The role of the *BDNF* Val^{66}Met polymorphism in synaptic plasticity and Recognition memory.

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Abstract

A single nucleotide polymorphism of the human BDNF gene (Val^{66}Met) may account for much of the variation in human memory performance. BDNF may influence memory via, either a modulation of acute plasticity (i.e. LTP), or a chronic influence on developing memory systems. Not only this, but BDNF could interact with different memory types in different ways. BDNF is concentrated most heavily in the hippocampus, and therefore would be likely to have a greater effect on hippocampal dependent memory. Recognition memory involves the contribution of two distinct retrieval processes, Recollection and Familiarity. Prior research suggests that Familiarity does not depend on the hippocampus, but Recollection does. Recent evidence has shown Recollection and Familiarity are associated with distinct event-related potentials (ERP): Familiarity with an early-onset effect called the FN400; and Recollection with a later positivity called the late positive component (LPC). Recent evidence suggests the successful recognition of famous faces is dependent on the hippocampus, whilst recognising a non-famous face is not.

Study 1 employed a recently developed human sensory LTP paradigm in the intact human brain, and found subjects carrying the Met allele (Val/Met and Met/Met) had significantly less LTP than Val/Val individuals. Met/Met individuals also performed significantly less well in a test of visual memory. Further, the degree of LTP was significantly correlated with the index of visual memory. Study 2 found no genotype differences in FN400 amplitude (evoked when correctly recognising a previously presented face) were found. However, Val/Val individuals generated a significantly more positive LPC when correctly identifying an old face after a period of consolidation of 24 hours. Study 3 found differences between famous and non-famous face recognition, as well as between Val homozygotes and Met carriers. Famous faces

elicited significantly greater activation in the hippocampus than non-famous faces. There was greater hippocampal activation seen for Val/Val individuals. There were no clear differences in the recognition of non-famous faces. These results suggest an exclusive role of BDNF in hippocampal dependent memory, with the mechanism of this likely to be a person's ability to display LTP.

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Abbreviations

AA	Arachidonic acid
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropinate
AMPAR	AMPA receptor
ANOVA	Analysis of variance
AP5	Aminophosphonovaleric acid
APP	Aβ precursor protein
APV	2-amino-5-phosphonovalerate
BDNF	Brain-derived neurotrophic factor
BOLD	Blood-oxygen-level-dependent (response)
c/deg	Cycles per degree (of visual angle)
Ca^{2+}	Calcium
CaM	Calmodulin
CaMKII	Calcium/calmodulin-dependent protein kinase II
cAMP	Cyclic adenosine 3',5'-monophosphate
CPP	3-(2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid
CREB	cAMP-response-element-binding
EEG	Electroencephalography
ERP	Event-related potential
fMRI	Functional magnetic resonance imaging
FN400	Frontal Negativity 400
FWHM	Full-width-at-half-maximum
GABA	Gamma-aminobutyric acid
ICA	Independent components analysis
K^{+}	Potassium
LGN	Lateral geniculate nucleus
LTD	Long-term depression
LTP	Long-term potentiation
LPC	Late positive component
MAPK	Mitogen-activated protein kinase
M-cell	Mauthner cell
MEP	Motor-evoked potential
Mg^{2+}	Magnesium
mGluRs	Metabotropic glutamate receptors
Na^{+}	Sodium
NMDA	N-methyl-D-aspartate
NMDR	NMDA receptor
NO	Nitric oxide
NT	Neurotransmitter
PKA	Protein kinase A
PKC	Protein kinase C
PTP	Post-tetanic potentiation
PVC	Primary visual cortex (human)
RF	Receptive field
R/K	'Remember/Know'
rTMS	Repetitive TMS
STP	Short-term potentiation
TBS	Theta-burst stimulation

TMS	Transcranial magnetic stimulation
TPO	Temporo-parieto-occipital junction
TrkB	Tyrosine-related kinase B
V1	Primary visual cortex (animal)

Disclaimers

Chapters 2, 3 and 4 of this thesis are either submitted or in the process of submission for publication.

Chapter 1. Introduction

The field of human cognitive neuroscience is often limited by a lack of direct data on molecular-level workings of the brain. The invasive nature of molecular neuroscience methods limits their use in human cognitive neuroscience, leaving the more fundamental workings of the brain to be inferred. However, the presence of genetic mutations in the form of single nucleotide polymorphisms (SNPs) provide an interesting potential window into the role of molecular level neural processes, and their effects on behaviour, in human participants. A number of genetic mutations affecting the function of certain neuroactive proteins, have been uncovered recently, and investigating behavioural differences in individuals who have these mutations has proved fruitful. One such protein that has garnered a lot of attention of late is brain-derived neurotrophic factor (BDNF). Some people have much better memory than others. This variation in memory ability may be due to the variation of a gene that controls secretion of brain-derived neurotrophic factor (BDNF). In humans, a frequent single nucleotide polymorphism (SNP) to the *BDNF* gene has recently been discovered (dbSNP number rs6265). This SNP is seen as a non-conservative amino acid substitution, switching a valine to a methionine, at codon 66 (val^{66}met). A large body of research from an NIH research group headed by Daniel Weinberger has shown that the Val^{66}Met polymorphism is associated with poorer memory. For example, Egan et al (2003) showed in both healthy individuals and schizophrenics, that individuals carrying a copy of the Met allele did significantly more poorly on a test of verbal episodic memory from the Wechsler Memory Scale revised version (WMS-R; Wechsler, 1987). However, many questions remain unanswered. Firstly, what is the mechanism by which BDNF is affecting memory processes? And are all forms of memory

affected equally by this polymorphism? Are behavioural differences supported by differences in brain activity? These are the key questions the following thesis will attempt to answer. The structure of this introduction is as follows: Firstly memory processes will be broadly described, with a particular focus on recognition memory. Long-term potentiation, a molecular mechanism that is thought to be the basis of memory, will also be described and discussed. Following on from this will be a description of measuring LTP in humans. Secondly, the *BDNF* Val66Met polymorphism will be looked at in more detail. Investigations into how *BDNF* polymorphism affects human behaviour (and memory processing in particular) will be outlined. Following from this will be an exploration into the different memory systems. Finally, a potential mechanism for how BDNF affects human behaviour will be examined. More specifically, the area of long term potentiation (LTP) will be introduced. BDNF is known to play a large part in the maintenance of LTP. As LTP is currently thought to be the best synaptic model of learning and memory, this will also be investigated.

1.1 Memory

There is a general consensus that there exist two distinct forms of memory. Implicit memory describes the retention of motor skills for instance the ability to walk. Explicit memory is the other form, which in itself can then be split into two separate categories. Semantic memory describes the retention of facts about the world and other non-autobiographical information, while episodic memory describes the retention of events in one's own life (Tulving, 1983). There is also evidence to suggest that these types of memory rely on different neural substrates. Ever since the case of H.M. (Scoville & Milner, 1957), there has been intense interest in the medial temporal lobe (MTL) as the site

for memory. Subsequent research has gone on to show that the MTL, more specifically the hippocampus, is an important structure for memory and is implicated in episodic/autobiographical (Rekkas & Constable, 2005; Ryan et al., 2001), semantic (Bartha et al., 2003; Bernard et al., 2004), and spatial memory (Maguire, Frackowiak, & Frith, 1997; Rosenbaum, Ziegler, Winocur, Grady, & Moskovitch, 2004). However, memory is not exclusively controlled by the hippocampus. Nor can memory be simply broken down into Tulving's definitions. Aggleton and Brown (1999) proposed a neuro-anatomical model of recognition memory, which implicates differential involvement of hippocampus and perirhinal cortex in recognition memory processes. Based on evidence from animal and neuropsychological studies, Aggleton and Brown argue that the hippocampal network system is involved in recollective recognition memory while the perirhinal network system is involved in mediation of familiarity-based recognition (Aggleton & Brown, 1999; Aggleton & Brown, 2006; Brown, Warburton, & Aggleton, 2010).

Recognition Memory

Humans are specialized stimuli detectors, as it often only takes a single exposure to a particular stimuli to activate neural pathways to encode and store this information. Irrespective of whether the information is in the form of a person, object, or an event, this information is able to be stored and subsequently retrieved in order to guide future interactions when the stimulus is encountered again. A casual interaction with someone at a grocery store is often enough to then subsequently remember that person again when then meeting them again when you bump into them at the movie theatre, regardless of whether or not you ever exchanged pleasantries. The ability to recognize something in this fashion

is known as recognition memory, which is a form of declarative memory (Milner, Squire & Kandel, 1998; Yonelinas, 2002). Recognition memory is thought to take two forms, Recollection and Familiarity (Yonelinas, 2002).

As noted by William James, there are often situations where in we "recognise but do not remember" (James, 1890, p. 673). The situation described earlier is an example of familiarity, a feeling that a particular thing has been experienced before but is free of any context. This involves a measure of memory strength, which can be described as knowledge of previously encountering a stimulus, without necessarily remembering the exact event or remembering the stimulus itself (Mandler, 1980). Opposed to this is Recollection, in which contextual details about a particular stimulus are remembered as well as the stimuli itself. (Gardiner, 1988; Jacoby, 1991; Mandler, 1980; Tulving, 1985; Yonelinas, 1999, 2001, 2002; Yonelinas et al., 1996; Yonelinas & Jacoby, 1996). Due to the contextual nature of recollection forms of memory, it is thought to depend on episodic memory processes (Aggleton & Brown, 2006).

Recollection and Familiarity have been shown to be functionally dissociable (Rugg & Yonelinas, 2003; Yonelinas, 2001, 2002). Recollection forms of memory are slower than familiarity-based recognition. When individuals are forced to make quick responses, performance for recollection based retrieval suffers significantly whilst familiarity performance does not (Yonelinas & Jacoby, 1994, 1996). Further, Recollection forms of memory are more sensitive to the effects of divided attention, being affected at the encoding and retrieval stages. Familiarity, in contrast is relatively unaffected by divided attention (Gardiner & Parkin, 1990; Gruppuso, Lindsay, & Kelley, 1997; Yonelinas, 2001). Conversely, performance accuracy of Familiarity significantly declines with an increase in time between the presentation of items and their subsequent retrieval, while accuracy for

Recollection does not (Hockley, 1992; Yonelinas & Levy, 2002). However, based on these behavioural results, the argument could be made the Familiarity represents a shallower, or less rich version of recollection, and that Recollection and Familiarity are not necessarily distinct memory processes, but represent different levels of the same process. Thus, a double dissociation still needs to be demonstrated wherein Familiarity and Recollection can be shown to be dependent on different brain structures.

Neuro-Anatomical Model of Recognition Memory

Aggleton and Brown (1999) suggest that retrieval processes of recognition memory are reliant on interacting, but dissociable neural circuits. The first hypothesised circuit known as the hippocampus-diencephalon system consists of the hippocampus that projects to the prefrontal cortex (PFC) via the anterior thalamus. Direct connections to the anterior hypothalamus and the mammillary bodies come via the fornix, and damage to any of these structures can lead to anterograde amnesia. It is this circuit that is thought to subserve recollection. However, detecting how familiar a stimuli is does not necessarily rely on the hippocampus. The perirhinal cortex projects to the PFC via the dorso-medial thalamic nucleus and this circuit is thought to subserve familiarity. (Aggleton & Brown, 1999; Aggleton & Brown, 2006; Aggleton, Nicol, Huston, & Fairbairn, 1988; Aggleton & Sahgal, 1993; Dusoir, Kapur, Byrnes, McKinstry, & Hoare, 1990; Markowitsch, 1982; Meunier, Bachevalier, Mishkin, & Murray, 1993; Suzuki, Zola-Morgan, Squire, & Amaral, 1993; Zola-Morgan, Squire, Clower, & Rempel, 1993). The key here is that this familiarity network is independent of the hippocampus proper. Aggleton & Brown (1999) contend that support for their model comes from animal studies as well as

neuropsychological case studies which will be discussed below

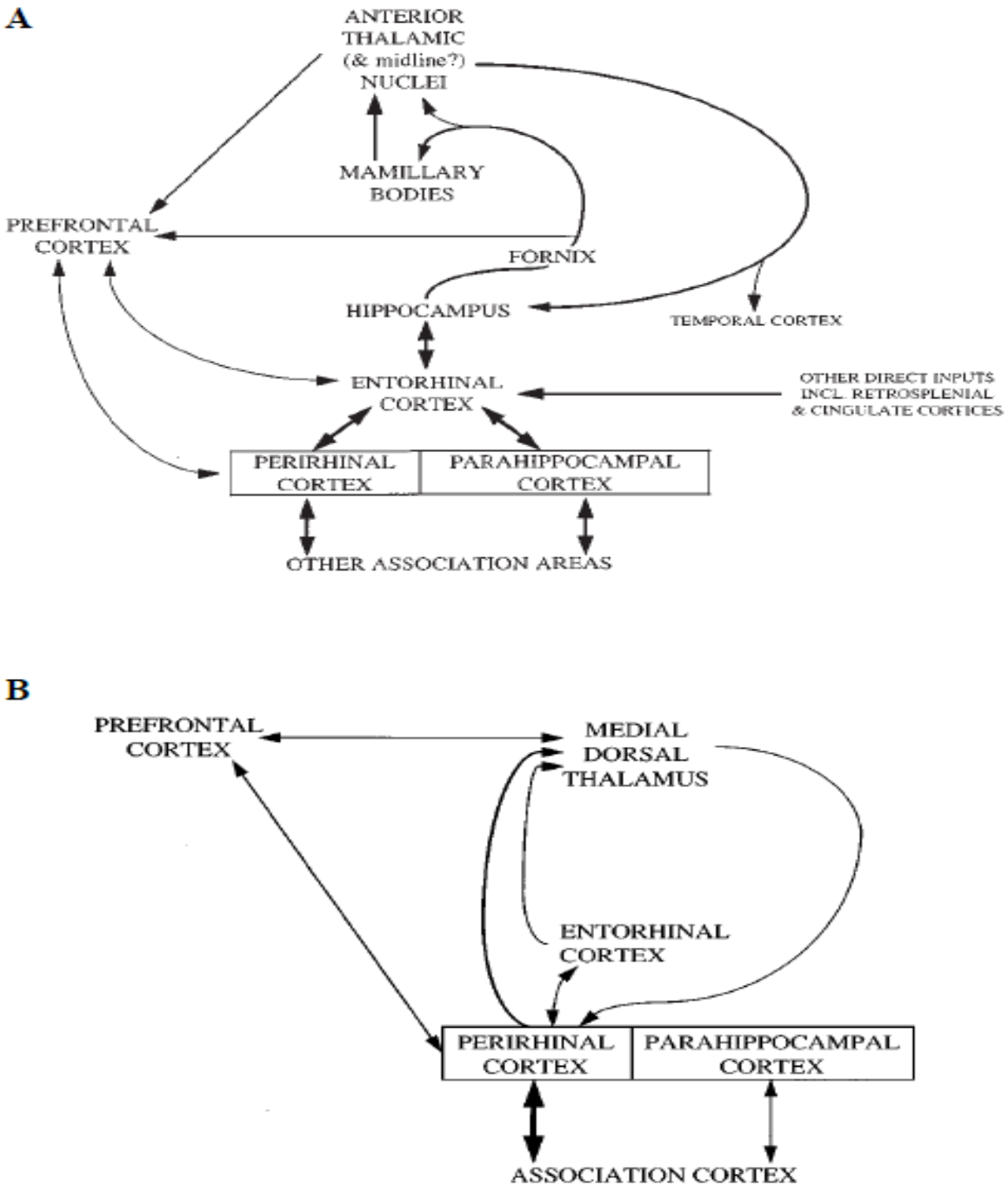

Figure 1.1 Schematic taken from Aggleton & Brown (1999) illustrating the networks underlying recollection (A) and familiarity (B) The thickness of the lines illustrate the importance of the connection.

Animal Studies

The delayed non-matching-to-sample task (DNMS) is the task of choice when studying an animal's ability to recognise previously presented stimuli (Mishkin and Delacour, 1975). The animal is first shown an object and after a time-delay is presented with a choice of two objects: the previously seen one and a novel object. The animal is required to select a novel object, which it learns to achieve through positive reinforcement training. The DNMS task is thought to be able to be solved by a familiarity- judgement alone and thus represents an opportunity to test whether hippocampal or perirhinal networks are implicated in an animal's ability to complete this task.

Monkeys with circumscribed bilateral lesions to the perirhinal cortex exhibit impaired performance on the DNMS task relative to a control-group (Meunier et al., 1993; Nemanic, Alvarado, & Bachevalier, 2004; Winters & Bussey, 2005). Lesions to perirhinal cortex also have a lasting impact on an animal's recognition memory, with deficits persisting more than two years post-surgery (Suzuki et al., 1993; Zola-Morgan et al., 1993). Further reinforcing the importance of the perirhinal cortex in familiarity judgements, monkeys with conjoint lesions of the hippocampus and perirhinal cortex have significantly greater deficits on the DNMS task than monkeys with lesions exclusively to the hippocampus proper (Alvarez, Zola-Morgan, & Squire, 1995; Zola-Morgan et al., 1993; Zola-Morgan, Squire, & Ramus, 1994; Zola-Morgan, Squire, Rempel, Clower, & Amaral, 1992). In general, in primates, the greatest deficit to familiarity is found following a lesion to the perirhinal cortex, compared to damage to any other single

structure within MTL alone, or in combination (Meunier et al., 1993; Murray & Richmond, 2001).

However, the role of the hippocampus in familiarity judgements is not as clear. Murray and Mishkin (1998) showed spared recognition memory performance on the DNMS following bilateral hippocampal ablations. This is contrasted by other studies have shown deficits on the DNMS task after lesions bilaterally to the hippocampus (Alvarez et al., 1995; Zola, Squire, Teng, Stefanacci, Buffalo, & Clark, 2000). Similar results were reported in earlier research (Zola-Morgan & Squire, 1986). Although the lesion was not circumscribed in this study, with the hippocampus, perirhinal and entorhinal cortices all being damaged. Further, ablation of the hippocampus has been shown to damage posterior perirhinal and entorhinal efferents projecting to thalamic structures, leading to the disruption of perirhinal and entorhinal cortical fields (Murray & Mishkin, 1998). Thus, recognition deficits following selective hippocampal ablation may be the result of collateral damage to perirhinal and entorhinal cortices in addition to the hippocampus (Murray & Mishkin, 1998). Differences in surgery procedures can lead to variability in the severity and extent of damage to neural structures. Inconsistency in research results may be due to variations across studies in the efficacy of surgical procedures utilized to fully ablate activity of neural structures (Zola-Morgan et al., 1992).

Irrespective of the inconsistencies in the research, overall findings from animal research tend to suggest a key role for the perirhinal cortex familiarity-based recognition in monkeys. However, evidence of hippocampal involvement in recollection is scant at best, as it is still debatable whether or not animals have a memory comparable to human episodic memory (Aggleton & Brown, 2006; Tulving, 1983).

Amnesic Patients

Studies of recognition memory in amnesic patients following medial temporal lobe lesions provide additional support for the Aggleton and Brown (1999) model. According to Aggleton & Brown's (1999) model, individuals with damage to the hippocampus and the associated recollection network should show an impairment to recollection, but not to familiarity. On the contrary, individuals with damage to the perirhinal cortex and associated familiarity network should show a selective impairment to the familiarity system. The combination of hippocampal lesioned and perirhinal lesioned patients provide an opportunity for a double dissociation and strengthen the argument that familiarity and recollection represent functionally distinct networks.

Yonelinas et al (2002) studied patients with damage to either the hippocampus (caused by hypoxia) or extensive damage to MTL regions, including the hippocampus and the surrounding perirhinal and entorhinal regions (caused by temporal lobectomy and hypoxia following extensive cardiac arrest). Using remember/know (R/K) responses and receiver operating characteristic (ROC) scores, Yonelinas et al (2002) found that patients with hippocampal damage showed a selective deficit in recollection ('know' judgements versus 'remember' judgements) while the patients with widespread MTL damage showed impairments on both Recollection and Familiarity ('remember' and 'know' judgements). Also, individuals who suffer hippocampal damage in early childhood show a similar pattern to those identified by Yonelinas et al (2002) in that tasks examining recall are

impaired, whilst performance on tests assessing familiarity-based recognition is preserved. (Vargha-Khadem, Gadian, Watkins, Connelly, Van Paesschen, & Mishkin, 1997). This finding was not found when this study was replicated with adult-onset selective hippocampal damage, with individuals showing impairments on both Familiarity and Recollection (Manns & Squire, 1999; Reed & Squire, 1997). Squire and colleagues explain Vargha-Khadem et al (1997) result as a structural and functional reorganization of the brain that can occur when damage is early, and thus the rhinal complexes learn to accommodate familiarity based judgements. However, Mayes, Holdstock, Isaac, Hunkin, and Roberts (2002) showed in a single patient with adult-acquired hippocampal damage with a similar pattern to earlier studies, that the ability to perform tasks relying on Familiarity was spared whilst recall was not. This ability was independent of memory modality (visual/verbal), and delay-intervals as long as 4 weeks did not affect the result.

Bowles and colleagues' (2010) investigated recognition memory discrepancies in 10 subjects who had undergone left or right hemisphere stereotaxic amgdalo-hippocampectomy (AH) as a surgical treatment for intractable temporal lobe epilepsy. On an RK paradigm, AH patients showed deficits in recollection, but familiarity was spared when results were compared to healthy controls. The problem with this study surrounds the damage also present to the amygdala, as this structure has also been implicated in memory. Although it is thought these memory processes the amygdala carries out are based on arousal rather than in the actual memory process itself (LaBar & Cabeza, 2006). This study provides further support for a double dissociation between recollection and familiarity-based recognition memory within the MTL (Bowles et al., 2010).

On balance, the majority of neuropsychological studies implicate dissociable involvement of the hippocampus and medial temporal lobe structures in retrieval of

recognition memory. Notably, however, patient-based research has seldom revealed instances of impaired familiarity and intact recollection to corroborate the role of perirhinal cortex in familiarity, possibly because discrete lesions to perirhinal cortex are extremely rare. For the reasons described above, there are concerns about the use of clinical patients, as one can never be confident that the damage is circumscribed. There is also no way of knowing how the diseased brain has subsequently developed as a response to that damage. Thus, research needs to be done on healthy subjects with neuroimaging, to attempt to functionally dissociate recall and familiarity forms of memory.

Neuro-Imaging Studies

Neuroimaging allows a way for the non-invasive investigation of human brain functioning, whilst allowing the identification of brain processes in the healthy brain. Whilst also not free of its own confounds, neuroimaging provides a way to avoid the use of clinical patients whose brain development is potentially unpredictable and unreliable. Several imaging studies using fMRI and EEG have been employed and show hippocampal activity in recollection and perirhinal activity in Familiarity.

An increase in hippocampal activity is observed during retrieval of successfully recognized words using a remember/know paradigm (for 'remember' responses). However, this increase is not seen for 'know' responses (Eldridge, Knowlton, Furmanski, Bookheimer, and Engel, 2000). Davachi, Mitchell and Wagner (2003) presented participants with a list of words and asked them to, either imagine a scene associated with that word or pronounce the word backwards. Participants were then brought back the next day and had to make old/new judgments on a new list of words. Subjects were then asked

which task ("imagine/read") they performed when studying the word. Increased hippocampal activity was seen during correct identifications of words together with the tasks accompanying their learning ("imagine/read"), whereas there was activation of perirhinal cortex for correct detection words only, without recollection of the associated tasks. Additionally, hippocampal activity during the encoding phase predicted correct recognition of words and the accompanying tasks, while perirhinal activity predicted correct identification of words only. These findings indicate the importance of hippocampal activity in recollective mechanisms (a representation of an item and its contextual material) of recognition memory and the significance of perirhinal activity in Familiarity-based recognition (a sense of a general experience of a prior encounter with an item).

