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Effect of the *BDNF V166M* polymorphism on working memory in healthy adolescents

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Brain-derived neurotrophic factor (BDNF) may play a role in modulating memory function and there is growing evidence that the *BDNF V166M* polymorphism may influence episodic memory in humans. However, previous association studies examining this polymorphism and working memory are inconsistent. The current study examined this association in a large sample of adolescent twin-pairs and siblings (785 individuals from 439 families). A range of measures (event-related potential, general performance and reaction time) was obtained from a delayed-response working-memory task and total association was examined using the quantitative transmission disequilibrium tests (QTDT) program. Analyses had approximately 93–97% power ($\alpha = 0.05$) to detect an association accounting for as little as 2% of the variance in the phenotypes examined. Results indicated that the *BDNF V166M* polymorphism is not associated with variation in working memory in healthy adolescents.

Keywords: delayed-response, event-related potentials, genes, QTDT, twins

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Brain-derived neurotrophic factor (BDNF) belongs to a family of highly conserved polypeptide growth factors and is an important component in the normal development of the central nervous system (CNS) and in modifying CNS structure and function in adults (Bibel & Barde, 2000; Huang & Reichardt 2001). In humans, it may play a role in the development of neurological conditions such as epilepsy (Scharfman 2005) and in psychiatric disorders such as schizophrenia and bipolar disorder (Fanous *et al.* 2005; Neves-Pereira *et al.* 2002).

Extensive studies in rats indicate that BDNF is essential for some forms of learning and memory (Yamada *et al.* 2002) and suggest an involvement in working memory. For instance, Mizuno *et al.* (2000) reported that infusion of antisense BDNF oligonucleotide (which was associated with a significant reduction of BDNF mRNA and protein levels in the hippocampus) impaired spatial memory formation, retention and/or recall in rats. Furthermore, frontal cortex BDNF levels are reported to correlate negatively with the number of working-memory errors in aged rats, but not young rats (Bimonte *et al.* 2003), and in Ts65D mice (an animal model of Down syndrome) (Bimonte-Nelson *et al.* 2003).

A growing number of studies suggest a role for BDNF in the function of human memory. Egan *et al.* were the first to examine the *V166M* polymorphism in the 5′ pro-region of the human BDNF protein in relation to human memory and hippocampal function (Egan *et al.* 2003) – this polymorphism has also been widely studied for a range of neurological and psychiatric conditions (e.g. Hall *et al.* 2003; Karamohamed *et al.* 2005). Egan and colleagues showed that relative to *Val*, the *Met* allele was associated with a qualitatively different hippocampal response, as assayed with functional magnetic resonance imaging during performance of an N-back working-memory task. More specifically, a robust and reliable hippocampal deactivation is normally produced in these tasks (Meyer-Lindenberg *et al.* 2001) and was observed in *Val/Val* individuals, but an abnormal pattern of increased activity was observed in *Val/Met* individuals. A subsequent study by Hariri *et al.* (2003) examined hippocampal and cortical function during the encoding and retrieval of novel, complex scenes – a task previously associated with increased hippocampal activation (Stern *et al.* 1996). They found hippocampal activity to be reduced in *Met*-allele carriers compared to *Val/Val* individuals during both encoding and retrieval. In addition to these observations of disrupted hippocampal function, reduced grey matter volumes for the hippocampus and dorsolateral prefrontal cortex (both regions known to subserve working memory (Fuster 1997)), have been reported for *Met*-allele carriers compared to *Val* homozygotes (Bueller *et al.* 2006; Pezawas *et al.* 2004; Szeszko *et al.* 2005).

Egan *et al.* also examined the association between the *V166M* polymorphism and a range of memory phenotypes (episodic, semantic, working) (Egan *et al.* 2003). They found

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an association with episodic memory [as assessed with the WMS but not the CVLT (Table 1)], such that *Met/Met* individuals performed poorly compared to *Val*-allele carriers. This was found in controls alone and also in their entire sample, which comprised individuals with schizophrenia, their siblings and controls. However, they found no association between the *BDNF* genotype and either semantic or working memory.

