Biological Psychology 80 (2009) 176–188

Contents lists available at ScienceDirect

Biological Psychology

journal homepage: www.elsevier.com/locate/biopsycho

Disturbances in selective information processing associated with the BDNF Val66Met polymorphism: Evidence from cognition, the P300 and fronto-hippocampal systems

Peter R. Schofield [a,b,c,1], Leanne M. Williams [d,1,*], Robert H. Paul [e], Justine M. Gatt [d], Kerri Brown [d], Agnes Luty [c], Nicholas Cooper [f], Stuart Grieve [f], Carol Dobson-Stone [a,b,c], Charlotte Morris [f], Stacey A. Kuan [d], Evian Gordon [d,f]

[a] Prince of Wales Medical Research Institute, Barker Street, Randwick, Sydney 2031, Australia
[b] University of New South Wales, Sydney 2052, Australia
[c] Garvan Institute of Medical Research, 384 Victoria St, Darlinghurst, Sydney 2010, Australia
[d] Brain Dynamics Centre, Westmead Millennium Institute and Western Clinical School (Psychological Medicine), University of Sydney, Westmead Hospital, Westmead, Sydney 2145, Australia
[e] Behavioral Neuroscience Program, Department of Psychology, University of Missouri-St. Louis, 412 Stadler Hall & 08 Weinman Bldg, One University Boulevard, St. Louis, MO 63121-4499, United States
[f] Brain Resource International Database, Brain Resource Company, 237 Jones St, Ultimo, Sydney 2007, Australia

ARTICLE INFO

Article history:
Received 20 September 2007
Accepted 3 September 2008
Available online 16 September 2008

Keywords:
Brain derived neurotrophic factor
BDNF Val66Met
Cognition
Hippocampus
Neuroimaging
P300 event-related potential
Grey matter

ABSTRACT

In this study, we examined whether the Met allele of the BDNF Val66Met polymorphism is associated with selective disruptions to task-relevant information processing. In 475 non-clinical participants for whom BDNF genotype status was determined we used the 'IntegNeuro' computerized battery of neuropsychological tests to assess cognitive performance, an auditory oddball task to elicit the P300 event-related potential (ERP) and, in smaller subsets of these subjects, high resolution structural MRI imaging to quantify fronto-hippocampal grey matter ($n = 161$), and functional magnetic resonance imaging to assess fronto-hippocampal BOLD activation ($n = 37$). Met/Met (MM) homozygotes had higher verbal recall errors, in the absence of differences in attention, executive function, verbal ability or sensorimotor function. Further, MM homozygotes demonstrated a slowed P300 ERP during the oddball task, with corresponding alterations in hippocampal and lateral prefrontal activation, and a localized reduction in hippocampal grey matter. These results are consistent with a subtle impact of the Met allele on fronto-hippocampal systems involved in selective information processing of stimulus context and memory updating within the normal population. The findings also indicate that heritable endophenotypes such as the P300 have value in elucidating genotype–phenotype relationships.

Functional brain imaging provides a powerful approach for investigating functional genomics because it can more directly capture the effects of genetic variation on information processing than behavioral measures (Hariri and Weinberger, 2003). Highly heritable measures, such as the P300 event-related potential (ERP), have already shown promise as brain imaging endophenotypes in studies of several clinical disorders (Bramon et al., 2005; Hesselbrock et al., 2001), while functional magnetic resonance imaging studies reveal variations in neural activity with cognition, in the absence of behavioral differences (Hariri and Weinberger, 2003).

Brain derived neurotrophic factor (BDNF) is a key neurotrophin involved in cell survival and context-dependent synaptic plasticity, and has been implicated in selective information processing relevant to learning and memory. BDNF is synthesized as pro-peptide and is cleaved intracellularly to release mature, secreted growth factor that selectively binds to members of the *Trk* family of receptor tyrosine kinases that promote survival and differentiation (Friedman and Greene, 1999). These synaptic changes have been demonstrated in a variety of cellular models, such as hippocampal long-term potentiation (LTP), and are associated with learning and adaptive behaviors in animals (Poo, 2001; Tyler et al., 2001).

Within the BDNF gene, a distinct haplotype containing a frequent single nucleotide polymorphism (SNP), located at nucleotide 196 (dbSNP rs6265), results in a valine to methionine (Val66Met) substitution in the pro-peptide of the BDNF molecule.

* Corresponding author. Tel.: +61 2 9845 8186; fax: +61 2 9845 8190.
E-mail address: lea_williams@wmi.usyd.edu.au (L.M. Williams).
[1] Equal authors.

0301-0511/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.biopsycho.2008.09.001

As a result, the BDNF methionine-containing variant (Met) fails to localize to secretory granules or synapses, resulting in inefficient activity-dependent secretion (Chen et al., 2004; Egan et al., 2003). The BDNF Met allele has been associated with a wide range of psychiatric and neurodegenerative disorders, which involve a loss of neuronal integrity, including Alzheimer's disease (Kunugi et al., 2001; Riemenschneider et al., 2002; Ventriglia et al., 2002), Parkinson's disease (Momose et al., 2002), bipolar disorder (Neves-Pereira et al., 2002; Sklar et al., 2002), neuroticism as a vulnerability trait for depression (Sen et al., 2003), anorexia nervosa (Ribases et al., 2003) and obsessive-compulsive disorder (Hall et al., 2003).

In addition to associations with various neuropsychiatric disorders, Met/Met (MM) homozygosity has been associated with cognitive impairments in otherwise healthy individuals. Impairments in episodic memory combined with exaggerated hippocampal function during an fMRI working memory task have been found (Egan et al., 2003). Differences in hippocampal function have also been observed in Met carriers during a declarative memory task (Hariri et al., 2003). Structural brain differences are also evident, with the BDNF Met allele being associated with reductions in the hippocampus and dorsolateral prefrontal cortex, regions responsible for memory and attention (Bueller et al., 2006; Pezawas et al., 2004; Szeszko et al., 2005).

To date, few studies have examined the specificity of BDNF effects on memory in contrast to other cognitive domains. One study of elderly individuals reported better non-verbal reasoning in BDNF MM homozygotes, which was also reflected in their childhood scores from a 1932 survey (Harris et al., 2006), suggesting that BDNF Val66Met may impact other domains of cognition in adulthood. The BDNF polymorphism has also not been examined in relation to highly heritable cognitive brain function endophenotypes, such as the P300 ERP (O'Connor et al., 1994).

In this study, we employed the auditory oddball task to elicit selective information processing. The P300 (also known as 'P3b') elicited by a standard two-tone oddball task reflects voluntary detection of task-relevant, salient stimuli and has been associated with both regional neural inhibition involved in processing expected stimuli and the updating of activity in corticolimbic circuits during attention and working memory (Donchin and Coles, 1998; Polich, 2003; Soltani and Knight, 2000; Verleger, 1998). Biophysical modeling also implicates corticothalamo-limbic feedback in generation of the P300 (Rennie et al., 2002). These concepts of selective attention and context updating are relevant to evidence for the involvement of the BDNF polymorphism in aspects of learning and memory (Egan et al., 2003; Poo, 2001; Tyler et al., 2001).

Oddball stimuli have been found to elicit hippocampal activation in both intracranial P300 recordings (Halgren et al., 1980; Heit et al., 1990; McCarthy et al., 1989) and functional magnetic resonance imaging (fMRI) (Crottaz-Herbette et al., 2005; Kiehl et al., 2001; Williams et al., 2007; Yoshiura et al., 1999). However, there is also contrary evidence of P300 preservation despite unilateral hippocampal lesions (Jonson, 1989; Rugg et al., 1991). Moreover, infarctions of the posterior hippocampus, as well as anterior and medial temporal lesions, may induce P300 deficits over temporal as well as frontal sites (Knight, 1996; McCarthy et al., 1989; Onofrj et al., 1992). While hippocampal activation has been observed in the context of both frontal and parietal activation in the oddball task (e.g., Williams et al., 2007), other fMRI studies have observed distributed cortical activation in the absence of hippocampal engagement (e.g., Downar et al., 2002; Menon et al., 1997; McCarthy et al., 1997). Variation in hippocampal activation in these studies might in part reflect task and methodological variation.

Here we sought to determine whether the BDNF polymorphism shows specific associations with the memory domain of cognitive function, and whether BDNF variants are associated with disruptions to the P300 and underlying hippocampal–prefrontal networks (as measured by fMRI), in the same cohort of non-clinical individuals.

1. Methods

1.1. Subjects

A total of 475 participants (51% females, mean age of 32.4 ± 12.7 years) provided voluntary and informed written consent, according to the relevant local human research ethics committee, to participate in testing and the Brain Resource International Database (BRID; Gordon et al., 2005). To achieve a representative community sample, recruitment was undertaken professionally via widespread advertisement. Participants older than 60 years were excluded, given evidence that BDNF Val66Met genotypes might have differential effects on cognition in older age (Harris et al., 2006). Additional exclusion criteria were determined using the BRID personal history and screening assessments, which include the SPHERES (Hickie et al., 1998) and Patient Health Questionnaire (PHQ9) (Kroenke and Spitzer, 2002) to screen

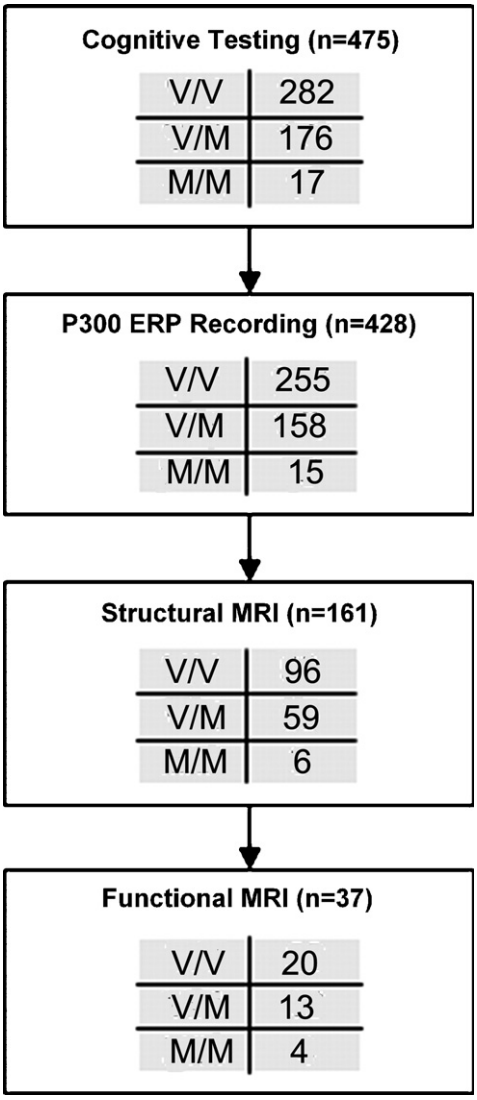

Fig. 1. A flow chart summarizing the distribution of BDNF Val66Met genotypes in the total sample, and in the subsamples for which ERP, MRI and functional MRI data were available. Importantly, each subsample was a subset of the total sample, and of each other (for instance, the functional MRI subsample was a subset of those completing MRI, ERPs and cognition). These subsamples were also matched in demographic factors to the total sample, such that the mean age, years of education and estimated IQ were the same (within one decimal place) within each subsample.