In another fMRI study, Kirk et al (2004) found further evidence of circumscribed perirhinal activation in a Familiarity task. In a picture or a word condition, participants were presented with a 50-item list of words or pictures. In the first block, 10% of the words or pictures are repeated, and in the second block, 50% of the words or pictures are repeated. The high repeat condition (argued to represent high familiarity) was contrasted with the low repeat condition. No BOLD activation was seen in the hippocampus. However, significant activation was seen in perirhinal areas, and the mediodorsal thalamus, which is also hypothesised to be part of the recognition circuit (see above). Engagement of the perirhinal cortex in familiarity tasks was also observed during other fMRI studies (Henson, Hornberger, & Rugg, 2005; Ranganath, Yonelinas, Cohen, Dy, Tom, & D'Esposito, 2004), although a number of studies with similar methodological approaches failed to identify perirhinal activation with familiarity (Henson, 2005; Yonelinas, Hopfinger, Buonocore, Kroll, & Baynes, 2001). Activation is seen in the parahippocampal

areas however, which could include entorhinal and perirhinal cortices (Yonelinas, Otten, Shaw, & Rugg, 2005). Interestingly, these differences illustrated with fMRI in recall and familiarity for remember/know decisions persist during the lifecycle. Angel et al (2013) showed that these networks existed for young and older adults. However, for recollection only, older adults showed an increase in precuneus activity.

Studying event-related brain potentials (ERPs) has provided another alternative for measuring the contributions of recollection and familiarity during recognition memory testing (Curran, 2000; Rugg & Curran, 2007). ERP studies have found two distinct ERP modulations associated with recollection and familiarity (Addante, Ranganath & Yonelinas, 2012; Curran, 2000; Düzel, Vargha-Khadem, Heinze & Mishkin, 2001; Rugg & Curran, 2007). Voltage recorded over parietal sites (often maximal on the left) 600-800 ms after stimulus onset is more positive when individuals successfully identify a stimulus as being 'old' as opposed to a 'new' stimulus (Curran, 2000). RK tasks show that 'remember' as opposed to 'know' judgements show an increase in amplitude of this component (coined late positive component (LPC): Düzel, Yonelinas, Mangun, Heinze & Tulving, 1997). This LPC component is associated with the recollection of specific information such as study modality (Wilding & Rugg, 1997) and temporal source (Trott, Friedman, Ritter & Fabiani, 1997).

On the other hand, familiarity can be indexed by an ERP component called the FN400. This component occurs earlier than the LPC, and is seen in more frontal areas. As with the LPC, when individuals recognise a stimuli as old, there is an increase in positivity between 400-600 ms after stimulus onset relative to new stimuli (Curran, 1999) (Figure 2b). Participants who correctly recognise stimuli as 'old' but have not encoded that item

particularly deeply (no contextual details about the stimuli) show an FN400 ERP, but no LPC as the LPC is only exhibited when 'deep' encoding occurs (Rugg & Curran, 1998).

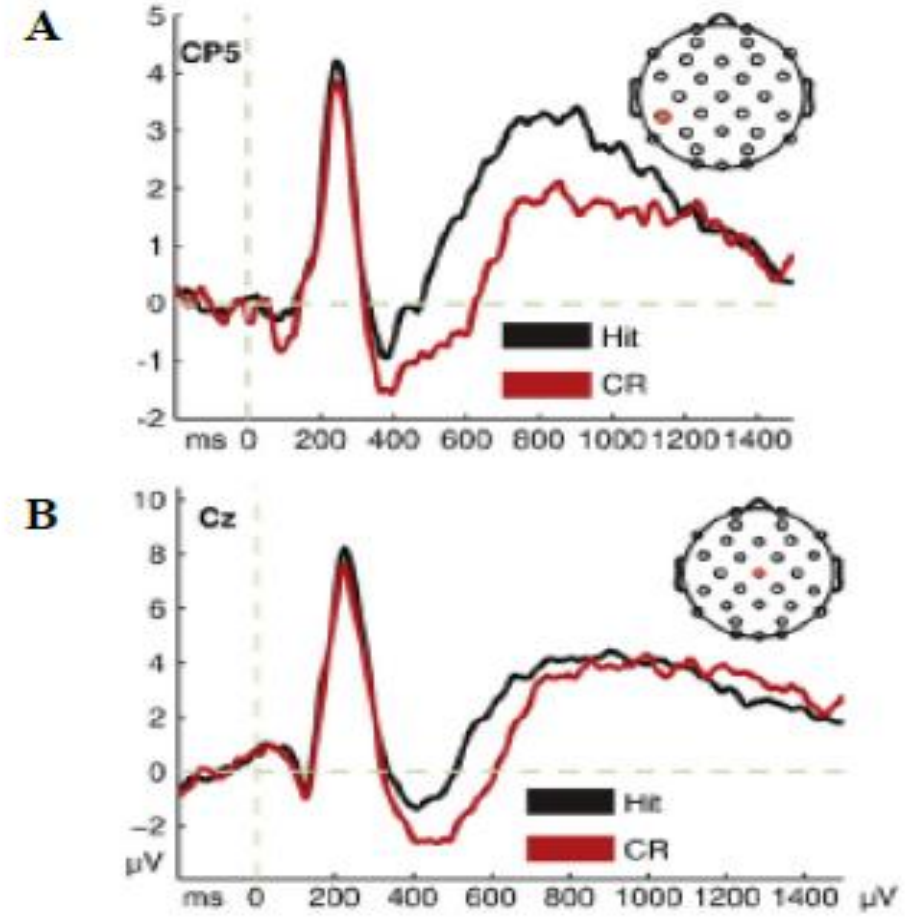

Figure 1.2. Recognition memory ERP effects taken from Addante et al., (2012) showing mean ERPs for hits and correct rejections plotted for electrodes CP5, showing the LPC effect (A), and Cz showing the FN400 effect (B).

Curran & Cleary (2003) showed that when individuals correctly recognised images, an LPC was elicited. However, Curran & Cleary (2003) modified their stimuli so that while some 'new' pictures were dissimilar to the 'old'/studied pictures, other 'new' pictures were the mirror-reversal of the 'old'/studied pictures. When the mirror-reversed pictures were incorrectly classified as 'old', a FN400 was seen. This suggests that the LPC ERP is linked to recollection-based recognition of specific information and the FN400 is related to familiarity-based recognition (Curran & Cleary, 2003).

Addante et al (2012) further investigated the role source recognition confidence plays in modulating the two ERP's associated with familiarity and recollection. By using measures of recognition confidence and source memory accuracy, they found that the FN400 and LPC were differentially related to familiarity and recollection respectively. Results show an increase in FN400 amplitude when item recognition confidence was high, irrespective of whether or not correct source judgements were made. LPC modulation was only seen when participants correctly judged the source of the memory, with increases in amplitude seen when this correct source judgement was coupled with the highest rating of item confidence. (Addante et al., 2012).

Finally, amnesic patients also provide support for these components, while also providing interesting links to the role of the LPC in hippocampal networks Düzel et al (2001) present the case study of Jon, who has isolated bilateral hippocampal damage, whilst his parahippocampus is intact. Jon showed no LPC ERP but does show the FN400 component. Interestingly, Jon also performs similar to controls in measures of familiarity, whilst showing poor performance on recollection tasks (Düzel et al., 2001).

The case for faces

Facial recognition memory presents an interesting way to further look into differences in memory systems discussed by Aggleton & Brown (1999). While facial recognition is an interesting topic of study, it is a separate field in itself. For the purposes of this thesis, only memory processes associated with successfully recognising/remembering a face will be analysed. An interesting distinction that has been found in the successful retrieval of faces is a difference between successfully remembering a famous or familiar face, and that of an unfamiliar face (Bird & Burgess, 2008, Trinkler et al., 2009) More specifically, the hippocampus is involved in the successful recollection of familiar faces, but not unfamiliar faces (Bird & Burgess, 2008, Trinkler et al., 2009). Bird & Burgess (2008) investigated the individuals with circumscribed damage to the hippocampus on tests of verbal and facial recognition memory. Performance on the Recognition Memory Test (RMT: Warrington, 1984) found that relative to controls, individuals with damage to the hippocampus performed significantly worse on the single-word recognition subset of this test. However, on the test which involves unfamiliar faces rather than words (with all other experimental procedure kept the same as the single-word recognition test) showed no difference between controls and individuals with hippocampal damage. This suggests that there is hippocampal involvement in the recognition of well-known stimuli, and this increase in successful recognition is driven by the activation of pre-experimental contextual factors (Bird & Burgess, 2008).

Further to this point, Trinkler et al's (2009) research with healthy subjects showed successful recollection of familiar faces (both famous and faces that are known to the subject) was associated with a different network than if those faces were unfamiliar (no

previous association with the subject). Trinkler et al (2009) presented subjects in an fMRI scanner with a collection of faces and had them judge whether the faces where either famous, known to them, or unknown. Subjects then had to successfully recognise previously presented faces in a basic face recognition memory paradigm where subjects had to indicate if the face was an old face or a new face. The results showed increased BOLD response for previously known faces, in both the study and testing phase, in the hippocampus, angular gyrus, amygdala, and lateral temporal cortices. Contrasting this, when looking at just correct rejections vs hits irrespective of what the face type was showed no activation of hippocampal networks, with activation seen in the mid/posterior cingulate and the ventral striatum instead (Trinkler et al., 2009). This study further backs up the earlier point raised by Bird & Burgess (2008) that hippocampal activation facilitates a stronger/better recollection experience via the retrieval of additional pre-experimental knowledge of the stimulus. This recruitment of contextual details into the recollection experience most likely accounts for the common finding of increased performance for well-known stimuli as opposed to novel stimuli (Trinkler et al., 2009; Westmacott & Moscovitch, 2003)

In summary, the majority of the research suggests recollection and familiarity are functionally dissociable processes and that different neural networks make distinct contributions to recognition memory. Evidence from neuropsychological, neuroimaging and neurophysiological studies of humans, rats and monkeys indicate a specific role of the hippocampus, mammillary bodies, anterior medial prefrontal cortex and anterior thalamic nuclei networks in recollection. The perirhinal cortex, lateral prefrontal cortex and medial dorsal thalamus networks are thought to modulate familiarity-based recognition memory. Further to this point, depth of processing and contextual information are important

modulators of hippocampal involvement, and lead to interesting possibilities of stimuli choice in attempting to design experimental paradigms to dissociate the two memory networks

1.2 Long Term Potentiation

Long term potentiation (LTP) is a long-lasting increase in synaptic efficacy that follows repeated co-activation of synaptically associated neurons in a network. LTP is the principal candidate as the mechanism of memory formation (Bliss & Collingridge, 1993; Cooke & Bliss, 2006). The characteristics of LTP were described, before the discovery of LTP, by Donald Hebb (Hebb, 1949). It is these characteristics nonetheless, that make LTP the prime candidate as the mechanism of memory. These characteristics are co-operativity, input-specificity, and associativity (Bliss & Collingridge, 1993; Cooke & Bliss, 2006). Co-operativity describes the requirement to achieve a threshold of activation in order for LTP to be induced. Input-specificity describes the process whereby LTP can be induced at a particular synapse without potentiating surrounding synapses, and associativity describes how weaker stimuli that cannot induce LTP on their own can be 'facilitated' by other stronger coactive stimuli which will trigger LTP at the site of the weak stimulus. Input-specificity and associativity are of particular importance when describing LTP as a memory mechanism. Associativity provides the necessary mechanism by which memories (or aspects of memories) are linked within the brain. Input-specificity provides the means for large-capacity storage of memories, with the unit of storage for memories being the

synapse rather than the neuron (Cook & Bliss, 2006). As well as these characteristics, LTP also consists of three distinct temporal phases (Bliss & Collingridge, 1993). Firstly, short term potentiation occurs, which lasts 15-30 minutes (Malenka, 1991), followed by early-LTP lasting approximately 1-3 hours and finally followed by late-LTP which lasts many hours, or possibly days and even years (Abraham, Logan, Greenwood, & Dragunow, 2002; Kelleher, Govindarajan, & Tonegawa, 2004). Both the properties and phases of LTP are attributed to specific cellular processes that will be discussed at a later point.

LTP was originally discovered *in vitro* in the rabbit hippocampus, and later described experimentally using *in vivo* experiments on the rabbit hippocampus (Lømo, 1966; Bliss & Gardner-Medwin, 1973; Bliss & Lømo, 1973). Bliss and colleagues (Bliss & Gardner-Medwin, 1973; Bliss & Lømo, 1973) found that when a high-frequency repetitive electrical stimulation (often referred to as "a tetanus") was applied to afferent projections of the dentate area in the rabbit hippocampus, there was a significant increase in field potentials in response to a single-pulse stimulation of that afferent to the dentate gyrus. This increase in potentiation lasted for 10 hours in anaesthetized animals and up to 16 weeks in unanaesthetized animals. Subsequent research has shown that this increased potentiation can potentially last for as long as a year (Abraham et al., 2002). The advent of the hippocampal slice preparation (Skrede & Westgaard, 1971), which involves maintaining slices of hippocampus in artificial cerebrospinal fluid, allowed a more detailed inspection of the hippocampus and thus a more detailed examination of the mechanisms of LTP (Cooke & Bliss, 2006). Research of this kind demonstrated the existence of mechanistically different forms of LTP, with the two main forms differing on their dependence on N-methyl-D-aspartate (NMDA) receptors (Cooke & Bliss, 2006; Huang & Kandell, 1998; Larkman & Jack, 1995; Malenka & Bear, 2004; Wang, Ferguson, Pineda,

Cundiff & Storm, 2004; Sweatt, 2007). For example, LTP in Schaffer-collateral/CA1 regions of the hippocampus is dependent on NMDA receptors, while mossy-fibre and cerebellar parallel fibre LTP is not dependent on NMDA receptors. Due to the ease of measuring potentials and drug application, most work on LTP is done on the rat hippocampus *in vitro* (Cooke & Bliss, 2006). LTP has also been demonstrated in neocortical tissue (Fox, 2002), as well as visual cortical areas (Heynen & Bear, 2001; Kirkwood & Bear, 1994; Komatsu, 1994). LTP also occurs in human cortical tissue and is mechanistically similar to LTP seen in non-human preparations (Chen et al., 1996; Beck, Goussakov, Lie, Helmstaedter, & Elger, 2000). The induction of this form of human LTP is also NMDA receptor dependent, as LTP induction was blocked after the application of DL-2-amino-5-phosphonovaleric acid, a NMDA receptor antagonist. (Chen et al., 1996; Beck et al., 2000). As NMDA receptor dependent LTP is more common and is present in human cortical tissue, the following will focus on NMDA receptor dependent LTP.

The phenomenon of LTP is the direct result of both pre and post synaptic activity and a complex interplay of neurophysiology. LTP occurs at glutamatergic synapses, with the first step involving an action potential arriving at the terminal button, stimulating release of glutamate, a neurotransmitter, into the synapse. Once in the synapse, glutamate binds to NMDA receptors on the post-synaptic membrane (see Figure 1.3). However, the binding of glutamate does not result in the activation of the NMDA receptors. This is due to the presence of a magnesium (Mg^{2+}) 'plug' which blocks the NMDA receptor channel and prevents the entry of sodium (Na^{+}) and calcium (Ca^{2+}) into the cell (Nowak, Bregestovski, Ascher, Herbet, & Prochiantz, 1984). In order for the NMDA receptors to become completely active, glutamate also binds to post-synaptic α-amino-3-hydorxy-5-

methyl-4-isoxazolepropinate (AMPA) receptors. The action of AMPA receptors allows Na^+ to enter the post-synaptic cell, which depolarizes it sufficiently to a point where the Mg^{2+} 'plug' is expelled from NMDA receptors. This then allows the influx of Na^+ and Ca^{2+} ions into the cell. It is the repeated influx of Ca^{2+} ions into the cell through multiple activations that triggers LTP (Lynch, Larson, Kelso, Barrionuevo, & Schottler, 1983; Malenka, Kauer, Zucker, & Nicoll, 1988).

The characteristics of co-operativity, input-specificity and associativity can be seen in the process of LTP induction (Bliss & Collingridge, 1993). Associativity can be seen through strong activation depolarizing nearby regions of the cell membrane, input specificity is illustrated through the localized influx of Ca^{2+}, and co-operativity is demonstrated through the need for significant depolarization in order for the Mg^{2+} 'plug' to be expelled, thus triggering LTP.

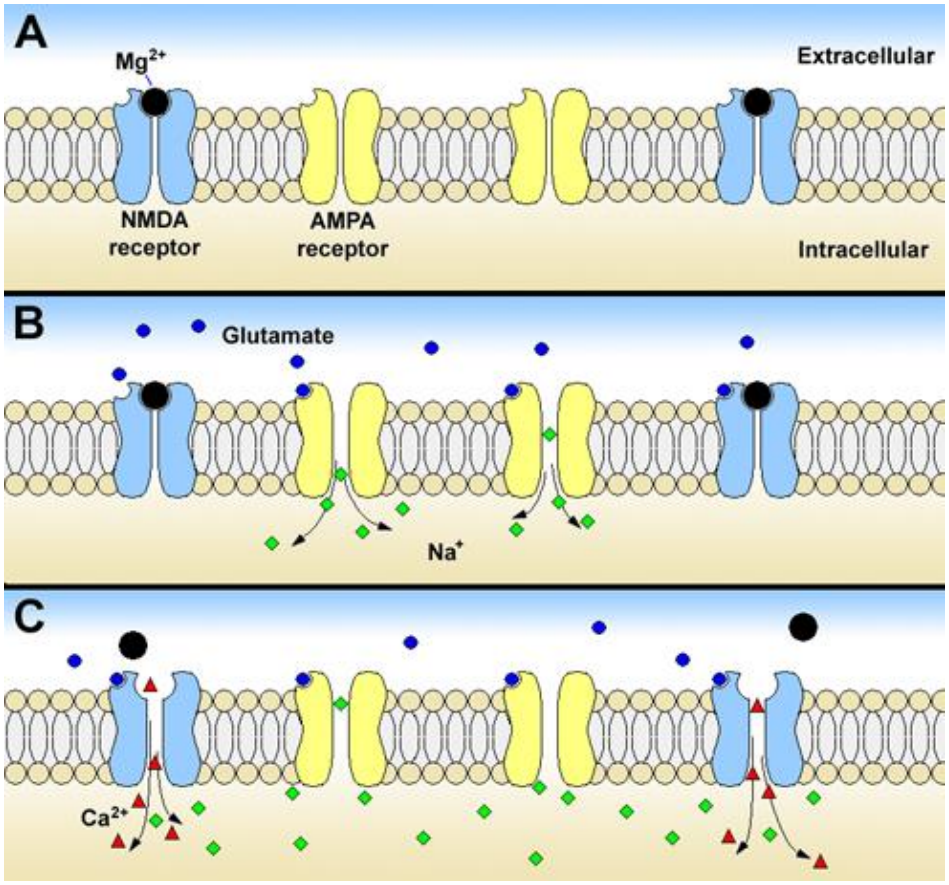

Figure 1.3. A) A post-synaptic cell membrane containing two subtypes of glutamatergic receptor: the NMDA receptor and the AMPA receptor. At rest, Mg^{2+} ions are attracted into the NMDA receptor channel by the negative transmembrane potential, blocking any ion movement through the channel. B) The pre-synaptic cell releases glutamate neurotransmitters that diffuse across the synapse and bind to the AMPA and NMDA receptors. Because of the Mg^{2+} 'plug', the binding of glutamate, by itself, is insufficient to open the NMDA receptor channel. The binding of glutamate to AMPA receptor opens the ion channel permitting Na^{+} to enter the cell, depolarising the cell membrane. C) If the depolarising current is strong enough, it expels the Mg^{2+} from the NMDA receptor channel. This allows the binding of glutamate to open the ion channel, and Na^{+} and Ca^{2+} ions to enter the cell. The influx of Ca^{2+} then triggers LTP.

LTP is the result of both pre and post-synaptic activity, with the literature suggesting LTP is primarily a post-synaptic phenomenon (Malenka & Bear, 2004; Malenka & Nicoll, 1999; Soderling & Derkach, 2000). However, processes taking place after LTP induction are not well understood, and there is still debate about particular steps in the cascade of events that characterizes LTP. After the influx of Ca^{2+} into the cell, the Ca^{2+} interacts with the protein Calmodulin (CaM; see Figure 1.4). CaM in turn activates both Calmodulin-dependent kinase II (CaMKII) and protein kinase C (PKC) (Akers, Lovinger, Colley, Linden, & Routtenberg, 1986; Fukunaga, Stoppini, Miyamoto, & Muller, 1993; Lovinger, Wong, Murakami, & Routtenberg, 1986; Malenka et al., 1988; Malinow, Shulman, & Tsien, 1989; Reymann, Brödemann, Kase, & Matthies, 1988). PKC phosphorylates (and therefore activating) NMDA receptors (Ben-Aro, Aniksztejn, & Bregestovski, 1992; Fitzjohn et al., 1996; Lu et al., 1999), while CaMKII phosphorylates AMPA receptors (Barria, Muller, Derkach, Griffith, & Soderling, 1997), and leads to an increase in the amount of post synaptic AMPA receptors via insertion of new receptors at the synapse (Liao, Scannevin, & Huganir, 2001; Shi, Hayashi, Esteban, & Malinow, 2001). CaM also activates cyclic adenosine monophosphate (cAMP) via the adenylyl cyclase enzyme. The activation of cAMP triggers a cascade of events, including the activation of protein kinase A (PKA). PKA then phosphorylates cAMP-response-element binding

(CREB) protein and mitogen-activated protein kinase (MAPK), both of which are thought to be involved in gene transcription and protein synthesis (Frey, Huang, & Kandel, 1993; Huang & Kandel, 1994; Huang, Martin, & Kandel, 2000; Nguyen & Kandel, 1996; Rosenblum, Futter, Jones, Hulme, & Bliss, 2000; Rosenblum et al., 2002; Waltereit & Weller, 2003).

The three temporal phases of LTP (STP, early-LTP, late-LTP) are also demonstrated by the molecular processes discussed above. STP relies on the activation of NMDA receptors and subsequent influx of Ca^{2+} and activation of CaM. Early-LTP is dependent on the subsequent activation of CaMKII, PKC and other related protein kinases, which in turn leads to subsequent activation of NMDA and AMPA receptors. Finally, late-LTP is dependent on the activation of MAPK and CREB and subsequent gene transcription and protein synthesis.

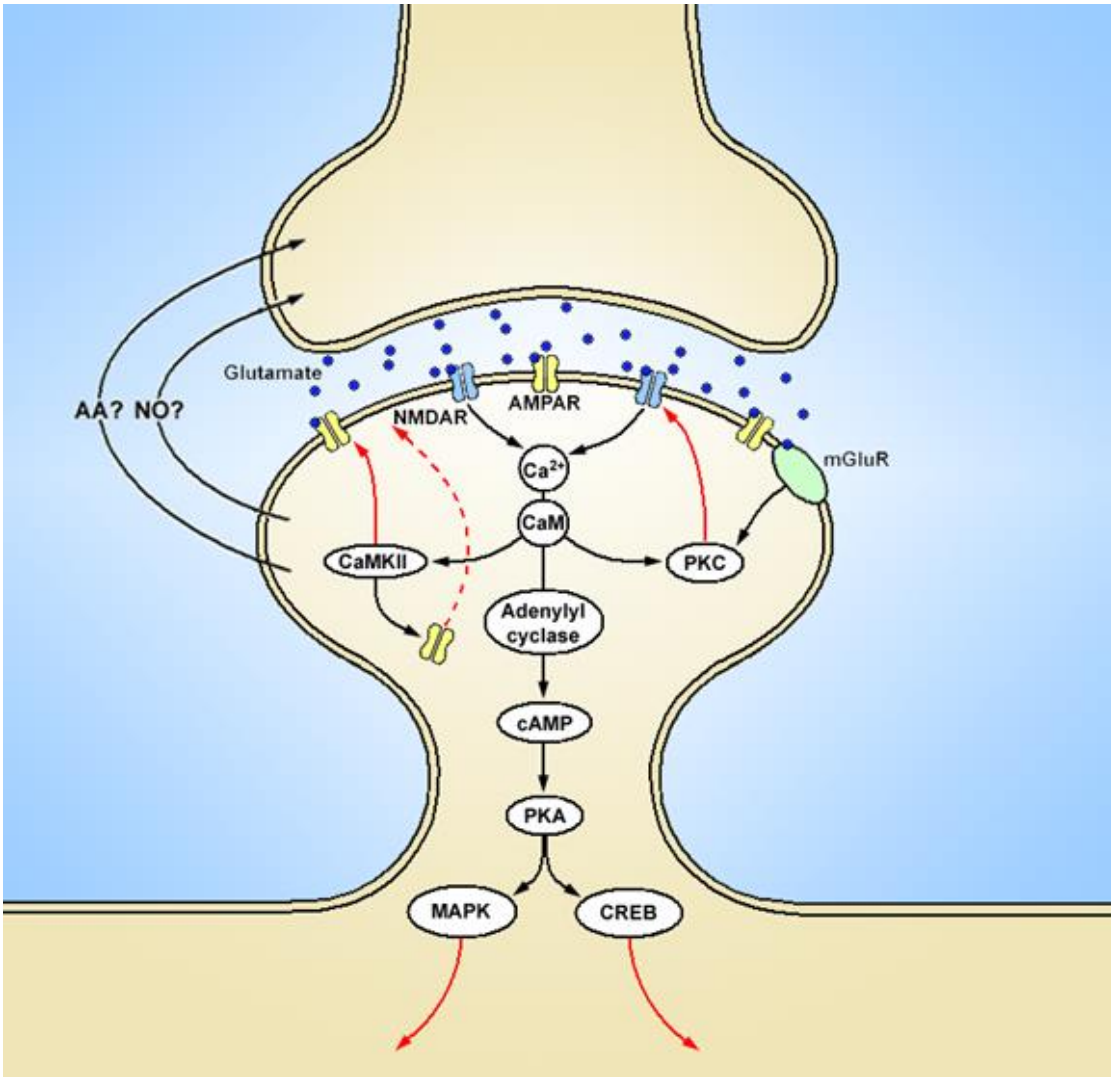

Figure 1.4. Post-synaptic molecular mechanisms involved in LTP following influx of Ca^{2+} from NMDA receptors (NMDAR). Influx of Ca^{2+} interacts with CaM to activate a number of protein kinases including CaMKII, PKC (mediated by mGluR activation), and PKA (via adenylyl cyclase and cAMP). CaMKII enhances conductance of AMPA receptors and also triggers insertion of new AMPA receptors (AMPAR) to synapse. PKC enhances conductance of NMDAR, while PKA activates the MAPK and CREB pathways leading to protein synthesis and gene expression. Possible pre-synaptic changes may be triggered by retrograde messengers including AA and NO.