As shown in Table 1, a number of studies have since found supporting evidence for an association between the *V166M* polymorphism and measures of episodic memory such that poorer episodic memory is found in *Met*-allele carriers compared to *Val* homozygotes (Dempster *et al.* 2005; Echeverria *et al.* 2005; Hariri *et al.* 2003; Tan *et al.* 2005), although there are some inconsistencies based on sample type (e.g. healthy vs. schizophrenia) and a lack of association was reported by Strauss *et al.* (Strauss *et al.* 2004). In contrast to the findings of Egan et al. (Egan *et al.* 2003), evidence of an association between working memory and the *BDNF V166M* polymorphism has since been reported (Echeverria *et al.* 2005; Rybakowski *et al.* 2003, 2006), although once again there are some inconsistencies.

Overall, the evidence suggests that the human *V166M* polymorphism may influence episodic memory performance but with fewer studies, and with the largest study reporting a lack of association (Egan *et al.* 2003), the evidence for an effect on working memory is less convincing. The present study therefore examined the association between the

Table 1: Studies investigating associations between the *BDNF V166M* polymorphism and memory phenotypes

Study	Sample (Age)	Memory phenotype (Test/Test Battery)*	Finding
Egan *et al.* (2003)	203 with schizophrenia 305 siblings 133 healthy controls (18–60 years)	Episodic (WMS) Episodic (CVLT) Semantic (WCST) Working (WCST)	*Met/Met* associated with worse scores for episodic memory obtained from WMS, but not CVLT. No association found with semantic or working memory.
Hariri *et al.* (2003)	64 healthy participants (30.9 $\pm$ 1.3 years *Val/Val*, 30.3 $\pm$ 1.6 years *Val/Met* & *Met/Met*)	Episodic (encode/retrieve fMRI paradigm)	*Met* allele associated with an increased number of recognition errors.
Rybakowski *et al.* (2003)	54 with bipolar (18–72 years, mean 46 years)	Working (WCST)	*Met* allele associated with significantly worse scores.
Strauss *et al.* (2004)	63 with major depression or dysthymic disorder (18.4 $\pm$ 2.5 years)	Episodic (WMS) Episodic (VPALT)	No association found.
Dempster *et al.* (2005)	92 with schizophrenia 114 healthy relatives (17–85 years)†	Episodic (WMS)	*Met* allele associated with significantly worse score in relatives.
Tan *et al.* (2005)	108 with schizophrenia	Episodic (WMS)	*Met/Met* associated with significantly worse scores.
Rybakowski *et al.* (2006)	111 with bipolar (18–72 years, mean 43 years) 129 with schizophrenia (18–65 years, mean 27 years) 92 healthy controls (19–58 years, mean 31 years)	Working (WCST)	*Met* allele associated with significantly worse scores in bipolar patients for three of five subtests. No differences found for schizophrenia or control groups.
Echeverria *et al.* (2005)	194 male dentists 233 female dental assistants (all with chronic low-level mercury exposure)	Episodic (BEES) Working (BEES)	*Met* allele associated with 1 Poorer episodic memory in males but not females, and 2 Reduced working memory performance in females but not males – although result was in expected direction.

*WMS, Wechsler Memory Scale; CVLT, California Verbal Learning Test; WCST, Wisconsin Card Sorting Test; VPALT, Verbal Paired-Associate Learning Test; BEES, Behavioral Evaluation for Epidemiologic Studies.

†Based on demographics reported in Toulopoulou *et al.* (2003)

Note: Episodic and semantic memory are both types of declarative memory. Some of the studies listed above (i.e. Echeverria *et al.* 2005; Hariri *et al.* 2003; Strauss *et al.* 2004) describe their memory phenotype as declarative rather than episodic as shown here. Declarative memory requires conscious recall, with episodic memory based on one's own experiences and semantic memory based on knowledge learned (Lezak 1995). Episodic and semantic memory are both types of long-term memory, while working memory is a type of short-term memory.

V166M polymorphism and working memory in a large sample, with greater power than previous studies to detect association in healthy individuals. Furthermore, in addition to measures of overall performance and reaction time, P300 and slow-wave event-related potential (ERP) measures of brain function (all obtained from a delayed-response working-memory task) were examined.

