our sample is composed of normal adults, these findings suggest the possibility of increased variability because some normal adults may have less available dopamine due to the alleles which they carry. Volkow et al. (2001) argue that dopamine is likely involved in attention because methylphenidate (used to treat ADHD) acts to increase

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[2]Note that in an ex-Gaussian distribution if μ represents the mean and σ the standard deviation of the normal component, and τ represents the mean of the exponential component, then the mean of the response times = $\mu + \tau$ and the variance of response times = $\sigma^2 + \tau^2$.

dopamine availability. In addition, subjects treated with methylphenidate have reduced RT variability on the Eriksen flanker task (Castellanos, et al., 2005; Eriksen & Eriksen, 1974). This has application to normal subjects because it shows that behavior on an attention task is normal when dopamine levels are normal (with methylphenidate treatment). If attention task performance is low when dopamine availability is more likely to be low (based on genotype) then this is evidence for the role of dopamine in normal attentional functioning.

The work just described (Castellanos et al., 2005; Volkow et al., 2001) points to biological contribution to RT variability in psychological processes, as do other studies. Lesion studies, for example, show that damage to the frontal lobes is accompanied by an increase in RT variability but that RT variability is not a simple consequence of general brain dysfunction—focal frontal lesions were associated with RT variability more than other lesion areas (Bellgrove, Hester & Garavan, 2004; Stuss, Murphy, Binns & Alexander, 2003). Of course, biologic pathways are also implied because RT variability is highly heritable. Kuntsi and Stevenson (2001) studied monozygotic and dizygotic twins for shared genetic effects on 1) hyperactivity as measured by the Conners' Rating Scale (rated by teachers; Goyette, Conners, & Ulrich, 1978) and 2) RT variability on the Stop Task (which measures the ability to inhibit a normally correct response when a tone is presented; Oosterlaan & Sergeant, 1998). They found a bivariate heritability index of 64%, indicating that hyperactivity and RT variability are influenced by the same set of genes (see Stevenson, Pennington, Gilger, DeFries, & Gillis, 1993).

That dopamine is probably related to RT variability is especially interesting because many of the genes we have studied are dopamine related. DAT1 produces a

protein involved in dopamine transport out of the synapse; COMT controls the catabolisis (degradation) of dopamine; and DRD4 encodes a receptor which is activated by dopamine (Swanson et al., 2001). Furthermore, our previous analysis of these data showed that normal individuals differed on mean RT depending on their genotype. The direction of the effect for each gene was consistent with the risk allele (lower dopamine availability) leading to worse outcomes on some measures. It seems advisable, therefore, to follow these results to determine if lower dopamine availability also increases RT variability.

As previously mentioned, a limited number of studies examine genes related to ADHD and RT variability. Kebir, Tabbane, Sengupta, and Joober (2009) reviewed 12 studies that considered RT variability while examining six genes (DRD4, DAT1, DBH, DRD5, ADRA2A, and TPH2). These studies used a variety of tasks including CPT, SART, go/no-go task, stop signal task, and Stroop. Association between high RT variability and the absence of the 7-repeat allele of DRD4 is a consistent result (4 studies). For DAT1 (4 studies), high RT variability seems to be most commonly associated with the 10-repeat homozygosity. DBH, DRD5, ADRA2A, and TPH2 each had one study that associated them with RT variability.

These reported findings involve sustained attention. Replication and extension with a reflexive orienting task is desirable because it would lead to a more complete picture of attention. Results from such a study might allow for earlier identification and more complete treatment of attentional problems. Although we do not have information on DRD5, ADRA2A, and TPH2 in our data set, we do have DRD4, DAT1, DBH and an additional gene, COMT (which degrades dopamine), for which no studies have been

reported. Since DBH has only one study associating it with RT variability, that study especially needs replication /extension. We also have information on APOE (which carries risk for Alzheimer's disease), which is not related to dopamine but has been related to cued orienting (Parasuraman, Greenwood, & Sunderland, 2002).

In the literature, genetic studies have sometimes found that there were no mean RT differences by genotype, but there were difference in variability. I wondered if this might be true for the genes for which we did not find mean differences; I also wondered if differences in RT variability might be found (in addition to differences in mean RT) for the genes for which we did find mean differences.

Research Questions

The literature review in the previous section suggests that those with risk alleles on DAT1 or DRD4 have increased variability, but that replication and extension to additional genes (and a new task) are needed. Because we found mean RT differences between normal subjects with different genotypes previously with this data, it makes sense to ask if there is increased RT variability as well. This research project also considers the relationship between mean RT and RT variability because RT variability often increases with increasing mean RT. If so increases in RT variability are only due to this relationship, then the explanation of mean RT differences is the more parsimonious explanation for how genes influence RT behavior.

A sequential multiple regression analysis was chosen to better control when and how predictors are entered into the models. In this way I can determine if there are genetic influences on the RT variability of a given outcome measure beyond the influence attributable to other predictors (including the mean RT for that outcome measure). The

genotypes of each genetic marker are coded so as to reflect the increasing "dose" of a risk allele. It is appropriate to look for linear relationships because the dose of an allele is associated with the greater or reduced availability of a neurotransmitter. Do any of the genetic markers selected impact RT variability in an orienting task? This general question leads to specific questions and hypotheses.

First Hypothesis

Will examining RT variability further clarify how genes contribute to attention? I suspect that individuals will show greater RT variability when they have genotypes that tend toward less available dopamine. In the current data set COMT, DAT1, DBH and DRD4 are related to dopamine availability. In addition, APOE, which is associated with the neurotransmitter acetylcholine, conveys enhanced risk of cognitive deficits in those who carry at least one copy of the ε4 allele and thus may also influence attentional abilities. The markers and their risk alleles are described in Table 1.

Second Hypothesis

Do any of the intake variables (see Appendix A) associate with increased RT variability? I suspect greater sleepiness will associate with more RT variability (because attentional arousal is lower) but particularly impact those with less dopamine. I also anticipate that as age increases, RT variability will also increase (because dopamine levels are lower; Erixon-Lindroth et al., 2005). Thus, I expect that age and sleepiness will each interact with risk alleles for lower dopamine availability to influence the effects we may see on RT variability.

Table 1. Rationale for genetic markers selected.

Genetic Marker	Risk Allele	Biological Effect	Functional Effect
COMT rs4680	G	G at rs4680 produces valine which is more active in catabolizing dopamine and so less dopamine is available[1]	Reduced cognitive funcion[1]
DAT1 intron 8 VNTR	6R	6R leads to more dopamine transporter and therefore less dopamine in the synapse[2] and this terminates the dopaminergic signal transmission[3]	Greater cuing costs for targets in the left hemifield[4]
DRD4 rs747302	C	C leads to fewer dopamine receptors via reduced transcription[5]	There is an association between rs747302 and ADHD[5]
APOE rs429358 + rs7412	ε4	ε4 reduces acetylcholine receptor number[6] and possibly diminished synthesis of acetylcholine via impaired regulation of phospholipids and/or fatty acid transport[7]	Middle age, nondemented carriers of ε4 showed deficits in spatially cued visual tasks[8]
DBH rs1108580	A	DβH converts dopamine to norepinephrine and the A allele is associated with lower levels of plasma DβH and therefore lower norepinephrine to dopamine ratios[9]	Lower levels of plasma DBH activity have been associated with attention deficit[9]

Note. 1) Starr, Fox, Harris, Deary, & Whalley, 2007; 2) Brookes et al., 2007; 3) Giros et al., 1992; 4) Bellgrove, Chambers, Johnson, Daibhis, Daly, Hawi, Lambert, Gill, & Robertson, 2007; note that Bellgrove et al. (2007) refer to 3R but according to Rommelse et al. (2008) 3R is now called 6R; 5) Lowe, Kirley, Mullins, Fitzgerald, Gill, & Hawi, 2004; 6) Parasuraman et al., 2002; 7) Poirier, 1996; 8) Greenwood, Sunderland, Fritz, & Parasuraman, 2000; 9) Kopečková, Paclt, & Goetz, 2006.

Methodology

Subjects and Data Set

Behavioral and genetic data were collected on 161 individuals. We tested normal subjects between the ages of 18 and 61 years (69 males). Most of the participants (n = 107) were Rice University students. A community sample was also obtained (n = 54) to increase the age range of the total sample. Prior to completing the visual orienting task, subjects signed a consent form and completed an intake questionnaire that included questions on basic demographics, attentional disorders in self and biological relatives, tobacco use, and the Epworth Sleepiness Scale (Johns, 1991). The data set also contains information on location of testing (Rice or community), gender, age, smoking habits, sleepiness, ethnicity, and genotypes for five genetic markers.

The cleaned data set contains data from 145 individuals who had complete behavioral data, whose error rates were less that 10%, who could be classified into a single ethnicity, and who had reported no history of neurological disorders. Of the original 161 subjects, two subjects had their data excluded for having data collected prior to luminance calibration. One subject had their data excluded for not being classifiable to a single ethnicity (other individuals with dual ethnicity were classifiable based on the pattern of their genes; no individuals reported more than two ethnicities). Three individuals had their data excluded for reporting prior strokes or seizures. Two individuals had their data excluded for having less than 200 trials. Eight subjects had

their data excluded for having an error rate over 10%.[3] The median error rate for those subjects whose data were excluded was 2% (range = 0% - 31%). The median age was 22 (range = 19 - 61). Fifty-three per cent were female. The median Sleepiness Scale score was 10 (range 2 – 15).

It was determined that subjects reporting ADHD need not necessarily be excluded (but outliers and solutions with and without these subjects were compared; see the Results section). Eight subjects in the cleaned data set reported this diagnosis, four of whom were on medication (one additional subject reporting ADHD was excluded for having fewer than 200 trials). However, fewer than half of these eight subjects had the high risk genotype on any given genetic marker so we reasoned that any results could not be driven by these subjects. The 145 subjects in the cleaned data set were between the ages of 18 and 61 years (64 males). Ninety-four of 107 participants in the Rice University sample had useable data (45.74% male) as did 51 of the 54 community participants (41.18% male). Overall, 90.06% of the subjects had useable data. The mean age for the university sample was 20.22 years (range 18 to 38 years), and for the community sample it was 35.45 years (range 18 to 61 years). Eleven subjects who were included in the cleaned data set reported dual ethnicity (no subjects reported more than two ethnicities). These subjects were given a code for a single ethnicity based on the pattern of their other genetic information.

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[3]This decision was based on the distribution of error rates (including catch trial errors). High error rates seem to indicate that subjects might not be motivated or understand the task.

Subjects completed a choice response task (responding to indicate either a left or right target). Participants were dark adapted before beginning the behavioral task and completed 20 practice trials before beginning data collection trials. Subjects viewed a 1024 x 768 pixel CRT monitor with a background luminance of 0.08 cd/m^2. A fixation cross, centered on the monitor, was always visible. Participants were instructed to fixate the central cross and to maintain fixation throughout data collection.

