Genetic Association Study of Spatial Working Memory

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ABSTRACT

GENETIC ASSOCIATION STUDY OF SPATIAL WORKING MEMORY

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Working memory (WM) is a collection of cognitive processes that include short term storage of task related information and manipulation of this information to facilitate the transformation of memory to action immediately. Age-related declines in WM performance have been attributed to dysfunction in dopamine and cholinergic neurotransmission. In this study, we applied a genetic approach to investigate how normal variation in genes controlling monoamine expression in PFC is linked to age-related decline in working memory.

One well-studied source of genetic variation in dopamine neurotransmission occurs in the gene controlling the enzyme Catechol-O-Methyltransferase (COMT). A well-studied 158 G/A polymorphism in the COMT gene (rs4680) is non-synonymous and results in a valine-to-methionine substitution. The methionine variant is associated with a 3-4 fold lower level of enzyme activity, compared to its valine counterpart. We analyzed two measures of performance (accuracy and reaction time measures) in terms of the

influence of two biological parameters (age and COMT genotype) and two spatial WM parameters (distance between the target and the probe dot, and memory load). For accuracy measures, a significant interaction of memory load x COMT x age in the "Match" task conditions was observed. For accuracy measures, we showed a significant memory load x COMT x age interaction in "match" task conditions and the age effect was most prominent in "non-match" short distance task conditions. For reaction time measures, the older val/val homozygotes showed longer reaction times than the met/met and val/met subjects. Taken together, our results support the idea that different levels of COMT enzyme activity may be optimal for different tasks and heritability of COMT becomes increasingly important in cognitive performance with advancing age.

The muscarinic cholinergic M2 receptor (CHRM2) belongs to the superfamily of G- protein coupled receptors, whose roles include modulation of cholinergic transmission, neuronal excitability, synaptic plasticity and feedback regulation of acetylcholine release. The CHRM2 A1890T polymorphism (rs8191992) which located in the 3' untranslated region has been repeatedly reported to be correlated to intelligence quotient (IQ). In this study, we also analyzed two measures of performance (accuracy and reaction time measures) in terms of the influence of two biological parameters (age and CHRM2 genotype) and two spatial WM parameters (distance between the target and the probe dot, and memory load). For the accuracy measures, we showed a significant memory load x CHRM2 x age interaction in the match task conditions. This interaction showed improved accuracies for CHRM2 AT heterozygotes in high memory loads. For reaction time measures, a significant CHRM2 x age interaction was also observed. This interaction

showed that the young AA homozygotes used shorter reaction times than the young AT heterozygotes and the TT homozygotes, while the older AA homozygotes used longer reaction times than the older AT heterozygotes and the TT homozygotes. The CHRM2 A1890T polymorphism also showed increased effects in non-match task conditions for older adults. Taken together, our results support the idea that the CHRM2 A1890T polymorphism associated with the performance of spatial working memory at different ages. The increased genetic effects were observed in older adults.

In this study, we showed the influence of normal gene variability on working memory. We also showed the increased gene effects in older adults. However, the cellular mechanism of how did these polymorphisms effect the neuronal activity is still not clear. Muscarinic neurotransmission has been implicated to play an important role in learning, attention and in Alzheimer's disease. Thus, further research is needed in this area. Cellular and molecular studies of rs8191992 may help to elucidate the molecular mechanism of this SNP.

Chapter 1

Introduction

With the sequencing of Human Genome Project, which provided a draft of 3 billion letters of DNA in human genome, individual differences in DNA sequences have created novel approaches to investigate the association of genes and human behaviors. Single nucleotide polymorphisms (SNPs) were considered to account for the majority of human genetic variation among individuals (Bentley, 2000) and are presumably responsible for the widespread heritability of behavior disorders (Goldberg and Weinberger, 2004). The significant genetic component of heritability of many cognitive disorders (such as bipolar disorder and schizophrenia) imply that many SNPs influence cognitive behaviors (Greenwood and Parasuraman, 2003; Bouchard, 2004; Goldberg and Weinberger, 2004). For many complex cognitive behaviors, quantitative traits (e.g., cognitive test scores) contain more information than the information provided by dichotomous traits (e.g., normal or abnormal). These quantitative traits can provide effective descriptions of cognitive behaviors that differ between normal people. In the present study, we analyzed a behavior test on working memory. We focused on the genetic association approach to study the influences of SNPs on working memory (WM) performance as well as the relationship with normal aging.

The dopaminergic neurotransmission in prefrontal cortex (PFC) is considered to be related to working memory performance (Braver and Barch, 2002; Bäckman et al., 2006). Working memory is a collection of cognitive processes that included short term storage of task related information and manipulation of this information to facilitate the immediate transformation of memory to action (Baddeley and Della Sala, 1996). This information manipulation process is also labeled as executive control and is highly related to cognitive processes mediated by PFC. It is well known that WM performance is related to catecholamine signaling, neuronal excitability and synaptic plasticity in PFC (Seamans and Yang, 2004; Arnsten and Li, 2005). Reduced WM ability is thought to be a principal contributor to age-related cognitive decline (Dobbs and Rule, 1989). As dopamine inputs to PFC play an important role in WM (Abi-Dargham, 2004), the decline of WM in normal aging may in turn reflect dysfunction in dopaminergic neurotransmission in PFC (Braver and Barch, 2002; Bäckman et al., 2006). Previous studies have shown that working memory decayed with advanced age. Normal aging was correlated with slower information processing speed and a decline in memory and attention abilities (Woodruff, 1997). Furthermore, spatial working memory has been shown to have greater age decrements in performance (Myerson et al., 1999) as well as slower reaction time (Hale et al., 1996; Lawrence et al., 1998) than verbal working memory.

One important enzyme that controls catecholamine (including dopamine, norepinephrine and epinephrine) methylation in the synaptic cleft is Catechol-O-Methyltransferase (COMT). The COMT val158met (rs4680) polymorphism, which

resulted in 3-4 fold differences in enzyme activity, has been suggested to be correlated to working memory performance (Egan et al., 2001; Mattay et al., 2003; Nolan et al., 2004). Therefore, we will focus on the association between WM performance, COMT rs4680 and aging in chapter 2.

The muscarinic acetylcholine M2 receptor (CHRM2) is also considered to be correlated to the working memory performance. In previous studies, several SNPs in CHRM2 gene has been implicated in human cognitive functions, especially the intelligence quotient (IQ) (Comings et al., 2003; Dick et al., 2006; Gosso et al., 2006; Gosso et al., 2007). Importantly, the CHRM2 A1890T polymorphism (rs8191992) which is located in the 3'-untranslated region (3'-UTR) has been consistently reported to be correlated to IQ, as measured by the Wechsler Adult Intelligence Scale-revised (WAIS-R) (Comings et al., 2003; Dick et al., 2006). This CHRM2 A1890T polymorphism explained 1% of the variance of Full scale IQ (measured by WAIS-R) in parents of twins from Minnesota Twins and Family study (Comings et al., 2003). The AA homozygotes of the CHRM2 A1890T polymorphism were reported to have approximately 5 performance IQ points higher than the TT homozygotes measured by WAIS-R (Dick et al., 2006). The performance IQ in WAIS-R test is primarily a measure of fluid intelligence which was considered to be highly correlated to working memory (Kyllonen and Christal, 1990; Kane and Engle, 2002; Conway et al., 2003). Animal models of the M2 receptor-deficient (M2-/-) mice also showed significant behavioral flexibility deficits in the Barnes circular maze as well as significant working memory deficits in the T-maze delayed alternation tests (Seeger et al., 2004). In addition, the M2-/- mice showed profound changes in

neuronal plasticity studied at hippocampal synapses (Seeger et al., 2004). These results suggested that the M_2 receptor-mediated modulation of hippocampal neuronal activities may be correlated to working memory performance as well. Thus, we will focus on the associations between working memory, CHRM2 rs8191992 and aging in chapter 3.

In preliminary analyses, we applied factor analysis followed by multivariate ANOVA to assess the between subject effects of these and other SNPs (within subject differences were analyzed later). The results suggested that (1) COMT val158met had substantial relation with age when the target dot and probe dot were in the same location, (2) BDNF (brain-derived neurotrophic factor) val66met and DBH (dopamine beta hydroxylase) C-1021T may interact with age in the most difficult task conditions, and (3) COMT val158met may interact with CHRM2 A1890T in some task conditions. In the following chapters, we applied repeated measure ANOVA to facilitate the analysis of within-subject effects. Since the BDNF met/met homozygotes are rare in European American populations, we chose to focus our analysis on COMT and CHRM2 polymorphisms and their interactions with age in this dissertation.

Based on the results of chapter 2 and chapter 3, we were able to perform a preliminary analysis of the interaction of COMT rs4680 and CHRM2 rs8191992 on working memory performance under the influence of age. This is presented in chapter 4. Chapter 5 is the conclusion and a general discussion of the problems we encountered in this analysis.

Chapter 2

The Effect of COMT on Working Memory Depends on Age, Task Difficulty and Memory Load

2.1 Introduction

Many cognitive functions, including decision making, problem solving, and reasoning, require active short-term storage (maintenance) and manipulation of information over time in order to be carried out efficiently. This ability -- termed working memory (WM) (Baddeley, 1992) -- is well known to undergo age-related decline (Salthouse et al., 1989; Park, 2000). Reduced WM ability has been claimed to be a principal contributor to age-related cognitive decline (Dobbs and Rule, 1989). Braver and Barch have argued that this decline is due to dysfunction within the dopaminergic neurotransmission system in prefrontal cortex (PFC) (Braver and Barch, 2002; Bäckman et al., 2006). As dopamine (DA) inputs to PFC play an important role in WM (Abi-Dargham, 2004), the decline of WM in normal aging may be attributable to such dysfunction. However, the cholinergic system has also been claimed to underlie cognitive aging (Bartus et al., 1982). To date, efforts to understand the underpinnings of cognitive aging have primarily used pharmacological manipulations to examine, for example, how age-related loss of function in dopaminergic systems might mediate

age-related decline in WM. An alternative approach is to investigate how normal variation in genes controlling monoamine expression in PFC is linked to age-related decline in WM.

One rationale for investigating the role of genetic variability in age-related decline in DA-mediated functions is that the heritability of general cognitive ability is known to increase with age - from about 20% in young children to 62% in very old age (McGue et al., 1993; McClearn et al., 1997; Ando et al., 2001). Moreover, the heritability of WM appears to be substantial (Ando et al., 2001). If, by extension, the effect of genetics on WM increases with age, then what is the mechanism? Normal variation is known to occur in genes controlling neurotransmission, and the cognitive consequences of that variation have been increasingly shown in recent years to affect information processing tasks, including WM and attention (Greenwood and Parasuraman, 2003; Greenwood et al., 2005; Parasuraman et al., 2005). Lower efficiency in DA neurotransmission due to normal genetic variation may be more consequential in older persons due to age-related declines in dopaminergic functionality (Nagel et al., 2008).

One well-studied source of genetic variation in DA neurotransmission occurs in the gene controlling the enzyme Catechol-O-Methyltransferase (COMT). The COMT enzyme breaks down catecholamines in the synaptic cleft. The COMT gene has been studied in relation to PFC-dependent functions, notably WM (Egan et al., 2001; Goldberg et al., 2003; Meyer-Lindenberg et al., 2005). A well-studied 158 G/A polymorphism in the COMT gene (rs4680) is non-synonymous and results in a valine-to-methionine substitution. The methionine variant is associated with a 3-4 fold lower level of enzyme

activity, compared to its valine counterpart (Lachman et al., 1996). The reduced enzyme activity is most likely due to the reduced protein thermostability of the met allele (Männistö and Kaakkola, 1999).

This variation in activity of the COMT enzyme due to variation in the gene appears to have consequences for cognition. A number of studies have examined associations between the COMT val158met polymorphism and individual differences in DA-mediated cognitive functions associated with PFC. Weinberger and colleagues examined associations between COMT genotype and WM performance by the Wisconsin Card Sorting Test (WCST) (a test of integrated cognitive functions associated with the frontal lobe, including attention, WM and visual processing). The subjects with the Val/Val genotype performed worse (higher perseverative errors) than those with the Val/Met and Met/Met genotypes (Egan et al., 2001). Other studies using WCST have also found that the met allele was associated with better WM performance (Malhotra et al., 2002; Goldberg et al., 2003; Diamond et al., 2004; Diaz-Asper et al., 2008). However, some studies that also used WCST reported only a nonsignificant trend toward better performance for met/met homozygotes (Bilder et al., 2002; Joober et al., 2002), or no relationship between COMT genotype and WCST performance (Tsai et al., 2003). Nevertheless, pharmacological and genetic studies in rats and mice have shown that COMT plays a significant role in the metabolism of released dopamine in PFC, but has a smaller role in other brain areas and with other catecholamines (Tunbridge et al., 2004; Yavich et al., 2007). Thus, it is possible that detectable effects of COMT on cognition are seen primarily when tasks involving dynamic changes in DA signaling in the PFC are

required. This is consistent with our observation of an association between another polymorphism (in dopamine beta-hydroxylase, an enzyme that converts DA to norepinephrine in adrenergic vesicles) and performance in a spatial WM task involving retention of 1-3 dots over a 3 sec period (Greenwood and Parasuraman, 2003; Parasuraman et al., 2005).

Another possible contributor to the complex COMT results in the literature is the inverted U-shaped curve which has been claimed to describe the relation between PFC function and increasing dopamine signaling (Goldman-Rakic et al., 2000). The inverted U-shaped curve hypothesis argues that cognitive processes mediated by the PFC are optimized within a range of dopamine activity. In this view, cognitive functions dependent on PFC may be disrupted when dopamine levels in PFC are either higher or lower than this optimal range. Floresco and Philips tested this hypothesis in rats using local injection of dopamine D1 receptor agonists into PFC at differing times following task acquisition (Floresco and Phillips, 2001). The results showed that the D1 receptor agonists disrupted the performance of the spatial win-shift test at times when the performance was optimal, but the same D1 receptor agonists improved performance 12 hr later, when dopamine levels were inferred to be lower. These results are consistent with the inverted U-shaped curve of WM performance with respect to DA signaling. In addition, evidence from a positron emission tomography (PET) ligand study in normal people revealed that COMT val/val homozygotes (presumably with lower PFC dopamine levels) had higher D1 receptor availability (and thus presumably lower D1 receptor activation) in PFC than subjects with the met allele, most likely due to differences in

dopamine binding (Slifstein et al., 2008). However, there was no difference in D1 receptor binding in striatum between the val/val homozygotes and subjects with the met allele. Thus, COMT is primarily involved in regulating DA neurotransmission in PFC but not in striatum. Based on (a) the inverted U-shaped curve of PFC performance and dopamine signaling and (b) the role of COMT in regulating dopamine neurotransmission in PFC, the variation of enzyme activity in COMT val158met polymorphism may help to assess the role of PFC dopamine neurotransmission in regulating cognitive functions associated with PFC.

The inverted U-shaped curve hypothesis and COMT val158met polymorphism has also been applied to variations of cognitive abilities in young and older people. Lindenberger et al. reported that older COMT val/val homozygotes had more preservative errors in WCST than individuals with met/met or val/met genotypes (Nagel et al., 2008). They also showed that older COMT val/val used longer reaction times than the val/met or met/met individuals, provided that they also carried the BDNF (Brain-derived neurotrophic factor) met allele. In another study, amphetamine (a drug that generally increases extracellular dopamine) was used in the n-back test in adults (mean age approximately 33.0 years old) to show that amphetamine (a) improved the performance of val/val homozygotes on both 2-back and 3-back tests, (b) did not improve the performance of met/met homozygotes on the 2-back test, and (c) actually made performance of met/met homozygotes worse on the 3-back test (Mattay et al., 2003). The authors' interpretation of these results was that dopamine signaling in val/val homozygotes was suboptimal (on the upward slope of the inverted U-shaped curve) and

was improved by amphetamine treatment. On the other hand, dopamine signaling in met/met homozygotes was inferred to be near optimal (near the peak of the inverted U-shaped curve), and was made worse by amphetamine treatment. The authors thus suggested that dopamine receptor activation might improve PFC functioning, but only if the individuals' baseline dopamine signaling was normally suboptimal for that particular task. For older adults, Deary and colleagues reported that older val/met heterozygotes (all 79 years old) performed better than either homozygote group on the Wechsler Memory Scale Logical Memory subtest (Harris et al., 2005). They interpreted their results as indicating that older val/met individuals were near the peak of the inverted U-shaped curve – in other words, they speculated that the optimal level of dopamine signaling is lower in older people, perhaps due to age-related declines in PFC dopamine receptors (Harris et al., 2005; Bäckman et al., 2006). However, a recent study also found better WM performance for COMT val/met heterozygotes than either homozygotes in the combined cohort of children (mean age 12.4 years) and their parents (mean age 36.2 years) (Gosso et al., 2008). The authors also showed interactions between dopamine D2 receptor and COMT on working memory performance. Thus, the optimum dopamine signaling of WM performance may be not only a function of genotype and age, but also a function of behavior tasks and interactions of genes as well.

Among studies associated with COMT, relatively few previous studies have focused specifically on the effects of variation in the COMT val158met on cognitive aging (De Frias et al., 2004; De Frias et al., 2005; Harris et al., 2005; Starr et al., 2007; Nagel et al., 2008). Moreover, those studies used relatively complex tasks which require

multiple cognitive functions such as hypothesis generation, conceptualization, and error correction. Such tasks may not be able to identify deficits in specific cognitive abilities. Therefore, we chose to assess the effects of COMT variation on cognitive aging by using an information-processing cognitive task aimed at assessing specific aspects of spatial WM performance. We have previously observed this task to be sensitive to the G444A polymorphism of dopamine beta-hydroxylase and the apolipoprotein E4 allele (Greenwood and Parasuraman, 2003; Greenwood et al., 2005). Based on the above evidence of (a) the importance of PFC dopamine signaling for WM performance (Goldman-Rakic et al., 2000; Egan et al., 2001; Mattay et al., 2003), (b) age-related declines in dopamine signaling (Volkow et al., 2000; Bäckman et al., 2006) and WM (Salthouse et al., 1989; Park, 2000), and (c) the increased heritability of cognitive ability with age (Plomin, 1986; McGue et al., 1993; McClearn et al., 1997; Ando et al., 2001), we hypothesized that differential WM performance caused by genetic variation of COMT would have greater effects on cognition in older than in young people. We analyzed two measures of performance (accuracy and reaction time measures) in terms of the influence of two biological parameters (age and COMT genotype) and two spatial WM parameters (distance between the target and the probe dot, memory load). Our results showed a trend towards greater COMT effects on WM performance in older compared to young individuals, as well as statistically significant interactions between memory load, COMT genotype, and age group.

2.2 Methods and materials

2.2.1 Participants

Six hundred and thirty individuals screened for medical and psychiatric health volunteered to participate. The data reported here was collected in the context of a large study of the effect of normal genetic variation on cognitive aging. Individuals were grouped based on their age. Young adults ranged in age from 18-25 years old and older adults ranged from 64-89 years old. Each individual was genotyped for the val158met SNP in the COMT gene. A few individuals (18/630) were excluded because of missing data (reaction time and/or working memory accuracy). The remaining 612 individuals were used in all analyses reported. For the young adult group, val/val/ = 88 subjects, val/met = 230 subjects and met/met = 111 subjects. For older adults, val/val/ = 45 subjects, val/met = 98 subjects and met/met = 40 subjects. The observed COMT genotype distribution was consistent with that expected under Hardy Weinberg equilibrium.

The self-reported racial and ethnic identities of the subjects were indicated on a questionnaire. Racial and/or ethnic identities were classified according to the NIH policy on reporting race and ethnicity (http://grants.nih.gov/grants/guide/notice~files/NOT-OD-01-053.html). Ethnic identities were reported as follows: 17 subjects in "Hispanic or Latino" group, and 595 subjects in "Not Hispanic or Latino" group. Racial identities were reported as follows: White (450 subjects), Black or African American (54 subjects), Asian (47 subjects), Native American (10 subjects), Native Hawaiian or other Pacific Islander (1 subject). The remaining 33 subjects did not fall into any of the racial categories recognized by the NIH -- 27 subjects did not answer the question of racial identity, and 6 subjects reported multiple racial categories (4 White + Asian and 2 Pacific

Islander + Asian).