Table 1
Summary of demographic information for BDNF VV, VM and MM genotypes.

Demographic variables	ANOVA/chi-square[a]		BDNF genotype		
	Statistic	p	VV	VM	MM
Full sample (n = 475)					
Age (mean/S.D. in years)	$F_{(2,473)} = 2.05$	0.130	33.24 ± 12.92	31.45 ± 12.59	28.01 ± 9.30
Sex distribution (male/female)	$\chi^2_{(2)} = 3.56$	0.169	138/144	93/83	5/12
Education (mean/S.D. in years)	$F_{(2,473)} = 1.95$	0.230	12.94 ± 4.23	13.18 ± 3.64	13.88 ± 2.96
Estimated IQ (mean/S.D.)	$F_{(2473)} = 1.11$	0.330	106.11 ± 6.40	107.08 ± 6.52	104.80 ± 6.79
Cigarettes smoked per day (median)	$\chi^2_{(6)} = 2.69$	0.847	1.0	1.0	1.5
P300 ERP sample (n = 428)					
Age (mean/S.D. in years)	$F_{(2,426)} = 1.56$	0.211	33.40 ± 12.94	32.07 ± 12.66	28.88 ± 4.29
Sex distribution (male/female)	$\chi^2_{(2)} = 3.10$	0.212	123/131	84/74	5/11
Education (mean/S.D. in years)	$F_{(2,426)} = 2.27$	0.104	12.88 ± 4.29	13.48 ± 3.58	14.69 ± 2.98
Estimated IQ (mean/S.D.)	$F_{(2,426)} = 0.61$	0.546	105.40 ± 8.02	105.84 ± 7.57	107.64 ± 5.53
Cigarettes smoked per day (median)	$\chi^2_{(6)} = 2.60$	0.857	1.0	1.0	1.5
Structural MRI (n = 161)[b]					
Age (mean/S.D. in years)	$F_{(2,159)} = 2.01$	0.138	33.62 ± 13.38	31.12 ± 12.75	30.83 ± 9.66
Sex distribution (male/female)	$\chi^2_{(2)} = 2.17$	0.339	47/49	36/23	3/3
Education (mean/S.D. in years)	$F_{(2,159)} = 0.17$	0.845	12.81 ± 3.32	13.46 ± 3.35	14.23 ± 1.00
Estimated IQ (mean/S.D.)	$F_{(2,159)} = 0.37$	0.690	106.42 ± 6.79	106.15 ± 6.27	106.02 ± 3.78
Cigarettes smoked per day (median)	$\chi^2_{(6)} = 1.17$	0.884	1.0	1.0	1.0
Functional MRI (n = 37)[b]					
Age (mean/S.D. in years)	$F_{(2,35)} = 0.04$	0.964	30.52 ± 9.01	29.87 ± 8.09	29.91 ± 4.14
Sex distribution (male/female)	$\chi^2_{(2)} = 0.26$	0.877	7/8	10/8	2/2
Education (mean/S.D. in years)	$F_{(2,35)} = 0.04$	0.961	13.10 ± 2.19	13.72 ± 2.69	14.87 ± 0.98
Estimated IQ (mean/S.D.)	$F_{(2,35)} = 0.32$	0.732	106.76 ± 4.98	107.02 ± 5.22	107.21 ± 3.52
Cigarettes smoked per day (median)	$\chi^2_{(2)} = 0.83$	0.659	1.0	1.0	1.0

[a] There were no significant differences between genotype groups in demographic measures.
[b] Note that VM and MM genotype groups were combined in a 'Met carrier' group for structural and functional MRI analyses.

for symptoms of Axis-1 disorder, the AUDIT (Alcohol Use Disorders Identification Test of the WHO) and Fagerstrom Tobacco Dependency Questionnaire, and items to screen for family history of psychiatric disorder (defined in terms of severity requiring medication and/or hospitalization), physical brain injury (causing loss of consciousness for 10 min or more), neurological disorder or other serious medical or genetic condition.

Fig. 1 presents a flow chart of the composition of the full sample and sub-samples according to BDNF genotype and type of measure (i.e., ERP, sMRI, and fMRI). Of the total of 475 participants, 428 completed the ERP testing session with artifact-free data and the remaining 47 were excluded due to non-completion or artifact. Within this subset, no genotype differences were evident in terms of age, gender, or education (Table 1). Structural MRI scans were conducted on a subset of 161 participants (matched for age, sex, education and genotype to the total sample), who agreed to take part in the MRI scanning, and were able to attend the MRI Unit at Westmead Hospital in Sydney, or at Wakefield Hospital in Adelaide (for which we have previously demonstrated cross-site consistency of MR images; Grieve et al., 2005) (Table 1). A smaller subset of 37 participants undertook functional MRI. Due to specialist radiographer needs, functional MRI scans were undertaken only on specific days of the week, such that the selected subjects were those who agreed to take part in the additional functional MRI scans, and were available on those days. While this was not a random procedure, these days were not predictable from week to week. Importantly, these subjects did not differ in age and genotype distribution from the total sample, and there were genotype differences on demographic measures (Table 1). Moreover, age and sex were included as covariates in analyses to ensure that results were apparent over and above any non-significant contributions of these demographic factors.

1.2. BDNF genotypes

DNA was extracted from cheek swab samples by a standard proteinase digestion and chloroform extraction procedure. PCR amplification of participant DNA was undertaken using primers 5′ TGTATTCCTCCAGCAGAAAGAGAA 3′ and 5′ AAAGAAGCAAACATCCGAGGAC 3′ using standard conditions. The amplified fragment was digested with the restriction enzyme *Afl*III, which cleaves the Val allele and includes a positive digestion control in the PCR amplicon. PCR products were separated on 4% agarose gels. Allele frequencies were V = 0.81 and M = 0.19, and genotype frequencies were 59.4% VV (n = 282), 37% VM (n = 176) and 3.6% MM (n = 17), which are in Hardy–Weinberg equilibrium. These BDNF genotypes did not differ in distribution of APOEε4/ε4 [$\chi^2_{(2)} = 0.22$, p = 0.895], which has also been associated with effects on memory. Moreover, the genotype groups did not significantly differ on demographic variables, including mean age, sex distribution, years of education, estimated IQ (derived from the Spot the Word Test; Baddeley et al., 1993), and nicotine intake in terms of number of cigarettes smoked per day (see Table 1).

1.3. Neuropsychological tests of cognitive performance

Subjects completed a standardized and computerized battery of neuropsychological tests assessing cognitive performance. This battery relies on a touchscreen format, and the standardized approach to its development has been described elsewhere (Gordon, 2003a,b; Gordon et al., 2005). The battery tapped five core domains of cognitive function, for which validity, reliability and cross-cultural consistency have been established (Paul et al., 2005, 2007; Williams et al., 2005). These domains are memory (as measured by verbal list learning, working memory, visual memory span, and digit span), executive function and planning (executive maze and word interference), attention (switching of attention), verbal processing (verbal and semantic fluency), and sensori-motor function (simple motor tapping and choice reaction time). Table 2 provides a detailed description of each of these tests.

Performance on the cognitive test battery was quantified in terms of accuracy and reaction time measures. Analyses were performed using ANOVA, with BDNF genotype as the between-groups factor and each of the behavioral measures of cognitive performance as the dependent measure. Given the relatively wide age range of subjects, and that cognition varies with age and sex (Clark et al., 2006), age and sex were taken into account as covariates. In addition to significance, the effect size (partial eta-square) of genotype differences was determined. Dunnetts contrasts, which do not assume equal variance between groups, were then undertaken to determine the direction of differences between VV, VM and MM genotypes for significant effects. We focused on effects significant at an alpha level of 0.01 or less, but undertook contrasts for ANOVA effects at trend level (alpha = 0.05–0.07) given that these tests have not previously been examined with BDNF genotypes.

Given the unequal numbers in the genotype groups, we confirmed focal ANOVA effects using follow-up ANOVAs with VV and VM groups matched to the smaller MM (n = 17) group.

1.4. Standard oddball task

ERP recording was undertaken during a standard auditory oddball task from the "NeuroMarker" testing battery (Williams et al., 2005), which reliably elicits the P300 to task-relevant 'target' stimuli (Polich and Kok, 1995; Soltani and Knight, 2000). A series of 60 target tones (1000 Hz, 16% probability) and 310 non-target tones (500 Hz) were presented, with an inter-stimulus interval of 1 s, intensity of 75 dB (SPL), duration of 50 ms, and rise and fall time of 5 ms. Subjects were instructed to button press with the index finger of each hand (to counterbalance for possible motor effects) to detection of target tones – and to not respond to non-target tones. Speed and

Table 2
Summary of cognitive tests in the IntegNeuro neuropsychological test battery.