Long-term Depression

Long-term Depression (LTD) is the converse process of LTP in that rather than a

long-lasting *increase* in the strength of synaptic communication, a long lasting *decrease* is observed. The induction of LTD is seemingly counter intuitive, in that it is unexpectedly similar to LTP. Repetitive stimulation is also used in the induction process of LTD, the difference being that, the frequency used (~0.5-3Hz) is much lower than that used to induce LTP (Dudek & Bear, 1992). Two distinct forms of LTD exist, which differ in both their molecular mechanisms and function (Cooke & Bliss, 2006). The first form, known as 'depotentiation', is the process of reversing of LTP. The second form, known as '*de novo*' LTD, is the process of a depression process from an unpotentiated baseline (Cooke & Bliss, 2006). In concordance with LTP, some forms of LTD are induced by a Ca^{2+} influx through activated NMDA receptors, with the concentration of post-synaptic Ca^{2+} necessary being lower than what is needed for LTP induction. (Kirkwood & Bear, 1994; Mulkey & Malenka, 1992; Nishiyama, Hong, Mikoshiba, Poo, & Kato, 2000). Phosphatases such as calcineurin are key molecules in regards to the mechanisms of LTD, by deposphorylating glutamate receptors (Morishita, Marie, & Malenka, 2005). In terms of the functional effects of LTD, studies show an association between LTP and reductions in dendritic length, branching, and spine density (Monfils & Teskey, 2004). It has also been suggested that LTD may play a key role in learning by serving as a homeostatic mechanism, ensuring that synapses in the CNS do not become saturated (Cooke & Bliss, 2006).

LTP and Memory in Animals

LTP has been established as an attractive candidate for the mechanism of memory because of the particular characteristics that define it. However, it is important to show that LTP has functional effects on learning and memory in order to confirm this hypothesis. Much research is carried out using the rat hippocampus, thus the induction and

measurement of LTP in rats is relatively non-controversial. The link between LTP and memory is achieved by interfering with hippocampal LTP and showing the subsequent effects such interference has on memory tasks in animals.

The use of NMDA receptor antagonists such as 2-amino-5-phosphonopentanoic acid (AP5) in rats blocks LTP induction and impairs their ability in hippocampal-dependent memory tasks, such as learning the location of a hidden platform in the Morris water-maze (Abraham & Mason, 1988; Morris, Anderson, Lynch, & Baudry, 1986). Interestingly, administration of AP5 has no effect on a potentiated response once LTP has been induced, and does not affect the ability of the animal to locate the water platform if the animal has already learnt the location of the water platform. This suggests that NMDA receptors are not as important for expression of a potentiated response as they are for the induction of LTP itself (Cooke & Bliss, 2006; Morris et al., 1986). Transgenic mice also provide evidence for the dual role of NMDA receptors in LTP and memory. Tsien, Huerta, & Tonegawa (1996) generated a 'conditional' knock out mouse, which deleted genes of NMDA receptor subunits in the CA1 subfield of the hippocampus. It was important to restrict the knock out of the receptor subunits to only the CA1 subfield, as NMDA receptors in other areas are vital for development of an organism (Cook & Bliss, 2006). These 'conditional' knockout mice showed profound deficits on spatial learning and memory on the Morris water-maze task, and were unable to exhibit LTP.

There is substantial evidence to suggest a role for NMDA receptor dependent LTP in hippocampal dependent memory and learning, and there is also a growing body of research showing the role of other cellular substrates of LTP in animal memory. Interference with regular function of PKA (Abel et al., 1997), adenylyl cyclase (Wu et al., 1995), and AMPA receptors (Lee et al., 2003) produce memory impairments similar to that

seen from interfering with NMDA receptors. Inactivation or reduced phosphorylation of MAPK and CREB also produces impairments to both spatial memory and the consolidation of recognition memory (Athos, Impey, Pineda, Chen, & Storm, 2002; Blum, Moore, Adams, & Dash, 1999; Bozon, Kelly, Josselyn, Silva, Davis, & Laroche, 2003).

Of particular importance is CaMKII. CaMKII is self-regulating and able to maintain its own activity via autophosphorylation, and due to its location in the synapse has been argued to be a possible self-perpetuating memory molecule (Lisman & Goldring, 1988; Lisman, Schulman, & Cline, 2002; Miller & Kennedy, 1986). A mutation to threonine 286 in the alpha CaMKII protein affects the proteins ability to autophosphorylate. Studies show mice carrying this mutation are unable to exhibit LTP and have profound deficits in hippocampal-dependent learning and memory (Cooke et al., 2006; Giese, Fedorov, Filipkowski, & Silva, 1998).

Further to this point, unpublished results from Goh & Manahan (in press) show that in transgenic mice which leads to chronic inhibitory autophosphorylation of alpha-CaMKII, object learning is intact. These mice also show a higher threshold for induction of hippocampal LTP as well as displaying greater LTD in response to learning of novel spatial configurations. These findings further back up the notion that CaMKII is key to LTP and memory, whilst also suggesting a qualitatively different role potentially of LTP and LTD in memory formation. Whereas LTP is important for the encoding of space, LTD may play a role in the encoding of the content of that space.

While there is a strong correlation between hippocampal LTP and hippocampal-dependent learning, there have been studies showing normal LTP but deficient memory (Fragkouli et al., 2005; Migaud et al., 1998; Norsten-Bertrand et al., 1996) and perhaps even more important, a study with a mouse with no exhibition of LTP but normal learning

and memory (Zamanillo et al., 1999). In this study, the researchers described a mutant lacking the GluR1 subunit of the AMPA receptor, and attempts to elicit LTP *in vitro* yielded no result. Nonetheless, the knockout mouse performed as well as wild type mice in standard tests of hippocampal-dependent memory. However, Cooke and Bliss (2006) note that this is not the same as inducing LTP *in vivo*, and thus perhaps using different methods, such as those used by Hoffman, Sprengel, & Sakmann (2002) would yield different results.

LTP and Memory in humans

There is clear a link between LTP and hippocampus-dependent memory tasks in animal models. However, the same cannot be said for humans. Animal models allow far greater experimental control, thus it is known where and how LTP occurs and which parts of the animal brain are important for memory.

One reason for the difficulty in linking memory to LTP is the sheer complexity of human memory when compared to animal memory, as described earlier. The second reason for the difficulty in linking memory to LTP in humans is the absence of a paradigm to elicit and measure LTP *in vivo* in humans. As mentioned earlier, LTP has been demonstrated in isolated preparations of human brain tissue (Chen et al., 1996; Beck et al., 2000). Unfortunately *in vitro* measurement and induction of LTP provides no insight into the functional significance of LTP. Much knowledge is therefore inferred on the basis that the molecular mechanisms of LTP and memory in animal models are analagous to humans. Administration of ketamine – a NMDA receptor anatagonist – to participants leads to impaired performance on a variation of the Rey verbal memory task (Grunwald et al., 1999). Goff et al (2001) also showed that application of CX-516, from the group of

molecules known as AMPAkines which are molecules that enhance LTP through facilitation of NMDA receptors (Arai, Xia, & Suzuki, 2004), has beneficial effects on learning and memory. In order to obtain detailed information on the functional significance of LTP in humans and whether LTP is in fact the basis of memory in humans, *in vivo* induction and measurement of LTP is required.

1.3 Non-invasive LTP in humans

The challenge of inducing and measuring LTP in humans is how to carry this out non-invasively. However promising advances in this regard have been made with transcranial magnetic stimulation (TMS), or by using sensory stimuli and measuring changes in brain activity with instruments such as electroencephalography (EEG) or functional magnetic resonance imaging (fMRI).

The first attempts at showing non-invasive induced plasticity in humans were made using TMS. TMS is a technique which uses a magnetic current applied to the scalp, and depending on the frequency can inhibit (1Hz) neural activity or facilitate (upwards of 5Hz) neural activity of the underlying cortical tissue (e.g. Hallett, 2000). This is usually carried out over the area of the scalp corresponding to the motor cortex, which results in motor output of the muscle fibres enervated by the particular population of neurons targeted. With the use of electromyography, resulting muscle movement in response to intensity of TMS pulse is used as a measure of cortical activity, and is known as motor evoked potentials (MEPs). Early attempts at using repetitive TMS (rTMS) to produce long-lasting LTP-like changes were not consistent and never lasted long enough to constitute an LTP-like process

(Maeda, Keenan, Tormos, Topka, & Pascual-Leone, 2000). This could be due to the fact that 5Hz stimulation (a common protocol used in TMS studies) was not strong enough to stimulate an LTP like response. Huang and Rothwell (2004) found that 50Hz could be safely used as the stimulation threshold, but these changes lasted no longer than hundreds of milliseconds. However, the results of this study allowed them to use a particular pattern of stimulation called 'theta burst' stimulation (TBS), where 3 pulses of 50Hz are given every 200ms (i.e. 5 Hz), thus falling in the theta oscillation range (~ 4 – 7 Hz). Using this protocol, the amplitude of MEPs significantly increased and lasted for at least 20 minutes (Huang, Edwards, Rounis, Bhatia, & Rothwell, 2005). The use of low frequency stimulation (1 Hz) can also lead to a LTD-like effect (Hallet, 2000). Another way in which LTP can be induced by TMS without using such high frequency stimulation is a technique called paired associative stimulation (PAS) which involves the simultaneous pairing of peripheral nerve activation while also applying TMS to the cortex. Using this protocol, MEPs were significantly increased compared to baseline, and were shown to be specific to the muscle group targeted and NMDA receptor dependent (Stefan, Kunesch, Cohen, Benecke, & Classen, 2000; Stefan, Kunesch, Benecke, Cohen, & Classen, 2002; Ridding & Uy, 2003). The problem with TMS studies is that the mechanism and locus of effects are unclear and unspecific. Also, using MEPs as a measure of cortical output is less direct than other methods such as EEG or fMRI, that are arguably more direct measures of activity in the brain.

The first direct demonstration of an "LTP-like" effect induced non-invasively and by sensory stimuli was performed by Teyler et al (2005). An earlier study showed how repetitive visual sensory stimulation led to LTP in the visual system of the developing tadpole (Zhang, Tao, & Poo, 2000). Following on from this, Teyler et al (2005)

hypothesized repetitive visual stimulation in humans would lead to LTP in the visual cortex, and that this could be measured by using EEG and looking at changes in amplitude to visual evoked potentials. EEG is a technique where in electrodes are placed on the scalp, which are then able to pick up electrical signals from the brain. These electrical signals are the result of neuronal firing. In order to measure to induce and measure the theorized LTP in humans, Teyler et al (2005) presented participants with a checkerboard stimulus presented to either the left or right visual field at a low rate of stimulus presentation (1 Hz). Following baseline measures of both left and right visual field presentations, either the left or the right stimuli was flashed at a higher presentation rate (9 Hz) which constituted the photic "tetanus", after which baseline was then reinstated. Baseline was reinstated at differing times in order to see if the "LTP-like" was stable and lasted over long periods of time. Analysis of event related potentials (ERP) using independent components analysis (ICA; Makeig et al., 1999) showed a significant increase following the photic tetanus to the N1b component of the short-latency components of the ERP (See Figure 1.5). The N1b component is a bilateral negative-going component, which occurs approximately 170-190 msec. after the onset of the stimulus. This was the only component of the ERP that showed a significant change in amplitude following the tetanus, and this change was shown to last up to an hour after presentation of the tetanus. The location of the "LTP-like" effect was localized to bilateral extra striate regions using low resolution source estimation (LORETA; Pascual-Marqui et al., 1994). To confirm this, Clapp et al (2005a) replicated this experiment but used the far greater spatial resolution capabilities of fMRI, and found that blood oxygen level dependency (BOLD) responses in area V2 of the visual cortex were significantly increased following administration of the photic tetanus. This phenomenon is not limited to visual sensory information alone, and has been shown in

different sensory modalities. Using essentially the same paradigm but with auditory stimuli, after an auditory tetanus the N1 components of auditory evoked potentials (AEP) were significantly increased and this increase was maintained over an hour (Clapp, Kirk, Hamm, Shepherd & Teyler, 2005). Thus, the results of Teyler et al (2005), Clapp et al (2005a), and Clapp et al (2005b) suggest it is possible to non-invasively induce and measure LTP-like changes in humans.

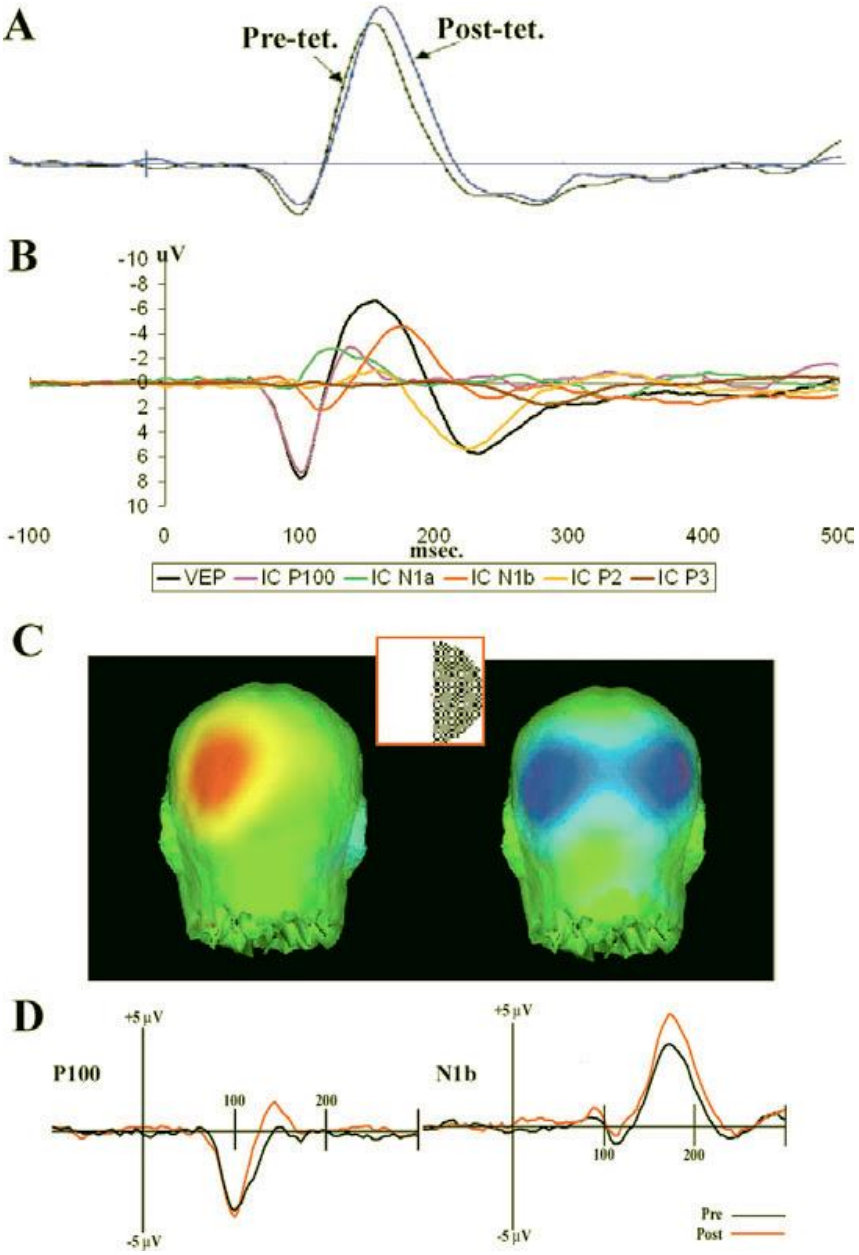

Figure 1.5 (A) Pre- and post-tetanus average evoked potentials recorded over the occipital cortex contralateral to the visual stimulus. (B) An independent components analysis identified five components of the visual evoked response (VEP) to checkerboard stimuli. (C) Checkerboard stimuli (subtending 4_ visual angle with three checks per degree) to the left or right visual hemifield elicited a contralateral P100 response (C, left panel) and a bilateral N1b response (C, right panel) in occipital cortex. (D) Repetitive presentation of the checkerboard (at 9 Hz) led to a significant change in only the N1b component (D, right panel). The amplitude of the P100 (D, left panel), for example, did not change significantly

However, as Cooke & Bliss (2006) duly note, the changes seen by Teyler et al (2005) and other TMS paradigms may not necessarily reflect LTP per se, but rather could reflect overall cell excitability, or a possible imbalance of excitation and inhibition in a

network. The significant increase in amplitude of only the N1b component and no other component suggests otherwise. However, more refinement to the paradigm developed by Teyler et al (2005) and other TMS paradigms is needed before it can be said with confidence that the changes elicited by these methods are in fact LTP. In order to do this, two important things need to be shown. Firstly, whether or not this paradigm can show input-specificity and secondly whether or not the effect seen from this paradigm is NMDA receptor dependent, both of which are essential to LTP.

Input-specificity cannot be assessed with the EEG and rTMS protocol discussed above (although this problem is somewhat solved by using PAS). That is because the population of neurons targeted by each respective stimulus is too large. However, subsequent studies have shown that it is possible using a modification of the paradigm developed by Teyler et al (2005) to target distinct populations of neurons. To do this, baseline readings of two stimuli that are very similar but differ just enough that they are encoded by different neurons are taken, and then one of the stimuli is tetanized. Following this, baseline readings of both stimuli are taken again, with the test being to see if only the tetanized stimuli is potentiated. This can be achieved by the use of sine gratings. Sine gratings are the result of performing a 2D Fourier analysis on a sine wave with the subsequent 2D realization of this transform being a sine grating. Sine gratings are optimal stimuli to use because they can differ in both their orientation and their spatial frequency, and there is considerable evidence that the human visual system is sensitive to these aspects of visual information (Blakemore & Campbell, 1969; De Valois, Yund, & Helper, 1982; Kamitani & Shinsuke, 1999; Kenemens, Kok, & Smulders, 1993; Tootell et al., 1998). McNair et al (2006) showed that using sine gratings of different spatial frequencies (1 cycle-per-degree and 5 cycles-per-degree) that the increase in potentiation following the

tetanus was specific to the spatial frequency that was tetanized. Following on from this, Ross et al (2008) showed that using sine gratings of different orientations (Horizontal and Vertical) that once again the increase in potentiation following the tetanus was specific to the orientation that was tetanized. These results strongly suggest that the changes being witnessed are not due to general cortical excitability nor altered attention or arousal, as you would expect both stimuli to be affected by these. The selective potentiation of only the tetanized stimuli strongly suggests that this effect is an input-specific process, and is therefore most likely LTP.

As described earlier, a dependence on NMDA receptors is what typifies the most prevalent form of LTP. In order to show if the paradigm developed by Teyler et al (2005) was also dependent on NMDA receptors, the same paradigm was applied to experiments that worked with anesthetized rats (Clapp, Eckert, Teyler, & Abraham, 2006). A urethane anesthetized rat was exposed to a high-frequency photic stimulus while intracortical field responses were recorded. It was found that a photic tetanus was able to induce cortical LTP of the visual-evoked potential. This duration lasted for up to 5 hours. In order to show whether or not that this potentiation was NMDAR dependent, the NMDA receptor antagonist CPP was applied. The application of CPP had no effect on the visual-evoked potentials themselves, but rats that had been exposed were no longer able to demonstrate LTP to the checkerboard tetanus, strongly suggesting that the potentiation is dependent on NMDA receptors. However, this finding was recently unable to be replicated (Eckert, Guevremont, Williams & Abraham, 2013). Eckert et al (2013) found that using a similar protocol did not lead to an increase in sensory LTP. However the study observed a significant increase in visual cortex glutamate receptor expression. To determine if the same mechanism is operating in humans, Cavus et al (2009) applied the same paradigm to

humans, using the NMDA receptor antagonist, ketamine. The subjects who were given ketamine did not show an LTP-like response to a photic tetanus. These two experiments demonstrate that sensory-induced cortical LTP is a process which is dependent on the action of NMDA receptors, and suggests that the cellular mechanisms underlying the potentiation in sensory cortex are similar to those extensively studied in the hippocampus and other areas. The results of these studies as well as those discussed earlier regarding input-specificity suggest there is now a reliable method of inducing and measuring LTP in humans. This means there exists a way to examine the claim made by Egan et al (2003) that BDNF affects memory via LTP. As outlined earlier, Egan et al (2003) suggest that the differences seen in different geno types of BDNF (hippocampal activation, and memory performance) could be due to the role of BDNF in LTP. However, there had previously never been a way of looking at this in the intact human brain. With the LTP assay developed by Teyler et al (2005), we are now able to test whether or not that differences in BDNF genotype cause differences in LTP in the intact human brain.

1.4 Brain derived neurotrophic factor

BDNF is a small dimeric protein belonging to the bigger family of signaling proteins known as the neurotrophins, and is thought to play a crucial role in early cell survival, development, and proliferation (Davies, 1994; Levi Montalcini, 1987; Lewin and Barde, 1996). The effects of BDNF are not limited to the developing nervous system, as BDNF has also been shown to be able to alter the properties of synapses in the mature nervous system (Gottmann et al., 2009; Park & Poo, 2012). This is further demonstrated by

Murer, Yan, & Raisman-Vozari (2001), who demonstrated that in the event of healthy aging, peak concetrations of BDNF observed after birth do not decrease across the lifespan. Due to the effect BDNF has on both neurite outgrowth and neuronal differentiation, it is a strong candidate for expressing functional change into structural change (Zagrebelsky & Korte, 2014). Despite being named brain derived neurotrophic factor, BDNF is relatively widespread through both the CNS and the PNS (Bramham & Messaoudi, 2005). In the CNS it is primarily located in secretory vesicles at the dendrites and axon terminals of glutamate synapses (Fawcett et al., 1997; Haubensak, Narz, Heumann, & Lessmann, 1998; Lu, 2003). More specifically, the highest concentrations of BDNF are found in the prefrontal cortex and the hippocampus (Pezawas et al., 2004).