Omnibus ANOVA analysis of the entire data set (see Results) gave essentially the same results as an omnibus ANOVA analysis that was restricted to White group only (not shown). Therefore, population stratification between racial groups did not affect any conclusions within the scope of the present study.

2.2.2 Task

A working memory task assessed accuracy of memory for location over a 3 sec delay. The task design included systematic variation of spatial distance and memory load. Participants were seated so their eyes were 60 cm from the computer screen. Each trial began with a fixation cross in the center of the display for 1 sec. One, two or three black target dots (0.67° in size) were displayed at random locations for 0.5 sec. Immediately following the disappearance of the black target dots, the centered fixation cross was displayed again for 3 sec – the WM maintenance interval. At the end of the delay, a single red probe dot (0.67°) appeared alone, either in the same location as a previous target dot or in a different location. Participants indicated their judgment of whether the probe was in the same location as one of the targets (or not) by pressing one specific button (or another). The measured response period began with the appearance of the red probe dot and lasted for 2 sec, after which responses were no longer recorded. Trials in which target and probe were at the same location were termed “Match”. Trials in which target and probe were at different locations were termed “Non-Match”. The distances between the probe dot and the target dot were either 0° (Match) or 2°, 4° and 8° of visual angle

(spatial distance on Non-Match). There were also three different levels of memory load (number of dot locations) at each distance. Considering both spatial distance and memory load, there were a total of twelve different task conditions. Thus, for example, the 1 dot VA2 task condition means memory load was 1 target dot location and the distance between this target dot and the probe dot was visual angle 2˚.

A total of thirty Match trials (zero degree visual angle) and fifty-four non-Match (non-zero degree visual angle trials, 18 for each visual angle) were presented. The accuracy and reaction time of responses were recorded.

2.3 Molecular genetics

The COMT val158met single nucleotide polymorphism (rs4680) was assayed by a combination of nested PCR and melting-curve analysis with T_m-shift primers (Wang et al., 2005). A 290 bp DNA fragment was preamplified from genomic DNA and used as a template for second round (allele-specific) PCR on a Bio-Rad MyiQ thermal cycler, which allows automated melting temperature analysis of the PCR products. One allele-specific primer was designed with a 5' GC tail, resulting in an easily detectable increase in the melting temperature of the PCR product. The forward and reverse primers used in the first PCR were 5’ ATCCAAGTTCCCCTC TCTC 3’ and 5’ CTTTTTCCAGGTCTGACAAC 3’. In the second round PCR, the primer specific to the ‘G’ allele was 5’ CGCCGCCGCCGACCGACCGCACACCTTGTCCTTTAC 3’, the primer specific to the ‘A’ allele was 5’ CGCACACCTTGTCCTTGAT 3’ and the common primer was 5’ CGCCTGCTGTCACCA 3’.

2.2.4 Statistical analysis

2.2.4.1 Omnibus analyses

Accuracy and reaction time measures of WM performance were analyzed. In order to analyze WM performance as functions of target-probe distance (TPD), memory load, COMT genotype and age, two separate omnibus analyses were conducted: (a) a factor analysis and (b) a mixed repeated measures ANOVA.

To investigate the possible causes of variation in accuracy and reaction time in different task conditions, we carried out a factor analyses using principle axis factoring with varimax rotation (Meyers et al., 2006). Factor analysis is a correlation-based approach that seeks to explore the inter-correlation among the variables. The goal was to allow factor analysis to look for structures in the data. In other words, if the same individuals performed well (or badly) on a certain cluster of test conditions, then those test conditions were likely to require similar cognitive functions. If so, then analysis focused on that specific cluster of related test conditions (a “factor”) may have greater resolving power to distinguish age and genotype effects vs. an omnibus analysis of a heterogeneous collection of test conditions that require multiple cognitive functions (see section 2.4.2)

Principle axis factoring utilizes shared variance between variables to assess the structures of the data. Varimax rotation is an orthogonal rotation that makes each factor as independent as possible. The number of reported factors was determined by the Kaiser rule (which retains only factors with eigenvalues > 1.0) and parallel analysis with data

permutation (which selects the factors whose eignevalues are greater than those obtained with comparable, but randomly permuted data) (O'Connor, 2000; Lance et al., 2006).

More conventional mixed model ANOVAs were also carried out to assess the overall within-subject and between-subject effects. Four levels of distance (0°, 2°, 4° or 8° of visual angle) and three levels of memory load (1, 2 or 3 target dots) were used as within-subject variables. The two categorical variables, COMT genotype and age group, were used as between-subject variables. The main effects of TPD and memory load as well as their interactions with COMT and age on WM performance were investigated.

2.4.2 Mixed repeated measure ANOVAs in each factor

Follow up mixed model ANOVAs was conducted in each factor based on the results of factor analyses. The task conditions that were clustered within a factor were used in the ANOVA for that factor, with minor exceptions (see results).

The Huynh-Feldt correction was used in the mixed model ANOVA when Mauchly's test of sphericity was significant (Meyers et al., 2006). This was used to correct the estimated number of degrees of freedom (Meyers et al., 2006). Effect sizes were expressed as eta squared from mixed model ANOVA. An alpha value of 0.05 was used to indicate statistical significance. The statistical analyses were performed within the Statistical Package for the Social Sciences, version 15.0 (SPSS Inc., Chicago, IL).

2.2.4.3 Speed-Accuracy trade-off

The speed of information processing (reaction time) has been reported to effect the

results of behavior tasks (Salthouse, 1996). We did a regression analysis of mean accuracy vs. mean reaction time (across memory load) in each factor to assess their relationship. The task conditions that were clustered to a factor were used to obtain mean accuracy and mean reaction time. For example, the 1 dot VA2, 2 dot VA2 and 3 dot VA2 task conditions that were clustered into short-TPD were used to get mean accuracy and mean reaction time of an individual in short TPD.

2.2.4.4 Dispersion analysis of genetic effects

The heritability of WM has been claimed to be greater in older than in young adults (McClearn et al., 1997; Ando et al., 2001). We assessed this heritability at the single-gene level by assessing the deviation of mean performance (including accuracy and reaction time) of a COMT genotype from the mean performance across genotypes in the following steps: (1) calculate $D_{ijk} = \frac{X_{ijk} - \overline{X_{ik}}}{\overline{X_{ik}}}$, where x_{ijk} = the mean performance (i.e., accuracy or reaction time) of each COMT genotypes in an age group and memory load (i = 1, 2 or 3 dot locations, j = met/met, val/met or val/val, k = old or young), $\overline{X_{ik}}$ = overall mean performance of all COMT genotypes in this age group at each memory load (i.e. $\overline{X_{ik}} = \frac{\sum_j x_{ijk}}{3}$). There were a total of 9 D_{ijk} values in each age group, 3 for each level of load and 3 for each genotype within load. (2) The F-test was applied to compare the variance of D_{ijk} between young and older adults, using full data set. Then, student's t-test

was applied to compare the mean of D_{ijk} between young vs. older adults (with correction for unequal variance, if needed) using full data set. (3) Apply bootstrap simulation to create 1000 different random samples of the young people which were matched in size to the sample of the older people for each COMT genotype. For example, assume we have 100 young COMT met/met, 120 young COMT val/met, 110 young COMT val/val and 25 old COMT met/met, 35 old COMT val/met, 30 old COMT val/val. At each iteration of bootstrap, we randomly selected 25 young COMT met/met from 100 young COMT met/met, 35 young COMT val/met from 120 young COMT val/met and 30 young COMT val/val from 110 young COMT val/val. Thus in each iteration, we obtain young and old groups, with equal sample size. After 1000 iterations, we had tested 1000 of these cases. (4) for each bootstrap iteration, we followed step (1) to calculate D_{ijk}. After we get 9 D_{ijk} values in each age group, we first applied *F*-test to verify the equity of variance between young and older people. Then, student's *t*-test was applied to compare the mean of D_{ijk} between young vs. older adults (with corrections for unequal variance, if needed). (5) among those statistically significant bootstrap cases in *F*-test, we calculate the *F* statistic ($F = \frac{\text{var}(older\,adults)}{\text{var}(young\,adults)}$), we concluded that the magnified COMT effect was observed in older adults if $F > 1$. For statistically significant student's *t*-test cases, we concluded that the magnified COMT effect was observed in older adults if the mean of D_{ijk} in older adults is larger than the mean of D_{ijk} in young adults.

2.3 Results

2.3.1 Analysis of accuracy measures

2.3.1.1 Factor analysis

Factor analysis based on principle axis factoring and varimax rotation was applied on WM accuracy measures. Three factors were extracted from the accuracy data (Tables 1-2, see also Methods and Materials). After varimax rotation, the first factor accounted for 34% of the variance, the second factor accounted for 19%, and the third factor accounted for 13% (Table 2.1). The factor loadings for each task condition are shown in Table 2.2. The factor loadings indicated the correlations between each task condition and the factor. Communality represents the total fraction of the variance (for each task condition) that was explained by the extracted factors.

The first factor was correlated primarily with the easier Non-Match task conditions in which target-probe distance was larger, making the discrimination easier (long TPD, visual angle 4˚ and 8˚). The second factor correlated primarily with the Match task conditions that had zero target-probe distance (zero TPD, visual angle 0˚). The third factor correlated primarily with the more difficult Non-Match task conditions that had relatively short distances between target and probe (short TPD, visual angle 2˚). In some task conditions, we did observe that a single task condition had factor loadings > 0.4 on two factors. This means that both factors were correlated to some extent with those particular task conditions [e.g. both zero-TPD (Match) and short-TPD (Non-Match, difficult) were correlated with 1 dot VA2 task condition] (Table 2.2).

2.3.1.2 Omnibus mixed model repeated measure ANOVA

A 4 (target-probe distance, including 0˚) x 3 (levels of memory load) x 3 (COMT genotypes) x 2 (age groups) mixed model ANOVA was conducted on accuracy measures (Table 2.4). Figure 2.1 A-C illustrates the significant distance x memory load x COMT interactions, while D-F illustrates the significant distance x memory load x age interactions. Within short TPD, the val/val homozygotes had lowest mean accuracy at all levels of memory load (Figure 2.1 A-C). With regard to age effects, the young adults were more accurate than older adults at all levels of memory load, but only in short TPD task conditions (Figure 2.1 D-F).

2.3.1.3 Follow-up ANOVAs

Based on the results of the factor analysis on accuracy, task conditions were organized into three clusters, corresponding roughly with target-probe distance. For each cluster, a follow-up 3 (levels of memory load) x 3 (COMT genotypes) x 2 (age groups) mixed model ANOVA was conducted to assess the influence of memory load and its interactions with COMT and age on accuracy measures (Table 2.5). The three task conditions (i.e., 1 dot VA0, 2 dot VA0 and 3 dot VA0) within the zero TPD cluster were used as repeated measures in ANOVAs for the zero TPD cluster. Likewise, the three task conditions with short TPD (i.e., 1 dot VA2, 2 dot VA2 and 3 dot VA2) were analyzed in repeated measure ANOVAs for the short TPD cluster. The six task conditions with visual angle large than 4˚ were analyzed in repeated measure ANOVAs for the long TPD cluster (see Table 2.2). Figure 2.2 illustrates the interaction of memory load x COMT x age in

each cluster of task conditions. Different patterns of interactions were observed between the three clusters.

2.3.1.3.1 Zero-TPD (Match) cluster

Overall, mean accuracy decreased as memory load increased, but the effect was modulated by both COMT genotype and age (Table 2.5, Figure 2.2A). A significant memory load x age x COMT interaction was observed in this cluster ($p < 0.05$). The interaction suggested that the patterns of memory load x age interaction were different across COMT genotypes. Further 3 (levels of memory load) x 2 (age groups) mixed model ANOVA for each COMT genotype in Zero TPD showed that significant memory load x age interactions were observed only in met/met homozygotes ($p < 0.001$), in other words not in subjects with val/met ($p = 0.21$) or val/val ($p = 0.14$) genotypes. As illustrated in Figure 2.2A, the young met/met showed reduced accuracy from medium memory load (2 dot locations) to high memory load (3 dot locations), while older met/met did not show this decline.

2.3.1.3.2 Short-TPD cluster

Overall, young adults showed higher mean accuracy measures than older adults. In this cluster of task conditions, mean accuracy was modulated by memory load and COMT genotype (Table 2.5). On average, this cluster showed the lowest mean accuracy measures among the three clusters of task conditions observed in the factor analysis (Table 2.2). The 2 dot task condition showed the highest mean accuracy, while the 1 dot

and 3 dot task conditions showed lower levels (Figure 2.2B). Differential age effects on mean accuracy were prominent in this cluster ($p < 0.001$), in which the young adults generally showed higher accuracies than the older adults (Figure 2.2B).

2.3.1.3.3 Long-TPD cluster

In this cluster of task conditions, mean accuracy generally decreased with increasing memory load, regardless of COMT genotype. Significant interactions of memory load x COMT ($p < 0.001$) was observed (Table 2.5). The val/val homozygotes showed higher mean accuracy than subjects with met/met and val/met genotypes, but only when memory load was high (3 target dot locations) (Figure 2.2C).

2.3.2 Analysis of reaction time

2.3.2.1 Factor analysis

Factor analysis based on principle axis factoring and varimax rotation was applied on WM reaction time measures. One factor was extracted by factor analysis from the reaction time measures (Table 2.3, see also Methods and Materials). This factor accounted for approximately 81% of the total variance (Table 2.1). The factor loadings of each task condition showed high correlations between this factor and all task conditions. Moreover, communality showed that this factor explained at least 75% of variance in each task condition. Therefore, we labeled this factor “reaction time factor” (RTF).

2.3.2.2 Omnibus mixed model repeated measure ANOVA

We followed the same procedure in performing a 4 (TPDs) x 3 (levels of memory load) x 3 (COMT genotype) x 2 (age group) mixed model ANOVA on reaction time measures to assess the possible interactions between these variables (Table 2.6). In general, older adults used longer reaction times than young adults ($p < 0.001$).

Figure 2.3A and Figure 2.3B illustrated the significant interaction of distance x COMT x age. Figure 2.3C and Figure 2.3D illustrated the significant interaction of memory load x COMT x age. The results showed that young participants with the val/met genotype consistently showed longer reaction times than participants with val/val or met/met genotypes at all levels of TPD (Figure 2.3A) and at all levels of memory load (Figure 2.3C), though the differences were small. Conversely, older val/met heterozygotes showed the shortest reaction times compared to the other genotypes, at all levels of TPD (Figure 2.3B) and at all levels of memory load (Figure 2.3D). In addition, older val/val showed a slight but consistent trend of longer reaction times than subjects with met/met or val/met genotypes at all levels of TPD and memory load (Figure 2.3B, Figure 2.3D).

2.3.3 Characterization of speed-accuracy tradeoffs

Many cognitive tasks show speed-accuracy tradeoffs (Salthouse, 1996; Salthouse et al., 2003; Ivanoff et al., 2008; James et al., 2008). The general concept of speed-accuracy tradeoff is that the less time you allot on a task, the more errors you will get, or vice versa, the more time you allot to a task, the better you will do at that task. However, in our tasks, we found that most ages and genotypes of participants exhibited a

negative tradeoff between processing speed (reaction time) *vs*. accuracy (Figure 2.4). In other words, scatter plots of WM accuracy *vs*. reaction time gave negative best-fit slopes that were significantly less than zero. This negative tradeoff was observed in both young and older adults. Older adults showed significantly more negative slopes than young adults in short TPD and long TPD. For zero TPD conditions, older adults also showed significantly larger intercepts than young adults (Figure 2.4). Longer reaction times were associated with lower accuracies. This pattern suggests that some individuals may have used longer times to respond if they were uncertain of the answer, but did not benefit from the additional time. Although we did not emphasize the processing speed in task instruction, we did limit the reaction time to two seconds (after which the next trial was presented). Therefore, the two second limit may have influenced both the young and older adult answering patterns.

2.3.4 Dispersion analysis of genetic effects

In our data set, the number of young adults was approximately 2.3 fold higher than the number of older adults because older adults were more difficult to recruit. In Figure 2.2 A-C, the variations of mean accuracy between COMT genotypes in older adults were more pronounced than in young adults. In order to test whether this was an artifact of sample size, we followed the procedures detailed in section 2.4.4 to compare the dispersion of mean accuracy and mean reaction time between young vs. older adults. The results are shown in Table 2.7. For accuracy measures, we first used the full dataset in the *F*-test to see whether the variance between young vs. older adults were statistically

equal or unequal. Then the *t*-test was performed to compare the mean value of *D* (see section 2.4.4). Both the *t*-test and *F*-test in short TPD using the full data set differed significantly between young vs. older adults and the older adults showed higher dispersion than the young adults.

In order to verify if the dispersion was influenced by smaller sample size in older adults, we did 1000 bootstrap simulations in young adult pool to randomly select equal numbers of young adults as in older adults, within each COMT genotype. By reducing the sample size, we also reduced the statistical power. Therefore, we obtained only 19% of bootstrap iterations with a significant *t*-test and 15% with a significant *F*-test, among the 1000 bootstrap cases in short TPD. However, within significant bootstrap iterations, 100% had greater dispersion in older adults than in young adults. We concluded that COMT did have greater genetic effects on older adults' performance in short TPD task conditions, than on young adults in the same task conditions.

We thought that it could be of interest to also examine the dispersion of COMT in young vs. older adults' reaction times in these same three clusters of task conditions. For reaction time measures, both the *t*-test and *F*-test of dispersion in zero TPD and long TPD using the full data set were significantly different. After bootstrap simulation, over 81% of bootstrap iterations that showed significant *t*-tests and/or *F*-tests had greater genetic dispersion in older adults.

2.4. Discussion

Based on evidence of increased heritability of cognitive performance with age

(Ando et al., 2001) and age-related declines in signaling in dopamine neuronal pathways (reviewed in (Bäckman et al., 2006)), we hypothesized that genetic variability in the dopamine biodegradation pathway, specifically involving COMT, would exert a stronger effect on the cognitive performance of healthy older individuals compared to younger individuals. Similar results were recently reported, although limited to preservative errors and reaction time (Nagel et al., 2008). Consistent with our predictions, we observed a significant memory load x COMT x age interaction in the zero TPD cluster of task conditions. The interaction was specific to met/met individuals, and indicated that older met/met individuals had higher WM accuracy under high memory load conditions (in comparison to younger met/met on the same task). More generally, our results with accuracy measures showed that higher memory loads gave the most prominent differences and gene effects were more prominent in older adults.

Interestingly, reaction time measures gave us another point of view. At all levels of TPD and memory load, young val/met individuals consistently showed longer reaction times than individuals with val/val or met/met genotypes. Conversely, older val/met used shortest reaction times (when compared to met/met and val/val). In addition, older val/val generally used longer reaction times than older val/met and met/met at all levels of TPD and memory load. These results are consistent with mean reaction times in correct responses reported by Lindenberger and colleagues (Nagel et al., 2008).

2.4.1 Tonic and phasic hypothesis and its relation to COMT

It has been suggested that the varied relationships between COMT genotype and

cognition may be due to differences among the cognitive tasks that depend primarily on PFC dopamine signaling (Bilder et al., 2004). Conventional tests of WM, along with blood oxygen level-dependent (BOLD) functional magnetic resonance imaging (fMRI) have shown that human subjects carrying the high-activity COMT val allele show a larger area that is specifically activated during WM tasks (greater engagement of cortical resources), along with generally lower behavior task performance. Conversely, individuals homozygous for the COMT met allele show smaller areas of specific activation during WM tasks (the most focused cortical engagement) and somewhat better behavioral task performance (Egan et al., 2001; Mattay et al., 2003; Bertolino et al., 2004; Blasi et al., 2005). The tonic/phasic dopamine hypothesis suggests that these differing patterns of cortical activation correspond to either greater sensitivity to sudden-onset dopamine signaling (less accumulation of extracellular dopamine) by the high-activity val allele, or more sustained dopamine signaling (greater accumulation of extracellular dopamine) by the low-activity met allele, respectively. If so, then the val allele may be better adapted to cognitive flexibility (better performance in task switching and updating working memory traces), but less well adapted to holding information (Bilder et al., 2004; Nolan et al., 2004). Moreover, some studies have suggested the optimal dopamine signaling may be related not only to dopamine levels but also the sensitivity of dopamine receptors in PFC, which may change with age (Bäckman et al., 2006; Gosso et al., 2008).