Test[a]	Description
Memory domain	
Verbal list learning & recall	A list of 12 words is presented in 'learning' trials 1–4. Trial 5 presents new 'distractor' words. Trial 6 assesses 'short delay' recall, and trial 7, 'long delay' recall (after 20 min) for the original word list. Recognition memory is assessed using the original list plus 12 'foil' words. Lists are matched on word length (4–7 letters), concreteness and frequency, and semantic or phonemic similarities are excluded (Clark et al., 2006). This test assesses similar constructs of word list learning and recall to those evaluated by the California Verbal Learning and Memory test (Geffen et al., 1990)
Working memory n-back	An n-back continuous performance test of sustained attention based on Clark et al. (1998). Subjects identify 85 'targets' (consecutive presentation of letters B, C, D or G) embedded in a pseudorandom series of 125 letters of 200 ms, with ISI 2.5 s. Speed and accuracy are stressed equally
Span of visual memory	A set of randomly arranged squares is highlighted sequentially on each trial, in both forward and reverse conditions. Subjects repeat the order of each sequence. This test is a computerized variant which assesses similar aspects of memory to the Corsi blocks test (Milner, 1970)
Digit Span	The order of a series of digits (e.g., 4, 2, 7 etc.), each 500 ms, is recalled using a numeric keypad. Number of digits is gradually increased from 3 to 9, and both forward and reverse order conditions are employed
Executive function/planning domain	
Maze	A hidden path within an 8 × 8 'maze' grid, with 24 is identified, requiring 24 consecutive correct moves. Incorrect moves elicit a tone and red cross at the bottom of screen, and correct moves a different tone and green tick. Test ends with two error-free completions (or time-out after 7 min). It is a computerized variation assessing similar constructs to those assessed by the Austin Maze (Walsh, 1985)
Verbal interference	Colored words with incongruent color-word combinations are presented. In Part 1, the name of each word, and in Part II the color of each word, are identified as quickly as possible. It assesses aspects of inhibition and interference which correspond to those indexed by the Stroop tes (Golden, 1978)
Attention domain	
Switching of attention	In Part I, 25 digits are identified in ascending numerical sequence (i.e., 1,2,3 . . .). In Part II, 13 digits (1–13) and 12 letters (A–L) are identified in ascending sequence of alternating digits and letters (i.e., 1 A, 2 B, 3 C. . .). It assesses constructs equivalent to those assessed by Trails Making A and B (Reitan, 1958)
Verbal processing domain	
Letter fluency	Subjects generate words that began with the letters F, A and S, recorded via 'wav' files, allowing 60 s for each letter. Proper nouns are not allowed. This test is a computerized variant, which assesses constructs corresponding to those assessed by the FAS test (Benton and Hamsher, 1989)
Semantic fluency	In this version, subjects generated animal names in a 60 s period
Sensori-motor domain	
Motor tapping	Subjects were required to tap a circle with the index finger of their dominant and then their non-dominant hand, as fast as possible, for 60 s each.
Choice reaction time	One of four target circles is illuminated pseudorandomly in 20 trials, and each illuminated circle is touched as quickly as possible

[a] More recent versions of IntegNeuro include Emotion Recognition and Go-NoGo tests.

accuracy of detection were stressed equally in the task instructions (presented via standardized 'wav' files). The two tones were presented in a quasi-random order, with the only constraint being that two task-relevant tones could not appear consecutively. Target stimuli were defined as salient by their low probability and intensity (high pitch) relative to the more frequent, lower-pitch non-target tones.

Functional MRI was undertaken during an equivalent standard auditory oddball task as used to elicit the P300 ERP, which has been shown to elicit hippocampal and fronto-parietal activation (Williams et al., 2007).

1.5. P300 ERP

All subjects were asked to refrain from drinking caffeinated beverages or smoking cigarettes 2 h before the testing session, due to the potential acute effects of these stimulants on ERP data.

During the auditory oddball task, subjects were seated in a comfortable, reclining chair in a dimly lit room. EEG data were recorded according to the International 10–10 electrode system using a Quikcap with sintered Ag/AgCl electrodes from 26 scalp electrode sites (Fp1, Fp2, F7, F3, Fz, F4, F8, FC3, FCz, FC4, T3, C3, Cz, C4, T4, CP3, CPz, CP4, T5, P3, Pz, P4, T6, O1, Oz and O2), and a NuAmps DC amplifier in which each channel was referenced to a ground within the instrument ('virtual ground'). A sampling rate of 500 Hz and lowpass filter of 100 Hz were employed, and skin resistance was $<5\ k\Omega$. The 22-bit digitized data were re-referenced offline to the average signal from mastoid sites. Horizontal eye movements were recorded with electrodes placed 1.5 cm lateral to the outer canthus of each eye. Vertical eye movements were recorded with electrodes placed 3 mm above the middle of the left eyebrow and 1.5 cm below the middle of the left bottom eye-lid. Ocular (horizontal, vertical, and blink) artifacts were corrected offline according to a protocol based on the Gratton et al. (1983) algorithm, with subsequent confirmation by visual inspection of the data.

An epoch rejection algorithm was employed, which rejected epochs in which three or more channels exceed a maximum threshold of 100 uV for all sites except Oz, O1 and O2, for which the threshold was 250 uV. We then averaged ERP epochs in which subjects correctly identified target stimuli. Across genotype groups, the mean correct recognition was very high (VV = 59.6, S.D. = 0.4; VM = 59.6, S.D. = 0.3; MM = 59.7, S.D. = 0.3) and did not differ between groups. For the P300 component peak amplitude and latency were extracted for the latency window 270–550 ms post-stimulus, using a previously established baseline to peak method (Williams et al., 2005). This window was consistent with observations from scalp-recordings, that the P300 component typically peaks 300–500 ms post-stimulus onset (Duncan-Johnson and Donchin, 1982; Soltani and Knight, 2000). For the standard oddball task used here, the P300 has also been referred to as the voluntary 'P3b' to distinguish it from the involuntary 'P3a', a positive potential generated 60–80 ms earlier to automatic detection of novel events in particular (Soltani and Knight, 2000).

To confirm that BDNF genotypes did not differ on behavioral performance on the oddball task, we first used ANOVA to compare genotypes on reaction time and accuracy for responses to target stimuli, with age and sex as covariates. Repeated measures ANOVAs were then undertaken to compare genotypes on P300 amplitude and latency. Three sets of ANOVAs were employed, each with BDNF genotype as the between-groups factor and with different regional groupings as within-subjects factors: (i) midline site (with repeated measures, Fz, Cz, Pz); (ii) frontal versus centro-temporal versus parieto-occipital regions (three repeated measures) as the first within-subjects factor and site (for the eight sites contributing to each of these regions) as the second within-subjects factor, and (iii) left versus right hemisphere (two repeated measures) as the first within-subjects factor and site (for the 10 sites contributing to each hemisphere) as the second within-subjects factor. As for cognitive measures, age and sex were included as covariates in each ANOVA model. Focal effects of interest were those involving genotype differences, and effect size (partial eta-squared; η^2) was determined for significant genotype effects. Results were reported for corrected degrees of freedom, using the Greenhouse-Geisser correction, in regard to the multiple repeated measures. Univariate ANOVA with Dunnett's contrasts were used to determine the direction of genotype differences at each site contributing to significant ANOVA effects.

Given the unequal numbers in genotype groups, we again confirmed focal ANOVA effects using matched-samples comparisons of VV and VM groups matched

Table 3
Summary of ANOVA and contrasts for VV, VM and MM genotypes on cognitive performance in memory, executive function, attention, verbal and sensori-motor function domains.

BRID test	ANOVA (d.f. = 2,470)[a]		Genotype mean[a]		
	F	*p*	VV	VM	MM
1. Memory domain					
Verbal list learning					
Memory recognition errors	1.24	0.291	1.22	1.12	1.54
Immediate recall errors	*2.85*	*0.059*	4.25	**3.88**	**5.39**
Delayed recall errors	0.81	0.447	4.40	4.55	5.36
Intrusive word errors	**5.18**	**0.006**	**1.31**	**1.55**	**2.55**
Repeated word errors	0.83	0.438	3.03	2.73	3.23
Working memory					
Average Response time (ms)	1.51	0.222	618	622	621
Errors of commission	2.33	0.099	0.44	0.37	0.62
Errors of omission	0.44	0.643	0.52	0.48	0.60
Total errors	1.63	0.200	0.86	0.76	0.98
Span of visual memory					
Longest correct sequence	0.48	0.621	5.46	5.55	5.57
Digit span (forwards)					
Longest correct sequence	2.48	0.085	6.33	6.48	6.82
Digit span (backwards)					
Longest correct sequence	1.41	0.244	4.83	4.94	4.40
2. Executive function/planning domain					
Word interference					
Total errors	0.07	2.65	0.80	0.72	1.17
Av. response time (ms)	0.332	0.722	1604	1575	1550
Executive maze					
Total errors	0.43	0.654	42.3	39.1	37.6
Total number of overruns	0.43	0.649	18.3	17.0	16.6
Av. time to complete (ms)	0.07	0.946	23719	23852	23121
3. Attention domain					
Switching of attention					
Total errors	0.37	0.691	1.28	1.11	1.20
Av. response time (ms)	0.44	0.647	46651	45507	46789
4. Verbal processing					
Verbal fluency					
Letters (total words beginning with F, A, S)	1.25	0.287	15.2	15.7	14.7
Semantic fluency					
Animals (total animal categories)	0.45	0.637	24.0	24.3	23.2
5. Sensori-motor domain					
Finger tapping (simple motor reflex)					
Dominant hand: number taps	0.18	0.838	165.9	164.5	164.0
Dominant hand: Av. RT (ms)	0.20	0.822	200.9	209.0	186.6
Choice reaction time (motor coordination)					
Av. RT (ms)	0.05	0.951	726.5	721.6	715.9

[a] Bolding indicates a significant group difference in ANOVA (covarying for age and sex), and significant pair-wise differences in Dunnetts contrasts. Italics indicate a trend towards a group difference.

to the smaller MM ($n = 15$) group. Corrected degrees of freedom, using the Greenhouse-Geisser correction, were again employed for these analyses.

1.6. Structural MRI

High resolution structural MRI images were acquired using a T1 (MPrage) sequence, in the saggittal plane, with 180 slices, 1 mm cubic voxels, 256 × 256 matrix: TR 9.7, TE 4, TI 200 and flip angle 12. Segmentation and spatial normalization of MRI data was performed using voxel-based morphometry (VBM) in SPM2 (http://www.fil.ion.ucl.ac.uk/spm), using a protocol described in detail elsewhere (Ashburner and Friston, 2000; Grieve et al., 2005). Images were smoothed using a FWHM 12 mm kernel, spatially normalized by transforming each brain to a standardized stereotactic space based on the ICBM 152 template (Montreal Neurological Institute). Images were segmented grey, white, CSF and non-brain portions based on a cluster analysis method to separate pixels based on intensity differences, together with *a priori* knowledge of spatial tissue distribution patterns in normal subjects (Friston et al., 1996). Customized templates created from the BRID subjects were used for normalization and segmentation processes (Grieve et al., 2005). A correction was made to preserve quantitative tissue volumes following normalization procedure (Ashburner and Friston, 2000).

Due to the comparatively small number of MM homozygotes, we examined grey matter variation for VV homozygotes contrasted to Met carriers (MM and VM combined), using planned contrast *t*-tests. Grey matter differences were examined relative to the whole brain using VBM, with corrections for multiple comparisons based on Gaussian random field theory (Friston et al., 1996; Worsley et al., 1996). Age, sex and global brain volume were included as covariates. To further elucidate the effect of the Met allele, we also undertook follow-up contrasts of VV compared to VM and MM considered separately. In these contrasts, we matched the VV and VM groups to the smaller MM group ($n = 6$), and these contrasts were considered in the context of the focal comparison between VV homozygotes and Met carriers.

1.7. Functional MRI

For fMRI acquisition we used a full brain coverage protocol and sought to confirm hippocampal engagement in the comparatively large group of 37 subjects.

