UNIVERSITY
OF TASMANIA

Environmental and genetic contributors to cognitive reserve

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Submitted in fulfilment of the requirements for the Degree of Doctor of Philosophy

University of Tasmania, January 2015

Declaration of Originality

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Paper 2 - ***APOE*** **and** ***BDNF*** **Val66Met polymorphisms combine to influence episodic memory function in older adults**

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Statement of Ethical Conduct

The research associated with this thesis abides by the international and Australian codes on human and animal experimentation, the guidelines by the Australian Government's Office of the Gene Technology Regulator, and the rulings of the Safety, Ethics and Institutional Biosafety Committees of the University.

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Publications from Thesis

Peer reviewed articles

Ward, D. D., Summers, M. J., Saunders, N. L., & Vickers, J. C. (2014). Modelling cognitive reserve in healthy middle-aged and older adults: The Tasmanian Healthy Brain Project. *International Psychogeriatrics*, FirstView.

Ward, D. D., Summers, M. J., Saunders, N. L., Janssen, P., Stuart, K. E., & Vickers, J. C. (2014). APOE and BDNF Val66Met polymorphisms combine to influence episodic memory function in older adults. *Behavioural Brain Research*, *271*, 309-315.

Papers and Posters Presented at Conferences

Ward, D. D., Summers, M. J., Saunders, N. L., & Vickers, J. C. (2013). *Cognitive reserve and Apolipoprotein E in healthy cognitive function*. Oral presentation at University of Tasmanian Health Science HDR Research Conference, Hobart (Australia).

Ward, D. D., Summers, M. J., Saunders, N. L., Valenzuela, M. J., Summers, J. J., Ritchie, K., Robinson, A., & Vickers, J. C. (2013). *The effects of cognitive reserve and APOE on healthy cognitive function*. Poster presented at Alzheimer's Association International Conference, Boston (USA).

Ward, D. D., Summers, M. J., Saunders, N. L., Janssen, P., Stuart, K. E., & Vickers, J. C. (2014). *APOE and BDNF Val66Met polymorphisms interact to predict episodic memory*. Poster presented at University of Tasmania Health Science HDR Research Conference, Hobart (Australia).

Ward, D. D., Summers, M. J., Saunders, N. L., & Vickers, J. C. (2014). *Cognitive reserve, APOE, and BDNF Val66Met in healthy adult cognitive function*. Oral presentation at International Psychogeriatric International Meeting, Beijing (China).

Vickers, J. C., **Ward, D. D.**, Stuart, K. E. Saunders, N. L., & Summers, M. J. (2014). *Effects of cognitive reserve and the BDNF Val66Met polymorphism on episodic memory, working memory, executive function and language processing in healthy older adults.* Poster presentation at Society for Neuroscience Annual Conference, Washington (USA).

Abstract

An individual's rate of age-related cognitive decline and risk for later-life dementia is influenced by environmental and genetic factors. One protective environmental factor that may mitigate cognitive decline is cognitive reserve (CR) and is generated by participation in tasks that involve complex cognitive engagement. Two genes that may influence CR through their associations with synaptic plasticity are apolipoprotein E (*APOE*) and brain-derived neurotrophic factor (*BDNF*). Participants from the on-going Tasmanian Healthy Brain Project, a longitudinal investigation into whether an intervention of later-life tertiary education increases CR, contributed to the data analysed in this thesis. It was aimed: to develop two operational measures of CR; to quantify the cognitive implications of variation in *APOE* and *BDNF* Val66Met; and to detect genetic interactions with CR. Comprehensive neuropsychological assessments were conducted as part of the baseline testing for Tasmanian Healthy Brain Project participants. *APOE* and *BDNF* Val66Met polymorphisms were determined through analysis of saliva samples. The statistical analyses yielded three main results. First, factor analysis was successful in identifying latent variables in both prior and current models of CR. Second, while variation in *APOE* or *BDNF* did not produce significant main effects in any assessed cognitive domain, an *APOE* x *BDNF* interaction was present in episodic memory function. Third, *BDNF* moderated the association between CR and executive function, with a significantly reduced association between the variables present in *BDNF* Met carriers. Overall, this investigation developed a method of assessing CR that can provide a comprehensive estimate of an individual's CR and furthered knowledge on the effect single genes exert on healthy cognitive function. The most important finding to emerge was a CR x *BDNF* interaction in cognitive function, which suggests that CR-based interventions aimed at delaying dementia onset may have a higher efficacy in *BDNF* Val homozygotes than in *BDNF* Met carriers.

Acknowledgements

I would like to thank my supervisors, Mathew, James and Nikki for their assistance, patience and guidance. Over the course of my candidature, each of you helped me develop professionally in different ways, and I now feel prepared for a career in science. Thank you for making time for me in your incredibly busy schedules - it has been greatly appreciated.

I would like to acknowledge the role my friends and family played during the creation of this thesis. In particular, my father for encouraging me to extend myself; my mother for imparting her sense of humour and exceptional coping skills; and my partner, Donnamay, for supporting me through it all. My friends and fellow students at Wicking, School of Medicine and Menzies – thank you for being a bunch of intelligent, supportive and funny people. You have been essential to the success of my PhD.

My appreciation goes to the University of Tasmania, the Australian Government, and the Wicking Dementia Research and Education Centre for their financial support during my candidature. I also extend my gratitude to all participants involved in the Tasmanian Healthy Brain Project – your willingness to contribute towards scientific research has been greatly valued.

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Chapter 1

Introduction

Australia's ageing population is typical of most developed countries. Over the previous 100 years, average life expectancy in Australia has increased by 24 years for males and 25 years for females, mainly due to reduced mortality rates as a result of medical, public health and social advances (ABS, 2010a). Consequently, the average life expectancy of a child born in 1901 was 55.2 years for males and 58.8 years for females and has increased for a child born in 2006 to 78.7 years for males and to 83.5 years for females (ABS, 2010a). Not only are Australians living longer, but the overall Australian population is also ageing proportionately, with the proportion of Australians aged 65 years and over increasing from 4% in 1901 to 13.5% in 2010 (ABS, 2010a).

The trend towards a progressively older Australia is not likely to halt, with the proportion of 65-85 year olds estimated to double, and the proportion of those aged 85 years and over expected to quadruple, by 2050 (ABS, 2011). Notably, the growth in older Australians will not be linear; the proportion of Australians aged over 65 years will increase from 13% in 2007 to 25% in 2050 (ABS, 2008). The significance of Australia's ageing population is pertinent when the rates of disability are considered, with 88% of Australians aged 90 years or older reporting a disability in 2011 (ABS, 2011). If similar rates of age-related disability are observed in the coming years, a greater proportion of Australians will be living with a disability over time. Such conclusions partially account for the projected increase in aged-care-related spending by the Australian government, from 0.8% of GDP in 2010 to 1.8% of GDP by 2050 (Australian Government, 2010).

One of the major causes of death and disability in older Australians is dementia (ABS, 2010b; Mathers, Vos, Stevenson, & Begg, 2001), with the incidence of dementia in Australia expected to rise from 266,574 in 2010 to 942,624 by 2050 (Access Economics, 2011). As Alzheimer's disease (AD) accounts for 50-70% of dementia cases (Hardy, 1997), a

considerable amount of research has been undertaken to identify psychosocial or pharmacological treatments that can halt or slow the progression of AD. If an intervention is successful in delaying the average onset of AD by five months, the incidence of new AD cases would be reduced by 5% per year (Access Economics, 2004). Similarly, a reduction in the incidence of AD would correspond to a reduction in the mortality attributable to AD (James et al., 2014). Recent reports have indicated that potentially modifiable risk factors, such as low education, may account for between a third (Norton, Matthews, Barnes, Yaffe, & Brayne, 2014) to a half (Barnes & Yaffe, 2011) of all dementia cases.

With a high degree of importance placed upon research into AD and other dementing illnesses, an often overlooked population are those ageing older Australians who do not develop dementia, but who will have to cope with non-pathological reductions in cognitive function associated with normal ageing. This demographic, as with that of dementia sufferers, is expected to rise considerably over the next 50 years (Access Economics, 2011).

Healthy ageing

Ageing is a complex biological process. With increasing age, the deterioration of physiological function and increasing vulnerability occurs (De Magalhaes, 2011). In addition to reductions in physiological function, cells in all regions of the nervous system are affected by ageing (Hofer, Berg, & Era, 2003) and the organism's ability to maintain cellular homeostasis is progressively impeded (Kowald, 2002). This leads to normal age-related declines in many cognitive domains, but not all (Deary et al., 2009). A contentious feature of ageing is that it is non-pathological, and normal ageing has been defined as the absence of an increase number of conditions (Brayne, 2007), while others note that the absence of illness and accidents allows the ageing process to occur naturally (Hansen-Kyle, 2005). This

distinction between normal ageing-related decline and pathology-induced decline is crucial for elucidating ageing processes and consideration of both processes separately defines the two-component model of ageing (Hedden & Gabrieli, 2004).

Cognitive ageing

Other than age-related conditions affecting physical function, reports of cognitive difficulties associated with increasing age are common. Certainly, an inevitable and gradual reduction in cognitive and mental capacities is associated with growing older (Salthouse, 2012; 2009). An important feature of cognitive ageing is that decline in cognitive function is not uniform across different cognitive domains. For example, while it is well established that general memory performance declines with age (Small, Stern, Tang, & Mayeux, 1999), not all components of memory are affected equally (Craik & McDowd, 1987). Although cross-sectional and longitudinal experiments report different patterns of cognitive decline onset and severity (Salthouse, 2009; Schaie, 1996), results from both methodologies show some overlap. Typically, age-related cognitive decline is observed in the capacity for episodic memory, working memory, and processing speed, whereas cognitive stability is generally observed among short-term memory, semantic knowledge, autobiographical memory, and emotional processing (Hedden & Gabrieli, 2004; Hoogendam, Hofman, Geest, Lugt, & Ikram, 2014).

Selective cognitive functions display decline across the lifespan, beginning early in adulthood (Salthouse, 2009). An early review identified robust age-related decline to working memory, processing speed, and episodic memory encoding (Craik, 1994) and has been supported by subsequent studies (Park et al., 2002; 1996; Schaie, 1996). For example, a cross-sectional investigation of 301 participants, aged 20-90 years, reported significant linear life-long

decline in the three cognitive domains reported by Craik, after having matched participants for education, health, and demographic variables (Park et al., 1996). Significantly, an interrelationship was identified between processing speed, working memory performance, and other memory tasks, indicating that the efficacy of these processes was a significant predictor of age-related decline in memory function, a result confirmed in a later study (Park et al., 2002). Cross-sectional data from the Seattle Longitudinal Study also identified linear age-related declines in processing speed, episodic memory, spatial ability, and reasoning (Schaie, 1996).

Although cross-sectional designs indicate life-long decline in some cognitive processes begins in early adulthood, longitudinal investigations tend to describe a later onset of decline. In addition to examining cross-sectional effects, the main longitudinal analyses from the Seattle Longitudinal Study reported no decline in verbal ability, inductive reasoning, numeric ability, verbal memory, and spatial orientation until the age of 60, after which, a curvilinear decline became apparent, with the rate of decline increasing with age (Schaie, 1996). Notably, processing speed showed steady and linear decline from the age of 25, consistent with cross-sectional results.

Age-related decline in well-practiced or knowledge-based tasks is minimal until very late-life (70+ years of age; Hedden & Gabrieli, 2004). In one cross-sectional study, verbal knowledge, a composite score composed from Shipley vocabulary, synonym vocabulary, and antonym vocabulary, showed slight improvements throughout life until decline began at age 70 (Park et al., 2002). Similarly, in both longitudinal and cross-sectional analyses from the Seattle Longitudinal Study, measures of verbal ability and semantic knowledge remained stable until very late in life (Schaie, 1996). For short-term memory, slight, but uniform, age-related decline was present in both forward and backward digit span, with the onset of accelerated

decline at age 70 (Gregoire & Van Der Linden, 1997). The very late-life declines in verbal ability, semantic knowledge, and short-term memory have been attributed to disease processes, with the suggestion that pathology-free ageing may result in either no late-life decline, or a more linear decline (Hedden & Gabrieli, 2004).

Ageing is commonly thought to be associated with universal cognitive decline, but some processes show patterns of stability over time. For example, implicit memory, or memory that is measured indirectly, shows minimal age-related reduction (La Voie & Light, 1994). Defined as the unconscious influence of previously encountered information on subsequent performance (Hedden & Gabrieli, 2004), implicit memory is an automatic memory process (Hasher & Zacks, 1979) and consequently should show minimal age-related decline. A meta-analysis of adult age differences in repetition priming, a method of assessing implicit memory, found no or minimal age-related change (La Voie & Light, 1994). Similarly, the processing of emotional information does not decline with age. Here, older adults are better at remembering emotional information, particularly positive memories (Charles, Mather, & Carstensen, 2003), and report less anxiety and depression (Jorm et al., 1998a), relative to younger adults.

Overall, despite disparities in the conclusions drawn from cross-sectional and longitudinal investigations, some broad patterns emerge. Measures of acquired knowledge tend to increase until 60 years of age and decline thereafter, whereas measures of efficiency or effectiveness of processing show a linear decline from early adulthood (Salthouse, 2011).

Age-related neural changes

The divergent patterns of age-related decline for separate cognitive processes indicate the potential selective vulnerability of specific neural circuits underlying these cognitive processes. With advances in brain-imaging techniques such as structural (MRI) and functional (fMRI) magnetic resonance imaging, and positron emission tomography (PET), it is now possible to quantify the brain's response to advancing age for discrete cognitive tasks *in vivo*. Structural MRI captures high resolution images that have been widely used to study anatomy and function (Cheng et al., 2006). When the voxel based morphometric analytic method is applied to MRI data, grey and white matter volume and density can be assessed (Ashburner & Friston, 2000). Both fMRI and PET assess neural activity during cognitive tasks and, while their temporal resolution is limited by the slow hemodynamic response, both methods can be used to identify neural networks responsible for cognitive processing (Cabeza & Nyberg, 2000).

Increasing age is associated with a progressive decline in whole-brain volume, in addition to the heightened vulnerability of specific brain regions (Kruggel, 2006). In addition to age-related differences in overall brain structures, grey matter and white matter have both shown differential ageing effects. For instance, whole-brain white matter increases linearly in volume until mid-life, after which an accelerated decline takes place, while whole-brain grey matter declines linearly with age beginning in early adulthood (Allen, Bruss, Brown, & Damasio, 2005; Sowell et al., 2003). Although the associations between regional volumes and cognitive functions tend to be weak to moderate, the strength of such correlations appears to increase with age (Greenwood, 2007).

Frontal lobe

Frontal cortices, as well as the hippocampus, have been found to show the greatest volumetric declines with age (Raz et al., 2005; Tisserand et al., 2002), particularly when compared to the relatively spared temporal and occipital lobes (Resnick, Pham, Kraut, Zonderman, & Davatzikos, 2003). Age-related cortical volume loss in the prefrontal cortex (PFC) has been estimated at about 5% per decade after the age of 20 (Raz et al., 2004), with the lateral structures undergoing the greatest volumetric declines with age (Tisserand et al., 2002). A longitudinal investigation estimated annual rates of volume loss in the lateral PFC at 0.91% and in the orbitofrontal PFC at 0.85% (Raz et al., 2005). Cortical thickness of the PFC also significantly declines with age, first evident in mid-life, while temporal and parahippocampal corticies remain intact (Salat et al., 2004).

A number of investigations have related these frontal volumetric effects to cognitive performance. Age-related shrinkage of the PFC has been associated with an increase in perseverative errors, reflecting a reduced control of executive functions (Raz, Gunning-Dixon, Head, Dupuis, & Acker, 1998). In support, one investigation reported that smaller frontal lobe volume, particularly white matter, was associated with a greater decline in executive functioning at one-year follow-up (Cardenas et al., 2011). Prefrontal volume has also been positively associated with fluid intelligence (Godfrey et al., 2000). Similarly, accuracy during a fast working memory task, a cognitive process associated with intelligence, was positively associated with grey matter volume of the right dorsolateral PFC (Takeuchi et al., 2012). Frontal regions have also been implicated in visual memory, with a positive association of left orbital PFC volume and performance (Steffens, McQuoid, Welsh-Bohmer, & Krishnan, 2003). The cognitive implications of variation in PFC volume are not limited to simple effects on performance, with PFC white matter volume negatively associated with

within-person variability in choice-reaction time and dorsolateral PFC volume negatively associated with within-person variability in episodic memory (Lövdén et al., 2013). Finally, atrophy and reduced PFC volume is strongly associated with dementia, and may predict clinical symptoms more sensitively than atrophy of the medial temporal lobe (Burgmans et al., 2009).

Although neuronal death was once erroneously considered to account for age-related declines in cortical volume (Brody, 1955), the loss of neurons due to advancing age is minimal (Pakkenberg & Gundersen, 1997; Yankner, Lu, & Loerch, 2008). Rather, age-related volume loss has been attributed to, for example, dendritic and axonal pruning (Dickstein et al., 2007; Pannese, 2011), reductions in synaptic density (Peters, Sethares, & Luebke, 2008), white matter loss (Gunning-Dixon, Brickman, Cheng, & Alexopoulos, 2009), and demyelination (Peters, 2002). Such changes likely account for the under- and over-recruitment of cortical regions during cognitive processing (Grady, 2008; Kalpouzos, Persson, & Nyberg, 2012) and the general reduction in the coordination of brain systems that occurs with age (Bishop, Lu, & Yankner, 2010). Specifically, the age-related disruption in white matter integrity may explain the non-specific recruitment of structures that is significantly more localised in younger adults (Andrews-Hanna et al., 2007; Bishop et al., 2010). Overall, the associations of regional brain volumes with age-related cognitive deficits are likely mediated by neural dysfunction and changes to functional recruitment that occur with age (Grady, 2008).

Functional MRI investigations have demonstrated the engagement of the PFC during the processing of a variety of cognitive tasks, particularly executive function, including decision-making and working memory (Cabeza, 2002; Cabeza & Nyberg, 2000). Research indicates that PFC recruitment is altered with advancing age. For working memory, ageing is associated with an increased prefrontal activity (Fakhri, Sikaroodi, Maleki, Ali Oghabian, &

Ghanaati, 2012; Grady, Yu, & Alain, 2007; Mattay et al., 2006), particularly in the left PFC. In support, a meta-analysis of 32 fMRI studies concluded that during working memory the cortical engagement by older adults is characterised by increased activation in bilateral regions of the dorsolateral PFC, as well as supplementary motor cortex and left inferior parietal lobule (Turner & Spreng, 2012). For another component of executive function, inhibitory control, a similar increase in the recruitment of regions of the PFC is observed with age (Mathis, Schunck, Erb, Namer, & Luthringer, 2009; Wood, Ischebeck, Koppelstaetter, Gotwald, & Kaufmann, 2009). Typically, the age-related over-recruitment of cortical regions within the PFC is localised in areas that are either not active in, or contralateral and homologous to those activated in, younger individuals (Grady, 2008).

It is uncertain whether increased cortical activation reflects compensatory processes or the non-selective recruitment of brain regions (Grady, 2008). However, the pattern of increased activity in older adults is often reported in those who perform better on tasks. Importantly, in one study the increased activity in the left inferior PFC was observed in older participants only during the successful encoding of information or during a working memory task with no evident ageing effects (Grady et al., 2007). Similarly, an age-related effect of increased bilateral dorsolateral PFC recruitment was present in older participants only when they performed similarly to the younger participants (Mattay et al., 2006). Here, older participants demonstrated reduced activation compared to younger when task difficulty was increased. Furthermore, even when activity in visual and auditory cortices is reduced in aged individuals, heightened activation of the PFC is still present, suggesting that the PFC may attempt to compensate for reduced function in other cortices (Fakhri et al., 2012).

The PFC also undergoes age-related alterations in neurotransmitters. Reduced efficiency of the dopamine system, which plays an important role in attention and executive control of

memory, is evident in the PFC with advancing age (Ghosh, Agarwal, & Haggerty, 2011). Dopamine concentration, transporter availability, and D1, D2 and D3 receptor density have all been shown to decline with age (Bäckman et al., 2011; Park & Reuter-Lorenz, 2009). As such, it has been proposed that many of the age-related cognitive deficits can be attributed to loss of such receptors (Li, Lindenberger, & Sikström, 2001). Further, it has been demonstrated that reductions in D2 dopaminergic receptor binding explain more of the variance in cognitive performance across the adult lifespan than chronological age (Bäckman et al., 2000). One investigation determined that age-related reductions in the PFC's capacity to synthesise dopamine, irrespective of cortical atrophy, were associated with reduced processing speed (Kalbitzer et al., 2012). Greater intra-individual variability was also associated with diminished D1 receptor in the dorsolateral prefrontal cortex, anterior cingulate gyrus, and parietal cortex, during a reaction time task (MacDonald, Karlsson, Rieckmann, Nyberg, & Bäckman, 2012).

Rather than simply affecting cognitive performance, D1 receptor binding also alters functional connectivity in healthy individuals. In one study of working memory, age was negatively associated with load-dependent left frontal recruitment; however, once D1 binding was controlled for, the age-related reduction in load-dependent blood-oxygen-level dependent signal in the left frontal cortex was eliminated (Bäckman et al., 2011). Similarly, the use of a D1 antagonist induced increased frontal bilateral connectivity in healthy young adults (Rieckmann, Karlsson, Fischer, & Bäckman, 2012), a pattern of cortical engagement characteristic of older age (Cabeza, 2002). In subjects who were administered the D1 antagonist, those who showed increased bilateral recruitment of the dorsal frontal network demonstrated a reduced negative effect of the dose on spatial working memory, leading the authors to suggest that this additional functional recruitment is consistent with the compensation hypothesis (Cabeza, 2001).

Alzheimer's disease

Although it was historically believed that AD is an inevitable, natural, consequence of the ageing process, a two-component model of ageing is generally accepted, in which the first step involves normal age-related decline in brain functioning and the second step involves separate, potentially avoidable, pathological changes (Ghosh et al., 2011). AD is the most common cause of dementia in older age (Hardy, 1997). Overall, dementia due to AD is characterised by early impairment to memory processes followed by an overall decline in global cognitive function, including executive dysfunction (Weintraub, Wicklund, & Salmon, 2012). The pathological hallmarks of AD are the accumulation of amyloid-beta (Aβ) fibrils into amyloid plaques, followed by the aggregation of hyperphosphorylated intracellular tau protein to form neurofibrillary tangles (NFT; Vickers et al., 2000).

Alzheimer's disease pathology

Consisting of fibrillar Aβ, amyloid plaques are the predominant feature of AD pathology (Glenner & Wong, 1984) and are derived via sequential proteolytic cleaves of the amyloid precursor protein (APP; Prox, Rittger, & Saftig, 2012). In the original amyloid cascade hypothesis, the deposition of Aβ was believed to cause neuronal dysfunction and cell death in the brain due to the toxic effect of the total amyloid load (Hardy & Higgins, 1992). However, more recent research has focused on how APP is differentially cleaved into Aβ peptides of different amino acid lengths (Hartmann et al., 1997). Here, Aβ peptides 42 amino acids in length are more likely to form into toxic insoluble fibres, which subsequently aggregate into amyloid plaques (Haass & Selkoe, 2007).

In addition to amyloid plaques, AD is characterised by aggregates of tau, a microtubule-associated protein, that take the form of NFTs within neurons (Vickers et al., 2000). In a non-pathological state, tau is expressed during neuronal development and becomes integrated into axons to maintain axon morphology (Caceres & Kosik, 1990). The formation of NFTs occur when tau becomes hyperphosphorylated, disassociates from the microtubules and aggregates into intraneuronal fibrillar inclusions (Gendron & Petrucelli, 2009). Although the formation of tau fibrils has been proposed to mediate the AD-related neuronal cell death, the exact mechanisms are unknown (Beharry et al., 2014; Chun & Johnson, 2007). Furthermore, why tau becomes hyperphosphorylated in AD is yet to be fully answered, but evidence suggests that accumulated Aβ may initiate the hyperphosphorylation process in tau (Huang & Jiang, 2009).

The toxicity of Aβ on synapses and neural networks has been established (Mucke & Selkoe, 2012) and Aβ correlates closely with the rate of other neurodegenerative biomarkers (Hyman, 2011). However, cognitive decline is only weakly associated with amyloid deposition (Jack et al., 2009). The characteristic early deficits in episodic memory are unlikely to be accounted for by amyloid pathology, which is typically deposited in regions comprising the default-mode network (DMN; Weintraub et al., 2012).The DMN encompasses functionally connected cortical areas that project onto structures within the medial temporal lobe (MTL; Buckner, Andrews-Hanna, & Schacter, 2008). Rather, reductions in memory function are more likely due to the formation of NFT in the MTL, which interrupt the neural networks responsible for memory function (Braak & Braak, 1991). Relative to amyloid load, NFT density is more closely related to both episodic memory function and risk for developing clinical AD (Bennett, Schneider, Wilson, Bienias, & Arnold, 2004; Guillozet, Weintraub, Mash, & Mesulam, 2003).

Dementia risk modification

An important finding that emerged from a post-mortem examination of aged-care residents was the identification of a group of individuals who displayed typical AD pathology yet retained cognitive performance similar to that of non-demented control participants (Katzman et al., 1988). In 137 participants, it was found that cognitively intact but pathologically compromised individuals had significantly higher brain weights than those that exhibited clinically reduced cognitive function. This led the authors to propose that there is not necessarily a direct relationship between neuropathological burden and cognitive outcome. Furthermore, the authors proposed that larger brain weight and subsequently more neurons confer a greater reserve that allows the extension of non-impaired function into old age (Katzman, 1993).

Other investigations have supported the association between measurements of brain size and risk for dementia. For example, a cross-sectional population-based study of ageing recorded neurological, psychological, and anthropometric measurements for 640 participants (Schofield, Logroscino, Andrews, Albert, & Stern, 1997). After adjusting for age, education, ethnicity, gender, and height, smaller head circumference was associated with increased risk for AD. Similarly, AD patients with smaller head circumferences were found to have either had the disease longer, or progressed more rapidly, than those with larger head circumferences (Graves et al., 1996).

More recent studies have provided support for these earlier findings. One study of AD patients demonstrated that participants with larger head circumferences showed a reduced negative association between level of brain atrophy and cognitive function, indicating a protective effect of brain size (Perneczky et al., 2010). Another study demonstrated that larger

intracranial volume was associated with a slower deterioration in cognitive function due to AD-related atrophy in patients with amnestic mild cognitive impairment (MCI; Guo et al., 2012). The finding that smaller head size is related to lower early-life socioeconomic status provides one explanation for such findings, as such associations may reflect the detrimental effect of economic disadvantage on brain and skeletal development (Kim et al., 2008).

Brain reserve capacity

Such associations have led researchers to propose the existence of a reserve that allows individuals with larger brains, and, potentially more neurons or synapses, to function at a non-impaired level for longer despite neuropathology (Satz, 1993). A critical review of the literature that related measurements of brain size to cognitive outcome following brain damage concluded that brain size may be a major factor in explaining the spectrum of outcomes (Satz, 1993). Satz proposed that a person has a critical level of brain reserve capacity and, once their reserve has been depleted, functional and clinical deficits may emerge. Although it is acknowledged that brain reserve is a hypothetical construct, measures of overall brain size and synapse count may help to operationally define the concept (Stern, 2002).

While brain size likely contributes to an individual's reserve, the brain reserve capacity model can only explain part of the variance in the association between neurological burden and cognitive ability. This is due to the lack of consideration of individual differences in how the brain's cognitive processing of a task is disrupted by neuropathology (Stern, 2002) and the wealth of literature that indicates that reserve is modifiable through adult life (Petrosini et al., 2009; Reed, Dowling, & Farias, 2011; Richards & Deary, 2005; Valenzuela & Sachdev, 2007). On the contrary, the brain reserve capacity theory suggests that reserve is not

influenced throughout adulthood, as maximal brain volume is generally achieved by five years of age (Reiss, Abrams, Singer, Ross, & Denckla, 1996). However, the modification of the brain's neurophysiological properties is possible. For instance, new neurons are continuously generated in the adult brain (Eriksson et al., 1998), with exercise, stimulating environments, and potentially diet, shown to promote neurogenesis (Stangl & Thuret, 2009; van Praag, Shubert, Zhao, & Gage, 2005; Wu et al., 2008). Despite this, it is unlikely that these mechanisms would lead to meaningful increases in intracranial volume (Valenzuela & Sachdev, 2007).

The brain capacity formulation of reserve is referred to as a 'passive' model of reserve, as whether a patient shows cognitive deficits due to neuropathology is determined by whether the degree of damage exceeds that patient's pre-determined neurological capacity (Stern, 2002). In contrast, the 'active' formulation of reserve, cognitive reserve (CR), allows for individual differences in the efficiency and effectiveness in which a task is processed in the presence of neural damage (Stern et al., 2005; 2003). Rather than simply considering anatomical data as conferring reserve, the 'active' model considers the efficacy in use of brain networks (Solé-Padullés et al., 2009).

Cognitive reserve

CR is a model of reserve that proposes that the brain actively attempts to cope with damage through pre-determined cognitive strategies, such as the efficient use, and differential recruitment, of brain networks (Stern, 2002). Changes in the recruitment of brain networks are a normal response to increased task demands (Stern et al., 2012), and individual differences in neural efficiency exist in non-pathological cognitive function (Solé-Padullés et al., 2009). Therefore, it is likely that, in addition to being clinically relevant, CR is a construct

that is developed throughout healthy adulthood and is present in older adults without disease. CR focuses on the processes that allow the brain to maintain function despite neural interruption and differs from 'passive' models that propose a fixed threshold at which impairment will occur. CR also offers an explanation as to how one patient can display clinical symptoms of AD where another, with the same head circumference, intracranial volume, or number of healthy neurons and synapses, can continue to function at a subclinical level (Stern, 2009).

Neurologically, CR has two main complementary facets that assist the individual to maintain cognitive function despite neural disruption by pathology: neural reserve and neural compensation (Stern et al., 2005). Neural reserve refers to the pre-existing differences in neural network efficiency that exist between individuals. Higher neural reserve manifests as brain networks that are either more efficient or have greater capacity when faced with increased task demand. In addition, individuals with higher neural reserve may more readily recruit additional brain networks to support performance when the original networks are insufficient. The other component of CR, neural compensation, is a response specific to brain damage that reflects the individual differences in the ability to use alternative brain structures or networks resulting from disruption to the original networks. Here, engaging compensatory neural networks may not result in improved performance, but may serve to maintain performance in the face of ageing or pathology (Stern et al., 2005).

CR provides one explanation for the individual differences in the relationship between neuropathological burden and cognitive outcome but, as a hypothetical construct, is inherently difficult to measure. One approach to CR measurement is to quantify the difference between observed and expected cognitive performance due to the pathology present in the brain, but accurate measures of both performance and neuropathology must be acquired (Jones et al.,

2011). One study estimated CR by the residual term in the regression of cognitive function on brain pathology, providing an operational measure of an individual's current reserve (Reed et al., 2011). However, such an approach requires *in vivo* estimates of brain pathology, the acquisition of such data being time-consuming and costly.

Other methods of measuring CR have been suggested, such as through the assessment of neurocomputational factors such as network efficiency, redundancy, and dynamic range (Valenzuela, 2008). However, for practicality, it is recommended that a behavioural approach, ascertaining how mentally active and engaged a person is, be adopted (Stern, 2002; Valenzuela, 2008). In the past two decades, multiple studies have endeavoured to identify factors that support subclinical cognitive performance in the presence of neuropathology. These studies have led to the identification of several potential proxy measures of CR. These proxy measures comprise estimates of lifetime exposures that display an inverse relationship with dementia risk, and include: educational attainment; the nature and complexity of occupational history; participation in cognitively stimulating leisure activities; and, general intelligence. Importantly, research has demonstrated that different lifetime exposure to CR proxies contribute independently to reserve capacity (Foubert-Samier et al., 2012; Reed et al., 2011).

Educational attainment

The notion that greater participation in education reduces the prevalence of AD was first given consideration after researchers recognised the importance of adjusting for education when screening for dementia (Kittner et al., 1986). A subsequent investigation identified significantly increased risk for dementia among individuals with either low education or low lifetime occupational attainment (Stern et al., 1994). The authors proposed that lifetime

educational or occupational attainment either increase the difficulty in detecting clinical AD or impart a level of CR that delays onset of clinical symptoms. Soon after, multiple studies provided support for the hypothesis that education provides protection from dementia and AD (Letenneur et al., 1999; Mortel, Meyer, Herod, & Thornby, 1995; Ott et al., 1995). However, such associations are not always found. One longitudinal investigation found that less educated adults had a later age of dementia onset, a similar load of neurodegenerative lesions (senile plaques, neurofibrillary tangles, Lewy bodies), but a greater number of cerebrovascular lesions, than higher educated patients (Del Ser, Hachinski, Merskey, & Munoz, 1999). The authors proposed that education modifies dementia risk through a reduction in cerebral infarcts rather than by enhancing CR, supporting a 'brain-battering model'. Other research has identified an independent contribution of education on healthy cognitive function, after adjusting for multiple socioeconomic status measures (Cagney & Lauderdale, 2002).

Many epidemiological investigations have provided support for the association between educational attainment and risk for dementia. For example, after adjusting for age and gender, education but not occupational attainment was negatively associated with dementia in two independent samples (Fritsch, McClendon, Smyth, & Ogrocki, 2002; Ravaglia et al., 2002). The possibility that greater educational attainment is associated with reduced dementia risk due to its status as a proxy variable for socioeconomic status has also been investigated. One study demonstrated that education reduces the risk for dementia even after adjusting for multiple demographic, socioeconomic, vascular, and lifestyle characteristics (Ngandu et al., 2007). Here, the authors proposed that unhealthy lifestyles might contribute to dementia risk due to the depletion of CR or through direct effects on pathological processes. Similarly, greater years of education reduced dementia risk in older female members of a religious order who lived together, effectively excluding the influence of environmental factors and lifestyle

(Bickel & Kurz, 2009). In 488 subjects who were demented at death, although education did not alter neurodegenerative or vascular pathologies, more years of education mitigated the impact of pathology on cognitive function, decreasing the risk for clinical symptoms of dementia (Brayne et al., 2010). Further, a faster AD-related cognitive decline was found in individuals with higher education compared to individuals with lower education (Bruandet et al., 2008). Higher education has also been related to protection from cognitive impairment resulting from stroke (Ojala-Oksala et al., 2012) and brain injury in professional fighters (Banks et al., 2014).

Rather than modifying the neurobiological processes of AD, education seems to act through the buffering of cognitive impairment associated with AD pathology (Brayne et al., 2010). Two separate investigations that quantified neuropathological markers at autopsy found that higher education was associated with a reduced impact of AD pathology upon cognitive function, while having no impact on the severity of the pathology present (Bennett et al., 2003; Koepsell et al., 2008). Studies that have determined *in vivo* estimates of amyloid plaque load using Pittsburgh Compound B (PiB) have reported similar findings (Rentz et al., 2010). In higher educated and lower educated participants matched on level of cognitive deterioration, a greater PiB uptake in the lateral prefrontal cortex was identified in those with more years of education (Kemppainen et al., 2008). Similarly, in participants with elevated PiB uptake, performance on multiple tests of cognitive function was increased with more years of education (Roe et al., 2008). Brain glucose metabolism, another pathological marker of AD, is also more severely reduced in higher educated individuals when compared to lower educated individuals, despite comparable cognitive impairment (Garibotto et al., 2008; Kemppainen et al., 2008). Structural pathology is also modified by education. Here, more education was associated with higher cognitive function at any level of medial temporal lobe atrophy (Perneczky et al., 2009) and, after being matched for dementia severity, higher

education was associated with more severely reduced white matter integrity in the medial temporal lobe and association fibre tracts (Teipel et al., 2009).

Although associations between education and risk for dementia are reliable, education has shown inconsistent protective effects on the rate of normal cognitive ageing (Christensen et al., 2009). A longitudinal study of 872 healthy individuals, aged 49-81 years at baseline, did not show any alteration of cognitive ageing trajectory due to education level after six years (Van Dijk, Van Gerven, van Boxtel, Van der Elst, & Jolles, 2008). Likewise, in 1,014 participants followed over 12 years, education did not alter rates of change in any cognitive domain (Zahodne, Glymour, & Sparks, 2011). One investigation showed a protective effect of education on the association between amyloid deposition and cognition in cognitively intact participants, albeit only when using an especially difficult memory test (Rentz et al., 2010). Overall, the protective effects of education on age-related cognitive decline are likely small or non-existent. However, one finding that is consistent across longitudinal studies is of higher baseline cognitive function as a result of education (Gottesman et al., 2014; Singh-Manoux et al., 2011; Zahodne et al., 2011). In this regard, one reason why education may delay the onset of dementia is due to higher cognitive function resulting in neuropsychological performance that reaches clinical significance later than those with lower education.