It has been suggested that the hippocampus may be an important generator of the P300 (Fushimi *et al.* 2005; Halgren *et al.* 1980; Nakajima *et al.* 1995; Okada *et al.* 1983). Thus, as the *BDNF V166M* polymorphism has been associated with hippocampal function during an N-back working-memory task (Egan *et al.* 2003), it may also be associated with variation in the P300 elicited during a delayed-response working-memory task. Similarly, the *V166M* polymorphism has been associated with variation in grey matter volume of the dorsolateral prefrontal cortex (Pezawas *et al.* 2004) and this may be reflected in P300 and slow-wave measures recorded over the prefrontal brain region. Therefore, it was hypothesized that the P300 and the prefrontal slow wave may be associated with the *V166M* polymorphism. ERP measures have generally shown little association with behavioural working-memory performance (Hansell *et al.* 2005) and studies have generally found no association between behavioural measures of working memory and the *V166M* polymorphism in healthy individuals (Egan *et al.* 2003; Rybakowski *et al.* 2006). Consequently, it was hypothesized that no association would be found between the behavioural measures of working memory and the *BDNF V166M* polymorphism.

Materials and Methods

Participants

BDNF genotypes and working-memory phenotypes were available for 785 adolescents (385 males and 400 females) from 439 families. These families comprised 193 dizygotic (DZ) twin-pair families with no other siblings, 42 families with a DZ twin-pair plus one other sibling, seven families with a DZ twin pair and two further siblings, six families with three non-twin siblings, 36 families with two non-twin siblings, and 155 single-participant families. [Note that for families with monozygotic (MZ) twin pairs, only one co-twin was included in this study.)] *BDNF* genotypes were also available for both parents for 292 of these families, for the father only in 26 families, for the mother only in 91 families, and for neither parent in 30 families.

Working-memory data were recorded as part of an ongoing study of cognitive function (Wright & Martin, 2004; Wright *et al.* 2001). Ethics approval for this study was obtained from the Human Research Ethics Committee at the Queensland Institute of Medical Research. Written, informed consent was obtained from all participants as well as from a parent or guardian. Participants were excluded if the parental report indicated a history of head injury, neurological or psychiatric conditions, substance abuse/dependence, and/or medication with significant CNS effects. All participants were instructed to avoid caffeine-containing foods and drinks in the 2 h before their visit. Testing occurred as close as possible to the participants' 16th birthday and for those in the present study, age ranged from 15.7 to 22.3 years (mean 16.4 years, SD 0.7 years), with 60 individuals aged 15 years at the time of testing, 649 aged 16 years, 47 aged 17 years, 20 aged 18 years, four aged 19 years, two aged 20 years, and three aged 22 years. Participant ancestry and allele frequencies are shown in Table 2. Of the *Val* homozygotes, 268 were male (mean 16.3 years, SD 0.7, range 15.7–22.3) and 252 were female (mean 16.4 years, SD 0.8, range 15.7–22.1). Similarly, for the *Val/Met* genotype, 103 were male (mean 16.4 years, SD 0.7, range 15.7–20.4) and 124 were female (mean 16.3 years, SD 0.5, range 15.7–18.7). Of the *Met* homozygotes, 14 were male (mean 16.2 years, SD 0.1, range 16.0–16.4) and 24 were female (mean 16.2 years, SD 0.2, range 15.9–16.8).

Working-memory task

Both ERP and behavioural performance measures were obtained from a computerized visuo-spatial delayed-response task, which has been described previously (Hansell *et al.* 2001). Briefly, ERP and behavioural data were collected while participants completed a task that required them to remember the location of a visual target. During each trial, participants were required to focus on a central fixation dot to reduce eye movement. Two hundred and fifty milliseconds after fixation onset, a single target (checkered dot, 1.5° visual angle) was presented peripherally. Target presentation was brief in memory trials (150 milliseconds), but in sensory control trials, the target remained on-screen until target location was indicated. Target presentation was followed by a 1- or 4-second delay. In 50% of memory and sensory trials a distracting stimulus (identical to the target but differing in location) was presented for 150 milliseconds during the delay period. The timing of the presentation of the distracting stimulus was random within the interval 300–700 milliseconds post target onset. The disappearance of the fixation dot signalled the end of the delay period and was the cue for participants to lift their preferred hand from a touch-sensitive pad and to indicate target location with a rubber-tipped pointer. In total, eight trial-type variations were presented (memory/sensory × distractor presence/absence × delay 1 second/4 seconds).