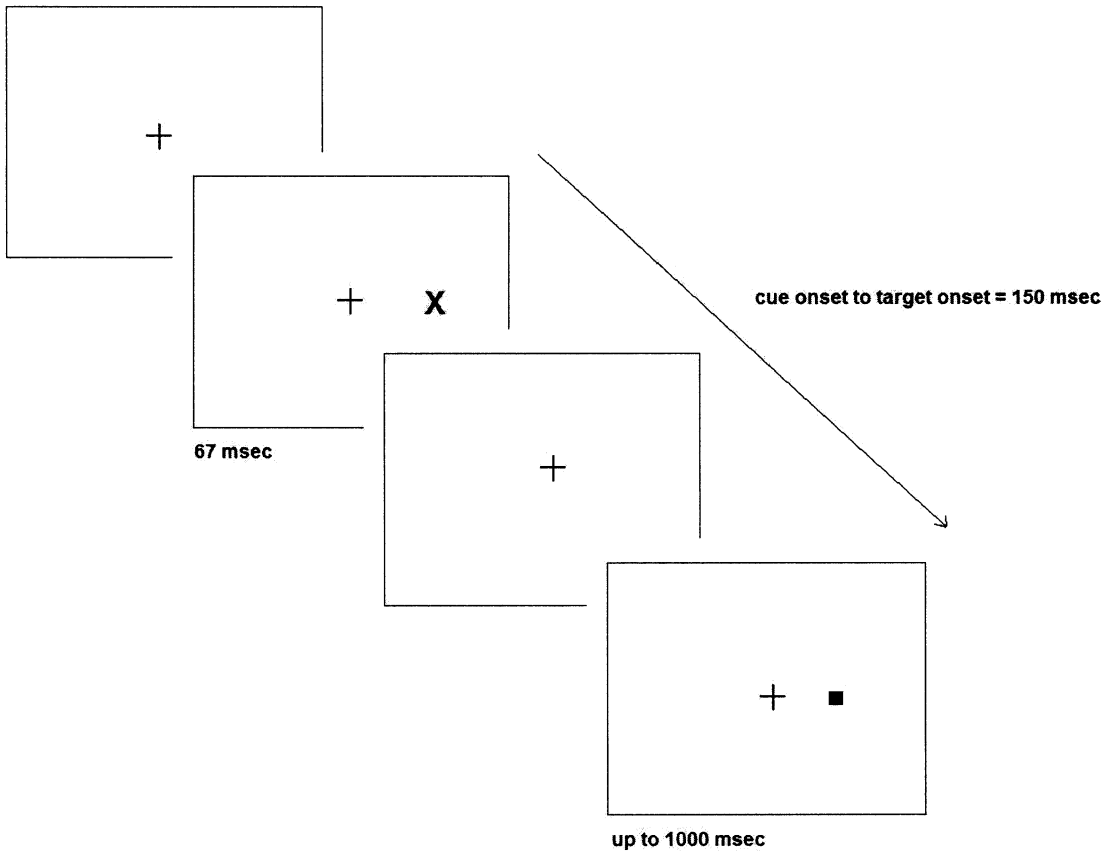

Figure 2. Representation of stimuli. The pre-cue stimulus flashed on for 67 msec. Targets were on the same side as the cue 50% of the time. The target remained on display until a response was made but for no longer than 1000 msec. Dual cues were identical except that a secondary cue ('X') appeared contralaterally.

The target appeared after a pre- cue appeared on the computer display. There was an 83 msec gap after the offset of the cues and prior to the onset of the target. The target remained on display for 1000 msec or until the participant made a key press (see Figure 2). Participants were asked to respond as quickly as possible while maintaining accuracy by making a key press to indicate a target either to the left (pressing 'a') or to the right (pressing 'l') of fixation. After the participant responded, there was a variable delay (1.3 to 1.8 sec), and the next trial began. No feedback was provided.

There were various trial types. That is, there were different pre-cue conditions on the display prior to the appearance of the target. A reflexive orienting task typically uses single pre-cues. To these we added unequal-luminance ("asymmetric"), dual-cue trials (used by Kean & Lambert, 2003) in order to examine the ability to benefit from either of two simultaneous pre-cues. Thus, for each trial one or two cues were presented for 67 msec. The cues could be valid (i.e., appear where the target would subsequently appear) or invalid (appear contralateral to where the target would subsequently appear); for dual cues, the target could appear near the brighter of the two cues or near the dimmer. The pre-cues are considered uninformative because the probability of the target appearing near where the pre-cue (or brighter cue) had appeared was only 50%. The target also appeared randomly on half the trials on the right side of the display independently of where the cues appeared. We averaged the left and right target presentations. These averaged RTs are termed 'primary measures' of which there are nine.

Single cue trials were intermixed with catch, dual neutral, and dual asymmetric cue trials. Participants were told 1) that the cues did not predict the target's location and 2) to ignore the cues as much as possible. Participants completed all trials within one

session with pauses as necessary. Response time (RT) was measured from the onset of the target.

Dual asymmetric cues comprised two cues of unequal luminance presented on either side of fixation. The brighter and dimmer cue luminances were 11.7 and 2.0 cd/m^2, respectively. The target (a square) always had a luminance of 15.5 cd/m^2. The centermost edge of the target appeared 5.5 deg to either side of the fixation cross. The cues were shaped like the letter X, measured 0.8 (width) x 1.0 (height) deg, and appeared 7.3 deg (innermost edges) to the left and right of the display's center.

The various primary measures have unique names. *Single dim valid* indicates a single cue of the dimmer luminance. The term 'valid' indicates that the target appeared near where the dim cue had been. Conversely, the configuration termed *single dim invalid* indicates a dim cue followed by a target on the side contralateral to where the cue was presented. There were corresponding valid and invalid configurations for the *single bright* cues. We also included *neutral bright* and *neutral dim* cues. On these trials, identical bright or dim cues were presented simultaneously on both sides of the fixation cross. These spatially neutral cues were used to calculate alerting effects (that is, reduced RT due to the temporal signal of the cue). When the *dual asymmetric* cues were presented, the target could appear either near the brighter cue (*dual asymmetric bright*) or near the dimmer cue (*dual asymmetric dim*). Targets could appear *uncued* without being preceded by any cues. Finally, *catch trials* were also presented, and subjects were instructed to withhold responding since no target appeared. Single cue trials were intermixed with catch, uncued, and dual cue trials. Each of the 10 conditions (nine target-

present plus one target-absent) was presented 20 times (10 times with a left and 10 with a right target), yielding 200 trials.

Participants were told 1) that the cues did not predict the target's location and 2) to ignore the cues as much as possible. Participants completed all trials within one session with pauses as necessary. RT was measured from the onset of the target. The different trial types were presented in random order within blocks of 20 trials. Because the order of the trial types is random by block, the trials of a given type are not equally spaced in time but can be examined as an ordinal variable by looking at block number.

Genetic Methods

Participants produced a saliva sample of approximately 2 ml in an Oragene-250 kit (DNA Oragene, Kanata, Ontario, Canada). DNA sequencing assays were performed to genotype known SNPs (see See Appendix B). Polymerase chain reaction (PCR) amplifications were carried out using HotStarTaq™ DNA polymerase (Qiagen Inc., Valencia, CA). PCR products were treated using Exo_SAP (Affymetrix, OH) to digest primers and followed with sequencing PCR using the BigDye™ sequencing reaction mix (Applied Biosystems, CA). The sequencing PCR products were purified using the BigDye XTerminator kit (Applied Biosystems, CA) and then loaded on an ABI3730xl sequencing instrument using the Rapid36 run module. The DNA sequencing results were analyzed using the Mutation Surveyor software (SoftGenetics, PA).

In the case of the DAT1 (SL6A3) exon 8 polymorphism, genotyping was performed using methods for microsatellite repeat polymorphisms. The fluorescently labeled PCR products were generated with a fluorescently labeled primer (see See Appendix B). The amplified products were analyzed on an ABI3130xl Genetic Analyzer.

The Genemapper 4.0 software was used to assign the allele distribution (Applied Biosystems).

To assess the reliability of the genotyping, we had seven of the participants submit second saliva samples. These samples were treated identically to all of the other samples, and the lab doing the genotyping did not know that they were duplicates of existing saliva samples. The agreement between the two genotyping runs was 97.5% (78 of 80 alleles agreed).[4]

Statistical Procedures

Multiple regression was used to create the nine outcome variables as well as to examine evidence for the main hypotheses. It can sometimes be difficult to determine which variables should be included as predictors in the model. Modeling more variables increases the ability to explain more residual variance. However, there is a caution. Models with unnecessary variables are termed 'over-specified' and can perform as unreliably as those with not enough explanatory variables (Wood, 2001). Since I am using existing data I can only include those variables for which we have data. Not all of these variables may be relevant. Conceptually speaking, it is best not to include variables for which I do not have a hypothesis about their need to be in the model. Therefore, I included only the variables for which I have conceptually important reasons for including in the model (see See Table 2 for a list of the model elements). Empirically, variables that are highly correlated with other variables may be redundant in the model. For example,

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[4]Two subjects contributed 10 alleles, and five subjects contributed 12 alleles to the reliability analysis. Each of the two subjects who contributed 10 alleles could not be genotyped on one genetic marker.

there was a correlation of .70 between age and site of testing. I had no hypothesis about location as a predictor (education did not appear substantially different), so I left location of testing out of the model.

Table 2. Elements of statistical models.

Variable	Purpose in Model
RT Variability for each of 9 measures	Outcome
Mean RT for the associated measure	Predictor (Covariate)
Sleepiness	Predictor (Covariate)
Age	Predictor (Covariate)
Ethnicity	Predictor (Covariate)
Genotype for each of 5 genes	Predictor

Results

Previous Analyses

Prior to considering analysis for RT variability we examined the influence of genotype on the mean for each derived measure. Derived measures are difference scores between various primary measures. Three of these derived measures are standard in a Posner-type cueing paradigm: alerting, costs, and benefits. The use of two different cue luminances yielded six of these three standard, derived measures. We derived four additional measures by using trials in which the dual, asymmetric luminance cues appeared. Table 3 shows the differences between primary measures which produced all 10 derived measures. For this previous analysis of means, we ran a two-step ANOVA to examine the mean of each derived measure for each gene separately. The first step used only ethnicity as an independent variable; the second step added genotype. The initial

ANOVA was used to correct statistically for possible population stratification (spurious genetic associations, discussed below). The second step added genotype as another independent variable, and the incremental R^2 was obtained. This strategy is similar to that recommended by Hutchison, Stallings, McGeary and Bryan (2004) to address potential stratification artifacts by using self-reported ethnicity as a proxy for genetic subpopulation. Stratification in this case is an artifact which occurs when there are systematic differences in a phenotype that have nothing to do with a marker under study, yet the association appears statistically significant. Spurious relationships are possible because genetic ancestry is related to ethnicity. Studies are more at risk for these spurious relationships when subjects from various ethnic groups are 1) combined in the same analysis, 2) differ on a phenotype, and 3) simultaneously differ for unrelated reasons on the frequencies of target genotypes. In a particular study it is often impossible to determine if group differences are due to ethnic group differences but population stratification may be suspected if a study fails to replicate. Genetics papers are often criticized for failing to address possible confounding between genes and ethnicity (Thomas & Witte, 2002).

Table 3. The calculation of derived measures.

Derived Measure	Primary Measures Used in Calculation
Alert Bright	No Cue - Neutral Bright
Alert Dim	No Cue - Neutral Dim
Benefit Bright	Neutral Bright - Single Bright Valid
Benefit Dim	Neutral Dim - Single Dim Valid
Cost Bright	Neutral Bright - Single Bright Invalid
Cost Dim	Neutral Dim - Single Dim Invalid
Congruence Benefit	Dual By Dim - Dual by Bright
Dual Asymmetric Cost Bright	Single Bright Valid - Dual by Bright
Dual Asymmetric Cost Dim	Single Dim Valid - Dual by Dim
Dim Better Than Nothing	Single Bright Invalid - Dual by Dim

Note. The RT differences between the primary measures in the second column are used to calculate the derived measure in the first column.

From the two-step ANOVA analysis for mean differences we were able to conclude that DAT1, COMT, and APOE each showed significant associations with the mean RT of a particular derived measure (*cost dim*; *cost dim* and *cost bright*; and *congruence benefit*, respectively). DRD4 approached significance on *cost dim* and *benefit dim* and DBH did not show association. The *cost dim* measure (derived from invalid trials and neutral trials) was the outcome most frequently associated with a particular gene.

Recently, I repeated the above steps for RT variability (using, in turn, each primary measure's standard deviation as the outcome). Primary measures (raw RTs for various display conditions) are described under Methodology. We did not use derived

measures to obtain RT variance because it is unclear which occurrence of one display condition to subtract from which occurrence of another display condition to use in the calculation of the difference scores.