Occupational attainment

Although education is the most commonly used proxy measure for CR, other life experiences have also been proposed to increase reserve. One of these life experiences is occupational attainment, with earlier research suggesting that occupational attainment and education independently or synergistically contribute to higher reserve (Mortel et al., 1995; Stern et al., 1994; 1995). Epidemiologically, the results are mixed, with some studies reporting significant

reductions in risk for dementia due to level of occupational attainment (Mortel et al., 1995; Qiu et al., 2003), while others have found no association between occupation and dementia risk (Fritsch et al., 2002; Jorm et al., 1998b). In both of these studies that reported negative results, the authors suggested that any relationship is likely due to premorbid cognitive differences, established prior to entering the workforce. A longitudinal study of 2,950 subjects, 251 of whom developed AD, found no support for the hypothesis that occupation alters the risk for AD (Helmer et al., 2001). Despite this, more recent evidence reports protective effects of occupation on dementia risk (Andel et al., 2005; Bickel & Kurz, 2009), particularly in those occupations that involve complex interactions and skills (Karp et al., 2009; Kröger et al., 2008). A meta-analysis of 12 studies found that a history of high occupational attainment was associated with a 44% decreased risk of incident dementia (Valenzuela & Sachdev, 2006).

Like education, data have implicated occupational attainment in influencing the clinical expression of AD. In AD patients matched on clinical impairment, a history of participation in occupations that involved more complexity, interpersonal skills, and physical demands was associated with greater cerebral blood flow deficits in the parietal lobe (Stern et al., 1995). Similarly, in patients with MCI or probable AD who were later diagnosed with AD, despite comparable cognitive function, a more severe reduction in glucose metabolism was found in patients with more education/occupational attainment when compared to patients with less (Garibotto et al., 2008). In both studies, the cognitive deficits associated with the pathological progression of AD were mitigated due to occupational history.

Cognitively stimulating leisure activities

Participation in cognitively stimulating leisure activities is a further life experience that may contribute to CR (Stern, 2002). Longitudinal investigations examining measures of physical, mental, and social activity have demonstrated a significantly lower risk of dementia in individuals who were more active (Paillard-Borg, Fratiglioni, Winblad, & Wang, 2009; Wang et al., 2013; Wilson et al., 2002b). In two of the studies, such associations remained even after adjusting for education and baseline cognitive function (Wang et al., 2013; Wilson et al., 2002b), indicating that these leisure activities provide a unique contribution to CR. Frequent cognitive activity has been reported to compress the cognitive morbidity associated with AD, such that frequent cognitive activity slows the annual rate of global cognitive decline by 52% in cognitively normal individuals, yet hastened the rate of decline by 42% in individuals with AD (Wilson et al., 2010). A cognitively inactive individual is 2.6 times more likely to develop AD than a cognitively active person, even after controlling for current social and physical activity (Wilson, Scherr, Schneider, Tang, & Bennett, 2007). More frequent participation in specific cognitive activities, such as completing crossword puzzles in later-life, is also related to a later memory decline in individuals who develop dementia (Pillai et al., 2011).

There is evidence suggesting that cognitively stimulating leisure activities modify the impact of AD pathology on cognitive function. One study investigated the associations between regional cerebral blood flow, cognitive function, and intellectual, social, and physical engagement (Scarmeas et al., 2003). After controlling for education and intelligence, greater reductions in regional cerebral blood flow, indicating a more advanced AD-related pathology, were found in participants with a higher activity score while matched for clinical severity. Another study quantified the difference between expected cognitive function due to AD pathology and actual level of performance, capturing CR as a latent variable (Reed et al.,

2011). Here, potential lifetime contributors of reserve (socioeconomic status, education, cognitive leisure activities at age 40, cognitive leisure activities in late life) were regressed onto the latent CR variable, with cognitive leisure activities at age 40 found to be the strongest correlate of CR. In this study, activities such as reading, writing letters, visiting a library, and keeping a journal were most effective at suppressing the detrimental cognitive outcomes of neuropathology.

Intelligence

Although less frequently investigated than other CR proxies, intellectual capacity may be another lifetime factor that exerts a protective influence against age-related or disease-related cognitive dysfunction (Stern, 2009). A large-scale longitudinal investigation of 2,063 elderly subjects, followed for four years, demonstrated that low IQ predicted incident dementia better than low level of education (Schmand, Smit, Geerlings, & Lindeboom, 1997). Another study found that low abstract reasoning capacity, a component of intelligence, was one of the strongest predictors of AD over 22 years of follow-up (Elias, Beiser, Wolf, & Au, 2000). Intellectual capacity does not just protect from cognitive deficits arising from dementing illnesses, but also from cognitive impairment resulting from obesity (Galioto, Alosco, Spitznagel, Stanek, & Gunstad, 2013). Here, the authors demonstrated that in participants with higher premorbid intellectual ability, the expression of obesity-related cognitive impairment was attenuated. Intelligence has also been demonstrated to moderate the association between AD pathology and cognitive function, with a higher CR level negating the detrimental association between amyloid deposition and cognitive performance (Rentz et al., 2010). However, in a separate investigation of 109 cognitively normal subjects, 192 amnestic MCI patients, and 98 AD patients, an independent additive effect of IQ was found

on cognitive function in the presence of AD biomarkers, rather than a compensatory interaction between the variables (Vemuri et al., 2011).

A lifetime of cognitive engagement

Rather than determining an individual's CR through measurement of a single CR proxy (education, occupational attainment, cognitively stimulating leisure activities, intelligence), examination of mental engagement over the lifespan that reflects multiple CR proxies may more accurately estimate an individual's CR. This approach has been suggested as a means of assessing CR effectively (Sánchez, Torrellas, Martín, & Barrera, 2011) and a meta-analysis that examined the protective effect of multiple CR proxies in combination reported a reduction in the risk of incident dementia of 46% for those with higher reserve (Valenzuela & Sachdev, 2006). However, although the construct validity of the *a priori* grouping of correlated CR proxies approach has been criticised (Satz, Cole, Hardy, & Rassovsky, 2011), recent investigations increasingly take into account multiple proxy measures when assessing CR (Foubert-Samier et al., 2012). Furthermore, a more precise estimate of CR may be obtained through combining several indicators (Jones et al., 2011).

The Lifetime of Experiences Questionnaire (LEQ) incorporated the lifetime approach to estimating CR (Valenzuela & Sachdev, 2007). The LEQ is a retrospective self-report tool that is designed to comprehensively assess complex mental activity over the lifespan. To achieve this, the LEQ records information relating to education, occupational attainment, and cognitive lifestyle in different life stages (early life: 13-30; midlife: 30-65; late life: 65+ years). An analysis of the inter-correlations between CR proxies in different life stages has underlined the importance of a lifespan approach to estimating CR (young adulthood and midlife, $r = 0.65$; midlife and late life, $r = 0.26$; Valenzuela & Sachdev, 2007). However, the

LEQ may be best suited to provide a measure of pre-existing cognitive lifestyle (Suo et al., 2012) rather than a point measure of CR, a form of measurement required for longitudinal research or for the examination of potential benefits of interventions and treatments. Furthermore, while the LEQ assesses multiple proxies for CR, it does not assess intellectual capacity, a previously identified contributor to CR (Galioto et al., 2013; Rentz et al., 2010; Vemuri et al., 2011) independent of other proxies (Stern, 2009).

Mechanisms of action

Although participation in cognitively stimulating activities develops cognitive strategies that offset dementia (Stern, 2002), neuroimaging studies have indicated that life experience preserves cognition above and beyond these strategies. Evidence supporting an overlap between CR and cerebral reserve hypotheses is provided by *in vivo* investigations that have demonstrated an association between CR proxies and the integrity of neurological structures (Barulli & Stern, 2013). In an MRI investigation of healthy adults, participants with more years of education had larger regional cortical thickness in transverse temporal cortex, insula, and isthmus of cingulate cortex than participants with low education (Liu, Julkunen, Paajanen, & Westman, 2012). Similarly, education has been positively correlated with both grey and white matter brain volumes (Foubert-Samier et al., 2012), as well as the integrity of white matter tracts (Teipel et al., 2009). Another CR proxy, intelligence, has demonstrated positive correlations with grey matter volume in multiple brain regions (Colom, Jung, & Haier, 2006; Haier, Jung, Yeo, Head, & Alkire, 2004).

In addition to the associations between CR proxies and larger regional brain volumes, individuals with more CR show slower rates of age-related cortical atrophy. In 37 healthy older adults who completed the LEQ and underwent MRI at baseline and at three years

follow-up, participants with more CR, as estimated by the LEQ, possessed both greater hippocampal volumes and experienced less hippocampal atrophy over the follow-up period (Valenzuela, Sachdev, Wen, Chen, & Brodaty, 2008). In a follow-up study with a larger sample of 1,037 older adults aged over 70 years, a similar effect was observed (Suo et al., 2012). After excluding participants with MCI at their three-year follow-up MRI, the rate of hippocampal atrophy in late-life was five-times slower for those participants with high level supervisory experience in midlife when compared to those with no midlife supervisory experience.

Functional MRI and PET investigations have identified altered patterns of neural activation that represent functional reorganisation of neural networks associated with more frequent participation in CR proxy variables (Barulli & Stern, 2013), even in younger adults (Stern et al., 2003). Such findings have supported the two main hypothesised neural implementations of CR: neural reserve and neural compensation (Stern, 2009; Stern et al., 2005). For example, during a memory task, education and frontal activity were negatively correlated in younger adults but positively correlated in older adults, implicating the frontal cortex as an alternative network that may be engaged by higher educated individuals in later life (Springer, McIntosh, Winocur, & Grady, 2005). In addition, a specific network has been identified in two separate cognitive tasks that increases in activation as a function of CR (Stern et al., 2005). More efficient use of the task-associated neural networks was also found in individuals with more CR (Morbelli & Nobili, 2014). For example, a higher score on a composite estimate of CR was related to reduced activity during cognitive processing of a visual encoding task in healthy adults (Solé-Padullés et al., 2009). Similarly, healthy older adults with low CR showed higher functional connectivity while performing a memory task when compared to participants with high CR, despite no difference in cognitive performance (Lopez et al., 2014). In comparison, active compensatory mechanisms have been identified in MCI and AD

patients, with higher CR associated with increased brain activity during cognitive processing (Solé-Padullés et al., 2009).

Rather than impacting solely on cognitive processing, higher neural efficiency associated with higher CR may also modify the rate of AD pathology accumulation (Jagust & Mormino, 2011). A recent imaging investigation assessed the association between lifetime participation in cognitively stimulating activities and cortical PiB uptake in 65 healthy older participants (Landau et al., 2012). The authors reported that greater early- and mid-life engagement was associated with a significantly reduced Aβ deposition in later life. Similarly, reductions in the levels of cerebral Aβ and amyloid deposits are found in mice exposed to an enriched environment when compared to mice raised in standard housing (Lazarov et al., 2005). Another investigation examined CR proxy variables and longitudinal change in AD biomarkers in 819 participants with normal cognitive function, MCI, and mild AD (Lo & Jagust, 2013). Here, cerebrospinal fluid Aβ 42 decline was slowed by higher levels of education, occupation, and premorbid intelligence in cognitively normal subjects, with an additional effect of preserved glucose metabolism present in AD patients with higher premorbid intelligence.

Genetic contributions to reserve

As genetic influences account for over 50% of the variability in adult cognitive function (Petrill et al., 2004), it is likely that a measurement of CR based on environmental exposures alone is incomplete (Lee, 2003). The notion of biological, or inherited, reserve has not come under the same level of exploration as environmental contributors to CR. Yet, high heritability has been identified in both general cognitive ability (Bouchard & McGue, 1981; Deary, Spinath, & Bates, 2006; Plomin, 2004) and specific cognitive functions, such as

working memory and verbal ability (Ando, Ono, & Wright, 2001; Blokland et al., 2008). Furthermore, the heritability of general cognitive ability has been demonstrated to increase with age rather than diminish (Davis, Haworth, & Plomin, 2009; Haworth et al., 2010). Research that examines the interaction between genes and CR proxies is justified. The most likely candidate genes for CR are those that interact with environmental factors to induce effects on cognitive functions (Lee, 2003).

Genetic associations with cognitive function

Although there is strong evidence from genome-wide investigations that variation in genetic makeup affects the cognitive function of a healthy adult (Plomin et al., 2013), replicable single gene associations with cognitive ability or cognitive ageing are rare (Houlihan et al., 2009). For example, one large-scale investigation examined 325 single nucleotide polymorphisms (SNPs) in 109 genes that have been previously implicated in oxidative stress and/or cognitive function in two separate cohorts totalling 922 participants (Harris, Fox, Wright, & Hayward, 2007). The authors failed to find support for any of the previous associations in both cohorts, but identified an intronic SNP in the APP gene (rs2830102) that was significantly associated with cognitive ageing in the first cohort alone and in a combined sample of both cohorts. Further candidate genes for cognitive ability and ageing are apolipoprotein e (*APOE*) and brain-derived neurotrophic factor (*BDNF*), both of which have shown inconsistent associations with cognitive functions (Foster et al., 2013; Stuart, Summers, Valenzuela, & Vickers, 2014). Despite this, synaptic plasticity is a key mechanism that facilitates the development of CR from participation in cognitively stimulating activities (Esiri & Chance, 2012), and both *APOE* and *BDNF* have been shown to impact upon components of plasticity (Chen, Durakoglugil, Xian, & Herz, 2010; Pattwell et al., 2012). As

such, investigations that examine whether *APOE* or *BDNF* are associated with an environmental estimate of CR are important.

Apolipoprotein E

One of the key genes that accounts for a major proportion of studies that investigate genetic associations with cognitive ability, ageing, and AD is *APOE*. The human *APOE* gene is located on chromosome 19 and is composed of 299 amino acids, with APOE isoforms differing due to the presence of cysteine or arginine at amino acid residues 112 and 158: *APOE* ε2 (Cys 112, Cys 158), *APOE* ε3 (Cys 112, Arg 158), *APOE* ε4 (Arg 112, Arg 158) (Mahley & Rall, 2000); a meta-analysis of worldwide prevalence rates for the three variants are reported to be 8.4%, 77.9% and 13.7%, respectively (Farrer et al., 1997). However, an Australian population reported slightly different prevalence rates of 14.7%, 73.5%, and 11.8%, respectively (Vickers et al., 2002).

The APOE protein is involved in regulating lipid homeostasis by transporting lipids between tissues or cells (Mahley & Rall, 2000). In the central nervous system, APOE is produced primarily by astrocytes and transports cholesterol to neurons through APOE receptors; however, the liver and macrophages also produce APOE (Liu, Kanekiyo, Xu, & Bu, 2013a). Although APOE isoforms differ by only one or two amino acids, the physiologic effects are particularly evident. For instance, *APOE* ε4 is associated with calcific valvular heart disease (Novaro, Sachar, Pearce, Sprecher, & Griffin, 2003), atherosclerosis (Mahley & Rall, 2000), hyperlipidemia (Ghiselli, Schaefer, Zech, Gregg, & Brewer, 1982), and glaucoma (Vickers et al., 2002); while *APOE* ε2 has been associated with Parkinson's disease (Huang, Chen, & Poole, 2004). However, the most robust finding is the link between *APOE* polymorphisms and late-onset sporadic AD (Verghese, Castellano, & Holtzman, 2011).

The *APOE* ε4 gene dose has been shown to be the largest genetic risk factor for late-onset AD (Lovati et al., 2010). An increased risk for developing AD and an earlier age of onset of clinical AD has been reported with an increasing number of ε4 alleles (Corder et al., 1993). Specifically, the frequency of AD and mean age of clinical onset are 91% and 68 years in ε4 homozygotes, 47% and 76 years in ε4 heterozygotes, and 20% and 84 years in ε4 noncarriers (Corder et al., 1993). In support, it has been reported that while the prevalence of ε4 in the general population was 13.7%, the prevalence of the ε4 allele was 40% in patients with AD (Farrer et al., 1997). Here, ε4 heterozygous participants had a 3-4 times increased risk for AD while ε4 homozygous participants had 10-12 times the risk of AD. Despite this, the presence of ε4 is not diagnostic, as ε4 alone is not sufficient to cause the pathogenesis of AD and some *APOE* ε4 carriers achieve old age without developing dementia (Tiraboschi et al., 2004).

The *APOE* ε4 allele may increase risk for AD due to an allele-specific increase in the rate of abnormal AD pathology accumulation in the brain (Corder et al., 1993; Morris et al., 2010). An autopsy study of 603 deceased subjects, aged 0 to 97 years, found that the presence of ε4 was associated with an earlier onset of AD-related brain changes (Kok et al., 2009). Here, ε4 carriers had more amyloid plaques compared to ε4 non-carriers in most age brackets, with a particularly strong effect in subjects aged 50-59 years: 40.7% of ε4 carriers had amyloid plaques compared to 8.2% in noncarriers. This may be due to *APOE* effects on the rate of Aβ deposition to form amyloid plaques (Liu et al., 2013a) or the disruptive effect of ε4 isoforms on mechanisms that clear Aβ peptide from the brain (Castellano et al., 2011; Deane et al., 2008). In addition to affecting amyloid pathways, it has been proposed that ε4 may also contribute to AD risk through the promotion of tau hyperphosphorylation (Kim, Basak, & Holtzman, 2009) and through driving atrophy to the medial temporal lobe (Manning et al., 2014; Pievani et al., 2011). Conversely, ε2 has demonstrated protective effects from the

accumulation of AD pathology (Castellano et al., 2011; Suri, Heise, Trachtenberg, & Mackay, 2013; Tiraboschi et al., 2004).

Although the link between *APOE* and risk for AD is established, the notion that *APOE* polymorphism accounts for variance in healthy non-pathological cognitive function is less clear. Some investigations have identified detrimental cognitive effects associated with the ε4 allele in healthy adults (Boardman, Barnes, Wilson, Evans, & Mendes De Leon, 2012; Deary et al., 2004; Ready et al., 2011), while others have failed to support such associations (Foster et al., 2013; Jorm et al., 2007). Two meta-analyses have demonstrated that the ε4 allele imparts a negative effect on a variety of cognitive functions in healthy older individuals (Small, Rosnick, Fratiglioni, & Bäckman, 2004; Wisdom, Callahan, & Hawkins, 2011): global cognitive ability, episodic memory, executive functioning, and perceptual speed were all significantly impaired in ε4 carriers. Despite this, more recent data refutes the notion that a specific ε4-related cognitive phenotype exists independent of dementia or MCI (Foster et al., 2013; Quintas et al., 2013).

Part of the reason why *APOE* polymorphisms may influence healthy cognitive function is due to the allele-specific effects on the structure and function of the brain. For example, in individuals aged 49-79 years, ε4 alleles were associated with significantly reduced right hippocampal volumes, with the most pronounced effect found before the age of 65 (Lind et al., 2006). Similarly, relative to ε3 homozygotes, ε4 carriers aged 51-59 years had a thinner cortex in superior frontal, left rostral, and right caudal midfrontal regions (Fennema-Notestine et al., 2011). The ε2 allele exerts a positive effect on brain volume, with healthy ε2 carriers showing thicker right parahippocampal cortex when compared to ε3 homozygotes (Fennema-Notestine et al., 2011). In patients with AD, ε2 alleles appear to be protective: relative to ε3 homozygotes, ε2 carriers show thicker entorhinal cortex, transverse temporal cortices, and

whole cerebral gray matter, and left hippocampal volumes (Liu et al., 2010; Pievani et al., 2011). *APOE* polymorphism also affects white matter, with less white matter volume present in ε4 carriers when compared to noncarriers (Ready et al., 2011).

Similarly to low CR, *APOE* ε4 has been associated with reduced neural efficiency when processing cognitive tasks. Even in young healthy individuals aged 20–35 years, ε4 alleles are associated with greater hippocampal activation during an encoding task, despite no difference in memory performance (Filippini et al., 2009). In the same investigation, brain activity at rest revealed increased activity in the default-mode network (DMN) in ε4 carriers relative to noncarriers. Two other studies have demonstrated greater neural activation in multiple components of the temporal lobes in ε4 carriers when compared to noncarriers (Dennis et al., 2010; Scarmeas, 2005). In healthy older individuals, aged 60-79 years, ε4 carriers exhibited increased functional connectivity but reduced complexity of spontaneous brain activity, a suggested hallmark of illness and ageing, indicating compensatory neural recruitment (Yang et al., 2013). Overall, a lifetime of heightened neural activity associated with ε4 may contribute to a faster rate of Aβ peptide accumulation (Jagust & Mormino, 2011).

Brain-derived neurotrophic factor

A second gene that warrants investigation for potential interactions with CR is brain-derived neurotrophic factor (*BDNF*). The *BDNF* gene is located on chromosome 11 and encodes a precursor peptide which, once cleaved, forms the mature *BDNF* protein (Egan et al., 2003). BDNF is a nerve growth factor that is expressed in the central nervous system and is vital for the maintenance, survival, and growth of neurons (Mattson, Maudsley, & Martin, 2004), but has also been well characterised for its role in synaptic plasticity (Yoshii & Constantine-Paton, 2010). Many SNPs have been identified within the region that codes for *BDNF*, yet a

valine (Val) to methionine (Met) substitution at position 66 in the pro-domain has been the most widely studied (Mizuno, Yamada, Olariu, Nawa, & Nabeshima, 2000). A US-based study reported Val66Met allele frequencies of 82% and 18% for Val and Met, respectively (Egan et al., 2003), although ethnicity-dependent frequencies have also been identified (Shimizu, Hashimoto, & Iyo, 2004).

As BDNF has been implicated in neurodegenerative diseases, factors that influence the rate at which BDNF is expressed have been the target of research. Reduced BDNF levels are found as a result of AD in frontal and parietal cortices and the hippocampus (Ferrer et al., 1999; Hock, Heese, Hulette, Rosenberg, & Otten, 2000). Similarly, normal ageing has been associated with a decreased BDNF signalling capacity, with age-related decreases in BDNF proposed to account for age-related impairments in cognitive function (Mattson et al., 2004). Of particular interest is the *BDNF* Val66Met polymorphism associated with protein expression (Egan et al., 2003). Not only does the Met variant of the polymorphism cause impaired activity/depolarisation-dependent secretion of BDNF in hippocampal neurons, but, where the Val variant results in secretory granules within synapses, the Met variant results in secretory granules that are accumulated in the cell body and rarely in synapses (Egan et al., 2003). As a consequence of these deficits, the *BDNF* Met variant has been linked to impairments in synaptic plasticity and transmission (Cheeran et al., 2008; Ninan et al., 2010; Pattwell et al., 2012).

The previously identified associations between BDNF and synaptic plasticity and growth indicate that the protein plays an important role in cognitive functions (Lu, Nagappan, Guan, Nathan, & Wren, 2013). Despite this, following an initial report that the Met variant impairs memory performance in healthy individuals (Egan et al., 2003), conflicting data have been reported. In 1063 Scottish individuals with a mean age of 70 years, variation in the Val66Met

polymorphism did not account for significant variance in any cognitive test (Houlihan et al., 2009). In two other investigations, the polymorphism was shown to not significantly alter learning and memory function in either healthy younger (Gong et al., 2012) or older (Stuart et al., 2014) individuals. However, other investigations have supported deficits in healthy cognitive function due to inheritance of the Met allele (Hariri et al., 2003). One study of 189 adults aged 18 – 89 years reported that carriers of the Met variant displayed reduced memory performance and slower processing speed when compared to Val homozygotes (Raz, Rodrigue, Kennedy, & Land, 2009). Another demonstrated slower processing speed in female Met carriers relative to female Val homozygotes (Laing et al., 2011).

Multiple pathways may account for data that have related the *BDNF* Val66Met polymorphism to alterations in healthy cognitive function. Long term potentiation, a process of synaptic plasticity that allows memories to be encoded (Bliss & Collingridge, 1993), is dependent on the presence of BDNF in the hippocampus (Ying et al., 2002). Consequently, the impaired expression of BDNF associated with the Met variant may lead to a disruption in the memory encoding process. In addition, abnormal morphology and activation is associated with the Met variant. A significantly reduced total hippocampal volume is present in Met carriers when compared to Val homozygotes (Frodl et al., 2007; Kauppi, Nilsson, Persson, & Nyberg, 2014) even in a young sample with a mean age of 34.2 years (Pezawas et al., 2004). In support, a recent meta-analysis of 5,298 healthy individuals found a small but significant reduction in hippocampal volumes related to the Met variant (Harrisberger et al., 2014). In addition, reduced activity in the medial temporal lobe and hippocampus during the encoding of information while performing memory tasks, an indicator of subsequently forgotten memories (Eichenbaum, Yonelinas, & Ranganath, 2007), has been identified in Met carriers (Kauppi et al., 2014; 2013).

The *BDNF* gene has been widely investigated for its role in brain development and for mediating the neurological benefits of environmental enrichment (Casey et al., 2009; Lu et al., 2013). For example, exposure to cognitively stimulating environments results in a widespread increase in BDNF expression in the brain (Ickes et al., 2000) and in the upregulation of BDNF, resulting in the promotion of neural plasticity (van Praag, Kempermann, & Gage, 2000). One study demonstrated that hippocampal neurogenesis resulting from environmental enrichment in wild-type mice was absent in *BDNF* heterozygote knock-out mice (Rossi et al., 2006). Such effects also impact upon cognitive function: when *BDNF* activity is blocked in the hippocampus, exercise is no longer associated with improvements in spatial memory (Vaynman, Ying, & Gomez-Pinilla, 2004). Similarly, dietary restriction, another modifiable variable that enhances hippocampal neurogenesis, does not positively influence neurogenesis in *BDNF* knock-out mice (Lee, Duan, & Mattson, 2002). In humans, the *BDNF* Val66Met polymorphism has been implicated in moderating the association between physical activity and risk of incident dementia (Kim et al., 2011).

Genetic interactions with CR

That individuals with higher CR display greater resistance to the effects of neuropathology is likely due to induced cortical plasticity caused by a prolonged mismatch between functional supply and task demands (Lövdén, Bäckman, Lindenberger, Schaefer, & Schmiedek, 2010), resulting in more flexible and denser neural networks (Barulli & Stern, 2013; Stern, 2009). As such, synaptic plasticity is a key mechanism that facilitates the development of CR (Esiri & Chance, 2012) and genes that impact upon the processes of synaptic and neuronal plasticity are viable candidates for genetic influences on CR. Both *APOE* (Chen et al., 2010; Levi, Jongen-Relo, Feldon, & Michaelson, 2005) and *BDNF* Val66Met (Cheeran et al., 2008; Egan et al., 2003) have shown such associations.

Although it would be convenient to assume that CR is determined solely by environmental factors, this does not appear to be the case, as studies have demonstrated interactions between genetic and environmental factors when determining risk for dementia (Wang et al., 2012). In this regard, a reliable finding is that higher CR can reduce the additional risk for dementia associated with *APOE* ε4 alleles due to higher education (Ferrari et al., 2013; Shadlen et al., 2005; Wang et al., 2012), occupational attainment (Garibotto et al., 2011), and participation in leisure activities (Ferrari et al., 2013). In one study, it is reported that higher education halved the risk of dementia associated with inheritance of *APOE* ε4 (Wang et al., 2012). Although these investigations indicate that CR can reduce an individual's genetic risk for dementia, a separate study has implicated that the impaired plasticity associated with *APOE* ε4 impacts on the association between CR and cognitive function (Runge, Small, McFall, & Dixon, 2014). Here, it was found that lifestyle activities involving cognitive effort predicted verbal fluency and semantic memory recall in *APOE* ε4 non-carriers but not in carriers.

Environmental enrichment in experimental animals and measures of CR in humans are unlikely to be analogous; however, a comprehensive review of the literature concluded that, as with human educational and occupational attainment, environmental enrichment develops reserve that can be used to offset the impact of cortical lesions (Petrosini et al., 2009). In *BDNF* Met carriers, the reduced presence of *BDNF* in the synapses (Egan et al., 2003) and an impaired capacity for long term potentiation/depression may cause a reduction in the ability of connected cells to undergo synaptic and/or neuronal plastic changes (Egan et al., 2003; van Praag et al., 2000). As a result, variation in the *BDNF* Val66Met polymorphism may affect the association between participation in cognitively stimulating activities and cognitive function: for two individuals exposed to the same level of extrinsic environmental

stimulation, the individual who carries the *BDNF* Met polymorphism may exhibit lesser cortical changes as a result.

Thesis aims and hypotheses

One of the major issues that the CR theory faces is how to measure an individual's reserve, but the development of an operational method of CR quantification could lead to practical advancements, such as more accurate diagnoses and prognoses offered to patients suffering from neurodegenerative disorders (Stern, 2013). However, no single approach to quantifying CR has been agreed upon, yet many attempts have been made. Another major issue that is present in ageing literature is the lack of reliable associations between single genes and cognitive processes (Harris & Deary, 2011). Studies that examine multiple genes, and that have substantial sample sizes, are the most likely to identify true and reliable genetic effects on cognitive function. Although genetic variation may also influence the rate of age-related cognitive decline and risk for dementia, it is possible that genes exert an effect on these processes through interaction with CR (Lee, 2003). As such, investigations aimed at identifying genetic interactions with CR are warranted, although are rarely seen in the literature.

The Tasmanian Healthy Brain Project (THBP) is an on-going longitudinal investigation into the hypothesis that later-life tertiary education may result in increased CR, potentially contributing to a reduction in the rate of age-related cognitive decline and risk for dementia (Summers et al., 2013). Participants were healthy and free of significant medical, psychological, or psychiatric illness at study entry. The experimental group, exposed to a minimum of 12 months part-time University level study, currently comprises approximately 500 participants; the control group, not exposed to the education intervention, currently

comprises approximately 100 participants. For the first four years of participation, participants were required to complete comprehensive neuropsychological assessments, after which, assessments are completed biennially.

While often applied to neurological disorders, such as AD, CR may also explain variance in premorbid cognitive function. The present investigation utilised a cross-sectional design to assess a number of aims. In healthy older adults participating in the longitudinal THBP (Summers et al., 2013), the aims were:

1. To explore the inter-relationships between CR proxy variables and develop two operational measures of CR: prior and current.
2. To determine whether common genetic variation in *APOE* or *BDNF* Val66Met is associated, either independently or through gene-gene interaction, with cognitive function.
3. To quantify the premorbid associations between CR and cognitive function.
4. To investigate the relationship between CR, cognitive function, and *APOE* and *BDNF* Val66Met polymorphisms.

In order to achieve these aims, seven hypotheses were proposed, that:

1. Traditional CR proxy variables (education, occupational attainment, intelligence, cognitively stimulating leisure activities) can be combined through factor analysis to provide a comprehensive operational estimate of CR prior to entering the THBP.
2. Flexible measures of cognitive ability that approximate traditional CR proxy variables (intelligence, academic ability) can be combined through factor analysis to provide an estimate of change in CR due to new life experiences.
3. *APOE* ε2 alleles are associated with better cognitive function and ε4 alleles are associated with worse cognitive function when compared to ε3 homozygotes.
4. *BDNF* Val homozygotes are associated with better cognitive function when compared to Met carriers.
5. A cumulative effect of the cognitive consequences associated with *APOE* and *BDNF* alleles results in the highest cognitive function in *APOE* ε2/*BDNF* Val homozygotes and the lowest cognitive function in *APOE* ε4/*BDNF* Met carriers.
6. That higher CR is associated with better premorbid cognitive performance.
7. That *BDNF* Val66Met and *APOE* polymorphisms independently moderate the association between CR and cognitive performance, with carriers of the putative detrimental alleles (Met and ε4, respectively) showing a reduced influence of CR when compared to non-carriers.

Chapter 2

General Methodology

Study population

A total of 467 healthy English-speaking participants from the longitudinal THBP completed the baseline neuropsychological battery. Participants were 50-79 years old at entry into the THBP and were community-residing and were recruited through a campaign that involved print, radio and television advertising, and community information presentations. All participants underwent comprehensive screening before being invited to participate in the THBP and were excluded if they had a history of any medical, psychiatric or psychological condition known to be independently associated with cognitive deficits. Conditions included dementia; multiple sclerosis; previous significant head injury requiring hospitalization; epilepsy; history of cerebro-vascular complications; diabetes – poorly controlled; blood pressure complications – poorly controlled; other neurological disorders; chronic obstructive pulmonary disease; heart disease; blindness; deafness; and psychiatric disorders.

Participants were not compensated for their time. However, those participating in the THBP experimental group were eligible to receive a waiver of individual course charges (Australian Higher Education Contribution Scheme) for a study load equivalent to a 12.5% unit per academic year at the University of Tasmania. The study had full approval from the Tasmania Human Research Ethics Network (Ref: H11070) and was conducted in accordance with the ethical guidelines of the National Health and Medical Research Council of Australia (NHMRC).

Neuropsychological stream

Materials

As part of the THBP, participants completed a comprehensive assessment battery each year. Full details of the administered tests can be found elsewhere (Summers et al., 2013). The assessment battery was designed to gain comprehensive measures of neuropsychological, health, and psychosocial function, as well as estimates of variables associated with CR.

Screening

For the screening of the sample, the Mattis Dementia Rating Scale-2 (DRS-2; Jurica, Leitten, & Mattis, 2001) was used to detect symptoms of dementia, the Hospital Anxiety and Depression Scale (HADS; Zigmond & Snaith, 1983) was used to assess psychological health, and the Medical Health Status questionnaire (Summers et al., 2013) collected data on health, medical conditions, medication use, and drug and alcohol use for the preceding 12 months. An experienced neuropsychologist reviewed performance on these tools to determine participant inclusion.

Mattis Dementia Rating Scale-2 (DRS-2; Jurica et al., 2001) - The DRS-2 is a screening task used to objectively assess the presence and progression of AD, vascular dementia, Parkinson's disease, and Huntington's disease. The DRS-2 consists of 36 tasks and 32-stimulus cards, is a neuropsychological measure of cognitive status for adults with cortical impairment, and is individually administered. The task yields five subscale scores (Attention, Initiation/Perseveration, Construction, Conceptualization, and Memory) as well as an overall score for cognitive functioning. Within each subscale the most difficult tasks are presented

first and if the subject performs well subsequent tasks in the subscale are credited with correct performance. Completion time for cognitively intact individuals is therefore shorter. Administration time takes, depending on the individual, 15-30 minutes (Strauss, Sherman, & Spreen, 2006).

Medical Health Status Questionnaire (MHSQ; Summers et al., 2013) – The MHSQ is a structured self-report questionnaire that assesses health, medical conditions, prescription medication use, and drug and alcohol use for the preceding 12 months. This questionnaire was developed for the THBP and also collects demographic data such as age, gender, handedness, height, weight, and marital status. The questionnaire is completed each year and a short-form version is used for subjects completing their third annual assessment and onwards. The Medical Health Status questionnaire takes 5-10 minutes to complete.

Hospital Anxiety and Depression Scale (HADS; Zigmond & Snaith, 1983) - The HADS is a screening measure of clinically significant symptoms of anxiety or depression disorders among hospital patients. The HADS consists of 14-items, half measuring anxiety and half measuring depressive symptoms, and allows for four different responses to each item. The participant is asked to underline the response that most accurately reflects how they have been feeling over the past week. Ordinal data is generated for each anxiety (HADS-A) and depression (HADS-D) subscale with higher scores indicating the presence of clinical symptoms. The HADS has been widely used, and one review from 2002 identified 747 papers that referred to HADS (Bjelland, Dahl, Haug, & Neckelmann, 2002).

Neuropsychological assessment battery

Although there are no established measures of CR in the individual, the THBP protocol included multiple proxy indicators previously used to estimate CR. To estimate prior CR, the Wechsler Test of Adult Reading (WTAR; The Psychological Corporation, 2001) was used as an estimate of premorbid IQ; the Lifetime of Experiences Questionnaire (LEQ; Valenzuela & Sachdev, 2007) was used to quantify prior lifetime experience; and the number of years of prior formal education were recorded on the Medical Health Status questionnaire (Summers et al., 2013). To estimate current CR, the Wechsler Adult Intelligence Scale, 3rd edition, Short Form 1 (WAIS-III-SF1; Donnell, Pliskin, Holdnack, Axelrod, & Randolph, 2007) was used to assess current intellectual capacity; and four components (spelling, math computation, sentence completion, word reading) from the Wide Range Achievement Test, 4th edition, Progress Monitoring Version (WRAT-4-PMV; Roid & Ledbetter, 2006), were used to assess current academic ability.

Tests of cognitive function were selected to assess learning and memory, working memory, executive function, and language processing. Episodic memory was assessed using the Rey Auditory Verbal Learning Test (RAVLT; Strauss et al., 2006), the Logical Memory test (LM; Wechsler, 1997b) and the visual Paired Associates Learning test (PAL; Cambridge Cognition Ltd., 2004) from the Cambridge Automated Neuropsychological Assessment Battery (CANTAB). Working memory was assessed using WAIS-III Digit Span (DSP; Wechsler, 1997a), WAIS-III Letter-Number Sequencing (LNS; Wechsler, 1997a), CANTAB Spatial Span (SSP; Cambridge Cognition Ltd., 2004), and CANTAB Spatial Working Memory (SWM; Cambridge Cognition Ltd., 2004). Executive function was assessed using CANTAB Rapid Visual Processing (RVP; Cambridge Cognition Ltd., 2004), 24-item Victoria version Stroop test (Lezak, Howieson, Bigler, & Tranel, 2004), and Trail Making Test (TMT; Lezak

et al., 2004). Language processing was assessed using WAIS-III Vocabulary (VOC; Wechsler, 1997a), WAIS-III Comprehension (COM; Wechsler, 1997a), and Boston Naming Test (BNT; Kaplan, Goodglass, & Weintraub, 1983).