ERP recording and processing

ERPs were recorded from 15 scalp locations (Fp1, Fp2, Fz, F3, F4, F7, F8, Cz, C3, C4, Pz, P3, P4, O1, O2) using the Electrocap system. However, in this instance only data recorded at prefrontal (Fp1, Fp2) and parietal (Pz, P3, P4) sites was examined because studies in humans and primates

Table 2: Distribution of genotypes and alleles for the *V166M BDNF* variant by ancestry

Ancestry	Number of genotypes (%)			Number of alleles (%)	
	Val/Val	*Val/Met*	*Met/Met*	*Val*	*Met*
British Isles (≥50%, $n = 598$)	406 (68)	165 (28)	27 (4)	977 (82)	219 (18)
Northern Europe (≥50%, $n = 99$)	60 (61)	33 (33)	6 (6)	153 (77)	45 (23)
Mediterranean (≥50%, $n = 42$)	25 (60)	14 (33)	3 (7)	64 (76)	20 (24)
Unknown (>50%, $n = 46$)	29 (63)	15 (33)	2 (4)	73 (79)	19 (21)
Total ($n = 785$)	520 (66)	227 (29)	38 (5)	1267 (81)	303 (19)

Northern European ancestors were from Britain, Denmark, Finland, the Netherlands, France, Poland, Germany, Austria and Switzerland. Mediterranean ancestors were from Italy, Greece, Yugoslavia, Portugal and Turkey.

have shown enhanced prefrontal and parietal activation during spatial working memory tasks (Batuev *et al.* 1985; Fuster 2001; Rowe *et al.* 2000). Impedances were kept below 5 Ω and linked ears served as reference. Eye movements and blinks were monitored through the placement of electrodes on the supra-orbital ridge and the outer canthus of the left eye. The electro-oculogram (EOG), Fp1 and Fp2 were amplified 5000 times and remaining EEG channels 20 000 times by Grass pre-amplifiers, with a band pass of 0.01–100 Hz. ERPs were sampled at 250 Hz from 100 milliseconds before fixation point onset to 200 milliseconds post fixation point offset and monitored on-line. EEG data exceeding 50 μV root mean squared (RMS) were automatically rejected. Eye blink artefacts were removed using a computerized algorithm developed by examining eye blinks during electroencephalogram (EEG) recordings and using those records as a digital template to detect and eliminate similar patterns from the recordings.

Following artefact rejection, trials were averaged separately for each trial type using a pre-target baseline of 350 ms. The acceptance criteria required that EOG/EEG rejections be less than 40% and that behavioural rejections (too slow, too fast, or spatially incorrect) be less than 30%. Data not meeting these criteria were visually inspected and accepted if the waveforms did not show significant drift and appeared stable (i.e. waveforms from the 1-second delay trials were comparable to those collapsed over the 1-second and 4-second delay trials).

ERP phenotypes

Slow-wave average amplitude, P300 average amplitude, and P300 latency were extracted from the electrophysiological data. Slow-wave average amplitudes were computed for the interval 650–1150 milliseconds post target onset (i.e. the last 500 milliseconds of the 1-second delay period). For the present analyses, slow-wave amplitudes recorded during memory trials in which a distracting stimulus was presented were examined. This trial type was chosen because working memory processes may be better reflected in distractor compared to non-distractor trials (Engle *et al.* 1999). The mean number of trials averaged for each individual was 62.9 (SD 16.4, range 11–94). Note that recording the slow wave over delay periods resulted in longer trials than is typical for ERP studies, leading to an increased possibility of trial rejection because of eye blinks and other artefacts.