In the two-step ANOVA analyzing for genotypic differences in variance, only COMT showed significance as a predictor of the standard deviation on one trial type (*dual by bright*; $F[1, 131] = 7.59, p = .007$). However, RT variance could be inflated if subjects get progressively faster or slower across the 20 presentations of a given trial type (see Figure 3). It also does not take into account the side on which the target appeared, which has sometimes been shown to make a difference (Heilman & Van Den Abell, 1980). Therefore, I reanalyzed the data using the standard deviation of the residuals after regressing RT on block (to account for trends over time) and side (to account for any response differences to left v. right targets). This process is described below.

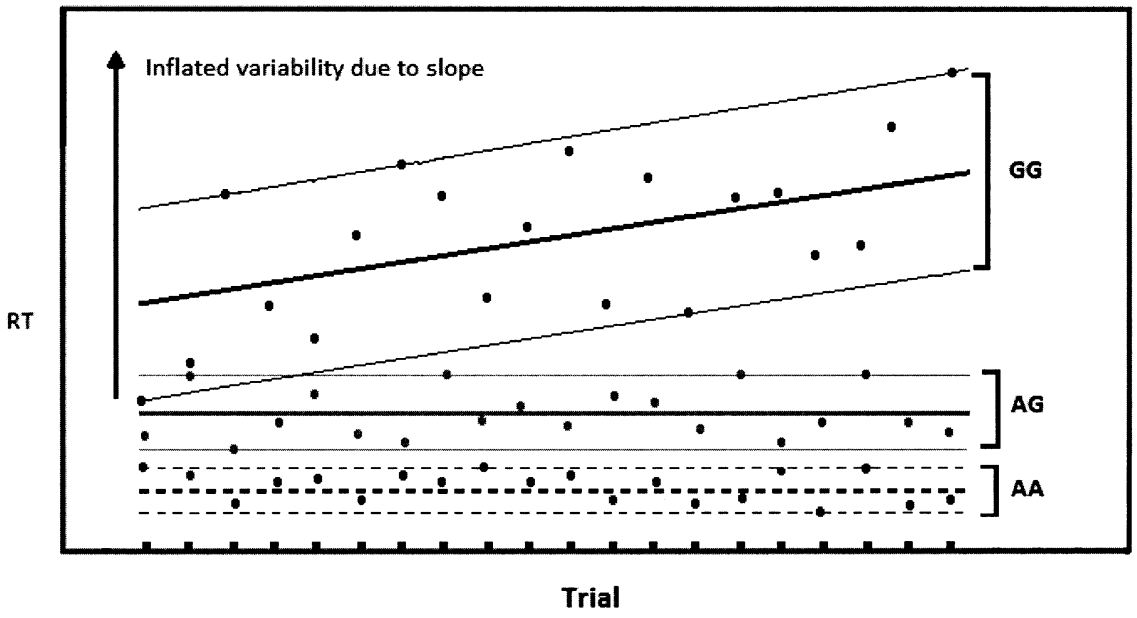

Figure 3. Standard deviations would be inflated if an individual responded faster (or slower) over the 20 presentations of a given trial type.

Data Preparation

A major question in this study is whether genotype is significantly associated with the outcome measure (RT variance). We examined markers on five genes, COMT, DAT1, DBH, DRD4, and APOE. The first four each have three genotypes based on two alleles (one inherited from each parent, such as AA, AG and GG for COMT). APOE is based on two SNPs from which we have created three groups based on the literature. For example, Hubacek (2008) did not consider individuals with the ε2/ ε4 genotype since one allele carries risk for cognitive deficits (even in middle aged adults without Alzheimer's; Parasuraman et al., 2002) and the other provides protection against cognitive deficits (Corder et al., 1994). In our data set two individuals had the ε2/ ε4 genotype and were therefore excluded from analysis on this gene. Other genotypes, such as ε2/ε2, are rare and do not occur in our data set. This leaves three genotype groups: ε2/ ε3, ε3/ ε3, and ε3/ ε4 or ε4/ ε4. The first group (ε2/ ε3) represents those with a protective allele; the second group represents those with typical risks, and the third group represents those with at least one risk allele (that is, ε4).

All genes except APOE were coded ordinally so that 0 represent no risk alleles, 1 represents one risk allele, and 2 represents two risk alleles. APOE was coded so that 0 indicates possession of a protective allele, 1 represents the most common "normal" variant, and 2 represents possession of either one or two risk alleles. The coding for APOE has a slightly different interpretation because it consists of two different markers (haplotype) in contrast to the other genes that each had only one marker. However the risk for cognitive deficits appears to be ordinal and can still be tested for linear effects

using similar coding. These genes will be entered as potential predictors of RT variability for each trial type in turn.

Other variables were also tested for their usefulness as predictors (covariates) in the models. These were: the mean RT associated with the outcome measure being tested, age, sleepiness, and ethnicity. I used mean RT as a covariate of RT variability since it is well known that RT variability increases with an increase in mean RT but I was not interested in this mathematical relationship. Age was entered as the number of years reported by a subject. The sleepiness variable represents the Epworth Sleepiness Scale score (Johns, 1991) which is a simple rating of sleepiness during certain hypothetical, common events (see Appendix A). Sleepiness is related to RT in the literature (e.g., Ogilvie, Wilkinson, & Allison, 1989) and so may be a useful predictor. Data were imputed for the twelve Rice University students who were tested before use of the sleepiness scale began. These students were given the mean sleepiness score for other Rice students in the sample. Ethnicity was coded into three dummy codes so that zero represents Caucasian.

Prior to the main analyses, I determined that assumptions of normality were not met (the RT variables had significant positive skew). Raw RT values were transformed using a base ten logarithm. No subjects were outliers to the regression or contributed undue influence to the solution according to Cook's distances or standardized beta scores. Seven subjects were identified as outliers according to Mahalanobis' distances (using a criterion based on $p = .001$ and $df = 7$ on a χ^2 distribution). The analyses were run with none of these subjects excluded and when only excluding those subjects ($n = 2$) who were the most extreme outliers at the next logical break in the outliers. The statistical decisions

were identical, so no subjects had their data removed due to their Mahalanobis distances. However, recall that 16 subjects had their data removed for reasons described under Subjects and Data Set. In addition to these exclusions, some subjects had missing genetic information due to the inability to obtain genotypic information from their saliva sample on a particular marker. For APOE, 12 subjects had missing genetic information. For COMT, two subjects had missing genetic information. For DAT1, seven subjects had missing genetic information. For DBH, three subjects had missing genetic information. Finally, for DRD4 the number of subjects with missing genetic information was 13.

Statistical Results

Sequential multiple regression was used to examine five genetic markers as potential predictors of RT variability for each of nine different outcome measures. RT variability was defined as the standard deviation of the residuals obtained from regressing a primary measure's RTs on block and side. Some intake variables were also evaluated for significance as predictors. Sequential multiple regression allows me to answer the question: After accounting for some reasons that RT variability may vary for a given display condition (primary measure), does genotype explain additional variance in RT variability?

I created a separate model predicting RT variability for each primary measure. This is because I thought that there could be different factors (especially different genes) associated with RT variability for the different trial types. Some trial types, for example, involve switching attention from one side of the display (where a pre-cue appeared) to the other (where a target appears). These trial types, termed *invalid* in the case of single cues or *incongruent* in the case of dual asymmetric cues might be related to a difficulty in

disengaging attention. *Valid* and *congruent* trials are not expected to have the same patterns of association as *invalid* and *incongruent* trials. These trial types might, therefore, be associated with dopamine availability in different ways.

In a sequential multiple regression, the order of a predictor's entry into a model is determined by the researcher. I first entered the mean RT for a given primary measure as a predictor of RT variability (as previously defined) for that measure. In the next step, intake variables were entered which were thought to influence the residual RT outcome. These were age, ethnicity, and the sleepiness scale score. Third, a gene was entered to determine if it explained any additional variability beyond these predictors. Thus, variance that was not unique to a predictor was first attributed to mean RT, then to intake variables, and finally to the gene. There were 45 models tested (nine for each of five genes; see Table 4). The analyses were run both with and without the eight subjects who reported diagnoses of ADHD and outliers and solutions were examined. Since ADHD subjects were not outliers (as determined by standardized residuals, Mahalanobis' distances, and Cook's distances) their data were ultimately included in the analyses reported below. The solutions with and without the ADHD subjects were substantively similar.

Correlations between selected variables are provided in Table 5. The need for using the standard deviation of the residuals is evidenced by fewer than the expected number of subjects with the CC genotype for DRD4 having significant slope (getting faster) over time in the *dual by bright* condition; χ^2 (2, N= 161) = 5.46, p = .07). Other slope differences were also investigated as a supplemental analysis (see Discussion section).

Table 4. A summary of key models evaluated for hypotheses one and two.

$RT_{\text{residual SD}}$ = constant + associated mean RT + error
$RT_{\text{residual SD}}$ = constant + b_1 * associated mean RT + b_2 * ethnicity . . . + b_5 * age + b_6 * sleepiness + error
$RT_{\text{residual SD}}$ = constant + b_1 * associated mean RT + b_2 * ethnicity . . . + b_5 * age + b_6 * sleepiness + GENE + error

Note. Separate models were created for each trial type and each gene. Ellipses indicate dummy coding.

Entering genotype third in the multiple regression model determines if a particular genetic marker explains any additional variance beyond that captured by predictors previously entered. In these analyses, genotype did not significantly explain any additional variance. Two genetic markers each had near significant results on one of the nine outcomes for which they were tested. Each of these is described below.

Table 5. Selected correlations between model elements.

Variable	1	2	3	4	5	6	7	8	9	10	11
1. Age	--										
2. Sleepiness	-.001	--									
3. Mean DBB	.10	.09	--								
4. Mean NBB	.13	.10	.95***	--							
5. COMT	-.06	.14	.10	.08	--						
6. DRD4	.13	-.02	-.10	-.06	.10	--					
7. DBB Variability	.01	.25**	.26**	.23**	.19*	.03	--				
8. NBB Variability	-.24**	.14	.32***	.38***	.04	-.17*	.43***	--			
9. Asian	-.22**	-.06	.08	.03	.16*	-.11	-.03	-.03	--		
10. Black	-.08	.01	.11	.07	.15*	-.07	.00	.11	-.10	--	
11. Hispanic	-.13	.10	.14*	.11	.07	-.14	.13	.06	-.17*	-.08	--

Note. Mean DBB (*dual by bright*), mean NBB (*neutral both bright*), DBB Variability, and NBB Variability are logarithm transformations. * $p < .10$. ** $p < .01$. *** $p < .001$.

COMT

Earlier I mentioned that a preliminary analysis of genetic association with each of nine outcome measures showed significance between COMT and *dual by bright*, F[1, 131] = 7.59, p = .007. In the new analysis, however, COMT just failed to reach conventional significance levels in its association with RT variability in the *dual by bright* condition (ΔR^2 = 2%, F(1, 135) = 3.46, p = .06) after entering the various covariates described above. The *dual by bright* condition is illustrated in Figure 4. The *dual by bright* condition is designed to measure the extra time it takes to respond to a bright cue if there was also a dim cue preceding the appearance of the target. The *dual by dim* condition showed somewhat less significant difference by genotype (p = .11). In the *dual by dim* condition it is the bright cue that is the distractor. The error rate for the *dual by dim* condition (M = .006, SEM = .002) is significantly higher (t[233.53] = -3.93, p < .001) than for the *dual by bright* condition (M = .02, SEM = .003). But it seems unlikely that error rate could be responsible for the different significance levels for these two conditions. Since only correct responses were analyzed. Furthermore, the significance levels are relatively close to each other and could represent similar processes.