Cognitive reserve

Wechsler Test of Adult Reading (WTAR; The Psychological Corporation, 2001) – The WTAR allows the estimation of premorbid intellectual functioning for individuals aged 16 to 89 years, takes less than 10 minutes to administer, and has extensive clinical validity with a number of groups including AD patients. The task requires the individual to read 50 words with atypical grapheme-phoneme translations aloud. Using words with irregular pronunciations minimises the individual's current ability to apply standard pronunciation rules to assess previous learning of the word, and capacity to read such words is preserved until late stages of dementia. Correct pronunciations are printed on the record form to assist in scoring; total score is the number of words read correctly. A raw score is converted to a standard score by using the participant's age at testing. Scores are then converted to WAIS-III Full Scale IQ estimates (Wechsler, 1997a).

Lifetime of Experiences Questionnaire (LEQ; Valenzuela & Sachdev, 2007) – Designed to assess complex lifespan mental activity in individuals aged over 65 years, the LEQ is a self-report questionnaire that assesses the full range of complex mental pursuits that build up over a person's lifetime from young adulthood (13-30 years of age), mid-life (30-65 years of age), and late life (65 years onwards) stages. For each life stage, respondents answer questions on frequency of mental activities that are specific to that life stage (e.g. educational, occupational activity) as well as questions on mental activities that are more general in nature (e.g. sport, hobbies, recreational activity, musical activities). Participants complete the LEQ on the basis

of retrospective recall of information from each of the age bands. Specific and non-specific subscores for each life stage are calculated, yielding an overall total LEQ score. Higher LEQ scores indicate that the respondents have undertaken more complex mental activity over their lifespan and has been shown to independently predict reduced age-related cognitive decline as well as a reduced rate of hippocampal atrophy (Valenzuela et al., 2008).

Wechsler Adult Intelligence Scale, 3rd edition, Short Form 1 (WAIS-III-SF1; Donnell et al., 2007) – The WAIS-III-SF1 provides an estimate of current intellectual capacity. This is achieved through the extrapolation of a full scale WAIS-III intelligence quotient from performance across four WAIS-III subtests. Perceptual organisation, processing speed, working memory, and verbal comprehension are assessed through completion of picture completion, digit symbol-coding, arithmetic, and similarities subtests, respectively. Summation of performance on these tests provides an accurate and reliable estimate of WAIS-III FSIQ and is validated in both normal and clinical samples (Donnell et al., 2007). The WAIS-III-SF1 can also be rapidly administered in less than 20 minutes.

Wide Range Achievement Test, 4th edition, Progress Monitoring Version (WRAT-4-PMV; Roid & Ledbetter, 2006) – The WRAT-4-PMV assesses word reading, sentence comprehension, spelling, and math computation in adults with higher secondary education. Each individual task (e.g. word reading) takes approximately 5-10 minutes to complete and includes four parallel versions to allow for effective retesting. Raw scores on each subtest can be converted to Level Equivalent Scores (LES), which provides a comparable score for forms (Roid & Ledbetter, 2006). Originally developed to assess the academic progress of individuals exposed to education, the WRAT-4-PMV is ideal for quantifying the cognitive benefits that the THBP experimental group obtain from participation in tertiary education (Summers et al., 2013).

Memory and learning

Rey Auditory Verbal Learning Test (RAVLT; Strauss et al., 2006) – The RAVLT assesses verbal episodic learning and memory and is useful in evaluating recent memory, susceptibility to (proactive and retroactive) interference, encoding versus retrieval, retention of information after a certain period of time, and recognition memory. The RAVLT is sensitive to memory decline in early AD and MCI (Saunders & Summers, 2010; 2011), as well as predicting risk for progressing to AD from MCI (Summers & Saunders, 2012). The examiner reads aloud a list of 15 words (List A) at the rate of one word per second. The subject must repeat as many words from List A that they remember in any order. List A is read aloud to the participant on four subsequent trials, with the participant recalling as many of the 15 words following each presentation trial. Following the fifth presentation of List A, the examiner verbally presents a second list of 15 words (List B), allowing the test-taker only one attempt at recall. Immediately following this, the subject must recall as many words from List A as they can. Finally, a recognition trial is completed whereby participants are presented with a block of 50 words and they must circle those that they recognise from List A. Parallel versions of the RAVLT are available that reduce the potential for practice effects.

WMS-III Logical Memory (LM; Lezak et al., 2004) – LM assesses verbal episodic memory through the free recall of two paragraphs and has been extensively used in the detection of MCI (Petersen et al., 1999). Participants are read two independent stories (A and B) with immediate recall after each story. After recalling story B, verbal presentation is repeated for that story and recall is again recorded. After a 30-minute delay, participants are asked to recall as much of the stories as possible, followed by a yes/no recognition test. Each story consists

of 25 bits of information and unit score and thematic score determine performance (Strauss et al., 2006).

Paired Associates Learning (PAL; Cambridge Cognition Ltd., 2004) – the PAL subtest of the CANTAB assesses visual memory, conditional learning of pattern-location associations, and can be used as a tool to assess age-related memory loss. Additionally, PAL is sensitive to memory decline in early AD (Ahmed, Mitchell, Arnold, Nestor, & Hodges, 2008) and MCI (Summers & Saunders, 2012). White boxes are displayed on the screen and are opened in a randomised order whereby one (or more) box contains a pattern (Figure 2.1). After all the boxes have opened (and subsequently closed) the subject is required to touch the box where the centrally displayed pattern was originally located. If the subject makes an error, the patterns are re-presented to remind the subject of their locations. The difficulty of the task increases from displaying just a single pattern to displaying eight. The task is ended if the subject repeatedly makes mistakes on any of the levels.

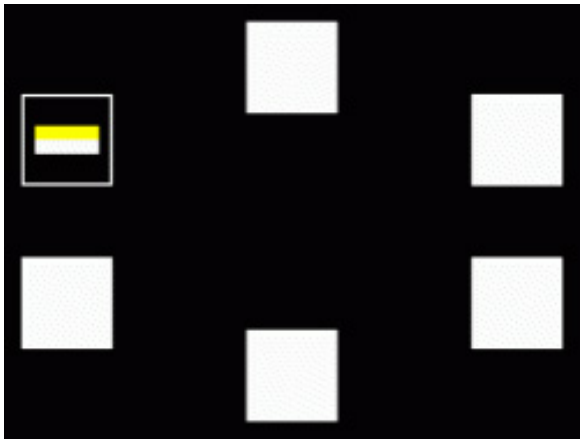

Figure 2.1. Display screen for Paired Associates Learning (PAL).

Working memory

WAIS-III Digit Span (DSP; Lezak et al., 2004) - DSP assesses verbal working memory through the measurement of the number of discrete sequential units that an individual can successfully recall. DSP is sensitive to age-related declines in working memory, with more pronounced deficits observed after the age of 70 (Gregoire & Van Der Linden, 1997). In the digits-forward condition, the assessor presents digits verbally to the participant (e.g. '4' '6' '2'), who is required to immediately repeat them back in the same order that they were presented in (i.e. "4,6,2). In the digits-backward condition, the participant is required to immediately repeat the sequence in reverse order (i.e. "2,6,4"). Either condition is discontinued after two consecutive incorrect responses are recorded on the same sequence length. DSP takes approximately 5-10 minutes for completion.

WAIS-III Letter-Number Sequencing (LNS; Lezak et al., 2004) – LNS assesses the capacity to manipulate verbally presented information in short-term memory. This auditory learning task requires the reordering of an initially unordered set of letters and numbers. The examiner reads out a group of alternating numbers and letters (e.g. '7' 'A' '2' 'G') and the subject must say the numbers back first, in lowest-highest numerical order, and then the letters in alphabetical order. As the subject successfully completes trials, the length of the presented sequence increases. A curvilinear age-related decline is evident on the LNS, indicating increasingly worse scores associated with advancing age (Myerson, Emery, White, & Hale, 2002). The task is discontinued if the subject responds erroneously to three trials of the same length. LNS takes approximately 5-10 minutes for completion.

Spatial Span (SSP; Cambridge Cognition Ltd., 2004) - SSP is a CANTAB subtest developed as a visuospatial version of the digit span test and computerised adaptation of the Corsi Block

Span test; as such it is designed to assess visuospatial working memory capacity. The test presents a pattern of white squares on the screen that change colour, one at a time, in a sequence (Strauss et al., 2006). The subject must correctly remember the sequence and touch each of the target boxes in the same order after the presentation phase has ended. After each correct trial, the sequence of boxes increases until either the subject can no longer respond with the correct sequence or the subject correctly responds to nine boxes. The test provides multiple measurements, including sequence length, number of errors, attempts, and delay time. The test takes approximately 5 minutes to complete.

Spatial Working Memory test (SWM; Cambridge Cognition Ltd., 2004) – SWM is a CANTAB subtest that requires participants to retain and manipulate spatial information in working memory (Strauss et al., 2006). While SWM is sensitive to frontal lobe damage, damage to the temporal lobe does not affect performance (Robbins & Sahakian, 1994). The aim of the test is for the participant to find a blue token in each of the white boxes and fill up the column to the right (Figure 2.2). The subject decides the order in which boxes are opened, with the key instruction being that each box will only contain one blue token per level. Returning to an empty box already sampled on the search is an error (Strauss et al., 2006). Each successive trial displays boxes differing in colour and position to the previous trial to discourage stereotyped search strategies. SWM takes approximately eight minutes to administer, with total errors providing a measure of monitoring errors, whereby the subject has returned to a box that was certain not to contain a token. SWM total errors measures aspects of the central executive.

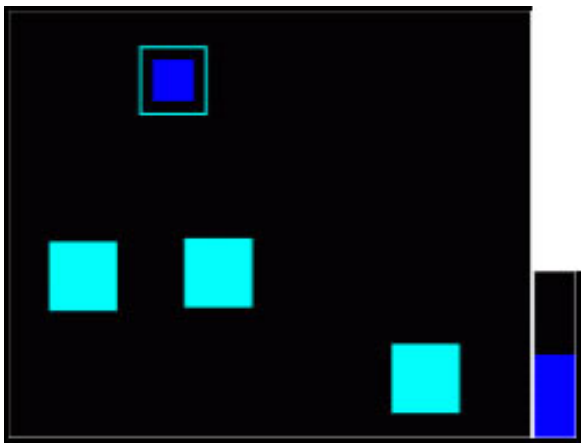

Figure 2.2. Display screen for Spatial Working Memory (SWM).

Executive function

Stroop test (Lezak et al., 2004)– The Stroop test provides a measure of executive functioning through assessment of cognitive control, the ease with which a person can maintain a goal in mind and suppress a habitual response in favour of a less familiar one (Strauss et al., 2006). The Victoria version is a 24-item short version assessing speed of information processing and impulse control for auditory-verbal information (Lezak et al., 2004). The test consists of three cards, each containing six rows of four items. The first card requires the subject to name, as quickly as possible, the colour of 24 dots printed in blue, green, red, or yellow. The second card is similar to the first, except the dots are replaced by common words (e.g. 'when', 'hand', 'over'), and the subject must name the colours of the words. The final card is similar to the previous two, but the coloured stimuli are colour names ('blue', 'green', 'red', 'yellow'). This requires the participant to inhibit an automatic reading response and to produce a more effortful colour-naming response. The extra time required to name colours in the interference task, compared to the time required to name colours in the control task, gives the interference score.

Rapid Visual Processing (RVP; Cambridge Cognition Ltd., 2004) – RVP is a test of sustained attention with a working memory component. Sensitive to dysfunction in the frontal and parietal lobes of the brain (Sahakian & Coull, 1993), the test displays a white box in the centre of the screen, inside which digits, from 2 to 9, appear in a pseudorandom order, at the rate of 100 digits per minute. The subject must detect three distinct digit sequences (3-5-7; 2-4-6; and 4-6-8), occurring at a rate of 16 every two minutes, and register responses by pressing a button on the response pad. The target digit sequences are displayed on the screen to the right of the central stimulus window, thereby reducing the memory load for task performances. The test takes approximately seven minutes to complete, and no cues or feedback are given regarding accuracy. Prior to commencing the assessed block, a practice phase is completed (Figure 2.3). Measures of target detection threshold and sustained attention are given by accuracy and mean latency, respectively, are obtained.

Figure 2.3. Display screen for Rapid Visual Processing (RVP).

Trail Making Test (TMT; Lezak et al., 2004) provides an assessment of processing speed, sequencing, mental flexibility and visual-motor skills (Bowie & Harvey, 2006). The test comprises Part A and Part B. In Part A, the subject is required to draw a line that connects numbered circles, sequentially, from '1 to 25'. In Part B, the subject is required to draw a line that connects alternating circles of numbers and letters. From '1', the subjects first draws to 'A', then to '2', then to 'B', and so on (Lezak et al., 2004). In both Part A and Part B, the subject is required to complete the task as quickly and as accurately as possible. While Part A is considered to be a test of visual search and motor speed skills, Part B is said to assess higher level cognitive skills, such as divided attention and mental flexibility (Bowie & Harvey, 2006).

Language processing

WAIS-III Vocabulary (VOC; Lezak et al., 2004) – VOC assesses the capacity to recognise common words and provide definitions for them in the English language. Words are presented visually (using a stimulus book) and verbally by the examiner simultaneously, participants are asked to correctly define each word with the words becoming progressively more difficult (i.e. less common usage). For example, easier words include 'winter' and 'breakfast', whereas more difficult words include 'encumber' and 'tirade'. Responses are recorded and matched to answers supplied in the scoring booklet. A complete answer gives two-points, a less complete answer gives one-point, and an irrelevant response scores zero. The assessor may prompt the subject for more information ('Can you tell me any more about it?') if a relevant but incomplete response is given. The task is discontinued if the subject scores zero on eight items in succession.

WAIS-III Comprehension (COM; Lezak et al., 2004) – COM assesses capacity to use language to express ideas and understand verbal communication. The task requires subjects to respond to questions that require understanding of concepts and social practices. For example, 'Why does the state require marriages to be officially registered?'. Responses are recorded and matched to answers supplied in the scoring booklet. A complete answer gives two-points, a less complete answer gives one-point, and an irrelevant response scores zero. The assessor may prompt the subject for more information ('Can you tell me any more about it?') if a relevant but incomplete response is given. The task is discontinued if the subject scores zero on four items in succession.

Boston Naming Test (BNT; Kaplan et al., 1983) – The BNT is a widely used naming test that requires the respondent to identify objects represented as line drawings. Impairments to word-finding ability is a characteristic of preclinical AD (Jacobs et al., 1995) and the BNT has been demonstrated to predict the rate of cognitive decline in AD (Rasmusson, Carson, Brookmeyer, Kawas, & Brandt, 1996). The test comprises 60 drawings with the respondent beginning on the 30th item and continuing forwards unless an error is recorded within the first eight items. As the test progresses, the objects that the respondent is required to identify become less frequently encountered and, as such, the items are more difficult. On average, the test takes approximately 5 minutes to complete.

Procedure

Trained assessors undertook the testing for baseline THBP assessments in a quiet room. The screening, neuropsychological, and CANTAB tests were administered in the following order: WTAR; LEQ; DRS-2; MHSQ; WAIS-III-SF1; WRAT; PAL; RAVLT; LM; SSP; SWM; DSP; LNS; VOC; COM; RVP; BNT; Stroop; TMT; HADS. The LEQ and WTAR were only

administered during the baseline assessment. As these tests were part of the THBP's larger test battery, baseline assessments took approximately 4 hours to administer, with subsequent assessments completed in approximately 3.5 hours. Subjects were encouraged to take 10-minute breaks whenever required to reduce fatigue. The CANTAB tests were administered through a standardised script on a Cambridge Cognition Ltd. supplied 17-inch touch-sensitive screen with response pad to measure reaction time. Participants were seated approximately 50cm from the touch-screen with the response pad positioned in a comfortable position approximately 15cm from the screen.

Genetic stream

In addition to the neuropsychological stream, genetic samples were collected from THBP subjects for *APOE* and *BDNF* genotyping. These genes were investigated due to their involvement in cognitive ageing, AD risk, and neuroprotective mechanisms. Although not an exhaustive list of potential genetic polymorphisms related to cognition, other candidate genes (e.g. *KIBRA, COMT*) were the subject of parallel investigations within the THBP and, as such, were not analysed in the present thesis.

Materials

DNA samples were collected with Oragene DNA self-collection kits supplied by Genotek. Instruments and reagents used for DNA extraction and purification were a microcentrifuge (Eppendorf), 1.5 mL microcentrifuge tubes (Astral Scientific: 11210-00), PrepIT.L2P reagent (Genotek: QUO-09611-KZNX), DNA TE storage buffer (pH 8.0), ethanol, and an air incubator.

BDNF Val66Met and *APOE* genotypes were determined through one-step amplified refractory mutation system polymerase chain reaction (ARMS-PCR; Little, 2001) and subsequent gel electrophoresis. Common instruments and reagents used for *APOE* and *BDNF* genotyping were a vortex, a Mastercycle gradient thermal-cycler (Eppendorf), Red-extract-N-Amp (Sigma: R4775), Diethyl Pyrocarbonate (DEPC) treated water (150903), Sybr-safe DNA gel stain (Invitrogen: 533102), a Chemi-do UV light source (Biorad), wide-mini gel electrophoresis trays (Biorad), Tris-acetate-EDTA (TAE) buffer, agarose powder (Bioline Australia: Bio-41025), and Hyperladder IV (Bioline: Bio-33031).

PCR primers specific to *APOE* genotyping were used from an established assay (Donohoe, Salomäki, Lehtimäki, Pulkki, & Kairisto, 1999): Cys158/Arg158 (5'-ATGCCGATGACCTGCAGAAT<u>T</u>-3')/(5'ATGCCGATGACCTGCAGAA<u>T</u>C-3'), Cys112/Arg112 (5'-CGCGGACATGGAGGACGT<u>T</u>T-3')/(5'-CGCGGACATGGAGGACGT<u>T</u>C-3'). The 3'-most nucleotide recognised either A or G corresponding to Arg or Cys at positions 112 or 158, respectively. To enhance primer specificity, the penultimate nucleotide (underlined) was mismatched. Additionally, a common primer was used (5'-GTTCAGTGATTGTCGCTGGGCA-3') that paired with Arg/Cys 158 or Arg/Cys 112 and produced an amplicon of 588 and 451 base pairs (bp), respectively. To act as an internal positive control, the α-antitrypsin gene was coamplified at 360-bp.

PCR primers specific to *BDNF* genotyping were used from an established assay (Sheikh, Hayden, Kryski, Smith, & Singh, 2010): P1 forward (CCTACAGTTCCACCAGGTGAGAAGAGTG); P2 reverse (TCATGGACATGTTTGCAGCATCTAGGTA); P3 G allele specific (5'-CTGGTCCTCATCCAACAGCTCTTCTAT<u>A</u>AC-3'); P4 A allele specific (5'-ATCATTGGCTGACACTTTCGAAC<u>C</u>CA-3'). The first set of primers (P1 and P2)

amplified the 401 bp region containing the SNP of interest (rs6265) and the second set (P3 and P4) were allele specific. Underlined nucleotides represent intentional mismatching to enhance primer specificity.

Procedure

Prior to taking part in the genetic component of the study, participants were supplied with information sheets and informed consent was obtained. Participation in the genetic component was not a requirement of the THBP. For those who consented (response rate: 92%), Oragene DNA self-collection kits were mailed to participants with instructions to complete and return. Alternatively, subjects could opt to donate their sample during their annual assessment. Before genotyping, the sample was extracted and purified. Genotyping was repeated on samples to ensure accuracy. DNA extraction, purification, and genotyping took place at the Menzies Research Institute laboratories.

DNA extraction and purification

Each Oragene kit contained a buffer solution which, when mixed with the sample, stabilised the DNA at room temperature. Samples were then air-incubated at 50^{o}C overnight to inactivate nucleases and to ensure DNA was adequately released. For each sample, 500 μL was transferred to a 1.5 mL microcentrifuge tube with 20 μL of PT-L2P added to precipitate impurities and inhibitors. The remaining 1.5 mL of sample stored in a secure location. Samples were then vortex-mixed and incubated on ice for 10 minutes, which assisted in impurity removal. Following this, centrifuge for 5 minutes at 13,000 rpm formed a pellet of impurities in each sample. The clear supernatant was transferred to a fresh microcentrifuge tube and the pellet discarded. To precipitate the DNA, 600 μL of room temperature 95% to

100% ethanol was added, and mixed gently by inversion 10 times. The samples were allowed to stand at room temperature for 10 minutes to allow the DNA to fully precipitate. After precipitation, the samples were centrifuged, in a known orientation, for 2 minutes at 13,000 rpm. A pellet of DNA was formed and the impurity-containing supernatant was carefully removed. The final steps involved an ethanol wash to removal residual inhibitors and the addition of 500 µL of Tris-EDTA buffer followed by vortex to ensure hydration of DNA. The DNA was stored in a refrigerator at 4°C for seven days before use.

APOE genotyping

Two SNPs (rs429358, rs7412) were assessed to determine *APOE* polymorphisms. Two separate PCR reaction mixtures were assembled using amplification-refractory mutation system (ARMS; Little, 2001). Reaction A contained 0.8 µmol/L Cys 158 (451 bp) and 0.4 µmol/L Cys112 (588 bp) primers while Reaction B contained 0.8 µmol/L Arg158 (451 bp) and 0.4 µmol/L Arg112 (588 bp) primers. Both reactions contained RED Extract-N-Amp PCR mix and ARMS primer. Depending on how many samples were being genotyped, 11 µL of Reaction A PCR mixture was added to each well in the first three to four rows of a 96 well plate. Similarly, 11 µL of Reaction B PCR mixture was added to wells in the lower rows of the plate. Subsequently, 1 µL of the participants DNA sample was pipetted into the Reaction A wells, with 1 µL of the same sample, corresponding to the first, added to the Reaction B wells. Each reaction had a no template control to exclude contamination. The DNA product was amplified by placing the 96 well plate in a thermal-cycler for initial denaturation at 95°C for 4 min, followed by 35 cycles of denaturation at 96°C for 45 s, annealing at 65°C for 45 s, and extension at 72°C for 45 s. Finally, one last cycle of extension at 72°C for 5 min completed the amplification.

For gel electrophoresis, 1 x TAE was mixed with agarose powder and dissolved in a microwave oven to yield a 1.5% concentration agarose solution. Eighty millilitres of agarose solution were combined with 5 μL of Sybr-safe DNA stain for visualisation and poured into a mould with a comb inserted. After the gel had set, the comb was removed revealing wells in to which amplified DNA product could be pipetted. To support conductivity, the gel was placed into a wide sub cell gel tray and covered with 1 x TAE. Using a pipette, 5 μL of 100 bp DNA ladder was loaded into the first well, with 9 μL of amplified DNA product loaded into subsequent wells, in order: Sample 1 Reaction A, Sample 1 Reaction B; Sample 2 Reaction A, Sample 2 Reaction B. The no template control samples were loaded last. The samples were electrophoresed for 30-40 minutes at 110 volts, with resulting bands visualised with UV light and Quantity One software (Figure 2.4).

To determine genotypes, banding was compared to previously established banding patterns (Donohoe et al., 1999). An ε2 ε2 genotype was identified by a Cys112 (588 bp) and Cys158 (451 bp) positive double band in Reaction A, with banding absent in Reaction B. An ε2 ε3 genotype was identified by a Cys112 (588 bp) and Cys158 (451 bp) positive double band in Reaction A, with a Arg112 (588 bp) positive single band in Reaction B. An ε3 ε3 genotype was identified by a Cys158 (451 bp) positive single band in Reaction A and an Arg112 (588 bp) positive single band in Reaction B. An ε3 ε4 genotype was identified by a Cys158 (451 bp) positive single band in Reaction A and an Arg112 (588 bp) and Arg158 (451 bp) positive double band in Reaction B. An ε4 ε4 genotype was identified by banding absent in Reaction A, with an Arg112 (588 bp) and Arg158 (451 bp) positive double band in Reaction B. Finally, an ε2 ε4 genotype was identified by a Cys112 (588 bp) and Cys 158 (451 bp) positive double band in Reaction A and an Arg112 (588 bp) and Arg158 (451 bp) positive double band in Reaction B.

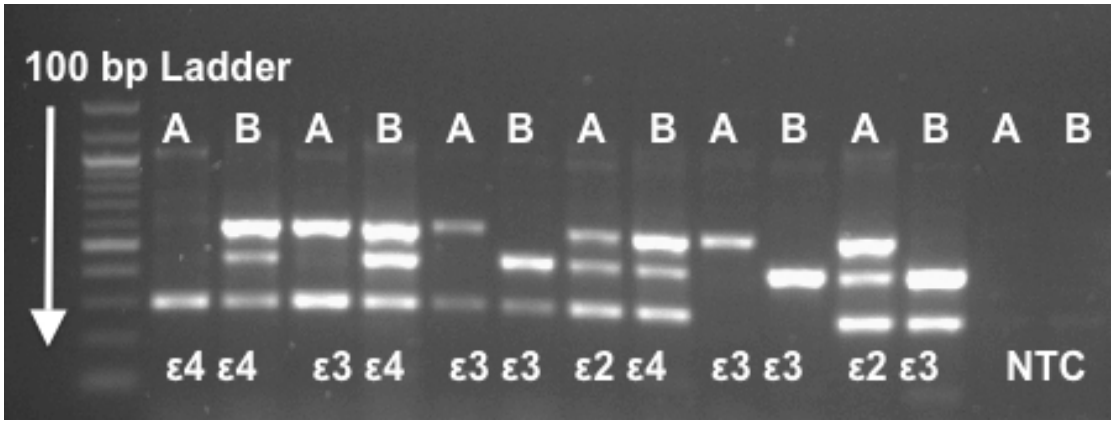

Figure 2.4. An imaged *APOE* gel electrophoresis for six DNA samples and a no template control sample. All *APOE* genotypes are present except ε2 ε2.

BDNF Val66Met genotyping

A SNP (rs6265) was assessed to determine BDNF polymorphisms. A PCR reaction mixture was assembled using ARMS (Little, 2001) that contained 1.5 µmol/L P1 (forward), 1.25 µmol/L P2 (reverse), 0.75 µmol/L P3 (G allele specific), 1.25 µmol/L P4 (A allele specific), and RED Extract-N-Amp PCR mix. For each 1.0 µL of DNA, 11 µL of PCR mix was added to a 96 well plate. The DNA product was amplified by placing the 96 well plate in a thermal-cycler for initial denaturation temperature of 94°C for 5 min, followed by 30 cycles of denaturation at 94°C for 45 s, annealing at 62.5°C for 60 s, and extension at 72°C for 60 s. Finally, one last cycle of extension at 72°C 5 min completed the amplification.

For gel electrophoresis preparation, an identical method was used as per *APOE* genotyping. After the gel had set it was placed into a wide sub cell gel tray and covered with 1 x TAE to support conductivity. Using a pipette, 5 µL of 100 bp DNA ladder was loaded into the first

well, with 9 μL of amplified DNA product loaded into subsequent wells. The no template control sample was loaded last. The samples were electrophoresed for 30-40 minutes at 110 volts, with resulting bands visualised with US light and Quantity One Software (Figure 2.5). A Val/Val genotype was identified by a single 253 bp band, a Met/Met genotype was identified by a single 201 bp band, and a double band (253 and 201 bp) identified a Val/Met genotype. The 400 bp line was the control amplicon.

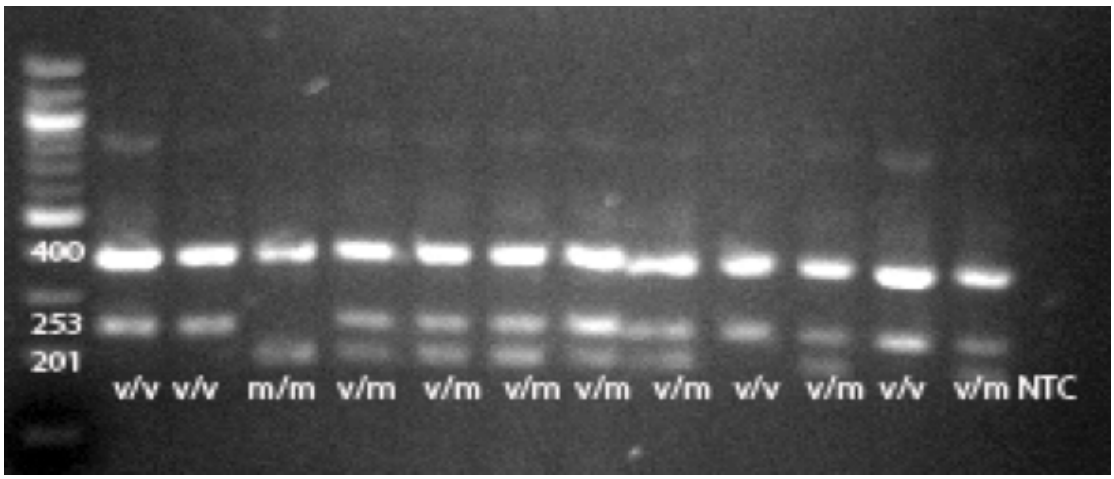

Figure 2.5. An imaged *BDNF* gel electrophoresis for 12 DNA samples and a no template control sample. V/V = Val/Val, V/M = Val/Met, M/M = Met/Met.

Chapter 3

Modelling cognitive reserve in healthy middle and older aged adults: The Tasmanian Healthy Brain Project

Abstract

Cognitive reserve (CR) is a protective factor that supports cognition by increasing the resilience of an individual's cognitive function to the deleterious effects of cerebral lesions. A single environmental proxy indicator is often used to estimate CR (e.g. education), possibly resulting in a loss of the accuracy and predictive power of the investigation. Furthermore, while estimates of an individual's prior CR can be made, no operational measure exists to estimate dynamic change in CR resulting from exposure to new life experiences. This chapter aimed to develop two latent measures of CR through factor analysis: prior and current, in a sample of 467 healthy older adults. The prior CR measure combined proxy measures traditionally associated with CR, while the current CR measure combined variables that had the potential to reflect dynamic change in CR due to new life experiences. The main finding of this chapter was that the analyses uncovered latent variables in hypothesised prior and current models of CR. The prior CR model supports multivariate estimation of pre-existing CR and may be applied to more accurately estimate CR in the absence of neuropathological data. The current CR model may be applied to evaluate and explore the potential benefits of CR-based interventions prior to dementia onset.

Introduction

The predicted epidemic increase in the incidence of dementing degenerative illnesses, such as AD over the next 10-20 years (Access Economics, 2011) has focused international research efforts on identifying potential pharmacological agents that can delay or prevent AD. An alternative, non-pharmacological approach receiving less attention is increasing understanding of what contributes to, and how to increase, the brain's ability to sustain effective cognitive function in the presence of cerebral lesions. CR is a hypothetical construct that describes neurological processes that support cognitive function in the face of pathology-related neural disruption (Stern, 2002). Two complimentary neural processes, neural reserve and neural compensation, result in brain networks being better able to resist or compensate for the effects of neurological degeneration (Stern et al., 2005). Individuals with higher CR, generated by a lifetime of cognitive engagement, have lower risk for dementia despite no decrease in their risk for accumulating frank AD pathology (Stern, 2002). Although CR has been researched intensively, few factor-supported operational measures exist for accurately estimating an individual's CR.

The notion that CR protects against the emergence of clinical symptoms in neurodegenerative disorders is supported by an autopsy investigation that found a subset of pathologically-confirmed AD cases with preserved cognition prior to death (Katzman et al., 1988). Subsequent investigations identified education, occupational attainment, intelligence, and participation in cognitively stimulating leisure activities as being lifetime contributors to CR (Stern, 2002). Excluding intelligence, the majority of proposed CR proxies are measures of environmental exposure (Stern, 2002), with measures of individual cognitive processes tending not to be used as estimates of CR, with the possible exception of executive function (Satz et al., 2011). Earlier studies demonstrating an inverse relationship between educational

or occupational attainment and risk for dementia (Ott et al., 1995; Stern et al., 1994) have been supported by more recent data (Basu, 2012; Brayne et al., 2010; Karp et al., 2009; Meng & D'Arcy, 2012). Intellectual factors have been shown to possess similar cognition-preserving effects (Galioto et al., 2013; Rentz et al., 2010; Vemuri et al., 2011). Importantly, CR is modifiable throughout life and cognitively stimulating leisure activities in adults over the age of 65 years are also associated with a reduced risk for dementia (Akbaraly et al., 2009; Norton et al., 2012; Wilson et al., 2010) and age-related cognitive decline (Small, Dixon, McArdle, & Grimm, 2012; Wang et al., 2012). However, such studies using longitudinal designs focused solely on a single-point measure of cognitive activities at baseline and neglect consideration of potential change in activities over time. Nonetheless, two systematic reviews have reported significant reductions in risk for dementia in people who lived cognitively engaged lives (Fratiglioni & Wang, 2007; Valenzuela & Sachdev, 2006).

Neuroimaging studies indicate that life experience contributes to the preservation of cognition above and beyond the development of cognitive strategies that offset dementia. For example, *in vivo* investigations have linked some CR proxies to the integrity of neurological structures, suggesting an overlap between CR and cerebral reserve hypotheses (Barulli & Stern, 2013). Healthy individuals with more education have larger regional cortical thickness in transverse temporal cortex, insula, and isthmus of cingulate cortex (Liu et al., 2012), and greater grey and white matter brain volumes (Foubert-Samier et al., 2012). As people with higher IQs obtain more education which, in turn, increases IQ (Brinch & Galloway, 2012; Ceci, 1991), the expected positive correlation between IQ and grey matter volume has also been reported (Colom et al., 2006; Haier et al., 2004). Life experience may also provide benefits through direct modification of detrimental age or disease processes, and data that relate lifetime cognitive engagement to reductions in Aβ deposition (Landau et al., 2012) and rates of

hippocampal atrophy (Valenzuela et al., 2008) have further underlined the importance of a lifespan approach to measuring CR.

As education, occupational attainment, and participation in leisure activities have been found to differently and independently contribute to CR (Foubert-Samier et al., 2012), a comprehensive estimate of CR must incorporate a number of contributors effectively (Sánchez et al., 2011). To date, the majority of research has focused on a single CR proxy when investigating potential relationships with dementia, whereas the assessment of multiple proxies over a lifespan may lead to research that improves understanding, and accurate application, of the theoretical construct. Despite criticism of the *a priori* grouping of CR proxies regarding construct validity (Satz et al., 2011), recent investigations increasingly take into account multiple proxy measures when assessing CR (Foubert-Samier et al., 2012). The method of combining several indicators of CR may provide a more precise estimate of reserve than can be obtained from the use of a single indicator (Jones et al., 2011).

Estimates of pre-existing CR that incorporate a range of proxy measures will most accurately reflect the individual's ability to compensate for neuropathology, yet commonly used CR variables do not allow for dynamic change in an individual's reserve due to new life experiences. Importantly, CR is not set in early life and, rather than being static, continues to develop and be affected by new life experiences across the lifespan (Richards & Sacker, 2003). Despite this, CR proxies commonly used in research do not have the capacity to reflect a dynamic developmental change in CR, with measures of early life education, occupational attainment, and intelligence being relatively resistant to modulation from later life experiences. Another retrospectively measured CR proxy variable is frequency of participation in cognitively stimulating activities (Wilson et al., 2010). However, participation in leisure activities in adult life is susceptible to change over time, and CR-based

interventions that determine the effect of modulation in cognitive activities are eagerly awaited (Stern, 2012). Although interventions that aim to increase CR in later life hold promise (Tucker & Stern, 2011), no operational measure exists to provide an estimate of dynamic change in CR resulting from exposure to new experiences. Such a measure might comprise cognitive tests that can detect changes in cognitive function due to exposure to environmental factors that contribute to prior CR (e.g., education). It is in the interest of the CR hypothesis that a validated measure of current CR is developed so that the efficacy of later life interventions can be assessed prior to dementia onset.

The THBP is an on-going longitudinal investigation into the hypothesis that later-life tertiary education may result in increased CR, potentially contributing to delayed expression of clinical dementia symptoms (Summers et al., 2013). The sample consists of cognitively healthy older community-residing subjects, aged 50-79 years at study entry, who will complete annual comprehensive neuropsychological assessments. The need for factor-supported operational measures of CR is high, and the present study aims to develop two latent constructs of reserve: prior and current. The prior CR factor was derived from proxy measures traditionally associated with CR: estimated pre-existing intellectual capacity; cognitive life experience; and prior education. The current CR factor was derived from measures that had the potential to reflect dynamic change in CR in response to later life activities that increase reserve: current intellectual capacity and academic ability. This measure of CR would allow preliminary examination of the efficacy of the later life education intervention central to the THBP, prior to dementia onset. Both latent CR variables, prior and current, were constructed through factor analysis with the principal component analysis extraction method. This generated weighted linear combinations of CR proxies, which, when added together, represented quantifiable estimates of CR, both prior and current, for each participant in the THBP.

Method

Study population

A total of 467 healthy English-speaking participants from the longitudinal THBP completed the baseline neuropsychological battery after informed consent was obtained. Trained assessors obtained measures of CR variables, neuropsychological function, and health and psychosocial function (Summers et al., 2013). Subjects were community residing 50-79 year olds at their first neuropsychological assessment, were predominantly female (68.1%), and were recruited through an on-going campaign that involved print, radio, television advertising, and community information presentations. All participants underwent screening as described in chapter 2. Participants were not compensated for their time; however, those participating in the THBP's experimental group were eligible to receive a waiver of individual course charges (Australian Higher Education Contribution Scheme) for a study load equivalent to a 12.5% unit per academic year at the University of Tasmania. The study had full approval from the Tasmania Human Research Ethics Network and was conducted in accordance with the ethical guidelines of the National Health and Medical Research Council of Australia (NHMRC).