P300 data were collapsed over all trial types because the delay task was not designed to differentiate P300 measures by trial type, and visual inspection of waveforms and preliminary analyses indicated no P300 amplitude or latency differences for trial type (Wright *et al.* 2002). P300 average amplitude was examined for the interval 150 to 450 milliseconds (post target onset). The mean number of trials per individual was 277.0 (SD 57.8, range 59–376) for P300 average amplitude and 277.7 (SD 57.3, range 59–376) for P300 latency. A detailed description of P300 latency detection has been previously published (Wright *et al.* 2002).

Preliminary analyses showed high correlations between ERPs recorded at prefrontal sites (0.95–0.97) and between ERPs recorded at parietal sites (0.85–0.93). Consequently, mean prefrontal and mean parietal measures were examined for slow-wave average amplitude, P300 average amplitude and P300 latency. Note that correlations between prefrontal and parietal measures were moderately low, ranging from 0.36 to 0.43 (Table 3).

Non-ERP phenotypes

Two measures of overall performance (Winnings, Trials Correct) and two measures of reaction time (Initiation Time, Movement Time) were examined. Winnings is the total amount of money won through participation in the delayed-response task – i.e. each correct response was rewarded 2–10 cents, dependent upon accuracy, and each incorrect response (inaccurate, too slow, too fast) was penalized 5 cents. Responses on all trial types contributed to Winnings. Only memory trials were used in the computation of Trials Correct, Initiation Time, and Movement Time. Trials Correct is the percentage of trials within pre-specified accuracy and time constraints (after trials affected by electro-oculo artefacts, as measured by EOG, are removed). Initiation Time is the latency between fixation offset and break of hand contact

Table 3: Means, standard deviations and Pearson correlation coefficients for the Working Memory Phenotypes*

	Prefrontal			Parietal			Wins ($)	Trials Correct (%)	ITime (ms)	MTime (ms)
	SW (μV)	P3 Amp (μV)	P3 Lat (ms)	SW (μV)	P3 Amp (μV)	P3 Lat (ms)				
n	661	660	626	655	661	627	754	775	774	775
Mean	−0.2	1.1	302	−2.5	1.2	308	25.74	78	381	676
SD	8.6	4.5	54	6.7	4.0	59	5.13	14	39	90
Prefrontal										
P3Amp	0.72									
P3Lat	0.27	0.05								
Parietal										
SW	0.40	0.31	0.20							
P3Amp	0.28	0.36	−0.08	0.67						
P3Lat	0.08	0.00	0.42	0.21	0.05					
Wins	−0.06	0.10	−0.44	−0.13	0.07	−0.10				
TCorrect	−0.06	0.12	−0.49	−0.13	0.12	−0.10	0.90			
ITime	0.00	−0.05	0.05	0.03	0.01	0.08	−0.16	−0.10		
MTime	−0.06	0.02	−0.16	−0.08	−0.06	−0.05	0.21	0.13	−0.08	

*Slow-Wave Average Amplitude (SW), P300 Amplitude (P3Amp), and P300 Latency (P3Lat)
Recorded at prefrontal and parietal sites, Winnings (Wins), Trials Correct (TCorrect), Initiation Time (ITime), and Movement Time (MTime)
Analyses are not corrected for twin/sibling relatedness.

with the response pad. Movement Time is the latency between break of contact with the response pad and the screen touch time. For a more detailed description of Trials Correct see Luciano *et al.* (2001) and for Winnings, Initiation Time, and Movement Time, see Luciano *et al.* (2004).

Zygosity determination and genotyping

Zygosity for same-sex twin pairs was determined using a commercial kit (AmpFISTR Profiler Plus Amplification Kit, Applied Biosystems, Foster City, CA, USA), which was further cross-checked with blood group and other phenotypic data. This method has an overall probability of correct assignment of greater than 99.99% (Nyholt 2006). Using the genotyped data, GRR (Graphical Representation of Relationships; http://bioinformatics.well.ox.ac.uk/grr) and Relpair were subsequently used to confirm zygosity determination.

Genotyping of single nucleotide polymorphisms (SNP) was performed using primer extension on the Sequenom MassArray system as described previously (James *et al.* 2004). The *V166M* polymorphism is identified in the dbSNP public database (http://www.ncbi.nlm.nih.gov/dbsnp/) as rs6265. Genotyping error rates were determined by replicate typing of several SNPs on over 3000 individuals and was <0.1% (James *et al.* 2004). The rs6265 SNP was multiplexed with seven other SNPs for unrelated projects which allowed additional quality checks.