BDNF is similar to many other neurotrophins in that it is the product of a precursor molecule, in this case pro-BDNF (Seidah et al., 1996). The gene is located on chromosome 11 (Liu et al., 2005). The protein is then cleaved to create pro-BDNF and the mature BDNF protein which activates distinct signaling pathways. (Dincheva, Glatt, & Lee, 2012; Matsumoto et al., 2008; Yang et al., 2009) and are both active extracellularly (Pang et al., 2004). Each of these signaling pathways have different functional outcomes, with pro-BDNF binding to the p75 neurotrophin receptor, leading to apoptosis and dendritic pruning (Roux & Barker, 2002; Yang et al., 2009), and mature BDNF binding to the tropomyosin-related kinase B (TrkB) receptor. This results in synaptic differentiation and plasticity (Bouelle et al., 2012; Dincheva et al., 2012). These functional outcomes of BDNF signaling pathways are thought to be crucial for cognitive processes such as learning and memory (Callaghan & Kelly, 2012; Pezawas et al., 2004).

BDNF is unique in its action as a neurotrophin as the release and regulation of BDNF are activity dependent as opposed to constitutive release, the more common form of

release seen in the other neurotrophins (Lu, 2003). This means that the regulation and release of BDNF is dependent on Ca^{2+} levels, with high frequency stimulation leading to an influx of Ca^{2+} into the cell through either NMDA receptors or voltage-gated Ca^{2+} channels (Aicardi et al., 2004; Balkowiec & Katz, 2002; Gartner & Staiger. 2002; Hartmann, Heumann, & Lessmann, 2001; Lever et al., 2001). As mentioned earlier, and can be seen in Figure 1.6, the mechanism of action of BDNF involves binding to both pre and post synaptic tyrosine-related kinase B (TrkB) receptors (Drake, Milner, & Patterson, 1999). Pre-synaptic binding of BDNF to TrkB receptors results in increased neurotransmitter release through an increase in mobilization and docking of NT vesicles in the terminal button (Jovanovic, Czernik, Fiendberg, Greengard, & Sihra, 2000; Pozzo-Miller et al., 1999). Post-synaptic binding of BDNF to TrkB receptors is associated with both NMDA receptors and PSD95 receptors, resulting in the phosphorylation of NMDA receptors (Levine & Kolb, 2000; Lin et al., 1998; Suen et al., 1997; Yoshii & Constantine-Paton, 2007). Post-synaptic binding of BDNF also leads to the phosphorylation and downstream activation of several protein kinase signalling pathways including PKC, MAPK, and CREB (Finkbeiner et al., 1997; Kaplan & Miller, 2000; Kaplan & Stevens, 1994; Greene & Kaplan, 1995; Minichiello et al., 2002; Patapoutian & Reichardt, 2001).

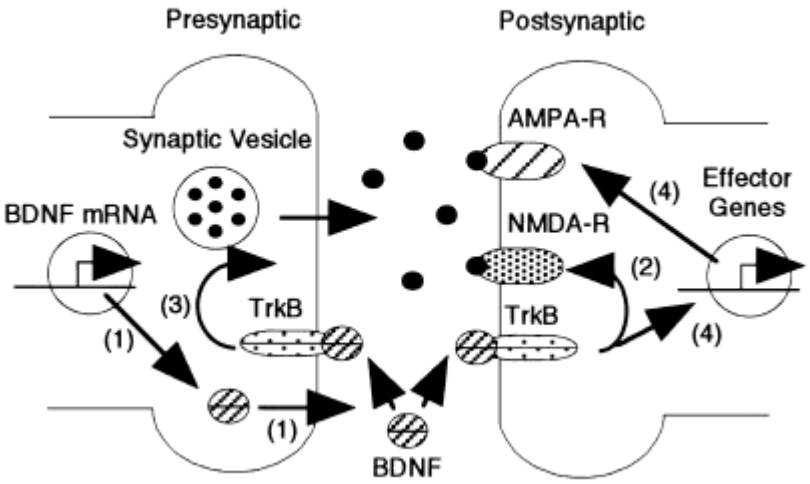

Figure 1.6. A model for the role of BDNF in synaptic plasticity, from Namada and Nabeshima (2003). Activity dependent release of BDNF from presynaptic sites (1). BDNF binds its receptor TrkB located pre- and postsynaptically which leads to the activation of signal transduction pathways, phorphorylation of NMDA receptors (2), an increase in the release of neurotransmitters from the presynapse (3), and an increase in protein synthesis (4).

Due to the abundance of BDNF and its receptor TrkB at glutamate synapses, it has emerged as a key orchestrator of synaptic plasticity, and is thought to play an important role in both early and late phase long-term potentiation (LTP: Lu, 2003; Lu, Christian, & Lu, 2007; Poo, 2001). The effects of immediately available endogenous BDNF and pre-synaptic binding of BDNF are thought to be important for early LTP through the mechanisms stated earlier (Lu, Christian, & Lu, 2007). However, this amount of BDNF is not sufficient to induce late LTP by itself (Lu, Christian, & Lu, 2007). The induction of late LTP triggers an enhancement to post-synaptic synthesis of BDNF, with in situ hybridization studies showing levels of BDNF mRNA to be increased 2-4 hours after late LTP induction (Castren et al., 1993; Dragunow et al., 1993; Patterson, Grover, Schwartzkroin, & Bothwell, 1992). LTP will be described in more detail in a subsequent

section of the thesis. One particular study showed disrupted late LTP in a rat with impaired activity-dependant BDNF, suggesting that BDNF is not just a key protein product of LTP but is in fact required for late LTP to occur, with a strong tetanus being insufficient (Lu, Christian, & Lu, 2007; Sakata, Woo, Wu, Shen, & Lu, 2005). The role of BDNF is not just limited to the hippocampus, with the infusion of BDNF inducing LTP of excitatory signals in the visual cortex of rats (Jiang et al., 2001).Studies have also shown that BDNF dependent retrograde effects are also required for the induction of LTP in visual cortical pyramidal neurons (Inagaki et al., 2008), and rats with implicated activity-dependent release of BDNF show implicated visual cortical LTP (Abidin et al., 2006).

As mentioned earlier, BDNF is thought to affect many brain processes. However, in terms of the impact BDNF has behaviourally, reduced expression of BDNF has been mainly implicated in the areas of memory and learning (Ma et al., 1998; Mu, Li, Yao, & Zhou, 1999). BDNF mRNA expression is highly correlated with behavioural performance in learning and memory tasks (Yamada, Mizuno & Nabeshima, 2002). More specifically, the levels of BDNF mRNA are elevated following 3 and 6 days of training in a water maze (Kesslak et al., 1998). Hippocampal-dependent learning in the Morris water maze, contextual fear conditioning, and passive avoidance are associated with a rapid increase in BDNF mRNA expression in the hippocampus (Ma, Wang, Wu, Wei, & Lei, 1998; Tyler, Alonso, Bramham & Pozzo-Miller, 2002; Cunha et al., 2010). The influence of BDNF on memory is not just limited to the hippocampus. Infusion of anti-BDNF antibodies into the parietal cortex of rats impaired inhibitory avoidance, by blocking cyclic adenosine monophosphate (cAMP) response element-binding (CREB) protein activation (Alonso et al., 2005). An interesting study conducted using genetically hypertensive rats with reduced expression of BDNF showed that this decrease in expression of BDNF correlated with

deficits in LTP (Hennigan, Callaghan, Kealy, Rouine, & Kelly, 2009). The study also measured the rats on an object recognition task, which involved initial exploration of two objects, then a 24-hour rest period, followed by an introduction of novel objects. Time in seconds spent exploring each object were expressed as a percentage of total time exploring and was used as the measure of recognition memory. The rats with decreased expression in BDNF and deficits in LTP also showed deficits in learning and recognition memory, suggesting BDNF is a key molecule in these processes. A recent study investigated the application of exogenous BDNF on memory consolidation and reconsolidation of day old chicks (Samartgis, Schachte, Hazi & Crowe, 2012). Results showed that chicks injected intracranially with recombinant BDNF following a single-trial training event exhibit increased memory retention up to 24 hours after training. The application of BDNF also enhanced retention following initial weak training. These results show that BDNF is involved in memory consolidation and reconsolidation.

1.5 *BDNF* Val^{66}Met polymorphism

In humans, a frequent single nucleotide polymorphism (SNP) to the *BDNF* gene has recently been discovered (dbSNP number rs6265). This SNP is seen as a non-conservative amino acid substitution, switching a valine to a methionine, at codon 66 (val^{66}met). Because the SNP is located in the 5' pro-BDNF sequence (the area of the gene which encodes the precursor peptide pro-BDNF), it is thought that the val^{66}met SNP does not affect function of the mature protein, but rather the intracellular processing and activity-dependent secretion of BDNF (Chen et al., 2004, Egan et al., 2003). This 'activity-dependent' release is in the form of post synaptic Ca^{2+}, which is also the first stage of LTP

induction. Thus, it is possible that this mutation may have an effect on LTP. All versions of the SNP are relatively common, with 65% of individuals in a Caucasian population Val^{66}Val homozygotes, and the remaining 35% of individuals Met carriers (Cheeran et al., 2008).

Individuals heterozygous for the polymorphism (at least carrying one copy of the met allele) have been shown to have an increased susceptibility to a range of disorders, such as Parkinson's disease (Momose et al., 2002) and Alzheimer's disease (Huang, Huang, Cathcart, Smith, & Podulso 2007; Ventriglia et al., 2002). The presence of a Met allele has also been linked with brain abnormalities, such as reductions to hippocampal volume and activation (Hariri et al., 2003; Pezewas et al., 2004), less gray matter in frontal, occiptial and temporal regions (Ho, Andreasen, Dawson, & Wassink, 2007; Ho et al., 2006), and lower hippocampal n-acetyl aspartate (NAA) which is a putative marker of neuronal integrity and synaptic abundance (Egan et al., 2003).

The finding that the Val^{66}Met polymorphism is associated with changes in hippocampal volume has been an area of vigorous debate. Original findings showed reductions in hippocampal volume (Pezewas et al., 2004). However, this supposed consistent finding has been the subject of numerous meta-analyses. The most recent of which by Harrisberger et al (2014) examined 643 young adults, and added this to a sample upwards of 5000 individuals in a meta-analysis. Whilst the study found no difference in hippocampal volume by genotype in their sample, the meta-analysis revealed a significant difference between individuals with a copy of the met allele and Val/Val homozygotes. However, they suggest this difference is mainly driven by studies with small sample sizes, which use manual tracing approaches rather than automatic tracing approaches. Thus, it is still not clear what effect this polymorphism has on hippocampal volume.

A large body of research from an NIH research group headed by Daniel Weinberger has also recently shown in multiple studies that the Val^{66}Met polymorphism is associated with poorer memory. Egan et al (2003) showed that in both healthy individuals and schizophrenics, that individuals carrying a copy of the Met allele did significantly poorer on measures from the Wechsler Memory Scale revised version (WMS-R; Wechsler, 1987) as a test of verbal episodic memory. This study also showed that genotype did not have a significant effect on a working memory task, the Wisconsin Card Sorting Test (WCST), which heavily relies on frontal lobe functioning, further suggesting the polymorphism has a more profound effect on episodic memory. Following up from this research, Hariri et al (2003) showed using an episodic memory task requiring the encoding of visual scenes, those individuals with a Met allele performed significantly poorer on this task than those without a met allele. Also, Goldberg et al (2008) have shown that this polymorphism significantly affects "hits" in a verbal recognition memory paradigm. Thus, this body of research taken together suggests that the Val^{66}Met polymorphism significantly impacts memory. It is interesting to note that these studies classified individuals into Val/Val and then Met carriers. This is likely due to a lack of power / small sample size. It is possible that there exists a dose response in BDNF genotypes, with Met/Met and Val/Met actually being functionally dissociable groups.

There is also evidence that the Val^{66}Met polymorphism affects brain plasticity that may or may not necessarily represent LTP. (Cheeran et al., 2008; Kleim et al., 2006). Kleim et al (2006) showed using TMS that the different genotypes had different degrees of experience-dependent plasticity in the motor cortex. Using TMS, baseline MEPs were taken of the first dorsal interosseous (FDI) of the right hand. The cortical representation area of the FDI was also calculated, as well as the centre of gravity. Following training on

three tasks (pinch grip, finger tapping, and nine hole peg board) Val/Val individuals showed increase MEP amplitude as well as increased FDI map area and greater shift in centre of gravity compared to individuals with Met versions of the Val^{66}Met polymorphism. Cheeran et al (2008) used theta burst stimulation, transcranial direct current stimulation, and paired associative stimulation to investigate whether the presence of a Met allele affected motor models of human plasticity. Individuals carrying the Met versions of theVal^{66}Met polymorphism showed significantly less plasticity as indexed by the three motor paradigms when compared to individuals who did not carry a Met allele. It should be noted that the plastic processes that are induced in these two studies use TMS, which is a technique which differs significantly to that used in this research. As discussed earlier, there is uncertainty as to both the mechanism and locus of the effects of TMS. Using MEPs is a less direct way to measure cortical activity than using EEG to measure sensory evoked potentials. Also, the paradigm employed by Kleim et al (2006) has not been shown to be either NMDA dependent or input specific, which as explained earlier are both key for something to be classified as LTP.

Taking all of this together, a re-evaluation of the initial work by Egan et al (2003) reveals some interesting ideas. Firstly, Egan et al (2003) argue that due to its role in long-term potentiation (LTP) and LTP's subsequent role in learning and memory, that the BDNF Val66Met polymorphism is affecting an individual's ability to induce LTP. The implication here is that if an individual's capacity to induce LTP is affected, then so will their ability to form memories. Whilst this is an attractive hypothesis, there do not exist many ways to measure LTP in humans. Whilst studies have shown that BDNF has the ability to affect forms of brain plasticity, these have not directly assessed brain activity. Secondly, Egan et al (2003) looked at what they considered to be a measure of episodic

memory. However, they did not look at any form of functional imaging to confirm the area of the brain that was responsible for their task. Therefore, it is possible that this polymorphism is important for some forms of memory but not others, which differ on the basis of brain area. As has been described earlier, recollection and familiarity represent two different memory systems which are subserved by different neural substrates, with the hippocampus being involved in recollection but not familiarity. BDNF is found in abundance all over the brain, but is highest in concentration in the hippocampus (Pezawas et al., 2004). Therefore, it is entirely possible that this polymorphism is more important for recollective forms of recognition memory and potentially does not play a part in familiarity-based memory judgements. Finally, if these the behavioural differences are seen between individuals of different genotypes, is this also reinforced by differences in brain functionality – are there differences in the pattern of brain activity between different genotypes for memory processes? And if there are functional differences in brain processing, are they present for all forms of memory? The aim of this thesis is therefore three fold.

1.6 Hypothesis/Aims

Due to the work described above from our laboratory in the Research Centre for Cognitive Neuroscience at the University of Auckland, paradigms to test human LTP induction and the ability to measure this induction now exist. It is now possible to look at the functional effects of LTP induction propensity in humans. Therefore, it is possible to directly look at the suggestion made by Egan et al (2003) that difference in levels of BDNF

as a result of being a Val^{66}Met variant result in lower memory due to lower LTP. However, we are not directly measuring serum levels of BDNF, one can infer from previous work that the different genotypes will lead to different levels of BDNF. Firstly, LTP will be assayed and compared between those individuals carrying at least one copy of the met allele, and those without a met allele. We hypothesise that individuals with the Val/Val genotype will show a greater magnitude of LTP. Most studies combine both Val/Mets and Met/Mets. However, it is of the opinion of the author that there may be valuable information lost by doing so. Thus, an attempt to recruit groups representing all three genotypes will be made in order to assess whether there exists any gene dose response functions (Met/Met being functionally different from Val/Met). LTP magnitude will be assessed just after induction and over 30 minutes after induction.

Secondly, electroencephalography (EEG) will be employed to assess the effect of the *BDNF* polymorphism on different event related potentials (ERP) related to independent memory systems. As described earlier, EEG allows the identification and observation of early brain processes related to familiarity and recollection forms of recognition memory. Thus, the differences in ERP modulation by genotype will also be investigated. We hypothesize in this instance that Val/Val's will show a greater modulation of the LPC, but there will be no difference in FN400 amplitude.

Lastly, as EEG does not allow for the localisation of the source of this activity. We will use fMRI to look at whether there is differential activation in hippocampal and extra hippocampal structures involved in memory processing. More specifically, through the use of famous vs non famous faces, we hypothesize that the network responsible for sub-serving successful famous face retrieval, involving particularly the hippocampus, will show greater activation in Val/Val homozygotes relative to Val/Met and Met/Met carriers. Thus

overall, the aim of this thesis is to assess the importance of BDNF in a variety of memory processes.

Chapter 2. *BDNF* Val^{66}Met and LTP

Brain-Derived Neurotrophic Factor Val^{66}Met Influences the Magnitude of Human Long-Term Potentiation which Predicts Memory Performance.

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2.1 Abstract

A single nucleotide polymorphism of the human BDNF gene (Val^{66}Met) may account for much of the variation in human memory performance. BDNF may influence memory via, either a modulation of acute plasticity (i.e. LTP), or a chronic influence on developing memory systems. Until recently, the link between BDNF and LTP has been difficult to assess in humans. Here however, by employing our recently developed human sensory LTP paradigm in the intact human brain, the effects of BDNF polymorphism on LTP and memory performance are investigated. Subjects carrying the Met allele (Val/Met and Met/Met) had significantly less LTP than Val/Val individuals. Met/Met individuals also performed significantly less well in a test of visual memory. Further, the degree of LTP was significantly correlated with the index of visual memory.

2.2 Introduction

Some people have much better memories than others. This variation in memory ability may be due to the variation of a gene that controls secretion of brain-derived neurotrophic factor (BDNF). In humans, a single nucleotide polymorphism (SNP) of the BDNF gene (Val^{66}Met; SNP rs6265) has been shown to influence episodic memory performance, and the degree of task-related hippocampal activation measured by fMRI. Specifically, it has been shown that individuals carrying the Met allele perform significantly worse in a test of episodic memory relative to those homozygous for the Val allele, and have significantly lower levels of neural activation in the hippocampal region (Egan et al., 2003; Hariri et al., 2003).

As cultured hippocampal neurons transfected with Met-BDNF have reduced activity dependent BDNF secretion (Egan et al., 2003; Chen et al., 2004), it was suggested that BDNF influences memory via an effect on a form of acute neural plasticity known as long-term potentiation (LTP) (Egan et al., 2003; Hariri et al., 2003). LTP is an enduring facilitation of synaptic transmission between neurons that follows repeated co-activation of the neurons in a network (Martin, Grimshaw, & Morris, 2000). Since its discovery (Bliss & Lomo, 1973), LTP has been studied extensively at the cellular and molecular level in laboratory animals, and remains the principal candidate mechanism underlying learning and memory (Teyler, 2000; Bliss, Collingridge, & Laroche, 2006; Whitlock, Heynen, Shuler, & Bear, 2006; Pastalkova et al., 2006). Certainly, there is extensive evidence that in animal preparations BDNF plays an important acute role in LTP induction (Lu & Gottschalk, 2000; Figurov, Pozzo-Miller, Olafsson, Wang, & Lu, 1996; Hennigan, Callaghan, Kealy, Rouine, & Kelly, 2009; Lu, 2003; Poo, 2001; Bramham & Messaoudi,

2005; Lu, Christian, & Lu, 2008; Cunha, Brambilla, & Thomas, 2010). Thus, the poorer mnemonic performance of carriers of the Met-BDNF allele may well be due to reduced BDNF release that results in diminished levels of LTP.

However, BDNF also has a variety of roles in long-term developmental processes such as neuronal migration, differentiation and survival, as well as neurogenesis and dendritic maintenance (Acheson et al., 1995; Huang & Reichardt, 2001; Hua & Smith, 2004). Indeed, it has been found that Met-BDNF carriers have reduced neural volumes in the hippocampus, as well as a variety of neocortical areas (Pezawas et al., 2001; Szeszko et al., 2005). It is possible therefore that the reduced hippocampal activation and poorer memory performance observed in Met-BDNF carriers (Egan et al., 2003; Hariri et al., 2003) may be due to chronic developmental adaptations in brain anatomy rather than acute changes in neural transmission (Pezawas et al., 2001).

Thus, the reported differences in mnemomic performance and hippocampal activation between Val homozygotes and Met carriers might be due either to acute LTP-like processes or chronic developmental mechanisms. In this study therefore, we investigated the extent to which Val66Met-BDNF polymorphism affects LTP measured non-invasively in humans in a paradigm that was recently developed in our laboratory (Teyler et al., 2005; Clapp et al., 2005; McNair et al., 2006). Further, we investigated the extent to which the magnitude of LTP correlated with previously employed tests of visual memory.

2.3 Materials and Methods

Genotyping

DNA extraction

DNA was extracted from blood samples using the method described in previous literature (36). The DNA samples were resuspended in Tris-EDTA buffer and quantified using Nanodrop ND-1000 1-poisition spectrophotometer (Thermo Scientific).

DNA amplification

All DNA samples were diluted to 50 ng/µL. Amplification was carried out using a modified version of the method described in previous literature (35) on the 113 bp polymorphic BDNF fragment, using the following primers BDNF-F 5'-GAG GCT TGC CAT CAT TGG CT-3' and BDNF-R 5'-CGT GTA CAA GTC TGC GTC CT-3'. Polymerase chain reaction (PCR) was conducted using 10x Taq buffer (2.5L µL), Taq polymerase (0.125 µL), dNTPs (5 nmol), primers (10 pmol each), Q solution (5 µL) and DNA (100 ng) made up to 25 µL with dH_2O. PCR conditions were as follows: denaturation at 95 °C for 15 min, 30 cycles on a thermocycler (denaturation at 94 °C for 30 s, annealing at 60 °C for 30 s, and extension at 72 °C for 30 s) with a final extension at 72 °C.

Enzyme digestion

PCR product (6.5 µL) was incubated with PmlI at 37 °C overnight. Digestion products were analysed using a High-res agarose gel (4%) with a Quick load 100bp ladder (BioLabs) and a GelPilot Loading Dye (QIAGEN). The DNA was visualized under UV

light after immersing in ethidium bromide solution for 10 min.

Genotyping

Digestion resulted in a 113 bp fragment for the Met^{66} allele and this was cut into 78 and 35 bp fragments for the Val^{66} allele (35). The genotyping resulted in 10 Val/Val subjects, 10 Val/Met subjects, and 9 Met/Met subjects. This did not differ from Hardy-Weinburg equilibrium ($\chi^2_{(2)} = 0.818$, $p = .664$), and there were no gender differences between genotypes ($\chi^2_{(2)} = 0.876$, $p = .645$).

LTP Induction and Measurement

Subjects

Twenty nine healthy participants with a mean age of 24.2 years (range 21-35; *SD* = 3.3 years) took part in this experiment. There were sixteen females (all right handed) and thirteen males (3 left handed). Handedness was determined using the Edinburgh Handedness Inventory (Oldfield, 1971). All participants had normal or corrected-to-normal vision. Subjects gave their informed consent to participate in the study and all experimental procedures were approved by the University of Auckland Human Subjects Ethics Committee.

Apparatus

Electroencephalogram (EEG) recordings were carried out in an electrically shielded room. EEG was recorded continuously (250 Hz sampling rate; 0.1 – 100 Hz analogue bandpass filter) using 128-channel Ag/AgCl electrode nets (Electrical Geodesics Inc.,

Eugene, OR, USA). All electrode impedances were below 50 kΩ (range 30 to 50 kΩ), an acceptable level for this system (Ferree & Luu, 2001). EEG was acquired using a common vertex (Cz) reference and later re-referenced to the average reference off-line.

Stimuli

Sine gratings with a spatial frequency of 1 cpd were used as the stimuli. The gratings were either horizontal or vertical in orientation and were generated using Matlab 6.5 software (The Mathworks Inc.). The gratings were circular, with the diameter subtending a visual angle of 8°, and presented at full contrast on a grey background (Figure 2.1). Stimuli were presented on a SVGA computer monitor (1024 x 768 pixel resolution; 60 Hz refresh rate) at a distance of 57cm. Stimulus presentation was controlled using E-Prime v1.1 (Psychology Software Tools). TTL pulses generated via the parallel port of the display computer provided synchronisation of stimulus events with EEG acquisition. Millisecond timing routines for the visual displays and pulse generation were conducted as outlined in the E-Prime User Guide (Psychology Software Tools).