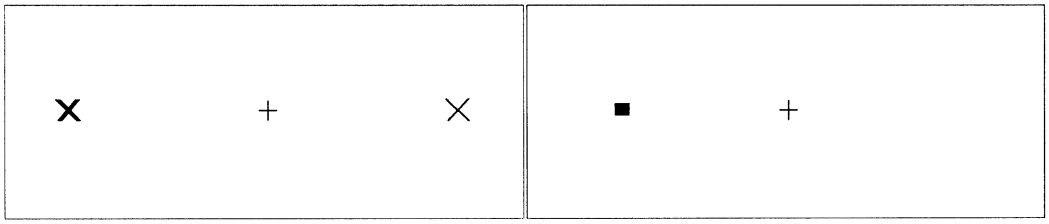

Figure 4. In the *dual by bright* condition, a two cues flash and a target subsequently appears near where the brighter cue had been.

As noted in Table 6, the R for the final regression model was significantly different from zero, R = .40, F(7, 135) = 3.43, p = .002. The results indicate that 15% of

the variability in *dual by bright* is predicted by this model. Seven percent of the variability in *dual by bright* is predicted by the mean of *dual by bright.* An additional 6% is predicted by the sleepiness scale score and ethnicity together. Age was not a significant predictor. Additional variability (2%) in *dual by bright* is predicted by the COMT genotype (see Table 6). The standardized coefficients suggest that the mean of the *dual by bright* condition is the most influential of the predictors ($\beta = .23$), followed by sleepiness scale score ($\beta = .21$), and COMT genotype ($\beta = .16$). Ethnicity is not a significant predictor but was included in the model to address concerns about population stratification (see Previous Analyses in the Results section) and to account for analyses performed *post hoc*; these analyses indicate that the coefficient estimates for gene effects are different for Caucasians than for non-Caucasian ethnic groups. The beta weight of COMT as a predictor in the *dual by bright* model was smaller ($\beta = .07$) for Caucasians than for other ethnic groups ($\beta = .29$). I therefore concluded that ethnicity should be included in the model.

The regression equation (with unstandardized weights and including predictors with $p < .10$) is $DBB_{var} = .08 * DBB_{mean} + .001 * SSS + .004 * COMT$. The abbreviation DBB stands for *dual by bright* and SSS stands for sleepiness scale score. Had COMT reached conventional significance ($p < .05$) then the direction of its effect is such that subjects with two copies of the risk allele (G) would be predicted to have greater variability on *dual by bright* by 0.008 log msec over subjects without any risk alleles at this marker when mean *dual by bright* and sleepiness scale score are held constant. See the Discussion section for further interpretation.

Table 6. Sequential multiple regression predicting variability in *dual by bright*.

	Model 1			Model 2			Model 3		
Variable	*B*	*SE B*	β	*B*	*SE B*	β	*B*	*SE B*	β
Constant	-0.17	0.07		-0.16	0.07		-0.16	0.07	
Mean	0.08	0.03	0.27**	0.07	0.03	0.24**	0.07	0.03	0.23**
Sleepiness				0.001	0.0004	0.23**	0.001	0.0004	0.21*
Asian versus Caucasian				NS	NS	NS	NS	NS	NS
Black versus Caucasian				NS	NS	NS	NS	NS	NS
Hispanic versus Caucasian				NS	NS	NS	NS	NS	NS
COMT							0.004	0.002	0.15*
R^2		.07			.13			.15	
F for ΔR^2		10.16			2.43			3.49	
n		142			142			142	

Note: Ethnicity was represented as three dummy variables with Caucasian serving as the reference group. For model two, ΔR^2 = .06*. For model three, ΔR^2 = .02*. * $p < .10$. ** $p < .01$. *** $p < .001$.

DRD4

The only other marker that I will discuss is DRD4, which also failed to achieve conventional significance levels. Nevertheless, I found weak association between DRD4 and RT variability in the *neutral both bright* condition after the covariates described above had been entered as predictors of the variance on this outcome measure, $\Delta R^2 = 2\%$, $F(1, 124) = 3.16, p = .08$. The *neutral both bright* condition is illustrated in Figure 5. The RTs to the *neutral both bright* condition are used in the calculation of the alerting effect. Please see the Discussion section for further details.

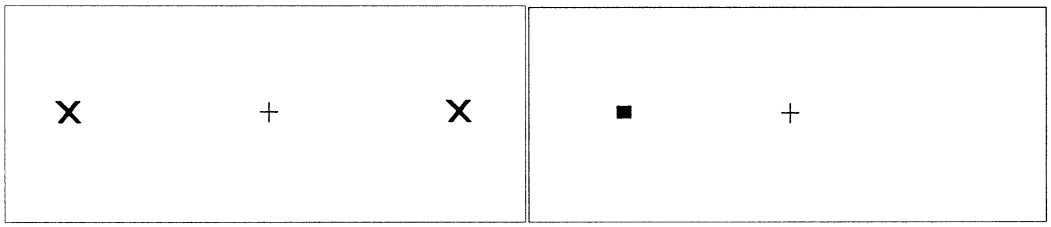

Figure 5. In the *neutral both bright* condition, two bright cues flash simultaneously. The target may subsequently appear near either of the two cues.

The R for the full regression model was significantly different from zero, $R = .54$, $F(7, 124) = 7.14, p < .001$ (see Table 7). This indicates that 29% of the variance in RT variability of *neutral both bright* is predicted by the full model. Sixteen percent is explained by the mean of *neutral both bright*, an additional 11% by age and ethnicity together, and 2% by DRD4 genotype. The standardized coefficients of the full model likewise indicate that the strongest predictors of variability in the *neutral both bright* condition are the associated mean ($\beta = 0.47$) and age ($\beta = -0.31$); however, ethnicity is also a significant predictor. Since ethnicity was dummy coded we can say that there is decreased variability in the RT of *neutral both bright* when we compare Asians to

Caucasians (β = -0.18, p =.03) and when we compare Hispanics to Caucasians, although this latter difference failed to reach conventional significance levels (β = -0.14, p = .09).

The regression equation (with unstandardized weights and including predictors with $p < .10$) is NBB_{var} = .07 * NBB_{mean} - .0005 * age - 0.007*Asian - .007 * Hispanic. Had DRD4 reached conventional significance then the direction of its effect is such that subjects with two copies of the risk allele (CC genotype) would be predicted to have reduced variability on *neutral both bright* by 0.003 log msec over subjects with the CG genotype, if mean *neutral both bright* is held constant and ethnicity is Caucasian. See the Discussion section for further interpretation.

Table 7. Sequential multiple regression predicting variability in *neutral both bright*.

	Model 1			Model 2			Model 3		
Variable	*B*	*SE B*	β	*B*	*SE B*	β	*B*	*SE B*	β
Constant	-0.24	0.06		-0.28	0.57		-0.27	0.54	
Mean	0.11	0.02	0.40***	0.13	0.22	0.72***	0.13	0.20	0.72***
Age				-0.001	0.0001	-0.32***	-0.0005	0.0001	-0.31***
Asian versus Caucasian				-0.007	0.003	-0.16*	-0.008	0.003	-0.18*
Black versus Caucasian				NS	NS	NS	NS	NS	NS
Hispanic versus Caucasian				NS	NS	NS	-0.007	0.004	-0.14*
DRD4							-0.003	0.002	0.14*
R^2		.16			.27			.29	
F for ΔR^2		25.28			4.12			3.16	
n		131			131			131	

Note: Ethnicity was represented as three dummy variables with Caucasian serving as the reference group. For model two, ΔR^2 = .10**. For model three, ΔR^2 = .02*. p * < .10. ** p < .01. p *** < .001.

Other Predictors

The previous genetic associations answer the primary question of this research and suggest that no significant contribution to RT variability on orienting measures. However, my second hypothesis was that other variables, such as age and sleepiness, would associate with greater RT variability. These are described below.

Mean RTs. In most of the 45 regression analyses of outcome variability, the mean associated with the outcome measure was the strongest predictor. This is not surprising given the positive nature of RT scores. All mean RTs were highly correlated with all other mean RTs (*P*s < .001). APOE was correlated with most mean RTs (.01 < Ps < .06) and age was correlated with mean *dual by dim,* mean *single bright valid*, mean *single dim valid.* The one situation where mean RT did not significantly predict the associated RT variability was for *single bright valid.*

Age and Sleepiness. Age was a significant predictor for *dual by dim* (β = -.26, p = .002), *neutral both bright* (β = -.32, p < .001), *neutral both dim* (β = - .21, p = .01), *single bright valid* (β = -.26, p = .004), *single bright invalid* (β = -.27, p = .001), *single dim valid* (β = -.17, p = .047), and *single dim invalid* (β = -.23, p = .005) variability outcomes. However, in *dual by bright* models sleepiness showed significance (β = .22, p = .01) rather than age (β = -.02, p = .86). Neither sleepiness nor age was significant in the *no cue* condition. These values were obtained after entering the associated mean RT in the first step and then ethnicity, age, and sleepiness scale scores in the second step of a regression. No genes were entered; therefore, individuals who did not have genetic information for a particular gene were included. Asian versus Caucasian ethnicity is correlated with age (r = - .22, p = .01) but no other ethnicity was correlated with age.

This is probably because the subjects recruited from a Houston suburb were much less likely to be Asian (1.96% versus 26.60% in the Rice University sample) and were more likely to be of older age (median = 35.00, with range 18 – 61 years versus median 20.00 with range 18 – 38 years in the Rice sample).

Ethnicity. This variable was not a significant predictor for most models (Black versus Caucasian was near significance on *single bright valid,* $\beta = .15$, $p = .08$; Hispanic versus Caucasian was significant on *single dim valid*, $\beta = .18$, $p = .03$). However, ethnicity was at or near significance in eight of 45 full gene models even when the gene was not a significant predictor of RT variability. Ethnicity was associated with the following gene models: APOE *neutral both bright* (Asian versus Caucasian, $\beta = -.13$, $p = .09$); APOE *single dim valid* (Hispanic versus Caucasian, $\beta = .17$, $p = .053$); COMT *single dim valid* (Hispanic versus Caucasian, $\beta = .18$, $p = .04$); DAT1 *single bright valid* (Black versus Caucasian, $\beta = .16$, $p = .06$); DAT1 *single dim valid* (Hispanic versus Caucasian, $\beta = .21$, $p = .01$); DBH *single bright valid* (Black versus Caucasian, $\beta = .15$, $p = .07$); DBH *single dim valid* (Hispanic versus Caucasian, $\beta = .18$, $p = .04$); and DRD4 *neutral both bright* (both Asian versus Caucasian, $\beta = -.17$, $p = .04$; Hispanic versus Caucasian, $\beta = -.15$, $p = .06$).

The above results are interesting because Black versus Caucasian ethnicity was correlated with DAT1 ($r = -.24$, $p = .005$). Although this gene did not significantly predict any outcome measure, the correlation further illustrates the need to address population stratification by including ethnicity in genetic models because significant differences do sometimes exist. For a comparison of the frequencies of alleles in our sample with that recorded in public databases, see Table 8. COMT allele frequencies in

our sample were not significantly different from those in a study from the NCBI data set. DBH allele frequencies were different, however, χ^2 (3, N= 302) = 15.28, $p \leq .05$. The NCBI database is a collection of studies, some of which have relatively small sample sizes so that the estimated allele frequencies are likely to change as the database grows. Some of the studies on the web site include HapMap populations. The HapMap samples are from specific populations. For example, a CEU HapMap sample relies on Caucasian subjects in Utah with Northern and Western European ancestry. A YRI HapMap sample relies on subjects from Yoruba in Ibadan, Nigerian (see http://hapmap.ncbi.nlm.nih.gov).

Table 8. Comparison of genotype or allele frequencies in this study and other studies.