Statistics

Preliminary analyses

Data were screened for univariate outliers using SPSS 13.0 for Windows (SPSS Inc., 1989–2004). Those data with *z*-score values greater than ± 3.3 (less than 1% of the dataset) were excluded from all reported analyses (note that results did not differ when analyses were run using a dataset that included outliers). Phenotypes were normally distributed with the exception of Trials Correct, which had moderate negative skewness and required square-root transformation (Tabachnick & Fidell 1989). Furthermore, means, standard deviations and Pearson correlation coefficients were computed.

Allele frequencies were compared for ancestral group using a hybrid approximation to Fisher exact test probabilities for a contingency table using the network algorithm of Mehta and Patel (Clarkson *et al.* 1993). Families with both parental genotypes available were tested for segregation distortion. Families were classified according to mating genotype (*Val/Val* × *Val/Met*, V*al/Met* × *Val/Me*t, or *Val/Met* × *Met/Met*) and offspring genotypes were determined. These were then compared to expected segregation ratios (1:1, 1:2:1, and 1:1 respectively) using a goodness of fit χ^2 test. Using MERLIN (Abecasis *et al.* 2002), genotype frequencies were examined for deviation from Hardy–Weinberg equilibrium and identity-by-descent (IBD) sharing probabilities were estimated.

Association analyses

Allelic means were evaluated in a family-based approach using the program QTDT (Abecasis *et al.* 2000a, 2000b). This approach involves maximum-likelihood modelling of the raw data using a variance-components framework that allows for the simultaneous modelling of the means and variances. Analyses tested for population stratification (which can lead to spurious association), locus dominance effects and total association.

To test for population stratification, association effects are partitioned into orthogonal between- and within-family components. Between-family effects (β_b) reflect both genuine and spurious association, while within-family effects (β_w) reflect only genuine association. Therefore, population stratification is indicated when $\beta_b \neq \beta_w$. The total association approach is not robust to population stratification, but was chosen in this instance because no evidence of population stratification was found and because it maximizes power by using all available information. Analyses were run as described at http://www.sph.umich.edu/csg/abecasis/QTDT/, with both sex and age included as covariates.

Genetic power calculations were performed using the package available at http://statgen.iop.kcl.ac.uk/gpc/ (Purcell *et al.* 2003). *P*-values for the association analyses can be Bonferroni corrected for a factor of 6 [the number of effective traits as obtained from a Principal Components Analysis (Eigenvalues > 1)]. However, as all association analyses were non-significant before correction for multiple testing, the α-value was left at 0.05 and uncorrected *P*-values were reported.

Results

Preliminary analyses

Means, standard deviations, and Pearson phenotypic correlation coefficients are shown in Table 3. The correlations show that the measures examined reflect a number of largely independent processes occurring during the performance of a working-memory task – i.e. processes underlying prefrontal amplitudes (slow wave and P300), parietal amplitudes (slow wave and P300), P300 latency (prefrontal and parietal), overall non-ERP performance (Winnings and Trials Correct), Initiation Time and Movement Time, (see also Hansell *et al.* 2005; Luciano *et al.* 2004; Wright *et al.* 2002).

Val allele frequencies were similar for all ancestral groups, ranging from 76% to 82% (Table 2). This is consistent with frequencies reported by the International Hapmap Project (The Consortium, 2003) for rs6265, which found a *Val* allele frequency of 0.825 for a sample of Utah residents with northern and western European ancestry. Allele frequencies were compared for ancestral group and no significant differences were found ($P > 0.3$).

For families with both parental genotypes, offspring genotypes did not vary significantly from expected segregation ratios (*P*-values ranged from 0.06 to 0.78). In addition, genotype frequencies did not deviate significantly from Hardy–Weinberg equilibrium in general ($\Delta\chi^2 = 1.19$, $P = 0.28$) or among founders ($\Delta\chi^2 = 0.05$, $P = 0.82$).