In real life situations, the interaction of multiple elements such as the specific tasks involved (Nolan et al., 2004), emotions (Bishop et al., 2006), stress (Stefanis et al., 2007), and genetic polymorphisms in additional genes in the dopamine pathway

(Bertolino et al., 2006) may modulate the performance of the individual. This implies that the function of different COMT polymorphisms is more complex than simply one allele being good or bad, but also depends on the demands of task conditions. Moreover, under certain task conditions, heterozygotes may have a unique phenotype – for example, male mice heterozygous for a COMT mutation (knockout +/-) show unusually high aggression, even though this aggression is absent from both +/+ and -/- homozygotes (Gogos et al., 1998).

2.4.2 The relation of genetic effects, memory loads and TPD in older adults

In our data set, we found that our ranking of the performance of the various COMT genotypes tended to be more consistent (between ages) at higher memory loads (Figure 2.2 A-C). Moreover, the difference between the best-performing and the worst-performing genotypes at the highest memory loads (3 dot locations) were greater in every case in older adults than in young adults. For older people in the high memory load (3 dots locations) task conditions of short-TPD, the difference between the highest-performing genotype (val/met) and the lowest-performing genotype (val/val) was statistically significant (*t*-test, $p < 0.02$). The corresponding difference was not significant in young adults, in spite of their larger sample size. In general, the rank order of performance (WM accuracy) between genotypes was more consistent under higher memory loads (than under lower memory loads) and at higher ages (elderly people) than in young adults.

In the previous literature, positron emission tomography (PET) scans of the

activation of brain areas during a spatial WM test found that young adults showed right lateralization in their PFC, but older adults showed activation in both left and right PFC, as if they were using cortical-cortical connections to compensate in ways that were not necessary in younger adults (Reuter-Lorenz et al., 2000). The activation of a greater cortical area in older adults might also imply a somewhat greater sensitivity to the tendency of tonic/phasic dopamine signaling to further focus or expand these areas of cortical activation.

A global COMT allele frequency survey (Palmatier et al., 1999) showed that the allele frequencies of the val allele were higher (about 76%) in Africa and eastern Asia, and somewhat lower (about 50%) in Europe. The subjects used in our study were primarily European-Americans, so it is not surprising that our data showed a val allele frequency of 48.5%, which is comparable to the previous published data (Palmatier et al., 1999). Our results showed that individuals with one or more met alleles tended to have higher accuracy (than val/val homozygotes) on the short-TPD cluster, which corresponded to our most difficult task conditions. In evolutionary terms, our closest relatives (nonhuman primates) all have valine at this position of the COMT protein, and so the val allele is believed to be ancestral, in other words the met allele apparently arose relatively recently in the human lineage (Palmatier et al., 1999). The fact that individuals with one or more met alleles perform better on the most difficult task conditions suggests the intriguing possibility that the human-specific met allele might be (at least partially) favored by natural selection for certain cognitive abilities. More specifically, heterozygotes (val/met) were consistently the best in the short-TPD cluster,

in both young adults and older adults, but were also consistently the worst, in both young adults and older adults, in the somewhat easier zero TPD and easiest long TPD clusters (compared to short TPD). The zero TPD and long TPD also showed virtually identical patterns of significant interactions in our ANOVA analysis (except a significant interaction of memory load x COMT x age in zero TPD but not in long TPD). This pattern was quite distinct from the pattern observed in the short TPD cluster (Table 2.5), perhaps because short TPD tasks were the most difficult (lowest percent correct).

2.4.3 Dispersion analysis of COMT effect

Using combined criteria of (i) statistical significance of the original data set in both *t*-test and *F*-tests AND (ii) greater dispersion of COMT in older adults from the majority of significant bootstrap resampling experiments with equalized sample sizes in BOTH *t*-test and *F*-test, we concluded that the genetic effects were indeed significantly greater in older adults for short-TPD accuracy measures, as well as zero TPD and long TPD test conditions for reaction time measures (Table 2.7) (although we had to reduce the statistical power in our simulations, and the number of significant simulations was reduced accordingly). We have not attempted to rule out the possibility that random variation in cognitive performance observed among older people (i.e., individual differences due to other causes) may contribute to the separation between COMT genotypes shown here. However, in our view, a simple explanation of separation of genetic means is that the COMT polymorphism has greater effects on cognition in older people.

2.4.4 Reaction time

Slowing in the speed of information processing has been hypothesized as a major mediator of age-related differences in working memory efficiency and other cognitive functions (Kail and Salthouse, 1994; Salthouse, 1996). Our results are generally consistent with this hypothesis, as older adults had generally longer reaction times and lower accuracies than young adults, particularly in the more difficult task conditions (Figure 2.1 D-F). However, there were also allele-specific effects. Among young adults, our data showed that val/met heterozygotes used slightly (but consistently) longer reaction times than either homozygote at all distances and memory loads. Conversely, for older adults, the val/val homozygotes used longer reaction times, met/met homozygotes used intermediate reaction times, and the val/met heterozygotes had the fastest reaction times. This is comparable to the results of a longitudinal aging study, which showed that the most significant impairment associated with older val/val individuals was on the digit symbol coding task, a measure of processing speed (Starr et al., 2007). If the most impaired individuals had both the longest reaction times and the lowest accuracies, this would explain the apparent "negative speed-accuracy tradeoff" that we observed in older val/val individuals (see Results).

We also found that val/met had the highest accuracy in short TPD cluster (a group of difficult task conditions) at higher memory loads for older adults. More generally, when comparing genotypes of older individuals, we observed an apparently negative correlation between accuracy and reaction time, in that genotypes with higher accuracy tended to have shorter reaction times and vice versa, particularly under the most difficult

task conditions (short-TPD with three dot locations, Figure 2.3 C-D). Given that val/val individuals are expected to have the lowest levels of extracellular dopamine (Lachman et al., 1996), and all individuals undergo age-related declines in dopamine signaling (Bäckman et al., 2006), it is tempting to speculate that dopamine signaling may decline in older val/val individuals to a point where working memory performance is compromised. A possible connection between speed of information processing and quality of performance is that the information processed in the early stages may be lost before the later processes are completed (Salthouse, 1996). If so, then attempts to recreate this information, and/or search for the connection(s) between intermediate results, might explain the greater areas of brain activation ("lesser efficiency") seen in functional MRI studies of val/val individuals performing a WM task (Egan et al., 2001).

2.4.5 Conclusions

Our findings confirm and extend previous findings that the COMT val158met polymorphism influences spatial WM performance, including WM accuracy and reaction time. We have conducted a detailed analysis of the effects of memory load and spatial distance on performance, and their interactions with COMT genotype and age. In terms of accuracy measures, we found highly significant interactions of COMT with memory load, as well as one significant interaction of Memory load x COMT x age in zero TPD. We used dispersion analysis to confirm the increasing cognitive effects of COMT with increasing age, particularly in the discrimination of small spatial distances. We also show highly significant effects of COMT on the voluntary reaction time of individuals during

WM tasks. Age had significant effects on reaction time measures as well as significant interactions with task difficulty and COMT genotype.

Taken together, our results support the idea that different levels of COMT enzyme activity may be optimal for different tasks. These variations in the COMT gene become increasing important in cognitive performance with advancing age, and may contribute to the increasing heritability of cognitive performance with age.

Table 2.1 Eigenvalue and percent of variance explained in each factor

Accuracy				
	Initial		after rotation	
Factor	Eigenvalues	% of Variance	Eigenvalues	% of Variance
1	6.8	56.5	4.1	34.0
2	1.4	11.2	2.3	19.5
3	0.9	7.7	1.6	13.5

Reaction Time		
	Initial	
Factor	Eigenvalues	% of Variance
1	9.9	80.9

Table 2.2 Factor loadings of WM accuracy measure

Task conditions	Factor 1	Factor 2	Factor 3	Communality	Cluster name of task conditions
1-dot, VA8	0.82	#[1]	#	0.85	Long TPD
2-dot, VA8	0.80	#	#	0.82	Long TPD
2-dot, VA4	0.79	#	#	0.81	Long TPD
3-dot, VA8	0.75	0.42	#	080	Long TPD
1-dot, VA4	0.72	#	#	0.77	Long TPD
3-dot, VA4	0.40	0.56	#	0.54	Long TPD
3-dot, VA0	#	0.91	#	0.93	Zero TPD
2-dot, VA0	0.41	0.52	#	0.54	Zero TPD
1-dot, VA0	0.61	0.44	#	0.63	Zero TPD
3-dot, VA2	#	#	0.72	0.60	Short TPD
2-dot, VA2	0.42	#	0.63	0.64	Short TPD
1-dot, VA2	#	0.50	0.47	0.44	Short TPD

Task conditions are described by memory load and target-probe distance (TPD). For example, 1-dot VA2 is the task condition that memory load is 1 dot location and TPD is represented by visual angle (VA) 2˚. Values in Factor 1, Factor 2 and Factor 3 columns represented factor loading, i.e. the weighted contribution of each factor to that task condition. Communality represents the decimal fraction of variance that was explained by the extracted factors jointly.

[1] "#" indicates factor loadings less than 0.4.

Table 2.3 Factor loadings of WM reaction time measure

Task conditions	RTF	Communality
2-dot, VA4	0.95	0.89
2-dot, VA8	0.92	0.85
2-dot, VA2	0.92	0.85
1-dot, VA4	0.91	0.83
3-dot, VA8	0.91	0.83
1-dot, VA8	0.91	0.82
2-dot, VA0	0.91	0.81
3-dot, VA4	0.90	0.86
3-dot, VA0	0.89	0.79
1-dot, VA2	0.87	0.76
3-dot, VA2	0.87	0.75
1-dot, VA0	0.86	0.75

Task conditions are described by memory load and target-probe distance (TPD). For example, 1-dot VA2 is the task condition that memory load is 1 dot location and TPD is represented by visual angle (VA) 2˚. Values in Factor 1, Factor 2 and Factor 3 columns represented factor loading, i.e. the weighted contribution of each factor to that task condition. Communality represents the decimal fraction of variance that was explained by the extracted factors jointly.

Table 2.4 Mixed model ANOVA (WM Accuracy measures)

Within-subject effect				
	Effect Size[#] (η^2)	*DF*[1]	*F*[1]	*P*[1]
Distance	0.50	2	751.54	***
Distance x COMT	0.08	4	5.87	***
Distance x Age	0.02	2	33.66	***
Distance x COMT x Age	< 0.01	4	1.16	0.33
Memory load	0.36	2	366.77	***
Memory load x COMT	0.01	4	5.50	***
Memory load x Age	< 0.01	2	2.09	0.13
Memory load x COMT x Age	< 0.01	4	2.07	0.08
Distance x Memory load	0.16	4	117.67	***
Distance x Memory load x COMT	0.01	7	5.05	***
Distance x Memory load x Age	0.01	4	5.54	***
Distance x Memory load x COMT x Age	< 0.01	7	0.93	0.48
Between-subject effect				
COMT	< 0.01	2	0.17	0.84
Age	< 0.01	1	3.40	0.07
COMT x Age	< 0.01	2	0.19	0.83

* represents $p < 0.05$, ** represents $p < 0.01$, *** represents $p < 0.001$

[1]DF = Degrees of freedom, *F* = *F* statistics, *P* = *P* value

[#]Eta squared was calculated with respect to the total within subjects sum of squares (within-subject effect) or the total between subjects sum of squares (between-subject effect).

Table 2.5 Mixed model ANOVA results for each cluster of task conditions

Zero-TPD					
	Within-subject Effect	Effect Size[#] (η^2)	*DF*[1]	*F*[1]	*P*[1]
	Memory load	0.37	2	368.41	***
	Memory load x COMT	0.02	4	9.75	***
	Memory load x Age	0.01	2	7.5	**
	Memory load x COMT x Age	0.006	4	2.49	*
	Between-Subject Effect				
	COMT	< 0.01	2	0.47	0.62
	Age	< 0.01	1	0.52	0.47
	COMT x Age	< 0.01	2	0.10	0.91
Short-TPD					
	Within-subject Effect				
	Memory load	0.11	2	81.39	***
	Memory load x COMT	0.01	4	2.86	*
	Memory load x Age	< 0.01	2	1.23	0.29
	Memory load x COMT x Age	< 0.01	4	0.47	0.74
	Between-subject Effect				
	COMT	0.01	2	3.88	*
	Age	0.06	1	40.42	***
	COMT x Age	< 0.01	2	0.93	0.40
Long-TPD					
	Within-subject Effect				
	Memory load	0.22	2	205.10	***

Memory load x COMT	0.01	3	7.24	***
Memory load x Age	< 0.01	2	4.55	*
Memory load x COMT x Age	< 0.01	3	1.09	0.36
Between-subject Effect				
COMT	< 0.01	2	1.04	0.35
Age	< 0.01	1	0.04	0.84
COMT x Age	< 0.01	2	0.23	0.80

* represents $p < 0.05$, ** represents $p < 0.01$, *** represents $p < 0.001$

[1]DF = Degrees of freedom, $F = F$ statistics, $P = P$ value

[#]Eta squared was calculated with respect to the total within subjects sum of squares (within-subject effect) or the total between subjects sum of squares (between-subject effect).

Table 2.6 Mixed model ANOVA (WM reaction time measures)

	Effect Size[#] (η^2)	DF^1	F^1	P^1
Within-subject effect				
Distance	0.25	2	221.08	***
Distance x COMT	< 0.01	5	1.55	0.18
Distance x Age	0.04	2	38.46	***
Distance x COMT x Age	0.01	5	3.73	**
Memory load	0.53	2	772.08	***
Memory load x COMT	< 0.01	4	2.52	*
Memory load x Age	0.02	2	32.15	***
Memory load x COMT x Age	< 0.01	4	2.77	*
Distance x Memory load	0.15	5	108.01	***
Distance x Memory load x COMT	< 0.01	10	2.10	*
Distance x Memory load x Age	0.01	5	10.97	***
Distance x Memory load x COMT x Age	< 0.01	10	1.50	0.13
Between-subject effect				
COMT	< 0.01	2	0.91	0.41
Age	0.27	1	233.07	***
COMT x Age	< 0.01	2	2.04	0.13

* represents $p < 0.05$, ** represents $p < 0.01$, *** represents $p < 0.001$

[1]DF = Degrees of freedom, F = F statistics, P = P value

[#]Eta squared was calculated with respect to the total within subjects sum of squares (within-subject effect) or the total between subjects sum of squares (between-subject effect).

Table 2.7 Summary of bootstrap resampling

clusters	Statistical test	Significant? (full dataset)[1]	Greater dispersion in older adults? (full dataset)[3]	% of significant cases (bootstrap)[2]	% of greater dispersion in older adults[3]
Accuracy					
zero-TPD	*t*- test	N	N	1%	75%
zero-TPD	*F*-test	Y	Y	20%	46%
short-TPD	*t*- test	Y	Y	19%	100%
short-TPD	*F*-test	Y	Y	15%	100%
long-TPD	*t*- test	Y	Y	18%	90%
long-TPD	*F*-test	N	N	5%	43%
Reaction time					
zero-TPD	*t*- test	Y	Y	33%	99%
zero-TPD	*F*-test	Y	Y	37%	100%
short-TPD	*t*- test	Y	Y	10%	91%
short-TPD	*F*-test	N	N	0.6%	97%
long-TPD	*t*- test	Y	Y	28%	81%
long-TPD	*F*-test	Y	Y	11%	82%

[1] Based on the full data set in *t*-test and *F*-test. Y=yes, N=no.

[2] The percentage of cases that achieved statistical significances among all bootstrap cases.

[3]For simulations that achieved statistical significances in *F*-test, in what percentage of simulations were $F = \frac{\text{var}(older\,adults)}{\text{var}(young\,adults)} > 1$. For simulations that achieved statistical significances in student's *t*-test, in what percentage of simulation has Mean $_{\text{older adults}}$ > Mean $_{\text{young adults}}$

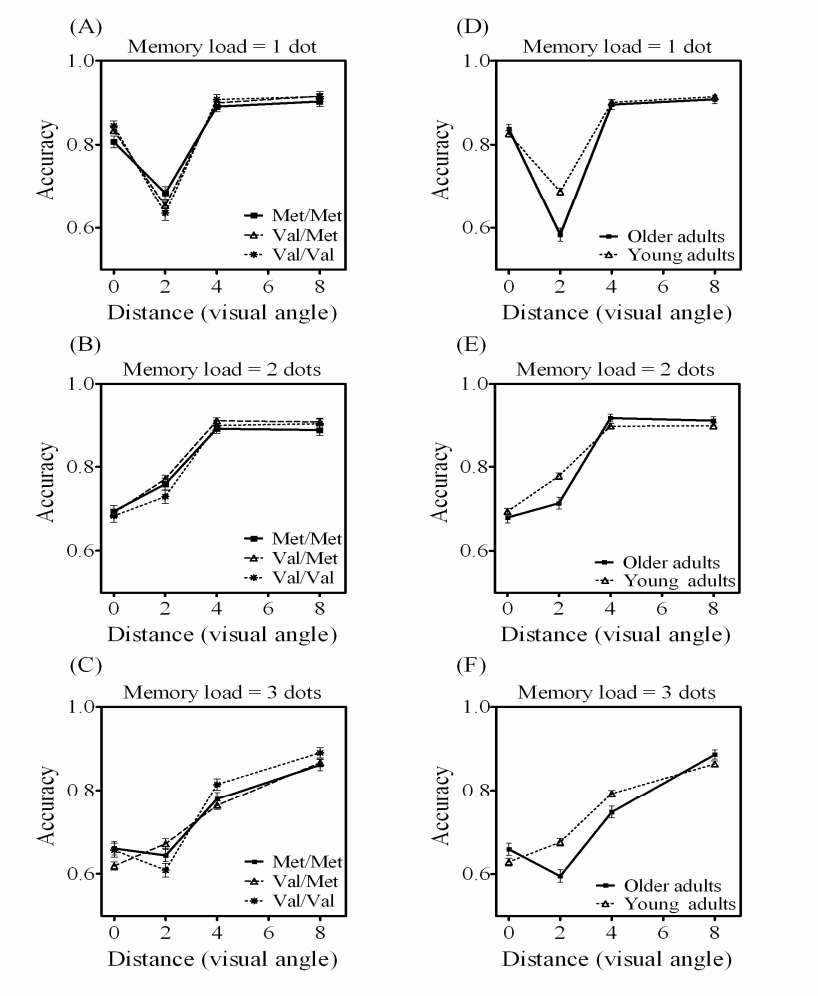

Figure 2.1 Working memory accuracy as a function of age, COMT genotype, distance (visual angle) and memory load (number of dots). **Panel (A-C)** show the mean accuracy (for all ages) as a function of distance (visual angle) and COMT genotype at various levels of memory load. **Panel (D-F)** show the mean accuracy (for all COMT genotypes) as a function of distance (visual angle) and age at various levels of memory load. The error bars represent the standard error of the mean. “Young adults” were 18-25 years old. “Older adults” were 64-89 years old.