		Allele Frequencies	
Marker	**Ethnicity**	**This Study**	**NCBI Database[1]**
APOE	Asian	7% e2, 80% e3, 13% e4	9% e2, 84% e3, 7% e4[2]
	Black	14% e2, 57% e3, 29% e4	10% e2, 69% e3, 20% e4[3]
	Caucasian	6% e2, 78% e3, 16% e4	7% e2, 80% e3,13% e4[3]
	Hispanic	0% e2, 93% e3, 7% e4	9% e2, 77% e3,14% e4[3]
COMT	Asian	70% G, 30% A	78% G, 22% A
	Black	75% G, 25% A	73% G, 27% A
	Caucasian	54% G, 46% A	52% A, 48% G
	Hispanic	61% G, 39% A	64% A, 36% G[4]
DAT1	Asian	16% 5R, 84% 6R	NA
	Black	57% 5R, 43% 6R	NA
	Caucasian	27% 5R, 73% 6R	23% 5R, 77% 6R[5]
	Hispanic	26% 5R, 74% 6R	55% 5R, 65% 6R[6]
DBH	Asian	30% G, 70% A	18% G, 82% A
	Black	56% G, 44% A	66% G, 34% A
	Caucasian	54% A, 46% G	54% A, 46% G
	Hispanic	32% A, 68% G	37% A, 63% G
DRD4	Asian	52% G, 48% C	NA
	Black	64% G, 36% C	42% C, 58% G
	Caucasian	38% G, 62% C	44% C, 56% G
	Hispanic	58% G, 43% C	NA

Note. [1]Based on HapMap samples unless noted; [2]See Hallman et al., 1991; [3]See Tang et al., 1998; [4]See Vargas-Alarcon et al., 2007; [5]See Brookes et al., 2006; [6]See Castillo, et al., 2010.

Discussion

COMT

While not obtaining conventional significance levels, the size and direction of the effect suggest that the AA genotype is weakly associated with the reduced variability on the *dual by bright* condition(see Figure 6) but that those with the GG genotype may also have increased risk for RT variability from sleepiness (see Figure 7). That is, those with the GG genotype are differentiated based on sleepiness scores. This means that while AA subject are similar to each other on RTs and to AG subjects (even when they vary on sleepiness scale scores), subjects with GG who have higher sleepiness scale scores are more variable in RTs than subjects with lower sleepiness scale scores.

Note that *dual by bright* cue variability was regressed on its own mean so that the effect could be illustrated in a single graph. Due to this, and the fact that RT variability was itself obtained by regression on block and side, the details of the scale can be difficult to understand. The main points to note in Figure 6 are that those with any copy of the risk (G) allele (n = 126) show more variability than those with no copies of the risk allele (n = 17). While type I error cannot be ignored, our findings are consistent with the risk of cognitive deficits in the literature (Starr et al., 2007). Figure 7 tells essentially the same story with the addition of sleepiness information. The effect is in the predicted direction with those with the risk allele showing more variability, possibly due to less dopamine availability as associated with this genotype.

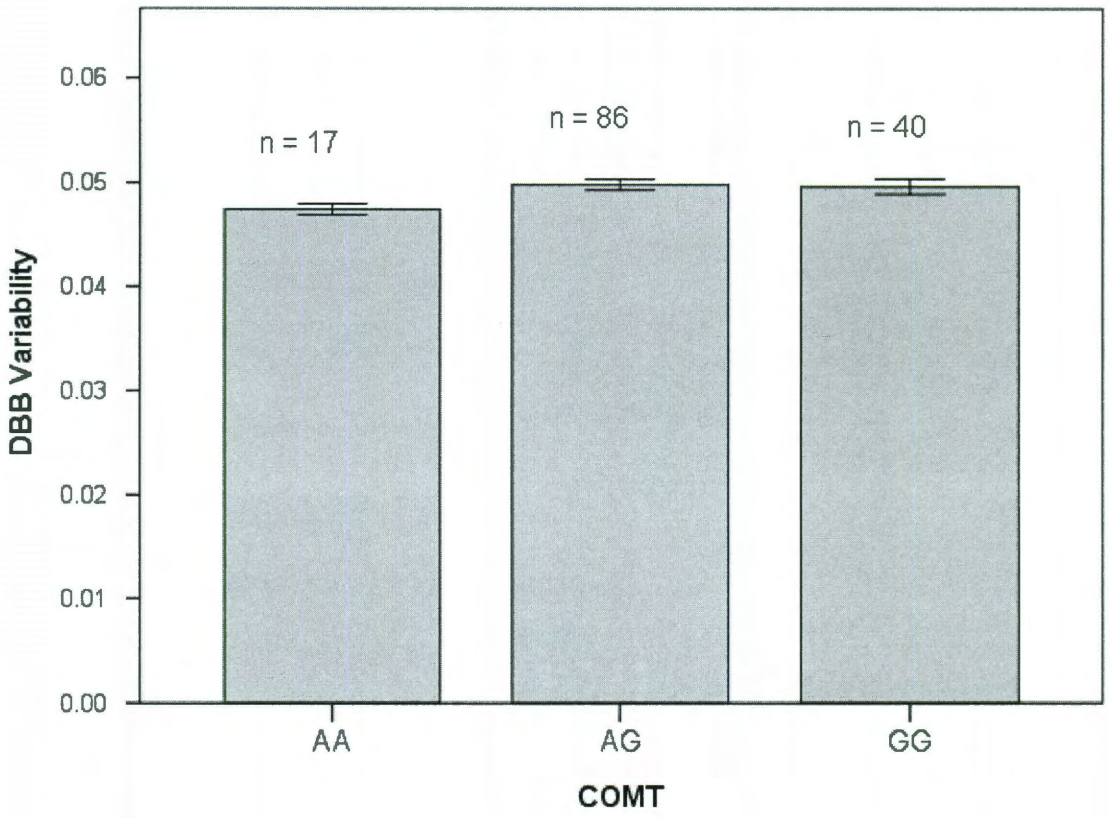

Figure 6. The effect of COMT genotype on variability in the *dual by bright* (DBB) condition. The bars represent the mean standard deviation of log RT adjusted for 1) any linear trend across repeated blocks and 2) the mean log RT of DBB.

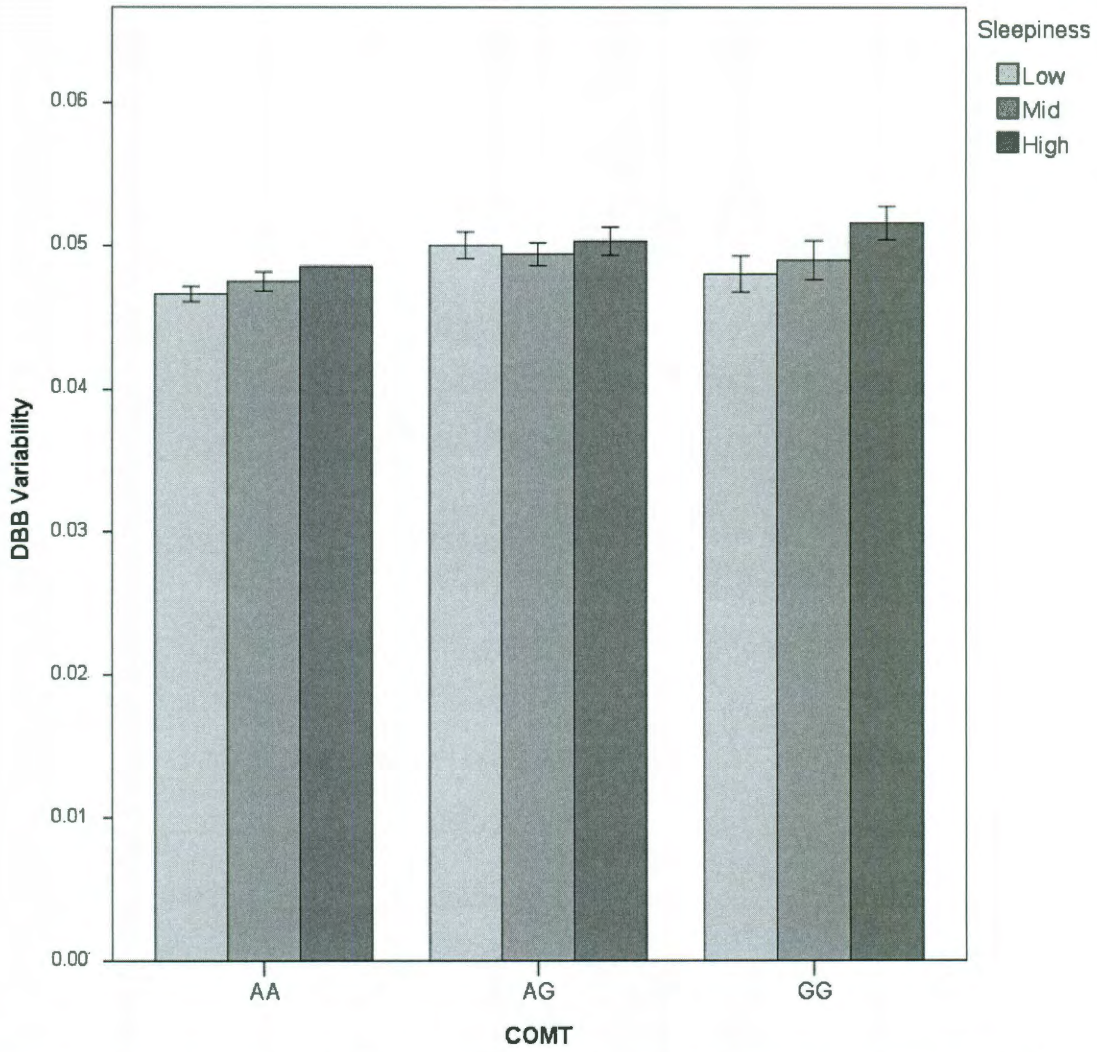

Figure 7. The effect of COMT genotype on variability in the *dual by bright* (DBB) condition with results separated by low, mid and high sleepiness scores. The bars represent the mean standard deviation of log RT adjusted for 1) any linear trend across repeated blocks and 2) the mean log RT of DBB.

These findings are difficult to compare to other studies. I could find no published studies examining genetic association between COMT and RT variability on a reflexive attention task. None of the studies reviewed by Kebir, Tabbane, Sengupta, and Joober (2009) examined COMT and variability. Of course, in our earlier study COMT was

associated with mean differences on the *cost dim* measure after controlling for ethnicity ($\Delta R^2 = 4\%$, $F\,[1, 131] = 5.57$, $p = .02$). This result is unlikely to be due to outliers since there was no significant skew and the most extreme outliers had previously been removed. Fossella et al., (2002) also found association (at $p < .10$) between COMT and performance on an endogenous orienting task (the Attention Network Test; ANT) in a sample of 200 adults. However, the ANT is even more different from both this current study and our previous one because the ANT is based on the Eriksen flanker task which uses a center arrow cue and requires use of more executive attentional function. Our task is more reflexive and so we might expect different results.

DRD4

The outcome measure that showed near significant association with DRD4 was *neutral both bright*. This display condition is used in the calculation of the alerting effect. That is, it helps answer the question of whether subjects are faster when there flash two pre-cues that do not bias attention to one side or the other as compared to when a target appears without being preceded by pre-cues. A target appears in both conditions and normally subjects are faster with the pre-cue than without them. However, there is no significant mean difference between genotype groups of DRD4 on the bright cue alerting effect, $F(2, 129) = .48$, $p = .62$. This suggests that the *no cue* condition has the same pattern of effects as the *neutral both bright* condition. However, there is no reason that variability could not differ by genotype. As described in the Results section, ethnicity was significant as a predictor of *neutral both bright* variability. However, this is likely to be spurious since ethnicity (in models without any gene as a predictor) was not a significant predictor on any outcome measure (on the *neutral both bright* variability

outcome, $\beta = -0.09$, $p = .15$ for Asian versus Caucasian; $\beta = 0.01$, $p = .90$ for Black versus Caucasian; and $\beta = -0.05$, $p = .47$ for Hispanic versus Caucasian). This suggests that the current improvement in significance comes from not including those individuals whose genetic assays did not produce a genotype at this marker. Perhaps those of different ethnicities vary in their ability to be genotyped at this location.