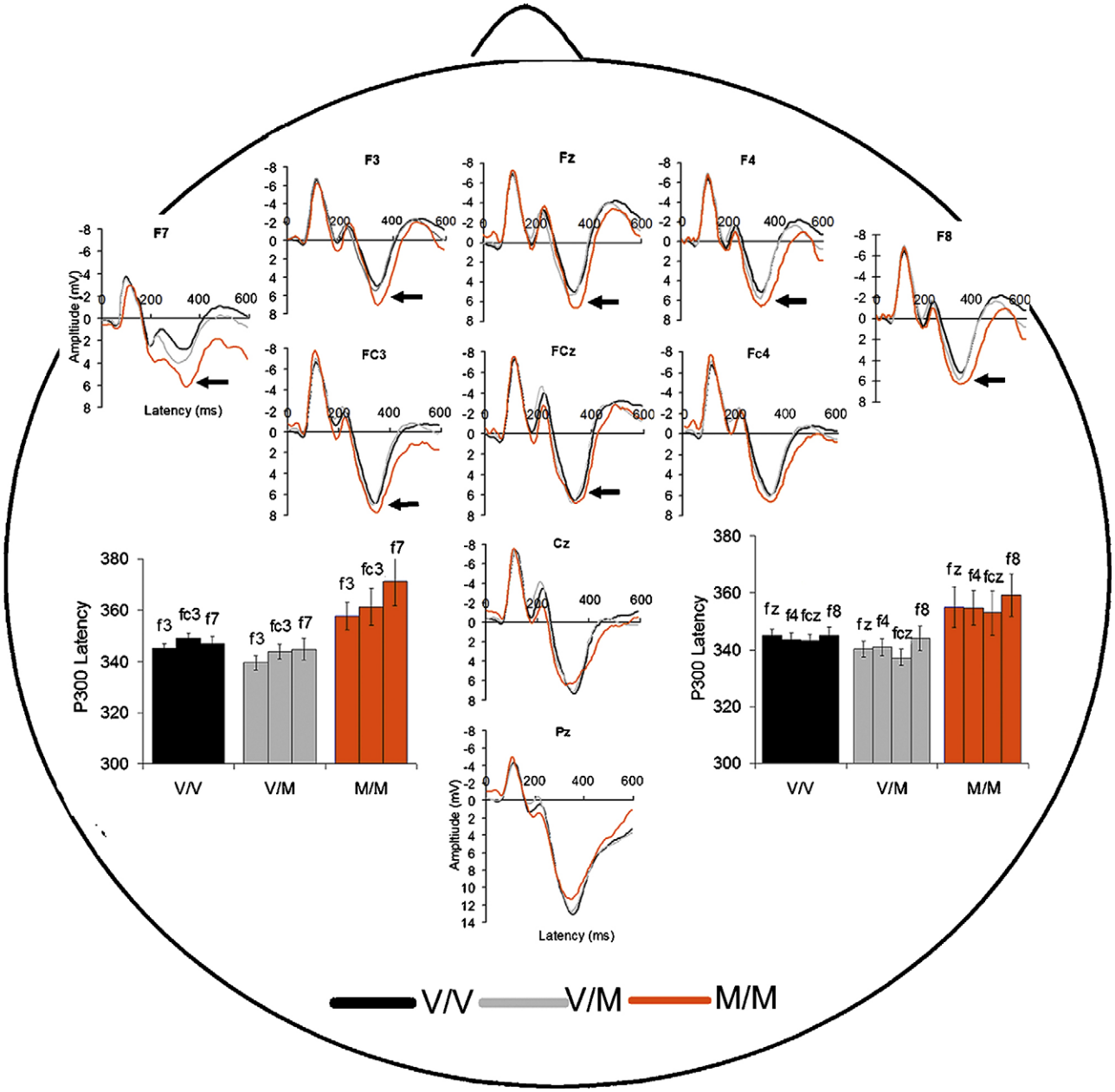

Fig. 2. Averaged ERP waveforms showing the significant delay in P300 latency (indicated by a black arrow) for the MM ($n = 15$) genotype relative to both V/V ($n = 255$) and V/M ($n = 158$) genotypes at the topographically represented fronto-central sites (F7, F3, Fz, FCz, FC3, F4 and F8 sites), but not at the Cz or Pz sites. Bar graphs show means (and standard error) for P300 latency at sites for which the M/M group showed a significant delay in P300 latency compared to V/M and V/V.

During the oddball task, stimuli were presented through headphones which also minimized scanner noise, as part of an MRI-compatible Avotec 'silent scan' system. Image data were collected on a 1.5 Tesla Vision Plus scanner, using gradient echo echoplanar imaging to depict blood oxygen level dependent (BOLD) contrast in response to the oddball task. We acquired 15 brain slices parallel to the AC-PC line (6 mm thick with 0.6 mm gap) for each volume, with 128 × 128 matrix: TR 3.5 s, TE 40 ms, and FOV of 250 × 250 mm. This EPI dataset provided complete coverage of the temporal lobes (including hippocampus and amygdala). A total of 125 volumes were acquired, with each volume corresponding to stimulus presentation. Three 'dummy' volumes were acquired prior to task commencement to ensure BOLD saturation.

Pre-processing and statistical analysis of fMRI data was conducted using SPM2 (http://www.fil.ion.ucl.ac.uk/spm/software/). Functional scans were realigned (including the unwarping routine for modeling residual movement-related variance), spatially normalised into standard anatomical MNI space, and smoothed using a Gaussian kernel (FWHM: 8 mm). An HRF-convolved boxcar model with temporal derivative was created to correspond to the experimental model. A high pass filter, set at the SPM2 default with a 128 s wavelength, was applied to remove low frequency fluctuations in the BOLD signal. BOLD signal change was based on the contrast of target versus non-target stimuli.

Paralleling the structural MRI analysis, we examined VV homozygotes contrasted to Met carriers in a combined VM and MM group. We undertook a whole-brain analysis, with a threshold of $p < 0.0001$, which determines significant clusters of activation relative to changes in BOLD activity across the whole brain. Planned contrast *t*-tests with a random effects procedure in SPM2 were used to compare between genotypes, with age and sex as covariates. We again undertook follow-up contrasts to further elucidate the impact of the Met allele in regard to MM and VM genotypes considered separately in comparison to VV homozygotes. Given the particularly small number of MM homozygotes ($n = 4$), we undertook these contrasts with matched groups of VM and VV genotypes.

1.8. Correlations between measures

Regression analyses were used to examine relationships between the measures of cognition, P300 and fMRI activation which showed significant BDNF genotype effects. Correlations between P300 and fMRI activation were undertaken with SPM2.

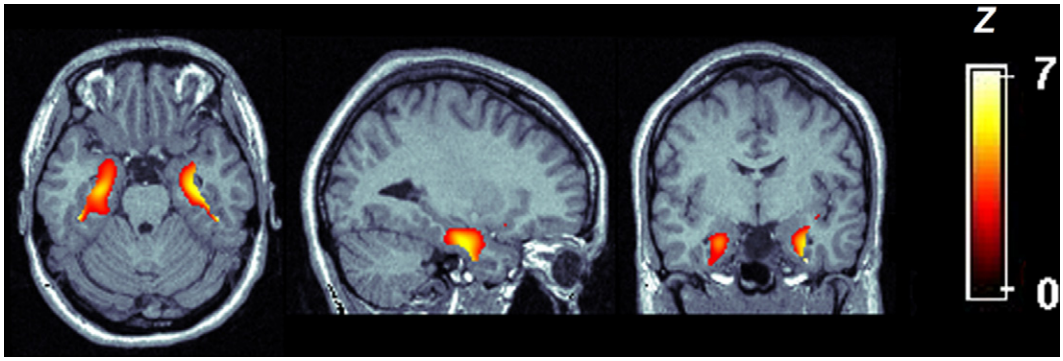

Fig. 3. Using voxel-based morphometry with structural MRI data, Met carriers (MM and VM genotypes combined) showed a significant bilateral decrease in hippocampal grey matter compared to VV homozygotes. This region of reduction is shown in axial, saggittal and coronal views, superimposed over a representative T1-weighted image drawn from the study, and the legend shows the T-score scale. The same region of grey matter reduction was identified in a follow-up contrast of MM and VV genotypes considered separately, compared to VV homozygotes, confirming the impact of the Met allele on the hippocampus.

2. Results

2.1. Cognitive performance and BDNF genotypes

BDNF genotypes differed significantly on intrusion errors during the first four learning trials of the verbal learning and recall test (Table 3). This effect was a moderate one (partial eta-squared = 0.047), and Dunnett's contrasts revealed higher errors in MM homozygotes compared to both VM and VV groups (Table 3). BDNF genotypes also demonstrated a trend effect in immediate recall errors (Table 3), with a small effect size (partial eta-squared = 0.019), and due to higher errors in MM homozygotes compared to the VM group (Table 3). These effects were confirmed in the ANOVAs for matched samples ($n = 17$) [intrusion errors, $F_{(2,49)} = 10.22$, $p < 0.0001$; immediate recall errors, $F_{(2,49)} = 3.90$, $p = 0.03$], indicating that they were not simply due to unequal group sizes.

Across other tests in the memory domain, and executive function, attention, verbal processing and sensori-motor domains, ANOVA did not reveal any further differences between BDNF genotypes. This pattern of findings remained when age and sex were included as covariates. The findings indicate that cognitive deficits associated with the Met allele, while subtle, may be specific to memory recall.

2.2. P300 ERP and BDNF genotypes

There were no differences between genotypes on behavioral responses to task-relevant oddball stimuli during ERP recording in terms of reaction time [$F_{(4,423)} = 1.64$, $p = 0.19$; mean RT: VV, 581 ms; VM, 583 ms; MM, 581 ms] or accuracy [$F_{(4,423)} = 0.11$, $p = 0.90$; mean errors: VV, 0.39; VM, 0.37; MM, 0.34].

Fig. 2 shows the P300 waveforms elicited by task-relevant oddball target stimuli, according to BDNF genotype group. The P300 peak around 350 ms, and was most prominent over the medial parietal (Pz) compared to frontal site (Fz), consistent with the characteristics of the 'P3b' subcomponent (Soltani and Knight, 2000).