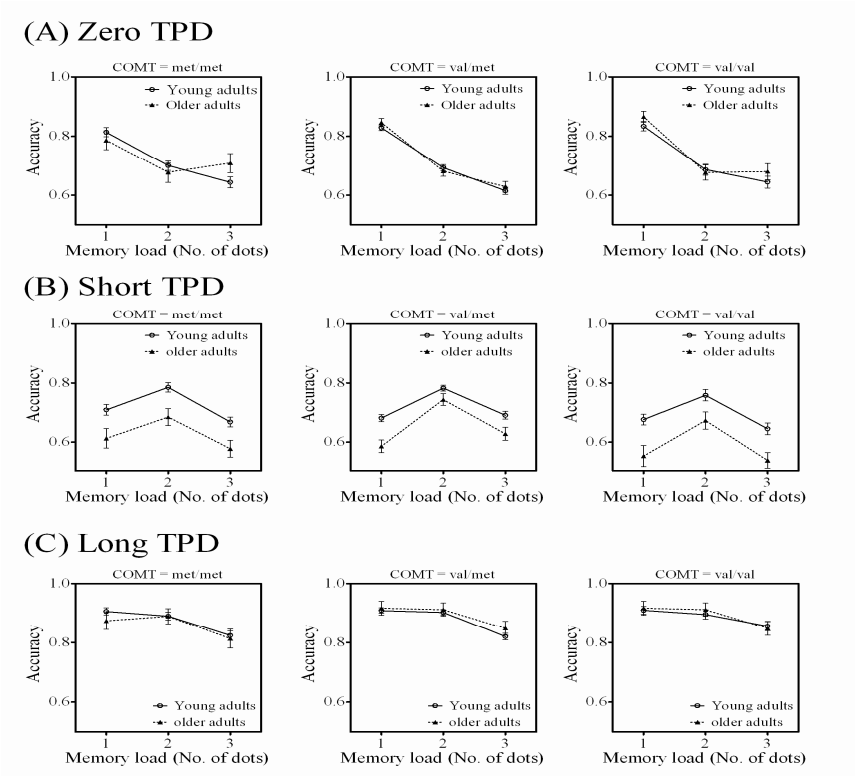

Figure 2.2 Working memory accuracy as a function of age, COMT genotype and memory load (number of dots) in each factor. The task conditions were grouped into three clusters ("factors") of related task conditions (see Tables 1-3). **Panel (A)** shows the mean accuracy as a function of memory load (number of dots) and age groups for the three COMT genotypes in zero TPD task conditions. **Panel (B)** shows the mean accuracy as a function of memory load (number of dots) and age groups for the three COMT genotypes in short TPD task conditions. **Panel (C)** shows the mean accuracy as a function of memory load (number of dots) and age groups for the three COMT genotypes in long TPD task conditions. The error bars represent the standard error of the mean. "Young adults" were 18-25 years old. "Older adults" were 64-89 years old.

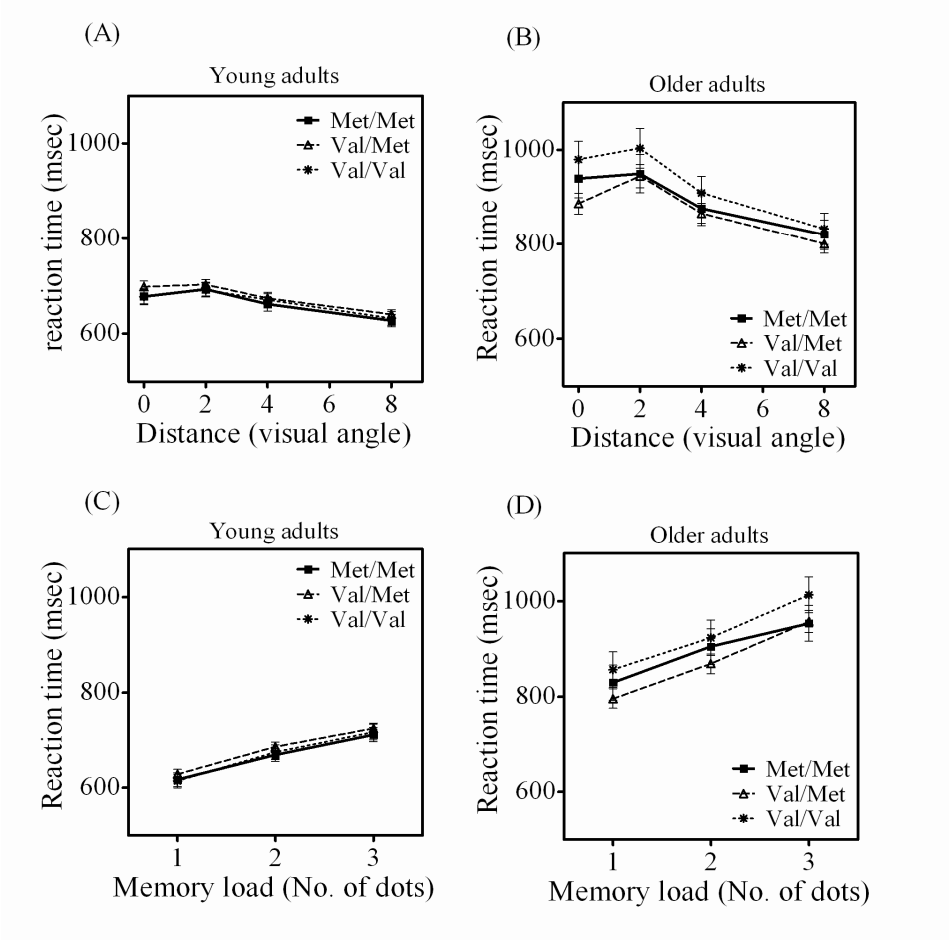

Figure 2.3 Reaction time as a function of age, COMT genotype, distance (visual angle) and memory load (number of dots). **Panel (A-B)** show the mean reaction times (across all levels of memory load) as a function of distance (visual angle) and COMT genotype for young adults and older adults. **Panel (C-D)** shows the mean reaction times (across all levels of distance) as a function of memory load (number of dots) and COMT genotype for young adults and older adults. The error bars represent the standard error of the mean. "Young adults" were 18-25 years old. "Older adults" were 64-89 years old.

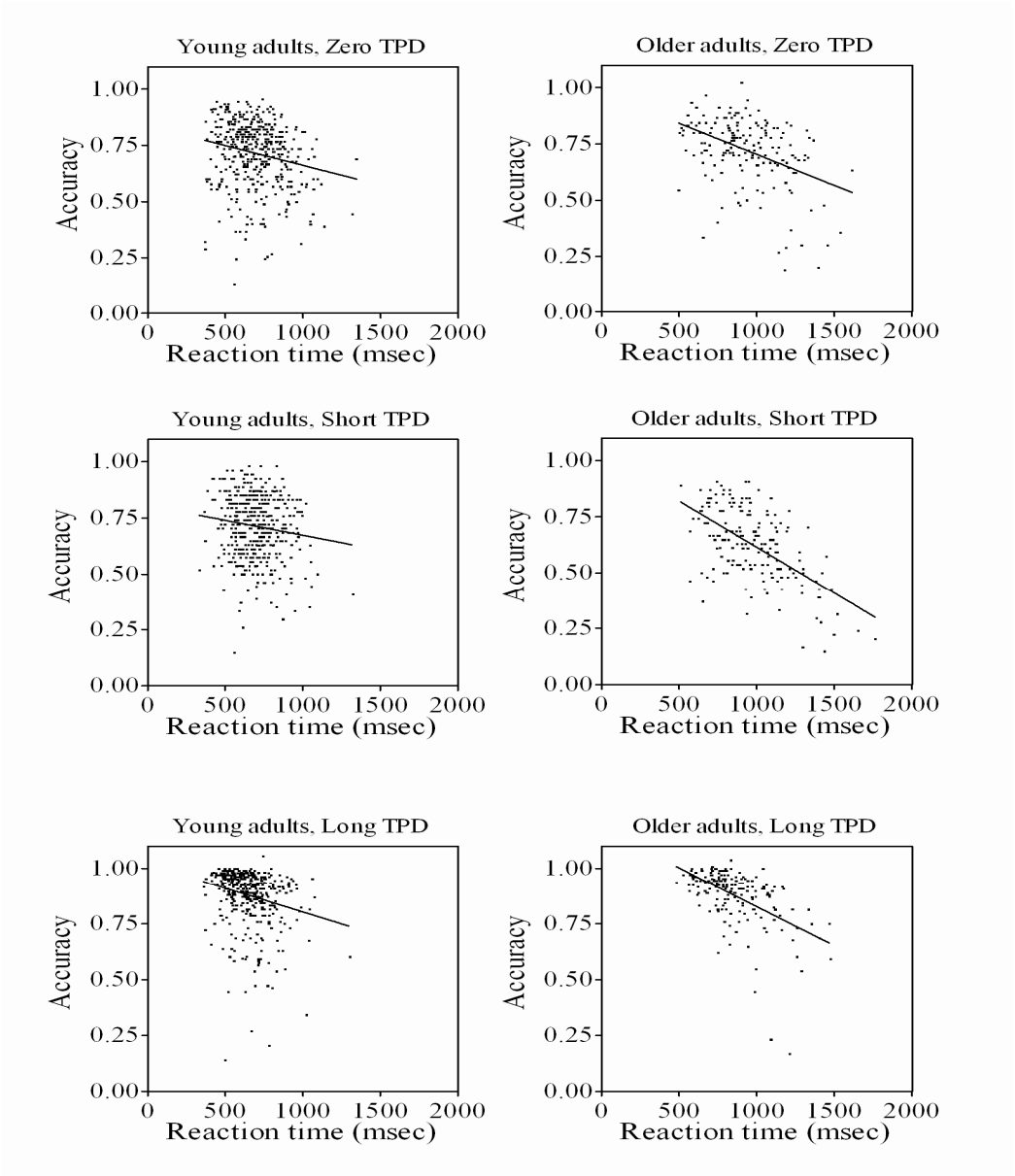

Figure 2.4 Regression analysis of accuracy vs. reaction time measures in each cluster. The Y axis is the mean accuracy measures of an individual in that cluster of task conditions. The X axis is the mean reaction time measures of an individual in that cluster of task conditions.

Chapter 3

The effect of CHRM2 on working memory and its relationship with age and task difficulty

3.1 Introduction

It is well known that the cholinergic muscarinic system plays an important role in memory processing and attention (Levin et al., 2006). This has been investigated using pharmacological methods and genetic mutant mice. Non-selective muscarinic antagonists such as scopolamine have been used to impair the acquisition of new information and to disrupt the processing of memory consolidation in normal human individuals (Drachman and Leavitt, 1974; Petersen, 1977; Jones et al., 1979; Bartus et al., 1982; Broks et al., 1988), as well as to impair performance in the tests of attention (Wesnes and Warburton, 1984; Broks et al., 1988). However, the non-selective activation or blockade of all or multiple muscarinic receptor subtypes also caused side effects which may limit the usefulness of these muscarinic drugs. In addition, these non-selective muscarinic drugs are unable to identify which specific muscarinic receptor subtypes are involved in mediating the various muscarinic action of acetylcholine (ACh). To overcome these obstacles, several groups have used gene knockout mice deficient in one or more muscarinic receptor subtypes to study the physiological role of the individual muscarinic

receptor subtypes, especially when the muscarinic actions of ACh is concerned (Hamilton et al., 1997; Duttaroy et al., 2002; Matsui et al., 2002; Struckmann et al., 2003). Animal studies carried out with the M2 receptor-deficient ($M2^{-/-}$) mice suggested that the M2 receptor is the key presynaptic muscarinic autoreceptor mediating the inhibition of hippocampal and cortical ACh release (Zhang et al., 2002; Tzavara et al., 2003). The $M2^{-/-}$ mice also exhibited significant deficits in working memory in the T-maze delayed alternation tests, as well as profound changes in neuronal plasticity studied at sliced hippocampal synapses (in vitro) (Seeger et al., 2004).

As an alternative to the gene knockout mice model, endogenous effects related to normal variation in genes controlling muscarinic receptors can be used to study ACh mediated action during cognitive tasks. Several genetic studies has reported that the cholinergic muscarinic 2 receptor (CHRM2) played key roles in facilitating cognitive functions (Jones et al., 2004; Gosso et al., 2006; Gosso et al., 2007; Ragozzino et al., 2009). Furthermore, several single nucleotide polymorphisms (SNPs) in the CHRM2 gene have been implicated in human cognitive functions (Comings et al., 2003; Dick et al., 2006; Gosso et al., 2006; Gosso et al., 2007). Among these SNPs, the CHRM2 A1890T polymorphism (rs8191992), which located in the 3' untranslated region, has been repeatedly reported to be correlated to intelligence quotient (IQ) (Comings et al., 2003; Dick et al., 2006). Comings et al. showed the rs8191992 polymorphism explained 1% variance in Full scale IQ (FSIQ) of the Wechsler Adult Intelligence Scale-revised (WAIS-R) test (Comings et al., 2003). Dick et al. reported that the rs8191992 polymorphism showed the highest influence on performance IQ (PIQ) than other SNPs

(Dick et al., 2006). The AA homozygotes of the rs8191992 polymorphism showed approximately 5 points of PIQ scores higher than the TT homozygotes, and the AT heterozygotes showed approximately 1 points of PIQ scores higher than the TT homozygotes. The PIQ in WAIS-R test is primarily a measure of fluid intelligence which refers to the ability to reason abstractly and solve novel problems (Cattell, 1987). In several studies, fluid intelligence was considered to be highly correlated to working memory, though they are not identical (Kyllonen and Christal, 1990; Kane and Engle, 2002; Conway et al., 2003). Salthouse and Pink (2008) found a correlation between working memory performance and fluid intelligence of approximately 0.7 after correction for age, although the correlation did not seem to be based on working memory capacity *per se* (Salthouse and Pink, 2008). But they do suggest that some sort of close relationship between fluid intelligence and working memory seems to exist. This evidence implicated a possible correlation between the rs8191992 polymorphism and working memory performance.

Working memory is a collection of cognitive processes that include short term storage of task relevant information and manipulation of this information to facilitate the immediate transformation of memory to action (Baddeley and Della Sala, 1996). This information manipulation process is also labeled as executive control and is highly related to cognitive processes mediated by PFC as well as striatum (Ungerleider et al., 1998; Seamans and Yang, 2004; Landau et al., 2009).

The high heritability of working memory has been independently confirmed by different groups (Johansson et al., 1999; Ando et al., 2001; Blokland et al., 2008). In

addition, the heritability of general cognitive ability is known to increase with age - from about 20% in young children to 62% in very old age (Plomin, 1986; McGue et al., 1993; McClearn et al., 1997). Thus if this rs8191992 polymorphism can influence the performance of fluid intelligence, then it is possible that this SNP may influence the individual differences in working memory performance as well, and this influence may vary with age.

In this study, we used the same spatial working memory task as described in chapter 2 and focused on the influence of task difficulty, CHRM2 rs8191992 polymorphism and age on working memory performance. Based on previous evidence that: (1) the CHRM2 rs8191992 polymorphism has substantial influences on fluid intelligence (Comings et al., 2003; Dick et al., 2006), (2) there was a substantial relationship between fluid intelligence and working memory (Engle et al., 1999; Kane and Engle, 2002; Colom et al., 2004), and (3) the genetic effect on intelligence was higher in older adults (Plomin, 1986; McGue et al., 1993; McClearn et al., 1997), we hypothesized that the interactions between task difficulty and CHRM2 rs8191992 polymorphism would be reflected in spatial working memory performance and might be more prominent in older adults.

3.2. Materials and Methods

3.2.1 Participants

The present data was collected in the context of a large study of the effect of normal genetic variation on cognitive aging. Individuals were divided into two groups

based on their age. Young adults ranged in age from 18-25 years old and older adults ranged from 64-89 years old. Each individual was genotyped for the A1890T polymorphism in the CHRM2 gene.

For working memory accuracy measures, five hundred and seventy-five individuals screened for medical and psychiatric health volunteered to participate. For young adults, the CHRM2 genotypes were AA = 118 subjects, AT = 207 subjects and TT = 86 subjects. For older adults, the CHRM2 genotypes were AA = 41 subjects, AT = 83 subjects and TT = 40 subjects. This dataset was 37 less than we had for COMT in chapter 2 because fewer participants were genotyped for CHRM2.

The self-reported racial and ethnic identities of the subjects were indicated on a questionnaire. Racial and/or ethnic identities were classified according to the NIH policy on reporting race and ethnicity (http://grants.nih.gov/grants/guide/notice~files/NOT-OD-01-053.html). Ethnic identities were reported as follows: 16 subjects in “Hispanic or Latino” group, and 559 subjects in “Not Hispanic or Latino” group. Racial identities were reported as follows: White (419 subjects), Black or African American (53 subjects), Asian (42 subjects), Native American (6 subjects), Native Hawaiian or other Pacific Islander (2 subjects). The remaining 37 subjects did not fall into any of the racial categories recognized by the NIH -- 27 subjects did not answer the question of racial identity, and 10 subjects reported multiple racial categories (4 White + Asian, 5 Pacific Islander + Asian and 1 White + African American).

Omnibus ANOVA analysis of the White group on accuracy measures showed similar results as omnibus ANOVA analysis of the entire dataset, with an exception that

the White group showed a significant CHRM2 x Age interaction (not shown) that was not observed when using entire dataset. This may be related to the main effect of age, which was highly significant for White individuals (124 older adults in White group, mean age 72.5±5.6), but was not significant for Asian (6 older adults in Asian group, mean age 68.0±3.4) or African Americans (20 older adults in African American group, mean age 68.0±6.3). We attribute the more significant main effect of age among the White group to the larger sample size and older average age in this group.

3.2.2 Task

The working memory task was the same as described in chapter 2.

3.2.3 Molecular genetics

The CHRM2 A1890T single nucleotide polymorphism (rs8191992) was assayed by a combination of nested PCR and melting-curve analysis with T_m-shift primers (Wang et al., 2005). A 300 bp DNA fragment was preamplified from genomic DNA and used as a template for second round (allele-specific) PCR on a Bio-Rad MyiQ thermal cycler, which allows automated melting temperature analysis of the PCR products. One allele-specific primer was designed with a 5' GC tail, resulting in an easily detectable increase in the melting temperature of the PCR product. The forward and reverse primers used in the first PCR were 5’ CAG TAT TAG GAG CAA TGA GA 3’ and 5’ CTT CTT TGA TTT TCT TTT TT 3’. In the second round PCR, the primer specific to the ‘A’ allele was 5’ CGC TGT ACG CAA GGG CTT CTC AA 3’, the primer specific to the ‘T’

allele was 5’ TGA AAT AGG GCT TCT CAT 3’ and the common primer was 5’ GTA ACA AAA AAG GAA CAA GG 3’.

3.2.4 Statistical analysis

Accuracy and reaction time were used as measures of WM performance. In order to assess the WM performance as functions of target-probe distance (TPD), memory load, CHRM2 genotype and age, two separate omnibus analyses were conducted: (a) factor analysis (b) omnibus mixed repeated measures ANOVA. After omnibus analyses, a follow up repeated measures ANOVA was conducted on each extracted factor.

The purpose of factor analysis was to examine whether the observed variables (e.g. WM task conditions) could be explained largely or entirely by smaller number of variables called “factors”. The method applied here was principle axis factoring (i.e., common factor analysis) in which extracted factors were based on the correlation matrix of the observed variables. Therefore, the extracted factors were based on the correlations between observed variables. After principle axis factoring, an orthogonal varimax rotation was applied to make each factor as independent as possible. Each factor represented one of the patterns among the patterns of relationships of observed variables. In order to know how many factors were needed to explain the pattern of relationships among the observed variables, both the Kaiser rule (which retains only factors with eigenvalues > 1.0) and parallel analysis with data permutation (which selects the factors whose eignevalues are greater than those obtained with comparable, but randomly permuted data) (O'Connor, 2000; Lance et al., 2006) were used. The extracted factors

may be seen as clusters of correlated task conditions that measure mostly similar cognitive functions.

In addition to factor analysis, a conventional omnibus mixed model repeated measure ANOVA was also conducted. The purpose of repeated measure ANOVA was to assess WM accuracy and reaction time measures as functions of target-probe distance (TPD), memory load, CHRM2 genotype and age group. Four levels of distance (0°, 2°, 4° or 8° of visual angle) and three levels of memory load (1, 2 or 3 target dot locations) were used as within-subject variables. The two categorical variables, CHRM2 genotype and age group, were used as between-subject variables.

In order to understand the nature of each factor, a follow up mixed model ANOVA was conducted on each factor. The tasks that were highly correlated to each factor were used in the ANOVA for that factor, with minor exceptions (see Table 3.2). After omnibus analyses, we found that TPD was the major variable that determined the way in which accuracy measures were divided into three factors. Therefore, the analysis in each TPD treated WM accuracy as a function of memory load, CHRM2 genotype and age. Memory load was used as within-subject variable, while CHRM2 genotype and age group were treated as between-subject variables.