Materials

Participants completed a comprehensive test battery as described in detail elsewhere (chapter 2). To ensure that the sample was free from dementia symptoms and in good physical and psychological health, the Mattis Dementia Rating Scale-2 (DRS-2; Jurica et al., 2001),

Hospital Anxiety and Depression Scale (HADS; Zigmond & Snaith, 1983), and the Medical Health Status questionnaire (Summers et al., 2013) were used . The DRS-2 objectively assessed the presence and progression of dementia of Alzheimer's type, vascular dementia, Parkinson's disease, and Huntington's disease. The HADS assessed symptoms of anxiety and depression via self-report and has been widely used to detect such disorders among respondents (Bjelland et al., 2002). A self-report medical health status questionnaire assessed health, medical conditions, prescription medication use, drug and alcohol use for the preceding 12 months (Summers et al., 2013). This questionnaire was developed for the THBP and also collected demographic information such as age, gender, handedness, height, weight, marital status, and educational and occupational history. An experienced neuropsychologist (MS) reviewed performance on these tools to determine participant inclusion.

Prior cognitive reserve variables

To generate factors associated with prior CR, the Wechsler Test of Adult Reading (WTAR; The Psychological Corporation, 2001) was used to estimate pre-existing intellectual capacity, the Lifetime of Experiences Questionnaire (LEQ; Valenzuela & Sachdev, 2007) was used to quantify prior lifetime experience, and number of years of prior formal education was recorded on the Medical Health Questionnaire (Summers et al., 2013). The WTAR provided a stable and reliable estimate of pre-existing intellectual capacity by assessing the capacity of the participant to correctly pronounce 50 atypical grapheme-phoneme words (e.g. 'ogre'). Using words with irregular pronunciations minimises the individual's current ability to apply standard pronunciation rules to assess previous learning of the word. As such, the WTAR can be applied to estimate intellectual function prior to traumatic brain injury (Green et al., 2008) and dementia onset (Donnell et al., 2007). Furthermore, tests of reading ability are frequently used to estimate CR (Lo et al., 2013; Vemuri et al., 2011). The LEQ is a self-report

retrospective questionnaire that assessed the full range of complex mental pursuits (educational, occupational, and leisure activities) that build up over a person's lifetime from young adulthood, mid-life, and late-life stages (Valenzuela & Sachdev, 2007). The LEQ has demonstrated adequate validity (Cronbach's $\alpha = 0.66$) and reliability (3-6 month test-retest $r = .98$; Valenzuela & Sachdev, 2007). For the purpose of factor analysis, only specific, non-specific, and continuing education sub-scores from Young Adulthood and Mid-Life sections of the LEQ were used. The LEQ Midlife Specific subscale, which provided a measure of occupational attainment, comprised a set of questions that determined the classification of each occupation engaged in and the managerial capacity of each occupation within the 30-65 years age bracket (Valenzuela & Sachdev, 2007). Total number of years of prior formal education was calculated as the sum of school education and post-secondary education reported by each participant.

Current cognitive reserve variables

To identify factors associated with current CR, the Wechsler Adult Intelligence Scale, 3rd edition Short Form 1 (WAIS-III-SF1; Donnell et al., 2007) was used to assess current intellectual capacity and two components from the Wide Range Achievement Test, 4th edition, Progress Monitoring Version (WRAT-4-PMV; Roid & Ledbetter, 2006), were used to assess current academic ability. The WAIS-III-SF1 extrapolates the full scale WAIS-III intelligence quotient from the performance across four WAIS-III subtests (picture completion, digit symbol coding, similarities, and arithmetic; Donnell et al., 2007). The WRAT-4-PMV assesses academic performance factors: word reading; sentence comprehension; spelling; and math computation ability in adults with higher secondary education (Roid & Ledbetter, 2006). Scores in each WRAT-4-PMV subtest increase with level of education, including into college/university level. The WRAT-4-PMV was selected for its capacity to detect increased

performance in subtests associated with increasing levels of education (Roid & Ledbetter, 2006). This differentiates education's contribution to prior CR, as measured by years, from current CR, by estimating future education-related change in cognitive function. For the current CR model, only spelling and math computation were included, as reading could confound with the WTAR, which comprises prior CR, and sentence comprehension reported a very low extracted communality value (.31). A previous version of the WRAT (WRAT-R) has been shown to correlate highly (r = .73) with the WTAR (Strauss et al., 2006), and in the present sample high correlations were found between WTAR and WRAT reading (r = .69) and WRAT spelling (r = .61), but not between WTAR and WRAT math computation (r = .30).

Procedure

Participants were administered the selected tests by trained assessors as part of the larger THBP assessment battery (Summers et al., 2013), which examined additional cognitive domains (memory and learning, working memory, language, executive function). The assessment process took four hours to complete, on average, and was undertaken in a quiet room. Subjects were encouraged to take 10-minute breaks as needed to reduce symptoms of fatigue.

Data analysis

The data's factorability was examined via Bartlett's test of sphericity and Kaiser-Meyer-Olkin measure of sampling adequacy. Factor analysis with the principal components extraction method was conducted separately for the prior CR factors and for the current CR factors. Principal components extraction was selected to maximize the likelihood that each variable

would contribute to final CR models. Varimax rotation was used to ensure that the extracted factors remained independent and because it produced the simplest factor structure, which allowed for increased interpretability - a priority when using factor analysis (Kline, 2002). For prior CR, the variables entered into the analysis were: Prior education (years); LEQ Young Adulthood Specific; LEQ Young Adulthood Nonspecific; LEQ Mid Life Specific; LEQ Mid Life Nonspecific; LEQ Midlife Continuing Education Bonus. For current CR, the variables entered into the analysis were: WAIS-III-SF1 FSIQ; WRAT-4-PMV Spelling Level Equivalent Score (LES); WRAT-4-PMV Math Computation LES. Factor scores for the prior CR factors and the current CR factor were generated for each participant using the regression method. For prior CR, a subject's individual scores on all factors were added together to yield their overall prior CR score. All data were analysed using SPSS v21.

Results

Participants

Participant demographics and characteristics of the final 467 THBP subjects that comprised the study population for this chapter are presented in Table 3.1, along with summaries of CR variables.

Table 3.1. Descriptive statistics for the study population (N = 467)

Variable	N	Mean	*SD*	Range
Characteristics				
Age (years)	467	60.64	6.81	50-79
Gender (% male/female)	467	31.7 / 68.3		
DRS-2 AEMSS	467	12.01	2.13	6-17
HADS anxiety	466	5.28	3.05	0-16
HADS depression	466	2.48	2.24	0-12
Prior CR				
WTAR Est. FSIQ	466	112.50	5.47	83-123
Prior education (years)	467	13.83	2.75	3-28
LEQ Young Adulthood Specific	467	15.69	7.67	0-46.20
LEQ Young Adulthood Nonspecific	467	24.84	5.52	11-41
LEQ Mid Life Specific	466	18.93	4.73	0-25.50
LEQ Mid Life Nonspecific	467	24.50	5.37	7.87-38
LEQ Mid Life Continuing Education	466	9.52	8.30	0-46.20
Current CR				
WAIS-III-SF1 FSIQ	466	118.97	13.42	85-155
WRAT-4-PMV Spelling LES	467	568.78	16.04	499-603
WRAT-4-PMV Math Computation LES	466	534.99	19.97	480-588

Note: Data represent proportions for categorical variables. DRS-2 = Dementia Rating Scale-2; AEMSS = Age- and education-corrected Mayo Older American normative Studies (MOANS) scaled score; HADS = Hospital Anxiety and Depression Scale; WTAR = Wechsler Test of Adult Reading; FSIQ = Full-scale IQ; LEQ = Lifetime of Experiences Questionnaire; WAIS-III-SF1 = Wechsler Adult Intelligence Scale (3rd edition) short form 1; WRAT-4-PMV = Wide Range Achievement Test-4 Progress Monitoring Version; LES = Level equivalent scores.

Prior cognitive reserve factor analysis

To develop a model of prior CR, the seven items were first examined for their factorability through widely used criteria. The sampling adequacy was verified by the Kaiser-Meyer-Olkin measure, KMO = .58. Bartlett's test of sphericity, $\chi^2_{(21)} = 519.74$, $p. < .05$, indicated that the variables were sufficiently correlated for factor analysis. Communality values were all above .4, confirming that the entered variables shared sufficient common variance. As a result of these preliminary tests, the data were deemed suitable for factor analysis. As the goal was to combine CR proxy variables into a single construct that represents prior CR with composite scores computed for each subject in the study, the principal components extraction method was used. In order to test whether the structure of the prior CR model was resistant to lifetime variables, initial factor analyses were compared across age group (under 65 years vs. 65 years and over) and employment status (still working vs. retired). The results indicated that, in both cases, the lifetime variables modulated the resulting structure of the prior CR model. In order to rectify this, an additional factor analysis was conducted that retained a fourth component and produced a factor structure that was stable across both age group and employment status. In combination, these factors explained 77.10% of the variance in the dataset. The structure of the final prior CR model with associated factor loadings following an orthogonal rotation (Varimax) is displayed in Figure 3.1, with variable loadings under .50 considered not essential to the final model. The clustering of variables suggested that factor 1 represents education and intelligence, factor 2 represents cognitive activities, factor 3 represents mid-life education, and factor 4 represents occupational attainment. A *z*-score was generated for each subject on each factor using the regression method, with an estimate of overall prior CR resulting from the addition of each factor score into a total. The equation that resulted in total prior CR = [0.370 (WTAR IQ) + 0.408 (Prior education) + 0.567 (LEQ Young Adulthood Specific)] + [0.565 (LEQ Young Adulthood Nonspecific) + 0.630 (LEQ Mid-Life

Nonspecific)] + [0.875 (LEQ Mid-Life Continuing Education Bonus)] + [1.004 (LEQ Mid-Life Specific)].

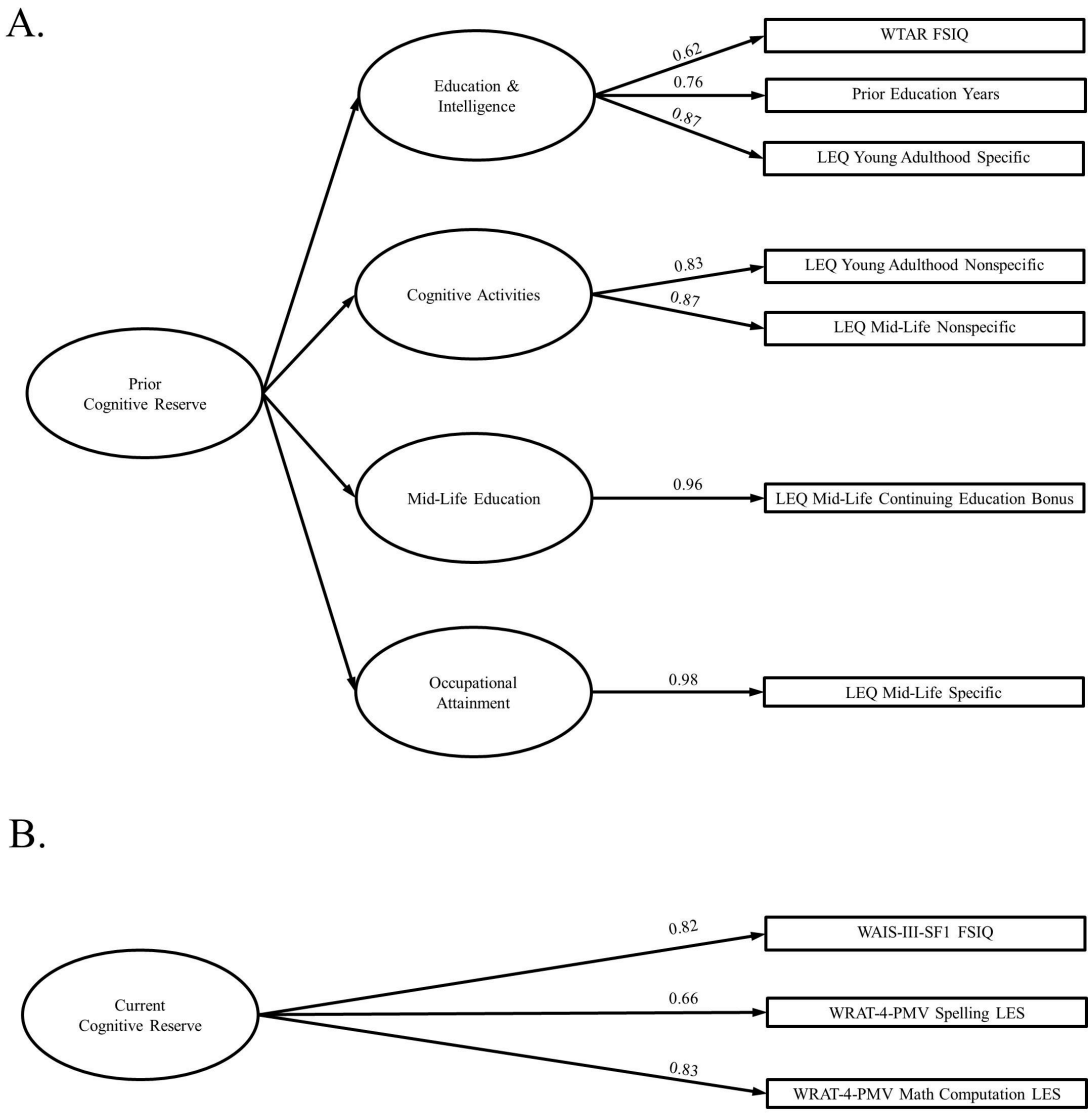

Figure 3.1. The results of the exploratory factor analyses that identified hypothesised (A) prior CR (N = 464) and (B) current CR (N = 465) models in a sample of healthy adults. Variables that were directly measured are denoted by squares and circles denote latent variables. The numbers along the lines represent rotated factor loadings. Prior CR was determined through the addition of an individual's scores on all three CR factors. WRAT-4-PMV = Wide Range Achievement Test-4 Progress Monitoring Version, level equivalent scores; LEQ = Lifetime of Experiences Questionnaire.

Current cognitive reserve factor analysis

To develop a model of current CR, the three items first had their factorability examined. The sampling adequacy was verified by the Kaiser-Meyer-Olkin measure, KMO = .62. Bartlett's test of sphericity, $\chi^2_{(3)} = 224.81$, $p. < .05$, indicated that the variables were sufficiently correlated for factor analysis. Communality values were all above .3, confirming that the entered variables shared enough common variance. As a result of these preliminary tests, the data were deemed suitable for factor analysis. The initial factor analysis that used the principal components extraction method identified a single underlying component with an eigenvalue above Kaiser's criterion of 1 (Kaiser, 1960). This factor explained 59.87% of the variance in the dataset. The structure of the current CR model with associated factor loadings is displayed in Figure 3.1, with variable loadings under .50 considered not essential to the final model. The clustering of variables suggested that the single component represents current CR. A *z*-score was generated for each subject for this factor using the regression method. The equation that resulted in total current CR = 0.454 (WAIS-III-SF1 FSIQ) + 0.369 (WRAT-4-PMV Spelling LES) + 0.463 (WRAT-4-PMV Math Computation LES).

Prior and current cognitive reserve differentiation

To test whether the proposed prior and current models of CR could be differentiated statistically, a final factor analysis was conducted that included all 10 variables from both models. The sampling adequacy was verified by the Kaiser-Meyer-Olkin measure, KMO = .67. Bartlett's test of sphericity, $\chi^2_{(45)} = 1047.51$, $p. < .05$, indicated that the variables were sufficiently correlated for factor analysis. An initial factor analysis that used the principal components extraction method identified three underlying components with eigenvalues above Kaiser's criterion of 1 (Kaiser, 1960). These factors explained 56.17% of the variance

in the dataset, in combination. The structure of the model with associated factor loadings following Varimax rotation is displayed in Figure 3.2. The clustering confirmed that the variables that comprised prior and current CR models could be statistically differentiated, with the exception of WTAR Est. IQ, which loaded on the current CR component containing other intelligence- and academic-related variables. Importantly, the participant scores for prior and current CR were significantly correlated ($r. = .40$, $p. < .01$). This indicated that both models assessed a similar construct.

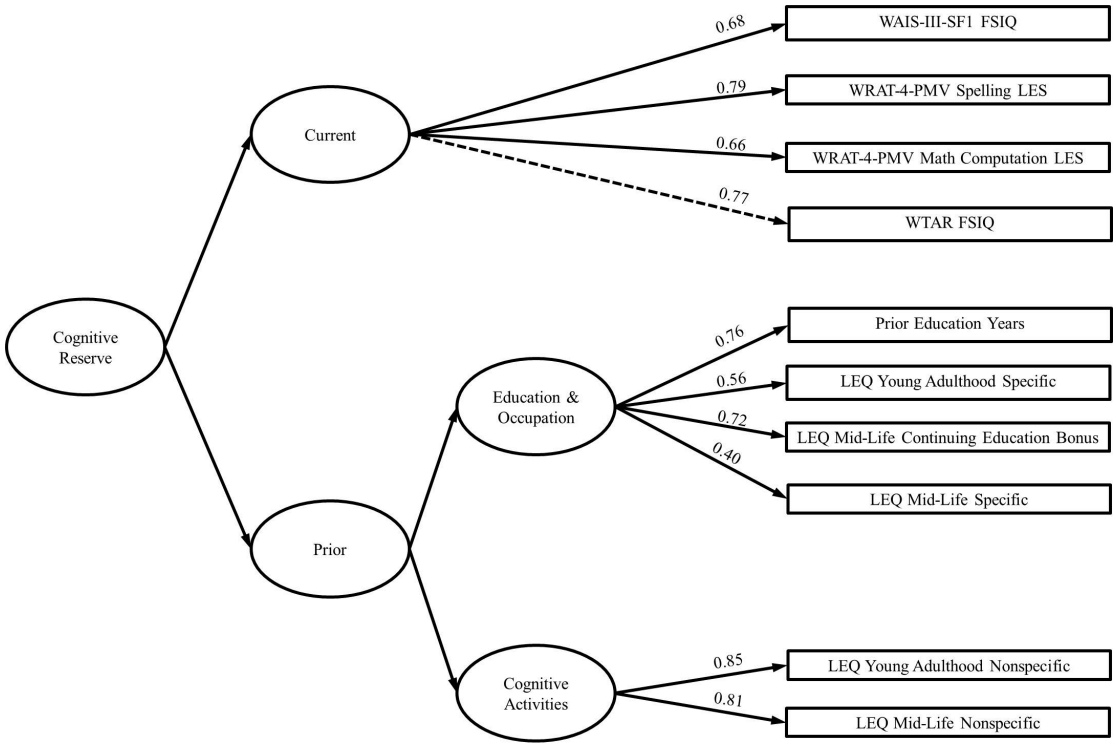

Figure 3.2. The results of the exploratory factor analysis that differentiated the prior and current models of CR (N = 462). Variables that were directly measured are denoted by squares and circles denote latent variables. The numbers along the lines represent factor loadings. The dotted lines denote an altered relationship from that which was hypothesised. WRAT-4-PMV= Wide Range Achievement Test-4 Progress Monitoring Version; LEQ = Lifetime of Experience Questionnaire.

Discussion

The present study was designed to use THBP baseline data to develop two factor-supported operational measures of CR, prior and current, using factor analysis with the principal components extraction method. Assessment of prior CR was based on the combination of traditional estimates of reserve: years of prior education; estimated pre-existing intellectual capacity; and lifetime of cognitively stimulating activities. Assessment of current CR was based on the combination of dynamic measures of current intellectual capacity and academic

ability. The results of the factor analyses indicate that significant latent variables were identified, which were labelled prior CR and current CR. Prior CR provided an operational measure of predominantly static CR, with the capacity to estimate an individual's increased protection from dementia due to prior education, intelligence, cognitive activities, and occupational attainment. Current CR provided an operational measure of dynamic CR, comprising cognitive variables that approximate traditional CR predictors but have an increased potential to reflect change in cognitive function due to exposure to CR-related activities. The investigation supports the notion that multiple CR proxy measures can be used to assess CR. Future use of this method may lead to research that improves understanding and accurate application of the theoretical construct.

Although the analyses produced prior and current CR models with interpretable extracted components, three issues that arose in the process warrant discussion. First, the initial attempts to identify a prior CR structure independent of lifetime factors were unsuccessful. Here, 3-factor models of prior CR were modulated by age and employment status, resulting in different structures of prior CR variables for different groups. However, the extraction of a 4-factor model of prior CR resulted in a structure that was stable between groups and a prior CR equation that could be applied equally to all participants in the THBP (Figure 3.1). Second, a factor analysis of whether the models could be statistically differentiated resulted in an unexpected relationship (Figure 3.2). Specifically, when all variables were analysed concurrently, WTAR IQ, a measure of intelligence that was originally retained in the prior CR model, loaded significantly on the current, rather than prior, CR model. This may reflect an issue of shared variance, indicating that WTAR IQ does not contribute uniquely to the estimation of prior CR. However, it was not possible to include WTAR IQ simultaneously in both factor models for current and prior CR due to a high degree of shared variance with the WAIS-III IQ measure, as reported in previous research ($r = .73$; Strauss et al., 2006).

Ultimately, it may be impossible to disentangle current CR fully from prior CR, as current CR likely reflects a combination of prior CR plus change in CR (i.e., through further education). Third, the number of components retained in the differentiating model differs from the number retained in the final prior and current CR models. This is due to the decision to force the retention of four components in the prior CR model, whereas the full model only retained components with eigenvalues above Kaiser's criterion of 1 (Kaiser, 1960).

Historically, studies of CR have relied upon a single indicator variable to estimate CR, but this may not be the best method. The present finding that factor analysis was successful in creating a prior CR model provides evidence that a multivariate approach to CR results in a more comprehensive estimate of an individual's prior CR. As the included proxy variables in the prior CR model have been shown to contribute to CR independently (Foubert-Samier et al., 2012; Stern, 2009), the factor analysis technique is likely to result in increased accuracy for assessing CR in the absence of neuropathological data. The present data support this notion, as the components extracted from the prior CR analysis were not highly correlated, indicating independent contributions to the overall CR model when each component was added together. If an investigation relies upon an estimate of CR derived from a single indicator, there is an increased risk of interpreting a factor with reduced predictive power. This is in line with a comprehensive review of the construct validity of the multiple indicator method, which concluded that techniques that combine a number of indicators, rather than an individual indicator, may increase the statistical and interpretive power of the CR measurement (Satz et al., 2011). However, as the present study does not include a measure of outcome (e.g., dementia onset), it is unknown whether this method produces an increase in predictive power when compared to a single indicator.

To date, there is no reliable or valid measure of changes to CR over time; such as might occur following interventions designed to enhance CR. While research continues to explore the potential benefits of various interventions to modify an individual's CR and thereby reduce the risk of dementia within the individual (Tucker & Stern, 2011), there is no available operational measure of CR to substantiate the efficacy of the intervention before dementia onset. It is of significance that the present study successfully generated, through factor analysis, a measure of current CR that encompasses dynamic variables known to display change over time. Rather than using predominantly static variables to create the current CR model, measures that had the potential for change due to new life experience were used, which also closely approximated traditional measures of CR. Education was approximated using the WRAT-4-PMV (Roid & Ledbetter, 2006), which can detect change in academic performance (math computation, spelling) associated with increasing levels of education (Roid & Ledbetter, 2006). A measure of intelligence, WAIS-III-SF1 (Donnell et al., 2007), which is more likely to show change in intellectual function than the measure of crystallised intelligence used for prior CR estimation (WTAR; The Psychological Corporation, 2001), was used. The WTAR provides a stable estimate of intellectual capacity that is resistant to brain damage arising from traumatic brain injury or dementia (Green et al., 2008), while the WAIS-III is not resistant to disease states and reflects reductions in FSIQ due to ageing (Ardila, 2007).

Fundamentally, CR is a hypothetical construct that is theorized to delay the expression of clinical deficits arising from cortical disease burden (Stern, 2002) and, although an estimate arising from multiple indicators is more likely to reflect this construct than a single variable/measure, this is still a limited approach. Empirical designs that incorporate an *in vivo* measure of brain damage/burden in addition to proxy measures of reserve and a measure of cognitive function will make the most influential contribution to CR literature. Such an

approach is becoming more popular as relevant technology becomes more widely available, and a number of studies have supported the hypothesis by demonstrating that higher CR is associated with comparable cognitive ability to those with less reserve despite having more severe AD pathology, such as cortical thinning (Querbes et al., 2009), medial temporal lobe atrophy (Perneczky et al., 2009; Teipel et al., 2009), and reduced cerebral blood flow (Scarmeas et al., 2003).

A number of limitations in the present study warrant examination. First, the LEQ (Valenzuela & Sachdev, 2007) and the Medical Health Status questionnaire (Summers et al., 2013) are retrospective self-report questionnaires. Here, the accuracy of information gained in such a manner cannot be verified (Bernard, Killworth, Kronenfeld, & Sailer, 1984). Second, it is important to note that variation in prior CR variables will be experienced in participants even after this measure is taken, and that the estimation of prior CR, while predominantly static, is not completely fixed, particularly in those participants whose age has not yet reached 65 years. Third, CR is a hypothetical construct that is mainly applied to samples experiencing traumatic brain injury or neurodegenerative disorders. The present sample was selected to be healthy, due to extensive screening of medical conditions and impairments prior to assessment and this may reduce the applicability of the data to less healthy samples. Fourth, although participant current CR scores at baseline THBP assessment likely reflect prior CR due to previous lifetime factors, it is expected that repeated assessment on this construct may demonstrate variance within scores attributable to the THBP intervention. With regard to the prior CR construct, no, or little, change is expected to occur due to exposure to the intervention.

The current CR model will be used to evaluate the efficacy of the THBP (Summers et al., 2013), with the equation applied annually to quantify the effects of the education intervention,

with increased scores thought to reflect increased CR. However, this observation cannot be confirmed until later in the project's life, when a subset of study participants begins to experience cognitive decline. Additionally, after the onset of cognitive decline, the predictive power of a multivariate approach to CR will be assessed and compared to the predictive power of a single indicator (e.g. education). Initially, the THBP aims to determine which factors (i.e., genetic makeup, prior CR) moderate the magnitude of gain in current CR following the education intervention, and ultimately whether the intervention delays onset of cognitive decline. Other future investigations should validate the present models of CR using confirmatory factor analysis and attempt to identify additional proxies of CR that can be used to increase the predictive power of reserve estimates.

In conclusion, the present study demonstrates that multiple CR proxy indicators can be successfully combined through factor analysis to yield two operational models of CR: prior and current. The prior CR model may more accurately reflect the CR hypothesis than a single indicator, while the current CR model provides a measure of dynamic change in CR over time. These methods of estimating CR will be used in the THBP to explore the modifiability of CR in later life due to participation in tertiary education, and will contribute data to elucidate why some older adults can temporarily suppress the detrimental effects of AD pathology, while others cannot.

Chapter 4

APOE and *BDNF* Val66Met polymorphisms combine to influence episodic memory function in older adults

Abstract

Genetic polymorphisms of *APOE* and *BDNF* have shown inconsistent associations with healthy adult cognitive functions. Recent investigations have suggested that *APOE* polymorphisms do not contribute to non-pathological cognitive function and that any effect is likely due to prodromal AD. Similarly, although *BDNF* Val66Met polymorphisms affect hippocampal morphology and function, associations with learning and/or memory have not always been found. This chapter sought to determine whether *APOE* and *BDNF* polymorphisms were associated, either independently or in combination, with adult cognition. Comprehensive neuropsychological assessments were conducted on 433 older adults, aged 50-79 years (M = 62.16, SD = 6.81), which yielded measures of episodic memory, working memory, executive function, and language processing. Participants underwent comprehensive neuropsychological assessment to ensure that only cognitively intact individuals comprised the sample. *APOE* and *BDNF* polymorphic data were used as predictors in general linear models that assessed composite cognitive domain variables, while covarying for education and age. Although no main effects for *APOE* or *BDNF* were found, the analysis identified a significant *APOE* x *BDNF* interaction that predicted episodic memory performance (p. = .02, η^2 = .02). Post-hoc analyses demonstrated that, in *BDNF* Val homozygotes, the cognitive consequences of *APOE* polymorphisms were minimal. However, in *BDNF* Met carriers, the hypothesised beneficial/detrimental effects of *APOE* polymorphisms were found. These data show that concurrent consideration of both *APOE* and *BDNF* polymorphisms is required in order to witness a cognitive effect in healthy older adults, with lowered episodic memory function present in *APOE* ε4/*BDNF* Met carriers.

Introduction

Genetic variance has been postulated to account for individual differences in adult cognitive capacity (Bouchard & McGue, 1981). Importantly, the heritability of cognitive function increases over the lifespan (Haworth et al., 2010). Although genome-wide investigations have supported the genetic basis for cognition (Plomin et al., 2013), replicable single gene effects are rare. However, two candidate genes for adult cognitive function are *APOE*, the largest known contributor to genetic risk for late onset AD (Corder et al., 1993), and *BDNF*, a gene implicated in synaptic plasticity and neurogenesis (Bath & Lee, 2006). While both genes have been associated with variance in healthy cognition (Harris & Deary, 2011), such associations have not been reliably replicated.

Inheritance of *APOE* ε4 is associated with an increased rate of accumulation of abnormal AD-like pathology and detrimental hippocampal morphology, among other negative effects (Espeseth et al., 2008; Kok et al., 2009; Ready et al., 2011). Conversely, *APOE* ε2 is associated with neurological benefits (Suri et al., 2013), such as protection from the accumulation of AD-like pathology (Castellano et al., 2011) and increased cortical thickness (Liu et al., 2010). Some studies suggest no effect of *APOE* genotype on healthy cognition (Jorm et al., 2007; Small, Basun, & Bäckman, 1998), while others report lowered cognitive functions associated with the *APOE* ε4 allele in aging populations (Deary et al., 2004; Wilson, Bienias, Berry-Kravis, Evans, & Bennett, 2002a; Wisdom et al., 2011). Recent data indicate that a specific *APOE* ε4-related cognitive phenotype does not exist independently from dementia or mild cognitive impairment (Foster et al., 2013; Quintas et al., 2013).

A second gene suggested to account for variance in healthy adult cognitive function is *BDNF*. The BDNF protein is a growth factor important to the maintenance, survival, and growth of

neurons (Lu et al., 2013), as well as synaptic plasticity, neurogenesis, and cell survival (Bath & Lee, 2006). The Met variant of the Val66Met human polymorphism has been linked to decreased activity-dependent secretion of BDNF at the synapse (Egan et al., 2003), and reduced hippocampal volume and function (Bath & Lee, 2006). Multiple investigations have reported *BDNF* polymorphism-related memory effects (Egan et al., 2003; Hariri et al., 2003; Raz et al., 2009). However, several other studies have reported negative results (Gong et al., 2012; Houlihan et al., 2009; Laing et al., 2011; Stuart et al., 2014).

This chapter investigated whether common variations in *APOE* and *BDNF* Val66Met are associated, either independently or through gene-gene interaction, with healthy cognitive function. Three hypotheses were examined using baseline data from the prospective longitudinal THBP (Summers et al., 2013): that *APOE* ε2 alleles are associated with better cognitive function and ε4 alleles are associated with worse cognitive function when compared to ε3 homozygotes; that *BDNF* Val homozygotes are associated with better cognitive function when compared to Met carriers; that a cumulative effect of the cognitive consequences associated with *APOE* and *BDNF* alleles results in the highest cognitive function in *APOE* ε2/*BDNF* Val carriers and the lowest cognitive function in *APOE* ε4/*BDNF* Met carriers.

Method

Study population

The sample consisted of community-residing adults, aged 50-79 years, who consented to comprehensive annual assessments of neuropsychological, health, and psychosocial function as part of the longitudinal THBP, as described in chapters 2 and 3. However, as participation

in the genetic component was not a requirement of the THBP, data from 433 participants were available for analysis in this chapter (response rate: 92%).

Materials

Participants were screened to ensure that they were cognitively intact and completed a comprehensive cognitive test battery as described in detail elsewhere (chapter 2).

Neuropsychological assessment battery

Tests of cognitive function were selected to assess learning and memory, working memory, executive function, and language processing. In order to simplify data analysis and interpretation, a composite summary measure was derived for each assessed cognitive domain. The episodic memory variable comprised Rey Auditory Verbal Learning Test (RAVLT; Strauss et al., 2006), Logical Memory test (LM; Wechsler, 1997b), and Paired Associates Learning test (PAL; Cambridge Cognition Ltd., 2004). The working memory variable comprised WAIS-III Digit Span (DSP; Wechsler, 1997a), WAIS-III Letter-Number Sequencing (LNS; Wechsler, 1997a), Spatial Span (SSP; Cambridge Cognition Ltd., 2004), and Spatial Working Memory (SWM; Cambridge Cognition Ltd., 2004). The executive function variable comprised Rapid Visual Processing (RVP; Cambridge Cognition Ltd., 2004), 24-item Victoria version Stroop test (Lezak et al., 2004), and Trail Making Test (TMT; Lezak et al., 2004). The language processing variable comprised WAIS-III Vocabulary (VOC; Wechsler, 1997a), WAIS-III Comprehension (COM; Wechsler, 1997a), and Boston Naming Test (BNT; Kaplan et al., 1983).

Genotyping

DNA samples were collected with Oragene DNA self-collection kits (DNA Genotek Inc., n.d.). *APOE* and *BDNF* genotypes were determined through one-step amplified refractory mutation system polymerase chain reaction (ARMS-PCR) and subsequent gel electrophoresis. For *APOE*, rs429358 and rs7412 were determined by following the method described by Donohoe and colleagues (Donohoe et al., 1999). For *BDNF*, Val66Met was determined by following the method described by Sheikha and colleagues (Sheikh et al., 2010). PCR amplifications were undertaken in a 12 μl reaction volume that contained approximately 50 ng of genomic DNA. PCR amplicons were resolved on 2% agarose gel. Genotyping was repeated on samples to ensure accuracy, with the proportion of concordance >99% for both polymorphisms. If a discrepancy in results was obtained, the samples were run a third time to establish genotype.

Procedure

Trained assessors performed the assessment of all participants at baseline THBP assessment (Summers et al., 2013). The assessment process took approximately four hours to complete and was undertaken in a quiet well-lit room. Subjects were encouraged to take 10-minute breaks when required to reduce fatigue.

Data analysis

In order to investigate whether *APOE* and *BDNF* genotypes were associated with healthy cognitive function, cognitive domain variables were first computed through factor (principal components extraction method). Factor coefficients for each of the test scores were combined

into a single factor score using a regression method for the episodic memory, working memory, executive function, and language processing domains. The extracted factor that explained the highest proportion of variance was retained to represent each domain (Table 4.1). General linear models were then fitted to each cognitive domain, using *APOE* and *BDNF* genotype data as predictors, while covarying for age and education. The independent *APOE* variable had three levels: ε2 carriers (genotypes: ε2/ε2 & ε2/ε3); ε3 homozygotes; and ε4 carriers (genotypes: ε3/ε4 & ε4/ε4). To assist in interpretation, participants with the ε2/ε4 genotype were excluded from the analysis. The independent *BDNF* variable had two levels: Val homozygotes and Met carriers. An alpha value of .05 was used for all statistical tests. All data were analysed in SPSS v21. As the four separate cognitive domain variables represent independent data, no multiple comparison corrections were applied to these analyses.

Table 4.1. Factor analysis results for composite cognitive domain variables

Cognitive domain	Initial eigenvalue	Variable	*N*	Mean	SD	Loading
Episodic memory	2.50 (62.39%)	RAVLT 1-5 total	418	52.95	8.86	.76
		LM I immediate recall total	418	48.45	8.02	.89
		LM II delayed recall total	418	30.30	6.24	.86
		PAL first trial memory score	418	18.33	3.48	.63
Working memory	2.06 (51.53%)	Digit span	418	18.66	3.93	.77
		Letter-number sequencing	418	11.61	2.40	.80
		SWM between errors	418	25.45	18.99	-.64
		SSP length	418	5.75	1.20	.65
Executive function	1.79 (59.50%)	Stroop trial C	414	26.30	7.65	.75
		RVP A’	414	0.91	0.05	-.81
		TMT trial B	414	59.16	19.09	.75
Language processing	1.89 (62.88%)	WAIS Vocabulary	432	56.59	6.28	.88
		WAIS Comprehension	432	26.20	3.33	.78
		Boston Naming Test	432	57.50	3.39	.71

Note: Data in parentheses represent the proportion of variance (%) explained by the resulting factor.

Results

Subjects

Eleven participants were excluded due to *APOE* ε2/ε4 genotype. The characteristics of the remaining 422 THBP participants are presented in Table 4.2. Participants in this chapter were 62.16 (*SD* = 6.81) years old and had an above average IQ (*M* = 112.47, *SD* = 5.52), overall.