For P300 latency, there was a significant BDNF genotype by region interaction for the P300 elicited over frontal versus centro-temporal versus parieto-occipital sites [$F_{(2.74,583.64)} = 6.06$, $p < 0.0001$; partial eta-squared = 0.042]. Estimated marginal means indicated this effect was due to slower P300 latency in MM genotypes over the frontal but not centro-temporal or parieto-occipital region. Univariate ANOVA confirmed the presence of significant genotype effects at individual frontal and fronto-central sites: spanning left frontal [F7, $F_{(2,423)} = 4.80$, $p = 0.009$; F3 ($F_{(2,423)} = 5.78$, $p = 0.003$), left centro-frontal [FC3, $F_{(2,423)} = 5.65$, $p = 0.004$], medial frontal [Fz, $F_{(2,423)} = 4.65$, $p = 0.010$], medial fronto-central [FCz, $F_{(2,423)} = 4.15$, $p = 0.014$] and right frontal [F4, $F_{(2,423)} = 4.66$, $p = 0.006$; trend level F8, $F_{(2,423)} = 3.36$, $p = 0.05$] sites (Fig. 2). Dunnetts post-hoc contrasts showed that P300 latency was delayed in MM genotypes compared to VV genotypes at each of these sites, and compared to VM genotypes for left frontal and centro-frontal regions ($p < 0.05$).

The interaction between BDNF genotype and regional effects (frontal versus centro-temporal versus parieto-occipital) for P300 latency was confirmed using matched samples ($n = 15$) repeated measures ANOVA [$F_{(3.01;75.18)} = 3.87$, $p = 0.033$]. Univariate ANOVA for the matched samples confirmed significant genotype differences for the individual sites: left frontal [F7, $F_{(2,43)} = 4.700$, $p = 0.012$; F3 ($F_{(2,43)} = 4.67$, $p = 0.013$), left centro-frontal [FC3, $F_{(2,43)} = 8.06$, $p = 0.001$], medial frontal [Fz, $F_{(2,43)} = 4.21$, $p = 0.019$], medial fronto-central [FCz, $F_{(2,43)} = 3.58$, $p = 0.020$] and right frontal [trend level F4, $F_{(2,43)} = 2.70$, $p = 0.070$; F8, $F_{(2,43)} = 2.74$, $p = 0.065$] sites (Fig. 2). Dunnetts post-hoc contrasts confirmed that P300 latency was delayed in MM genotypes compared to VV genotypes at these sites, and compared to VM genotypes for left frontal, centro-frontal and right frontal (F4) sites ($p < 0.05$).

By contrast, ANOVAs did not reveal any significant genotype effects for midline sites, or for laterality (left versus right hemisphere) for P300 latency, and these null effects were also confirmed using matched-samples analyses.[2]

There were also no significant BDNF genotype effects revealed by parallel ANOVAs of P300 amplitude, and these null effects were also revealed by matched-samples ANOVAs.

2.3. Structural MRI and BDNF genotypes

VBM showed that the MM genotypes had significantly smaller grey matter in small bilateral clusters of the hippocampus (Fig. 3). Grey matter volume for Met carriers was significantly smaller than that of VV genotypes for both the left ($p = 0.012$) and right ($p = 0.012$) hippocampus. These reductions represented an approximate 5% difference for each hemisphere, and were present relative to whole brain grey matter. BDNF genotypes did not differ on grey matter in any other brain region.

[2] Additional follow-up ANOVAs for the earlier ERP components elicited by oddball target stimuli (N100, P200 and N200) also showed no significant effects involving BDNF genotype group for either amplitude or latency, such that earlier differences in neural activity are unlikely to account for the group differences in P300 latency.

Genotype effects on hippocampal grey matter were confirmed in matched sample ($n = 6$) ANOVAs [left hippocampus $F_{(2,15)} = 7.74$, $p = 0.005$; right hippocampus $F_{(2,15)} = 7.62$, $p = 0.005$]. Dunnett's contrasts showed that grey matter reductions were most apparent in MM homozygotes compared to both VM and VV groups ($p < 0.05$).

2.4. Functional MRI and BDNF genotypes

There were similarly no differences between genotype groups (Met carriers versus VV) on behavioral responses to oddball stimuli during fMRI, in terms of reaction time ($t_{(10)} = 0.10$, $p = 0.92$; mean RT: VV, 595 ms; VM/MM, 605 ms) or accuracy ($t_{(10)} = 0.06$, $p = 0.95$; mean errors: VV, 0.80; VM/MM, 0.75).

We first confirmed that the oddball task elicited robust hippocampal activity. For the combined subject group, the contrast of target versus non-target data revealed a robust and significant ($p < 0.0001$) increase in activation for target stimuli in the bilateral hippocampi and parahippocampal gyri. Consistent with the total group finding, both VV subjects and Met carriers were also found to elicit activity in hippocampal and parahippocampal gyrus regions in response to oddball targets (Fig. 4A).

However, the contrast of Met carriers to Val homozygotes revealed enhanced hippocampal activity in the Met group, which extended to the boundary of the amygdala. Notably, the most significant effect was observed in the parahippocampal gyrus ($p < 0.0001$), with relatively enhanced activity in Met carriers (Fig. 4A; Table 4). At the whole-brain level, Met carriers also showed enhanced activity relative to Val homozygotes in the bilateral fusiform gyrus (Table 4). By contrast, Met carriers showed comparatively reduced ($p = 0.001$) activity in the dorsolateral prefrontal cortex (Fig. 4B; Table 4).

We confirmed the above contrasts using matched samples ($n = 4$) of VV, VM and MM genotypes. These contrasts confirmed that the significant ($p < 0.05$) enhancement of bilateral hippocampal and parahippocampal gyrus activity was present in MM subjects compared to both VV and VM subjects (Fig. 4A; Table 4). VM subjects also showed significantly ($p < 0.05$) greater activity than VV subjects in the right hippocampus (Fig. 4A; Table 4). We also confirmed the reduction in dorsolateral prefrontal activation for MM subjects compared to both VV and VM subjects (Fig. 4B; Table 4).

2.5. Correlations between measures

In regression analyses of the measures which showed significant genotype effects, P300 latency predicted behavioral performance on the oddball task. While oddball task performance did not differ between BDNF MM genotypes, slower P300 latency (averaged across frontal sites showing a significant genotype effect) predicted lower accuracy for detecting oddball target stimuli [$F_{(1,427)} = 3.34$, $p = 0.038$]. By contrast, P300 latency did not predict immediate recall or intrusion errors on the cognitive test of verbal learning and recall.

Regression analysis in SPM2 revealed a significant positive correlation between P300 latency at the frontal F3 site (where genotype effects were most significant) and hippocampal activation (left, $Z = 2.72$, $p = 0.003$, cluster size = 45 voxels; coordinates $x = -16$, $y = -32$, $z = -6$; right, $Z = 2.72$, $p = 0.004$, cluster size = 35 voxels; coordinates $x = -14$, $y = -34$, $z = -6$; Fig. 5). By contrast, there was a significant negative correlation between P300 latency and activation in the dorsal lateral prefrontal cortex ($Z = 2.98$, $p = 0.001$, cluster size = 122 voxels; coordinates $x = -40$, $y = 28$, $z = 32$).

There were no significant correlations between hippocampal grey matter and cognitive performance or P300 latency.

Table 4
Activation for the within- and between-group contrasts of BDNF genotypes revealed by functional MRI in response to oddball target stimuli.

Group	Side	MNI coordinates			Z-score
		x	y	z	
Across all genotypes ($n = 37$)					
Hippocampus	L	−28	−32	−6	2.98
	R	30	−32	−6	2.26
Parahippocampal gyrus	L	−22	−26	−14	3.35
	R	28	−20	−14	2.91
Dorsolateral prefrontal cortex	R	38	54	26	2.54
	L	−36	20	46	2.19
Amygdala	R	22	−8	−14	2.43
Thalamic pulvinar	R	4	−20	12	3.87
	L	−24	−32	4	3.23
Met carriers ($n = 17$) versus VV ($n = 20$)					
Met carriers > VV					
Parahippocampal gyrus	L	−32	−6	−26	2.90
	R	16	−28	−12	2.64
Hippocampus	L	−24	−20	−16	2.06
Met carriers < VV					
Thalamic pulvinar	L	−16	−24	10	2.74
Dorsolateral prefrontal cortex	L	46	24	42	3.50
MM versus VM versus VV ($n = 4$ per matched group)					
MM > VV					
Parahippocampal gyrus	L	−38	−32	−12	1.98
	R	22	−14	−24	2.83
Hippocampus	L	−24	−34	−2	2.78
	R	16	−26	−10	2.76
MM > VM					
Parahippocampal gyrus	L	−26	−32	−18	2.41
	R	16	−20	−18	1.92
Amygdala (extending from parahippocampal gyrus)	R	14	−2	−14	2.21
MM < VV					
Thalamic pulvinar	L	−16	−24	10	2.74
Dorsolateral prefrontal cortex	L	48	26	44	2.96
	R	46	32	42	2.64
MM < VM					
Dorsolateral prefrontal cortex	L	−42	22	40	2.74

These regions of activation were present at the whole-brain level of analysis ($p < 0.0001$), and confirmed in region of interest analysis ($p < 0.05$ small volume corrected).

3. Discussion

Here we provide new evidence that the BDNF Met allele impacts selective information processing, indexed by the P300 and involving hippocampal-lateral prefrontal systems. While Met homozygotes also showed poorer performance on the cognitive test of verbal recall, these effects were far more subtle than the alterations evident in measures of brain function and structure.

3.1. BDNF MM genotype and verbal recall errors

In terms of cognitive performance, MM homozygotes demonstrated more errors due to intrusive words and a trend towards more errors in immediate recall than VM and VV genotype groups on the verbal list learning and recall test, effects which have not previously been observed in non-clinical subjects using an equivalent California Verbal Learning Test (Egan et al., 2003). We did not observe corresponding reductions in recognition memory in MM subjects, which have been found for tests of visual