Mauchly's test was applied in mixed model ANOVAs to assess the sphericity of the observed variables (Meyers et al., 2006). When Mauchly's test was significant (which indicated that the homogeneity of data did not hold), we used a correction of degrees of freedom to provide more conservative *F* statistics. Based on the epsilon value of the Huynh-Feldt and the Greenhouse-Geisser adjustment for degrees of freedom, when the

epsilon value was larger than 0.75, the Huynh-Feldt correction was applied. Otherwise, the Greenhouse-Geisser correction was applied to give a more conservative adjustment of degrees of freedom. An alpha value of 0.05 was used to indicate statistical significance. The statistical analyses were performed within the Statistical Package for the Social Sciences, version 15.0 (SPSS Inc., Chicago, IL).

3.2.5 Dispersion analysis of genetic effect

We followed similar procedures as in section 2.2.4, replacing COMT genotypes with CHRM2 genotypes. The detailed concept and steps were addressed in section 2.2.4.

3.3 Results

3.3.1 WM accuracy measures

3.3.1.1 Factor analysis

The results of factor analysis were similar to what we observed in chapter 2, although the data set used here had 37 fewer individuals than we used in chapter 2. Three factors were extracted from the accuracy measures (Table 3.1). After varimax rotation, the first factor accounted for 34% of the variance, the second factor accounted for 20%, and the third factor accounted for 14% (Table 3.1). Table 3.2 displays the factor loadings of each task conditions, with loadings less than 0.40 omitted for clarity. The task conditions were divided into 3 clusters as in chapter 2 (zero TPD, short TPD and long TPD).

3.3.1.2 Omnibus mixed model ANOVAs

A 4 (distances) x 3 (levels of memory loads) x 3 (CHRM2 genotypes) x 2 (age groups) mixed model ANOVA was conducted on working memory accuracy measures (Table 3.4). A significant age effect ($p < 0.05$) was observed. The young adults in general showed higher accuracies than the older adults, particularly in the visual angle 2˚ task conditions. The mixed model ANOVA also showed significant distance x memory load x age x CHRM2 ($p < 0.05$) interactions and were illustrated in Figure 3.1. This significant 4-way interaction showed that the pattern of interactions between distance and age for different memory load is not the same across CHRM2 genotypes. Further 4 (distances) x 3 (levels of memory load) x 2 (age groups) mixed model ANOVA for each CHRM2 genotype were conducted to assess the difference of distance x memory load x age patterns between CHRM2 genotypes. The AA homozygotes and the AT heterozygotes showed significant distance x memory load x age interactions ($p < 0.01$), but the TT homozygotes did not (Figure 3.1). Thus, from Figure 3.1, the patterns of interactions between distances and age were different across memory loads for the AA homozygotes and the AT heterozygotes, but not for the TT homozygotes. The AA homozygotes also showed a significant age effect ($p < 0.05$) on accuracy, which means that the young AA homozygotes generally showed higher accuracies than the older AA homozygotes (Figure 3.1A). This age effect was not observed in the AT heterozygotes or TT homozygotes.

The panels of Figure 3.1 may also be regarded as vertical columns of panels, in order to compare the effects of memory loads and distances. Each panel in Figure 3.1 can be divided into two parts: (left half) visual angle 0˚ to visual angle 4˚; (right half) visual

angle 4˚ to visual angle 8˚. In the left half of each panel, the U curves observed from visual angle 0˚ to visual angle 4˚ in low memory load (1 dot location) were not consistently observed in medium (2 dot locations) or high memory load (3 dot locations)(Figure 3.1). In the right half of each panel, the trend from visual angle 4˚ to visual angle 8˚ was nearly parallel in low (1 dot location) and medium (2 dot locations) memory loads, but tended upward in high memory loads (3 dot locations)(Figure 3.1).

3.3.1.3 Follow up mixed repeated measures ANOVA in each factor

Based on the results of the factor analysis on accuracy, task conditions were organized into three clusters, corresponding roughly with target-probe distance. For each cluster, a follow-up mixed model ANOVA [3 (levels of memory load) x 3 (CHRM2 genotype) x 2 (age group)] was conducted to assess the influence of memory load and its interactions with CHRM2 and age on accuracy measures (Table 3.5). The three task conditions (i.e., 1 dot VA0, 2 dot VA0 and 3 dot VA0) within zero TPD cluster were used as repeated measures in ANOVAs for the zero TPD cluster. Likewise, the three task conditions with short TPD (i.e., 1 dot VA2, 2 dot VA2 and 3 dot VA2) were analyzed in repeated measure ANOVAs for the short TPD cluster and the six task conditions with visual angle large than 4˚ were analyzed in repeated measure ANOVAs for the long TPD cluster (see Table 3.2).

3.3.1.3.1 Zero TPD (Match) cluster

A 3 (levels of memory load) x 3 (CHRM2 genotype) x 2 (age group) mixed model

ANOVA was conducted to assess the influence of memory load and its interactions with CHRM2 and age on accuracy measures in the Zero TPD cluster. We observed a significant memory load x age x CHRM2 interaction in the Zero TPD cluster of task conditions (Table 3.5, Figure 3.2A). This interaction means that the patterns of interaction between memory load and age were not the same across CHRM2 genotypes. Further 3 (levels of memory load) x 2 (age groups) mixed model ANOVA showed significant memory load x age interactions for the AT heterozygotes ($p < 0.001$), but not for the AA and the TT homozygotes. This means that the influence of memory loads on accuracy was age dependent, but only for the AT heterozygotes. Based on Figure 3.2A, older AT heterozygotes showed improved mean accuracy in high memory loads (3 dot locations), but improved mean accuracy in high memory load was not observed in young AT heterozygotes.

3.3.1.3.2 Short-TPD

The 3 (levels of memory load) x 3 (CHRM2 genotype) x 2 (age group) mixed model ANOVA in the short TPD cluster of task conditions did not show significant memory load x age x CHRM2 interaction, which means that the patterns of interactions between memory load and age were the same across the CHRM2 genotypes. The mixed model ANOVA showed a significant age effect ($p < 0.001$) (Figure 3.2B). The young adults generally showed higher mean accuracy than the older adults.

3.3.1.3.3 Long-TPD

The 3 (levels of memory load) x 3 (CHRM2 genotype) x 2 (age group) mixed model ANOVA in the long TPD cluster of task conditions did not show significant memory load x age x CHRM2 interactions (Table 3.5). The patterns of interactions between memory load and age were the same across the CHRM2 genotypes.

3.3.2 WM reaction time measures

3.3.2.1 Factor analysis

One factor was extracted by factor analysis from the reaction time measure (Table 3.1). This factor accounted for approximately 82% of the total variance (Table 3.1). The factor loadings of each task condition showed high correlations between this factor and reaction time in all task conditions (Table 3.2). Moreover, communality showed that this factor explained at least 75% of variance in every task condition. Therefore, we labeled this factor "reaction time factor" (RTF).

3.3.2.2 Omnibus mixed model ANOVA

We followed a procedure similar to section 3.1.2 to run a 4 (levels of distances) x 3 (levels of memory loads) x 3 (CHRM2 genotypes) x 2 (age groups) mixed model ANOVA on reaction time measures (Table 3.6). In general, older adults used longer reaction times than young adults in all task conditions (age effect, $p < 0.001$). An interesting CHRM2 x age interaction was observed ($p < 0.05$)(Figure 3.3). The older adults showed *decreasing* reaction times with an *increasing* number of T alleles. Conversely, young adults showed somewhat *increasing* reaction times with an *increasing*

number of T alleles (Figure 3.3). Regression analysis was applied to compare the slopes of the two lines in Figure 3.3, based on scatter plots of individual reaction times vs. genotypes (not shown). The results showed that the slopes were significantly different between the young and the older adults ($p < 0.01$).

3.3.3 Dispersion analysis of genetic effect

We followed similar procedures as in section 2.4.4 to assess the genetic dispersion between young vs. older adults, under the influence of CHRM2 A1890T genotypes. For accuracy measures, *t*-tests and *F*-tests using the full data set in short and long TPD showed that the dispersion of accuracies caused by CHRM2 genotypes was significantly greater in older adults than in young adults. After bootstrap resampling of WM accuracy data (to test various combinations of equal numbers of young and older adults), we found that over 88% of the cases that were significant in *t*-tests and *F*-tests had higher dispersion for older adults (than young adults) in both short and long TPD (Table 3.7). For reaction time measures, higher dispersion was also observed in older adults (than young adults) in both short and long TPD (Table 3.7). Based on the combined criteria of (i) statistical significance of original data set in both *t*-test and *F*-test and (ii) greater dispersion of older adults in the majority of significant bootstrap resampling experiments with equalized sample sizes (between young vs. older adults) in BOTH *t*-test and *F*-test, we concluded that higher dispersion of older adults than young adults, under the influence of CHRM2 A1890T polymorphism, was most significant in the non-match difficult task conditions (short TPD) as well as in the non-match easy task conditions

(long TPD).

3.4 Discussion

In the present work, we investigated spatial working memory performance as functions of TPD, memory load, CHRM2 genotypes and age groups. We found that (i) older adults with AA genotypes generally showed lower accuracies than young adults with AA genotypes ($p < 0.05$)(Figure 3.1). (ii) The older AA homozygotes showed a trend of using longer reaction times than the older TT homozygotes. Conversely, the young AA homozygotes showed a trend of using shorter reaction times than the young TT homozygotes (Figure 3.3). (iii) in zero TPD task conditions, the older AT heterozygotes showed improved accuracy in high memory loads (3 dot locations), but not observed for young AT heterozygotes. (iv) greater dispersion of accuracy and reaction time measures in older adults than young adults, under the influence of CHRM2 A1890T polymorphism, was observed in this spatial working memory task (especially in short TPD and long TPD).

3.4.1 Translational and post-transcriptional control of the 3'UTR

Our results suggested that AA homozygotes were more vulnerable to age effects than AT or the TT individuals (Figure 3.1). The exact mechanism of how A and T allele affect WM performance is not clear as yet. We can say that A is the ancestral base at this location (according to dbSNP). Because this polymorphism is located in the AU-rich region (approximately 64% AU) of 3'UTR, one possibility is that this polymorphism is

related to translational or post-transcriptional control.

Previous studies had indicated that 3'UTR may be related to the stability and localization of mRNA translation (Jansen, 2001; Mitchell and Tollervey, 2001; Mazumder et al., 2003). In terms of stability, an example in yeast may help to understand how 3'UTR affect translational control. In yeast, biochemical data showed that the proteins bound to the mRNA cap (eIF-4F) and poly(A) tail (Pab1p) are physically associated with each other in yeast extracts. In addition, this cap-poly(A) tail complex also interacts with the cap binding protein eIF-4E, resulted in an end-to-end mRNA-protein complex (Tarun and Sachs1, 1996; Mitchell and Tollervey, 2001). This mRNA secondary structure may cause early termination of protein synthesis, triggering rapid mRNA degradation and reduced synthesis of the target protein. SNPs in the 3' UTR of neurotransmitter related genes also have been reported to change the mRNA expression. For example, polymorphisms in the 3'UTR of serotonin transporter gene caused differential mRNA levels through the modulation of mRNA stability (Vallender et al., 2008).

In terms of localization, the 3'UTR may contain signals that regulate subcellular localization of mRNA. The transport of mRNA to dendrites is well accepted (Martin and Zukin, 2006). For example, long-term potential (LTP) that related to memory storage are believed to require the local synthesis of proteins at postsynaptic sites (Schuman, 1997; Schuman, 1999; Martin and Zukin, 2006). Furthermore, the rate of protein synthesis may also be regulated by the neuronal activity at synaptic site, which in term related to the synaptic plasticity (Link et al., 1995; Lyford et al., 1995; Knowles and Kosik, 1997; Muslimov et al., 1998). Several studies have suggested that mRNA localization in

dendrites or synaptic sites is not limited to a small group of proteins but covers hundreds of proteins including integral membrane proteins in dendritic layers of the hippocampus and at postsynaptic densities of hippocampal neurons (Davis et al., 1987; Tian et al., 1999; Moccia et al., 2003; Sung et al., 2004). If one of the CHRM2 A1890T alleles caused reduced expression of the M2 receptor, it may cause reduced signal strength and/or signal transduction speed.

In our results, the older AA homozygotes not only showed lower accuracies than the young AA homozygotes, but also showed longer reaction times than the young AA homozygotes in this spatial working memory task. The AT heterozygotes and the TT homozygotes did not have significant age effect across target-probe distances and memory loads (see results 3.1.2). This implies that the normal aging processes influences the ability of AA individuals to respond to the task stimulation more than AT or TT individuals.

With the high resolution positron emission tomography (PET), older adults showed a greater rate of decline of muscarinic receptors in brain regions that have a relatively high number of muscarinic receptors (Dewey et al., 1990). In those high decline rate areas, older adults (82 years old) showed approximately 50% reduction of M2 receptor binding compared to young adults (19 years old) (Dewey et al., 1990). It is not yet known how this rs8191992 SNP influences the expression of M2 receptor in older adults. However, our data suggested that age has a greater effect on the older AA homozygotes than the older AT or TT individuals.

3.4.2 Future perspectives

In our results, the AA homozygotes showed both lower accuracy and longer reaction times than the AT or the TT homozygotes in older adults. Dick et al. reported that the AA homozygotes had approximately 5 points of PIQ scores higher than the TT homozygotes (Dick et al., 2006), while the age of participants was not reported. Nevertheless, our results showed that the effects of CHRM2 on working memory increased in older adults, but the AA homozygotes paradoxically did not show better performance in older adults. Therefore, it would be interesting to do a WAIS-R test on young and older adults to see if the IQ difference of the AA homozygotes and the TT homozygotes differ in the expected direction.

There were many reports of cognitive tests of SNPs in CHRM2 gene (Comings et al., 2003; Dick et al., 2006; Gosso et al., 2006; Gosso et al., 2007). However, the cellular mechanism of how these polymorphisms effect neuronal activity is still not clear. Muscarinic neurotransmission has been implicated to play an important role in learning, attention and in Alzheimer's disease (Bartus et al., 1982; Levey et al., 1995; Levey, 1996). Thus, further research is needed in this area. Cellular and molecular studies of rs8191992 may help to elucidate the molecular mechanism (if any) of this SNP.

Table 3.1 Eigenvalue and percent of variance explained in each factor

Accuracy				
	Initial		after rotation	
Factor	Eigenvalues	% of Variance	Eigenvalues	% of Variance
1	6.8	56.6	4.0	33.7
2	1.4	11.2	2.4	19.9
3	0.9	7.7	1.7	13.8
Reaction Time				
	Initial			
Factor	Eigenvalues	% of Variance		
1	9.8	82.0		

Table 3.2 Factor loadings of WM accuracy measure

	Factor 1	Factor 2	Factor 3	Communality	Cluster name of task conditions
1-dot, VA8	0.79	#[1]	#	0.81	Long TPD
2-dot, VA4	0.79	#	#	0.78	Long TPD
2-dot, VA8	0.79	#	#	0.80	Long TPD
3-dot, VA8	0.79	#	#	077	Long TPD
1-dot, VA4	0.75	#	#	0.75	Long TPD
3-dot, VA4	0.44	0.50	#	0.49	Long TPD
1-dot, VA0	0.57	0.49	#	0.64	Zero TPD
2-dot, VA0	#	0.91	#	0.52	Zero TPD
3-dot, VA0	#	0.57	#	0.94	Zero TPD
1-dot, VA2	#	0.42	0.41	0.40	Short TPD
3-dot, VA2	#	#	0.75	0.94	Short TPD
2-dot, VA2	0.42	#	0.63	0.52	Short TPD

Task conditions are described by memory load and target-probe distance (TPD). For example, 1-dot VA2 is the task condition that memory load is 1 dot location and TPD is represented by visual angle (VA) 2˚. Values in Factor 1, Factor 2 and Factor 3 columns represented factor loading, i.e. the weighted contribution of each factor to that task condition. Communality represents the decimal fraction of variance that was explained by the extracted factors jointly.

[1] "#" indicates factor loadings less than 0.4.

Table 3.3 Factor loadings of WM reaction time measure

	RTF	Communality
2-dot, VA4	0.95	0.89
2-dot, VA8	0.92	0.85
2-dot, VA2	0.92	0.84
1-dot, VA4	0.91	0.83
3-dot, VA8	0.91	0.83
1-dot, VA8	0.90	0.81
2-dot, VA0	0.89	0.80
3-dot, VA4	0.89	0.79
3-dot, VA0	0.88	0.77
3-dot, VA2	0.87	0.76
1-dot, VA2	0.87	0.76
1-dot, VA0	0.85	0.73

Task conditions are described by memory load and target-probe distance (TPD). For example, 1-dot VA2 is the task condition that memory load is 1 dot location and TPD is represented by visual angle (VA) 2˚. Values in Factor 1, Factor 2 and Factor 3 columns represented factor loading, i.e. the weighted contribution of each factor to that task condition. Communality represents the decimal fraction of variance that was explained by the extracted factors jointly.

Table 3.4 Mixed model ANOVA (WM Accuracy measures)

Within-subject effect				
	Effect Size[#] (η^2)	*DF*[1]	*F*[1]	*P*[1]
Distance	0.54	2	697.41	***
Distance x CHRM2	< 0.01	4	1.05	0.38
Distance x Age	0.02	2	27.61	***
Distance x Age x CHRM2	< 0.01	4	0.40	0.40
Memory load	0.41	2	392.40	***
Memory load x CHRM2	< 0.01	4	0.41	0.79
Memory load x Age	< 0.01	2	2.46	0.09
Memory load x Age x CHRM2	< 0.01	4	1.03	0.39
Distance x Memory load	0.18	4	126.31	***
Distance x Memory load x CHRM2	< 0.01	7	1.76	0.09
Distance x Memory load x Age	0.01	4	6.51	***
Distance x Memory load x Age x CHRM2	0.01	7	2.20	*
Between-subject effect				
CHRM2	< 0.01	2	0.63	0.53
Age	0.01	1	5.02	*
CHRM2 x Age	< 0.01	2	1.33	0.27

[1]DF = Degrees of freedom, *F* = *F* statistics, *P* = *P* value of the *F* test

* represents $p < 0.05$, ** represents $p < 0.01$, *** represents $p < 0.001$

[#]Eta squared was calculated with respect to the total within subjects sum of squares (within-subject effect) or the total between subjects sum of squares (between-subject effect).

Table 3.5 Mixed model ANOVA results for each cluster of task conditions

Zero-TPD					
	Within-subject Effect	Effect Size[#] (η^2)	DF^1	F^1	P^1
	Memory load	0.40	2	391.39	***
	Memory load x CHRM2	< 0.01	4	0.92	0.92
	Memory load x Age	0.01	2	3.13	*
	Memory load x Age x CHRM2	0.01	4	3.08	*
	Between-subject Effect				
	CHRM2	< 0.01	2	0.30	0.74
	Age	< 0.01	1	0.01	0.97
	CHRM2 x Age	< 0.01	2	1.05	0.35
Short-TPD					
	Within-subject Effect				
	Memory load	0.13	2	74.89	***
	Memory load x CHRM2	< 0.01	4	2.05	0.09
	Memory load x Age	< 0.01	2	3.03	*
	Memory load x Age x CHRM2	< 0.01	4	1.66	0.16
	Between-subject Effect				
	CHRM2	< 0.01	2	1.61	0.20
	Age	0.07	1	39.95	***
	CHRM2 x Age	< 0.01	2	0.83	0.44
Long-TPD					
	Within-subject Effect				
	Memory load	0.30	2	246.43	***
	Memory load x CHRM2	< 0.01	3	1.15	0.33

Memory load x Age	0.01	2	8.23	**
Memory load x Age x CHRM2	< 0.01	3	2.27	0.07
Between-subject Effect				
CHRM2	< 0.01	2	0.49	0.61
Age	< 0.01	1	0.35	0.56
CHRM2 x Age	< 0.01	2	1.31	0.27

[1]DF = Degrees of freedom, *F* = *F* statistics, *P* = *P* value of the *F* test

* represents $p < 0.05$, ** represents $p < 0.01$, *** represents $p < 0.001$

[#]Eta squared was calculated with respect to the total within subjects sum of squares (within-subject effect) or the total between subjects sum of squares (between-subject effect).