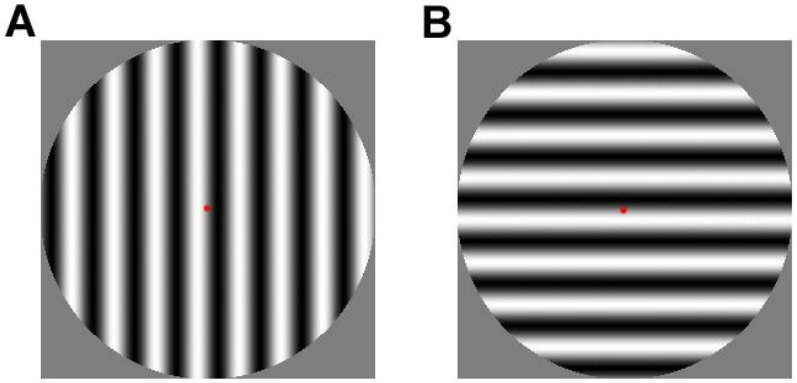

Figure 2.1. Experimental stimuli in the present study consisted of either A) horizontal or B) vertical circular sine gratings with a spatial frequency of 1cpd. Both gratings were presented on a grey background at full contrast.

Procedure

Subjects were asked to passively fixate on a circular red dot in the middle of the screen for the duration of the experiment. The experiment itself consisted of five phases: two baseline blocks, a photic tetanus, two more baseline blocks, a 30-min rest period, and finally two more baseline blocks. Each baseline block consisted of 120 presentations of the horizontal sine gratings and 120 presentations of the vertical sine gratings. The order in which stimuli were presented (horizontal or vertical orientation) during baseline blocks was randomized. The stimuli were presented centrally for 33ms with a randomly jittered inter-stimulus interval of 1000-1500ms – a temporal frequency of 1 Hz. The photic tetanus consisted of 1000 presentations of either the horizontal stimuli or the vertical stimuli. As baseline readings of horizontal and vertical sine gratings do not differ, subjects were randomly allocated to either the horizontal or vertical condition, which differed on which grating was used as the tetanus. During the tetanus, stimuli duration was 33ms with a randomly jittered inter-stimulus interval of 67-100ms – a temporal frequency of 8.6 Hz.

This frequency was chosen as it is a relatively high flicker rate, but not so high in that perceptual fusion occurs. During the rest period, participants had to rest with their eyes closed for 30 min.

Analysis

After data collection, EEG readings were then segmented into 600ms epochs – 100ms before the onset of the stimulus and 500ms post-stimulus onset. Automatic eye-movement correction was made on all segments according to the methods developed by Jervis and colleagues (Jervis et al., 1985). The data within each subject was then averaged according to orientation (horizontal and vertical) and block (pre-tetanus, early post-tetanus, late post-tetanus). The amplitude of the N1b component was measured by following validated procedures (Teyler et al., 2005; McNair et al., 2006; Ross et al., 2008). In order to non-arbitrarily measure the amplitude of the N1b component, individual N1b components were analysed. The N1b was defined as the part of the waveform extending from the peak of the N1 component to halfway between the N1 component and the P2 component (Figure 2.2). Averages were generated over this time period by analyzing clusters of seven electrodes situated around P7 and P8 (Figure 2.3) under the 10-10 system (Luu & Ferree, 2000), due to previous ICA work (Teyler et al., 2005). LTP amplitude was measured for each block (pre-tetanus, early post-tetanus, late post-tetanus) and each condition (horizontal, vertical). Amplitude increase was measured by subtracting pre-tetanus N1b amplitudes from early post-tetanus amplitudes and late post-tetanus amplitudes. This was done for both the tetanized and non-tetanized grating. There were no significant differences between pre-tetanus amplitudes and early post-tetanus amplitudes and late post-tetanus amplitudes for the non-tetanized condition ($p > .05$).

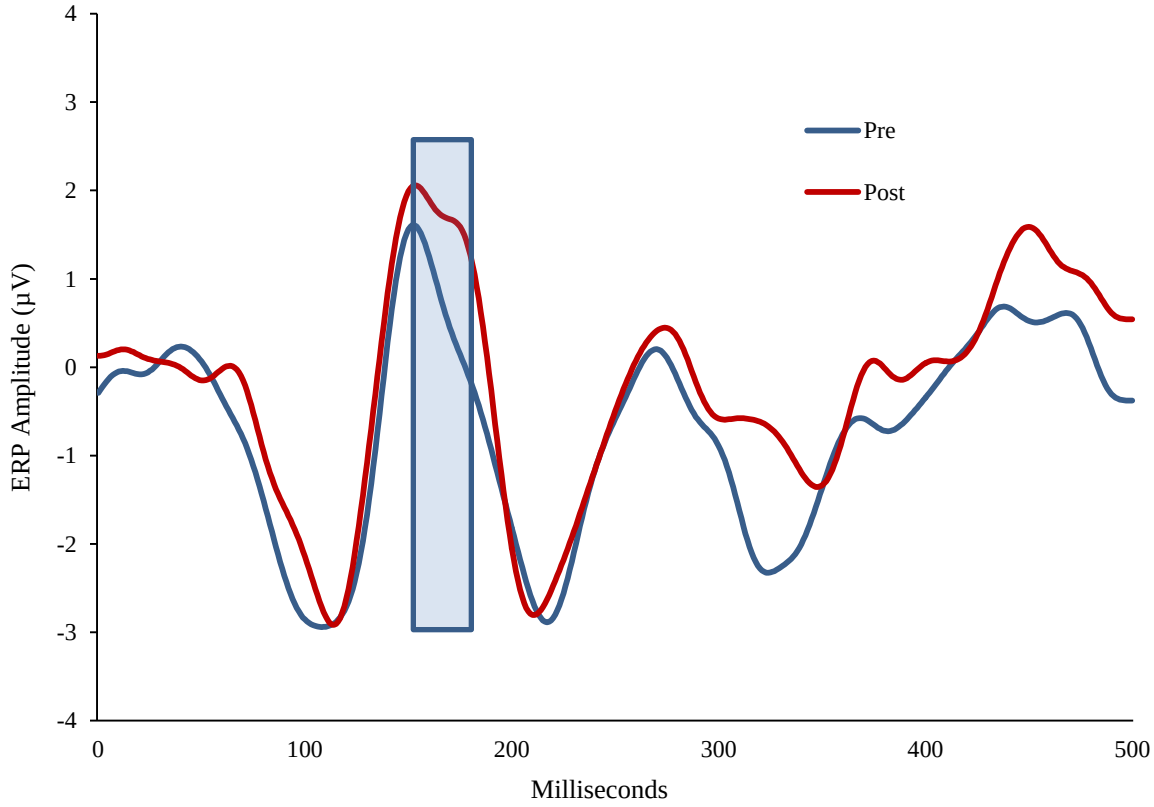

Figure 2.2. ERP waveform demonstrating potentiated post-tetanic N1b amplitudes for tetanized gratings Data is the average of the clusters surrounding P7 and P8. The blue box denotes the time period for where the N1b is taken. Data is taken from a representative subject.

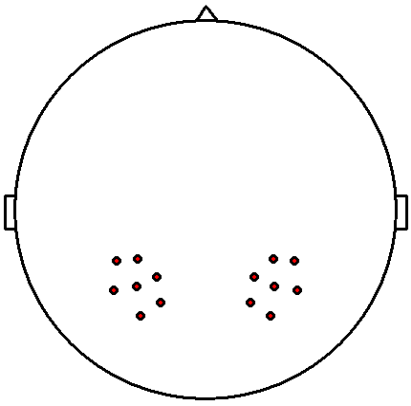

Figure 2.3. Approximate location of left and right hemisphere electrodes (centred on approximately P7 and P8 under the 10-10 system) used to measure amplitude of N1b component, due to this being earlier identified (Teyler et al., 2005).

Memory testing

Procedure

To test memory function, three subtests of the Wechsler Memory Scale Edition 3 (40) were used. Long-term memory was assessed by using two visual memory subtests (Faces and Family Pictures). On the Faces task, participants were presented with a set of twenty four faces for two sec each that they were asked to remember. Participants then had to pick the original faces (make an "old/new" decision) from a selection of forty eight faces which included the twenty four original faces as well as twenty four new faces. Participants then had to wait 30 min and were once again asked to pick the original faces from a new selection of forty eight faces. For the Family Pictures task, individuals were presented pictures of a family in a variety of scenes for 10 sec. Participants were then asked to recall as much detail as they could about each scene. Just as with the faces, participants had to wait 30 min, and were then asked to recall as many details as possible from each scene. Index scores of both immediate and delayed visual memory were taken.

Statistical Analyses

All non-tetanized gratings showed no significant difference following tetanus and thus were excluded from statistical analysis. For all statistical tests, subjects were grouped into

their respective genotypes (Val/Val, Val/Met, & Met/Met). In order to examine whether the differences in LTP were significant, a 3 x 2 Split-Plot analysis of variance (ANOVA) was conducted, using Genotype (Val/Val, Val/Met, and Met/Met) as the between-subjects independent variables and Time (Early LTP, Late LTP) as the within-subjects dependent variable, with early LTP being LTP recorded straight after tetanus, and late LTP being LTP recorded 30 minutes after tetanus. An earlier ANOVA revealed that the gender of participants played no part as there were no significant differences in LTP between genders, and thus was not included in the final ANOVA. The main effect of interest was whether or not there was a significant difference in LTP amplitude (μV) between the three Genotypes (a main effect of Genotype on LTP).

In order to examine whether or not the differences in memory scores were significant, a 3 x 2 Split-Plot ANOVA using Genotype (Val/Val, Val/Met, Met/Met) as the between-subjects independent variable and Time (Immediate memory, Delayed memory) as the within-subjects dependent variable was conducted. The main effect of interest was to examine whether there was a significant difference in memory test scores between the three Genotypes (a main effect of Genotype on Memory score). The interaction between Memory and Genotype was also examined, in order to see whether differences existed across both Immediate and Delayed memory. In order to examine whether or not individual levels of LTP (Early, Late) could predict memory performance as indexed by memory scores (Immediate and Delayed memory), two multiple regressions were conducted. The first multiple regression used Early LTP and Late LTP as the two predictor variables, and Immediate Memory as the criterion (dependent variable), with the second using Delayed Memory as the dependent variable. All statistical analyses were run using the computer program SPSS version 17, and an alpha of .05 was used for all analyses. Greenhouse-

Geisser corrections were applied to all *F*-statistics, and Bonferroni adjustments were used in any pair wise comparisons.

2.4 Results

Table 2.1 *Mean values of LTP* (*Early and Late*) *and Memory* (*Immediate, Delayed, and Working*) *for the two Genotype groups* (*Val/Val and Polymorphism*).

	LTP (µV)		**Memory**	
Group	**Early**	**Late**	**Immediate**	**Delayed**
Val/Val	0.49	0.61	85.70	86.43
Val/Met	-.01	0.08	77.52	81.45
Met/Met	.04	-.35	74.22	77.65

Genotype and LTP

The results from the 3 x 2 split plot ANOVA (seen in Table 2.1) revealed a significant main effect of Genotype on LTP ($F_{(2, 26)} = 10.88$, $p < .05$). Also, the interaction between Genotype and LTP was significant ($F_{(2, 26)} = 10.88$, $p < .05$; Figure 2.4). Post-hoc analysis revealed that the differences were between Val/Val individuals and both other groups. At LTP measured directly after tetanus (Early LTP) Val/Val individuals ($M = .49$, $SE = .12$) had significantly higher amplitude increase than Val/Met ($M =$.-.01, $SE = .12$; p

< .05) and Met/Met individuals (M = .04, SE = .13; p < .05). Similar findings were found for LTP measured 30 minutes after tetanus (Late LTP) with Val/Val (M = .61, SE = .12) individuals showing significantly higher amplitude increase than Val/Met (M = .07, SE = .12; p < .05) or Met/Met individuals (M = -.35, SE = .13; p < .05). Whilst there is a visible difference between Val/Met and Met/Met's in Late LTP, with Val/Met individuals seemingly having a higher amplitude increase, this was not, nor were any other effects significant (p > .05).

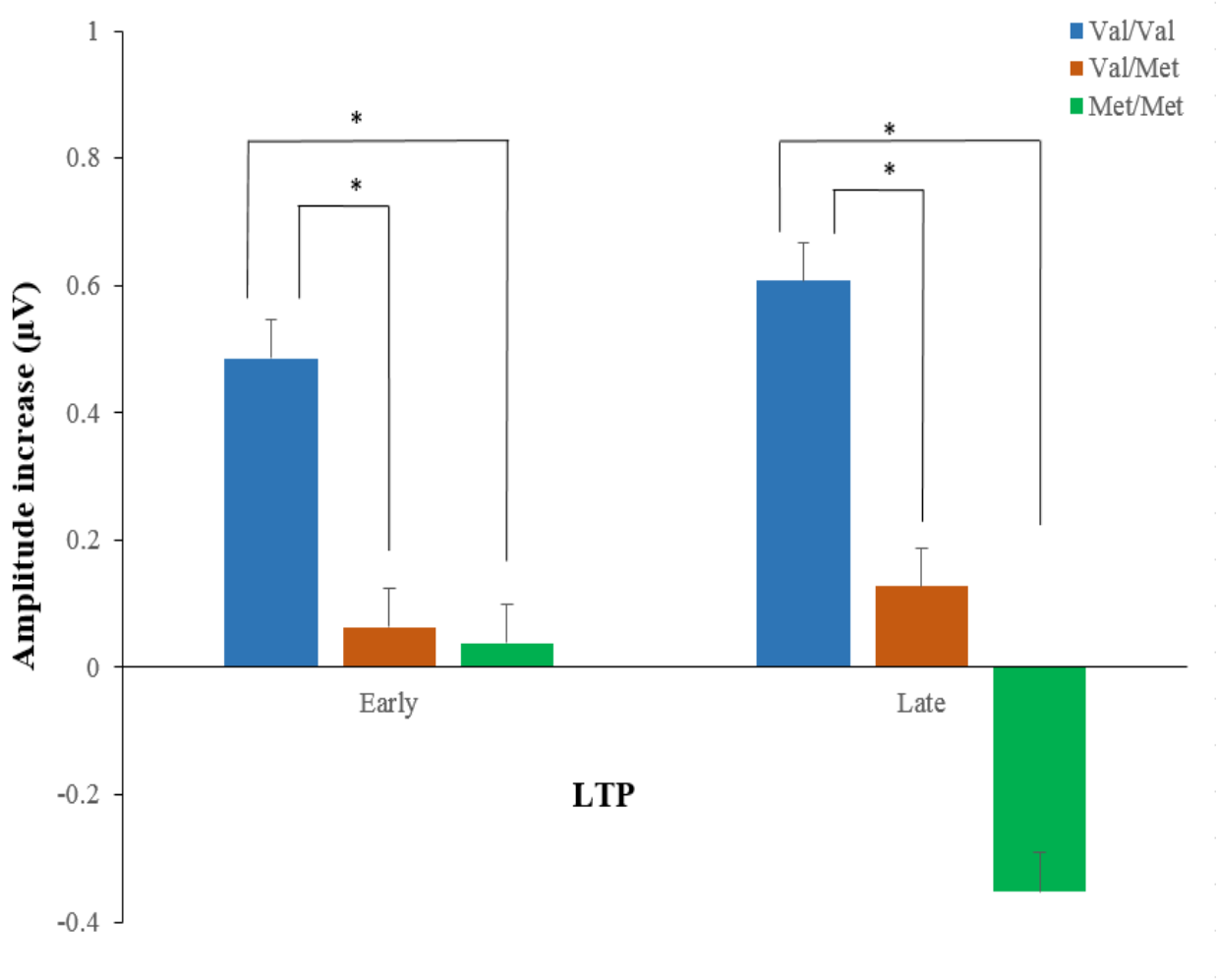

Figure 2.4. The interaction between BDNF genotype and LTP. Val/Val individuals show significantly more LTP both at earlier stages and at later stages of measurement. Val/Mets also show more LTP than Met/Mets at later stage, this however is not significant.

Genotype and Memory

The results of the 3 x 2 split plot ANOVA (seen in Table 1), revealed a significant main effect of Genotype on Memory performance ($F_{(1, 22)} = 7.65$, $p < .05$; Figure 2.5). Post hoc analysis revealed that Val/Val ($M = 86.07$, $SE = 2.38$) performed significantly better than Met/Met individuals ($M = 75.93$, $SE = 3.08$; $p < .05$). Whilst there appeared to be

differences in Immediate and Delayed memory between the three genotypes, there was no significant interaction between Time and Genotype ($F_{(2, 22)} = 1.22$, $p > .05$). No other significant main effects or interactions were found ($p > .05$)

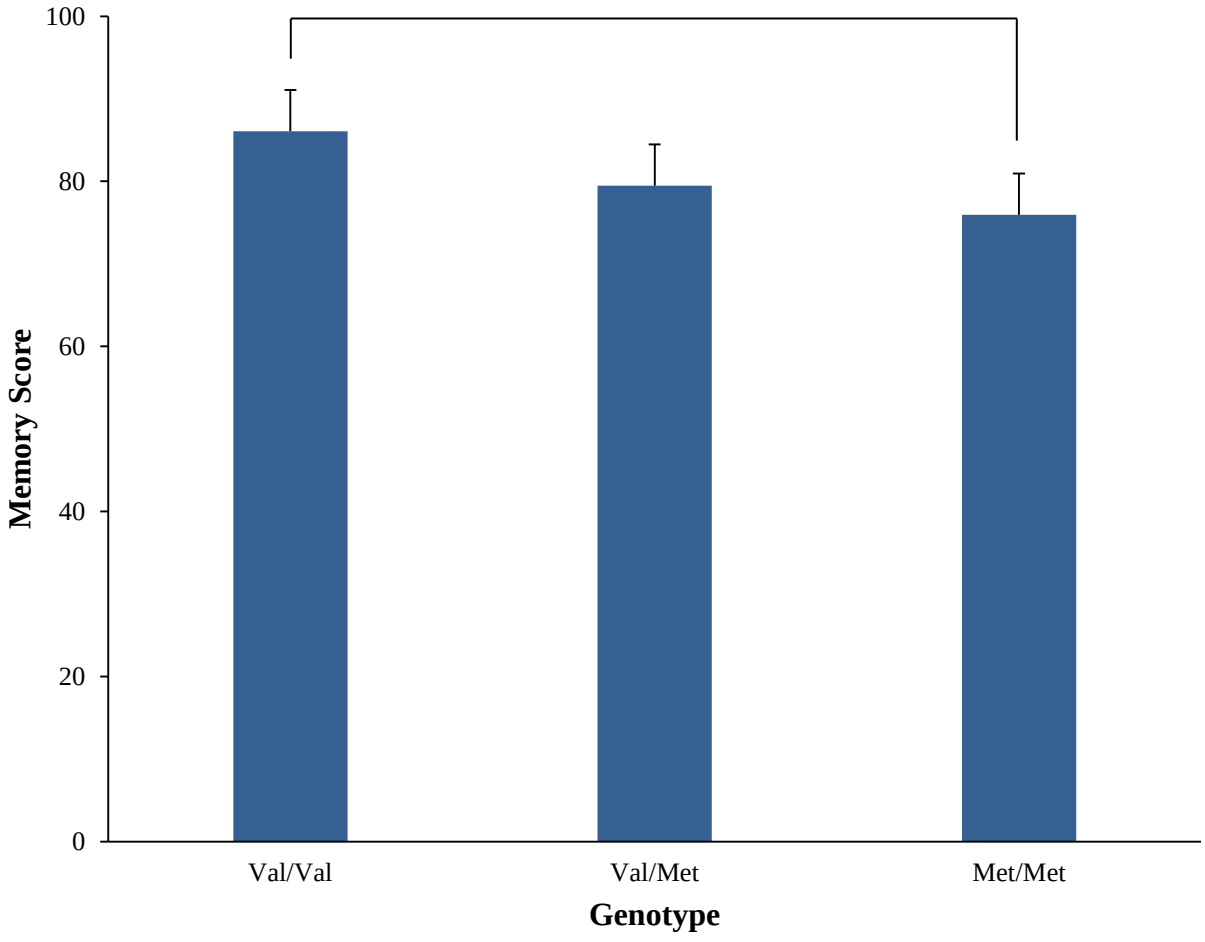

Figure 2.5. The main effect of Genotype on Memory scores. Val/Val individuals perform significantly better than Met/Met individuals on visual memory as indexed by scores on the WMS-III. While there is a

difference between Val/Val's and Val/Met's and Val/Mets and Met/Mets, this was not significant. Asterisks show significant differences at the .05 level.

LTP and Memory

Table 2.2 Pearson *Correlations between the predictor variables* (*Early and Late LTP*) (*predictor variable*) *and the two dependent variables* (*Immediate and Delayed*).

	1.	2.	3.	4.
1. Early LTP	1.00	.69*	.15	-.06
2. Late LTP	.69*	1.00	.43*	.39
3. Immediate memory	.15	.43*	1.00	.86*
4. Delayed memory	-.06	.39	.86*	1.00

Note * $p < .05$

As shown in Table 2.2, early LTP is significantly and positively correlated with late LTP, while late LTP is significantly and positively correlated with immediate memory. Immediate memory is also significantly and positively correlated with delayed memory. No other correlations were significant. The first analysis showed that 22% of the variability in immediate memory was accounted for by the two predictors, which however was not significant ($R^2 = .22$; $F_{(2, 22)} = 3.98$, $p > .05$). However, if early LTP was removed, the model became significant, which suggests a mediating effect of Early LTP. The second multiple regression used Early LTP and Late LTP as the two predictor variables, and Delayed Memory as the criterion. The resulting model explained 36% of the variance seen in memory scores, which was significant ($R^2 = .36$; $F_{(2, 22)} = 6.14$, $p < .05$). Late LTP was a

better predictor of memory (β = .83; t = 3.49, p < .05) than early LTP (β = -.63; t = -2.67 p < .05), in that for every one µV increase in late LTP there was an average increase in delayed memory scores by over 13 percent (B = 13.02, p < .05). These trends can be observed in Figure 2.6.