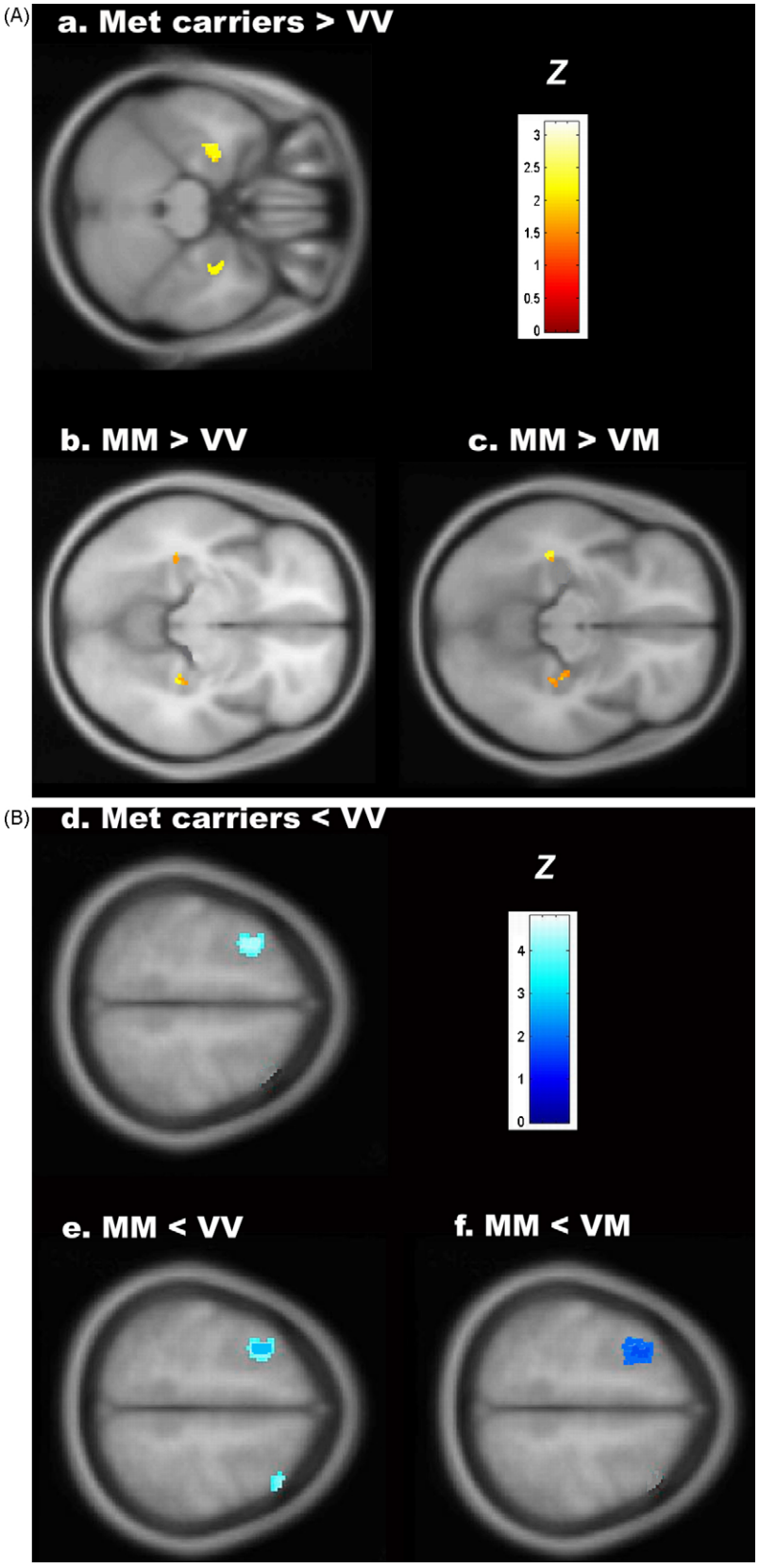

Fig. 4. (A) For functional MRI data, activation to oddball targets differed between genotypes in both the hippocampus and dorsolateral prefrontal cortex. Met carriers (MM and VM genotypes combined) showed significantly *exaggerated* activation in the hippocampus and parahippocampal gyrus compared to VV homozygotes (a: Met carriers > VV). In follow-up contrasts of MM and VM considered separately, this enhancement of activation was found to be present in MM genotypes compared to both VV homozygotes (b: MM > VV) and VM genotypes (c: MM > VM). In the contrast of MM > VM, enhanced activation in the parahippocampal gyrus extended into the amygdala (c). The legend shows the t-test scale for these contrasts, and coordinates for the regions of activation in each contrast are presented in Table 4. (B) By contrast, Met carriers (MM and VM genotypes combined) showed significantly *reduced* activation in the left dorsolateral prefrontal cortex compared to VV homozygotes (d: Met carriers < VV). In the follow-up

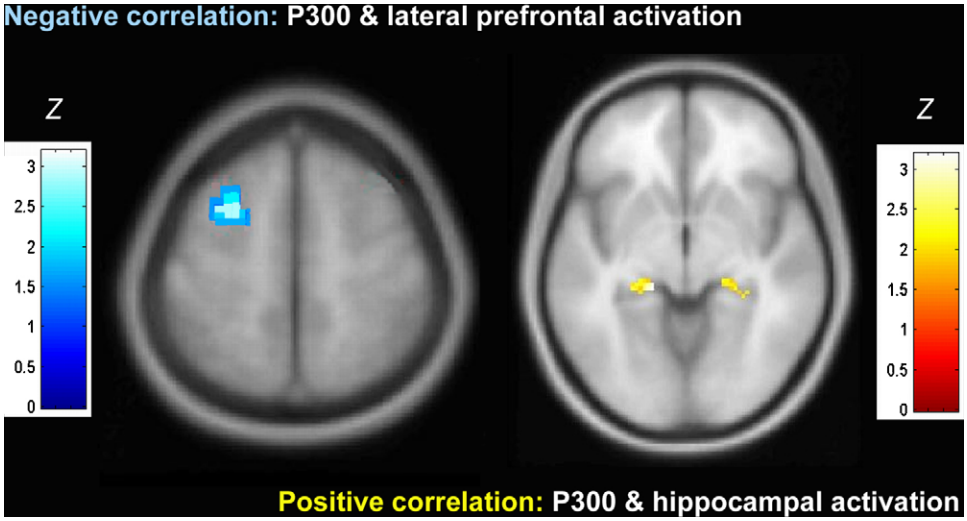

Fig. 5. Regression analysis for the prediction of functional MRI activation by P300 latency (at the exemplar F3 recording site). P300 latency significantly and positively predicted activation in the hippocampus, bilaterally (image on right), and significantly and negatively predicted activation in the left dorsolateral prefrontal cortex (image on left).

declarative memory (Hariri et al., 2003). However, consistent with previous findings, the effect of BDNF MM status was specific to the test of recall, and not present for other tests of memory (memory span, working memory, or semantic memory) or cognitive domains more generally (i.e., executive function, attention, verbal processing or sensori-motor abilities). It is possible that variations in the impact of MM status on different aspects of recall (immediate or delayed for instance) might reflect variation in the demands of the task used in each study. Nonetheless, these findings suggest that the overt impact of the BDNF Met allele on behavior is subtle in otherwise healthy people. Moreover, the presence of a significant finding within a battery of multiple cognitive tests warrants replication, to rule out the possibility it is a chance effect. In this study, stringent exclusion criteria were used to identify a healthy sample. A related possibility is that, if Met carrier status is associated with increased risk for mental disorder, screening for healthy status in this study may have contributed to a bias towards an absence of cognitive impairment in Met carriers.

3.2. BDNF MM genotype and delayed fronto-central P300 latency

Brain function measures may provide a more sensitive index of genetic effects than behavioral measures of cognitive performance. In this study, MM homozygotes showed a delay in P300 latency to task-relevant target stimuli in the oddball task, which was most apparent over fronto-central sites. The fronto-central locus of the delayed P300 in MM subjects is compatible with evidence that hippocampal, as well as anterior and medial temporal cortex lesions, are associated with a deficit in the P300 over far frontal as well as temporal regions (Knight, 1996, McCarthy et al., 1989; Onofrj et al., 1992). From these findings, it has been suggested that medial temporal (including hippocampal) structures may be involved in generating the field potentials that propagate to the surface (and are reflected in scalp recordings over frontal regions, for instance), or provide modulatory input which is necessary to generate the P300 over these regions (Soltani and Knight, 2000). From this view, delayed P300 latency could reflect a detrimental impact of BDNF Met on the propagation or modulation of hippocampal-prefrontal potentials such that updating of activity in these circuits during attention and working memory is slowed. Compatible with this interpretation, we have previously demonstrated that a delay in the P300 is a marker of Alzheimers disease which involves deficits to hippocampal-prefrontal circuits (Kraiuhin et al., 1989). Of course, we emphasize that the current findings are based on a correlational design, and specific experimental manipulations and longitudinal designs are required to elucidate these proposals.

Nonetheless, other studies have failed to observed hippocampal effects on the P300 (Jonson, 1989; Rugg et al., 1991). An alternative possibility is that the delayed P300 reflects the impact of BDNF MM status on the 'P3a', which is prominent frontally (Soltani and Knight, 2000), rather than the P3b. However, this account is unlikely, given that the P300 elicited in this study is more compatible with a P3b. The P3a is typically elicited by task-irrelevant novel stimuli (embedded within oddball targets and non-targets), and peaks within 240–300 ms (Polich, 2003). In this study, the P300 was elicited by oddball targets, peaked between 330 and 390 ms, and was more prominent over parietal than frontal sites (Fig. 2), characteristics which define the P3b. Slower P300 latency also predicted poorer detection of target stimuli in the oddball task, consistent with the proposed role of the P3b as an index of voluntary detection of task-relevant stimuli (Soltani and Knight, 2000).

3.3. BDNF MM genotype and alterations in hippocampal-lateral prefrontal activation

Functional MRI analysis provided complementary findings, suggesting that the Met allele is associated with alterations of hippocampal-prefrontal activation during selective processing of task-relevant target stimuli. For the combined sample, oddball targets elicited prominent activation in the hippocampus, hippocampal formation and dorsolateral prefrontal cortex, consistent with previous studies (Crottaz-Herbette et al., 2005; Kiehl et al., 2001; Williams et al., 2007; Yoshiura et al., 1999). There was a highly significant *exaggeration* of hippocampal activity for Met

contrast of MM and VM genotypes considered separately, this reduction was also present in MM genotypes compared to both VV homozygotes (e: MM < VV) and VM genotypes (e: MM < VM). The contrast of MM < VV revealed an additional reduction in the right dorsolateral prefrontal cortex (f). Regions of enhanced activation are shown in yellow, and regions of reduced activation in blue. The legend shows the *t*-test scale for these contrasts, and coordinates for the regions of activation in each contrast are presented in Table 4.

carriers (MM and VM subjects) but concomitant *reduction* in dorsolateral prefrontal cortex activation relative to VV homozygotes, and which was confirmed in equal-sized genotype groups.

This pattern of activation was predicted by P300 latency, such that slower latency predicted greater hippocampal but less lateral prefrontal activation to oddball targets, providing direct support for the convergence of fMRI and P300 findings. Again, the findings are correlational, warranting specific manipulations and designs to determine the nature of the association.

Nonetheless, this convergence of results suggests that the Met allele might contribute to a slowing of the P300, which involves dsyregulation of a distributed network comprising the hippocampus and its projections to the prefrontal cortex. MM homozygotes may require greater hippocampal capacity than VV homozygotes to selectively attend to significant stimuli, and to update them in memory, which may in turn interfere with resources available for prefrontal-dependent 'read out' of these stimuli. That is, Met carriers may take longer and draw on more blood oxygen within hippocampal circuits than VV subjects to selectively process stimuli and update their memory, with a concomitant loss of prefrontal function. This proposal may accord with the lack of hippocampal suppression observed previously in Met carriers during a working memory task, which requires a prefrontal contribution (Egan et al., 2003). The notion of dysregulation in these networks may also account for reports of reduced hippocampal activation in Met carriers for more complex visuospatial recall (Hariri et al., 2003).