Table 3.6 Mixed model ANOVA (WM reaction time measure)

	Effect Size[#] (η^2)	DF^1	F^1	P^1
Within-subject effect				
Distance	0.25	2	178.13	***
Distance x CHRM2	< 0.01	5	1.72	0.13
Distance x Age	0.04	2	25.01	***
Distance x CHRM2 x Age	0.01	5	1.38	0.23
Memory load	0.53	2	676.97	***
Memory load x CHRM2	< 0.01	4	2.35	0.06
Memory load x Age	0.02	2	37.60	***
Memory load x CHRM2 x Age	< 0.01	4	1.54	0.19
Distance x Memory load	0.15	5	105.05	***
Distance x Memory load x CHRM2	< 0.01	10	0.75	0.68
Distance x Memory load x Age	0.01	5	11.82	***
Distance x Memory load x CHRM2 x Age	< 0.01	10	1.13	0.33
Between-subject effect				
CHRM2	< 0.01	2	1.09	0.44
Age	0.27	1	233.07	***
CHRM2 x Age	0.01	2	4.23	*

[1]DF = Degrees of freedom, *F* = *F* statistics, *P* = *P* value of the *F* test

* represents $p < 0.05$, ** represents $p < 0.01$, *** represents $p < 0.001$

[#]Eta squared was calculated with respect to the total within subjects sum of squares (within-subject effect) or the total between subjects sum of squares (between-subject effect).

Table 3.7 Summary of bootstrap resampling

Task	Statistical test	Significant? (Full dataset)[1]	Greater dispersion in older adults? (full dataset)	% of significant cases (bootstrap)[3]	% of greater dispersion in older adults[2]
Accuracy					
zero-TPD	*t*- test	N	N	4%	12%
zero-TPD	*F*-test	Y	Y	10%	99%
short-TPD	*t*- test	Y	Y	23%	100%
short-TPD	*F*-test	Y	Y	12%	99%
long-TPD	*t*- test	Y	Y	17%	88%
long-TPD	*F*-test	Y	Y	20%	99%
Reaction time					
zero-TPD	*t*- test	N	N	29%	39%
zero-TPD	*F*-test	N	N	14%	25%
short-TPD	*t*- test	Y	Y	29%	90%
short-TPD	*F*-test	Y	Y	15%	95%
long-TPD	*t*- test	Y	Y	55%	96%
long-TPD	*F*-test	Y	Y	21%	99%

[1] Based on the full data set in *t*-test and *F*-test. Y=yes, N=no.

[2] The percentage of cases that achieved statistical significances among all bootstrap cases.

[3] For simulations that achieved statistical significances in *F*-test, in what percentage of simulations were $F = \frac{\text{var}(older\,adults)}{\text{var}(young\,adults)} > 1$. For simulations that achieved statistical significances in student's *t*-test, in what percentage of simulation has Mean $_{\text{older adults}}$ > Mean $_{\text{young adults}}$

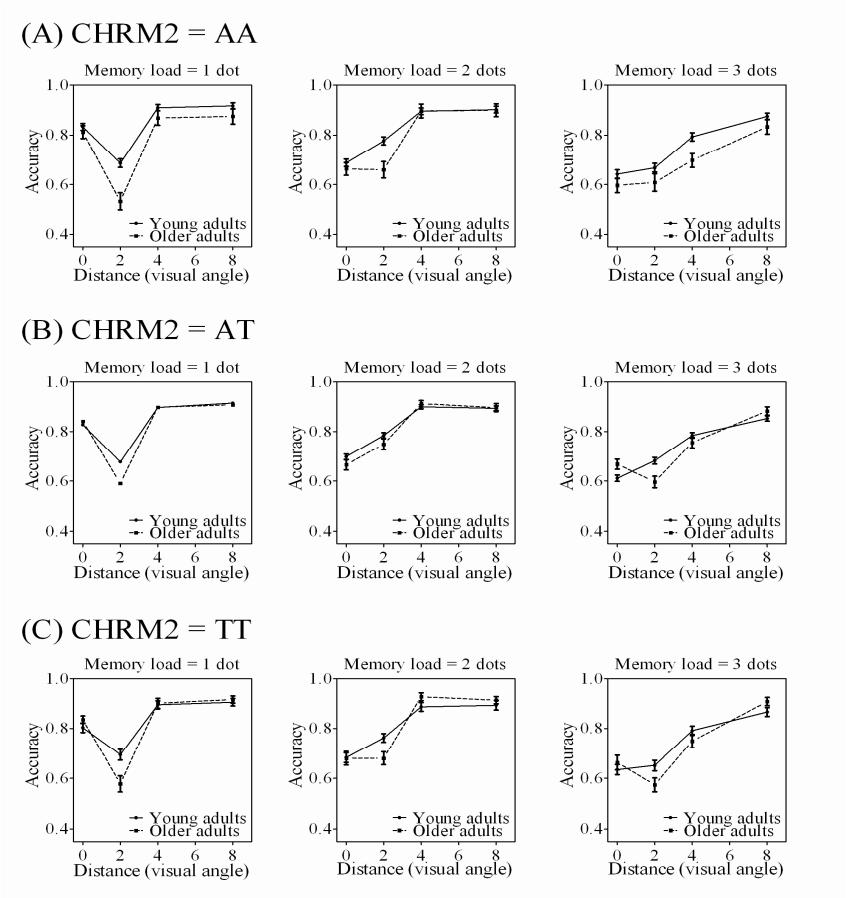

Figure 3.1 Working memory accuracy as a function of age, distance (visual angle), memory load (number of dots) and CHRM2 genotype. **(A)** the mean accuracy as a function of distance (visual angle) and age group at various levels of memory load for CHRM2 AA homozygotes. **(B)** the mean accuracy as a function of distance (visual angle) and age group at various levels of memory load for CHRM2 AT heterozygotes. **(C)** the mean accuracy as a function of distance (visual angle) and age group at various levels of memory load for CHRM2 TT homozygotes. The error bars represent the standard error of the mean. “Young adults” were 18-25 years old. “Older adults” were 64-89 years old.

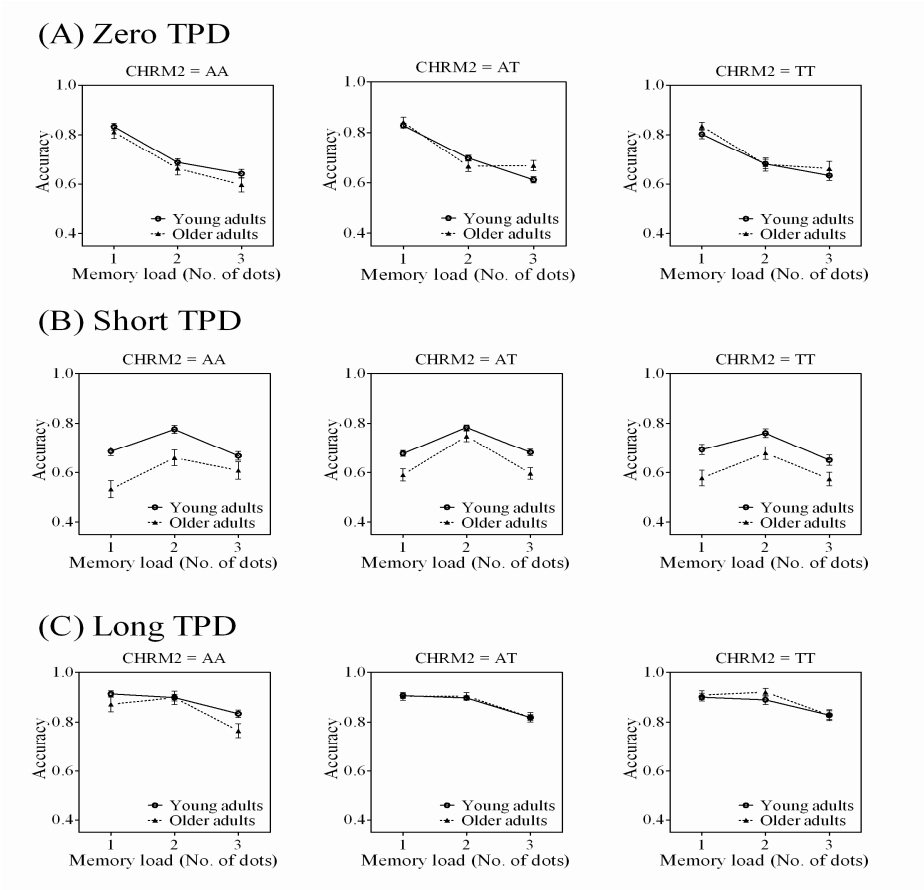

Figure 3.2 Working memory accuracy as a function of memory load (number of dot locations), age and CHRM2 genotypes. The task conditions were grouped into three clusters ("factors") of related task conditions (see Tables 1-3). Panel **(A)** shows the mean accuracy as a function of memory load and age for the three CHRM2 genotypes in zero-TPD cluster. Panel **(B)** mean accuracy as a function of memory load and age for the three CHRM2 genotypes in short TPD cluster. Panel **(C)** mean accuracy as a function of memory load and age for the three CHRM2 genotypes in long TPD cluster. The error bars represent the standard error of the mean. "Young adults" were 18-25 years old. "Older adults" were 64-89 years old.

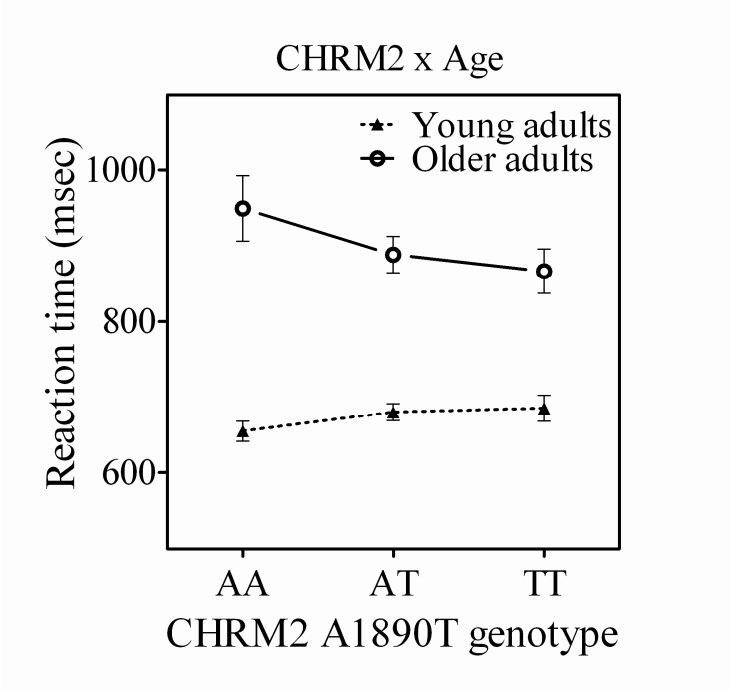

Figure 3.3 Reaction time as a function of age and CHRM2. The error bars represent the standard error of the mean. “Young adults” were 18-25 years old. “Older adults” were 64-89 years old.

Chapter 4

The interactions of CHRM2 A1890T, COMT val158met, age and task difficulty on working memory

4.1 Introduction

The dopamine (DA) system is known to play a role in cognitive processes, including working memory (Braver and Barch, 2002; Abi-Dargham, 2004). Pharmacological studies also have shown that cholinergic system played a important role in memory processing (Drachman and Leavitt, 1974; Petersen, 1977; Jones et al., 1979; Bartus et al., 1982; Broks et al., 1988), attention (Wesnes and Warburton, 1984; Broks et al., 1988), as well as working memory (Seeger et al., 2004). In chapter 2, effects related to normal variation in the COMT gene have shown the importance of dopaminergic system on the performance of spatial working memory. The COMT val158met polymorphism interacted with target-probe distances, memory loads and age to influence the spatial working memory performance. Moreover, greater effects of COMT genotypes in older compared to younger adults were observed both for accuracy measures and reaction time measures. In chapter 3, we observed that normal variation in a muscarinic receptor gene also interacted with working memory performance. Thus, it is of interest to use the COMT val158Met polymorphism and the CHRM2 A1890T polymorphism to

investigate the interaction of the dopaminergic system and the cholinergic system in working memory performance.

From the study of Dick et al., the AA homozygotes of the CHRM2 rs8191992 polymorphism showed approximately 5 points of PIQ scores higher than the TT homozygotes, and the AT heterozygotes showed approximately 1 points of PIQ scores higher than the TT homozygotes (Dick et al., 2006). In chapter 3, the CHRM2 AA homozygotes were more vulnerable to age effect (observed in reaction time measures) than individuals with AT or TT genotypes. Because the AT and the TT genotypes have shown similar IQ scores which was distinct from the AA genotype in previous study (Dick et al., 2006), and shown distinct reaction times from the AA genotype in our study, it is possible that the AT and TT genotypes provide similar influences to task performance. Thus, in this chapter we assessed the interactions between age, COMT (met/met, val/met and val/val genotypes) and CHRM2 (divided into two groups, TT or AT individuals vs. AA homozygotes) and their influence on working memory accuracy and reaction time measures. The grouping of CHRM2 genotypes will be discussed in more detail below. We hypothesized that the interactions between target-probe distances, memory loads, COMT genotypes and age will be different under the differential influences of the two CHRM2 groups.

4.2 Methods and materials

4.2.1 Participants

After excluded the data with missing values, the number of participants in each

category was as follows: (1) for young adults with at least one CHRM2 T allele, COMT met/met = 81, val/met = 151 and val/val = 57 participants; (2) for young adults with CHRM2 AA genotype, COMT met/met = 26, val/met = 68 and val/val = 23 participants; (3) for older adults with at least one CHRM2 T allele, COMT met/met = 31, val/met = 70 and val/val = 22 participants; (4) for older adults with CHRM2 AA genotype, COMT met/met = 8, val/met = 22 and val/val = 11 participants. The total number of participants was 570. Young adults were 18-25 years old. Older adults were 64-89 years old.

4.2.2 Statistical analysis

We focused on using mixed model repeated measure ANOVA to analyze the accuracy and reaction time measures in this section. Since the data we used is quite similar to what we used in chapter 3, the results of factor analysis did not change and is not shown here. An omnibus 4 (levels of target-probe distances) x 3 (levels of memory load) x 3 (COMT genotypes) x 2 (2 CHRM2 genotypes, AT or TT vs. AA) x 2 (age group, young vs. old) mixed model ANOVA was conducted on accuracy and reaction time measures. The target-probe distances and memory loads were used as within-subject variables. The grouping of CHRM2 genotypes is discussed below under results. COMT, CHRM2 and age were used as between-subject variables. A follow up mixed model ANOVA was conducted in each cluster as in chapter 2. For each cluster, a 3 (levels of memory load) x 3 (COMT genotype) x 2 (2 CHRM2 genotypes, AT or TT vs. AA) x 2 (age group, young vs. old) mixed model ANOVA was conducted. Memory load was used as within-subject variables and COMT, CHRM2 and age were used as between-subject

variables.

4.3 Results

4.3.1 Preliminary test of grouping CHRM2 genotypes

Because the AT and TT genotypes showed similar IQ scores in previous study (Dick et al., 2006) and reaction times in our results, it is possible that these two genotypes may show similar interactions with COMT. Before separating the CHRM2 into two groups (AA vs. AT + TT), we conducted a 4 (levels of target-probe distances) x 3 (levels of memory loads) x 3 (COMT genotypes) x 3 (CHRM2 genotypes) x 2 (age groups) mixed model ANOVA to assess the influence of COMT and CHRM2 on working memory performance. The results did not show any significant COMT x CHRM2 interactions among all possible combinations between distances, memory loads, COMT, CHRM2 and age.

We also tried to separate CHRM2 into two groups in several other ways (AA vs. TT, or AA+AT vs. TT), and conducted a 4 (levels of target-probe distances) x 3 (levels of memory loads) x 3 (COMT genotypes) x 2 (CHRM2 genotypes, AA vs. TT) x 2 (age groups) mixed model ANOVA on working memory performance. The results also did not show significant COMT x CHRM2 interaction. When we aggregated the CHRM2 AT and TT individuals together, many significant interactions of COMT x CHRM2 were observed. We concluded that AT and TT individuals, in addition to having similar IQ scores (Dick et al., 2006) and reaction times (chapter 3), may also have similar interactions with COMT. In any case, that assumption was used for the remaining

analysis in chapter 4.

4.3.1 Omnibus mixed model ANOVA for accuracy measures

A 4 (levels of target-probe distances) x 3 (levels of memory loads) x 3 (COMT genotypes) x 2 (CHRM2 genotypes, AA vs. AT+TT) x 2 (age groups) mixed model ANOVA was conducted on working memory accuracy measures. The results of this omnibus ANOVA is illustrated in Table 4.1. Among the significant interactions in Table 4.1, a significant distance x memory load x CHRM2 x COMT x age interaction was observed ($p < 0.05$). This complex interaction is illustrated in Figure 4.1A and Figure 4.1B. The patterns of interaction between the distances, memory loads, COMT and age were not the same across the two CHRM2 groups.

4.3.2 Mixed model ANOVA for accuracy measures in each cluster

For each cluster of task conditions, a follow-up 3 (levels of memory load) x 3 (COMT genotypes) x 2 (CHRM2 groups, AA vs. AT+TT) x 2 (age groups) mixed model ANOVA was conducted to assess the influence of memory load and its interactions with COMT, CHRM2 and age on accuracy measures. Table 4.2 showed the interactions in each cluster. The ANOVAs showed a series of significant interactions in short TPD. The short TPD represented the most difficult cluster of task conditions.

Figure 4.2 illustrated the 4 way interactions of memory load x COMT x CHRM2 x age ($p < 0.05$). In general, the COMT AT heterozygotes showed higher accuracies than the AA or the TT homozygotes in this cluster of task conditions, regardless of CHRM2

genotypes. One exception was the older CHRM2 AA + COMT val/val individuals, who showed a trend of increased mean accuracies as memory load increased.

4.3.3 Mixed model ANOVA for reaction time measures

A 4 (levels of target-probe distances) x 3 (levels of memory loads) x 3 (COMT genotypes) x 2 (CHRM2 genotypes, AA vs. AT+TT) x 2 (age groups) mixed model ANOVA was conducted on working memory reaction time measures. The results of mixed ANOVAs for reaction time measures are shown in Table 4.3. The ANOVAs showed a series of significant interactions. Figure 4.3 illustrates the 5 way interactions of distance x memory load x COMT x CHRM2 x age ($p < 0.01$).

From Figure 4.3A, the older CHRM2 AA + COMT val/met individuals and the older CHRM2 AA + COMT val/val individuals showed similar trends of variations across all levels of TPD in each level of memory load. For the trend of variations in the older CHRM2 AA, COMT met/met individuals were different from the trends of variations of the COMT val/met and the COMT val/val, across all levels of TPD in each level of memory load. Young adults did not show differences between COMT genotypes for CHRM2 AA individuals.

Interesting interactions were also observed in Figure 4.3B. For older individuals with CHRM2 AT or TT genotype, the older COMT met/met and COMT val/met individuals showed similar trends of variations, but the older COMT val/val homozygotes were different from COMT met/met and COMT val/met (Figure 4.3B).

4.4 Discussion

In this study, we observed the interactions of CHRM2 and COMT polymorphisms in a spatial working memory task. For accuracy measures in the short TPD cluster of task conditions, the older CHRM2 AA + COMT val/val individuals showed a trend of increased mean accuracies as memory load increased. The significance of this result is unclear. The sample size was relatively small (for older adults with CHRM2 AA genotype, COMT met/met = 8, val/met = 22 and val/val = 11 participants), but the profile was strikingly different from all other groups (Figure 4.2A).