The majority were female (66.7%) and had completed a mean of 13.97 (*SD* = 2.73) years of education. The *APOE* genotype distribution was 11.11% ε2 carrier, 56.4% ε3 homozygote, and 32.5% ε4 carrier, and did not differ significantly from Hardy-Weinberg equilibrium ($\chi^2_{(1, N = 433)} = 0.02$, $p. = 0.90$). The *BDNF* Val66Met genotype allele distribution was 67% Val homozygote and 33% Met carrier, and also did not differ significantly from Hardy-Weinberg equilibrium ($\chi^2_{(1, N = 433)} = 0.37$, $p. = 0.54$). Univariate ANOVAs were conducted on demographic and screening variables, with a single significant gene-related effect identified in education (years) measure ($F_{(1,431)} = 6.48$, $p. = .01$, $d = .26$, power = .726). Here, more years of education were reported in *BDNF* Met carriers ($M = 14.45$, $SD = 2.74$) than in *BDNF* Val homozygotes ($M = 13.74$, $SD = 2.70$), overall.

Table 4.2. Descriptive statistics for study population stratified by *APOE* and *BDNF*

		APOE ε2+		*APOE* ε3/ε3		*APOE* ε4+	
	N	Val/Val	Met+	Val/Val	Met+	Val/Val	Met+
Demographics							
N		34	13	158	80	90	47
Age (years)	422	61.09 (6.56)	64.85 (7.26)	62.49 (6.96)	62.05 (6.61)	61.98 (6.78)	61.09 (6.54)
Gender (male/female)	422	10/24	5/8	50/108	26/54	29/61	20/27
Education (years)	422	13.38 (2.82)	15.46 (2.57)	13.86 (2.79)	14.76 (2.75)	13.62 (2.56)	13.60 (2.65)
WTAR IQ	421	112.09 (4.80)	113.31 (3.73)	112.49 (5.87)	112.81 (4.91)	112.37 (5.08)	111.76 (7.39)
Screening							
DRS-2 AEMSS	422	11.94 (2.12)	12.31 (1.49)	11.97 (2.17)	11.76 (2.14)	12.19 (1.99)	12.11 (2.12)
HADS anxiety	421	5.03 (2.83)	5.15 (2.64)	5.28 (3.27)	5.16 (3.05)	5.59 (3.20)	4.89 (2.44)
HADS depression	421	2.79 (2.26)	2.46 (1.27)	2.30 (2.22)	2.30 (2.03)	2.51 (2.33)	2.70 (2.39)
Cognitive performance							
Episodic memory	407	-0.15 (0.92)	0.49 (0.67)	0.01 (1.04)	0.03 (0.92)	0.05 (1.01)	-0.22 (1.13)
Working memory	407	-0.07 (0.72)	0.18 (0.86)	-0.01 (1.06)	-0.09 (0.96)	0.09 (1.00)	-0.02 (1.01)
Executive function	403	-0.25 (1.06)	0.15 (0.83)	-0.03 (1.04)	-0.02 (0.96)	0.11 (0.90)	0.07 (1.10)
Language processing	421	-0.14 (1.08)	0.52 (0.57)	-0.02 (1.03)	-0.01 (1.00)	0.11 (0.85)	-0.14 (1.14)

Note: Data represent mean values (SD) for continuous variables and proportions for categorical variables.

Genotype and cognitive function

General linear models, using *APOE* and *BDNF* as predictors, were fitted to the composite cognitive variables while covarying for education and age. The education covariate showed a significant association with every cognitive domain ($p. < .05$), while the age covariate was significantly associated with each cognitive domain ($p. < .01$), excluding language processing ($p. = .06$). When each gene was considered independently, the univariate ANCOVA did not detect any simple main effects within cognitive domains, after controlling for age and education (Table 4.3).

Table 4.3. Summary statistics for the general linear models that assessed the cognitive implications of variation in *APOE* and *BDNF* genotypes, independently and in combination

Cognitive domain	Predictor	*N*	df	F	*P.*	η^2	Obtained power
Episodic memory	*APOE*	407	2, 399	1.28	.28	.01	.268
	BDNF	407	1, 399	0.76	.38	.00	.147
	APOE x *BDNF*	407	2, 399	3.87	**.02**	.02	.707
Working memory	*APOE*	407	2, 399	0.51	.60	.00	.152
	BDNF	407	1, 399	0.01	.93	.00	.064
	APOE x *BDNF*	407	2, 399	1.08	.34	.00	.228
Executive function	*APOE*	403	2, 395	0.71	.49	.00	.188
	BDNF	403	1, 395	0.46	.50	.00	.097
	APOE x *BDNF*	403	2, 395	1.39	.25	.01	.304
Language processing	*APOE*	421	2, 413	0.83	.44	.00	.195
	BDNF	421	1, 413	0.01	.92	.00	.065
	APOE x *BDNF*	421	2, 413	1.47	.23	.01	.316

Note: Results were derived from analysis of covariance (ANCOVA) models after adjusting for age and education.

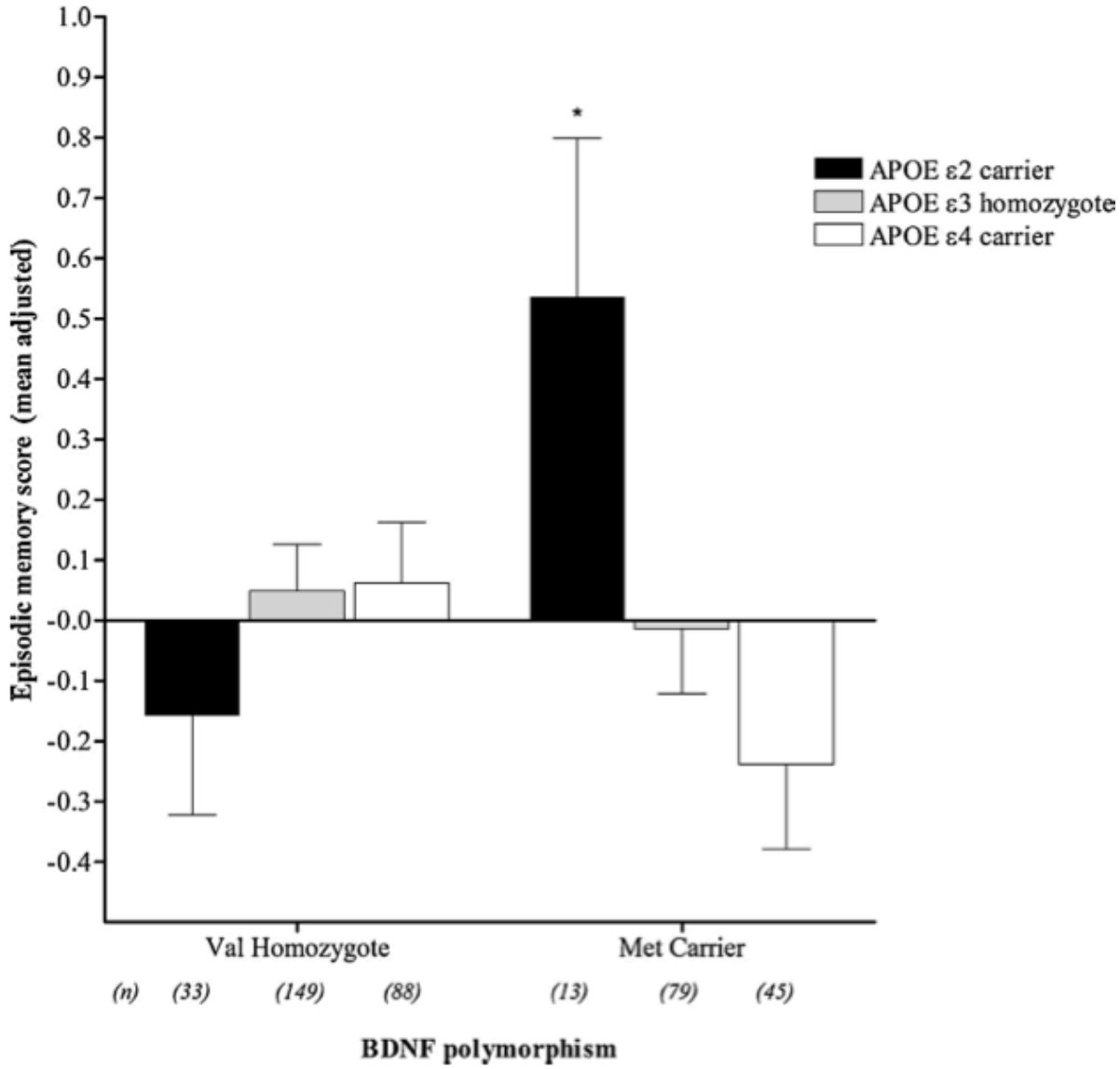

Figure 4.1. The significant *APOE* x *BDNF* Val66Met interaction in age- and education-adjusted episodic memory scores (N = 407). * = significant post-hoc group difference (p. < .05).

Gene-gene cognitive interactions

When gene-gene interactions were examined, a significant *APOE* x *BDNF* effect was detected by ANCOVA only for episodic memory, with age and education entered as covariates (Table 4.3). Subsequent follow-up univariate ANCOVAs were conducted to test for *APOE*-related differences for *BDNF* Val homozygotes and Met carriers separately, after controlling for age and education. The results showed that, for *BDNF* Val homozygotes, no differences in episodic memory were identified as a result of variation in *APOE* genotype ($F_{(2, 265)} = 0.63$, $p. = .54$, $\eta^2 < 0.01$, power = .228). However, for *BDNF* Met carriers, *APOE* genotype was associated with significant variation in age- and education-adjusted episodic memory scores ($F_{(2, 132)} = 5.12$, $p. < .01$, $\eta^2 = 0.06$, power = 1.00). Post-hoc analysis by least squares difference (LSD) revealed that for *BDNF* Met carriers, those who were also *APOE* ε2 carriers had significantly higher ($p. < .05$) episodic memory scores than those who were *APOE* ε3 homozygotes or *APOE* ε4 carriers. Although *APOE* ε3 carriers had higher episodic memory scores than *APOE* ε4 carriers, the difference did not reach significance (Figure 4.1).

Discussion

The present investigation sought to determine whether common genetic variations in the *APOE* and *BDNF* genes were associated with normal non-pathological cognitive function in older adults. Data from the THBP were used to generate composite variables of episodic memory, executive function, working memory, and language processing, with general linear models subsequently fitted. The first and second hypotheses, that variation in *APOE* or *BDNF* genotype is independently associated with cognitive function, were not supported. The third hypothesis, that a cumulative effect of the alleles' cognitive consequences resulted in the highest cognitive function in *APOE* ε2/*BDNF* Val carriers and the lowest cognitive function

in *APOE* ε4/*BDNF* Met carriers, was partially supported. Although the lowest mean episodic memory performance was found in *APOE* ε4/*BDNF* Met alleles carriers, unexpectedly high episodic memory performance was observed in *APOE* ε2/*BDNF* Met carriers. The main finding of this study is that the predicted effect of variation in *APOE* was identified in our dataset, but only in carriers of the *BDNF* Met allele.

The hypothesis that *APOE* is independently associated with a distinct cognitive phenotype in healthy adults has not received consistent support. Earlier studies described an *APOE* ε4-related impairment in episodic memory (Deary et al., 2004; Wilson et al., 2002c) that was not seen in the present investigation. Methodological differences may account for this. The sample in the present study is comprised of older adults with extensive cognitive assessment confirming that they were all cognitively intact and medical screening to ensure that they were physically healthy. The extensive screening of the present sample to ensure all were healthy may have reduced the likelihood that we would detect cognitive effects attributable to *APOE* ε4's effect on risk for dementia. Furthermore, if *APOE* ε4 is associated with accelerated cognitive ageing rather than simply conferring reduced cognitive processing (Harris & Deary, 2011; Wisdom et al., 2011), then it is possible that the present sample may be younger than the age at which ageing-related differences can be identified by cross-sectional analysis. The present sample was, on average, 10 years younger than those cohorts in studies reporting *APOE*-related differences (Deary et al., 2004; Wilson et al., 2002c). Despite this, even at this younger age, the accumulation of toxic Aβ would have been initiated, particularly in those who carry the *APOE* ε4 allele (Kok et al., 2009). The findings of this chapter add to an increasing body of evidence from recent investigations that report no cognitive differences in healthy individuals due to *APOE* genotype, even in subjects aged above 70 years (Foster et al., 2013; Quintas et al., 2013). The present data provide further

evidence that an association between *APOE* genotype and cognition in cognitively intact subjects may instead reflect emerging prodromal AD in these samples (Laukka et al., 2013).

Similarly, support for the hypothesised cognitive effect associated with variation in *BDNF* Val66Met was not found in the present dataset. Although the *BDNF* Met variant is associated with deficits to hippocampal morphology and function (Bath & Lee, 2006; Frodl et al., 2007), no significant differences were found between Val homozygotes and Met carriers on any cognitive domain. This supports previous investigations reporting no differences in cognitive processing and memory due to *BDNF* polymorphic variation (Gong et al., 2012; Houlihan et al., 2009; Stuart et al., 2014). However, the notion that *BDNF* is associated with memory function is not likely to be spurious, with a number of studies finding that a deficit in memory function is associated with the *BDNF* Met variant (Egan et al., 2003; Hariri et al., 2003; Raz et al., 2009). Disparities in results may be due to the profound differences in effects that variation in *BDNF* exerts across the lifespan (Casey et al., 2009). Such gene by environment interactions indicate that the neurological effects conferred by the *BDNF* polymorphism are complex, and ensure that differences in sample demographics, particularly age, diminish the idea of a simple risk allele for cognitive function.

The present investigation identified a significant *APOE* x *BDNF* gene-gene interaction that was associated with episodic memory function. The basis for this interaction was the *BDNF* genotype-dependent effects that variation in *APOE* exerted. Here, the hypothesised beneficial and detrimental effects on episodic memory function associated with the *APOE* ε2 and *APOE* ε4 polymorphisms, respectively, were found only in *BDNF* Met carriers, with no cognitive implication associated with variation in *APOE* in *BDNF* Val homozygotes. Two possible explanations for this effect are given. First, there is a biological interaction related to the systems or ageing-related roles of the encoded proteins. Second, the additive effects of the

polymorphisms caused the analyses to reach statistical significance. The 'additive' explanation does not, however, account for the present finding that *APOE* ε2/*BDNF* Met carriers had the highest episodic memory function, as *BDNF* Met polymorphisms were hypothesised to be associated with decreased cognitive function.

Interactions between *APOE* and *BDNF* have been previously investigated with mixed results. One study demonstrated a negative association between *BDNF* Met polymorphisms and working memory capacity, but found no influence of *APOE* on cognition, either independently or in combination with *BDNF* (Richter-Schmidinger et al., 2010). Another investigation showed an additive effect of *APOE* and *BDNF* on hippocampal activity during a memory encoding task: although reduced medial temporal lobe activation showed a dose-dependent relationship with *APOE* ε4 and *BDNF* Met polymorphisms, the combined effect of both genes explained more variance than when they were considered individually (Kauppi et al., 2014). The researchers concluded that both genes exert converging, but independent, effects on memory function. Finally, a recent study identified an interaction between *APOE* and *BDNF* in the putative preclinical phase of AD associated with Aβ deposition in the brain (Adamczuk et al., 2013). In cognitively normal older adults, *BDNF* Met carriers showed a significantly higher amyloid load than *BDNF* Met non-carriers, but only if they were *APOE* ε4 carriers. Notably, variation in *BDNF* polymorphisms did not have an effect on amyloid load in *APOE* ε4 non-carriers. These investigators also found an inverse relationship between episodic memory and amyloid burden that was only present in *APOE* ε4/*BDNF* Met carriers. Similarly, in cognitively normal or MCI individuals with high Aβ, relative to *BDNF* Val homozygotes, *BDNF* Met carriers had a larger rate of decline in episodic memory (Lim et al., 2013; 2014). Moreover, *APOE* and *BDNF* have been suggested to combine to affect the progression of pathological morphological AD-related changes in the preclinical phase (R. Hashimoto et al., 2009). Taken together, these findings suggest that the present data may

reflect a combination of an interaction of *APOE* and *BDNF* effects on medial temporal lobe activation, amyloid load, and atrophy, which culminate in cognitive outcomes.

With interactions between *APOE* and *BDNF* reliably identified in previous research, knowledge of accurate population-based genotype frequencies is important for predicting the efficacy of possible future interventions. In the present sample, the *BDNF* Val66Met polymorphism influenced whether variation in *APOE* exerted cognitive effects. Consequently, as *BDNF* Met carriers comprised 33% of the sample, a third of the participants were exposed to the cognitive implications of polymorphic variation in *APOE*. A meta-analysis of 643 subjects of varying ethnicities reported that 37% of their sample were *BDNF* Met carriers (Harrisberger et al., 2014). Similarly, a meta-analysis of 3322 subjects of majorly European descent reported that 36% of their sample were *BDNF* Met carriers (Brandys et al., 2013). The present data show that, in *BDNF* Met carriers, 6% of the variance in episodic memory performance was accounted for by variation in *APOE* genotype. Although initially a small effect, if an individual's genetic makeup accounts for 50% of overall adult cognitive function (Bouchard & McGue, 1981), 12% of the genetically-derived episodic memory function in *BDNF* Met carriers is accounted for by the interaction identified in the present study. Overall, the genotype frequencies are consistent with those previously identified in the literature and, as a result, the present findings indicate that a large proportion of individuals in the greater population may show affected episodic memory due to variation in *BDNF* Val66Met and *APOE*.

Increased understanding of complex gene-environment (Casey et al., 2009) and epigenetic (Egger, Liang, Aparicio, & Jones, 2004) interactions weakens the notion that simple risk alleles cause deterioration in cognitive function. In investigations that do find significant main effects for gene polymorphisms on cognitive function, the effect size is often very small. For

example, a meta-analysis of 77 studies comprising 40,942 cognitively normal adults identified an association of *APOE* ε4 with reduced episodic memory (d = -0.14), reduced global cognitive ability (d = -0.05), reduced executive function (d = -0.06), and slowed perceptual speed (d = -0.07; Wisdom et al., 2011). Similarly, Deary and colleagues found that the *APOE* ε4 polymorphism accounted for just 2% of the variance in verbal episodic memory performance (Deary et al., 2004). Investigations that examine single gene cognitive effects, and ignore environmental or gene product interactions, are unlikely to produce significant or translatable results. However, such studies can be useful in identifying potential targets for more complex investigations. In the present study, no cognitive effect was identified due to genetic variation unless genes were considered in combination. Large-scale investigations that utilize whole-genome sequencing may be best suited to answer questions relating to the genetics of human cognition (Davies et al., 2011), provided interpretations of any association identified take account of the magnitude of the effect size of the association rather than just statistical significance of an effect.

A limitation of the present research is that physical activity and diet were not measured or controlled for. Specifically, the general linear models did not covary for such variables while detecting the cognitive effects of polymorphic variation. This is particularly important when investigating *BDNF*, as evidence from animal studies reliably describes elevated BDNF secretion in hippocampal regions of mice after exercise (Farmer et al., 2004). BDNF signalling may also mediate the protective effects of diet and exercise on neurodegeneration (Mattson et al., 2004). Moreover, the inhibition of BDNF action has been demonstrated to negate the cognitive and synaptic benefits of exercise (Vaynman et al., 2004). A recent study demonstrated that exercise provided protection against diet induced cognitive decline, an effect likely due to the increased presence of BDNF in hippocampal CA3 region (Noble et al.,

2014). Future *BDNF* investigations should consider measures of physical activity in the design of their statistical analyses.

In conclusion, the present study did not support the hypotheses that variation in *APOE* or *BDNF* is associated with cognitive function in healthy older adults. However, a significant *APOE* x *BDNF* interaction that was associated with episodic memory capacity was identified. This effect demonstrated that, in *BDNF* Val homozygotes, the cognitive implications of *APOE* genotype were minimal, whereas, in *BDNF* Met carriers, the hypothesised beneficial/detrimental association with *APOE* polymorphisms was found. The data show the effects of single genes on cognition are neither simple nor reliable, and that consideration of multiple genes and interactions is necessary.

Chapter 5

The *BDNF* Val66Met polymorphism moderates the relationship between cognitive reserve and executive function

Abstract

Cognitive reserve (CR) describes the inherent cognitive resilience and flexibility that an individual has. The level of CR an individual attains has been proposed to be due to a product of intrinsic (biologic) and extrinsic (environmental) factors, and may mitigate against risk of developing dementing illnesses such as AD. In 433 healthy older adults, aged 50-79 years, it was investigated whether common polymorphic variation in *APOE* or *BDNF* Val66Met influenced the association between a multivariate estimate of prior CR and healthy cognitive function in older adults. Results indicated that prior CR was positively associated with performance in each assessed cognitive domain (episodic memory, working memory, executive function, language processing), while age showed a negative association with each domain, excluding language processing. It was also found that *BDNF* Val66Met moderated the association between prior CR and cognitive function. Prior CR accounted for 8.5% of the variance in executive function in *BDNF* Val homozygotes, but prior CR was a non-significant predictor in *BDNF* Met carriers. *APOE* polymorphisms were not linked to the influence of prior CR on cognitive function. This result implicates *BDNF* in playing an important role in capacity for building or accessing CR, and may indicate that *BDNF* Met carriers have a reduced capacity to increase cognitive resilience due to participation in cognitively stimulating activities over their lifetime.

Introduction

CR is a hypothetical construct of compensatory neurobiological processes that support cognitive function in the presence of cerebral lesions (Stern, 2002). Individuals with higher CR, generated by a lifetime of cognitive engagement, have lower risk for dementia despite having the same degree of AD pathology as individuals with lower CR (Valenzuela & Sachdev, 2006). Although CR is difficult to directly quantify, proxy variables used to estimate an individual's CR include those factors found to display an inverse relationship with dementia risk, such as education (Basu, 2012); intelligence (Rentz et al., 2010); occupational attainment (Karp et al., 2009); and cognitively stimulating leisure activities (Wilson et al., 2010). That individuals with higher CR display greater resistance to the effects of neuropathology is likely due to induced cortical plasticity caused by a prolonged mismatch between functional supply and task demands (Lövdén et al., 2010), resulting in more flexible and denser neural networks (Barulli & Stern, 2013). As adult cognitive function and cognitive ageing show significant heritability (Harris & Deary, 2011), research examining the interaction between genes and CR proxies are justified. The most likely candidate genes for CR are those that interact with environmental factors to induce effects on cognitive functions (Lee, 2003).

One gene that potentially interacts with CR is apolipoprotein E (*APOE*). While it has been established that specific allelic variants in the *APOE* gene are associated with risk for late-onset AD (Verghese et al., 2011), it is less clear whether allelic variants of the *APOE* gene impart healthy cognitive function (Foster et al., 2013). For instance, it was demonstrated in chapter 4 that variation in *APOE* was not independently associated with any of the assessed cognitive functions. However, *APOE* polymorphisms have been shown to exert divergent neuroprotective effects (Gokhale & Laskowitz, 2013; Liu et al., 2013a). For example, the

presence of *APOE* ε4 is associated with an increased rate of AD-related hippocampal atrophy (Manning et al., 2014), and impaired synaptic plasticity (Chen et al., 2010), when compared to *APOE* ε4 non-carriers. Lifestyle activities that require cognitive effort (e.g. completing puzzles, playing chess) predict verbal fluency and semantic memory recall in *APOE* ε4 non-carriers but not in carriers (Runge et al., 2014).

A second gene that may be associated with CR is brain-derived neurotrophic factor (*BDNF*). The encoded neurotrophin is crucial to neuronal survival, maintenance, neurogenesis, and synaptic plasticity (Bath & Lee, 2006; Mattson et al., 2004). The *BDNF* Val66Met polymorphism affects activity-dependent secretion of BDNF, with the Met allele associated with reduced depolarization-induced BDNF release into the synapse (Egan et al., 2003). *BDNF* Met alleles have been associated with reduced memory capacity (Egan et al., 2003; Hariri et al., 2003), but not consistently (Gong et al., 2012). In the present thesis, similar to *APOE*, it was demonstrated that variation in *BDNF* did not account for any significant variance in cognitive function (chapter 4). However, like *APOE*, impaired synaptic plasticity is a feature of *BDNF* Met alleles (Ninan et al., 2010; Pattwell et al., 2012).

Synaptic plasticity is a key mechanism that facilitates the development of CR (Esiri & Chance, 2012). Research to date has examined CR in the presence of pathology; however, as CR is developmentally acquired through both intrinsic (biologic) factors such as genetic heritability as well as extrinsic (environmental) factors such as education, it is imperative to examine the development of CR prior to the onset of clinical symptomatology. It was aimed to use baseline data from the THBP and a previously developed model of prior CR (chapter 3) to investigate the relationship between CR, cognitive function, and *APOE* and *BDNF* Val66Met polymorphisms in healthy older adults (50-79 years). Two hypotheses were tested. First, that higher prior CR is associated with better cognitive performance. Second, that

BDNF Val66Met and *APOE* polymorphisms independently moderate the association between prior CR and cognitive performance, with carriers of the putative detrimental alleles (Met and ε4, respectively) showing a reduced influence of CR when compared to non-carriers.

Method

Study population

The participants were the same 433 community-residing healthy older adults that were investigated in chapter 4.

Materials

A comprehensive test battery was completed by subjects as described in detail elsewhere (Summers et al., 2013). Multiple objective measures were used to screen participants for: symptoms of dementia, clinically significant symptoms of depression or anxiety, general health, medical conditions, prescription medication use, drug and alcohol use, handedness, height, weight, marital status, educational and occupational history.

Neuropsychological assessment battery

Cognitive functions were assessed using tests that measured learning and memory, working memory, executive function, and language processing, and are described in detail in chapter 2.

Assessment of cognitive reserve

To generate an estimate of CR, the prior CR equation that had been developed in chapter 3 was used.

Genotyping

DNA samples were collected with Oragene DNA self-collection kits (DNA Genotek Inc., n.d.). *APOE* and *BDNF* genotypes were determined through the methods outlined in chapter 2.

Procedure

Trained assessors carried out the neuropsychological testing as part of the baseline THBP assessments (Summers et al., 2013). The assessment process took approximately four hours to complete and was undertaken in a quiet room. Subjects were encouraged to take 10-minute breaks when required to reduce fatigue.

Data analysis

Prior to the main analyses, variables of prior CR and cognitive function were generated. To represent CR, a previously developed prior CR equation was used to create a comprehensive single-point measure of the construct (chapter 3). Standardised scores of prior CR variables (Table 5.1) were entered into the prior CR equation to yield total CR for each subject. Composite cognitive domain variables were also computed through the method of factor

analysis employed in chapter 4. The extracted factor that explained the highest proportion of variance was retained to represent each cognitive domain (Table 5.2). The *APOE* and *BDNF* predictor variables were coded as carriers or non-carriers of the detrimental allele, ε4 and Met, respectively. To assist in interpretation, subjects with the *APOE* ε2/ε4 polymorphism were excluded from the analysis.

The primary analyses used PROCESS v2.11 (Hayes, 2013) to test whether CR was associated with cognitive function, either independently or through CR x *APOE*/CR x *BDNF* interaction. PROCESS is a computational tool for path analysis-based moderation and mediation analysis that provides coefficient estimates for total, direct, and indirect effects of variables using OLS regression. Prior to analysis, the continuous independent predictor (CR) was mean-centred to reduce potential multicollinearity. Subsequent analyses involved testing for direct effects of predictors (CR, *APOE*, *BDNF*) and indirect effects of possible CR moderators (*APOE*, *BDNF*) on cognitive function domains while covarying for age. First, regression equations were fitted to cognitive domain data and the predictive capacity of age and CR were assessed. Second, *APOE*/*BDNF* data and corresponding CR-gene product variables were entered to test for CR moderation in separate models. An alpha value of .05 was used for all statistical tests and all data were analysed in SPSS v21.

Table 5.1. Descriptive statistics for study population stratified by *APOE* and *BDNF* genotype

Characteristic	*N*	*APOE* status			*BDNF* status		
		ε4-	ε4+	*p.*	Met-	Met+	*p.*
Demographics							
Age (years)	422	62.31 (6.83)	61.67 (6.69)	.53	62.16 (6.85)	61.99 (6.68)	.81
Gender (male/female %)	422	31.9/68.1	35.8/64.2		31.6/68.4	36.4/63.6	
Genotype (%)	422	67.5	32.5		66.8	33.2	
Screening							
DRS-2 AEMSS	422	11.93 (2.12)	12.16 (2.03)	.56	12.04 (2.10)	11.93 (2.08)	.61
HADS anxiety	421	5.21 (3.12)	5.35 (2.97)	.39	5.35 (3.19)	5.07 (2.80)	.38
HADS depression	421	2.36 (2.14)	2.58 (2.34)	.24	2.42 (2.26)	2.45 (2.10)	.91
Prior cognitive reserve							
WTAR Est. FSIQ	421	112.57 (5.39)	112.16 (5.94)	.86	112.40 (5.49)	112.51 (5.76)	.85
Prior education (years)	422	14.13 (2.81)	13.61 (2.59)	.19	13.73 (2.72)	14.44 (2.75)	.01
LEQ Young Adulthood Specific	421	16.19 (7.93)	15.51 (7.65)	.41	15.56 (7.61)	16.78 (8.25)	.13
LEQ Young Adulthood Nonspecific	421	24.71 (5.54)	24.93 (5.11)	.19	24.42 (5.45)	25.52 (5.23)	.05
LEQ Midlife Specific	420	19.07 (5.01)	19.06 (4.93)	.45	19.09 (4.82)	19.02 (5.28)	.90
LEQ Midlife Nonspecific	420	24.37 (5.55)	24.36 (5.60)	.46	24.08 (5.72)	24.93 (5.20)	.14
LEQ Continuing Education Bonus	418	10.22 (8.79)	9.38 (8.01)	.46	9.95 (8.45)	9.94 (8.75)	.99

Note: Data represented are mean values (*SD*) for continuous variables and proportions for categorical variable. DRS-2 = Dementia Rating Scale-2; AEMSS = Age- and education-corrected Mayo Older American normative Studies (MOANS) scaled score; HADS = Hospital Anxiety and Depression Scale; WTAR = Wechsler Test of Adult Reading; FSIQ = Full-scale IQ; LEQ = Lifetime of Experiences Questionnaire; ε4-/Met- = participants not carrying the *APOE* ε4/*BDNF* Met alleles; ε4+/Met+ = participants carrying at least one copy of the *APOE* ε4/*BDNF* Met alleles.

Results

Study population

The sample comprised 433 participants with a mean age of 62.16 years (*SD* = 6.81) and an above average estimated full-scale IQ (*M* = 112.47, *SD* = 5.52). Participants were mostly female (66.7%) and had completed an average of 13.97 (*SD* = 2.73) years of formal education. To assist in interpretation, 11 participants were excluded due to possessing the *APOE* ε2/ε4 genotype. The characteristics of the remaining participants are presented in Table 5.1. The *APOE* ($\chi^2_{(1, N = 422)} = 0.02$, *p.* = 0.90) and *BDNF* Val66Met ($\chi^2_{(1, N = 422)} = 0.31$, *p.* = 0.58) genotype distributions did not differ significantly from Hardy-Weinberg equilibrium.

Table 5.2. Factor analysis results for composite cognitive domain variables

Cognitive domain	Initial eigenvalue	Variable	*N*	Mean	*SD*	Loading
Episodic memory	2.50 (62.46%)	RAVLT 1-5 total	407	52.96	8.92	.76
		LM I immediate recall total	407	48.43	8.07	.89
		LM II delayed recall total	407	30.29	6.28	.86
		PAL first trial memory score	407	18.35	3.46	.63
Working memory	2.04 (50.93%)	Digit span	407	18.63	3.90	.76
		Letter-number sequencing	407	11.59	2.40	.79
		SWM between errors	407	25.36	18.64	-.63
		SSP length	407	5.75	1.20	.66
Executive function	1.80 (59.83%)	Stroop trial C	403	26.26	7.63	.76
		RVP A’	403	0.91	0.05	-.81
		TMT trial B	403	59.09	18.82	.75
Language processing	1.88 (62.81%)	WAIS Vocabulary	421	56.62	6.23	.88
		WAIS Comprehension	421	26.21	3.33	.77
		Boston Naming Test	421	57.49	3.42	.72

Note: Data in parentheses represent the proportion of variance (%) explained by the resulting factor. RAVLT = Rey Auditory Verbal Learning Test; LM = Logical Memory; SWM = Spatial Working Memory; SSP = Spatial Span; RVP = Rapid Visual Processing; TMT = Trail Making Test; WAIS = Wechsler Adult Intelligence Scale.

Cognitive reserve and age

PROCESS was used to fit linear regression models to the cognitive domain data. With the age and CR predictors entered, significant models were produced for all assessed cognitive functions: episodic memory ($F_{(2, 398)} = 22.15$, $p < .01$, $R^2 = .10$); working memory ($F_{(2, 398)} = 28.96$, $p < .01$, $R^2 = .13$); executive function ($F_{(2, 394)} = 52.88$, $p < .01$, $R^2 = .21$); and language processing ($F_{(2, 412)} = 34.15$, $p < .01$, $R^2 = .14$). The CR variable had a significant positive association with each cognitive domain ($p < .05$) while the age variable had a significant negative association with each cognitive domain ($p < .01$), excluding language processing ($p = .62$). The individual contribution that each predictor made to the model is detailed in Table 5.3.

Table 5.3. Results of the regression analyses for cognitive domain data

Cognitive domain	Predictor	*N*	B	SE	t	*p.*	R^2 change	F
Episodic memory	Age	401	-0.05	0.01	-6.51	**< .01**		
	CR	401	0.04	0.02	2.01	**.05**		
	APOE	401	-0.08	0.10	-0.84	.40		
	BDNF	401	-0.03	0.10	-0.25	.80		
	CR x *APOE*	401	-0.02	0.05	-0.35	.72	0.00	0.12
	CR x *BDNF*	401	-0.06	0.04	-1.41	.16	0.00	1.99
Working memory	Age	401	-0.05	0.01	-7.11	**< .01**		
	CR	401	0.07	0.02	3.37	**< .01**		
	APOE	401	0.07	0.10	0.70	.48		
	BDNF	401	-0.11	0.10	-1.12	.26		
	CR x *APOE*	401	0.04	0.04	0.99	.32	0.00	0.99
	CR x *BDNF*	401	0.04	0.04	0.99	.32	0.00	0.98
Executive function	Age	397	-0.06	0.01	-9.40	**< .01**		
	CR	397	0.10	0.02	5.23	**< .01**		
	APOE	397	0.12	0.10	1.25	.21		
	BDNF	397	-0.02	0.10	-0.17	.86		
	CR x *APOE*	397	0.02	0.04	0.40	.69	0.00	0.16
	CR x *BDNF*	397	-0.08	0.04	-1.98	**.05**	0.01	3.94
Language processing	Age	415	0.00	0.01	0.49	.62		
	CR	415	0.16	0.02	8.15	**< .01**		
	APOE	415	0.08	0.10	0.87	.38		
	BDNF	415	-0.07	0.10	-0.69	.49		
	CR x *APOE*	415	0.03	0.04	0.62	.54	0.00	0.38
	CR x *BDNF*	415	-0.05	0.04	-1.30	.19	0.00	1.70

Note: Apolipoprotein E and Brain-derived neurotrophic factor main and interaction effects were tested in separate models. CR = Prior cognitive reserve; *APOE* = Apolipoprotein E; *BDNF* = Brain-derived neurotrophic factor.

Gene-cognitive reserve interactions

It was then assessed whether the inclusion of *APOE*/*BDNF* Val66Met allelic carrier data significantly improved the fit of the models. Notably, no significant main effects of genetic predictors, *APOE* or *BDNF* Val66Met, were identified for any cognitive domain. A moderation analysis (PROCESS) was then conducted examining whether the inclusion of CR x *APOE* or CR x *BDNF* interaction terms significantly improved the fit of regression models. Results indicated that a single genetically based moderation effect on CR was present (Table 5.3). Specifically, inclusion of the CR x *BDNF* Val66Met interaction term led to a significant increase in the amount of variance in executive function explained by the model ($\Delta R^2 = .01$, $p = .05$). Simple slopes analysis was conducted in order to determine the basis of the moderation effect of BDNF polymorphism on the conditional effect between CR and executive function and tested whether the slopes statistically differed from zero for *BDNF* Met carriers and non-carriers, separately. These analyses indicate that a significant positive relationship between CR and executive function was identified in *BDNF* Val homozygotes ($B = 0.13$, $t = 5.44$, $SE = 0.02$, $p < .01$), but was not evident in *BDNF* Met carriers ($B = 0.05$, $t = 1.41$, $SE = 0.03$, $p = .16$). In *BDNF* Val homozygotes, CR accounted for a significant 8.5% of variance in executive function performance. In *BDNF* Met carriers, CR accounted for a non-significant 1.2% of variance in executive function performance. Simple slopes are presented in Figure 5.1.