3.4. BDNF MM genotype and hippocampal grey matter loss

In this study, structural MRI provided a means to explore whether BDNF genotypes are associated with neuroanatomical as well as functional alterations in hippocampal and lateral prefrontal network. Voxel-based morphometry revealed a significant reduction in hippocampal grey matter volume in Met carriers, consistent with previous morphologic and region-of-interest studies (Bueller et al., 2006; Pezawas et al., 2004; Szeszko et al., 2005). In between genotype comparisons of equal-sized groups, this grey matter reduction was most pronounced in MM homozygotes. However, a corresponding voxel-wise reduction in prefrontal volume was not observed in Met carriers, unlike Pezawas et al. (2004). Although prefrontal volume declines over age (Grieve et al., 2005), the absence of a prefrontal reduction in Met carriers is unlikely to be due to this factor, given that genotypes did not differ on mean age, and the inclusion of age as a covariate in focal analyses controlled for the range of ages.

It is possible that the impact of the BDNF Met allele is first observed in the hippocampus, consistent with its molecular effects, and that structural effects involving the prefrontal cortex (unlike functional effects) occur only with the progression towards disease status, such that they are not necessarily apparent in stringently screened healthy individuals. Variations in hippocampal grey matter have been associated with atrophy in degenerative memory conditions such as Alzheimer's disease, exemplifying the role that neuroanatomical change can play in impaired cognitive function (Scheltens and Korf, 2000). As grey matter variation did not correlate directly with cognitive or P300 latency variations, the effect of the BDNF Met allele on grey matter volume may impact the neural system via altered synaptic connections of hippocampal-prefrontal projections, or other cellular mechanisms.

3.5. Linking BDNF genotypes, neural processes and cognition: Implications for mechanism

The BDNF gene has been implicated in multiple neurodegenerative and psychiatric disorders (Hall et al., 2003; Kunugi et al., 2001; Momose et al., 2002; Neves-Pereira et al., 2002; Ribases et al., 2003; Riemenschneider et al., 2002; Sklar et al., 2002; Ventriglia et al., 2002), suggesting that the association is with an underlying neural trait rather than a disease specific marker. Our data indicate that highly heritable brain function endophenotypes, such as the P300, may have particular utility in capturing the association between the BDNF MM genotype and the underlying trait. On the other hand, MM genotype status may have more subtle and less direct effects on overt behavior, reflected in cognitive performance.

A mechanism has been proposed by which the Met allele results in lower activity-dependent secretion of BDNF from hippocampal and cortical neurons (Egan et al., 2003; Chen et al., 2004). This reduction may in turn lead to alter neuronal signaling processes in Met carriers, and particularly MM homozygotes, compared to VV homozygotes. This alteration may contribute to a dysregulation in activation of the hippocampus and its prefrontal projections, reflected in our fMRI data, and the slowing of neuronal activity, reflected in the delayed P300. It may only be when alterations in neuronal signaling reach the point of more marked disturbance that primary effects on behavior, reflected in more robust and widespread effects on cognitive test performance, become apparent. Investigation of the long-term cellular consequences of the BDNF Val66Met polymorphism is required to verify this proposed mechanism.

3.6. Limitations and future research

As the first study to examine BDNF genotypes, brain structure, ERPs and cognition in the one study, the findings raise a number of issues requiring further investigation. Our observation that BDNF Met allele impacts P300 latency in particular warrants confirmation in independent samples, as well as by the inclusion of other selective attention tasks. Dissociation studies which examine BDNF variants along with other variants likely to be unrelated to the P300 would be particularly valuable in determining the specificity of the findings. Further elucidation of the normative relationships between gene, brain function and cognitive behavioral performance will also be important in establishing a framework from which to assess the specific role of BDNF variants in risk for disease states. Disturbances in the P300 are a well-established marker of psychiatric illness (Baguley et al., 1997; Brown et al., 2000; Felmingham et al., 2002; Kraiuhin et al., 1989; Lagopoulos et al., 1998; Landau et al., 1998; Lazzaro et al., 1997; Williams et al., 2003), and future studies are needed to examine the contribution of the BDNF Met allele to the P300 endophenotype and complementary measures of brain function in these psychiatric conditions.

Acknowledgments

Supported by an Australian Research Council (ARC) Linkage grant (LP0455104) to LMW, PRS and RHP, with Brain Resource Company as industry partner. LMW holds a peer-reviewed Pfizer foundation Senior Research Fellowship, and PRS held an NHMRC Senior Principal Research Fellowship (no. 157209). CDS was supported by a European Molecular Biology Organisation post-doctoral fellowship (ALTF 166–2004). We acknowledge BRC's in-kind support in providing access to the Brain Resource International Database (BRID). Access to the BRID is administered via a scientific network independent of BRC's operations, known as BRAINnet (http://www.brainnet.net).

References

Ashburner, J., Friston, K.J., 2000. Voxel-based morphometry – the methods. Neuroimage 11, 805–821.

Baddeley, A., Emslie, H., Nimmo-Smith, I., 1993. The Spot-the-Word test: a robust estimate of verbal intelligence based on lexical decision. The British Journal of Clinical Psychology 32, 55–65.

Baguley, I.J., Felmingham, K.L., Lahz, S., Gordan, E., Lazzaro, I., Schotte, D.E., 1997. Alcohol abuse and traumatic brain injury: effect on event-related potentials. Archives of Physical Medicine and Rehabilitation 78, 1248–1253.

Benton, A.L., Hamsher, K., 1989. Multilingual Aphasia Examination. AJA Associates, Iowa City, Iowa.

Bramon, McDonald, E.C., Croft, F R.J., Landau S., Filbey, F., Cruzelier, J.H., Sham, P.C., Frangou, S., Murray, R.M., 2005. Is the P300 wave an endophenotype for schizophrenia? A meta-analysis and a family study. Neuroimage 27, 960–968.

Brown, K., Gordon, E., Williams, L.M., Bahramali, H., Harris, A.W.F., Gray, J.A., et al., 2000. Misattribution of sensory input reflected in dysfunctional target: Non-target ERPs in schizophrenia. Psychological Medicine 30, 1443–1449.

Bueller, J.A., Aftab, M., Sen, S., Gomez-Hassan, D., Burmeister, M., Zubieta, J.K., 2006. BDNF Val66Met allele is associated with reduced hippocampal volume in healthy subjects. Biological Psychiatry 59, 812–815.

Chen, Z.Y., Patel, P.D., Sant, G., Meng, C.X., Teng, K.K., Hempstead, B.L., et al., 2004. Variant brain-derived neurotrophic factor (BDNF) (Met66) alters the intracellular trafficking and activity-dependent secretion of wild-type BDNF in neurosecretory cells and cortical neurons. Journal of Neuroscience 24, 4401–4411.

Clark, C.R., Orr, R.S., Wright, E.K., Weber, D.L., 1998. Working memory updating to visual verbal stimuli: a high resolution ERP study. In: Koga, Y., Nagata, K., Hirata, K. (Eds.), Brain Topography Today. Elsevier, Tokyo, pp. 173–178.

Clark, C.R., Paul, R.H., Williams, L.M., Arns, M., Fallahpour, K., Handmer, C., Gordon, E., 2006. Standardized assessment of cognitive functioning during development and aging using an automated touchscreen battery. Archives of Clinical Neuropsychology 21, 449–467.

Crottaz-Herbette, S., Lau, K.M., Glover, G.H., Menon, V., 2005. Hippocampal involvement in detection of deviant auditory and visual stimuli. Hippocampus 15, 132–139.

Donchin, E., Coles, M.G.H., 1998. Is the P300 component a manifestation of context updating? The Behavioral and Brain Sciences 11, 357–374.

Downar, J., Crawley, A.P., Mikulis, D.J., Davis, K.D., 2002. A cortical network sensitive to stimulus salience in a neutral behavioral context across multiple sensory modalities. The Journal of Neurophysiology 87 (1), 615–620.

Duncan-Johnson, C.C., Donchin, E., 1982. The P300 component of the event-related potential as an index of information processing. Biological Psychology 14, 1–52.

Egan, M.F., Kojima, M., Callicott, J.H., Goldberg, T.E., Kolachana, B.S., Bertolino, A., et al., 2003. The BDNF val66met polymorphism affects activity-dependent secretion of BDNF and human memory and hippocampal function. Cell 112, 257–269.

Felmingham, K.L., Bryant, R.A., Gordon, E., Kendall, C., 2002. Event-related potentials in posttraumatic stress disorder: The role of numbing. Psychiatry Research 109, 171–179.

Friedman, W.J., Greene, L.A., 1999. Neurotrophin signaling via Trks and p75. Experimental Cell Research 253, 131–142.

Friston, K.J., Holmes, A.P., Poline, J.B., Price, C.J., Frith, C.D., 1996. Detecting activations in PET and fMRI: levels of inference and power. NeuroImage 4, 223–235.

Geffen, G.M., Moar, K.J., Hanlon, A.P., Clark, C.R., Geffen, L.B., 1990. Performance measures of 16- to 86-year-old males and females on the Auditory Verbal Learning test. The Clinical Neuropsychologist 4, 45–63.

Golden, C.J., 1978. Stroop Color and Word Test. Stoelting Company, Wood Dale, IL.

Gordon, E., 2003a. Integrative neuroscience and psychiatry. Neuropsychopharmacology 28 (1), S2–S8.

Gordon, E., 2003b. Integrative neuroscience in psychiatry: the role of a standardized database. Australasian Psychiatry 11, 156–163.

Gordon, E., Cooper, N., Rennie, C., Hermens, D., Williams, L.M., 2005. Integrative neuroscience: the role of a standardized database. Clinical EEG and Neuroscience 36, 64–75.

Gratton, G., Coles, M.G., Donchin, E., 1983. A new method for off-line removal of occular artifact. Electroencephalography and Clinical Neurophysiology 55, 468–484.

Grieve, S., Clark, C.R., Williams, L.M., Gordon, E., 2005. Limbic and paralimbic preservation with aging. Human Brain Mapping 25, 391–401.

Halgren, E., Squires, N.K., Wilson, C.L., Rohrbraugh, J.W., Babb, T.L., Crandall, P.H., 1980. Endogenous potentials generated in the human hippocampal formation and amygdala by infrequent events. Science 210, 803–805.

Hall, D., Dhilla, A., Charalambous, A., Gogos, J.A., Karayiorgou, M., 2003. Sequence variants of the brain-derived neurotrophic factor (BDNF) gene are strongly associated with obsessive-compulsive disorder. American Journal of Human Genetics 73, 370–376.