For reaction time measures, under the influence of CHRM2 AA, the older COMT met/met homozygotes showed different trends of variations from the older COMT val/val and COMT val/met individuals (Figure 4.3A). Furthermore, under the influence of the CHRM2 AT or TT genotypes, the older COMT val/val showed different trends of variations from the older COMT met/met and CHRM2 AT or TT + COMT val/met individuals (Figure 4.3B).

4.4.1 Future directions

Given the evidence that COMT is abundant in prefrontal cortex (Männistö and Kaakkola, 1999; Tunbridge et al., 2006) and M2 receptor is abundant in hippocampus (presynaptic and postsynaptic) (Levey, 1993; Levey et al., 1995; Rouse et al., 1997), the interactions we observed may reflect the hippocampus to PFC pathway in working memory. Previous studies showed that the hippocampal-PFC connection is involved in a spatial delayed non-matching to position task in the radial-arm maze (Floresco et al.,

1997; Burette et al., 2000). Their findings indicated that synaptic depression of hippocampal-PFC pathway occurs during the decay of spatial working memory. It is also well accepted that the sustained firing of cortical neurons during the delay period is an essential mechanism for holding information on line for the time necessary to respond to task stimulation (Fuster, 1984; Goldman-Rakic, 1987; Fuster, 1993). The tonic-phasic dopamine hypothesis (Bilder et al., 2004) has postulated that the met allele has a better ability to maintain the current working memory representation, while the val allele was postulated to have better ability to facilitate the switching or updating of working memory traces in an ongoing behavior program. These hypotheses were related to met/met has lower enzyme activity (higher PFC dopamine level) and val/val has higher enzyme activity (lower PFC dopamine level) which may help to sustain cortical firing. Because the M2 autoreceptor helps regulate the release of acetylcholine, variations in the CHRM2 gene may increase or decrease the release of acetylcholine. Perhaps acetylcholine release is also involved in sustained neuronal firing in the hippocampal-PFC circuit.

Table 4.1 Mixed model ANOVA (WM Accuracy measures)

Within-subject effect				
	Effect Size[#] (η^2)	DF[1]	F^1	P^1
Distance	0.46	2	515.76	***
Distance x CHRM2	< 0.01	2	0.09	0.93
Distance x COMT	0.005	5	3.00	*
Distance x Age	0.02	2	20.25	***
Distance x CHRM2 x COMT	< 0.01	5	1.00	0.41
Distance x CHRM2 x Age	< 0.01	2	0.62	0.56
Distance x COMT x age	< 0.01	5	0.68	0.63
Distance x CHRM2 x COMT x age	< 0.01	5	1.12	0.35
Memory load	0.31	2	259.43	***
Memory load x CHRM2	< 0.01	2	0.06	0.93
Memory load x COMT	0.01	4	5.68	***
Memory load x Age	< 0.01	2	0.01	0.28
Memory load x CHRM2 x COMT	< 0.01	4	1.48	0.21
Memory load x CHRM2 x Age	< 0.01	2	0.56	0.57
Memory load x COMT x Age	< 0.01	4	1.70	0.15
Memory load x CHRM2 x COMT x age	< 0.01	4	1.78	0.13
Distance x Memory load	0.13	4	84.44	***
Distance x Memory load x CHRM2	< 0.01	4	1.18	0.32
Distance x Memory load x COMT	0.01	7	4.47	***
Distance x Memory load x Age	0.01	4	3.59	**
Distance x Memory load x CHRM2 x COMT	< 0.01	7	1.72	0.10

Distance x Memory load x CHRM2 x Age	0.01	4	2.52	*
Distance x Memory load x COMT x Age	< 0.01	7	1.10	0.36
Distance x Memory load x CHRM2 x COMT x Age	0.005	7	1.96	*
Between-subject effect				
CHRM2	< 0.01	1	1.16	0.28
COMT	< 0.01	2	0.51	0.60
Age	0.01	1	6.99	**
CHRM2 x COMT	< 0.01	2	1.61	0.20
CHRM2 x age	< 0.01	1	1.84	0.18
COMT x age	< 0.01	2	0.60	0.55
CHRM2 x COMT x Age	< 0.01	2	0.21	0.81

[1] DF = Degrees of freedom, *F* = F statistics, *P* = P value

* represents $p < 0.05$, ** represents $p < 0.01$, *** represents $p < 0.001$

[#]Eta squared was calculated with respect to the total within subjects sum of squares (within-subject effect) or the total between subjects sum of squares (between-subject effect).

Table 4.2 Mixed model ANOVAs in each factor

Zero-TPD				
Within-subject effect	Effect Size[#] (η2)	DF[1]	F[1]	P[1]
Memory load	0.32	2	271.42	***
Memory load * CHRM2	< 0.01	2	0.17	0.83
Memory load * COMT	0.02	4	7.22	**
Memory load * Age	< 0.01	2	2.43	0.09
Memory load * CHRM2 * COMT	< 0.01	4	1.28	0.27
Memory load * CHRM2 * Age	< 0.01	2	2.34	0.09
Memory load * COMT * Age	< 0.01	4	2.02	0.09
Memory load * CHRM2 * COMT * Age	< 0.01	4	0.84	0.49
Between-subject effect				
CHRM2	< 0.01	1	0.97	0.33
COMT	< 0.01	2	0.25	0.78
Age	< 0.01	1	0.35	0.55
CHRM2 * COMT	< 0.01	2	2.47	0.09
CHRM2 * Age	< 0.01	1	2.41	0.12
COMT * Age	< 0.01	2	0.33	0.72
CHRM2 * COMT * Age	< 0.01	2	0.33	0.72
Short-TPD				
Within-subject effect				
Memory load	0.08	2	51.48	***
Memory load * CHRM2	< 0.01	2	1.18	0.31
Memory load * COMT	0.01	4	3.69	**
Memory load * Age	< 0.01	2	1.15	0.32
Memory load * CHRM2 * COMT	0.01	4	2.64	*

Memory load * CHRM2 * Age	< 0.01	2	2.12	0.12
Memory load * COMT * Age	< 0.01	4	0.85	0.49
Memory load * CHRM2 * COMT * Age	0.01	4	3.20	*
Between-subject effect				
CHRM2	< 0.01	1	1.09	0.30
COMT	0.01	2	3.92	*
Age	0.06	1	36.32	***
CHRM2 * COMT	< 0.01	2	0.28	0.75
CHRM2 * Age	< 0.01	1	0.24	0.62
COMT * Age	< 0.01	2	1.28	0.28
CHRM2 * COMT * Age	< 0.01	2	0.39	0.68
Long-TPD				
Within-subject effect				
Memory load	0.21	2	154.34	***
Memory load * CHRM2	< 0.01	2	1.10	0.33
Memory load * COMT	0.02	3	6.42	***
Memory load * Age	0.01	2	5.84	**
Memory load * CHRM2 * COMT	< 0.01	3	.54	0.68
Memory load * CHRM2 * Age	< 0.01	2	2.78	0.07
Memory load * COMT * Age	< 0.01	3	1.27	0.28
Memory load * CHRM2 * COMT * Age	< 0.01	3	1.10	0.35
Between-subject effect				
CHRM2	< 0.01	1	0.72	0.40
COMT	< 0.01	2	0.02	0.98
Age	< 0.01	1	1.36	0.24

CHRM2 * COMT	< 0.01	2	1.49	0.23
CHRM2 * Age	< 0.01	1	1.83	0.18
COMT * Age	< 0.01	2	0.31	0.74
CHRM2 * COMT * Age	< 0.01	2	0.51	0.60

[1] DF = Degrees of freedom, *F* = F statistics, *P* = P value

* represents $p < 0.05$, ** represents $p < 0.01$, *** represents $p < 0.001$

[#]Eta squared was calculated with respect to the total within subjects sum of squares (within-subject effect) or the total between subjects sum of squares (between-subject effect).

Table 4.3 Mixed model ANOVA on WM reaction time measures

Within-subject effect				
	Effect Size[#] (η^2)	DF[1]	*F*[1]	*P*[1]
Distance	0.18	2	130.4	***
Distance * CHRM2	< 0.01	2	1.65	0.19
Distance * COMT	0.01	5	3.20	**
Distance * Age	0.03	2	19.02	***
Distance * CHRM2 * COMT	< 0.01	5	1.85	0.10
Distance * CHRM2 * Age	< 0.01	2	1.28	0.28
Distance * COMT * Age	0.02	5	5.78	***
Distance * CHRM2 * COMT * Age	0.01	5	3.62	**
Memory load	0.46	2	497.5	***
Memory load * CHRM2	< 0.01	2	0.25	0.77
Memory load * COMT	< 0.01	4	1.41	0.23
Memory load * Age	0.02	2	26.70	***
Memory load * CHRM2 * COMT	< 0.01	4	.36	0.83
Memory load * CHRM2 * Age	< 0.01	2	1.19	0.30
Memory load * COMT * Age	< 0.01	4	1.97	0.10
Memory load * CHRM2 * COMT * Age	< 0.01	4	1.34	0.25
Distance * Memory load	0.10	5	66.10	***
Distance * Memory load * CHRM2	< 0.01	5	1.06	0.39
Distance * Memory load * COMT	0.01	11	2.56	**
Distance * Memory load * Age	0.01	5	5.97	***
Distance * Memory load * CHRM2 * COMT	0.01	11	2.29	*

Distance * Memory load * CHRM2 * Age	< 0.01	5	1.02	0.41
Distance * Memory load * COMT * Age	0.006	11	2.10	*
Distance * Memory load * CHRM2 * COMT * Age	0.01	11	2.83	**
Between-subject effect				
CHRM2	< 0.01	1	2.38	0.12
COMT	< 0.01	2	2.94	0.05
Age	0.26	1	197.1	***
CHRM2 * COMT	< 0.01	2	1.78	0.17
CHRM2 * Age	0.007	1	5.58	*
COMT * Age	< 0.01	2	2.66	0.07
CHRM2 * COMT * Age	< 0.01	2	0.12	0.88

[1] DF = Degrees of freedom, *F* = F statistics, *P* = P value

* represents $p < 0.05$, ** represents $p < 0.01$, *** represents $p < 0.001$

[#]Eta squared was calculated with respect to the total within subjects sum of squares (within-subject effect) or the total between subjects sum of squares (between-subject effect).

Figure 4.1A

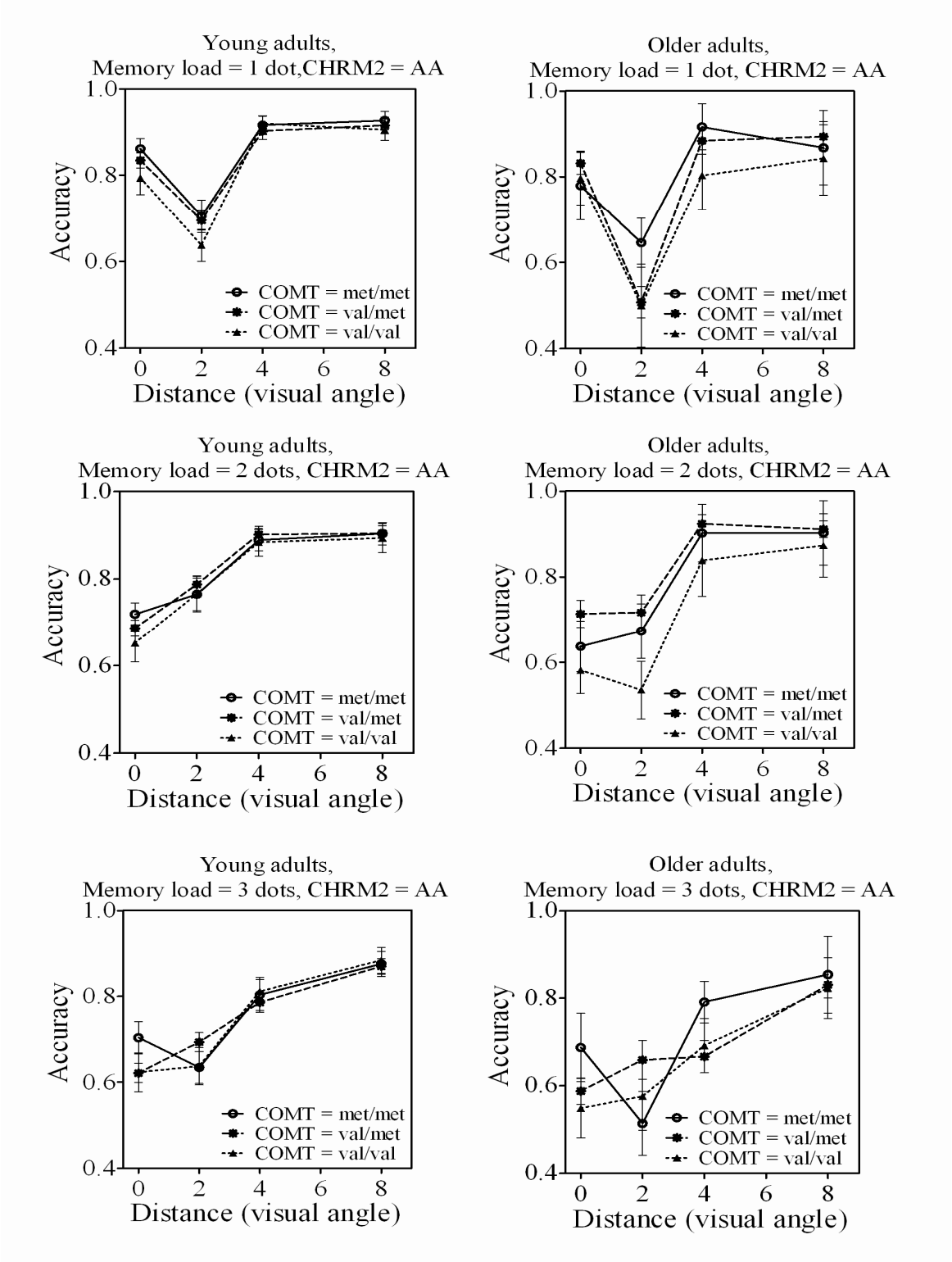
Young adults,
Memory load = 1 dot,CHRM2 = AA
Older adults,
Memory load = 1 dot, CHRM2 = AA
Young adults,
Memory load = 2 dots, CHRM2 = AA
Older adults,
Memory load = 2 dots, CHRM2 = AA
Young adults,
Memory load = 3 dots, CHRM2 = AA
Older adults,
Memory load = 3 dots, CHRM2 = AA
Accuracy
1.0
0.8
0.6
0.4
0
2
4
6
8
Distance (visual angle)
COMT = met/met
COMT = val/met
COMT = val/val

Figure 4.1B

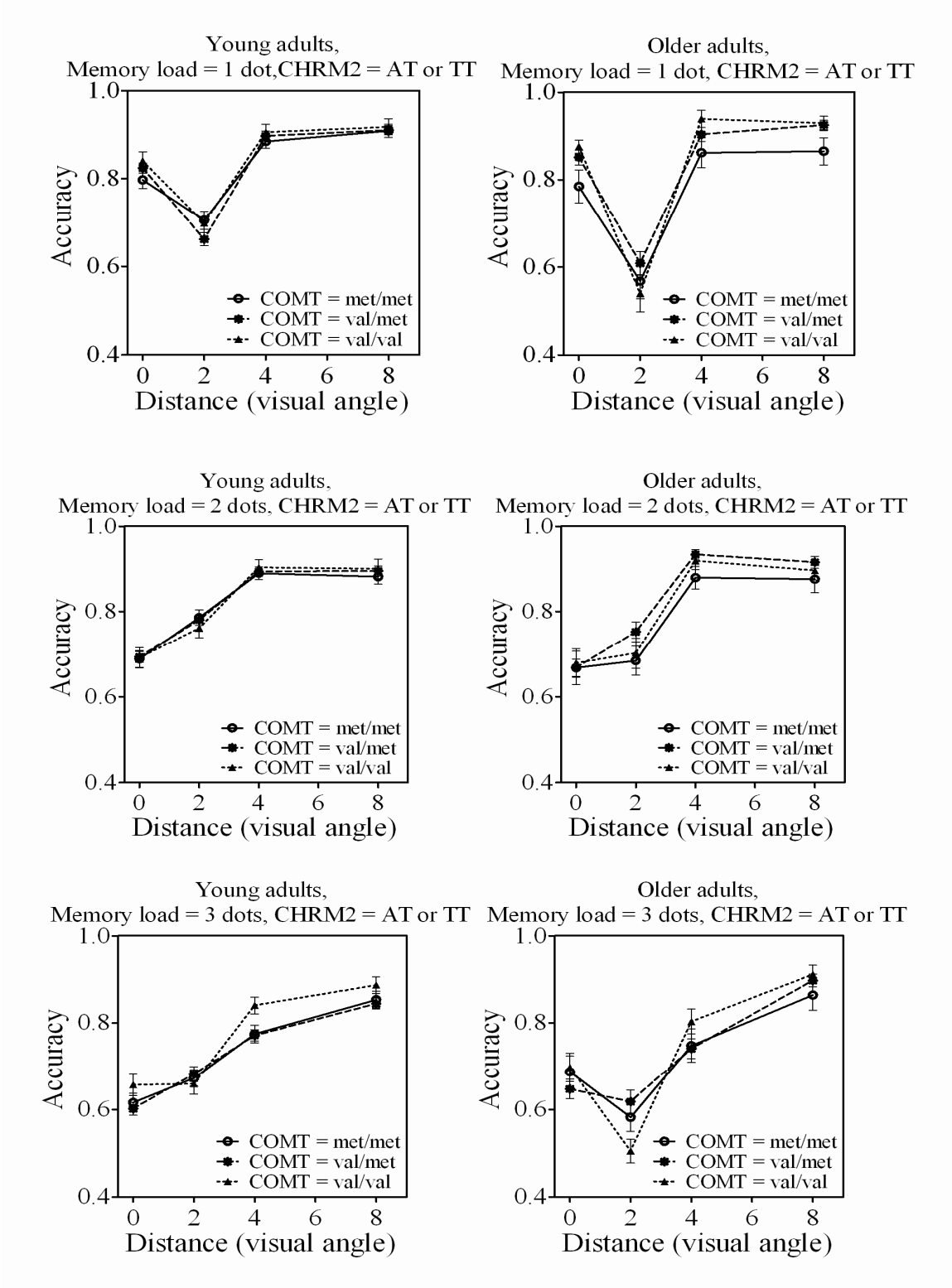
Young adults,
Memory load = 1 dot,CHRM2 = AT or TT
Older adults,
Memory load = 1 dot, CHRM2 = AT or TT
Young adults,
Memory load = 2 dots, CHRM2 = AT or TT
Older adults,
Memory load = 2 dots, CHRM2 = AT or TT
Young adults,
Memory load = 3 dots, CHRM2 = AT or TT
Older adults,
Memory load = 3 dots, CHRM2 = AT or TT
Accuracy
1.0
0.8
0.6
0.4
0
2
4
6
8
Distance (visual angle)
COMT = met/met
COMT = val/met
COMT = val/val

Figure 4.1 Accuracy as functions of TPD, memory load, COMT, CHRM2 and age. **Panel (1A)** shows the interactions of TPD, memory load, COMT and age, when CHRM2 genotype is AA. **Panel (1B)** shows the interactions of TPD, memory load, COMT and age, when CHRM2 genotype is AT or TT. The number of participants in each category is listed in section 4.2.1. The error bars represent the standard error of the mean.