Figure 8 illustrates the effect of the DRD4 genotype on variability in the *neutral both bright* condition. Note that *neutral both bright* cue variability was regressed on its own mean and on ethnicity so that the effect could be illustrated in a single graph. Recall that RT variability was itself obtained by regression on block and side. Note that those with two copies of the risk allele (CC) show less variability than those in either of the two other genotype groups. We might expect increased RT variability on this measure for subjects with the CC genotype because several studies show that individuals with attentional risk alleles on this gene (but at a different marker location) tend to respond more variably (Kebir, Tabbane, Sengupta, and Joober, 2009). However, the direction of the effect in our data is not consistent with these findings in that subjects with the CC genotype show *less* RT variability. It is also unlikely that outcomes seen in Figure 8 could be predicted by extra-optimal levels dopamine (Monte-Silva, Liebetanz, Grundey, Paulus & Nitsche, 2010; Takahashi et al., 2008; Williams-Gray, Hampshire, Robbins, Owen & Barker, 2007). These theories would predict the opposite trend noticed in Figure 8 (namely, increased variability in homozygote groups over the heterozygote group). Note that in Figure 9 the oldest group of subjects has less variability in the no risk (GG) group but that this pattern reverses for the two risk allele (CC) group, with the oldest group showing the most variability. One thing this might suggest is that RT variability in

general is impacted by many variables (genes, environmental factors). It is possible, in this case, that age (with the prediction of decreasing dopamine levels) impacts variability differently depending on initial levels of dopamine (as predicted by genotype). That is, if a person starts with relatively high levels of dopamine (as might occur for those with the GG genotype) then with age their RT variability may decrease but if they start with relatively low levels of dopamine then age (which is associated with further reductions in dopamine) may increase RT variability. Something similar to these trends might, in fact, be predicted by theories of optimal dopamine levels. Nevertheless, it does not conform cleanly with our original predictions and this recent hypothesis should be evaluated in future research.

Like COMT, DRD4 does not meet conventional significance levels as a predictor for RT variability. Unlike COMT, the biological explanation for the direction of the effects is not completely satisfying. The most likely explanation may be that other variables (e.g., other genes, environmental factors) may simply be more important. Some of those variables appear to be the mean for the outcome measure, age, and ethnicity.

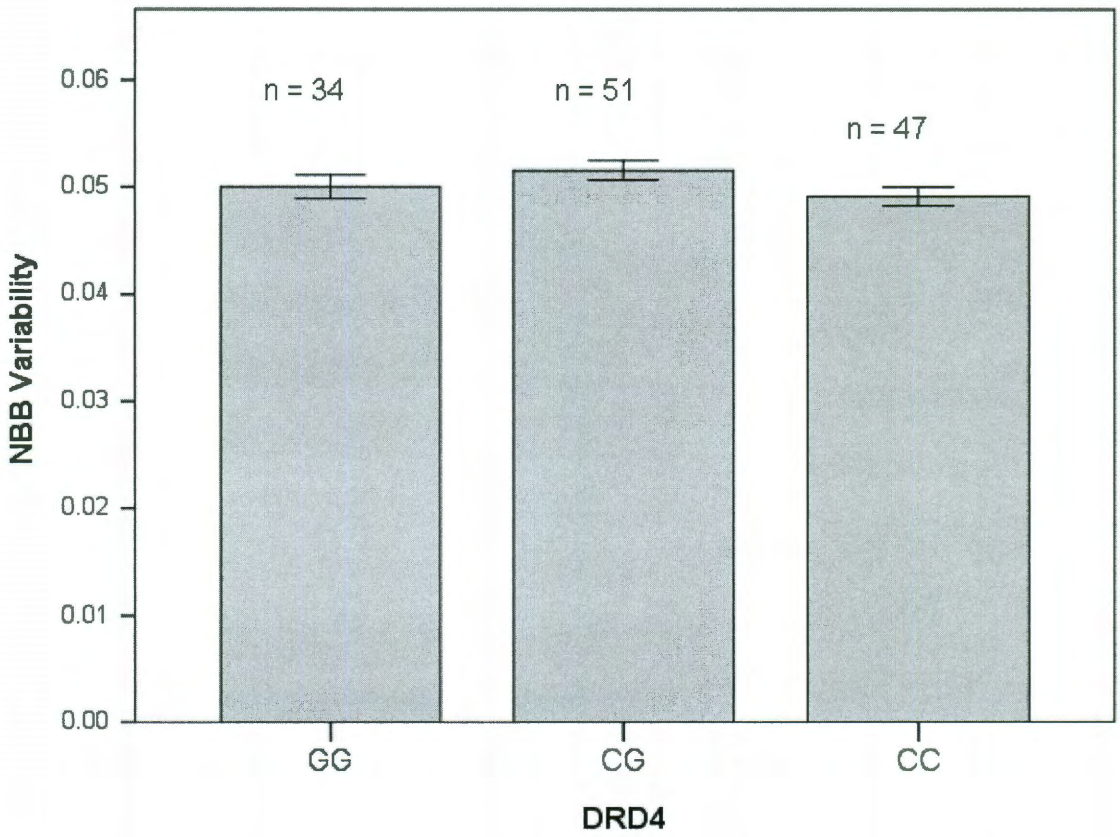

Figure 8. The effect of DRD4 genotype on variability in the *neutral both bright* (NBB) condition. The bars represent the mean standard deviation of log RT adjusted for 1) any linear trend across repeated blocks; 2) the mean log RT of DBB; and 3) ethnicity.

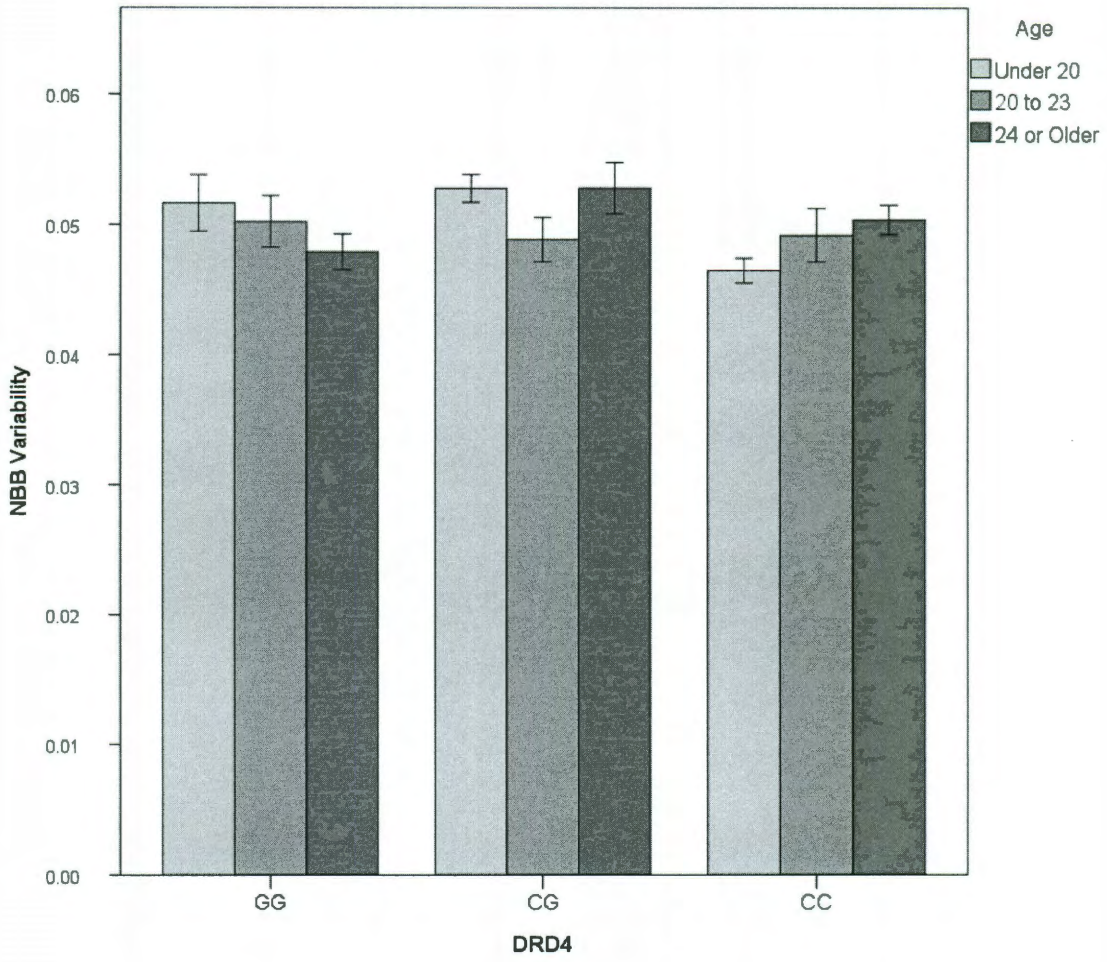

Figure 9. The effect of DRD4 genotype on variability in the *neutral both bright* (NBB) condition with results separated by age category. The bars represent the mean standard deviation of log RT adjusted for 1) any linear trend across repeated blocks; 2) the mean log RT of DBB; and 3) ethnicity.

Other Predictors

Several predictors seem important to include in future studies. Age was significant in seven of the nine outcomes (all except for *no cue* and *dual both bright* conditions). Interestingly, however, in every case where age was at or near significance (even in models where the gene was not significant), increased age was associated with

decreased RT variability even after I accounted for the associated mean RT and ethnicity. This is counter to my hypothesis and is puzzling because dopamine levels (which decline with age) predict an opposite trend and sleepiness cannot account for the trend because age and sleepiness scale score are not correlated (r = -.001, p = .99). It is therefore unlikely that middle and older adults experience less sleepiness during common activities than do college aged subjects. It appears that other influences, besides dopamine related factors, must be acting to decrease variability over the ages that we included in our sample. One possibility is greater motivation (or conscientiousness) in older subjects.

It is also interesting to note where age was not a significant predictor. That is, sleepiness scale score was more important than age (which was not significant) in the *dual by bright* condition. Here, variability increased as sleepiness increased, as hypothesized. It is puzzling, however, as to why this occurred for the *dual by bright* condition and not for other conditions. In addition to the *dual by bright* situation, neither age nor sleepiness was significant in the *no cue* condition. One might think that this would occur if the mean RT was a much stronger predictor in the *no cue* condition. This might be the case if it did not depend as much on attention as on motor speed. However, mean RT for the *no cue* condition was no more important a predictor for *no cue* variability (β = .36) than other condition means were for their respective variability measures (βs range from .23 to .47, except for *single bright valid* variability for which the mean RT was not a significant predictor).

Slope Differences

Recall that each person's raw RTs for the twenty occurrences of a given display condition were regressed on trial block (1-10)[5] to eliminate linear trends across blocks when calculating the SDs of those RTs (see Figure 3). Those slopes represent subjects getting faster or slower across blocks for a given condition. It was not my intention to examine differences in these slopes between the different genotype groups. Nevertheless, I performed an ancillary analysis and found that several genetic markers showed significant associations with these slopes. In particular, there were significant slope differences between genotypes on DAT1 for eight of the nine primary measures (ranging from $p < .001$ to $p = .04$; *neutral both bright* just failed to reach conventional significance with $p = .07$). On DBH there were significant slope differences on two measures (*single bright valid* with $p = .02$ and *single dim valid* with $p = .04$). APOE genotypes differed significantly for the slopes of one primary measure (*single bright invalid;* $p = .001$), and six additional measures had slopes with nearly significant differences across genotypes (ranging from $p = .08$ to .052). COMT was not associated with any of the primary measure slopes, and DRD4 had one measure (*dual by bright*) with nearly significant differences ($p = .09$).

The directions of the slopes are interesting. For DAT1, in all eight of the outcomes that were at or near significance, the slope increases with an increasing dose of the DAT1 risk allele (6R). That is, the slope is negative for those who carry no copies of the risk allele (i.e., the 5R/5R group) and becomes more positive (or closer to zero) for those with two copies of the risk allele (i.e., the 6R/6R group). This indicates that

[5] Each condition occurred twice within a block with the target appearing once on each side.

subjects without risk alleles are getting faster over time while subjects with two risk alleles are not. Those in the heterozygous 5R/6R genotype group have slopes between those of the two homozygous groups in all but one case.