Hariri, A.R., Weinberger, D.R., 2003. Imaging genomics. British Medical Bulletin 65, 259–270.

Hariri, A.R., Goldberg, T.E., Mattay, V.S., Kolachana, B.S., Callicott, J.H., Egan, M.F., et al., 2003. BDNF val66met polymorphism modulates memory-related hippocampal activity and predicts memory performance. Journal of Neuroscience 23, 6690–6694.

Harris, S.E., Fox, H., Wright, A.F., Hayward, C., Starr, J.M., Whalley, L.J., et al., 2006. The brain-derived neurotrophic factor Val66Met polymorphism is associated with age-related change in reasoning skills. Molecular Psychiatry 11, 505–513.

Heit, G., Smith, M.E., Halgren, E., 1990. Neuronal activity in the human medial temporal lobe during recognition memory. Brain 113, 1093–1112.

Hesselbrock, V., Begleiter, H., Porjesz, B., O'Connor, S., Bauer, L., 2001. P300 event-related potential amplitude as an endophenotype of alcoholism-evidence from the collaborative study on the genetics of alcoholism. Journal of Biomedical Science 8, 77–81.

Hickie, I., Hadzi-Pavlovic, D., Scott, E., Davenport, T., Koschera, A., Naismith, S., 1998. SPHERE: a national depression project. Australasian Psychiatry 6, 248–250.

Jonson Jr., R., 1989. Auditory and visual P300s in temporal lobectomy patients: evidence for modality-dependent generators. Psychophysiology 26, 633–650.

Knight, R.T., 1996. Contribution of human hippocampal region to novelty detection. Nature 383, 256–259.

Kraiuhin, C., Yiannikis, C., Coyle, S., Gordon, E., Rennie, C., Howson, A., et al., 1989. The relationship between reaction time and latency of the P300 event-related potential in normal subjects and Alzheimer's disease. Clinical and Experimental Neurology 26, 80–88.

Kroenke, K., Spitzer, R.L., 2002. The PHQ-9: a new depression and diagnostic severity measure. Psychiatric Annals 32, 509–521.

Kunugi, H., Ueki, A., Otsuka, M., Isse, K., Hirasawa, H., Kato, N., et al., 2001. A novel polymorphism of the brain-derived neurotrophic factor (BDNF) gene associated with late-onset Alzheimer's disease. Molecular Psychiatry 6, 83–86.

Lagopoulos, J., Clouston, P., Barhamali, H., Gordon, E., Li, W.M., Lesley, J., et al., 1998. Late components of the event-related potentials and their topography in Parkinson's disease. Movement Disorders 13, 262–267.

Landau, P., Gordon, E., Kraiuhin, C., Sara, G., Singer, A., Zurynski, Y., et al., 1998. The use of regional cerebral blood flow and the P300 event-related potential (ERP) in the diagnosis of dementia and depression. Australia New Zealand Journal of Medicine 18, 421–431.

Lazzaro, I., Anderson, J., Gordon, E., Clarke, S., Leong, J., Meares, R.A., 1997. Single trial variability within the P300 (250–500 ms) processing window in adolescents with attention deficit hyperactivity disorder. Psychiatry Research 73, 91–101.

McCarthy, G., Wood, C.C., Williamson, P.D., Spencer, D.D., 1989. Task-dependent field potentials in human hippocampal formation. Journal of Neuroscience 9, 4253–4260.

McCarthy, G., Luby, M., Gore, J., Goldman-Rakic, P., 1997. Infrequent events transiently activate human prefrontal and parietal cortex as measured by functional MRI. Journal of Neurophysiology 77, 1630–1634.

Menon, V., Ford, J.M., Lim, K.O., Glover, G.H., Pfefferbaum, A., 1997. Combined event-related fMRI and EEG evidence for temporal-parietal cortex activation during target detection. NeuroReport 8, 3029–3037.

Milner, B., 1970. Memory and the medial temporal regions of the brain. In: Pribram, K.H., Broadbent, D.E. (Eds.), Biology of Memory. Academic Press, New York.

Momose, Y., Murata, M., Kobayashi, K., Tachikawa, M., Nakabayashi, Y., Kanazawa, I., et al., 2002. Association studies of multiple candidate genes for Parkinson's disease using single nucleotide polymorphisms. Annals of Neurology 51, 133–136.

Neves-Pereira, M., Mundo, E., Muglia, P., King, N., Macciardi, F., Kennedy, J.L., 2002. The brain-derived neurotrophic factor gene confers susceptibility to bipolar disorder: evidence from a family-based association study. American Journal of Human Genetics 71, 651–655.

O'Connor, S.O., Morzorati, S., Christian, J.C., Li, T.-K., 1994. Heritable features of the auditory oddball event-related potential: peaks, latencies, morphology and topography. Electroencephalography and Clinical Neurophysiology 59, 238–248.

Onofrj, M., Fulgente, T., Nobilio, D., Malatesta, G., Bazzano, S., Colamartino, P., Gambi, D., 1992. P3 recordings in patients with bilateral temporal lobe lesions. Neurology 42, 1762.

Paul, R.H., Lawrence, J., Williams, L.M., Clark, C.R., Cooper, N., Gordon, E., 2005. A standardized battery for computerized neurocognitive assessment: Methods and construct validity of the 'IntegNeuro™'. International Journal of Neuroscience 115, 1549–1567.

Paul, R.H., Gunstad, J., Cooper, N.J., Williams, L.M., Clark, R.C., Cohen, R.A., Lawrence, J.J., Gordon, G., 2007. Cross-cultural assessment of neuropsychological performance and electrical brain function measures: Additional validation of an international brain database. International Journal of Neuroscience 117, 549–568.

Pezawas, L., Verchinksi, B.A., Mattay, V.S., Callicott, J.H., Kolachana, B.S., Straub, R.E., et al., 2004. The brain-derived neurotrophic factor val66met polymorphism and variation in human cortical morphology. Journal of Neuroscience 10, 10099–10102.

Polich, J., 2003. Theoretical overview of P3a and P3b. In: Polich, J. (Ed.), Detection of Change: Event-Related Potential and fMRI Findings. Kluwer Academic, Boston, pp. 83–98.

Polich, J., Kok, A., 1995. Cognitive and biological determinants of P300: an integrative review. Biological Psychology 41, 103–146.

Poo, M.M., 2001. Neurotrophins as synaptic modulators. Nature Review Neuroscience 2, 24–32.

Reitan, R.M., 1958. Validity of the Trail Making Test as an indicator of organic brain damage. Perceptual and Motor Skills 8, 271–276.

Rennie, C.J., Robinson, P.A., Wright, J.J., 2002. Unified neurophysical model of EEG spectra and evoked potentials. Biological Cybernetics 86, 457–471.

Ribases, M., Gratacos, M., Armengol, L., de Cid, R., Badia, A., Jimenez, L., et al., 2003. Met66 in the brain-derived neurotrophic factor (BDNF) precursor is associated with anorexia nervosa restrictive type. Molecular Psychiatry 8, 745–751.

Riemenschneider, M., Schwarz, S., Wagenpfeil, S., Diehl, J., Muller, U., Forstl, H., et al., 2002. A polymorphism of the brain-derived neurotrophic factor (BDNF) is associated with Alzheimer's disease in patients lacking the Apolipoprotein E epsilon4 allele. Molecular Psychiatry 7, 782–785.

Rugg, M.D., Pickles, C.D., Potter, D.D., Roberts, R.C., 1991. Normal P300 following extensive damage to the left medial temporal lobe. Journal of Neurology, Neurosurgery, and Psychiatry 54, 217–222.

Scheltens, P., Korf, E.S., 2000. Contribution of neuroimaging in the diagnosis of Alzheimer's disease and other dementias. Current Opinion in Neurology 13, 391–396.

Sen, S., Nesse, R.M., Stoltenberg, S.F., Li, S., Gleiberman, L., Chakravarti, A., et al., 2003. A BDNF coding variant is associated with NEO personality inventory domain neuroticism, a risk factor for depression. Neuropsychopharmacology 28, 397–401.

Sklar, P., Gabriel, S.B., McInnis, M.G., Bennett, P., Lim, Y.M., Tsan, G., et al., 2002. Family-based association study of 76 candidate genes in bipolar disorder: BDNF is a potential risk locus. Molecular Psychiatry 7, 579–593.

Soltani, M., Knight, R.T., 2000. Neural origins of the P300. Critical Reviews in Neurobiology 14, 199–224.

Szeszko, P.R., Lipsky, R., Mentschel, C., Robinson, D., Gunduz-Bruce, H., Sevy, S., et al., 2005. Brain-derived neurotrophic factor val66met polymorphism and volume of the hippocampal formation. Molecular Psychiatry 10, 631–636.

Tyler, W.J., Alonso, M., Bramham, C.R., Pozzo-Miller, L.D., 2001. From acquisition to consolidation: on the role of brain-derived neurotrophic factor signaling in hippocampal-dependent learning. Learning & Memory 9, 224–237.

Ventriglia, M., Bocchio Chiavetto, L., Benussi, L., Binetti, G., Zanetti, O., Riva, M.A., et al., 2002. Association between the BDNF 196 A/G polymorphism and sporadic Alzheimer's disease. Molecular Psychiatry 7, 136–137.

Verleger, R., 1998. Event-related potentials and cognition: a critique of the context updating hypothesis and an alternative interpretation of P3. The Behavioral and Brain Sciences 11, 343–356.

Walsh, K.W., 1985. Understanding Brain Damage: A Primer of Neuropsychological Evaluation. Churchill Livingstone, Edinborough, pp. 234–237.

Williams, L.M., Bahramali, H., Hemsley, D.R., Harris, A.W.F., Brown, K., Gordon, E., 2003. Electrodermal responsivity distinguishes ERP activity and symptom profile in schizophrenia. Schizophrenia Research 59, 115–125.

Williams, L.M., Simms, E., Clark, C.R., Paul, R., Rowe, D., Gordon, E., 2005. The reproducibility of a standardized and integrated neurophysiological and neuropsychological test battery. International Journal of Neuroscience 115, 1605–1630.

Williams, L.M., Felmingham, K., Kemp, A.H., Rennie, C., Brown, K.J., Bryant, R.A., Gordon, E., 2007. Mapping frontal-limbic correlates of orienting to change detection. NeuroReport 18, 197–200.

Worsley, K.J., Marrett, S., Neelin, P., Vandal, A.C., Friston, K.J., Evans, A.C., 1996. A unified statistical approach for determining significant signals in images of cerebral activation. Human Brain Mapping 4, 58–73.

Yoshiura, T., Zhong, J., Shibata, D., Kwok, W., Shrier, D.A., Numaguchi, Y., 1999. Functional MRI study of auditory and visual oddball tasks. Neuroreport 10 (8), 1683–1688.