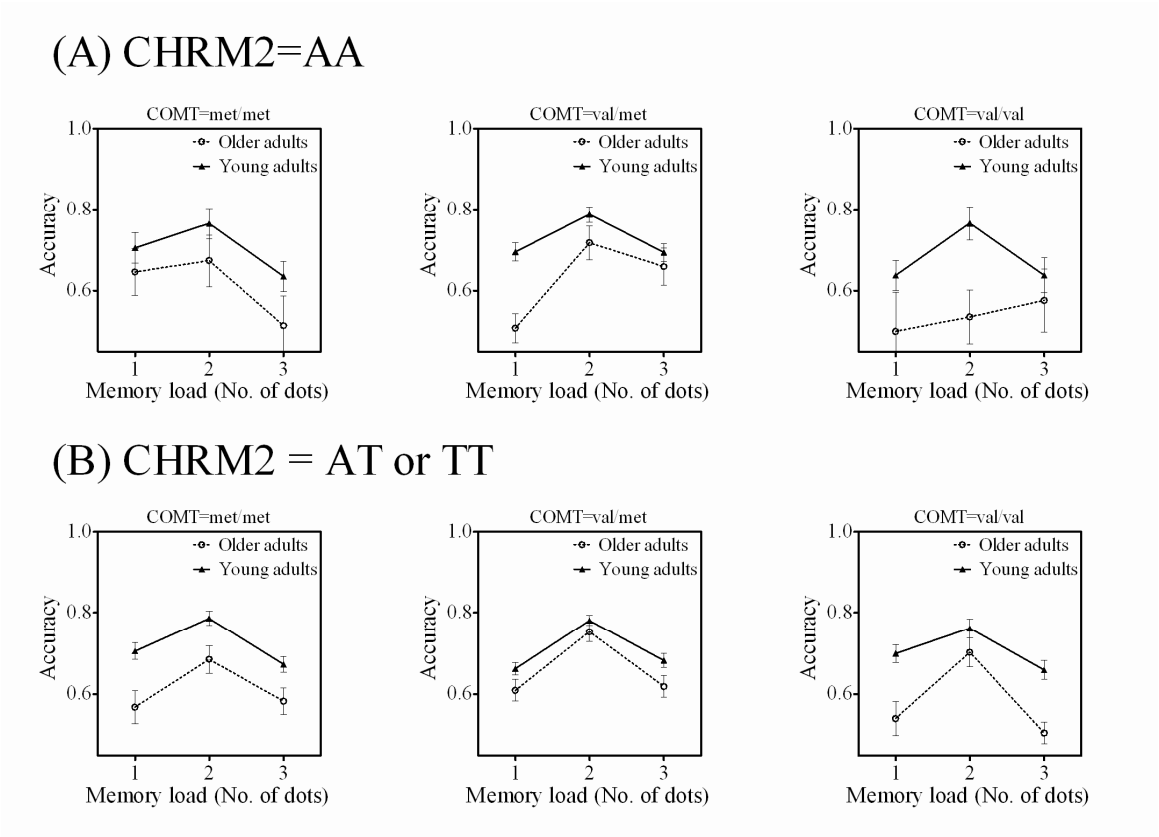

Figure 4.2 Accuracy as Functions of memory load and age. **Panel (A)** shows the accuracy measures as functions of memory load, COMT genotype and age, for individuals with CHRM2 AA genotype. **Panel (B)** shows the accuracy measures as functions of memory load, COMT genotype and age, for individuals with CHRM2 AT or TT genotypes. The number of individuals with each genotype is listed in section 4.2.1. The error bars represent the standard error of the mean.

Figure 4.3A

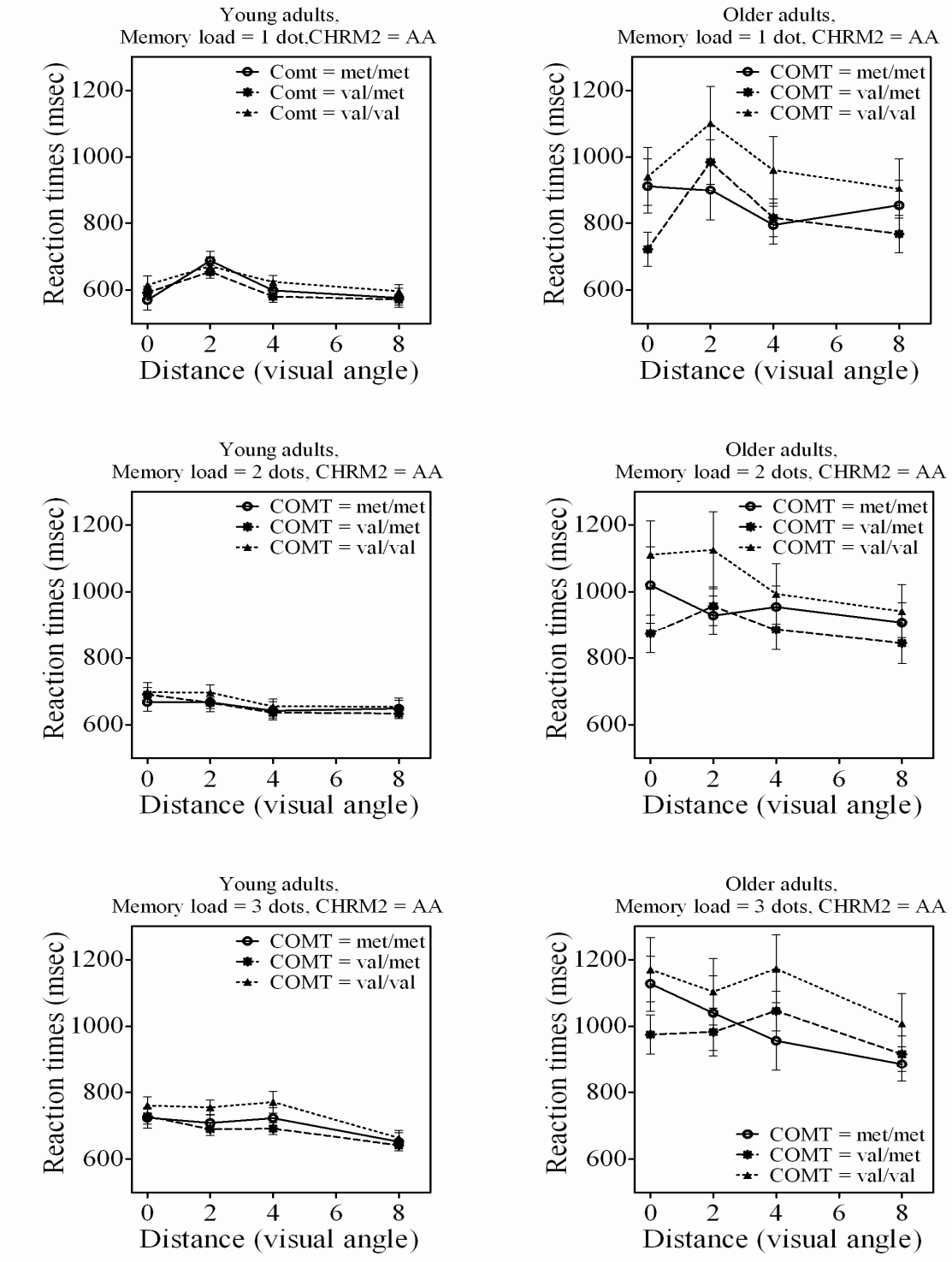
Young adults,
Memory load = 1 dot,CHRM2 = AA
Comt = met/met
Comt = val/met
Comt = val/val
Reaction times (msec)
Distance (visual angle)
Older adults,
Memory load = 1 dot, CHRM2 = AA
COMT = met/met
COMT = val/met
COMT = val/val
Young adults,
Memory load = 2 dots, CHRM2 = AA
Older adults,
Memory load = 2 dots, CHRM2 = AA
Young adults,
Memory load = 3 dots, CHRM2 = AA
Older adults,
Memory load = 3 dots, CHRM2 = AA

Figure 4.3B

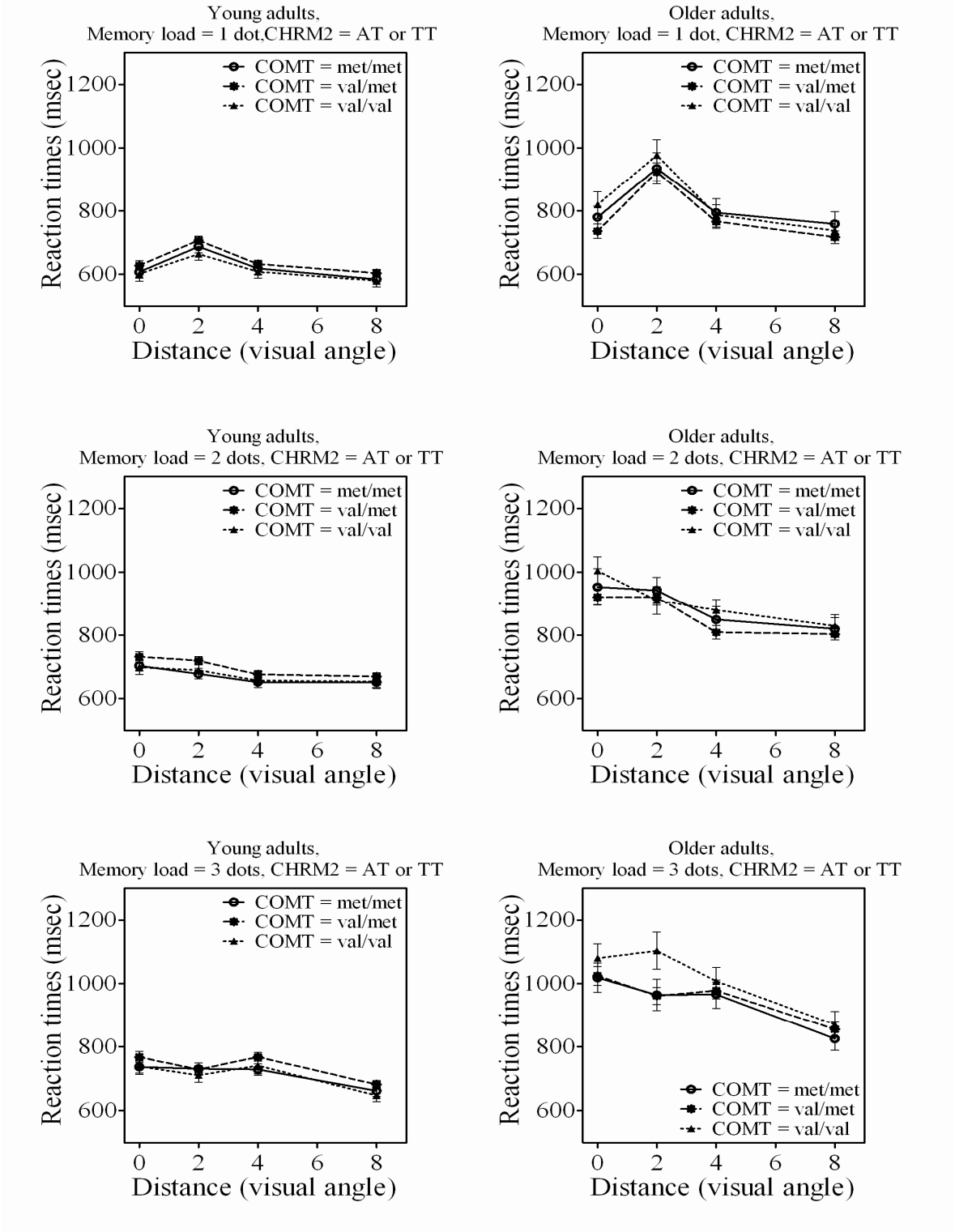
Young adults,
Memory load = 1 dot,CHRM2 = AT or TT
Older adults,
Memory load = 1 dot, CHRM2 = AT or TT
Young adults,
Memory load = 2 dots, CHRM2 = AT or TT
Older adults,
Memory load = 2 dots, CHRM2 = AT or TT
Young adults,
Memory load = 3 dots, CHRM2 = AT or TT
Older adults,
Memory load = 3 dots, CHRM2 = AT or TT
COMT = met/met
COMT = val/met
COMT = val/val
Reaction times (msec)
1200
1000
800
600
0
2
4
6
8
Distance (visual angle)

Figure 4.3 Reaction time measures as functions of TPD, memory load, COMT, CHRM2 and age. **Panel (1A)** shows the interactions of TPD, memory load, COMT and age, when CHRM2 genotype is AA. **Panel (1B)** shows the interactions of TPD, memory load, COMT and age, when CHRM2 genotype is AT or TT. The number of participants in each category is listed in 4.2.1. The error bars represent the standard error of the mean.

Chapter 5

General discussion and conclusions

Our results displayed the complex interactions between task difficulty, genotype and age (e.g., the variant performance of individuals with COMT met/met, val/met and val/val under different task difficulties). This complexity reflected the fact that working memory performance is an integrated phenotype contributed by many genes and each gene only contributes a small part to the variance of performance (Plomin and Kovas, 2005). Thus, the SNPs we used here are probably not the only cause of variation in spatial working memory performance. In this chapter, we will focus on why we adopted factor analysis and repeated measure ANOVAs to analyze these weak interactions. Because we observed that aging is a general cause of longer reaction times, regardless of the genotype, we will also briefly discuss the interactions between reaction time measures and normal aging.

A. The rationale of factor analysis

In this study, we applied two omnibus analyses on reaction time and accuracy measures of working memory performance. One is factor analysis and the other is repeated measures ANOVA. One may ask why we use factor analyses in addition to the

more traditional repeated measures ANOVA? What are the benefits of factor analysis in this study? We will discuss that point in the following sections.

Factor analysis is used to express the observed variables as a linear combination of a smaller number of unobserved variables called “factors”. Our factor analysis is based on principle axis factoring (PAF), which is a method based on the correlation matrix of variables (Meyers et al., 2006). PAF is mathematically similar to principle component analysis (PCA). The difference between PAF and PCA can be explained in two respects: conceptually and mathematically.

Conceptually, PCA derives a principle component that is a linear combination of several observed variables (e.g., accuracy measures in some task conditions). Therefore, PCA is simply a dimension reduction method used to derive principle components that can be used to explain most of the variances in the data.

Instead, PAF is more like a causal modeling method to derive the underlying constituents that can be used to explain the variance in the observed variables (Meyers et al., 2006). When we applied PAF, an observed variable (e.g., accuracy measures in a task condition) is considered to be a linear combination of the factors plus error terms. In our case, these factors may reflect unknown cognitive functions.

Mathematically, the difference between PAF and PCA is that PAF considers only the variance shared among a set of observed variables while PCA considers the total variance of a set of observed variables (Widaman, 1993). In other words, PAF postulates that the variance in a variable can be divided into unique variance (belongs the variable itself) and shared variance (common variance between variables) while PCA considered

that total variance is unique to the variable itself only. In a real world case, if we assume that the reliability of measured variables is in the range of 0.6 to 0.85 and adequate for research purposes, that would be equivalent to accepting the proportion of error variance of a measured variable in the range of 0.15 to 0.4. As a result of its emphasis on shared variance, PAF may effectively avoid the measurement errors in empirical data. Conversely, PCA may require more accurate measurement of variables (Widaman, 1993).

In the preliminary test of the accuracy and reaction time measures of this working memory task, the pairwise correlation (Pearson's correlation coefficient) between each task condition was moderate to high (0.4 – 0.8, data not shown). Therefore, the correlation between each task condition was not negligible. We believe PAF is a better method than PCA in this case.

The major concerns in statistics are (1) difference, such as ANOVA to assess the difference of means between different groups; (2) correlation, such as linear regression to assess the relation between variables. Just as other methods in the data mining field are used to find correlations between different objects (e.g., pattern recognition), factor analysis with PAF is used here to find the correlations between observed variables (i.e., measures in different task conditions). In other words, we assume there are some common basic factor shared between participants that caused their performance to be similar under certain task conditions. Clustering these task conditions is similar to the concept of clustering in microarray analyses, though the methods used were different (Lee and Williams, 2008).

B. Aging, working memory and reaction time

Older adults may use different brain areas to achieve comparable performance as young adults. At the cognitive level, our understanding of aging related memory processing indicates that working memory does have substantial association with normal aging (Cabeza and Nyberg, 1997). For example, positron emission tomography (PET) scans of the activation of brain areas during a spatial working memory test found that young adults showed right lateralization in their PFC, but older adults showed activation in both left and right PFC, as if they were using cortical-cortical connections to compensate in ways that were not necessary in younger adults (Reuter-Lorenz et al., 2000). In addition, during verbal working memory tasks, young adults showed left PFC lateralization while older adults showing bilateral activation (as in spatial working memory task)(Reuter-Lorenz et al., 2000). Therefore, these observations suggest (1) lateral dissociation of verbal and spatial working memory in young adults; (2) older adults may need to use more brain areas than young adults to achieve comparable performance (Cabeza et al., 1997). These results provided some evidence of aging after completion of brain development.

Before the completion of brain development, the visual components and spatial components of working memory mature at different times (Logie and Pearson, 1997; Logie, 2003; Pagulayan et al., 2006). With regard to child development of working memory, Pagulayan and colleagues conducted a study of spatial working memory on a group of 7-14 year old children and young adults (mean age = 21 years old) (Pagulayan et al., 2006). They observed that spatial working memory capacities increases with

maturation throughout childhood and the upper development plateau of working memory capacities was reached in early adolescence. Logie and Pearson studied a group of children age ranged from 5-12 years old on the developmental speed of visual and spatial components of working memory. They observed that the maturation of temporary storage capacity of visual information occurs before maturation of spatial information (Logie and Pearson, 1997; Logie, 2003). The same pattern of results was also observed by Pickering and colleagues (Pickering et al., 2001). These results suggested that visual working memory matured before spatial working memory and began in early childhood.

One of the important measurements of working memory performance is reaction time. Salthouse had proposed two reaction time hypotheses to explain the possible mechanisms of performance reduction in general cognition during aging (Salthouse, 1996). The first is the limited time hypothesis. The basic idea underlying the limited time hypothesis is that early steps in cognition use relatively larger amounts of time in older adults, and so the later processes were unable to be processed before time was up. This hypothesis is primarily applicable when the restricted external time is available or when concurrent demands on processing were needed. In other words, the slower speed on execution caused fewer processes to be completed in a limited time. The second mechanism is the simultaneity hypothesis. This hypothesis is based on the idea that the product of early processes was lost when the later processes is needed. The lost of relevant information may be caused by many reasons (e.g. decay, displacement, or frustration caused by wrong answer).

In our working memory task, the decision time was two seconds. If we adopted

the limited time hypothesis in this case, that would mean that two seconds was too short for all processes to be completed, in which case we should see a positive trade off for accuracy vs. reaction time. In other words, people who used longer reaction times should have better accuracy. However, in our regression analysis of accuracy vs. reaction time measures, a negative tradeoff was consistently observed.

The main postulate of the simultaneity hypothesis is that the availability of information is subject to decay, displacement or external interferences. Thus, based on the simultaneity hypothesis, longer reaction times in each sub-process could reduce the availability of previous information. If so, then longer reaction times should be correlated with lower accuracies. Therefore, the simultaneity hypotheses could explain the negative trade-off between accuracy and reaction time that we observed in spatial WM tasks.

Another alternative explanation of the negative trade-off (between accuracy and reaction time) could be based on differences in the rate of memory decay itself. Memory decay could lead to additional memory retrieval processes, in attempt(s) to recover the necessary information or integrate several pieces of residual memory together. An increased number of memory retrieval processes may increase the reaction time, and would also be correlated with lower accuracy if the additional rounds of memory retrieval were unsuccessful. Strictly speaking, the “simultaneity hypothesis” is distinct from this “memory decay” hypothesis because the latter does not (necessarily) require differences in processing speed. However, these two hypotheses are not mutually exclusive, and both may contribute to cognitive aging.

As an example from our genetic results, we found that older CHRM2 AA

homozygotes used longer reaction times and had lower accuracies than young CHRM2 AA homozygotes. In this case, the aging process may produce a negative trade-off (between speed and accuracy), at least in CHRM2 AA homozygotes. On the other hand, for the other CHRM2 genotypes, we did consistently observe longer reaction times among the older adults, but we did not observe any consistent age effect on accuracy (which may be lower or higher in older adults, depending on task conditions, see Chapter 3, Figure 3.1). Young adults did tend to perform slightly better on the most difficult task conditions (short TPD) (see Chapter 3, Figure 3.2).

According to the "memory decay" hypothesis, an increased number of memory retrieval attempts could lead to the activation of additional brain areas, which if successful could eventually reconstruct the correct (accurate) response. In other words, the "memory decay" hypothesis may explain why some older adults manage to maintain a good working memory capacity on most task conditions but still show dramatically slower response times. It may also explain why older adults show a tendency to activate additional brain areas during certain cognitive tasks (Reuter-Lorenz et al., 2000; Egan et al., 2001).

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