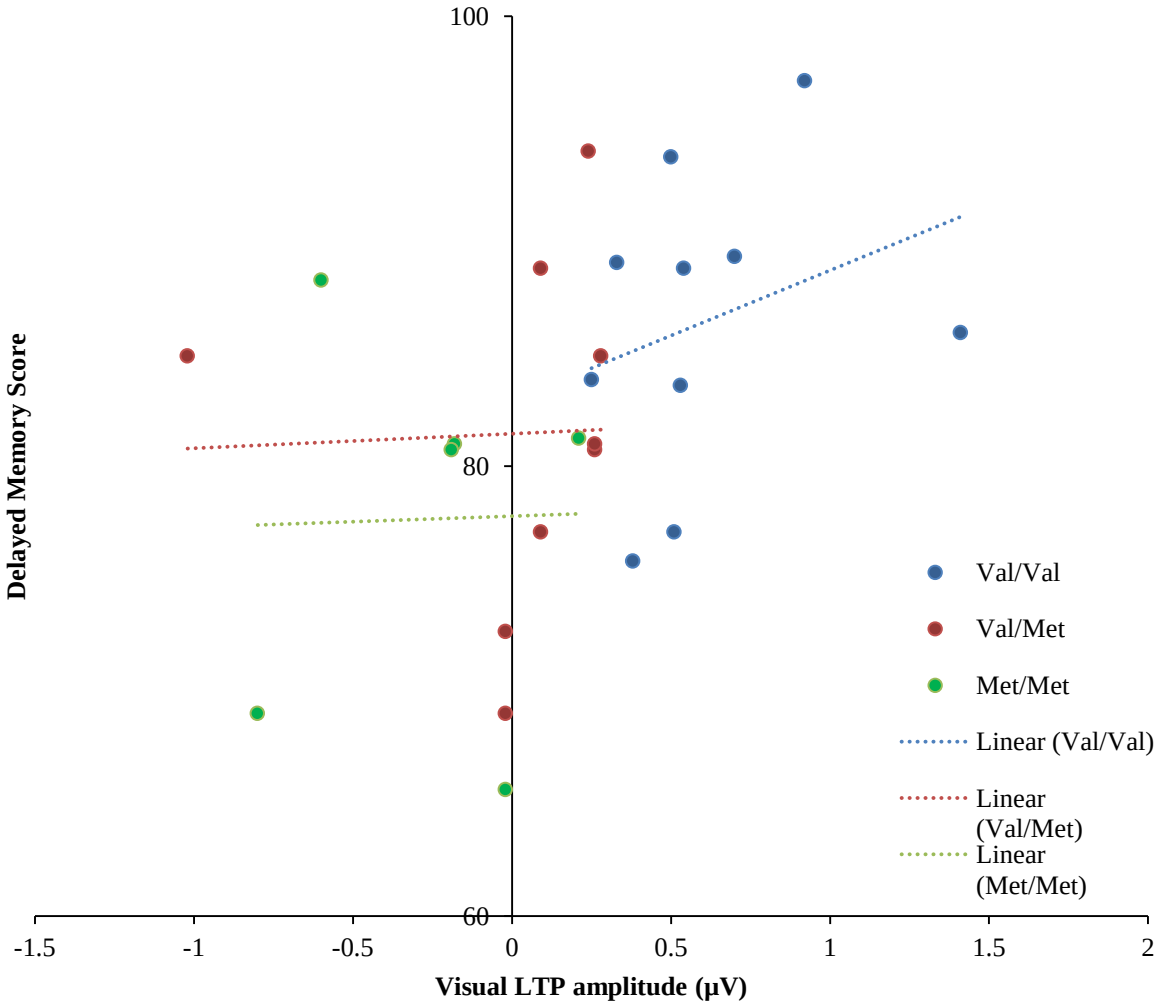

Figure 2.6. WMS-III delayed memory performance plotted against Late LTP amplitude. Visual LTP amplitude significantly predicts WMS-III visual memory index (p < .05; r=.60)

2.5 Discussion

Consistent with previous work (Egan et al., 2003; Hariri et al., 2003), we found that individuals carrying a Met-BDNF allele have significantly poorer performance on tests of visual memory relative to those homozygous for the Val-BDNF allele. However, this was only significant when individuals contained two copies of the Met allele as opposed to just one. However, we also show for the first time that the Val^{66}Met polymorphism for BDNF significantly affects LTP in human sensory LTP. Individuals carrying the Met-BDNF allele had significantly lower levels of LTP, as indexed by changes in amplitude to visual evoked potentials. Once again, individuals with two copies of the Met allele show an even greater disparity in LTP, with LTP measured at 30 minutes after tetanus actually being lower than baseline. Further, we show that the degree of LTP is a significant predictor of memory performance. That the delayed measure of LTP is a better predictor of mnemonic function than LTP measured immediately after its induction is consistent with the idea that a delayed measure of LTP is a more accurate indicator of the degree of the persistent increase in synaptic efficacy than an immediate measure. BDNF is nevertheless believed to have differential effects at various temporal stages of LTP induction (Braham & Messaoudi, 2005).

Thus, these data provide compelling evidence that differences in memory task performance between BDNF genotypes are to a considerable extent due to differences in acute or rapid LTP-like changes in synaptic transmission in mnemonic networks, as has been suggested previously (Egan et al., 2003; Hariri et al., 2003), rather than

developmental differences in the neural substrates. It is still possible however that, as suggested previously (Pezawas et al., 2004), chronic developmental differences between carriers of different BDNF polymorphisms do have some influence on memory performance. It remains a distinct possibility, for example, that different levels of hippocampal BOLD activation (Egan et al., 2003; Hariri et al., 2003) are due to differences of this sort.

Generally, these data also show that a measure of human LTP significantly correlates with mnemonic performance. A similar relationship between the extent of hippocampal LTP and memory task performance has been notoriously difficult to demonstrate in experimental animals. That we have done so here may be due to the relative homogeneity of our sample, and/or that accurately quantifying mnemonic performance is more easily achieved in tests of humans. Regardless, it is clear that the human sensory LTP paradigm used here has considerable potential in the study of LTP-memory relationships per se.

It should be noted however, that the types of memory task used here are generally assumed to be primarily dependent on hippocampal processes. It is perhaps surprising, therefore, that sensory LTP measured over P7 and P8 electrodes indexes memory performance so reliably. There are two possible explanations. Firstly, it is possible that the sensory LTP paradigm employed here indexes a general propensity for an individual to exhibit LTP. Thus, the degree of sensory LTP determined in the visual neocortex reflects the potential for similar plasticity in the hippocampus. As such, visual LTP is a window on the general potential for LTP, perhaps of the brain as a whole.

The second possible explanation for the correlation between visual cortical plasticity and memory performance that we describe here is the possible integral involvement of visual system circuitry in networks subserving visual memory formation. It has been

suggested for example that visual system plasticity may be an integral part of mnemonic processing of visual information (Tsanov & Manahan-Vaughan, 2008; Ji & Wilson, 2007), and that plastic processes in the visual system influence subsequent processing in hippocampus (Tsanov & Manahan-Vaughan, 2008).

Finally, it is widely believed that defects in synaptic plasticity are associated with a variety of affective and neurocognitive disorders (Kirk et al., 2010; Cooke & Bliss, 2006; Teyler & Cavus, 2007; Normann, Schmitz, Furmaier, Doing, & Bach, 2007; Hariri, 2010; Branchi, 2011; Erikson et al., 2008), and that BDNF polymorphisms may influence susceptibility to a number of these disorders (Hariri, 2010; Branchi, 2011; Erikson et al., 2008). Combining the assessment of plasticity with genotyping as described here may provide potential clinical applications in the diagnosis and assessment of a variety of perceptual and cognitive disorders, including Alzheimer's disease, depression and schizophrenia.

Chapter 3 *BDNF* Val^{66}Met and Recognition Memory: EEG

Brain-Derived Neurotrophic Factor Val^{66}Met affects Recollection but not Familiarity ERP components of human recognition memory.

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3.1 Abstract

A single nucleotide polymorphism of the human BDNF gene (Val^{66}Met) may account for much of the variation in human memory performance. However, it is not clear if this polymorphism affects all forms of memory. BDNF is concentrated most heavily in the hippocampus, and therefore would be likely to have a greater effect on hippocampal dependent memory. Recognition memory involves the contribution of two distinct retrieval processes, Recollection and Familiarity. Prior research suggests that Familiarity does not depend on the hippocampus, but Recollection does. Recent evidence has shown Recollection and Familiarity are associated with distinct event-related potentials (ERP): Familiarity with an early-onset effect called the FN400; and Recollection with a later positivity called the late positive component (LPC). The current research investigated whether different BDNF genotypes differ with respect to their generation of the FN400 and the LPC. Using ERP generation in a facial recognition memory paradigm, no genotype differences in FN400 amplitude (evoked when correctly recognising a previously presented face) were found. However, Val/Val individuals generated a significantly more positive LPC when correctly identifying an old face after a period of consolidation of 24 hours. These findings suggest that the Val^{66}Met polymorphism may play an exclusive role for hippocampal dependent memory.

3.2 Introduction

Some people have much better memories than others. This variation in memory ability may be due to the variation of a gene that controls secretion of brain-derived neurotrophic factor (BDNF). In humans, a single nucleotide polymorphism (SNP) of the BDNF gene (Val^{66}Met; SNP rs6265) has been shown to influence episodic memory performance, and the degree of task-related hippocampal activation measured by fMRI. Specifically, it has been shown that individuals carrying the Met allele perform significantly worse in a test of episodic memory relative to those homozygous for the Val allele, and have significantly lower levels of neural activation in the hippocampal region (Egan et al., 2003; Hariri et al., 2003).

However, it is not known whether this difference in memory seen by genotype holds for all forms of memory. Recognition memory in particular has been shown to be two functionally dissociable systems, known as Familiarity and Recollection (Rugg & Yonelinas, 2003; Yonelinas, 2001, 2002). Familiarity describes a feeling that a particular stimulus may have been experienced before but is devoid of contextual information, whereas Recollection is the recognition of a stimulus as well as the contextual details of the environment accompanying the original presentation of the stimulus (Gardiner, 1988; Jacoby, 1991; Mandler, 1980; Tulving, 1985; Yonelinas, 1999, 2001, 2002; Yonelinas et al., 1996; Yonelinas & Jacoby, 1996). These two systems have been shown through numerous animal, clinical and imaging studies to also be dependent on different neural substrates, namely Recollection being dependent on the hippocampus, whilst Familiarity involving the perirhinal cortex (Vargha-Khadem, Gadian, Watkins, Connelly, Van Paesschen, & Mishkin, 1997; Aggleton & Brown, 1999; Aggleton & Brown, 2006;

Yonelinas et al., 2002; Davachi, Mitchell and Wagner, 2003). As BDNF is most strongly concentrated in the hippocampus (Pezawas et al., 2005), it is possible that the BDNF polymorphism affects Recollection forms of memory, but not Familiarity. Thus, this could lead to differences in the neurological brain processes underlying these two forms of memory.

Recent evidence has suggested that Familiarity and Recognition are associated with two distinct ERPs. Familiarity is associated with an early frontally distributed ERP called the FN400, whilst Recollection is associated with a later parietal positivity called the LPC (Addante, Ranganath & Yonelinas, 2012; Curran, 2000; Duzel, Vargha-Khadem, Heinze & Mishkin, 2001; Rugg & Curran. 2007). Addante and colleagues (2012) showed that when individuals successfully recognized a stimuli as 'old' but were unable to remember contextual information about the stimulus (measured by source accuracy – whether the stimuli came from one condition or another) a clear FN400 ERP was seen. However, when the subject could accurately locate the source of the memory an LPC effect was seen. This suggests the recruitment of contextual elements to the recognition of stimuli is a function of the LPC. As patients with hippocampal damage are able to show an FN400 response but not an LPC response, it is argued that the LPC is dependent on the hippocampus (Duzel et al., 2001).

The aim of the current study was to see whether or not the BDNF Val^{66}Met polymorphism played a significant role in modulating the FN400 and LPC components of recognition memory. As the LPC is associated with contextual information, we employed a method where we had subjects study a set of stimuli and return a day later to recall this information. These responses would be compared with individuals responding to stimuli immediately after initial presentation, which would show a FN400. We hypothesized that

individuals with a copy of the Met allele would show less of an LPC response, but the polymorphism would have no effect on FN400 response.

3.3 Materials and Methods

Genotyping - A full description of the genotyping process can be found in Chapter 2

EEG Procedure

Subjects

An initial cohort was recruited and genotyped. From this cohort, eighteen healthy participants with a mean age of 23.5 years (range 21-29; *SD* = 2.5 years; 9 females) were selected such that they formed two groups defined by Val^{66}Met genotype (9 Val/Val, and 9 carrying at least one Met allele – this group consisted of 4 Met/Met participants). Some of these participants were individuals from the Chapter 2 study on LTP, but not enough to be able to correlate the results of that study with this study. The cohort did not differ from Hardy-Weinburg equilibrium ($\chi^2_{(2)} = 0.818$, $p = .664$), and there were no gender differences between genotypes ($\chi^2_{(2)} = 0.876$, $p = .645$). Two participants chose not to participate between the first EEG and second EEG, therefore only 16 subjects data was available to be analysed for the second part of the EEG. All participants had normal or corrected-to-normal vision. Subjects gave their informed consent to participate in the study and all experimental procedures were approved by the University of Auckland Human Subjects

Ethics Committee.

EEG Acquisition

A full description of the EEG acquisition parameters can be found in Chapter 2.

Stimuli

Two hundred faces obtained from the FEI Face database (Thomaz & Giraldi, 2010) were used in this experiment. All faces were on white backgrounds and were resized to 480 x 480 pixels using Adobe Photoshop. There was always an equal number of female to male faces in all parts of the experiment. Fifty faces were used in the study phase of each memory experiment ('old'). During the recall phase participants identified the 50 studied/'old' objects from 100 object images (50 'old' and 50 'new'). Four sets of faces were counterbalanced between 'old' and 'new' and counterbalanced between participants so each set were alternated between the Familiarity and Recollection tasks. Subjects were seated 57 cm from the display screen monitor (measured from the computer screen to the participant's face). Stimuli were presented on a SVGA computer monitor (1024 x 798 pixel resolution; 60Hz refresh rate). Stimulus presentation was controlled using E-Prime v1.1 (Psychology Software Tools). TTL pulses generated via the parallel port of the display computer provided synchronisation of stimulus events with EEG acquisition. Millisecond timing routines for the visual displays and pulse generation were conducted as outlined in the E-Prime User Guide

(Psychology Software Tools, Pittsburgh, PA, USA).

Experimental Procedure

The methods for the Familiarity and Recollection experiments are described together. The only methodological differences between the experiments was the time between the study and recall phases. In the Familiarity Task the recall phase came immediately after the study phase. In the Recollection Task the recall phase took place 24 hours after the study phase.

Procedure.

Two tests of recollection- and familiarity-based recognition memory were employed as measures of overall object recognition memory. In the Recollection Task the study phase and subsequent recall phase were separated by 24 hours, whereas they were consecutive in the Familiarity Task. In the study phase of the Familiarity Task participants were asked to actively put-to-memory 50 faces presented individually with a fixation cross 1500-2500 second jittered delay between each face. After the study phase, participants then had to make 'old-new' judgments for 100 faces (50 from the study phase ('old') and 50 novel objects ('new')). Subjects were advised to use the keyboard to indicate '1' for 'old' and '2' for 'new'. Participants were then removed from the EEG machine and testing equipment, and taken to a separate testing room where they were given 30 minutes to learn another set of 50 faces. These were presented on a computer using Microsoft Office Powerpoint. Participants were advised that they would be required to remember these faces for the recall phase of the Recollection Task, which was to occur the following day. The logic of this was to allow for a deeper processing and encoding of the faces to occur. As the hippocampus is involved in contextual information about a stimulus, we hypothesized that recalling these faces a day later would tap into a different memory network. Twenty-four hours after this study phase, participants completed the 'old-new' task to identify the

'old' objects they had learned the previous day and the 'new' objects.

EEG Analysis

EEG analyses were performed using custom software from The University of Auckland, School of Psychology. EEG readings were then segmented into 1100ms epochs – 100ms before the onset of the stimulus and 1000ms post-stimulus onset. Automatic eye-movement correction was made on all segments according to the methods developed by Jervis and colleagues (Ferree, Luu, Russell, & Tucker, 2001). A priori defined time windows were used based upon established literature of familiarity and recollection-related effects (Addante et al., 2012; Curran, 2000; Rugg & Curran, 2007). As per the literature we selected the following time windows for analysis: 400-600 ms (Figure 3.1) for the Familiarity Task, and 600-800 ms (Figure 3.2) for the Recollection Task. Averages were generated over this time period by analyzing clusters of seven electrodes situated around Cz and Cp5 (Figure 3.3) under the 10-10 system (Luu & Ferree, 2000). The electrodes to identify the FN400 and LPC components were selected for analysis on the basis of previous literature (Addante et al., 2012; Curran, 2000; Rugg & Curran, 2007). FN400 and LPC components were measured for both familiarity and recollection experiments, and amplitude increase was measured by looking at increases for successful 'old' responses versus successful 'new' responses. Misses and false alarms were removed from the analysis. The difference in component amplitude was taken ('old' minus 'new') and used for statistical analysis.

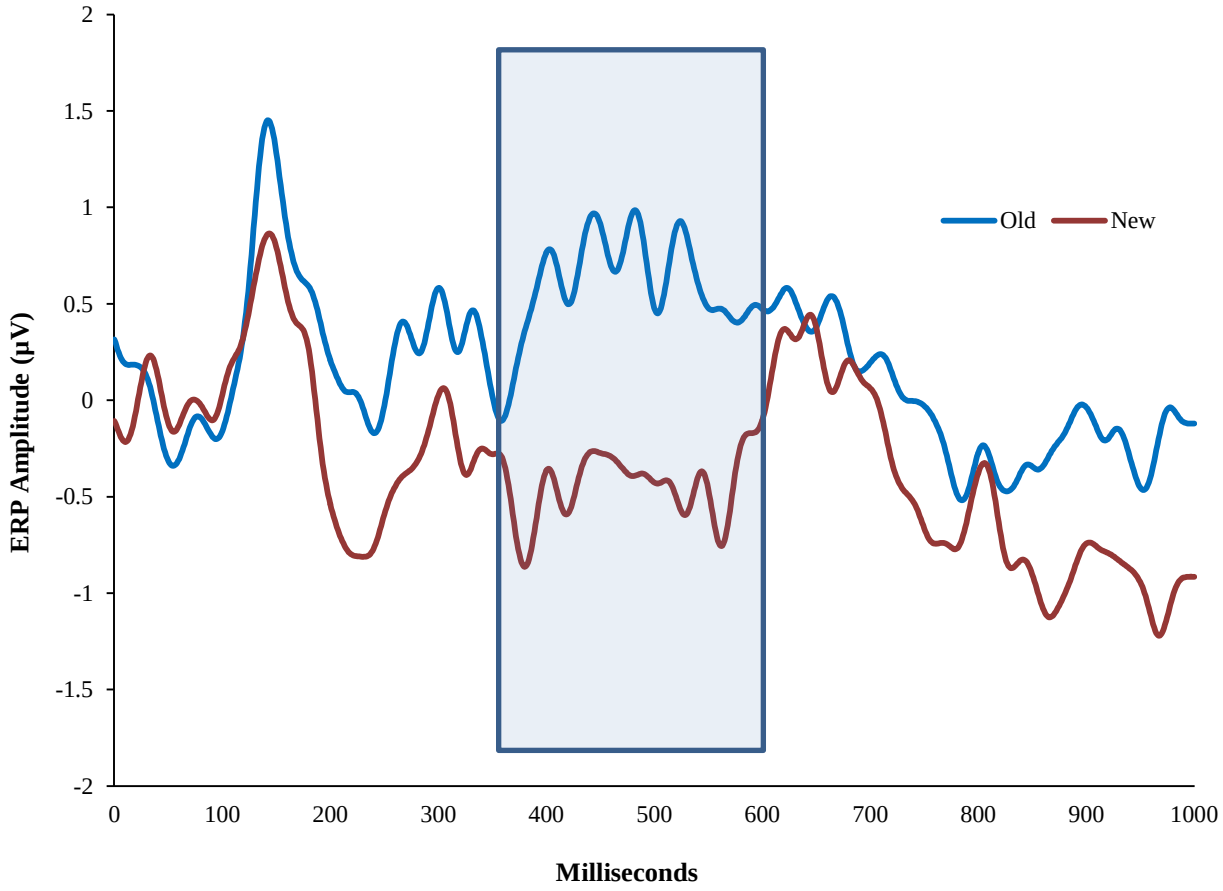

Figure 3.1 ERP waveforms demonstrating increased positivity seen for successful recognition of 'old' stimuli. The blue box shows the time period that the averages were generated from. Data is taken from a representati

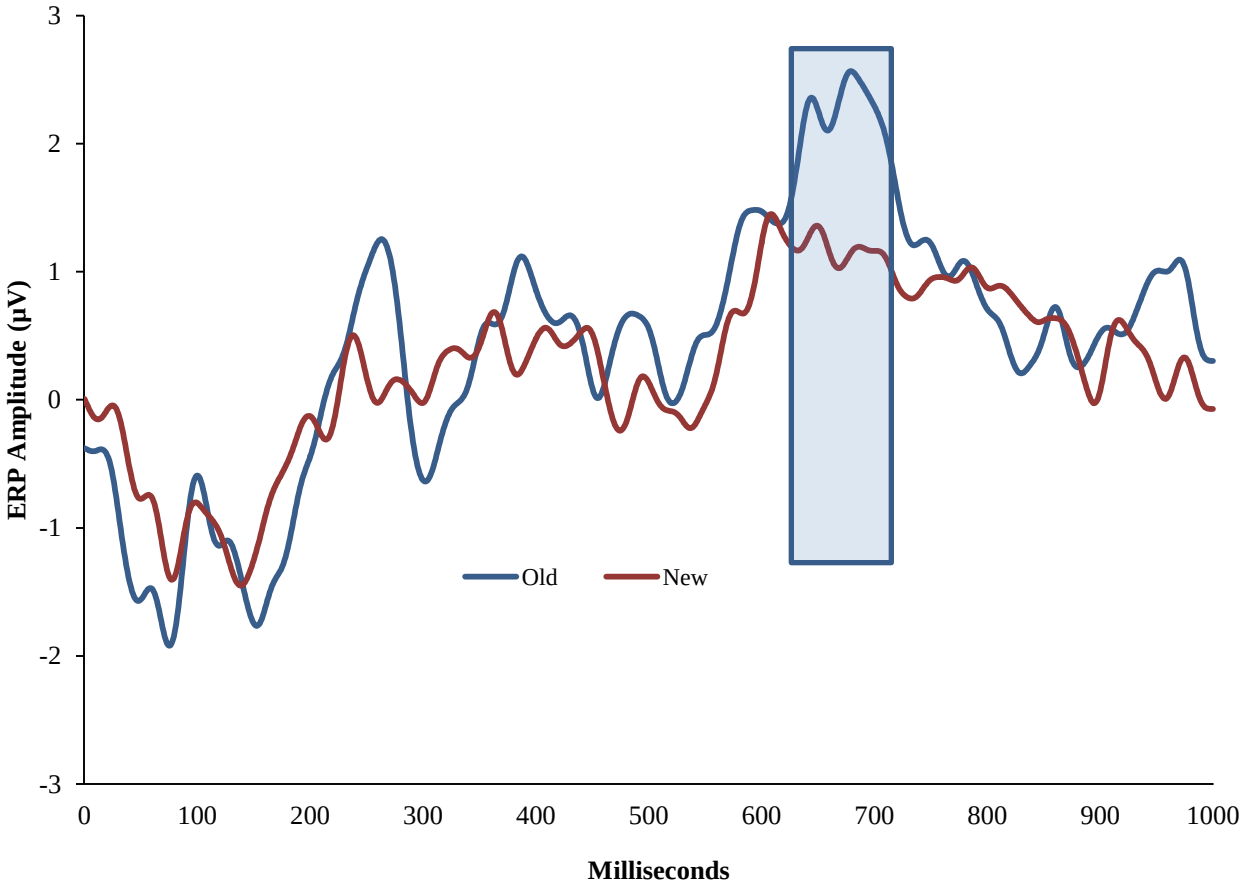

Figure 3.2 ERP waveforms demonstrating increased positivity seen for successful recognition of 'old' stimuli. The blue box denotes the time period for where averages were generated. Data is taken from a representative subject.

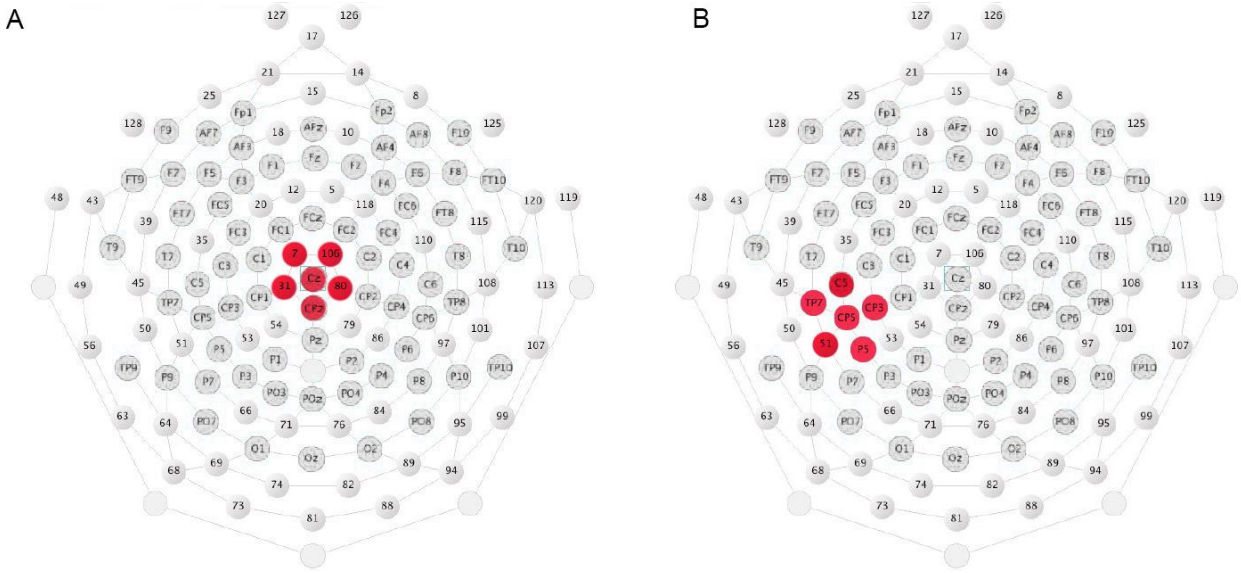

Figure 3.3. Approximate location of electrodes used for analysis. **A** shows the electrodes centred on Cz (under the 10-10 system) and were the electrodes used to identify the FN400 component, whilst **B** shows the electrodes centred around Cp5 (under the 10-10 system) and were the electrodes used to identify the LPC components.