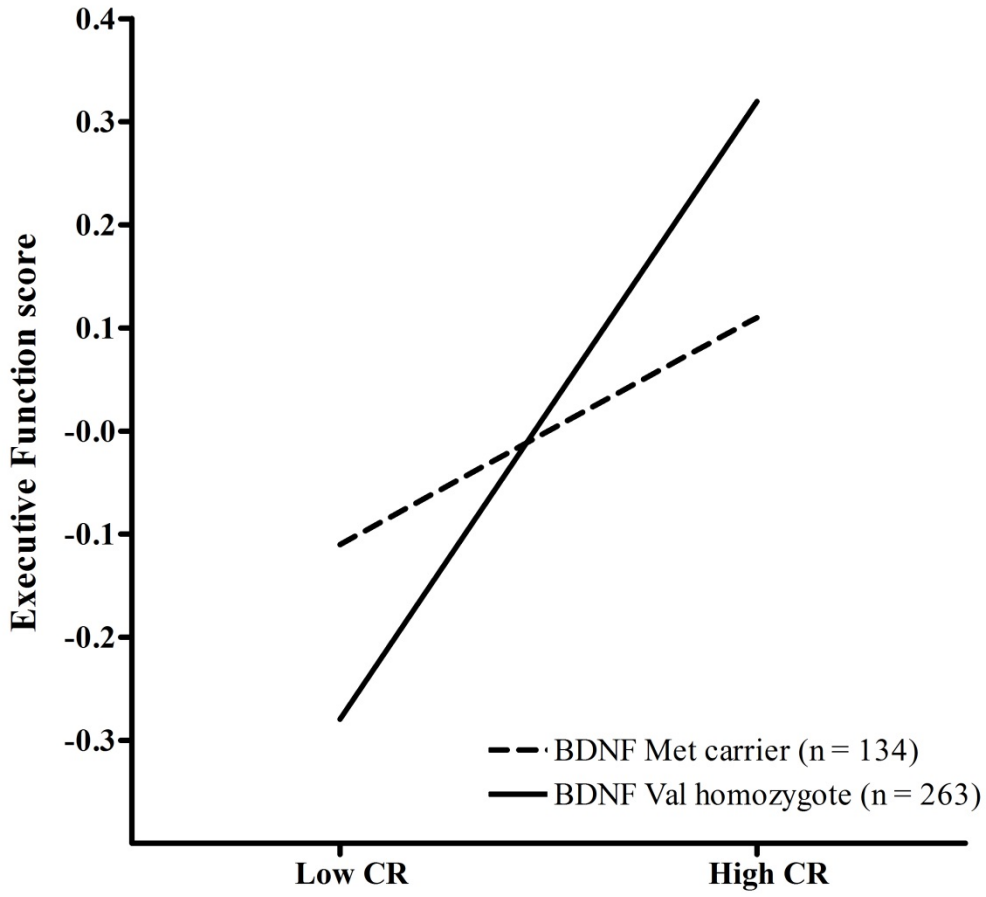

Figure 5.1. *BDNF* Val66Met moderates the relationship between CR and executive function scores. Plot represents age-adjusted executive function performance as predicted by the composite CR proxy variable for those with low CR (CR scores less than the mean – 1 standard deviation) and high CR (CR scores greater than the mean + 1 standard deviation) for *BDNF* Met carriers and Val homozygotes, separately.

Discussion

This chapter was designed to investigate whether a composite measure of CR was associated with healthy cognitive function, either independently or through an interaction with genetic *APOE*/*BDNF* Val66Met polymorphic data, in participants of the THBP. The first hypothesis, that higher CR is associated with better cognitive performance, was supported. The sample showed significant positive relationships between CR and cognitive function across all cognitive domains, after accounting for the effects of age. The second hypothesis, that *BDNF* Met carriers and *APOE* ε4 carriers would display a lower relationship between CR and cognitive function when compared to their respective non-carriers, was partially supported. The analyses revealed a single significant CR x *BDNF* interaction in predicting age-adjusted executive function performance. In this regard, the relationship between CR and executive function was moderated by *BDNF* genotype, with a positive association between the variables present in *BDNF* Val homozygotes that was absent in *BDNF* Met carriers.

CR is of interest due to its potential role in varying the age at which an individual develops dementia and the subsequent rate of dementia progression experienced (Barulli & Stern, 2013), as well as mitigating against the effect of other CNS insults. However, while previous research frequently highlights the relationship between increased CR and reduced dementia incidence (Brayne et al., 2010), other investigations have suggested that CR is also involved in non-pathological cognitive function. For instance, during a memory task, higher functional connectivity, indicative of heightened cognitive effort, is found in subjects with lower CR, despite achieving the same level of performance as those with higher CR (Lopez et al., 2014). Similarly, CR was positively associated with brain volume but negatively associated with cortical activity during a visual encoding task (Solé-Padullés et al., 2009). Such findings are consistent with the CR hypothesis, which posits that CR is implicated in non-pathological

cognitive function (Stern, 2002), particularly in relation to cerebral network efficiency (Stern, 2012). The finding from the present investigation, that CR was positively associated with all four assessed cognitive domains, adds further evidence that CR accounts for healthy cognitive function and that cognitive associations with CR are not limited to protection from the clinical expression of pathological processes. At the very least, higher scores on CR proxy variables would result in a delayed onset of dementia due to the pre-existing cognitive advantage afforded to high CR individuals through greater education, occupational attainment, intelligence, and participation in cognitively stimulating lifestyle activities. In high CR individuals a greater loss of cognitive function, resulting from greater levels of neuropathology, would be required to produce functional deficits of sufficient severity to meet diagnostic criteria for dementia.

A comprehensive review of the evidence for genetic contributions to CR noted that those genes that interact with environmental factors to produce a cognitive effect are the most likely candidates (Lee, 2003). In this study, a significant moderating influence of *BDNF* Val66Met polymorphism on the relationship between CR and executive function was identified. In those individuals who were *BDNF* Val homozygotes, a one point increase in CR was associated with a 0.13 point of increase in executive function. Comparatively, in those individuals who were *BDNF* Met carriers, a one point of increase in CR was associated with a non-significant 0.05 point increase in executive function. This finding is of particular importance due to the overlap between CR and executive function (Siedlecki et al., 2009). Both executive function and CR have an underlying reliance on cognitive flexibility (Miyake, 2000; Stern, 2002) and resources associated with frontal lobe activity (Alvarez & Emory, 2006), with a positive association of education and frontal engagement present in older adults that is not seen in younger adults (Springer et al., 2005). Clinical studies confirm that there is an age-related decline in executive function (Deary et al., 2009), and that the onset of dementia is often

characterised by early impairment of executive function (Silveri, Reali, Jenner, & Puopolo, 2007). Overall, the present findings indicate that while *BDNF* Val homozygotes show normal positive associations between CR and executive function, *BDNF* Met carriers have a reduced influence of CR on executive function.

No CR-*APOE* interaction was found in the current study. Previous longitudinal investigations have reported interactions between *APOE* ε4 alleles, education, and the rate of age-related cognitive decline (Seeman et al., 2005; Shadlen et al., 2005). However, a recent study found an interaction of CR and *APOE* polymorphism in cross-sectional but not longitudinal data (Runge et al., 2014). This investigation found that verbal fluency and semantic memory recall performance was predicted by participation in cognitively stimulating lifestyle activities in *APOE* ε4 non-carriers but not in carriers (Runge et al., 2014). The discrepancy between the results of the present study and those of Runge and colleagues may reflect differences in the method used to quantify lifetime cognitive stimulation. They assessed low (e.g. singing, traveling) and high (e.g. puzzles, viewing educational television) cognitive demand lifestyle activities, whereas the present study took into account a wider range of CR measures, including years of formal education and occupational attainment (chapter 3).

One explanation for the significant moderation of CR by *BDNF* Val66Met lies within differences in biochemical responses to cognitively stimulating environments. Namely, individuals with *BDNF* Met alleles may receive a lesser impact on neurological function of exposure to environmental activities that contribute to CR, whereas, for Met non-carriers there is a greater beneficial effect of exposure to environmental activities on neurological function. Previous research has demonstrated that long-term exposure to cognitively stimulating environments is associated with a widespread increase in BDNF expression in the brain (Ickes et al., 2000), but *BDNF* Met carriers have both reduced activity-dependent

secretion of BDNF (Egan et al., 2003) and impaired synaptic capacity for long term potentiation/depression (Cheeran et al., 2008). Consequently, for two individuals exposed to the same level of extrinsic environmental stimulation, the individual who carries the *BDNF* Met polymorphism exhibits reduced synaptic plasticity and neuronal restructuring subsequent to environmental stimulation and consequently displays lowered functional capacity due to the negative intrinsic effect of *BDNF* Met polymorphism on development of CR. Studies examining the influence of environmental enrichment in rodents report that EE only confers benefits in the presence of BDNF protein. For example, environment-induced hippocampal neurogenesis occurs only in the presence of BDNF (Rossi et al., 2006) and upregulation of BDNF by stimulating environments promotes neural plasticity (van Praag et al., 2000). Although environmental enrichment in experimental animals and measures of CR in humans are unlikely to be analogous, a comprehensive review concluded that, as with human educational and occupational attainment, environmental enrichment develops reserve that can be used to offset the impact of cortical lesions (Petrosini et al., 2009).

The results of the present study have several implications. First, as higher CR was associated with higher overall cognitive function, CR exerts an impact on healthy cognitive function, independent of dementia. While the cross-sectional analyses employed in the present study do not allow comment on the relationship between CR and rate of age-related cognitive decline, the results indicate that, at the very least, CR may benefit cognitive function in older age due to the persistent effects of improved cognitive function. Second, the results of the present study demonstrate that a genetic factor moderates the effect that CR has on cognitive function. Specifically, *BDNF* Met carriers showed a reduced relationship between CR and executive function when compared to non-carriers. This result provides evidence that *BDNF* Met carriers have a partial ‘disconnect’ between CR and cognitive function, and future

longitudinal analyses will examine whether *BDNF* Val66Met mediates environmentally generated protection from dementia.

Chapter 6

Discussion

CR describes the ability of an individual to compensate for disruptions to neural networks caused by pathological processes and, potentially, age-related neural changes (Stern, 2002). To achieve this, it is proposed that two complementary neural processes mediate the benefits of higher CR: neural reserve and neural compensation (Stern et al., 2005). Although further research into the construct has increased our understanding of what increases CR (Valenzuela & Sachdev, 2006), conceptual aspects of CR (Barulli & Stern, 2013), and how CR may be implemented into brain networks (Bozzali et al., 2014; Lopez et al., 2014), CR remains a nebulous concept. Similarly, inconsistent associations between variation in single genes and cognitive function are reported in the literature, despite strong evidence for the heritability of healthy cognitive function (Harris & Deary, 2011). The most ambiguity, however, is present in relation to genes that may be involved in CR (Lee, 2003), either through their effect on the development of CR or their effect on the ability to use CR.

Thesis aims

Overall, the present thesis had four overarching aims. First, to elucidate and provide specificity to the CR theory by operationally defining factor-supported prior and current CR constructs. Second, to determine whether common genetic variation in *APOE* or *BDNF* Val66Met is associated, either independently or through gene-gene interaction, with cognitive function. Third, to quantify the premorbid associations between CR and cognitive function. Fourth, to investigate *APOE* and *BDNF* Val66Met polymorphisms for potential genetic interactions with CR.

Modelling cognitive reserve in healthy older adults (chapter 3)

The aim of the first study was to explore the inter-relationships between CR proxy variables and to develop two operational factor-supported measures of CR: prior and current. This was an essential first step in the investigation of the CR theory, as future investigations (i.e. chapter 5) relied upon an estimate of CR to allow for tests of genetic associations. To achieve this aim, a multivariate latent approach was adopted for the development of both prior and current CR variables. Two hypotheses were proposed. First, traditional CR proxy variables (education, occupational attainment, intelligence, cognitively stimulating leisure activities) can be combined through factor analysis to provide a comprehensive operational factor-supported estimate of CR prior to entering the THBP. Second, flexible measures of cognitive ability that approximate traditional CR proxy variables (intelligence, academic ability) can be combined through factor analysis to provide an estimate of change in CR due to new life experiences.

A sample of 467 healthy older adults that had completed their baseline assessment for the THBP (Summers et al., 2013) participated in the present study. Participants were aged 50-79 years old and were mostly female (68.3%). They were cognitively normal and had undergone screening for symptoms of anxiety, depression and dementia. To develop a model of prior CR, data were used from seven items: WTAR Est. FSIQ; Prior education (years); LEQ young adulthood specific; LEQ young adulthood non-specific; LEQ midlife specific; LEQ midlife non-specific; LEQ midlife continuing education. To develop a model of current CR, data were used from five items: WAIS-III-SF1 FSIQ; WRAT-4-PMV spelling LES; WRAT-4-PMV math computation LES; WRAT-4-PMV word reading; WRAT-4-PMV sentence comprehension. Statistical analyses involved principal components analysis (PCA) with Varimax rotation to combine variables into separate models of prior and current CR. A further

PCA was used that included all CR variables in an attempt to differentiate contributors to prior from current CR models.

Key findings

These results suggest that multiple CR proxies can be combined to provide an estimate of reserve that likely better reflects the CR theory than the use of a single indicator (Stern, 2002). For the prior CR model, PCA was successful in combining measures of intelligence, education, occupational attainment, and participation in cognitively stimulating leisure activities. In this analysis, a four-factor prior CR model was extracted, which produced a factor structure independent of the effects of age and employment status. The clustering of the variables suggested that factor 1 represented education and intelligence, factor 2 represented cognitive activities, factor 3 represented midlife education, and factor 4 represented occupational attainment. In combination, these factors explained 77.10% of the variance in the dataset. The final equation for prior CR = 0.370 (WTAR FSIQ) + 0.408 (Prior education in years) + 0.567 (LEQ Young Adulthood Specific) + 0.565 (LEQ Young Adulthood Non-specific) + 0.630 (LEQ Midlife Non-specific) + 0.875 (LEQ Midlife Continuing Education Bonus) + 1.004 (LEQ Midlife Specific).

For the current CR model, PCA was successful in combining measures of intelligence and academic ability. However, in this analysis, two of the proposed contributors to the model were removed prior to finalising the current CR equation. WRAT-4-PMV reading was removed due to its potential for confounding with the WTAR, which contributes to prior CR; a previous version of the WRAT (WRAT-R) has been shown to correlate highly (r = 0.73) with the WTAR (Strauss et al., 2006). WRAT-4-PMV sentence comprehension was also removed from the model due to a very low extracted communality value in a preliminary PCA (0.31). The final PCA extracted a one-factor current CR model, with the factor explaining 59.87% of the variance in the dataset. The final equation for current CR = 0.454

(WAIS-III-SF1 FSIQ) + 0.369 (WRAT-4-PMV spelling LES) + 0.463 (WRAT-4-PMV math computation LES).

To validate the prior and current models of CR, a third PCA was conducted that attempted to statistically differentiate the two models. This analysis included the ten variables that comprised both prior and current CR models. Results indicated that the PCA was partially successful in differentiating the models; three underlying factors were extracted: current CR, education and occupation, and cognitive activities. Of importance, the variables in both hypothesised models were successfully separated, with the exception of WTAR FSIQ, which loaded on the current CR factor that contained other intelligence- and academic-related variables. Participant scores for prior and current CR were significantly correlated ($r = 0.40$, $p < 0.01$).

Genetic associations with cognitive function in healthy older adults (chapter 4)

The aim of the second study was to determine whether common genetic variation in *APOE* or *BDNF* Val66Met is associated, either independently or through gene-gene interaction, with cognitive function. In addition to CR, genetic variation has been proposed to account for differences in age-related cognitive decline (Harris & Deary, 2011) and risk for dementia (Verghese et al., 2011). However, earlier studies that reported differences in cognitive performance due to variation in *APOE* (Small et al., 2004) and *BDNF* Val66Met (Egan et al., 2003) were not supported by more recent work (Foster et al., 2013; Mandelman & Grigorenko, 2011). In order to provide clarity to these discrepancies, three hypotheses were proposed. First, *APOE* ε2 alleles are associated with better cognitive function and ε4 alleles are associated with worse cognitive function when compared to ε3 homozygotes. Second, *BDNF* Val homozygotes are associated with better cognitive function when compared to Met carriers. Third, a cumulative effect of the cognitive consequences associated with *APOE* and

BDNF alleles results in the highest cognitive function in *APOE* ε2/*BDNF* Val homozygotes and the lowest cognitive function in *APOE* ε4/*BDNF* Met carriers.

The participants who were included in chapter 4 were a subgroup of 433 members of the larger THBP cohort who had consented to genetic analysis. The health of the participants was particularly important, as this study aimed to investigate genetic associations with healthy, non-pathological, cognitive function. In this regard, it has been suggested that previously identified associations between *APOE* ε4 and reduced cognitive function were due to prodromal dementia onset (Foster et al., 2013). The sample's *APOE* allele frequencies were 11.11% ε2 carrier, 56.4% ε3 homozygote, and 32.5% ε4 carrier; *BDNF* Val66Met allele frequencies were 67% Val homozygote and 33% Met carrier. Allele frequencies for both genes did not differ significantly from Hardy-Weinberg equilibrium and were consistent with those previously reported (Egan et al., 2003; Small et al., 2004; Vickers et al., 2002). Prior to investigating associations between polymorphisms and cognitive function, PCA was used to compute cognitive domain variables from multiple tests of separate cognitive processes that assessed a similar domain. General linear models were fitted to each composite cognitive domain variable, using *APOE* and *BDNF* polymorphic data as predictors, while covarying for age and education.

Key findings

The results of this study, in combination with previous investigations into the genetic associations of healthy cognitive function (Foster et al., 2013; Gong et al., 2012; Houlihan et al., 2009; Quintas et al., 2013; Stuart et al., 2014), indicate that specific genes that account for the heterogeneity of cognitive function are elusive. This is despite evidence that shows the substantial heritability of cognitive function (Haworth et al., 2010; Plomin et al., 2013). The analysis first examined whether variation in *APOE* or *BDNF* Val66Met was independently

associated with variation in episodic memory, working memory, executive function, or language processing. The results indicate that variation in neither *APOE* nor *BDNF* polymorphisms influenced cognitive performance in any domain. This data adds to the growing body of evidence suggesting that variation in *APOE* or *BDNF* Val66Met does not independently cause differences in healthy adult cognitive function.

Although independent effects of the genes were not found in the present study, a significant *APOE* x *BDNF* effect was detected in the episodic memory domain. Follow-up analysis of age- and education-adjusted episodic memory scores revealed that, for *BDNF* Val homozygotes, no differences in function were identified as a result of variation in *APOE*. However, for *BDNF* Met carriers, *APOE* genotype was associated with significant variation in function. For *BDNF* Met carriers, *APOE* ε2 carriers had significantly higher episodic memory performance than *APOE* ε3 homozygotes and *APOE* ε4 carriers. Despite *APOE* ε3 carriers displaying better episodic memory performance than *APOE* ε4 carriers, the difference did not reach significance. This finding is consistent with previous work identifying significant cumulative effects of these genes on hippocampal function (Kauppi et al., 2014), amyloid deposition (Adamczuk et al., 2013), and the progression of AD-related morphological changes (Hashimoto et al., 2009). In all cases, including the present study, it was found the largest detrimental effects were present in carriers of both the *APOE* ε4 and *BDNF* Met alleles.

Genetic interactions with cognitive reserve in healthy older adults (chapter 5)

The third study was undertaken with two aims in mind, both of which involved the use of the prior CR equation that was developed in chapter 3. The first aim was to quantify the premorbid associations between CR and cognitive function. The CR theory posits that differences in neural efficiency exist in non-pathological functioning (Stern, 2002), with

recent data supporting this notion (Lopez et al., 2014; Solé-Padullés et al., 2009). It was hypothesised that higher prior CR is associated with better cognitive performance in healthy individuals. The second aim was to investigate *APOE* and *BDNF* Val66Met polymorphisms for potential genetic interactions with CR. Both of these genes have shown previous associations with cognitive function and play important roles in the process of synaptic reorganisation (Cheeran et al., 2008; Chen et al., 2010). Here, it was hypothesised that *BDNF* Val66Met and *APOE* polymorphisms independently moderate the association between CR and cognitive performance, with carriers of the putative detrimental alleles (Met and ε4, respectively) showing a reduced influence of CR when compared to non-carriers.

The participants who provided data for this chapter were the same healthy 433 THBP participants who had consented to genetic analysis in chapter 4. In contrast to the analysis in chapter 4, however, the potential benefit of the *APOE* ε2 allele was not examined in this investigation; subjects were coded as carriers or non-carriers of the detrimental alleles. To test the hypotheses, an estimate of CR was calculated by entering standardised scores of CR variables into the prior CR equation (chapter 3). Composite cognitive domain variables of episodic memory, working memory, executive function, and language processing (developed in chapter 4) were used as the dependent variables. The primary analyses used PROCESS v2.11 (Hayes, 2013) to test whether CR was associated with cognitive function, either independently or through CR x *APOE*/CR x *BDNF* interaction.

Key findings

The first hypothesis, that higher prior CR is associated with better cognitive performance in healthy individuals, was supported. When the predictors of age and CR were entered, significant models were produced for all assessed cognitive functions. Age was negatively correlated with each ageing-susceptible cognitive domain, consistent with previous literature

demonstrating age-related cognitive declines in episodic memory, working memory, and executive function/processing speed (Craik, 1994; Park et al., 2002; Schaie, 1996). However, a noteworthy finding was that each point of prior CR was associated with increases in performance in all assessed cognitive domains, after accounting for the effects of age. Given the clear cognitive benefits of participation in activities said to increase CR, in particular education (Brinch & Galloway, 2012; Ceci, 1991), this effect was not surprising. Other lines of evidence have also implicated CR in healthy neurological functioning, such as those that have demonstrated higher neural efficacy due to CR proxy variables (Lopez et al., 2014; Solé-Padullés et al., 2009). However, the present investigation was one of the first to quantify the premorbid cognitive effects of an operationally defined factor-supported variable of reserve that is consistent with the CR theory.

The second hypothesis, that *BDNF* Val66Met and *APOE* polymorphisms independently moderate the association between CR and cognitive performance, was then tested. Here, partial support was found, as a significant interaction between CR and *BDNF* Val66Met was present only in the executive function domain. Specifically, the expected significant positive relationship between CR and executive function that was present in *BDNF* Val homozygotes was not evident in *BDNF* Met carriers. In *BDNF* Val homozygotes, CR accounted for a significant 8.5% of variance in executive function performance; however, in *BDNF* Met carriers, CR accounted for a non-significant 1.2% of the variance in executive function performance. This result indicates that individuals with the most common *BDNF* polymorphism (Val/Val) demonstrate the normal benefits of CR on cognitive function, whereas individuals who carry a *BDNF* Met allele have a weakened association between CR and cognitive function.

General discussion

Modelling prior cognitive reserve

CR provides one explanation as to how two individuals with a similar level of neuropathology can express very different levels of clinical impairment (Stern, 2002; 2006). The conceptualisation of the CR theory, as well as the brain reserve capacity theory (Satz, 1993), was prompted by an early autopsy investigation that identified a subset of subjects who, prior to death, had significant AD pathology despite no clinical impairments to cognitive function (Katzman et al., 1988). Since CR was proposed, many investigations have reported that participation in activities that involve complex cognitive stimulation protects and preserves cognitive function after stroke (Glymour, Weuve, Fay, Glass, & Berkman, 2008; Ojala-Oksala et al., 2012; Sachdev, Brodaty, Valenzuela, Lorentz, & Koschera, 2004) and traumatic brain injury (Fay et al., 2010; Kesler, Adams, Blasey, & Bigler, 2003). The major disability that CR has been implicated in is dementia stemming from neurodegenerative diseases (e.g. Andel et al., 2005; Bickel & Kurz, 2009; Brayne et al., 2010; Fritsch et al., 2002; Ngandu et al., 2007; Ravaglia et al., 2002; Valenzuela & Sachdev, 2006). However, most of these investigations have large variations in their methods of measuring CR and, as such, it is difficult to equate the conclusions of one investigation with another.

Numerous methods of assessing CR have been suggested. One is to estimate neurocomputational factors, such as network efficiency, redundancy, and dynamic range (Valenzuela, 2008). Other methods involve the measurement of the discrepancy between expected cognitive outcome and actual cognitive outcome due to the presence of pathology (Reed et al., 2011). However, for practicality reasons, an estimate of CR can be established by determining how cognitively engaged an individual has been over their lifespan. It follows then, that a comprehensive estimate of CR should examine multiple lifetime factors that

involve complex cognitive stimulation (Sánchez et al., 2011), particularly since these different lifetime factors contribute independently to reserve capacity (Foubert-Samier et al., 2012; Reed et al., 2011). However, this is, more often than not, not the case, and many investigations have used just education (Batterham, Mackinnon, & Christensen, 2011; Bozzali et al., 2014; Brayne et al., 2010; Duda, Puente, & Miller, 2014; Guzzetti & Daini, 2014; Kesler, Tanaka, & Koovakkattu, 2010; Liu, Cai, Xue, Zhou, & Wu, 2013b), occupation (Ghaffar, Fiati, & Feinstein, 2012; Karp et al., 2009; Kröger et al., 2008; Potter, Helms, Burke, Steffens, & Plassman, 2007; Suo et al., 2012), intelligence (Albanese et al., 2012; Galioto et al., 2013; Koerts, Tucha, Lange, & Tucha, 2012; Vemuri et al., 2011), or cognitive activities (Booth et al., 2013; Wilson et al., 2007; 2010; Wilson, Barnes, & Bennett, 2003; Wirth, Haase, Villeneuve, Vogel, & Jagust, 2014) as an estimate of CR.

Although the use of single proxy variables to represent CR is common (e.g. education), this may not be the best method. In this regard, multiple indicators should be taken into account when calculating an estimate of an individual's CR (Sánchez et al., 2011). In support, one meta-analysis found that the combined protective effect of multiple CR indicators reduced risk for dementia by approximately 50% (Valenzuela & Sachdev, 2006). Reserve is a hypothetical construct that, at this point, cannot be directly measured (Stern, 2002). This is due to the fact that CR is not tied to any one of these CR proxy variables – it is a latent variable (Stern, 2006). Until a direct measure of CR is identified, it has been suggested that latent variable approaches be implemented to test CR hypotheses (Jones et al., 2011). Here, some important theoretical considerations have been proposed. Mainly, the *a priori* conceptualisation of CR factor models should be avoided (Jones et al., 2011; Satz et al., 2011). Instead, data-driven approaches should be implemented to decide how CR indicators should be grouped (Satz et al., 2011). Statistically, principal components analysis (PCA) is preferable for grouping CR indicators than a true factor analysis (e.g. maximum likelihood),

as PCA may be better suited to handle issues of shared covariance among predictors (Jones et al., 2011).

In line with such recommendations (Jones et al., 2011; Satz et al., 2011), the present investigation took a multivariate approach to constructing latent models of prior CR and current CR. For prior CR, quantifying previous educational, occupational, and cognitive activity, as well as including an estimate of premorbid intelligence, gave an estimate of an individual's existing CR. For current CR, a model that could reflect change in CR was constructed through the quantification of current intellectual and academic ability. For both models, the variables were combined using exploratory factor analysis (EFA) with the PCA method, which allowed us to use both shared and unique variance to construct new latent variables of reserve (Jones et al., 2011).

Other investigations have implemented similar approaches when investigating the predictive capacity of a CR variable that reflects data from multiple proxy variables. Most similar to the method used in the present investigation, one study used EFA using PCA to combine data of premorbid IQ, education-occupation history, and a measure of lifetime participation in leisure and cognitive activities (Arenaza-Urquijo et al., 2011). The total variance explained by the extracted latent variables in combination was also similar: 62.3% in their investigation compared to 77.10% in the present study's. In the study by Arenaza-Urquijo and colleagues, their multivariate estimation of CR correlated significantly with a number of different anatomic white matter areas in healthy older adults and patients with amnestic MCI, indicating an increased resilience of white matter in individuals with more CR.

In contrast to the data-driven, *post hoc*, approach recommended by Jones et al. (2011) and Satz et al. (2011), which was implemented in the present study and in the Arenaza-Urquijo et

al. (2011) study, some researchers have used an *a priori* approach to developing multivariate models of CR. For example, one study, which investigated whether CR predicted longitudinal cognitive performance in children and adolescents with schizophrenia, used confirmatory factor analysis (CFA) to test a three factor model of CR (la Serna et al., 2013). Here, the CR model comprised estimates of premorbid IQ, education-occupation history, and lifetime leisure activities; the results of the CFA indicating a good fit between the model and the observed data. Similarly, CFA has been used to test a four factor model of CR, comprising factors of memory-language, processing speed/executive function, attention, and CR factors (Mitchell, Shaughnessy, Shirk, Yang, & Atri, 2012). Again, support was found for the hypothesised model in both cognitively normal and cognitively impaired older adults. In contrast to Mitchell et al. (2012), others have tested and found support for two factor models (Lojo-Seoane, Facal, Guardia-Olmos, & Juncos-Rabadan, 2014) and three factor models (Giogkaraki, Michaelides, & Constantinidou, 2013).

Large variability is evident in the CR factor models that have resulted from multivariate estimations, regardless of the method of factor analysis used (i.e. EFA or CFA). Given the heterogeneity of datasets that were used to develop these models, such discrepancies are not surprising. In order to resolve potential inconsistencies in CR models, it is recommended that a data-driven exploratory approach should be applied. This method would avoid erroneous *a priori* groupings of CR indicators and allows the dataset to determine how CR indicators should be grouped (Jones et al., 2011; Satz et al., 2011).

Interventions and current cognitive reserve

Recent investigations have examined the consequences of CR in dementia (Andel et al., 2005; Bickel & Kurz, 2009; Brayne et al., 2010; Fritsch et al., 2002; Ngandu et al., 2007; Ravaglia et al., 2002; Valenzuela & Sachdev, 2006) or brain trauma (Fay et al., 2010; Glymour et al.,

2008; Kesler et al., 2003; Ojala-Oksala et al., 2012; Sachdev et al., 2004), but it is important to model CR and investigate the associations of CR and cognitive function in healthy midlife. This is the phase of life where prolonged participation in cognitively demanding activities can occur, the like of which is theorised to be responsible for the development of CR's beneficial compensatory neurological effects (Lövdén et al., 2010; Stern, 2002). In addition, healthy midlife is also the life phase where cognition-preserving interventions based on cognitive stimulation can readily occur.

Interventions aimed at providing a cognitive advantage, or buffer, to negate or protect from age-related cognitive decline or dementia usually take the form of randomised controlled trials (RCT). These trials typically involve exposing an experimental group of participants to an intervention of on-going computer-based cognitive training for the duration of the trial. The benefit of such a design is the ability to reduce allocation bias due to the randomisation of participants to treatment groups, allowing strong inferences about cause and effect to be made (Moher et al., 2010). However, one of the major criticisms directed at cognitive training interventions relates to the limited generalisability and longevity of the cognitive gains (La Rue, 2010), and evidence indicating that this form of mental exercise results in increased CR is weak (Gatz, 2005). This may relate to the distinction between specific training aimed at individual cognitive processes and the complex, multifaceted, stimulation obtained from education and demanding occupations.

Another trial that is currently on-going that aims to increase CR due to participation in an intervention is the THBP (Summers et al., 2013). The THBP has the advantage of using an intervention that involves complex cognitive stimulation; university study involves social, educational, and intellectual components. This more complex brand of cognitive stimulation, when compared to that used in RCTs, fits within the framework proposed by Loveden et al.

(2010), in which a prolonged mismatch between supply and demand must occur before plastic changes take place within the brain. However, a related disadvantage is the inability to use randomisation of participants, introducing a possible confounder to the intervention outcome (Moher et al., 2010).

One of the major problems with prospective investigations is the lack of an estimate of intervention efficacy prior to the end point – particularly with interventions that aim to reduce dementia risk. In this regard, it is in the interest of the CR hypothesis that an operationalised estimate of current/change in CR be developed (Tucker & Stern, 2011). The present study aimed to fill this gap by developing a model of CR comprised of variables that approximate traditional CR indicators, but can change dynamically to reflect change in cognitive function due to exposure to new CR-building activities. To achieve this, estimates of current intellectual and academic ability were combined using PCA, producing a latent current CR variable that accounted for 59.87% of the variance in the dataset. Notably, this current CR model demonstrated a moderate-strong correlation with the model of prior CR, indicating that both models assessed a similar underlying construct.

Although the model of current CR has the potential to reflect change in function due to participation in CR-related activities, it also reflects long-acquired cognitive capacity. In this regard, it is unlikely that any cognitive measure of current/change in CR will be unaffected by previous function, generated by prior lifetime exposures. As is the nature of CR, even estimates of change in network efficiency or redundancy (Valenzuela, 2008) would be tempered by the pre-existing individual differences in these factors. One of two approaches may allow for the capture of a 'pure' measure of change in CR. First, if a cognitive approach is adopted, researchers may consider entering prior CR as a covariate to control for the influence of pre-existing activity/function. Second,

researchers may consider prospectively recording the frequency with which an individual engages in activities that involve mental engagement. Ultimately, it may be that attempting to estimate change in a hypothetical construct, such as cognitive reserve, is associated with more drawbacks than benefits; an altered risk for dementia may be the only observation that can support the occurrence of a change in CR due to an intervention.

Genetic factors in healthy adult cognitive function

The notion that an individual's genetic makeup contributes to the capacity of cognitive function as an adult is recognised, but the exact genes that account for the heritability are elusive. One review of 111 studies that examined the familial basis of cognitive function concluded that approximately 50% of the variability in adult cognitive function is inherited (Bouchard & McGue, 1981). In addition, the heritability of cognitive function seems to increase with age, rather than decline (Davis et al., 2009; Haworth et al., 2010). For example, one study demonstrated linear increases in the heritability of general cognitive ability from 41% in childhood, to 55% in adolescence, to 66% in young adulthood (Haworth et al., 2010). Recent genome wide association studies have provided further evidence that genetic variation affects cognitive processing, with an estimated 40% of crystallised intelligence and 51% of fluid intelligence accounted for by both common SNP markers and unknown causal variants (Davies et al., 2011). Despite this, the replication of single gene effects on cognitive function is rare (Houlihan et al., 2009).

The first gene that was examined in the present study for effects on cognitive function was *APOE*. *APOE* ε4 has been associated with a faster age-related cognitive decline (Wilson et al., 2002c), and ε2 a slower decline (Wilson et al., 2002a), when compared to ε3 homozygotes. However, cross-sectional differences are also found. After adjusting for sex and childhood IQ,

ε4 was associated with reduced, and ε2 increased, verbal episodic memory, when compared to ε3 homozygotes at age 79 years (Deary et al., 2004). At age 56 years, ε4 has also been related to slower performance on an executive function and processing speed measure (Ready et al., 2011). Two meta analyses have identified converging results of impaired episodic memory, executive functioning, and overall global cognitive ability due to ε4 (Small et al., 2004; Wisdom et al., 2011). In line with such findings, it was hypothesised that *APOE* ε2 alleles are associated with better cognitive function and ε4 alleles are associated with worse cognitive function when compared to ε3 homozygotes.

In the present investigation, no evidence was found to support premorbid effects of *APOE* on cognitive function. Here, variation in *APOE* genotype was not associated with performance in any of the assessed cognitive domains (episodic memory, working memory, executive function, language processing). However, this finding is not novel, as more recent data have refuted the notion that *APOE* exerts a specific non-pathological cognitive phenotype (Quintas et al., 2013). For instance, no baseline differences and only greater negative 3-year change in recognition memory for faces and words were found in an earlier investigation of 74 very old adults without dementia (Small et al., 1998). Another investigation found no *APOE*-related differences in cognitive function in multiple age groups: 20-24, 40-44, and 60-64 years (Jorm et al., 2007). One recent study found *APOE* ε4-related cognitive deficits when MCI and healthy controls were examined concurrently; however, when the 764 healthy controls were tested separately, no significant *APOE* effects were found (Foster et al., 2013).

Overall, if variation in *APOE* is found to be associated with cognitive functions, it is likely that this is due to prodromal dementia. Support for this opinion is provided by the lack of significant *APOE*-related cognitive effects in investigations that implemented strict cognitive screening procedures (Foster et al., 2013). In the present study, all participants underwent

comprehensive screening before being invited to participate in the THBP and were excluded if they had a history of any condition independently associated with impairments to cognitive function. In addition, an experienced neuropsychologist reviewed performance on the Mattis Dementia Rating Scale-2 (DRS-2; Jurica et al., 2001), the Hospital Anxiety and Depression Scale (HADS; Zigmond & Snaith, 1983) and the Medical Health Status questionnaire (Summers et al., 2013) to determine participant inclusion. In this regard, the accidental inclusion of individuals with MCI into samples of otherwise healthy participants could lead to the detection of emerging prodromal AD attributable to the increased risk for AD associated with *APOE* ε4 (Verghese et al., 2011).

The second gene that the present study investigated was *BDNF*. The Val66Met polymorphism, characterized by a Val to Met substitution at position 66 in the pro-domain of the gene (Mizuno et al., 2000), has been extensively researched for potential effects on adult cognitive function, with mixed results. However, due to previously reported associations between variation in *BDNF* Val66Met and cognitive functions, it was hypothesised that individuals that are *BDNF* Val homozygotes have superior cognitive function compared to Met carriers. For example, Met alleles were associated with poorer memory function within 641 subjects tested on the Wechsler Memory Scale (Egan et al., 2003). In a smaller sample of 64 subjects, Val homozygotes were significantly more accurate than Met carriers at recognising both 'new' and 'old' scenes during the retrieval phase of a declarative memory paradigm (Hariri et al., 2003). Even in a younger sample of 135 healthy individuals with a mean age of 24.6 years, Met carriers have displayed significantly lower working memory function (Richter-Schmidinger et al., 2010). In addition to *BDNF* effects on memory processing, Met alleles have also been associated with reduced speed of processing (Laing et al., 2011; Raz et al., 2009).