Association analyses

No evidence of population stratification was found for any of the phenotypes ($\Delta\chi^2_1$ range 0.00–1.25, critical value 3.84). Likewise, no indication of locus dominance was found ($\Delta\chi^2_1$ range 0.00–0.91).

Phenotypic means for the *Val/Val*, *Val/Met* and *Met/Met* genotypes are shown in Fig. 1. No significant findings of association were found between the working memory phenotypes and the *BDNF* variants (Table 4). Total association maximized the information examined and thus maximized the power to find an association. For these analyses, power ranged from approximately 93% to 97% ($\alpha = 0.05$) to identify an association accounting for just 2% of the variance.

Discussion

BDNF has been previously identified as a potential mediator of memory function (e.g. Yamada & Nabeshima, 2003). There is growing evidence that the *V166M* polymorphism and episodic memory are associated, with a number of studies reporting poorer episodic memory in *Met*-allele carriers compared to *Val/Val* individuals (Dempster *et al.* 2005; Echeverria *et al.* 2005; Egan *et al.* 2003; Hariri *et al.* 2003; Rybakowski *et al.* 2003; Tan *et al.* 2005). However, studies examining the *BDNF V166M* polymorphism and its association with working memory have not been as consistent in their findings. Studies finding an association in bipolar patients (Rybakowski *et al.* 2003, 2006) and healthy individuals (albeit with chronic low-level mercury exposure; Echeverria *et al.* 2005) were offset by studies finding no association in healthy individuals (Egan *et al.* 2003; Rybakowski *et al.* 2006), or in individuals with schizophrenia (Egan *et al.* 2003; Rybakowski *et al.* 2006).

The present study examined the association between the *V166M* polymorphism and working memory in healthy adolescents and had considerable power to do so (approximately 93–97% power to detect an association accounting for 2% of the variance of the phenotypes examined). A range of measures obtained from a delayed-response working-memory task, including both event-related potential and behavioural performance measures, were examined. No associations were found.

A finding of no association was consistent with that hypothesized for the non-ERP behavioural measures, which was based upon previous findings (Egan *et al.* 2003; Rybakowski *et al.* 2006). However, a lack of association was contrary to that hypothesized for the ERP measures, in particular, for the P300 measures and for slow wave recorded over the prefrontal brain regions.

The hippocampal formation is thought to be a major generator of the P300 (e.g. Okada *et al.* 1983) and *Met*-allele carriers are reported to have reduced hippocampal volumes (e.g. Bueller *et al.* 2006) and abnormal hippocampal function during an N-back working-memory task (Egan *et al.* 2003). However, the results indicate that any BDNF-related hippocampal deficits did not affect the generation of the P300 component during a delayed-response task. This may be because the P300 has multiple generators (e.g. Halgren *et al.* 1998) and/or because BDNF-related differences in hippocampal structure and function are not sufficient to affect

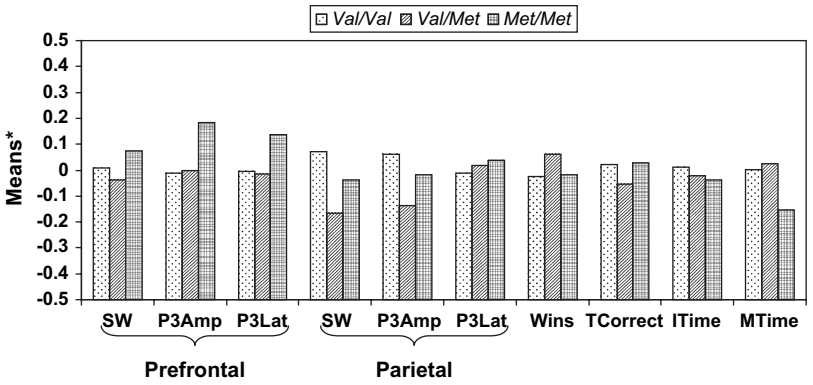

Figure 1: Means for *Val/Val, Val/Met,* and *Met/Met* genotypes for all phenotypes. Slow Wave average amplitude (SW), P300 Amplitude (P3Amp), and P300 Latency (P3Lat) recorded at prefrontal and parietal scalp locations, Winnings (Wins), Trials Correct (TCorrect), Initiation Time (ITime), and Movement Time (MTime) are represented. *Note that phenotypes were standardized to have a mean of zero and a standard deviation of one for ease of comparison.