Statistical Analyses

T-tests showed no differences for FN400 for the recollection experiment, and no differences for LPC for the familiar experiment, and thus were excluded from all further analyses. For all statistical tests, subjects were grouped into two groups, either not having a Met allele (Val/Val individuals) or having a copy of the met allele (Val/Met, & Met/Met). For the FN400 and LPC analysis, 2 x 2 ANOVAs were employed to see whether the amplitude for an 'old' and 'new' response was different, and whether this difference was higher or lower for one group than the other. An independent t test was employed to see whether or not there were behavioural differences between the two groups. Task performance was calculated as sensitivity (d'), which is the difference between hit rate and false alarm rate. All statistical analyses were run using the computer program SPSS version 17, and an alpha of .05 was used for all analyses. Bonferroni corrections were used for post

hoc analyses.

3.4 Results

Behavioural Results

On the Familiarity task there were no significant performance differences between Val/Val (M = 1.47, SE = .29) and Met carriers (M = 1.49, SE = .27; t (16) = -.07, p > .05). On the Recollection task there were no significant performance differences between Val/Val (M = 3.36, SE = .18) and Met carriers (M = 2.97, SE = .28; t (13) = 1.15, p > .05). Thus there was no significant performance difference between the genotype groups in either of the facial recognition memory tests.

Recognition memory – Familiarity

Table 3.1 *Mean values of FN400* (*Successful 'Old' responses and successful 'New' responses*) *for the two Genotype groups* (*Val/Val and Met Carrier*).

	FN400 (µV)	
Group	**'Old'**	**'New'**
Val/Val	.94	.16
Met Carriers	1.68	.79

The results of the 2x 2 split plot ANOVA (seen in Table 3.1), revealed a significant main effect of Memory on ERP amplitude ($F_{(1, 16)}$ = 11.58, p < .05). The amplitude of the FN400 for 'Old' responses (M = 1.31, SE = 0.28) was significantly more positive than

'New' responses. (M = .47, SE = 0.34; Figure 3.4). This shows the paradigm successfully elicits a FN400 component. However, there was no significant interaction between Memory and Genotype ($F_{(1, 16)}$ = .04, p > .05) and the main effect of genotype was not significant (p > .05). Thus, the increase in amplitude of the FN400 component seen for successful recognition of an 'old' face versus a new 'face' does not differ between genotype.

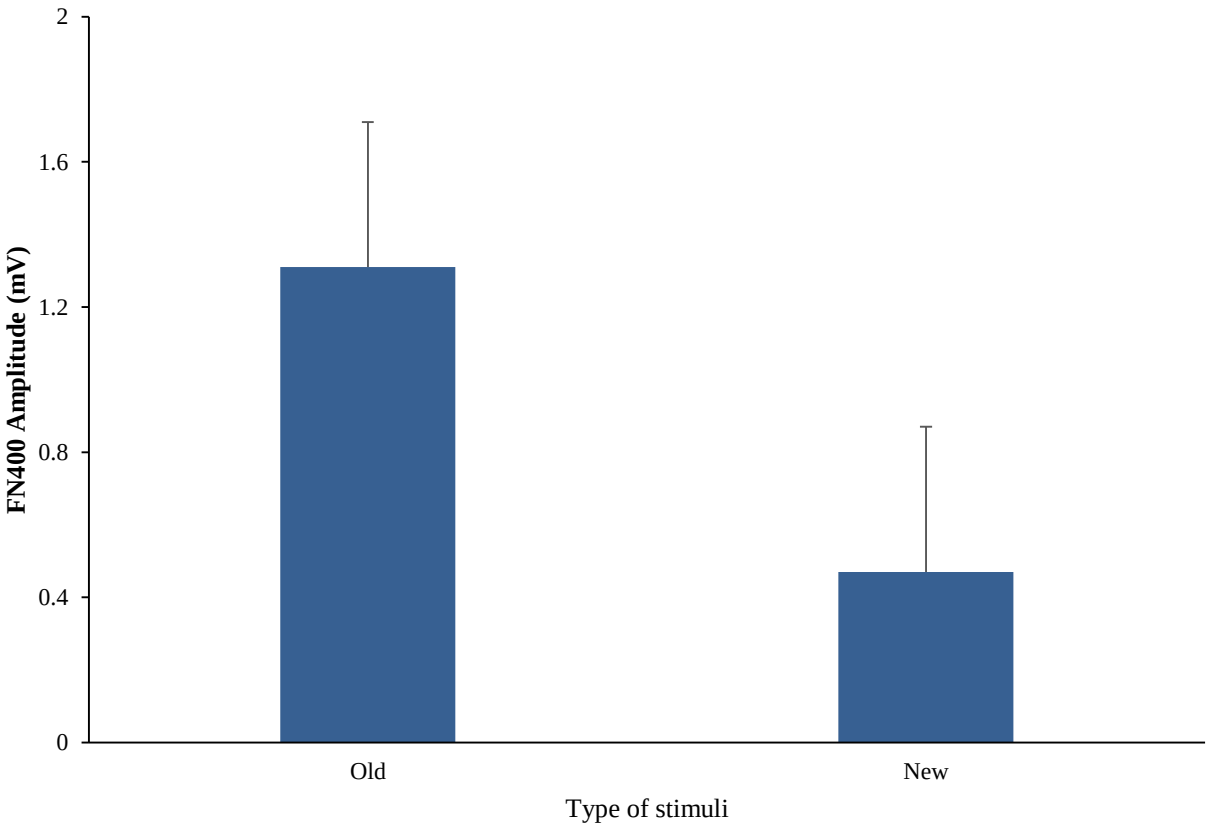

Figure 3.4. The main effect of Memory type on FN400 amplitude. Correctly recognizing an 'old' face elicits a significantly more positive FN400 than recognizing a 'new' face.

Recognition memory – Recollection

Table 3.2 *Mean values of LPC* (*Succesful 'Old' responses and successful 'New' responses*) *for the two Genotype groups* (*Val/Val and Met Carrier*).

	LPC (μV)	
Group	**'Old'**	**'New'**
Val/Val	1.75	.86
Met Carriers	.79	1.04

The results of the 2x 2 split plot ANOVA (seen in Table 3.2), revealed a significant main effect of Memory on ERP amplitude ($F_{(1, 14)} = 4.63$, $p < .05$). The amplitude of the LPC for 'old' responses ($M = 1.27$, $SE = .21$) was significantly more positive than 'new' responses. ($M = .95$, $SE = .19$; $p < .05$). This shows the paradigm successfully elicits an LPC component. The interaction between Memory and Genotype was also significant ($F_{(1, 16)} = .04$, $p < .05$; Figure 3.5). Post hoc analysis revealed for Val/Val individuals, the amplitude of the LPC for 'old' responses ($M = 1.75$, $SE = .32$) was significantly more positive than 'new' responses. ($M = .79$, $SE = .28$; $p < .05$). For Met carriers, the amplitude

of the LPC for 'old' responses (M = .86, SE = .29) was not significantly more positive than 'new' responses. (M = 1.04, SE = .26; p > .05). Lastly, when looking at the amplitude of the LPC for 'old' responses, Val/Val individuals were significantly more positive than Met carriers (p < .05). The results show the increase in amplitude of the LPC component seen for successful recognition of an 'old' face versus a new 'face' differed between genotype. The main effect of genotype was not significant (p > .05).

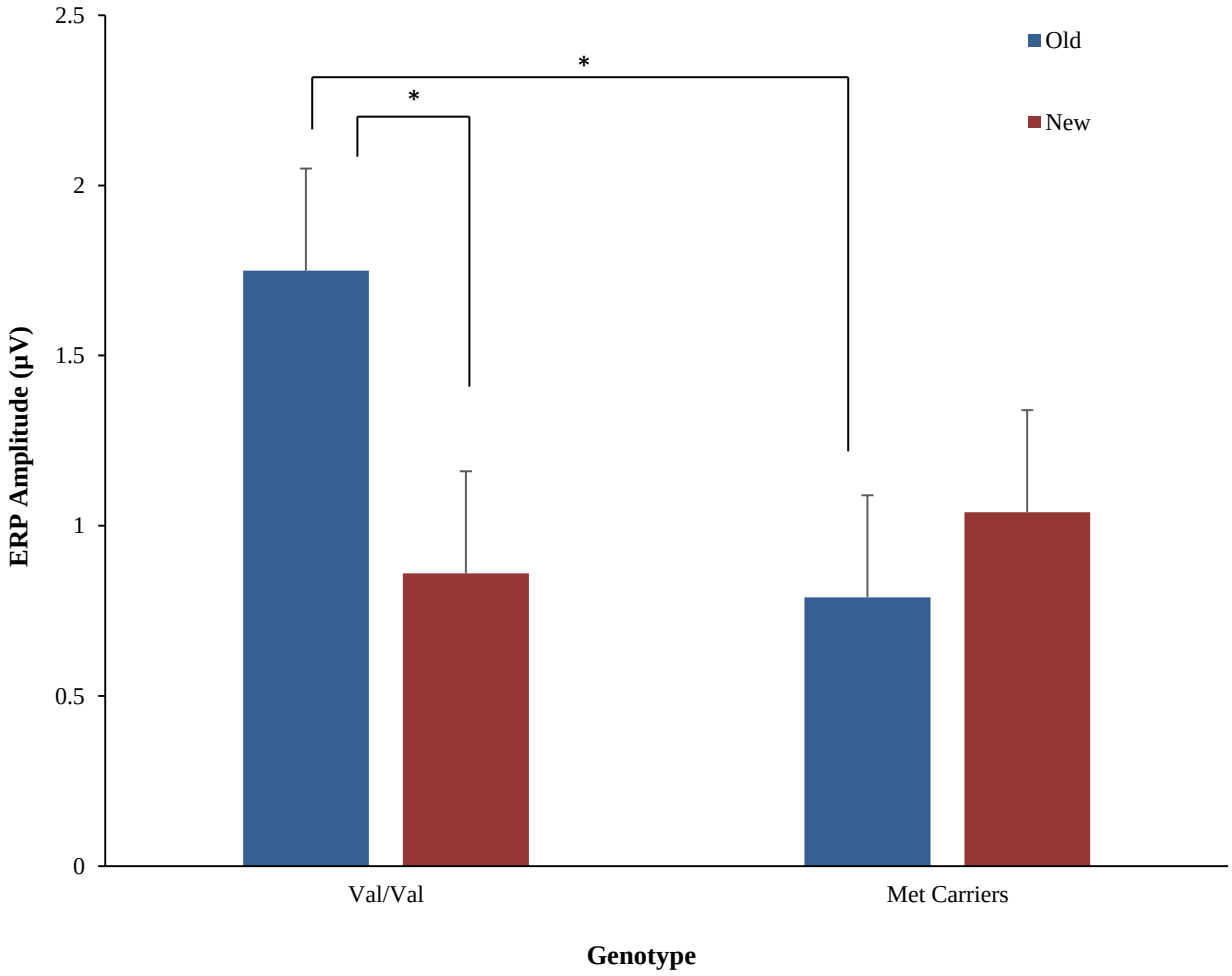

Figure 3.5. The significant difference in LPC modulation by Genotype. When correctly recognizing an 'old' face versus correctly recognizing a 'new' face, Val/Val individuals show a more positive LPC component, whereas Met carriers show no significant difference in LPC amplitude. Val/Val's also show a significantly more positive LPC for 'old' responses (asterisks label significances at p <.05).

3.5 Discussion

Consistent with previous work (Addante et al., 2012; Duzel et al., 2001) a significant increase in positivity in the FN400 component was found when participants successfully recognized a stimulus as 'old'. However, the resulting increase in positivity did not differ significantly by genotype. Individuals with a copy of a Met allele showed the

same increase in FN400 amplitude as Val/Val individuals. As there was no difference in behavioural scores for Familiarity, this suggests there is no effect of the BDNF polymorphism on this type of memory.

In contrast, a significant difference between genotypes for LPC amplitude was found. Relative to Met carriers, Val/Val individuals showed a significantly larger increase in positivity of the LPC component when successfully identifying a stimuli as 'old' as opposed to 'new'. Not only this, but Met carriers did not show a significant difference in LPC amplitudes. This suggests the BDNF polymorphism plays a part in this type of memory. This may be a subtle effect as no significant differences between genotypes was found in behavioural performance on Recollection.

It should also be noted that for the Familiarity experiment, no LPC old/new difference was generated, and there was no FN400 modulation generated in the Recollection experiment. This is further support for the body of work suggesting that these paradigms index respectively, the familiarity and recollection forms of recognition memory.

As there was no difference in FN400 modulation by genotype in the Familiarity experiment, this strongly suggests that the BDNF Val^{66}Met polymorphism does not play a role in the type of memory. As shown in previous research, the FN400 ERP is associated with Familiarity forms of recognition memory (Addante et al., 2012; Duzel et al., 2001). This type of memory has been shown to be exclusive of the hippocampus (Vargha-Khadem, Gadian, Watkins, Connelly, Van Paesschen, & Mishkin, 1997; Aggleton & Brown, 1999; Aggleton & Brown, 2006; Yonelinas et al., 2002; Davachi, Mitchell and Wagner, 2003). Whilst BDNF is widely spread throughout the CNS, it is most highly concentrated in the hippocampus (Pezawas et al., 2004). It is possible that this is the reason

the BDNF polymorphism is not having an effect on Familiarity forms of memory.

On the other hand, in the Recollection experiment, LPC modulation was significantly influenced by genotype, suggesting that the BDNF Val^{66}Met polymorphism affects this ERP. Previous research has shown the LPC to be associated with Recollection forms of memory, particularly memory that is rich in contextual detail. As mentioned earlier, BDNF is most highly concentrated in the hippocampus (Pezawas et al., 2004). As BDNF plays an important role in long-term potentiation (LTP), and the implication of hippocampal LTP, the BDNF polymorphism may affect hippocampal dependent forms of memory via a disruption to LTP (Lu, 2003; Lu, Christian, & Lu, 2007; Poo, 2001; Egan et al., 2003; (Athos, Impey, Pineda, Chen, & Storm, 2002; Blum, Moore, Adams, & Dash, 1999; Bozon, Kelly, Josselyn, Silva, Davis, & Laroche, 2003). Hariri and colleagues (2003) showed Met carriers had decreased hippocampal activation combined with their behavioural performance deficiencies. Whilst our cohort does not mirror the behavioural data of Hariri et al (2003), it is interesting that the BDNF Val^{66}Met polymorphism affects both electrical and haemodynamic properties of the brain. These results show the effect of the BDNF Val^{66}Met polymorphism has on the brain may be subtle.

Interestingly, whilst Val/Val individuals show a more positive LPC in the direction expected (more positive for 'old' stimuli), Met carriers actually show the reverse pattern, with the LPC trending more positive for 'new' stimuli. However, this is not significant. As there are no behavioural differences between the two groups, this suggest that the BDNF Val^{66}Met polymorphism could potentially lead to different memory strategies and subsequently different brain activity. Addante et al (2012) show that the LPC is modulated by source accuracy of the memory; that is to say memory confidence. This suggests Met carriers may be more certain for 'new' faces, whilst Val/Val show the same certainty for

faces that they have already encountered. The two groups could be employing different strategies to reach a similar result. The results suggest Val/Val individuals form stronger memories for the studied face. Introducing a measure of memory confidence into the paradigm (such as a remember/know judgment, or a temporal source judgment such as whether the face came from the initial familiarity experiment or was one of the faces studied subsequent to the initial study) could discover potential behavioural differences.

Chapter 4. *BDNF* Val^{66}Met and Recognition Memory: fMRI

Brain-Derived Neurotrophic Factor val^{66}met affects hippocampal activation in the successful recognition of a famous face.

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4.1 Abstract

A single nucleotide polymorphism of the human BDNF gene (Val^{66}Met) may account for much of the variation in human memory performance. However, it is not clear if this polymorphism affects all forms of memory equally. BDNF is concentrated most heavily in the hippocampus, and therefore would be likely to have a greater effect on hippocampal dependent memory. Recognition memory involves the contribution of two distinct retrieval processes, Recollection and Familiarity. Research suggests that Familiarity does not depend on the hippocampus, but Recollection does. Recent evidence suggests the successful recognition of famous faces is dependent on the hippocampus, whilst recognising a non-famous face is not. The current fMRI study looked at individuals who had a copy of the Met allele and whether they showed differential activation in the hippocampus using a facial recognition memory paradigm with famous and non-famous faces. Differences were found between famous and non-famous face recognition, as well as between Val homozygotes and Met carriers. Famous faces elicited significantly greater activation in the hippocampus than non-famous faces. There was greater hippocampal activation seen for Val/Val individuals. There were no clear differences in the recognition of non-famous faces. These results suggest an exclusive role of BDNF in hippocampal dependent memory.

4.2 Introduction

Some people have much better memories than others. This variation in memory ability may be due to the variation of a gene that controls secretion of brain-derived neurotrophic factor (BDNF). In humans, a single nucleotide polymorphism (SNP) of the BDNF gene (Val^{66}Met; SNP rs6265) has been shown to influence episodic memory performance, and the degree of task-related hippocampal activation measured by fMRI. Specifically, it has been shown that individuals carrying the Met allele perform significantly worse in a test of episodic memory relative to those homozygous for the Val allele, and have significantly lower levels of neural activation in the hippocampal region (Egan et al., 2003; Hariri et al., 2003).

However, it is not known whether this genotype-mediated difference in memory holds for all forms of memory. Recognition memory in particular can be dissociated into two functionally dissociable systems, known as Familiarity and Recollection (Rugg & Yonelinas, 2003; Yonelinas, 2001, 2002). Familiarity describes a feeling that a particular stimulus may have been experienced before but is devoid of contextual information, whereas Recollection is the recognition of a stimulus as well as the contextual details of the environment accompanying the original presentation of the stimulus (Gardiner, 1988; Jacoby, 1991; Mandler, 1980; Tulving, 1985; Yonelinas, 1999, 2001, 2002; Yonelinas et al., 1996; Yonelinas & Jacoby, 1996). These two systems have been shown through numerous animal, clinical and imaging studies to also be dependent on different neural substrates, with Recollection being dependent on the hippocampus, and Familiarity involving the perirhinal cortex (Vargha-Khadem, Gadian, Watkins, Connelly, Van Paesschen, & Mishkin, 1997; Aggleton & Brown, 1999; Aggleton & Brown, 2006;

Yonelinas et al., 2002; Davachi, Mitchell and Wagner, 2003). As BDNF is most strongly concentrated in the hippocampus (Pezawas et al., 2005), it is possible that the BDNF polymorphism affects Recollection forms of memory, but not Familiarity. This could lead to differences in the neurological brain processes underlying these two forms of memory.

The aim of the present fMRI study was to examine differences in familiarity and recollection networks. It is hypothesized that Val/Val individuals will show an increased BOLD response when Recollection networks are activated, but not necessarily when Familiarity networks are activated. In order to examine these networks, a facial recognition memory paradigm will be employed. Research has shown the hippocampus is involved in the successful recollection of familiar faces, but not unfamiliar faces (Bird & Burgess, 2008, Trinkler et al., 2009). Therefore, an increased BOLD response when successfully remembering a familiar face for Val/Val individuals compared to individuals with a Met allele is expected. No differences in activation between genotypes is hypothesized for the successful recognition of unfamiliar faces.

4.3 Materials and Methods

Genotyping - A full description of the genotyping process can be found in Chapter 2

Experiment procedure

Subjects

An initial cohort was recruited and genotyped. From this cohort, eighteen healthy participants with a mean age of 24.0 years (range 21-33; *SD* = 3.1 years) were selected such that they formed two groups defined by Val^{66}Met genotype (8 Val/Val, and 9 carrying at least one Met allele – this group consisted of 4 Met/Met participants). Neither the initial cohort, nor the subsequently selected sub-group differed significantly from Hardy-Weinberg equilibrium. All participants had normal or corrected-to-normal vision. Subjects gave their informed consent to participate in the study and all experimental procedures were approved by the University of Auckland Human Subjects Ethics Committee.

Stimuli

Two hundred famous faces and two hundred non famous faces were obtained using a Google image search and were used in this experiment. All faces were on white backgrounds and were resized to 480 x 480 pixels using Adobe Photoshop. There was always an equal number of female to male faces in all parts of the experiment, as well as equal number of famous to non-famous faces. Two hundred faces were used in the encoding phase of each memory experiment. During the recall phase participants identified

the 200 studied/'old' faces from 400 (200 'old' and 200 'new'). Faces were counterbalanced between 'old' and 'new' and counterbalanced between participants so each set were alternated between the 'old' and 'new' conditions. To counter the fact that famous people often have better photos taken of themselves, non-famous faces actually consisted of German actors and actresses. Because of this fact, any subjects who had either spent time in Germany or were from Germany were excluded. Stimuli were projected onto a screen using a projector (1024 x 768 pixel resolution; 60Hz refresh rate). Subjects viewed the stimuli through a mirror attached to the head coil. Stimulus presentation was controlled using E-Prime v1.2 (Psychology Software Tools). Millisecond timing routines for the visual displays was conducted as outlined in the E-Prime User Guide (Psychology Software Tools, Pittsburgh, PA, USA).

Procedure.

In the Encoding phase, participants were presented with 200 faces, 100 of which were famous, and presented individually with a fixation cross 1500-2500 second jittered delay between each face. Participants had to respond as to whether they thought a face was a famous face or a non-famous face. Subjects were advised to use the response box and to use their index finger to indicate a 'famous' face and their middle finger for a 'Non Famous' face. These faces were presented to subjects in two blocks, with a rest period in between. Following this was a consolidation phase of 10 minutes, during which time structural MRI was carried out. The Retrieval phase was next, with participants then being presented with 400 faces, 200 of which were famous with the other 200 being non famous. 200 of these faces were the faces originally presented to subjects. These were again

presented individually with a fixation cross 1500-2500 second jittered delay between each face. Subjects then had to respond using the response box as to whether the face being presented was an 'old' face (a face that was presented in the first phase) or a 'new' face (this is the first time observing the face), using their index finger for 'old' and middle finger for 'new'. This step was also split into two blocks.

Image Acquisition

Images were acquired using a 3 Tesler Siemens Magnetom Skyra MRI scanner (Erlangen, Germany). T1-weighted structural volumes, were taken during the 10-minute waiting period following the initial presentation of faces using 3D MP-RAGE sequence (TR: 1900ms; TE: 2.07ms; Flip angle: 9°; FOV: 20.8cm x 25.6cm; 176 sagittal slices; matrix size: 208x 256; voxel size: 1x1x1mm). Scanning sessions began with acquisition of 272 T2*-weighted images (136 per block) (EPI sequence parameters: TR: 2300ms; TE: 27ms; Flip angle: 90°; FOV: 19.2cm x 19.2cm; 45 transverse slices approximately oblique to the superior temporal gyrus, using interleaved sequence beginning at the back; matrix size: 64x64; voxel size: 3.5x3.5x3.5cm). A further 536 scans (268 per block) using the same parameters as previous, were recorded during the recall blocks.

Pre-Processing

Image pre-processing was performed using SPM8 software (Wellcome Trust Centre for Neuroimaging, London, UK; http://www.fil.ion.ucl.ac.uk/spm). To correct for head movement between scans, functional images were spatially realigned to the first image in the session using a spline interpolation (5mm FWHM Gaussian kernel). For each participant, the T1-weighted structural volume was co-registered with the mean of their

functional images. Normalisation parameters were estimated using the unified segmentation procedure (Ashburner & Friston, 2005), and used to normalise the structural and functional images to the Montreal Neurological Institute template (MNI152 T1). Finally, the functional volumes were spatially smoothed using an isotropic 8x8x8mm FWHM Gaussian filter.