Despite evidence that supports the link between *BDNF* Met alleles and reduced performance on tests of cognitive function, the analysis from the present investigation failed to identify any effect of variation in *BDNF* Val66Met on cognitive function. In the present study, the *BDNF* Val homozygote group performed similarly to the *BDNF* Met carrier group in all four assessed cognitive domains. Again, this finding is not unusual, as others have also reported negative findings (Gong et al., 2012; Houlihan et al., 2009; Stuart et al., 2014). In 1,063 Scottish individuals of mean age 70 years, variation in the polymorphism did not account for any of the variance in cognitive function variables (Houlihan et al., 2009). Similarly, in 700, 19-21 year olds, *BDNF* Val66Met had no observable effects on either semantic or episodic memories (Gong et al., 2012). One recent meta-analysis of 23 publications encompassing 7,095 subjects found no association of the polymorphism with any cognitive domain (Mandelman & Grigorenko, 2011).

While the results of the present investigation did not support a role for *BDNF* Val66Met in memory function, it is widely accepted that the gene and, specifically, the polymorphism, critically alters the neural networks that support memory processing (Lu et al., 2013). In particular, the memory encoding process, which is dependent on the presence of BDNF in the hippocampus (Ying et al., 2002), may be disrupted due to the Met variant's impaired activity-dependent secretion of BDNF in hippocampal neurons (Egan et al., 2003). Met alleles are also associated with reduced hippocampal volume (Frodl et al., 2007; Kauppi et al., 2014), even in young adults (Pezawas et al., 2004), when compared to Val homozygotes. In addition, reduced activity in the medial temporal lobe and hippocampus during the encoding of information, indicative of subsequently forgotten memories (Eichenbaum et al., 2007), has been found in Met carriers (Kauppi et al., 2013; 2014). Such evidence strongly supports an association of *BDNF* Val66Met and memory function, and discrepancies in results are likely

due to the profound differences in the effects that variation in *BDNF* exerts across the lifespan (Casey et al., 2009).

Interactive effects of *APOE* and *BDNF* on episodic memory

As the detrimental cognitive effects of the putative negative alleles could combine to produce a larger decrease in cognitive function in carriers of both *APOE* ε4 and *BDNF* Met, a third hypothesis was proposed. Specifically, it was expected that the highest cognitive function would be observed in *APOE* ε2 and *BDNF* Val homozygote carriers and the lowest cognitive function in *APOE* ε4 and *BDNF* Met carriers. Previous investigations have examined interactions between *APOE* and *BDNF* Val66Met in terms of brain morphology and function (Adamczuk et al., 2013; Hashimoto et al., 2009; Kauppi et al., 2014; Richter-Schmidinger et al., 2010), with mixed results. Such reports underline the importance of investigating the cognitive consequences of variation in both genes concurrently.

Partial support was found for the hypothesis of an *APOE* x *BDNF* interaction. For *BDNF* Met carriers, the expected beneficial and detrimental effects of *APOE* ε2 and ε4 alleles, with relation to *APOE* ε3 homozygotes, were found for episodic memory performance. For *BDNF* Val homozygotes, no differences were found for episodic memory due to *APOE* groups. In relation to the hypothesis, the lowest episodic memory was found to be associated with inheritance of both *BDNF* Met and *APOE* ε4, consistent with the hypothesis; but, unexpectedly, the highest episodic memory performance was associated with inheritance of *BDNF* Met and *APOE* ε2. That the lowest episodic memory function was found in *BDNF* Met/*APOE* ε4 can be attributed to an additive effect of both genetic variants. By comparison, the finding that the highest episodic memory function was found in *BDNF* Met/*APOE* ε2 is unable to be accounted for by an additive effect, as previous reports have implicated *BDNF* Met with reduced memory function across a range of age groups (Egan et al., 2003; Hariri et

al., 2003; Raz et al., 2009; Richter-Schmidinger et al., 2010). However, in the present study, the *BDNF* Met/*APOE* ε2 group comprised the lowest number of participants ($N = 13$), due to uncommon allele occurrence rates (Egan et al., 2003; Farrer et al., 1997). As a result, this result should be interpreted with caution, due to the high intra-group variance in the *BDNF* Met/*APOE* ε2 group.

Interactions, both additive and non-additive, have been identified between *APOE* and *BDNF* Val66Met in previous work. In one investigation into hippocampal activation during a memory task in healthy older adults, decreased activation in the bilateral hippocampus and parahippocampus was identified as a function of the number of *APOE* ε4 and *BDNF* Met alleles (Kauppi et al., 2014). An additive effect meant that, in this study, the combined effect of both genes was stronger than either of the individual effects. Similarly, a study of the morphological changes associated with preclinical AD found that, in addition to the commonly occurring atrophy in the medial temporal areas, the presence of either *APOE* ε4 and *BDNF* Met was associated with additional atrophy in the precuneus, and anterior and posterial cingulate cortices (Hashimoto et al., 2009). In cognitively normal older adults, individuals carrying *BDNF* Met have shown a higher amyloid load than individuals who are *BDNF* Val homozygotes, but only in the presence of the *APOE* ε4 allele (Adamczuk et al., 2013). In this study, variation in *BDNF* Val66Met was not associated with amyloid load in *APOE* ε4 non-carriers. Furthermore, the authors identified a significant inverse relationship between amyloid load and episodic memory function, but only in the *BDNF* Met group/*APOE* ε4 group.

The finding from the present investigation, that *APOE*-related variation in episodic memory function occurs only in the presence of a *BDNF* Met allele, appears novel but consistent with previous research. The present data likely reflect a combination of an interaction of *APOE*

and *BDNF* effects on medial temporal lobe activation (Kauppi et al., 2014), atrophy (Hashimoto et al., 2009), and amyloid load (Adamczuk et al., 2013), which culminate in differential cognitive outcomes. One of the most likely explanations involves the negative impact of amyloid load on healthy episodic memory function only in the presence of both *BDNF* Met and *APOE* ε4 alleles (Adamczuk et al., 2013). The presence of *APOE* ε2 is associated with reduced amyloid deposition and more efficient clearance (Castellano et al., 2011; Suri et al., 2013; Tiraboschi et al., 2004), whereas *APOE* ε4 is associated with increased amyloid deposition and less efficient clearance (Castellano et al., 2011; Deane et al., 2008; Kok et al., 2009), when compared to *APOE* ε3 homozygotes. In the present study, *BDNF* Met may have conferred a vulnerability to the differential amyloid-related effects of variation in *APOE*.

Cognitive reserve and premorbid cognitive function

Evidence has consistently demonstrated that the clinical symptoms of AD can be delayed due to increased CR (Amieva et al., 2014; Brayne et al., 2010; Valenzuela & Sachdev, 2006); however, few investigations have examined the premorbid impact of CR on cognitive function. Such investigations are important to the CR theory, as a main conceptualisation of CR involves the presence of neural reserve in healthy adult brain networks (Stern, 2002; Stern et al., 2005). Stern (2002) noted a distinction between neural reserve, the ability to optimise non-pathological function, and neural compensation, the ability to recruit additional brain structures or networks to maximise function in the presence of pathology. Consequently, variation in CR should result in changes to performance in cognitive processing. A primary aim of this thesis was to quantify the premorbid associations between prior CR and cognitive function.

Multiple behavioural investigations have suggested that engagement in CR-building activities is associated with higher baseline cognitive function (Gottesman et al., 2014; Singh-Manoux et al., 2011; Zahodne et al., 2011), despite little to no protective effects of education on age-related cognitive decline (Christensen et al., 2009; Van Dijk et al., 2008; Zahodne et al., 2011). In addition, neuroimaging studies have identified morphological (Foubert-Samier et al., 2012; Liu et al., 2012; Suo et al., 2012) and neural (Lopez et al., 2014; Morbelli & Nobili, 2014; Solé-Padullés et al., 2009) benefits associated with life experience in healthy individuals. As such, it was hypothesised that higher CR is associated with better premorbid cognitive performance. To test this hypothesis, a prior CR equation (developed in chapter 3) was used to compute an estimate of CR for each participant. This was subsequently regressed against age-adjusted composite cognitive domain variables (episodic memory, working memory, executive function, language processing), which were developed in chapter 4.

In the present study, compelling evidence was found implicating CR in cognitive functioning in healthy older adults; the sample showed significant positive relationships between CR and cognitive function across all assessed cognitive domains, after accounting for the effects of age. This finding is consistent with previous literature that has reported that increased participation in cognitively stimulating activities is accompanied by better cognitive processing (Gottesman et al., 2014; Singh-Manoux et al., 2011; Zahodne et al., 2011). For instance, in a 20-year longitudinal analysis of 14,020 individuals, aged 48-67 years at baseline, higher education was associated with substantial persistent increases in global, episodic memory, processing speed, and language processing function (Gottesman et al., 2014). Similarly, more education was related to increased function in a range of cognitive domains in 1,014 participants aged 54-95 years (Zahodne et al., 2011). Another study examined the 10-year effect of three markers of reserve (height, education, and occupation) on cognitive change in 7,454 individuals (Singh-Manoux et al., 2011). Although the authors

reported that reserve did not alter the rate of cognitive decline, they found that cognitive performance was remarkably higher in the high reserve groups.

Whether the finding of a positive relationship between CR and healthy cognitive function is consistent with the neural implications of the CR theory (Stern, 2002), or simply a reflection of the cognitive benefits associated with living a mentally engaged lifestyle, remains unknown. Without functional neuroimaging data, it is not possible to test whether the relationship between CR and cognitive function is mediated by differences in neural efficiency, which would be consistent with CR theory (Stern et al., 2005). However, due to evidence reported by other groups, it is plausible that CR-related differences in neural network recruitment and efficiency are present in this dataset (Morbelli & Nobili, 2014). In this regard, functional MRI and PET investigations have identified altered patterns of neural activation associated with CR proxy variables (Barulli & Stern, 2013), even in younger adults (Stern et al., 2003). For example, in older adults with greater levels of education, it has been found that there is greater recruitment of the frontal cortex during performance on a memory task (Springer et al., 2005). Individuals with more years of education and occupational experience also show lowered functional connectivity during a memory task, indicative of more efficient networks, despite no difference in behavioural accuracy (Lopez et al., 2014).

Another explanation for the increased cognitive function associated with more CR relates to possible morphological and pathological effects. Previous studies indicate that individuals with greater CR have larger regional cortical thickness in transverse temporal cortex, insula, and isthmus of cingulate cortex (Liu et al., 2012), more grey and white matter brain volumes (Foubert-Samier et al., 2012), better integrity of white matter tracts (Teipel et al., 2009), and less 3-year hippocampal atrophy (Valenzuela et al., 2008), when compared to those with less CR. More CR may also alter the rate at which AD pathology is accumulated (Jagust &

Mormino, 2011). For instance, higher levels of early- and mid-life engagement in cognitive activities is associated with a significantly reduced Aβ deposition in later life (Landau et al., 2012).

Genetic interactions with cognitive reserve

Although the development of CR is mainly achieved through sustained participation in activities that involve complex cognitive engagement (Lövdén et al., 2010; Stern, 2002; 2009), it is likely that genetic makeup impacts upon either the development or use of CR (Lee, 2003). This is assumed due to the large heritability of cognitive function in adult life (Bouchard & McGue, 1981; Plomin et al., 2013) and the significant impact that variation in genes can have on processes vital to CR, such as synaptic plasticity (Esiri & Chance, 2012). Two genes that show polymorphisms that impact upon both cognitive function and synaptic plasticity are *APOE* and *BDNF* Val66Met (Cheeran et al., 2008; Chen et al., 2010; Egan et al., 2003; Levi et al., 2005). Consequently, an aim of the present study was to investigate the relationship between CR, cognitive function, and *APOE* and *BDNF* Val66Met polymorphisms. It was hypothesised that *BDNF* Val66Met and *APOE* polymorphisms independently moderate the association between CR and cognitive performance, with carriers of the putative detrimental alleles (Met and ε4, respectively) showing a reduced influence of CR when compared to non-carriers.

To test this hypothesis, PROCESS (Hayes, 2013) was used to examine whether CR x *APOE* or CR x *BDNF* interaction terms explained significant variance in cognitive domain variables, after controlling for CR, age, and corresponding genetic main effects. The results indicate partial support for the hypothesis, as a significant CR x *BDNF* interaction was present for executive function. This result indicates that the relationship between CR and executive function was moderated by the *BDNF* Val66Met polymorphism, with a positive association

between the variables present in *BDNF* Val homozygotes and absent in *BDNF* Met carriers. However, no evidence was found to implicate variation in *APOE* in altering the relationship between CR and cognitive function.

Although investigations that attempt to quantify the impact of genetic variation on CR are rare, some *APOE*-related interactions have been previously reported. In most of the cases, the basis for the CR x *APOE* interactions were that higher education seemed to reduce the increased risk of dementia associated with *APOE* ε4 (Ferrari et al., 2013; Shadlen et al., 2005; Wang et al., 2012). For example, a steeper 6-year age-related decline in cognitive function due to the presence of two *APOE* ε4 alleles was reduced due to greater education in 2,168 non-demented elderly (Shadlen et al., 2005). Other studies have demonstrated a reduced association between CR and cognitive function in the presence of *APOE* ε4 (Runge et al., 2014; Seeman et al., 2005), potentially due to the allele-specific impaired synaptic plasticity (Chen et al., 2010; Levi et al., 2005). For instance, cognitively stimulating lifestyle activities was found to impact on verbal fluency and semantic memory recall in *APOE* ε4 non-carriers but not in *APOE* ε4 carriers (Runge et al., 2014). In any case, the present study does not provide evidence of such associations.

Surprisingly, few studies have examined the effect of variation in *BDNF* Val66Met on the relationship between CR and cognitive function, despite the clear and vital role the neurotrophin plays in many developmental processes and adult plasticity (Casey et al., 2009). However, it has been theorised that the increased neuronal plasticity associated with cognitive stimulation is mediated by the upregulation of BDNF (Stern, 2009; 2012). In this case, it follows that a polymorphism associated with reduced trafficking of BDNF into the regulated secretory pathway (Egan et al., 2003), leading to impairments in activity-dependent release of BDNF, may affect the association between lifetime cognitive activities and cognitive

function. In the present study, it was determined that the normal relationship between CR and executive function was present in *BDNF* Val homozygotes but absent in *BDNF* Met carriers. Such a result supports a role of *BDNF* in mediating the benefits associated with more CR, potentially due to reduced synaptic restructuring following participation in cognitively stimulating activities associated with inheritance of *BDNF* Met.

The finding that *BDNF* Val66Met moderated the association between CR and executive function is particularly important, given the overlap between these constructs. Overall, both CR and executive function have an underlying reliance on cognitive flexibility (Miyake, 2000; Stern, 2012) and a reliance on frontal lobe activity (Alvarez & Emory, 2006; Springer et al., 2005). For instance, although frontal activity was negatively related to education in young adults, frontal lobe activity was increased in highly educated older adults, potentially serving as an alternative network that may be engaged to aid cognitive function (Springer et al., 2005). One investigation examined whether CR was a distinct construct, separate from other cognitive domains, using structural equation modelling (Siedlecki et al., 2009). Here, the authors found evidence to suggest that CR was separate to other cognitive variables, but that CR was closely related to executive function. This may have been due to their approach to estimating CR, as only intellectual variables (reading ability, vocabulary, and years of education) comprised their model of CR, rather than variables of lifetime cognitive stimulation (e.g. occupation, leisure activities). Executive function is also clinically relevant, as reductions in executive function are found in response to ageing (Deary et al., 2009) and characterise early dementia onset (Silveri et al., 2007).

Effects of *BDNF* on the frontal lobe, a structure vital to cognitive processes associated with executive function (Kane & Engle, 2002), may also account for the gene-CR interaction witnessed in the present study. BDNF is expressed in significant quantities within the

prefrontal cortex, with reduced levels of BDNF and trkB protein in this region occurring due to the presence of AD (Allen, Wilcock, & Dawbarn, 1999; Ferrer et al., 1999). Recent investigations have also found an effect of the Val66Met polymorphism on frontal functioning. For instance, inheritance of *BDNF* Met leads to impairments to NMDA and GABA receptor transmission and plasticity in the infralimbic medial prefrontal cortex (Pattwell et al., 2012). Met alleles are also associated with a higher resting-state functional connectivity between the anterior insular and the dorsolateral prefrontal corticies (Wang et al., 2014). As lower resting-state metabolic activity in the default-mode network is present in individuals with higher CR (Bastin et al., 2012), the finding of Wang and colleagues may be explained through less efficient neural networks, indicative of lower CR (Morbelli & Nobili, 2014; Stern, 2002), in Met carriers.

Integration of results

This thesis has identified a biological target for the basis of individual differences in cognitive resilience. Overall, the data presented in this thesis are consistent with the suggestion that inheritance of *BDNF* Met is associated with a vulnerability of cognitive function to pathology, probably through the genetic modulation of an individual's CR. Although this hypothesis is yet to be tested, the results of the present investigation, in conjunction with previous literature, suggest that this may be the case. In order to support this theory, two criteria must be met. First, that *BDNF* Met is associated with a reduced influence of CR on cognitive function, when compared to *BDNF* Val homozygotes. Second, that *BDNF* Met is associated with a poorer cognitive outcome in the presence of neuropathology that CR is unable to buffer against, when compared to *BDNF* Val homozygotes.

The first criterion, that *BDNF* Met is associated with a reduced influence of CR on cognitive function, was demonstrated in this study. It was shown that a multivariate estimate of prior

CR had a positive association with all four assessed cognitive domains. However, when the impact of *BDNF* Val66Met on this association was examined, it was found that *BDNF* Met carriers lacked an association between CR and executive function, whereas the association was preserved in *BDNF* Val homozygotes. One possible explanation for this finding is that the impaired synaptic plasticity associated with *BDNF* Met (Cheeran et al., 2008; Ninan et al., 2010; Pattwell et al., 2012) led to a reduced neurological impact imparted due to participation in cognitively stimulating leisure activities.

The second criterion, that *BDNF* Met is associated with a poorer cognitive outcome in the presence of neuropathology, indicative of low CR, when compared to *BDNF* Val homozygotes, has been demonstrated by previous recent research (Adamczuk et al., 2013; Lim et al., 2013; 2014). In 165 healthy adults enrolled in the Australian Imaging, Biomarkers and Lifestyle (AIBL) study, *BDNF* Val66Met was found to interact with amyloid load resulting in an alteration of cognitive function (Lim et al., 2013). In the AIBL study, individuals with high Aβ and *BDNF* Met showed moderate-to-large declines in episodic memory, executive function, and language processing when compared to Val homozygotes; in comparison, *BDNF* Val66Met did not alter rates of cognitive change in individuals with low Aβ. Similarly, another investigation of 34 adults with aMCI recruited from the AIBL study found that, in individuals with high Aβ, *BDNF* Met carriers had larger declines in episodic memory and hippocampal volume at three-year follow-up than non-carriers (Lim et al., 2014). In a different study of 64 cognitively intact older adults, a detrimental effect of amyloid burden on episodic memory function was present only in the participants who carried both *APOE* ε4 and *BDNF* Met alleles (Adamczuk et al., 2013). In these studies, inheritance of *BDNF* Met conferred a vulnerability of cognitive function to the detrimental effects of AD-like pathology.

Overall, it may be that reduced functional CR is present in *BDNF* Met carriers, causing an inability to compensate for amyloid accumulation. This explanation accounts for the data that describe a poorer cognitive outcome associated with increased amyloid deposition only in the presence of *BDNF* Met (Adamczuk et al., 2013; Lim et al., 2013; 2014). It may also partially account for the finding of the present study that the lowest episodic memory function was present in *APOE* ε4/*BDNF* Met carriers: the increased rate of amyloid accumulation associated with *APOE* ε4, in conjunction with a reduced ability to compensate for pathology due to *BDNF* Met, culminates in reduced memory function.

Limitations

The present investigation possessed a number of limitations, which should be considered when interpreting the reported results. Although a model of prior CR comprised from only variables that were prospectively assessed would have been preferable, the LEQ and Medical Health Status questionnaire collected retrospective data. As such, the accuracy of the information that was obtained cannot be verified (Bernard et al., 1984) and the prior CR model may possess slight inaccuracies as a result. Another limitation of the prior CR estimate is that, even though the variables that were entered into the model were predominantly static, some variability in the items is expected. This is particularly the case for those participants who completed their baseline THBP assessment prior to being aged 65 years, as LEQ data for midlife subscales would have been incomplete. One of the limitations of the current CR model is that longitudinal changes in this measure may be particularly prone to reflecting change in CR due to exposure to education, but less likely to reflect change in CR due to participation in other cognitive activities, such as playing a music instrument or learning a second language. This is due to the current CR model comprising cognitive variables that assess mainly intelligence and academic ability. However, it must be noted that there are inherent significant barriers to quantifying and then developing reliable and valid objective

measures of lifestyle factors associated with lifetime cognitive stimulation (e.g. playing music, social conversation, learning a language, etc.).

When evaluating the cognitive implications of variation in *APOE* and *BDNF* Val66Met polymorphisms, previous education and age were controlled for in the analysis; however, other covariates, such as physical activity and diet, were not. These covariates are particularly important when investigating *BDNF*, a gene that has shown associations with such lifetime variables in previous investigations. For instance, the secretion of BDNF in the hippocampus of mice is elevated after participation in voluntary exercise (Farmer et al., 2004) and the inhibition of BDNF action in the hippocampus has been shown to negate the cognitive and synaptic benefits of exercise (Vaynman et al., 2004). Overall, it has been suggested that BDNF signalling mediates the protective effects of diet and exercise on neurodegeneration (Mattson et al., 2004). In support of this notion, a recent investigation showed that the alleviation of diet-induced cognitive decline by exercise was likely due to the increase presence of BDNF in the hippocampus (Noble et al., 2014). Future investigations should control for diet and exercise when examining the effects of *BDNF* on cognitive or neurological function.

The aforementioned limitations may have had slight impacts upon the accuracy or reliability of the respective investigations, but the strength of the findings in this thesis would have been considerably increased by the availability of a subgroup of dementia patients and longitudinal data. The compensatory effect of CR to delay clinical dementia is well documented (Stern, 2013), and the inclusion of participants with symptoms of dementia to test and validate the two models of CR would have been a major benefit. If longitudinal data were available at the time of analysis, hypotheses could have examined whether the models of CR or variation in *APOE* or *BDNF* Val66Met predicted age-related change in cognitive function. The ability to

test whether *BDNF* Val66Met moderated the association of CR on the rate of age-related cognitive decline would have been advantageous.

Overall, CR is a hypothetical construct that is mainly applied to samples experiencing traumatic brain injury or neurodegenerative disorders (Stern, 2002). In this investigation, the CR theory was examined in a sample of healthy, extensively screened, older adults. This may reduce the applicability of these results to less healthy samples, particularly those experiencing symptoms of dementia.

Clinical implications

In examining the association between prior CR and the four assessed cognitive domains in this study, positive correlations were identified for all cognitive processes. This indicates that a history of higher involvement in the lifetime factors that comprised prior CR is associated with better global cognitive ability. Previously, research has investigated whether higher CR protects from symptoms of dementia (e.g. Basu, 2012; Norton et al., 2012; Vemuri et al., 2011), however, the results of this thesis imply that CR exerts an impact on healthy cognitive function, independent of dementia. This finding is consistent with longitudinal investigations that have shown higher baseline cognitive function associated with more years of education (Gottesman et al., 2014; Singh-Manoux et al., 2011; Zahodne et al., 2011). Due to the nature of the cross-sectional design used in the present thesis, there is no opportunity to examine whether CR exerts a protective effect on the rate, or time of onset, of age-related cognitive decline. However, previous research has found negative results in relation to the hypothesis that CR protects from normal age-related declines in cognitive function (Christensen et al., 2009; Van Dijk et al., 2008; Zahodne et al., 2011). Regardless, these results indicate that, at the very least, CR may benefit cognition and delay dementia onset in older age due to the persistent effects of higher premorbid cognitive function.

A second clinical implication of the present data is that the effects of single genes on cognitive function are minimal and that previous positive associations of *APOE* (Boardman et al., 2012; Deary et al., 2004; Ready et al., 2011) or *BDNF* (Egan et al., 2003; Hariri et al., 2003; Raz et al., 2009) are not easily replicable, even with a substantial sample size. Investigations into single genes are unlikely to produce translatable results; however, they can be used as preliminary investigations to identify potential targets for follow-up studies. Despite this, an *APOE* x *BDNF* interaction was identified in the present study, which accounted for 6% of the variance in episodic memory. If 50% of adult cognitive function is inherited (Bouchard & McGue, 1981), then potentially 12% of the genetically-determined episodic memory is accounted for by this interaction. This effect size is likely a result of the cumulative effects of *APOE* and *BDNF* polymorphisms on cognitive function and the notion of simple risk alleles that have clinical importance for healthy adult cognitive function is unlikely; particularly with consideration of the increased understanding of complex gene-environment (Casey et al., 2009) and epigenetic (Egger et al., 2004) interactions. Overall, the results of the present investigation suggest that individuals who carry both *APOE* ε4 and *BDNF* Met alleles are at a higher risk for cognitive impairment and decline.

Perhaps the most important and clinically relevant finding from the present study was the identification of a genetic moderator of the influence that CR has on cognitive functioning. Although previous reports have identified genetic interactions between *APOE* and CR (Ferrari et al., 2013; Runge et al., 2014; Shadlen et al., 2005; Wang et al., 2012), no evidence of such an effect was found in the present thesis. Rather, the data demonstrated a significantly different relationship between CR and executive function for *BDNF* Met carriers compared to that observed in *BDNF* Val homozygotes. This implies that, in healthy older adults, *BDNF* Met carriers have reduced cognitive benefit from participation in cognitively stimulating

activities. Moreover, highly educated *BDNF* Met carriers may not develop the additional neurological resources associated with more CR to the same extent as *BDNF* Val homozygotes, and may be at a similar risk of dementia to those Met carriers who have not engaged in such CR-related activities.

Experimental design recommendations

That an estimate of prior CR that combines multiple independent proxy variables was successfully developed in this study has implications for the CR theory. Previously, single indicators (e.g. education) have been used to represent an individual's CR (Basu, 2012; Brayne et al., 2010; Meng & D'Arcy, 2012; Ott et al., 1995), but the results of the present thesis indicate that this may not be the best method. Indeed, an estimation of CR that takes into account several indicators may supply the researchers with a more precise estimate (Jones et al., 2011), particularly as the proxy variables of education, occupational attainment, and participation in leisure activities contribute differently and independently to CR (Foubert-Samier et al., 2012). In future, a practical suggestion for research is that the multiple indicator method be implemented when examining the CR theory. Specifically, the principal components analysis method, rather than a true factor analysis, should be used to increase the likelihood of separate CR contributors contributing to the final model (Jones et al., 2011; Satz et al., 2011). If authors fail to implement this method, they run the risk of interpreting a CR variable with reduced predictive power (Satz et al., 2011), potentially limiting the significance and interpretability of their results.

While attempts have been made to better operationalise a model of pre-existing CR (Lojo-Seoane et al., 2014; Reed et al., 2011; Valenzuela & Sachdev, 2007), very few attempts have been made at producing a reliable and valid measure of change in CR over time. CR is a dynamic construct that is continuously updated or developed by new life experiences across

the lifespan (Richards & Sacker, 2003). In addition, the importance of interventions that increase CR is well-known (Tucker & Stern, 2011), but the efficacy of such interventions is unknown until the onset of symptoms of dementia. It is of significance then, that an operational measure of CR was successfully generated in the present study. The measure of current CR was developed with consideration to those variables that closely approximate traditional measures of CR, but also have a documented potential to change due to new life experience (Donnell et al., 2007; Roid & Ledbetter, 2006). It is recommended that such a method be implemented into the design of clinical trials that aim to increase CR, to allow for an estimate of the intervention's efficacy prior to dementia onset.

Due to the identification of a moderating effect of *BDNF* Val66Met on the relationship between CR and cognitive function, it may be necessary to perform *BDNF* genotyping in research that aims to substantiate a clinical intervention or elucidate CR mechanisms. For instance, there is an abundance of randomised clinical trials whose intention is to demonstrate the efficacy of cognitive exercise on longitudinal cognitive performance in healthy older adults (Valenzuela & Sachdev, 2009). However, the findings of the present thesis imply that a commonly occurring genetic polymorphism in *BDNF* may influence the degree to which environmental exposure to cognitive stimulation results in changes to cognitive function. It is therefore recommended that *BDNF* Val66Met polymorphism data be specified in the statistical models of such research, otherwise important genetic interactions may be overlooked.

Future research

Although the present study has advanced our knowledge of how CR can be modelled, genetic influences on healthy cognitive function and a genetic basis for CR, there are some obvious unanswered questions. Many of these issues will be addressed in future THBP investigations.

The first key area that should be the target of future research is to validate the multiple indicator method of assessing prior CR. Although the method implemented in the current investigation was efficacious in constructing a composite estimate of CR, it is not yet known whether the predictive power of the prior CR model is higher than that of simply using a single indicator. One way of assessing this would be to conduct an analysis of longitudinal data to determine whether a multivariate approach or that of a single indicator accounts for a greater proportion of the variance in, immediately, age-related cognitive decline and, later, time of onset of dementia symptoms. Similarly, the model of current CR has been developed from theory, and is yet to be validated longitudinally. For this, it will be necessary to show a significant increase in current CR due to the education intervention, which, in turn, demonstrates a reduced risk of dementia.

The second key area that future investigations should explore is the interaction between *BDNF* Val66Met and CR that was identified in the present study. Primarily, if the modulating effect of the polymorphism on CR could be demonstrated in a longitudinal design, it would reinforce the notion that *BDNF* is a biological pathway through which CR is mediated. Such findings would have to demonstrate that variation in the *BDNF* Val66Met polymorphism influences the protection conferred by proxy CR from the rate of age-related cognitive decline and/or risk for dementia. The testing of this hypothesis is a priority of the THBP, with the relevant analyses to be conducted as longitudinal data become available.

Conclusion

In conclusion, CR remains an imprecise concept that is difficult to define. Estimates of participation in cognitively stimulating activities act as proxy measures for CR (Stern, 2002; Valenzuela & Sachdev, 2006), but *in vivo* assessments of neurological functioning, for

example neural efficiency (Lopez et al., 2014; Morbelli & Nobili, 2014), have been proposed as alternative approximations of CR. In this regard, the results of the present thesis have provided data that further knowledge on how to operationalise and estimate CR before the onset of dementia symptoms. These results also depict the impact of environmental and genetic factors on the cognitive function of healthy older adults. Here, higher CR was associated with higher cognitive functioning in all cognitive domains – a clear demonstration of the premorbid cognitive benefits of CR-related proxy variables that has been reported in other work (Gottesman et al., 2014; Singh-Manoux et al., 2011; Zahodne et al., 2011). Similarly, an interaction between *APOE* and *BDNF* Val66Met polymorphisms, consistent with previous investigations into the genes (Adamczuk et al., 2013; Kauppi et al., 2014), accounted for a substantial proportion of the genetically-derived variance in episodic memory. However, the most novel finding that emerged from the present research was that of a partial 'disconnect' between CR and cognitive function in approximately one third of the population who carry a *BDNF* Met allele. Future investigations should aim to verify CR's involvement in normal ageing and elucidate the biological mechanisms underpinning the moderating effect of *BDNF* Val66Met on CR.

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Appendix A – Ethics Approval Letter

Social Science Ethics Officer
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Tasmania 7001 Australia
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Marilyn.Knott@utas.edu.au

MEMORANDUM

HUMAN RESEARCH ETHICS COMMITTEE (TASMANIA) NETWORK

UTAS

FULL COMMITTEE ETHICS APPLICATION APPROVAL

25 March 2010

Dr Mathew Summers
Psychology
Private Bag 1342
Launceston

Dear Dr Summers

Ethics Reference: **H11070**

Project Title: **The Tasmanian Healthy Brain Study.**

The Tasmania Social Sciences HREC Ethics Committee approved the above project on 24 March 2010.

Please note that this approval is for four years and is conditional upon receipt of an annual Progress Report. Ethics approval for this project will lapse if a Progress Report is not submitted.

The following conditions apply to this approval. Failure to abide by these conditions may result in suspension or discontinuation of approval.

1. It is the responsibility of the Chief Investigator to ensure that all investigators are aware of the terms of approval, to ensure the project is conducted as approved by the Ethics Committee, and to notify the Committee if any investigators are added to, or cease involvement with, the project.

2. Complaints: If any complaints are received or ethical issues arise during the course of the project, investigators should advise the Executive Officer of the Ethics Committee on 03 6226 7479 or human.ethics@utas.edu.au.

3. Incidents or adverse effects: Investigators should notify the Ethics Committee immediately of any serious or unexpected adverse effects on participants or unforeseen events affecting the ethical acceptability of the project.

4. Amendments to Project: Modifications to the project must not proceed until approval is obtained from the Ethics Committee. Please submit an Amendment Form (available on our website) to notify the Ethics Committee of the proposed modifications.

5. Annual Report: Continued approval for this project is dependent on the submission of a Progress Report by the anniversary date of your approval. You will be sent a courtesy reminder closer to this date. **Failure to submit a Progress Report will mean that ethics approval for this project will lapse.**

6. Final Report: A Final Report and a copy of any published material arising from the project, either in full or abstract, must be provided at the end of the project.

Yours sincerely

Ethics Executive Officer

Appendix B – Ethics Amendment 1

Social Science Ethics Officer
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Fax: (03) 6226 7148
Katherine.Shaw@utas.edu.au

HUMAN RESEARCH ETHICS COMMITTEE (TASMANIA) NETWORK

UTAS

15 August 2011

Dr Mathew Summers
School of Psychology
Locked Bag 1342
Launceston Tasmania

Dear Dr Summers

Re: APPROVAL FOR AMENDMENT TO CURRENT PROJECT
Ethics Ref: **H0011070 - The Tasmanian Healthy Brain Study**

Amendment to change age range for participation from 50-70 to 50-79.
Revised participant information sheet.

We are pleased to advise that the Chair of the Tasmania Social Sciences Human Research Ethics Committee approved the Amendment to the above project on 13 August 2011.

Yours sincerely

Katherine Shaw
Acting Executive Officer

Appendix C – Ethics Amendment 2

Social Science Ethics Officer
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HUMAN RESEARCH ETHICS COMMITTEE (TASMANIA) NETWORK

22 December 2011

Dr Mathew Summers
School of Psychology
Locked Bag 1342
Launceston Tasmania

Dear Dr Summers

Re: APPROVAL FOR AMENDMENT TO CURRENT PROJECT
Ethics Ref: **H0011070 - The Tasmanian Healthy Brain Study**

Amendment Form dated 22 November 2011, revised 13 December 2011.
Collection of salivary samples from consenting participants for testing of genetic markers associated with elevated risk for dementia.
Oragene DNA Self-Collection Kit User Instructions.
Genetic Testing Information Sheet and Consent Form Version 1 dated 2011, revised 21 December 2011.
Response to NEAF questions regarding genetic testing.

We are pleased to advise that the Tasmania Social Sciences Human Research Ethics Committee approved the Amendment to the above project on 21 December 2011.

Yours sincerely

Katherine Shaw
Acting Executive Officer

Appendix D – Ethics Amendment 3

Social Science Ethics Officer
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HUMAN RESEARCH ETHICS COMMITTEE (TASMANIA) NETWORK

14 August 2012

Dr Mathew Summers
School of Psychology
University of Tasmania
Locked Bag 1342

Sent via email

Dear Dr Summers

Re: APPROVAL FOR AMENDMENT TO CURRENT PROJECT
Ethics Ref: **H0011070 - The Tasmanian Healthy Brain Study**

- Addition of investigator Dr Nikki Saunders.
- Revised participant information sheet.
- Revised participant information sheet for genetic testing.

We are pleased to advise that the Chair of the Tasmania Social Sciences Human Research Ethics Committee approved the Amendment to the above project on 10 August 2012.

Yours sincerely

Katherine Shaw
Ethics Officer
Tasmania Social Sciences HREC

Appendix E – Ethics Amendment 4

Social Science Ethics Officer
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HUMAN RESEARCH ETHICS COMMITTEE (TASMANIA) NETWORK

UTAS

3 January 2013

Dr Mathew Summers
School of Psychology
Locked Bag 1342

Sent via email

Dear Dr Summers

Re: APPROVAL FOR AMENDMENT TO CURRENT PROJECT
Ethics Ref: **H0011070 - The Tasmanian Healthy Brain Study.**

Merge of ethics applications H10947 and H10742 into principal application H11070.

We are pleased to advise that the Chair of the Tasmania Social Sciences Human Research Ethics Committee approved the Amendment to the above project on 2 January 2013.