For DBH, the situation is the opposite of what might be predicted. That is, subjects with two copies of the risk allele have negative slopes (are getting faster over time) and those with zero copies are essentially showing no trend in RT across the 10 blocks. This is also the case for the one outcome measure on APOE that showed significant slope differences. Those in the highest risk group got faster over the 10 blocks while those in the "protective allele" group got slower across the 10 blocks I plan to investigate genetic associations with these slope differences in a future project.

Conclusions

This thesis contributes to the understanding of exogenous orienting in several ways. First, studying additional genes beyond those reviewed by Kebir et al., (2009) has been useful. COMT has not been previously associated with RT variability. It has also been useful to extend RT variability studies to reflexive attention tasks since this gives a more complete picture of attentional deficits.

Second, it is useful to know that there is limited evidence of genetic effects on increased RT variability in normal individuals for the task we used and the genetic markers that we examined. However, if the effects exist then they may be too small to detect in the sample sizes we obtained. Replication with larger samples may be desirable, however, and is planned in a future study.

Alternatively, the genetic effects on RT variability may require a longer task to detect. It may be useful to lengthen the task beyond 20 minutes so that variability may be

better detected. If variability depends on the lack of consistent responding (perhaps through occasional failures of neural transmission) then increased task duration might increase the opportunity to detect this variability. Several researchers (Castellanos et al., 2005; Johnson et al., 2007) have suggested the investigation of patterns of rising and falling attention over the course of the task. Their methods are more appropriate with a longer task. An alternative may be to reduce the number of trial types so that the number of trials per type can be substantially increase. While the CPT is not much longer (approximately 30 minutes as opposed to 20 minutes) it uses trials of all one type and therefore it is possible to perform time series analysis and examine patterns of increasing and decreasing variability.

The third way this thesis contributes is by clarifying the variables useful in predicting RT variability. Age and the associated mean RT for a given outcome measure of variability seemed to be particularly important and need to be included in future research. Ethnicity, while not statistically significant for most models, seemed to be useful to include since models comparing Caucasian versus non-Caucasian samples seemed to differ in their coefficient estimates. Sleepiness was a significant predictor in *dual by bright* condition and may be useful in studying additional genes.

Additional genes that impact dopamine systems are good candidates for future studies in addition to those used in this study. For example, MAOA, SNAP25, DRD1IP, DRD2, and DRD5 are all genes that influence the dopamine system and have been shown to influence attention (Bellgrove & Mattingley, 2008; Corradini, Verderio, Sala, Wilson, & Matteoli, 2009; Fisher et al., 2002; Xu et al., 2007). It may be useful to examine select combinations of genetic as well as environmental influences on RT variability. In a large

enough sample (Long and Langley [1999] suggest approximately 500 subjects) gene x gene (and gene x environment) interactions can be studied. In addition, some genes may have an additive effect and examining a combination of genes may be found to have a greater influence on RT variability than any gene alone.

Naturally, the results of this study support the need to look for polygenetic, epistatic, environmental and gene-environment interaction influences because the genes examined explain 2%of the variance in RT variability (or less, for the non-significant genes). The results also suggest that RT variability on orienting tasks is less important than mean RT differences in distinguishing between those with genotypic differences. However, RT variability differences are suggested and may be important to understanding attentional deficits. Therefore, it appears that this study merits a replication attempt (with a larger sample, additional genes, and a longer task) because there are very few studies that report on any association between genes and RT variability on an exogenously cued orienting measure and effects for two RT variability outcomes were suggestive of genotypic differences.

References

Barkley, R. A. (1997). Behavioral inhibition, sustained attention, and executive functions: Constructing a unifying theory of ADHD. *Psychological Bulletin, 121*, 65-94.

Bellgrove, M. A., Chambers, C. D., Johnson, K. A., Daibhis, A., Daly, M., Hawi, Z., . . . Robertson, I. H. (2007). Dopaminergic genotype biases spatial attention in healthy children. *Molecular Psychiatry, 12*, 786-792.

Bellgrove, M. A., Hawi, Z., Kirley, A., Gill, M., & Robertson, I. H. (2005). Dissecting the attention deficit hyperactivity disorder (ADHD) phenotype: Sustained attention, response variability and spatial attentional asymmetries in relation to dopamine transporter (DAT1) genotype. *Neuropsychologia, 43*, 1847-1857.

Bellgrove, M. A., Hester, R., & Garavan, H. (2004). The functional neuroanatomical correlates of response variability: Evidence from a response inhibition task. *Neuropsychologia, 42*, 1910-1916.

Bellgrove, M.A., & Mattingley, J. B. (2008). Molecular genetics of attention. *Annals of the New York Academy of Sciences, 1129*, 200-212.

Brookes, K. J., Mill, J., Guindalini, C., Curran, S., Xu, X., Knight, J., . . . Asherson P. (2006). A common haplotype of the dopamine transporter gene associated with attention-deficit/hyperactivity disorder and interacting with maternal use of alcohol during pregnancy. *Archives of General Psychiatry,63*, 74-81.

Brookes, K. J., Neale, B. M., Sugden, K., Khan, N., Asherson, P., & D'Souza, D. M. (2007). Relationship between VNTR polymorphisms of the human dopamine transporter gene and expression in post-mortem midbrain tissue. *American Journal of Medical Genetics Part B 144B*, 1070-1078.

Castellanos, F. X. & Tannock, R. (2002). Neuroscience of attention-deficit/hyperactivity disorder: The search for endophenotypes. *Nature Reviews Neuroscience, 3*, 617-628.

Castellanos, F. X., Sonuga-Barke, E. J. S., Scheres, A., Di Martino, A., Hyde, C., & Walters, J. R. (2005). Varieties of attention-deficit/hyperactivity disorder-related intra-individual variability. *Biological Psychiatry, 57*, 1416-1423.

Cohen, J., & Cohen, P. (1975). Applied Multiple Regression/Correlation Analysis for the Behavioral Sciences. Lawrence Erlbaum Associates, Hillsdale, NJ

Conners, C.K. (1992). *Continuous Performance Test Computer Program* (Version 2.0). North Tonawanda, NY: Multi-Health Systems.

Corder, E. H., Saunders, A. M., Risch, N. J., Strittmatter, W. J., Schmechel, D. E., Gaskell, P. C., . . . Pericak-Vance, M. A. (1994). Protective effect of apolipoprotein E type 2 allele for late onset Alzheimer disease. *Nature Genetics, 7*, 180-184.

Corradini, I., Verderio, C., Sala, M., Wilson, M. C., & Matteoli, M. (2009). SNAP-25 in neuropsychiatric disorders. Annals of the New York Academy of Sciences, 1152, 93-99.

Dresler, T., Ehlis, A. C., Heinzel, S., Renner, T. J., Reif, A., Baehne, C. G., . . . Fallgatter A. J. (2010). Dopamine transporter (SLC6A3) genotype impacts neurophysiological correlates of cognitive response control in an adult sample of patients with ADHD. *Neuropsychopharmacology, 35*, 2193-2202.

Eriksen, B. A., & Eriksen, C. W. (1974). Effects of noise letters upon the identification of a target letter in a nonsearch task. *Perception &Psychophysics, 16*, 143-149.

Erixon-Lindroth, N., Farde, L., Wahlin, T-B. R., Sovago, J., Halldin, C., & Bäckman, L. (2005). The role of the striatal dopamine transporter in cognitive aging. *Psychiatry Research: Neuroimaging, 138*, 1-12.

Fisher, S. E., Francks, C., McCracken, J. T., McGough, J. J., Marlow, A. J., MacPhie, L., . . . & Smalley, S. L. (2002). A genomewide scan for loci involved in attention-deficit/ hyperactivity disorder. *American Journal of Human Genetics, 70*, 1183-1196.

Fossella, J.A., Sommer, T., Fan, J., Wu, Y., Swanson, J.M., Pfaff, D.W. & Posner M.I. (2002). Assessing the molecular genetics of attention networks. *BMC Neuroscience, 3*, 14.

Giros, B., El Mestikawy, S., Godinot, N., Zheng, K., Han, H., Yang-Feng, T., & Caron, M. G. (1992). Cloning, pharmacological characterization, and chromosome assignment of the human dopamine transporter. *Molecular Pharmacology, 42*, 383-90.

Goyette, C. H., Conners, C. K., & Ulrich, R. F. (1978). Normative data on revised Conners' parent and teacher rating scales. *Journal of Abnormal Child Psychology, 6*, 221-236.

Greenwood, P. M., Sunderland, T., Friz, J. L., & Parasuraman. R. (2000). Genetics and visual attention: Selective deficits in healthy adult carriers of the epsilon 4 allele of the apolipoprotein E gene. *Proceedings of the National Academy of Sciences of the United States of America, 97*, 11661-11666.

Hallman, D. M., Boerwinkle, E., Saha, N., Sandholzer, C., Menzel, H. J., Csazar, A., & Utermann, G. (1991). The apolipoprotein E polymorphism: A comparison of

allele frequencies and effects in nine populations. *American Journal of Human Genetics, 49*, 338 - 349.

Heilman, K. M. & Van Den Abell, T. (1980). Right hemisphere dominance for attention: The mechanism underlying hemispheric asymmetries of inattention (neglect). Neurology, 80, 327 - 330.

Hubacek, J. A., Lánská, V., Skodová, Z., Adámková, V., & Poledne, R. (2008). Sex-specific interaction between APOE and APOA5 variants and determination of plasma lipid levels. *European Journal of Human Genetics, 16*, 135-138.

Hurks, P. P., Adam, J. J., Hendriksen, J. G., Vles, J. S., Feron, F. J., Kalff, A. C., . . . Jolles J. (2005). Controlled visuomotor preparation deficits in attention-deficit/hyperactivity disorder. *Neuropsychology, 19*, 66-76.

Hutchison, K. E., Stallings, M., McGeary, J., & Bryan, A. (2004). Population stratification in the candidate gene study: Fatal threat or red herring? *Psychological Bulletin, 130*, 66-79.

Johns, M. W. (1991). A new method for measuring daytime sleepiness: The Epworth sleepiness scale. *Sleep, 14*, 540-545.

Johnson, K. A., Kelly, S. P., Bellgrove, M. A., Barry, E., Cox, M., Gill, M., & Robertson, I. H. (2007). Response variability in attention deficit hyperactivity disorder: Evidence for neuropsychological heterogeneity. *Neuropsychologia, 45*, 630-638.

Kean, M., & Lambert, A. (2003). The influence of a salience distinction between bilateral cues on the latency of target-detection saccades. *British Journal of Psychology, 9*, 373- 388.

Kebir, O., Tabbane, K., Sengupta, S., & Joober, R. (2009). Candidate genes and neuropsychological phenotypes in children with ADHD: Review of association studies. *Journal of Psychiatry & Neuroscience, 34*, 88-101.

Kopecková, M., Paclt, I., & Goetz, P. (2006). Polymorphisms of dopamine-beta-hydroxylase in ADHD children. *Folia Biologica, 52*, 194-201.

Kuntsi, J. & Stevenson, J. (2001). Psychological mechanisms in hyperactivity: II. The role of genetic factors. *Journal of Child Psychology and Psychiatry, and Allied Disciplines, 42,* 211-219.

Leth-Steensen, C., Elbaz, Z. K., & Douglas, V. I. (2000). Mean response times, variability, and skew in the responding of ADHD children: A response time distributional approach. *Acta Psychologica, 104*, 167-190.

Long, A.D. & Langley, C. H. (1999). The power of association studies to detect the contribution of candidate genetic loci to variation in complex traits. *Genome Research, 9*, 720-731.