P300 generation, at least in healthy adolescents. Similarly, any BDNF-related differences in grey matter volumes in the dorsolateral prefrontal cortex (Pezawas *et al.* 2004) did not appear to influence ERPs recorded over the prefrontal regions.

Consistent findings of an association between the *V166M* polymorphism and episodic but not working memory may reflect a greater reliance on hippocampal function for episodic versus working memory. A large literature indicates that the hippocampus plays a critical role in episodic memory (Squire *et al.* 2004). For instance, a recent study by Kramer et al. (2005) examined episodic memory in a sample of neurodegenerative patients and normal older controls, and showed that hippocampal volume predicted recall and recognition memory accuracy, but that frontal lobe, anterior temporal lobe and posterior cortex volumes did not.

In contrast, while the hippocampus is considered critical to the formation of memory networks in general (Fuster 1997), the prefrontal cortex appears to be of vital importance to working-memory function and its role has been widely studied in this context (Fuster 2001). Van Asselen *et al.* (2006) recently showed the importance of both the prefrontal cortex and the hippocampus to working memory. They examined working memory in stroke patients and healthy controls and found that performance was impaired by damage to the right dorsolateral prefrontal cortex and the bilateral hippocampal formation, in addition to the right posterior parietal cortex. Interestingly, patients with bipolar disorder, for whom an association between the *V166M* polymorphism and working memory has been found (Rybakowski *et al.* 2003, 2006), are reported to show impaired prefrontal and hippocampal function during memory-related encoding (Deckersbach *et al.* 2006). It is also interesting to note that there is some evidence to suggest that the effect of the *BDNF V166M* genotype may be greater in patient groups than in healthy volunteers (for influence on volume of the hippocampal formation see Szeszko *et al.* 2005; and for influence on working memory and executive functions see Rybakowski *et al.* 2006).

Table 4: Tests of association between working-memory phenotypes and *BDNF V166M* alleles.

	$\Delta\chi^2$ (1df)	*p*
Prefrontal		
Slow Wave	0.04	0.84
P300 Amplitude	0.72	0.40
P300 Latency	0.47	0.49
Parietal		
Slow Wave	3.53	0.06
P300 Amplitude	2.31	0.13
P300 Latency	0.84	0.36
Winnings	0.64	0.42
Trials Correct	0.45	0.50
Initiation Time	0.03	0.86
Movement Time	0.03	0.86

A limitation of the current study may be its focus on adolescents. The limited age range provides insight into the genotype/phenotype association in adolescents, but these insights may not be transferable to other age groups. Working memory tasks engage the prefrontal cortex (Fuster 2001) and the human prefrontal cortex is not functionally or structurally mature until early adulthood (Giedd *et al.* 1999; Hudspeth & Pribram 1992; Levin *et al.* 1991; Sowell *et al.* 1999). Furthermore, Webster *et al.* have shown that in relation to total mRNA, BDNF mRNA levels in the dorsolateral prefrontal cortex are relatively low in adolescents compared to young adults, adults, and aged individuals (Webster *et al.* 2002). In addition, during adolescence BDNF may be more involved in regulating neuronal morphology and synaptic pruning than in the maintenance of connectivity and synaptic plasticity as in the mature cortex (Webster *et al.* 2002). A further limitation of the study may be that the delayed-response task is not a standard task used to measure working memory at a behavioural level.

In conclusion, of studies examining the association between the *BDNF V166M* genotype and memory, this is the first to examine the following:

1 Phenotypes from a working-memory delayed-response task
2 Event-related potential measures of memory function, and
3 Data from a large sample of healthy adolescents.

Furthermore, the study had considerably more power to examine this association in healthy individuals than previous studies. For all measures examined (electrophysiological, general performance and reaction time) no association was found. Therefore, while the loss of efficiency in CNS function associated with the *Met* allele (Chen *et al.* 2004) may affect longer-term memory processes underlying episodic memory, in healthy adolescents this loss of efficiency does not appear to affect the shorter-term processes underlying working memory.

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