Yours sincerely

Katherine Shaw
Ethics Officer
Tasmania Social Sciences HREC

Appendix F – Information Sheet

UTAS FACULTY OF SCIENCE, ENGINEERING & TECHNOLOGY School of Psychology

WICKING DEMENTIA RESEARCH & EDUCATION CENTRE

PARTICIPANT INFORMATION SHEET
Genetic Testing in the Tasmanian Healthy Brain Study

Invitation
You are invited to provide a sample of saliva for DNA analysis as part of the Tasmanian Healthy Brain Study

The study is being conducted by:
- Dr Mathew Summers, Senior Lecturer/Research Fellow, School of Psychology, UTAS;
- Professor James Vickers, Professor of Pathology, School of Medicine, UTAS;
- Dr Nikki Saunders, Research Fellow/Project Coordinator, WDREC

1. 'What is the purpose of this study?'
The purpose is to investigate whether genetic markers are associated with the rate of age-related cognitive decline and rate of dementia in older adults, and whether late-life education minimizes the impact of the genetic risk factors.

2. 'Why have I been invited to participate in this study?'
You are eligible to participate in this study because you are currently participating in the Tasmanian Healthy Brain Study.

3. 'What does this study involve?'
A donation of a 2 ml sample of saliva, which can be made either in the privacy of your own home or at the Tasmanian Healthy Brain Research centres in Hobart, Launceston or Burnie. Donations made at home can be mailed back to the researchers using prepaid specially designed postal kits.

The DNA samples will be analysed by the Menzies Research Institute of Tasmania. Each sample will only be identified by your unique Tasmanian Healthy Brain Study participant ID code. Only Dr Mathew Summers and Prof James Vickers are authorized to match the participant ID code with your name and contact details.

Unfortunately, we are unable to provide you with the results of the DNA analysis performed. To date there are no known genetic markers that indicate you will develop dementia. At best, there are some genetic markers that appear to be associated with an increased risk for developing dementia, but just as many persons with these genetic markers do not develop dementia as do develop dementia. Therefore, provision of this information to you would be misleading and may result in unwarranted stress and anxiety.

The sample you donate will be stored indefinitely. This will enable researchers to reexamine the DNA you have donated as new genetic markers for different illnesses are uncovered. All DNA samples collected will be destroyed by incineration at the conclusion of the Tasmanian Healthy Brain Study.

In consenting to genetic sampling, you are consenting to not being informed of the results of your genetic testing and to permitting your DNA sample to be retained until the completion of the Tasmanian Healthy Brain Study. If you do not wish to consent to this, you will still be able to participate in the Tasmanian Healthy Brain Study without prejudice. In the *highly unlikely* event that future research identifies a genetic marker that accurately identifies which person will develop dementia with absolute certainty, we will take steps to screen all DNA samples for these markers and provide this information to each individual participant who provides written consent to receiving this information.

4. Are there any possible benefits from participation in this study?

The DNA sample you provide may assist researchers better understand the role of genetic factors in cognitive function and disease in old age. As such, the benefit from the study may be for future generations rather than an immediate benefit to yourself.

5. Are there any possible risks from participation in this study?

There are no known risks associated with salivary sample collection.

6. What if I have questions about this research?

If you would like to discuss any aspect of this study please feel free to contact either Dr Mathew Summers on ph 6324 3266 or Professor James Vickers on ph 6226 4830. Either of us would be happy to discuss any aspect of the research with you.

This study has been approved by the Tasmanian Social Science Human Research Ethics Committee. If you have concerns or complaints about the conduct of this study should contact the Executive Officer of the HREC (Tasmania) Network on (03) 6226 7479 or email human.ethics@utas.edu.au. The Executive Officer is the person nominated to receive complaints from research participants. You will need to quote **H1070**.

Thank you for taking the time to consider this study.
If you wish to take part in it, please sign the attached consent form.
This information sheet is for you to keep.

Appendix G – Consent Form

UTAS FACULTY OF SCIENCE, ENGINEERING & TECHNOLOGY School of Psychology

WICKING DEMENTIA RESEARCH & EDUCATION CENTRE

CONSENT FORM FOR GENETIC TESTING

Title of Project: **Tasmanian Health Brain Study – Genetic Testing**

1. I have read and understood the 'Information Sheet' for this project.
2. The nature and possible effects of the study have been explained to me.
3. I understand that the study involves collection of a 2ml sample of my saliva. I understand I can collect this sample at home and post it back to the researchers or I can donate a sample at the Tasmanian Healthy Brain Study research centres in Hobart, Launceston or Burnie.
4. I consent to not being informed of the results of my genetic testing. I understand that this is because there are no reliable genetic markers for dementia and that the provision of such information would be misleading to me. In the *highly unlikely* event that future research identifies a genetic marker that accurately identifies which person will develop dementia with absolute certainty, I understand that the researchers will screen all DNA samples for these markers and provide this information to each participant who consents to receiving this information.
5. I consent to the storage of my DNA sample until the conclusion of the Tasmanian Healthy Brain Study for use by researchers in the Tasmanian Healthy Brain Study. I understand that my DNA sample will be stored in a secure DNA storage facility at the Menzies Research Institute Tasmania. I understand that at the conclusion of the Tasmanian Healthy Brain Study my DNA sample will be destroyed by incineration.
6. I understand that my DNA sample will not be used by any research other than the Tasmanian Healthy Brain Study.
7. I understand that my DNA sample will be identified only by my unique participant ID code. I understand that only Dr Mathew Summers and Prof James Vickers are able to match my participant ID code with my name and contact details.
8. I understand that all research data will be securely stored on the University of Tasmania premises for at least five years following publication of the results, and will be destroyed when no longer required.
9. Any questions that I have asked have been answered to my satisfaction.
10. I agree that research data gathered from me for the study may be published provided that I cannot be identified as a participant.
11. I understand that the researchers will maintain my identity confidential and that any information I supply to the researcher(s) will be used only for the purposes of the research.
12. I agree to participate in this investigation and understand that I may withdraw at any time without any effect, and if I so wish, may request that any data I have supplied to date be withdrawn from the research.

UTAS FACULTY OF SCIENCE, ENGINEERING & TECHNOLOGY School of Psychology

WICKING DEMENTIA RESEARCH & EDUCATION CENTRE

Name of Participant:

Signature: Date:

Statement by Investigator

☐ I have explained the project & the implications of participation in it to this volunteer and I believe that the consent is informed and that he/she understands the implications of participation

If the Investigator has not had an opportunity to talk to participants prior to them participating, the following must be ticked.

☐ The participant has received the Information Sheet where my details have been provided so participants have the opportunity to contact me prior to consenting to participate in this project.

Name of Investigator

Signature of Investigator

Name of investigator _____

Signature of investigator _____ Date _____

Appendix H – Data Analyses for Chapters 3, 4 & 5

These articles have been removed for copyright or proprietary reasons.

Ward, D. D., Summers, M. J., Saunders, N. L., & Vickers, J. C., (2014), Modelling cognitive reserve in healthy middle-aged and older adults: The Tasmanian Healthy Brain Project, International psychogeriatric, 27(4), 579-589.

Ward, D. D., Summers, M. J., Saunders, N. L., Janssen, P., Stuart, K. E., Vickers, J. C., (2014), APOE and BDNF Val66Met polymorphisms combine to influence episodic memory
function in older adults, Behavioural brain research, 271, 309-315

Appendix K – Manuscript Submission for Chapter 5

The BDNF Val66Met polymorphism moderates the relationship between cognitive reserve and executive function

David D. **WARD**, *BPsych(Hons)* [1,2], Mathew J. **SUMMERS**, *PhD* [2,3], Nichole L. **SAUNDERS**, *PhD* [2], Karen **RITCHIE**, *PhD* [4,5], Jeffery J. **SUMMERS** *PhD* [1,6], & James C. **VICKERS**, *PhD* [1,2]

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[4] Neuroepidemiology of Ageing Research Unit, Imperial College, London, United Kingdom
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[5] Research Institute for Sport and Exercise Sciences, Liverpool John Moores University, Liverpool, United Kingdom.

RUNNING TITLE **BDNF Val66Met moderates CR and executive function**

Corresponding author: Assoc. Prof. Mathew Summers, School of Social Science, University of the Sunshine Coast, Locked Bag 4, Maroochydore DC, Queensland, Australia, 4558. Tel +61 7 5456 3758; email: msummers@usc.edu.au

ABSTRACT = 151 words
INTRODUCTION = 587 words
ARTICLE = 3100 words
TABLES = 3
FIGURES = 1

Abstract

The concept of cognitive reserve (CR) has been proposed to account for observed discrepancies between pathology and its clinical manifestation. Hypothesized to reduce the consequences of both ageing-related and pathology-related cognitive changes in the elderly, CR is assumed to be a functional consequence of underlying differences in brain structure and function. We investigated whether common polymorphic variations in apolipoprotein E (*APOE)* or brain-derived neurotrophic factor (*BDNF)* influenced the association between cognitive reserve contributors and healthy executive function in older adults. We show that *BDNF* Val66Met moderates the association between cognitive reserve and cognitive function. Cognitive reserve accounted for 8.5% of the variance in executive function in *BDNF* Val homozygotes, but cognitive reserve was a non-significant predictor in *BDNF* Met carriers. APOE polymorphisms were not linked to the influence of cognitive reserve on cognitive function. This result implicates BDNF in playing an important role in capacity for building or accessing cognitive reserve.

KEYWORDS: brain-derived neurotrophic factor; BDNF; executive function: cognitive reserve; moderation; aging

Observations of significant heterogeneity in the clinical manifestation of underlying brain pathology gas led to the development of the concept of cognitive reserve (CR) [1]. CR level is considered to be determined by both biological and environmental exposures, notably intelligence [2], education [3] and occupational attainment [4]. Epidemiological evidence further supports the notion of CR by demonstrating consistent associations between lifestyle characterized by intellectual and social engagement and slower cognitive decline [5]. In older persons, fMRI studies of neural networks suggest differences in functionally connected regions in persons with high CR may enhance the compensatory capacity of individuals in the face of both normal and pathological brain ageing [6]. Alzheimer's disease (AD) patients with higher educational and occupational attainment have more rapid cognitive decline that those with lower attainment, consistent with the idea that at a given common level of severity, underlying AD pathology is more advanced in patients with more CR [7]. That individuals with higher CR display greater resistance to the effects of neuropathology is likely due to induced cortical plasticity caused by a prolonged mismatch between functional supply and task demands [8], resulting in more flexible and denser neural networks [9]. As adult cognitive function and cognitive ageing show significant heritability [10], research examining the interaction between genes and CR proxies are justified. The most likely candidate genes for CR are those that interact with environmental factors to induce effects on cognitive functions [11].

One gene that potentially interacts with CR is apolipoprotein E (*APOE*). While it has been established that specific allelic variants in the *APOE* gene are associated with risk for late-onset AD [12], it is less clear whether other allelic variants of the *APOE* gene impart healthy cognitive function [13, 14]. However, *APOE* polymorphisms have been shown to exert divergent neuroprotective effects [15, 16]. For example, the presence of *APOE* ε4 is associated with an

increased rate of AD-related hippocampal atrophy [17], and impaired synaptic plasticity [18] when compared to *APOE* ε4 non-carriers. Lifestyle activities that require cognitive effort (e.g. completing puzzles, playing chess) predict verbal fluency and semantic memory recall in *APOE* ε4 non-carriers but not in carriers [19].

A second gene that may be associated with CR is brain-derived neurotrophic factor (*BDNF*). The encoded neurotrophin is crucial to neuronal survival, maintenance, neurogenesis, and synaptic plasticity [20, 21]. The *BDNF* Val66Met polymorphism affects activity-dependent secretion of BDNF, with the Met allele associated with reduced depolarization-induced BDNF release into the synapse [22]. *BDNF* Met alleles have been associated with reduced memory capacity [22], but not consistently [14, 23]. Like *APOE*, impaired synaptic plasticity is a feature of *BDNF* Met alleles [24, 25].

Synaptic plasticity is a key mechanism that facilitates the development of CR [26]. Research to date has examined CR in the presence of pathology; however, as CR is developmentally acquired through both intrinsic (biologic) factors such as genetic heritability as well as extrinsic (environmental) factors such as education, it is imperative to examine the development of CR prior to the onset of clinical symptomatology. We aimed to use baseline data from the Tasmanian Healthy Brain Project (THBP) [27] to investigate the relationship between CR, cognitive function, and *APOE* and *BDNF* Val66Met polymorphisms in healthy older adults (50-79 years). We tested two hypotheses. First, that higher CR is associated with better cognitive performance. Second, that *BDNF* Val66Met and *APOE* polymorphisms independently moderate the association between CR and cognitive performance, with carriers of the putative detrimental alleles (Met and ε4, respectively) showing a reduced influence of CR when compared to non-carriers.

[INSERT TABLE 1 HERE]

Methods

Participants

The participants were 433 community-residing healthy older adults, aged 50-79 years, who were all native-English speakers or spoke English as a primary language. They had consented to participate in the THBP, which involves comprehensive annual assessments of neuropsychological, health, and psychosocial function. The THBP is a prospective longitudinal study examining whether late-life education provides protection from dementia through enhancement of cognitive reserve [27]. Subjects were excluded from participating if they had a history of any condition independently associated with impairments to cognitive function (dementia; multiple sclerosis; previous significant head injury requiring hospitalization; epilepsy; history of cerebro-vascular complications; diabetes – poorly controlled; blood pressure complications – poorly controlled; other neurological disorders; chronic obstructive pulmonary disease; heart disease; blindness; deafness; psychiatric disorder). The THBP was conducted with full approval from the Tasmania Human Research Ethics Network and in accordance with the ethical guidelines of the National Health and Medical Research Council of Australia (NHMRC).

Materials

A comprehensive test battery was completed by subjects as described in detail elsewhere [27]. Multiple objective measures were used to screen participants for: symptoms of dementia,

clinically significant symptoms of depression or anxiety, general health, medical conditions, prescription medication use, drug and alcohol use, handedness, height, weight, marital status, educational and occupational history.

Neuropsychological assessment battery

Cognitive functions were assessed using tests that measured learning and memory, working memory, executive function, and language processing, and are described in detail elsewhere [27]. The test battery incorporated standardised neuropsychological measures with established reliability and validity in the measurement of: visual and verbal episodic memory, visual and verbal immediate memory span, visual and verbal working memory capacity, word knowledge, semantic memory recall, language comprehension, and multiple executive functions including attention, concentration, information processing speed, decision making capacity, and reaction time.

Assessment of cognitive reserve

To generate an estimate of CR, we used a previously developed equation to combine measures of lifetime education, occupational attainment, intelligence, and participation in cognitively stimulating leisure activities into a single variable [28]. The Wechsler Test of Adult Reading (WTAR) [29] was used to estimate premorbid intellectual capacity, the Lifetime of Experiences Questionnaire (LEQ) [30] to quantify prior lifetime participation in cognitive activities, and the Medical Health Screening questionnaire to record the number of years of prior formal education [27].

Genotyping

DNA samples were collected with Oragene DNA self-collection kits (Genotek Inc., 2012). *APOE* and *BDNF* genotypes were determined through one-step amplified refractory mutation system polymerase chain reaction (ARMS-PCR) [31] and subsequent gel electrophoresis. For *APOE*, rs429358 and rs7412 were determined by following the method described by Donohoe, Salomaki [32]. For *BDNF*, Val66Met was determined by following the method described by Sheikha, Hayden [33]. PCR amplifications were undertaken in a 12 µl reaction volume that contained approximately 50 ng of genomic DNA. PCR amplicons were resolved on 2% agarose gel. Genotyping was repeated on samples to ensure accuracy.

Procedure

Trained assessors carried out the neuropsychological testing as part of the baseline THBP assessments [27]. The assessment process took approximately four hours to complete and was undertaken in a quiet room. Subjects were encouraged to take 10-minute breaks when required to reduce fatigue.

Data analysis

Prior to the main analyses, variables of CR and cognitive function were generated. To represent CR, we used a previously developed equation to create a comprehensive single-point measure of the construct [28]. Standardised scores of CR variables (Table 1) were entered into the CR equation to yield total CR for each subject. Composite cognitive domain variables were also computed through a principal components analysis that combined measured variables (raw scores) into specific domains (episodic memory, working memory, executive function, language processing). The extracted factor that explained the highest proportion of variance was retained to represent each cognitive domain (Table 2). The *APOE* and *BDNF* predictor variables were coded as carriers or non-carriers of the detrimental allele,

ε4 and Met, respectively. To assist in interpretation, subjects with the *APOE* ε2/ε4 polymorphism were excluded from the analysis.

The primary analyses used PROCESS v2.11 [34] to test whether CR was associated with cognitive function, either independently or through CR x *APOE*/CR x *BDNF* interaction. PROCESS is a computational tool for path analysis-based moderation and mediation analysis that provides coefficient estimates for total, direct, and indirect effects of variables using OLS regression. Prior to analysis, the continuous independent predictor (CR) was mean-centered to reduce potential multicollinearity. Subsequent analyses involved testing for direct effects of predictors (CR, *APOE*, *BDNF*) and indirect effects of possible CR moderators (*APOE*, *BDNF*) on cognitive function domains while covarying for age. First, regression equations were fitted to cognitive domain data and the predictive capacity of age and CR were assessed. Second, *APOE*/*BDNF* data and corresponding CR-gene product variables were entered to test for CR moderation in separate models. An alpha value of .05 was used for all statistical tests and all data were analysed in SPSS v21.

Results

Participants

The sample comprised 433 participants with a mean age of 62.16 years (*SD* = 6.81) and an above average estimated full-scale IQ (*M* = 112.47, *SD* = 5.52). Participants were mostly female (66.7%) and had completed an average of 13.97 (*SD* = 2.73) years of formal education. To assist in interpretation, 11 participants were excluded due to possessing the *APOE* ε2/ε4 genotype. The characteristics of the remaining participants are presented in

Table 1. The *APOE* ($\chi^2_{(1, N = 422)} = 0.02$, $p. = 0.90$) and *BDNF* Val66Met ($\chi^2_{(1, N = 422)} = 0.31$, $p. = 0.58$) genotype distributions did not differ significantly from Hardy-Weinberg equilibrium.

[INSERT TABLE 2 HERE]

Cognitive reserve and age

PROCESS was used to fit linear regression models to the cognitive domain data. With the age and CR predictors entered, significant models were produced for all assessed cognitive functions: episodic memory ($F_{(2, 398)} = 22.15$, $p < .01$, $R^2 = .10$); working memory ($F_{(2, 398)} = 28.96$, $p < .01$, $R^2 = .13$); executive function ($F_{(2, 394)} = 52.88$, $p < .01$, $R^2 = .21$); and language processing ($F_{(2, 412)} = 34.15$, $p < .01$, $R^2 = .14$). The CR variable had a significant positive association with each cognitive domain ($p < .05$) while the age variable had a significant negative association with each cognitive domain ($p < .01$), excluding language processing ($p = .62$). The individual contribution that each predictor made to the model is detailed in Table 3.

Gene-cognitive reserve interactions

We then assessed whether the inclusion of *APOE*/*BDNF* Val66Met allelic carrier data significantly improved the fit of the models. Notably, no significant main effects of genetic predictors, *APOE* or *BDNF* Val66Met, were identified for any cognitive domain. A moderation analysis (PROCESS) was then conducted examining whether the inclusion of CR x *APOE* or CR x *BDNF* interaction terms significantly improved the fit of regression models. Results indicated that a single genetically based moderation effect on CR was present (Table

3). Specifically, inclusion of the CR x *BDNF* Val66Met interaction term led to a significant increase in the amount of variance in executive function explained by the model ($\Delta R^2 = .01$, p =.05). Simple slopes analysis was conducted in order to determine the basis of the moderation effect of BDNF polymorphism on the conditional effect between CR and executive function and tested whether the slopes statistically differed from zero for *BDNF* Met carriers and non-carriers, separately. These analyses indicate that a significant positive relationship between CR and executive function was identified in *BDNF* Val homozygotes ($B = 0.13$, $t = 5.44$, $SE = 0.02$, $p < .01$), but was not evident in *BDNF* Met carriers ($B = 0.05$, $t = 1.41$, $SE = 0.03$, $p = .16$). In *BDNF* Val homozygotes, CR accounted for a significant 8.5% of variance in executive function performance. In *BDNF* Met carriers, CR accounted for a non-significant 1.2% of variance in executive function performance. Simple slopes are presented in Figure 1.

[INSERT FIGURE 1 HERE]

Discussion

The present study was designed to investigate whether a composite measure of CR was associated with healthy cognitive function, either independently or through an interaction with genetic *APOE*/*BDNF* Val66Met polymorphic data, in participants of the Tasmanian Healthy Brain Project. The first hypothesis, that higher CR is associated with better cognitive

performance, was supported. Our sample showed significant positive relationships between CR and cognitive function across all cognitive domains, after accounting for the effects of age. The second hypothesis, that *BDNF* Met carriers and *APOE* ε4 carriers would display a lower relationship between CR and cognitive function when compared to their respective non-carriers, was partially supported. The analyses revealed a single significant CR x *BDNF* interaction in predicting age-adjusted executive function performance. In this regard, the relationship between CR and executive function was moderated by *BDNF* genotype, with a positive association between the variables present in *BDNF* Val homozygotes that was absent in *BDNF* Met carriers.

CR is of interest due to its potential role in varying the age at which an individual develops dementia and the subsequent rate of dementia progression experienced [9], as well as mitigating against the effect of other CNS insults. However, while previous research frequently highlights the relationship between increased CR and reduced dementia incidence [35], other investigations have suggested that CR is also involved in non-pathological cognitive function. For instance, during a memory task, higher functional connectivity, indicative of heightened cognitive effort, is found in subjects with lower CR, despite achieving the same level of performance as those with higher CR [36]. Similarly, CR was positively associated with brain volume but negatively associated with cortical activity during a visual encoding task [37]. Such findings are consistent with the CR hypothesis, which posits that CR is implicated in non-pathological cognitive function [1], particularly in relation to cerebral network efficiency [38]. Our finding, that CR was positively associated with all four assessed cognitive domains, adds further evidence that CR accounts for healthy cognitive function and that cognitive associations with CR are not limited to protection from the clinical expression of pathological processes. At the very least, higher scores on CR proxy variables would result in a delayed

onset of dementia due to the pre-existing cognitive advantage afforded to high CR individuals through greater education, occupational attainment, intelligence, and participation in cognitively stimulating lifestyle activities. In high CR individuals a greater loss of cognitive function, resulting from greater levels of neuropathology, would be required to produce functional deficits of sufficient severity to meet diagnostic criteria for dementia.

A comprehensive review of the evidence for genetic contributions to CR noted that those genes that interact with environmental factors to produce a cognitive effect are the most likely candidates [11]. In our study, we identified a significant moderating influence of *BDNF* Val66Met polymorphism on the relationship between CR and executive function. In those individuals who were *BDNF* Val homozygotes, a one point increase in CR was associated with a 0.13 point of increase in executive function. Comparatively, in those individuals who were *BDNF* Met carriers, a one point of increase in CR was associated with a non-significant 0.05 point increase in executive function. This finding is of particular importance due to the overlap between CR and executive function [39]. Both executive function and CR have an underlying reliance on cognitive flexibility [1, 40] and resources associated with frontal lobe activity [41], with a positive association of education and frontal engagement present in older adults that is not seen in younger adults [42]. Clinical studies confirm that there is an age-related decline in executive function [43], and that the onset of dementia is often characterised by early impairment of executive function [44]. Overall, our findings indicate that while *BDNF* Val homozygotes show normal positive associations between CR and executive function, *BDNF* Met carriers have a reduced influence of CR on executive function.

No CR-*APOE* interaction was found in the current study. Previous longitudinal investigations have reported interactions between *APOE* ε4 alleles, education, and the rate of age-related

cognitive decline [45, 46]. However, a recent study found an interaction of CR and *APOE* polymorphism in cross-sectional but not longitudinal data [19]. This investigation found that verbal fluency and semantic memory recall performance was predicted by participation in cognitively stimulating lifestyle activities in *APOE* ε4 non-carriers but not in carriers [19]. The discrepancy between our results and those of Runge, Small [19] may reflect differences in the method used to quantify lifetime cognitive stimulation. Runge, Small [19] assessed low (e.g. singing, traveling) and high (e.g. puzzles, viewing educational television) cognitive demand lifestyle activities, whereas we took into account a wider range of CR measures, including years of formal education and occupational attainment [28].

One explanation for the significant moderation of CR by *BDNF* Val66Met lies within differences in biochemical responses to cognitively stimulating environments. Namely, individuals with *BDNF* Met alleles may receive a lesser impact on neurological function of exposure to environmental activities that contribute to CR, whereas, for Met non-carriers there is a greater beneficial effect of exposure to environmental activities on neurological function. Previous research has demonstrated that long-term exposure to cognitively stimulating environments is associated with a widespread increase in BDNF expression in the brain [47], but *BDNF* Met carriers have both reduced activity-dependent secretion of BDNF [22] and impaired synaptic capacity for long term potentiation/depression [48]. Consequently, for two individuals exposed to the same level of extrinsic environmental stimulation, the individual who carries the BDNF Met polymorphism exhibits reduced synaptic plasticity and neuronal restructuring subsequent to environmental stimulation and consequently displays lowered functional capacity due to the negative intrinsic effect of BDNF Met polymorphism on development of CR. Studies examining the influence of environmental enrichment (EE) in rodents report that EE only confers benefits in the presence of BDNF protein. For example,

environment-induced hippocampal neurogenesis occurs only in the presence of BDNF [49] and upregulation of BDNF by stimulating environments promotes neural plasticity [50]. Although EE in experimental animals and measures of CR in humans are unlikely to be analogous, a comprehensive review concluded that, as with human educational and occupational attainment, EE develops reserve that can be used to offset the impact of cortical lesions [51].

The results of the present study have several implications. First, as higher CR was associated with higher overall cognitive function, CR exerts an impact on healthy cognitive function, independent of dementia. While the cross-sectional analyses employed in the present study do not allow comment on the relationship between CR and rate of age-related cognitive decline, the results indicate that, at the very least, CR may benefit cognitive function in older age due to the persistent effects of improved cognitive function. Second, the results of the present study demonstrate that a genetic factor moderates the effect that CR has on cognitive function. Specifically, *BDNF* Met carriers showed a reduced relationship between CR and executive function when compared to non-carriers. This result provides evidence that *BDNF* Met carriers have a partial 'disconnect' between CR and cognitive function, and future longitudinal analyses will examine whether *BDNF* Val66Met mediates environmentally generated protection from dementia. In relation to non-carriers, the identification of persons with very high resistance to the functional consequences of brain pathology may provide a cohort for the development of future epigenetic strategies to reduce dementia in the face of AD disease progression.

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Acknowledgements

This research was supported by National Health and Medical Research Council (NHMRC) of Australia Project Grant (No: 1003645) and the J.O. and J.R. Wicking Trust (Equity Trustees).

Author contributions

DW – performed analyses and prepared manuscript

MS – oversaw analyses and prepared manuscript

NS – manuscript preparation and data collation

KR – manuscript preparation

JS – manuscript preparation

JV – manuscript preparation

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Conflicts of interest

Assoc. Prof. Summers reports personal fees from Eli Lilly (Australia) Pty Ltd, grants from Novotech Pty Ltd, outside the submitted work. All other authors declare no conflicts of interest.

Table 1. **Descriptive statistics for study population stratified by *APOE* and *BDNF* genotype**

Characteristic	*N*	*APOE* status			*BDNF* status		
		ε4-	ε4+	*p*.	Met-	Met+	*p*.
Demographics							
Age (years)	422	62.31 (6.83)	61.67 (6.69)	.53	62.16 (6.85)	61.99 (6.68)	.81
Gender (male/female %)	422	31.9/68.1	35.8/64.2		31.6/68.4	36.4/63.6	
Genotype (%)	422	67.5	32.5		66.8	33.2	
Screening							
DRS-2 AEMSS	422	11.93 (2.12)	12.16 (2.03)	.56	12.04 (2.10)	11.93 (2.08)	.61
HADS anxiety	421	5.21 (3.12)	5.35 (2.97)	.39	5.35 (3.19)	5.07 (2.80)	.38
HADS depression	421	2.36 (2.14)	2.58 (2.34)	.24	2.42 (2.26)	2.45 (2.10)	.91
Cognitive reserve [28]							
WTAR Est. FSIQ	421	112.57 (5.39)	112.16 (5.94)	.86	112.40 (5.49)	112.51 (5.76)	.85
Prior education (years)	422	14.13 (2.81)	13.61 (2.59)	.19	13.73 (2.72)	14.44 (2.75)	.01
LEQ Young Adulthood Specific	421	16.19 (7.93)	15.51 (7.65)	.41	15.56 (7.61)	16.78 (8.25)	.13
LEQ Young Adulthood Nonspecific	421	24.71 (5.54)	24.93 (5.11)	.19	24.42 (5.45)	25.52 (5.23)	.05
LEQ Midlife Specific	420	19.07 (5.01)	19.06 (4.93)	.45	19.09 (4.82)	19.02 (5.28)	.90
LEQ Midlife Nonspecific	420	24.37 (5.55)	24.36 (5.60)	.46	24.08 (5.72)	24.93 (5.20)	.14
LEQ Continuing Education Bonus	418	10.22 (8.79)	9.38 (8.01)	.46	9.95 (8.45)	9.94 (8.75)	.99

Note: Data represented are mean values (*SD*) for continuous variables and proportions for categorical variable. DRS-2 = Dementia Rating Scale-2; AEMSS = Age- and education-corrected Mayo Older American normative Studies (MOANS) scaled score; HADS = Hospital Anxiety and Depression Scale; WTAR = Wechsler Test of Adult Reading; FSIQ = Full-scale IQ; LEQ = Lifetime of Experiences Questionnaire; ε4-/Met- = participants not carrying the *APOE* ε4/*BDNF* Met alleles; ε4+/Met+ = participants carrying at least one copy of the *APOE* ε4/*BDNF* Met alleles.

Table 2. **Factor analysis results for composite cognitive domain variables**

Cognitive domain	Initial eigenvalue	Variable	*N*	Mean	*SD*	Loading
Episodic memory	2.50 (62.46%)	RAVLT 1-5 total	407	52.96	8.92	.76
		LM I immediate recall total	407	48.43	8.07	.89
		LM II delayed recall total	407	30.29	6.28	.86
		PAL first trial memory score	407	18.35	3.46	.63
Working memory	2.04 (50.93%)	Digit span	407	18.63	3.90	.76
		Letter-number sequencing	407	11.59	2.40	.79
		SWM between errors	407	25.36	18.64	-.63
		SSP length	407	5.75	1.20	.66
Executive function	1.80 (59.83%)	Stroop trial C	403	26.26	7.63	.76
		RVP A’	403	0.91	0.05	-.81
		TMT trial B	403	59.09	18.82	.75
Language processing	1.88 (62.81%)	WAIS Vocabulary	421	56.62	6.23	.88
		WAIS Comprehension	421	26.21	3.33	.77
		Boston Naming Test	421	57.49	3.42	.72

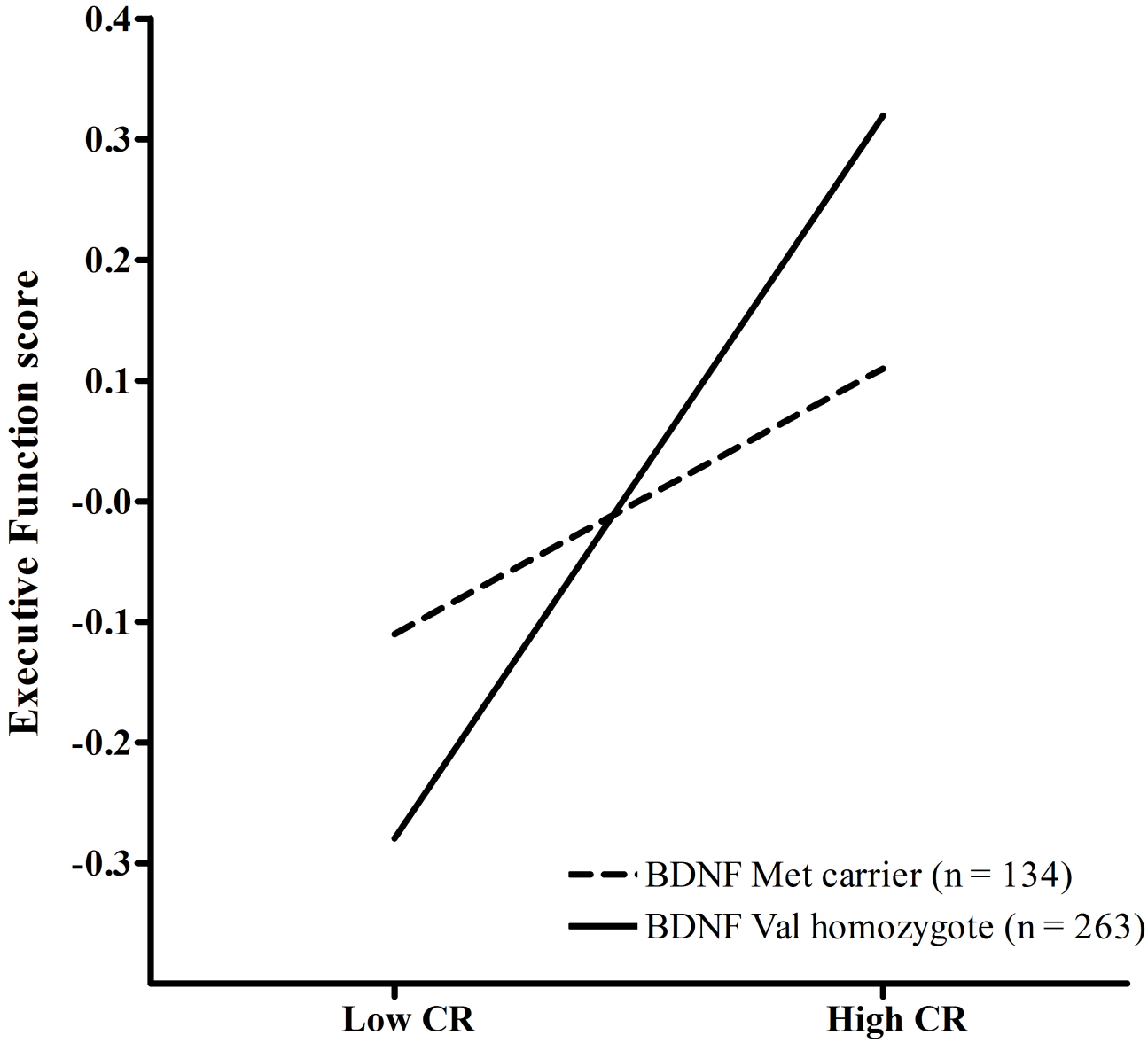
Note: Data in parentheses represent the proportion of variance (%) explained by the resulting factor. RAVLT = Rey Auditory Verbal Learning Test; LM = Logical Memory; SWM = Spatial Working Memory; SSP = Spatial Span; RVP = Rapid Visual Processing; TMT = Trail Making Test; WAIS = Wechsler Adult Intelligence Scale.

Table 3. **Results of the regression analyses for cognitive domain data**

Cognitive domain	Predictor	*N*	B	SE	t	*p*.	R^2 change	F
Episodic memory	Age	401	-0.05	0.01	-6.51	**< .01**		
	CR	401	0.04	0.02	2.01	**.05**		
	APOE	401	-0.08	0.10	-0.84	.40		
	BDNF	401	-0.03	0.10	-0.25	.80		
	CR x APOE	401	-0.02	0.05	-0.35	.72	0.00	0.12
	CR x BDNF	401	-0.06	0.04	-1.41	.16	0.00	1.99
Working memory	Age	401	-0.05	0.01	-7.11	**< .01**		
	CR	401	0.07	0.02	3.37	**< .01**		
	APOE	401	0.07	0.10	0.70	.48		
	BDNF	401	-0.11	0.10	-1.12	.26		
	CR x APOE	401	0.04	0.04	0.99	.32	0.00	0.99
	CR x BDNF	401	0.04	0.04	0.99	.32	0.00	0.98
Executive function	Age	397	-0.06	0.01	-9.40	**< .01**		
	CR	397	0.10	0.02	5.23	**< .01**		
	APOE	397	0.12	0.10	1.25	.21		
	BDNF	397	-0.02	0.10	-0.17	.86		
	CR x APOE	397	0.02	0.04	0.40	.69	0.00	0.16
	CR x BDNF	397	-0.08	0.04	-1.98	**.05**	0.01	3.94
Language processing	Age	415	0.00	0.01	0.49	.62		
	CR	415	0.16	0.02	8.15	**< .01**		
	APOE	415	0.08	0.10	0.87	.38		
	BDNF	415	-0.07	0.10	-0.69	.49		
	CR x APOE	415	0.03	0.04	0.62	.54	0.00	0.38
	CR x BDNF	415	-0.05	0.04	-1.30	.19	0.00	1.70

Note: Apolipoprotein E and Brain-derived neurotrophic factor main and interaction effects were tested in separate models. CR = cognitive reserve; APOE = Apolipoprotein E; BDNF = Brain-derived neurotrophic factor.

Figure 1. *BDNF* Val66Met moderates the relationship between cognitive reserve (CR) and executive function scores. Plot represents age-adjusted executive function performance as predicted by the composite CR proxy variable for those with low CR (CR scores less than the mean – 1 standard deviation) and high CR (CR scores greater than the mean + 1 standard deviation) for *BDNF* Met carriers and Val homozygotes, separately.


0.4
0.3
0.2
0.1
-0.0
-0.1
-0.2
-0.3
Executive Function score
Low CR
High CR
BDNF Met carrier (n = 134)
BDNF Val homozygote (n = 263)