Loo, S. K., Specter, E., Smolen, A., Hopfer, C., Teale, P. D., & Reite, M. L. (2003). Functional effects of the DAT1 polymorphism on EEG measures in ADHD. *Journal of the American Academy of Child and Adolescent Psychiatry, 42*, 986-993.

Lowe, N., Kirley, A., Mullins, C., Fitzgerald, M., Gill, M., & Hawi, Z. (2004). Multiple marker analysis at the promoter region of the DRD4 gene and ADHD: Evidence of linkage and association with the SNP -616. *American Journal of Medical Genetics B: Neuropsychiatric Genetics, 131*, 33–37.

Ogilvie, R. D., Wilkinson, R. T., & Allison, S. (1989). The detection of sleep onset: Behavioral, physiological, and subjective convergence. *Sleep, 12*, 458-474.

Oosterlaan, J. & Sergeant, J. A. (1998). Response inhibition and response re-engagement in ADHD, disruptive, anxious and normal children. *Behavior Brain Research, 94*, 33-43.

Parasuraman, R., Greenwood, P. M., & Sunderland, T. (2002). The apolipoprotein E gene, attention, and brain function. *Neuropsychology, 16*, 254-274.

Poirier, J. (1996). Apolipoprotein E in the brain and its role in Alzheimer's disease. *Journal of Psychiatry and Neuroscience, 21*, 128–134.

Posner, M. I. (1980). Orienting of attention. *The Quarterly Journal of Experimental Psychology, 32*, 3-25.

Rommelse, N. N., Altink, M. E., Arias-Vásquez, A., Buschgens, C. J., Fliers, E., Faraone, S. V., . . . Oosterlaan, J. (2008). A review and analysis of the relationship between neuropsychological measures and DAT1 in ADHD. *American Journal of Medical Genetics. Part B, Neuropsychiatric Genetics, 147B*, 1536-1546.

Sagvolden, T., Johansen, E. B., Aase, H., & Russell, V. A. (2005). A dynamic developmental theory of attention-deficit/hyperactivity disorder (ADHD) predominantly hyperactive/ impulsive and combined subtypes. *The Behavioral and Brain Sciences, 28*, 397-419.

Sergeant, J. A. (2005). Modeling attention-deficit/hyperactivity disorder: A critical appraisal of the cognitive-energetic model. *Biological Psychiatry, 57*, 1248-1255.

Söderlund, G. B. W. (2007). Noise improves cognitive performance in children with dysfunctional dopaminergic neurotransmission. (Doctoral Thesis). Retrieved from su.diva-portal.org.

Starr, J. M., Fox, H., Harrris, S., Deary, I. J., & Whalley, L. J. (2007). COMT genotype and cognitive ability: A longitudinal aging study. *Neuroscience Letters, 421*, 57-61.

Stevenson, J., Pennington, B. F., Gilger, J. W., DeFries, J. C., & Gillis, J. J. (1993). Hyperactivity and spelling disability: Testing for shared genetic aetiology. *Journal of Child Psychology and Psychiatry*, *34*, 1137-1152.

Stuss, D. T., Murphy, K. J., Binns, M. A., & Alexander, M. P. (2003). Staying on the job: The frontal lobes control individual performance variability. *Brain, 126*, 2363-2380.

Svensson, O., Arvestad, L., & Lagergren, J. (2006). Genome-wide survey for biologically functional pseudogenes. *PLoS Computational Biology*, *2*, e46.

Swanson, J., Posner, M. I., Fusella, J., Wasdell, M., Sommer, T., & Fan, J. (2001). Genes and attention deficit hyperactivity disorder. *Current Psychiatry Reports, 3*, 92-100.

Tang, M.-X., Stern, Y., Marder, K., Bell, K., Gurland, B., Lantigua, R., . . . Mayeux, R. (1998). The APOE-ε4 allele and the risk of Alzheimer disease among African Americans, Whites, and Hispanics. *Journal of the American Medical Association, 279*, 751-755.

Thomas, D. C. & Witte, J. S. (2002). Point: Population stratification: A problem for case-control studies of candidate-gene associations? *Cancer Epidemiology, Biomarkers & Prevention, 11*, 505-511.

Vargas-Alarcon, G., Fragoso, J. M., Cruz-Robles, D., Vargas, A., Vargas, A., Lao-Villadoniga, J. I., Garcia-Fructuoso, F., Ramos-Kuri, M., Hernandez, F., Springall, R., Bojalil, R., Vallejo, M., & Martinez-Lavin, M. (2007). Catechol-O-methyltransferase gene haplotypes in Mexican and Spanish patients with fibromyalgia. *Arthritis Research & Therapy*, *9*, R110.

Volkow, N. D., Wang, G-J., Fowler, J. S., Logan, J., Gerasimov, M., Maynard, L., . . . Franceschi, D. (2001). Therapeutic doses of oral methylphenidate significantly increase extracellular dopamine in the human brain. *The Journal Neuroscience, 21,* RC121.

Wood, S. N. (2001). Partially specified ecological models. *Ecological Monographs, 71*, 1-25.

Xu, X., Brookes, K., Chen, C. K., Huang, Y. S, Wu, Y. Y., & Asherson, P. (2007). Association study between the monoamine oxidase A gene and attention deficit hyperactivity disorder in Taiwanese samples. *BMC Psychiatry, 7*, 10.

APPENDIX A

Procedures for Experiment

Subjects were tested individually. The experimenter read the following statement to the subject "We are interested in possible genetic contributions to differences between individuals in visual attention. For that reason we are collecting some measures of visual attention and also a saliva sample that contains cells that can be used to do genetic analyses looking at specific genes that have been identified in the literature as being related to visual attention. Because this is basic research, the results of these analyses will not available to you."

The experimenter read a copy of the consent form to the subject prior to requesting a signature or the completion of intake forms. Written instructions for the behavioral portion of the experiment were reviewed with the subject. The lights were turned off so that the subjects eyes could adjust to the darkened room. While waiting, the chin/ forehead rest was adjusted to a comfortable height for the subject while maintaining the subject's eyes are 57 cm from the screen. After behavioral testing, the subject was instructed on how to produce a high quality saliva sample. When a sufficient saliva sample was produced, the subject was thanked and given either Rice University Psychology course credit or $10.

Written Instructions for Subjects

If you think you are going to need a drink in the next 30 minutes, you need to get one now because eating or drinking anything will dilute the DNA available in the saliva sample. Also, please turn off our cell phone for the approximately 20 minutes this portion of the experiment will take.

The computer portion of this study is an experiment on rapidly detecting and responding to a visual target. The target in this case is a small white square. Your task is to respond as quickly and accurately as possible to the location of this target. Please fixate your eyes on (look directly at) the central cross at the beginning of each trial, and maintain central fixation during the experiment. One or two Xs may flash on the screen, on the left, right, or on both sides of the display. The target will not appear at the same time as the cues. The location of the target is unrelated to the Xs. You are to respond as quickly and accurately as possible only to the location of the target (small square). If it appears on the left of the fixation cross, press the 'a' key. If it appears on the right, press the 'l' key. Again, do not respond to the Xs (if they appear); only respond to the white square. Sometimes a target will not appear after the Xs. These are catch trials. On these trials, please do not press any key. After a brief period a new target will appear (possibly preceded by Xs). Once again, respond to the small white square with a key press as before.

If you hit a key other than 'a' or 'l' the computer will give a short beep. If you need a break in order to find the 'a' and 'l' keys again (or for any other reason) you may press the space bar right after you see the small white square. The Velcro guides on the keyboard and/or the flashlight located just outside the shield can be used to re-locate the correct keys to press. When you are ready to continue with the experiment, simply press the spacebar.

First, there will be a practice set of 20 trials. The practice is over when you hear a series of beeps. Please take a short break when you hear those beeps. If you hear beeps at any other times than those I've mentioned, please stop responding and come and get me.

When you are ready, begin again by pressing the spacebar. When the attention portion of the experiment is over you will hear music. At that point, just step into the hall and call my name.

Do you have any questions?

Intake form

Code # _____

Age _____

Gender _____

Ethnicity (please check as many as apply)

___ American Indian or Alaskan Native

___ Asian

___ Black or African American

___ Hispanic

___ Native Hawaiian or Other Pacific Islander

___ White or Caucasian (not of Hispanic origin)

Have you ever been diagnosed with ADHD? Yes No

If yes, do you currently have a diagnosis of ADHD? Yes No

If yes, are you currently on medications for treatment of ADHD? Yes No

If yes, please list those medications._____

_____

Have you ever had a neurological disorder (examples are stroke, visual neglect, migraines, or Parkinson's disease)? Yes No

If yes, what neurological disorder? _____

If yes, have you had problems (symptoms) in the past 30 days?

If yes, are you currently on medications for treatment of the neurological disorder? Yes No

If yes, please list those medications. _____

First-degree relatives: Biological: mother, father, siblings, children

Second-degree relatives: Biological: grandparent, grandchild, uncle, aunt, nephew, niece, half-sibling

Do you have a first-degree or second-degree relative with a diagnosis of (please circle):

Attention Deficit Hyperactivity Disorder ADHD Yes No Don't know

If yes, please list the relative(s) _____

Alzheimer's Disease Yes No Don't know

If yes, please list the relative(s) _____

Schizophrenia Yes No Don't know

If yes, please list the relative(s) _____

Autism Yes No Don't know

If yes, please list the relative(s) _____

Obsessive-Compulsive Disorder (OCD) Yes No Don't know

If yes, please list the relative(s) _____

Tobacco Use

Have you ever smoked cigarettes for more than a month? Yes No

Do you currently smoke cigarettes? Yes No

If yes, please indicate the average number of cigarettes that you smoke per day

_____

If yes, how long ago was your last cigarette _____hours

_____minutes

If you have ever smoked cigarettes for more than a month but are not currently smoking, how long ago were you regularly smoking cigarettes?

_____years _____months _____days

Epworth Sleepiness Scale

How likely are you to doze off or fall asleep in the following situations, in contrast to feeling just tired?

This refers to your usual way of life in recent times.

Even if you haven't done some of these things recently try to work out how they would have affected you.

Use the following scale to choose the most appropriate number for each situation:

0 = would never doze

1 = slight chance of dozing

2 = moderate chance of dozing

3 = high chance of dozing

It is important that you answer each question as best you can.

Situation	**Chance of Dozing (0 – 3)**
Sitting and reading	_____
Watching TV	_____
Sitting, inactive in a public place (e.g., a theatre or a meeting)	_____
As a passenger in a car for an hour without a break	_____
Lying down to rest in the afternoon when circumstances permit	_____
Sitting and talking to someone	_____
Sitting quietly after a lunch without alcohol	_____
In a car, while stopped for a few minutes in traffic	_____

THANK YOU FOR YOUR COOPERATION

APPENDIX B

Genetics Protocol

Detailed information on the genetics protocol can be obtained from the Oragene website at www.dnagenotek.com.

Table B-1. The nucleotide sequences ("primers") used to isolate the polymorphisms analyzed.

Polymorphism	Strand	Primer sequence
rs429358 (APOE)	Sense	5'-GAACTGGAGGAACAACTGAC
	Antisense	5'-CGCTCGCGGATGGCGCTGA
rs7412 (APOE)	Sense	5'-GAACTGGAGGAACAACTGAC
	Antisense	5'-CGCTCGCGGATGGCGCTGA
rs4680 (COMT)	Sense	5'-GCTACTCAGCTGTGCGCATG
	Antisense	5'-ACGTGGTGTGAACACCTGGT
SL6A3 repeat (DAT1)	Sense	5'-TGTGTGCGTGCATGTGG[a]
	Antisense	5'-GCTTGGGGAAGGAAGGG
rs1108580 (DBH)	Sense	5'-ACGCCTGGAGTGACCAGAAG
	Antisense	5'-CCATCCTCCTTGGCTTTCTC
rs747302 (DRD4)	Sense	5'-CGGAGGGAATGGAGGAGGGA
	Antisense	5'-AGACCTGAGCTCAGGCTCTG

Note. [a] Primer with 5'-Fam fluorescent label