Analysis

Following preprocessing, the General Linear Model was employed to identify significant activations resulting from each condition. Within subject (1st level) design matrices were produced and estimated, followed by the formulation of contrasts to individually assess a number of conditions. These contrast images were then used to create a between subject (2nd level) design matrix. Two flexible factorial models were used for the Encoding phase, and then the Retrieval phase. For the Encoding phase with subject, group (2 levels, G1: Val/Val, G2: Met carriers), and condition (2 levels, Famous Hit, Non-Famous Hit) as factors. For the Retrieval phase with subject, group (2 levels, G1: Val/Val, G2: Met carriers), and condition (4 levels, Old Famous Hit, Old Non-Famous Hit, New Famous Hit, New Non Famous Hit) as factors. The main effects of group and condition, as well as the group X condition interaction were specified. This was then used to create contrasts to individually assess the different conditions and create statistical parametric maps. Contrasts were evaluated using a $p<0.05$ Family Wise Error (FWE) significance threshold to correct for multiple comparisons. These analyses were conducted across the whole brain and evaluated at the cluster level. Significant MNI coordinates were then converted into Talairach coordinates using the Talairach Applet (http://www.talairach.org/applet.html). Contrasts of interest and significance are laid out in the results section. Task performance for the memory recall phase was calculated as

sensitivity (d'), which is the difference between hit rate and false alarm rate. An independent samples t test was used to see if Val/Val's or Met carriers differed as groups on their task performance.

4.4 Results

Behavioural Results

There were no significant performance differences, in terms of d' scores for the Retrieval phase, between Val/Val (M = 1.97, SD = .37) and Met carriers (M = 1.90, SD = .50; t (15) = .30, p > .05).

Encoding phase

Analysis of the interaction between Group and Condition established that, compared to the Non-Famous faces, Famous faces had larger magnitude BOLD signal in the Left fusiform gyrus (Brodmanns area 37), right parahippocampal gyrus, right superior temporal gyrus and most importantly the left hippocampus. There were no areas associated with greater BOLD for non-famous faces compared to the famous faces. When comparing Val/Val individuals to Met carriers there was increased activation seen in right parahippocamal gyrus and the hippocampus, however this was not significant. There were no significant differences comparing that contrast the other way (see Figure 4.1 and Table 4.1 for all contrasts).

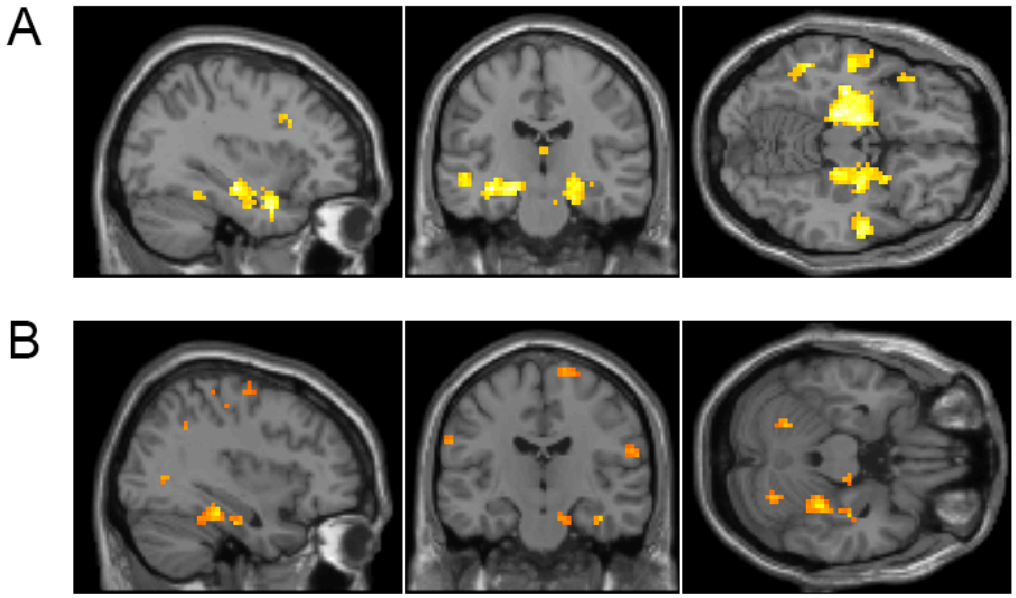

Figure 4.1 A) Comparison of increases of BOLD in the Encoding condition for Famous faces versus Non famous faces. Areas of significant increased activation can be seen in this slice in the hippocampus and the parahippocampal gyrus. B) Comparison of increases of BOLD in the Encoding condition for Famous faces, looking at Val/Val individuals versus Met carriers. Areas of increased activation can be seen in this slice in the hippocampus for Val/Val individuals. This was not significant.

Table 4.1 *Brain areas showing significant clusters of activation between conditions. Approximate locations are given, along with Brodmann's area, cluster size in voxels (3x3x5mm), and MNI coordinates and p-value for peak activation voxel. These results refer to the Encoding condition.*

Location	Brodmann's Area	Cluster size (voxels)	x y z (mm; MNI)	*p*-value
Famous face > Non famous face				
Left Fusiform gyrus	BA 37	56	-51 -43 -11	.022
Left Hippocampus	BA 54	1054	-33 -19 -14	<.001
Right Parahippocampal	BA 36	23	21 -22 -20	<.001
Right Superior Temporal Gyrus	BA 21	76	-51 -13 -11	.005
Non famous face > Famous face				
[No significant activation]				
Val/Val Famous face > Met carrier Famous face				
[No significant activation]				
Met carrier Famous face > Val/Val Famous face				
[No significant activation]				

Retrieval phase

Once again, a significant increase in BOLD was seen in the right parahippocampal gyrus, right superior temporal gyrus and the right hippocampus when comparing Famous faces with Non famous faces. Analysis of the interaction between Group and Condition established that Val/Val individuals when compared to Met carriers when observing a famous face (irrespective of it was an old or new face) had larger magnitude BOLD signal in the hippocampus which was significant. The converse contrast (activation seen for Met carriers and not Val/Val individuals) yielded no significant differences. When comparing Val/Val individuals to Met carriers successfully identifying an old famous face, there was increased activation seen in right parahippocampal gyrus and the hippocampus, however this was not significant. The converse contrast (activation seen for Met Carriers and not Val/Val individuals) showed no significant activation. When comparing Val/Val individuals to Met carriers successfully identifying an old non famous face, there was increased activation seen in the right thalamus/midbrain. The converse contrast (activation seen for Met Carriers and not Val/Val individuals) showed no significant activation (Refer to Figure 4.2 and Table 4.2 for all contrasts)

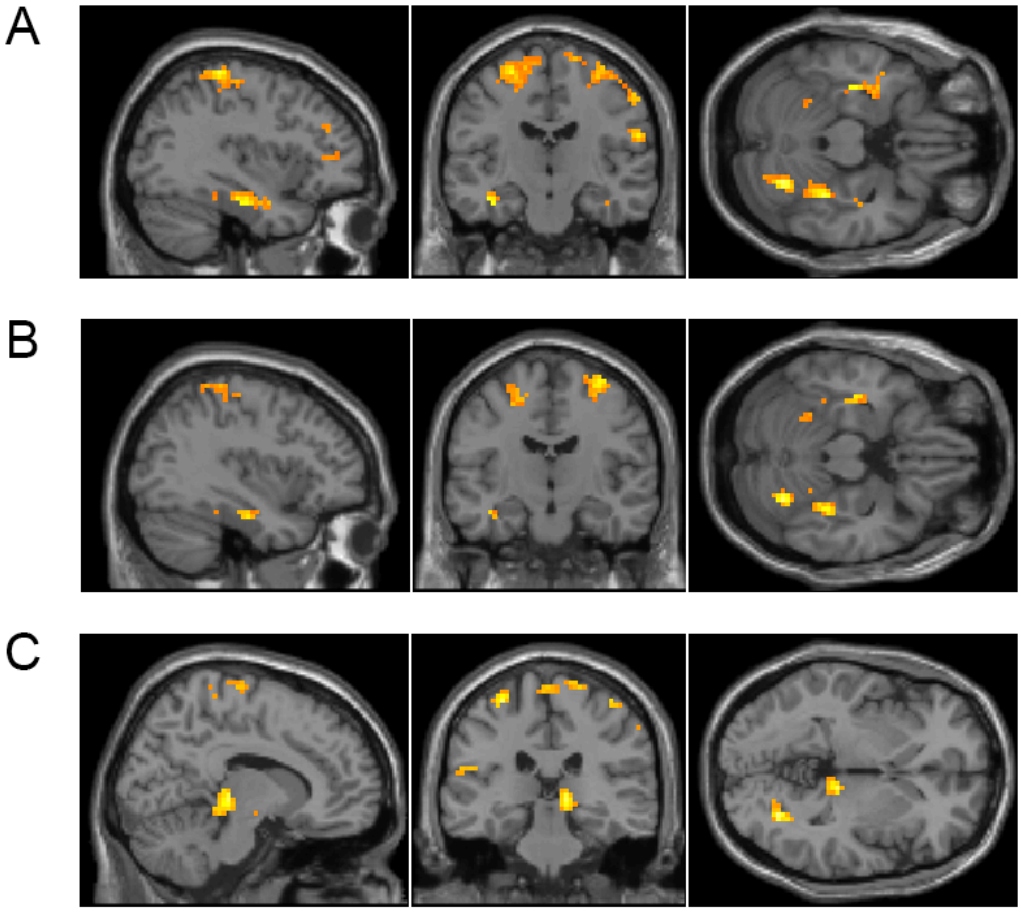

Figure 4.2 A) Comparison of increases of BOLD in the Retrieval condition for Famous faces, looking at Val/Val individuals versus Met carriers. Areas of significant increased activation can be seen in this slice in the hippocampus for Val/Val individuals. B) Comparison of increases of BOLD in the Retrieval condition for 'Old' Famous faces, looking at Val/Val individuals versus Met carriers. Areas of increased activation can be seen in this slice in the hippocampus for Val/Val individuals. This was not significant. C) Comparison of increases of BOLD in the Retrieval condition for 'Old' Non-Famous faces, looking at Val/Val individuals versus Met carriers. Areas of significant increased activation can be seen in this slice in the midbrain for Val/Val individuals.

Table 4.2. *Brain areas showing significant clusters of activation between conditions. Approximate locations are given, along with Brodmann's area, cluster size in voxels (3x3x5mm), and MNI coordinates and p-value for peak activation voxel. These results refer to the Retrieval condition*

Location	Brodmann's Area	Cluster size (voxels)	x y z (mm; MNI)	*p*-value
Famous faces > Non famous faces				
Left middle temporal gyrus	BA 21	153	-54 -13 -14	<.001
Right Hippocampus	BA 54	49	30 -13 -17	.018
Right Parahippocampal	BA 35	30	36 -37 -23	.030
Right Superior Temporal Gyrus	BA 21	40	54 -7 -17	.005
Met carrier Famous face > Val/Val Famous face				
[No significant activation]				
Val/Val Famous face > Met carrier Famous face				
Left Hippocampus	BA 54	60	-36 -16 -23	.021
Val/Val Old Famous face > Met carrier Old Famous face				
[No significant activation]				
Met carrier Old Famous face > Val/Val Old Famous face				
[No significant activation]				
Val/Val Old Non famous > Met carriers Old Non famous				
Midbrain	BA 50	57	12 -28 -2	.026
Met carrier Old Non Famous face > Val/Val Old Non Famous face				
[No significant activation]				

4.5 Discussion

Consistent with previous work (Trinkler et al., 2009), the identification of famous faces activated the hippocampus. Here we further show that this activation differed by genotype, with Val/Val individuals showing significantly stronger BOLD activation in the hippocampus for famous faces compared to those individuals who have a copy of the Met allele. We also showed that there was no difference in familiarity-related structures between the two genotypes, when successfully recognizing a non-famous face, supporting the hypothesis that the Val^{66}Met polymorphism will affect hippocampal dependent memory but not familiarity. Whilst there was also increased activation in the hippocampus for Val/Val individuals when successfully identifying an 'old' famous face, this was not significant.

There are clear differences in hippocampal activation between the two conditions. The famous face condition activated the hippocampus significantly beyond what is activated by the non-famous face condition, whereas there was no significant MTL activation specifically associated with non-famous faces. Identification of famous faces elicits the automatic retrieval of long term representations and contextual information associated with that identity (Leveroni et al., 2000). The famous faces are utilizing a network that even recently presented non-famous faces are not, therefore this activation is probably not due to Familiarity but rather to a Recollection of the identity of the famous person depicted in the photo. Thus, the activation of the hippocampus in relation to famous faces seen in the current study supports the idea that recollection is supported by the hippocampal-anterior thalamic circuit as proposed by Aggleton Brown (1999), and seen in other findings (Trinkler et al., 2009).

Critically, there are differences in hippocampal activation seen between the two groups, in that there is an increased BOLD response for Val/Val individuals. As mentioned earlier, BDNF is most highly concentrated in the hippocampus. (Pezawas et al., 2004). As BDNF plays an important role in long-term potentiation (LTP), and hippocampal LTP in particular it is possible that the BDNF polymorphism is affecting hippocampal dependent forms of memory via the disruption of hippocampal LTP (Lu, 2003; Lu, Christian, & Lu, 2007; Poo, 2001; Egan et al., 2003; (Athos, Impey, Pineda, Chen, & Storm, 2002; Blum, Moore, Adams, & Dash, 1999; Bozon, Kelly, Josselyn, Silva, Davis, & Laroche, 2003). Hariri and colleagues (2003) also showed in a study of hippocampal based memory that Met carriers had decreased hippocampal activation combined with behavioural performance deficiencies. Whilst the cohort in the current study does not mirror the behavioural data of Hariri et al (2003), the BDNF Val^{66}Met polymorphism is affecting the BOLD response in a different memory modality (face memory vs scene learning). As stated earlier, identification of famous faces elicits the automatic retrieval of long term representations and contextual information associated with that identity (Leveroni et al., 2000). It could be the case that Val/Val individuals are able to pull in more contextual details, or have stronger long term representations of the famous faces. This may be reflected as an increased BOLD response.

Whilst there is increased activation in the hippocampus for Val/Val individuals when successfully identifying an 'old' famous face, this was not significant. This may be due to the sample size. A relatively small sample necessitated the combination of individuals with a copy of the Met allele into a single group. Whilst this is common practice amongst BDNF researchers (Hariri et al., 2003; Suriyaprom, Tungtrongchitr, Thawnashom, & Pimainog, 2013), there is the potential for quantitative differences

between these genotypes to be lost. The sample in this population consisted of 5 Val/Mets and 4 Met/Mets. This may explain why the increased activation seen in the hippocampus was not significant. Val/Met's could represent an intermediary group between the more extreme Val/Val and Met/Met's. It is possible that Met/Mets are significantly more affected than Val/Mets and Val/Vals. Work on serum levels of BDNF in the Val66Met polymorphism also combine Val/Met and Met/Met users (Ozan et al., 2010; Suriyaprom et al., 2012). It is therefore still unclear whether or not Val/Met and Met/Mets are functionally different from one another.

Secondly, BDNF also has a variety of roles in long-term developmental processes such as neuronal migration, differentiation and survival, as well as neurogenesis and dendritic maintenance (Acheson et al., 1995; Huang & Reichardt, 2001; Hua & Smith, 2004). Indeed, it has been found that Met-BDNF carriers have reduced neural volumes in the hippocampus, as well as a variety of neocortical areas (Pezawas et al., 2004; Szeszko et al., 2005). It is possible therefore that the differences hippocampal activation observed in Met-BDNF carriers in some conditions or lack of differences in others may be due to chronic developmental adaptations in brain anatomy rather than acute changes in neural transmission (Pezewas et al., 2004).

Chapter 5 General Discussion

The aims of this thesis were three fold. Firstly, *BDNF* Val^{66}Met polymorphism was examined to see whether different genotypes affected LTP. More specifically, it was hypothesized that individuals carrying at least one copy of the Met allele (Val/Met or Met/Met) would have significantly lower LTP (either immediately after LTP-inducing stimulus ('early LTP'), or 30 minutes after the LTP-inducing stimulus ('late LTP')) than individuals who did not have a copy of the Met allele. Secondly, the BDNF Val^{66}Met polymorphism was examined to see if it played a significant role in familiarity and recollection memory networks. Specifically, Chapter 3 was concerned with whether the presence of a Met allele was involved in the modulation of the FN400 and LPC components - two ERP's associated with recognition memory. Finally, Chapter 4 investigates the effect of the BDNF polymorphism on recognition memory using fMRI. More specifically, the investigation assesed whether the presence of a Met allele affected the level of hippocampal activation when processing a famous face, and the level of extra hippocampal activation when successfully remembering a non-famous face.

The results from Chapter 2 showed that Val/Val individuals have significantly 'more' LTP (as indexed by EEG recordings) than individuals with at least one copy of the Met allele (both Val/Met and Met/Met participants. Here, 'more' is defined as having a greater amplitude increase to sine gratings following the photic tetanus, whereas 'less' would be defined as having a lower amplitude increase to sine gratings following the photic tetanus). These differences were seen for both early and late LTP. The magnitude of the difference between all the genotypes was largest between Val/Val and Met/Met individuals in late LTP. While there was a trend for Val/Met carriers to have more LTP

than Met/Mets, providing a dose-response curve, these differences were not significant. Previous findings regarding decreases memory performance seen with the presence of the Met allele were also replicated with performance differences seen on the WMS-III (Egan et al., 2003; Hariri et al., 2003; Goldberg et al., 2008). Critically however, these differences only existed between Val/Val's and Met/Met individuals, with Val/Mets performing at an intermediary level between the two. In our study, LTP was also seen to be a significant predictor of memory performance, with late LTP being the strongest predictor of both immediate and delayed memory performance. An examination of trend lines show this to be mainly driven by Val/Val individuals.

Lastly, the results from Chapter 4 replicate findings that suggest that the successful identification of a famous face over a non-famous face requires the hippocampus (Bird & Burgess, 2008, Trinkler et al., 2009). Whilst no differences existed in hippocampal activation between genotypes in the encoding condition, differences did arise in the retrieval condition. When observing a famous face (regardless of if it's an 'old' face or a 'new' face), Val/Val individuals showed significantly greater hippocampal activation than individuals with a Met allele. Interestingly, when successfully identifying an 'old' non-famous face, thought to be dependent on structures outside of the hippocampus (Bird & Burgess, 2008, Trinkler et al., 2009), there were no differences seen between genotype. Nor were there any behavioural differences between genotype, once again suggesting that the effect the BDNF Val^{66}Met polymorphism has on the brain may be quite subtle.

Taken together, the results of the thesis suggest an important role of the BDNF Val^{66}Met polymorphism in human EEG and fMRI correlates. Whilst behavioural differences weren't always observed, differences in underlying brain signalling (both electrical and blood flow) were always seen, suggesting that the role the polymorphism

plays may be relatively subtle and not readily measured by current behavioural techniques. Further to this point, the results of the experiments show a 'dose response' in both memory scores and brain processing. Val/Val individuals out performed both Val/Mets and Met/Mets in some measures of memory performance and LTP amplitude, however the magnitude of difference relative to Val/Vals was greater for Met/Mets, with Val/Mets being closer in all respects to Val/Val individuals. This suggests that Met/Mets are qualitatively different than Val/Mets, with Met/Met being the worst performing and Val/Mets presenting an intermediary stage of both memory performance and brain processing. Research that combines these two groups could be missing out on valuable information, and consequences of this will be discussed in further parts of this discussion. It is likely that the studies in general lack statistical power due to the smaller sample size present. An increase to the sample size could see an increase in statistical significance.

5.1 *BDNF* val^{66}met polymorphism and LTP

Individuals with the Met allele in the val^{66}met polymorphism on the *BDNF* gene had significantly lower LTP than individuals without a Met allele. Further, individuals with two copies of the Met allele had even lower LTP than Val/Met individuals. BDNF has been identified as a key regulator of synaptic plasticity. Immediately available endogenous BDNF, as well as later synthesis of post synaptic BDNF play key roles in both early and late LTP (Lu, Christian, & Lu, 2007). The effects of BDNF are not limited to hippocampal LTP. Infusion of BDNF enhanced electrically stimulated LTP in the visual cortex of rats (Jiang et al., 2001). BDNF-dependent retrograde effects are also required for the induction of LTP in visual cortical pyramidal neurons (Inagaki et al., 2008), and rats with impaired activity-dependent release of BDNF show deficient visual cortical LTP (Abidin et al.,

2006). The *BDNF* val^{66}met polymorphism affects the activity-dependent release of BDNF, and may also affect serum levels of LTP (Chen et al., 2004; Egan et al., 2003; Lang, Hellweg, Sander, & Gallinat, 2009; Ozan et al., 2010; Suriyaprom et al., 2013). Thus, it has been hypothesised that the polymorphism affects LTP in humans (Egan et al., 2003). However, further research into this has been hampered by the lack of a human model of LTP. Recent work suggests that it is possible to non-invasively induce LTP using sensory stimuli (Clapp et al., 2005b; Teyler et al., 2005), and that the effect is both NMDA receptor dependent (Cavus et al., 2009a; Clapp et al., 2006), and input specific (McNair et al., 2006; Ross et al., 2008). Using this paradigm, we showed that Val/Met and Met/Met individuals had significantly less amplitude increase in their visual-evoked potentials following "tetanic" stimulation than individuals without the Met variant of the val^{66}met polymorphism. The results show a significant main effect of genotype on an LTP-like phenotype, confirming the hypothesis that the *BDNF* val^{66}met polymorphism does affect LTP and this is most likely due to the fact that different versions of the polymorphism differentially affect the activity-dependent release of BDNF.

Not only was the main effect significant, but we also found a significant interaction between Genotype and LTP. Post-hoc analysis revealed that Val/Val individuals had significantly more LTP at both time periods than Val/Met and Met/Met individuals. This difference was most pronounced at late LTP between Val/Val and Met/Met participants, whilst there were no differences between Val/Met and Met/Met individuals at any time point. Met/Mets show a small increase immediately after tetanus, but actually show a decrease from baseline when LTP is measured 30 minutes after. Measurements taken immediately after the induction of LTP and 30 minutes after LTP induction could reflect different phases of LTP, with the immediate measurement possibly reflecting early LTP

and the delayed measurement possibly reflecting late LTP. As stated earlier, immediately available BDNF is important for early LTP. While BDNF is important for early LTP, the induction of late LTP also triggers an enhancement to post-synaptic synthesis of BDNF, with the action of BDNF thought to be more important for late LTP (Castren et al., 1993; Dragunow et al., 1993; Patterson, Grover, Schwartzkroin, & Bothwell, 1992). Sakata et al (2005) showed that impairing the activity-dependent release and regulation of BDNF in rats prevented the induction of late LTP. This finding suggests that BDNF is not just a key modulator of LTP, but is in fact required for late LTP to occur regardless of the intensity of inducing stimulation (Lu, Christian, & Lu, 2007; Sakata et al., 2005). The increased difference of LTP amplitudes between the three groups 30 minutes after induction, relative to immediately after induction, can be explained in terms of the findings of these studies. Individuals with the Met versions of val^{66}met polymorphism, particularly the Met/Met genotype, may not release BDNF (or release less) in response to activity, and thus may not have enough BDNF required to trigger late LTP. Research suggests that there may actually be an increase in serum levels for Met carriers (Lang, Hellweg, Sander, & Gallinat, 2009). As BDNF may be less important for early LTP (Figurov et al., 1996; Kramar et al., 2004; Lu, Christian, & Lu, 2007), early LTP can be induced in individuals with the Met allele via endogenous stores of BDNF. As BDNF may be more important for late LTP (Sakata et al., 2005), individuals with less activity-dependent release of BDNF as a result of having the Met versions of the val^{66}met polymorphism may not show late LTP, or at least have lower levels of late LTP. This would explain the increased difference between the three groups at 30 minutes after LTP induction as opposed to immediately after induction. Caution does have to be exercised with this conclusion as the results regarding serum levels of BDNF are inconsistent, with some studies finding increased levels for Met carriers, whilst others

finding it for Val/Val individuals, and others finding no differences (Lang, Hellweg, Sander, & Gallinat, 2009; Ozan et al., 2010; Suriyaprom et al., 2013). Also, there is no evidence to suggest a significant difference between Met/Met and Val/Met individuals. Research tends to group these two genotypes together into one group of Met carriers, and thus this information is not available (Egan et al., 2003; Hariri et al., 2004; Suriyaprom et al., 2012). Another point that is not easily answerable is the decrease from baseline seen in Met/Met individuals. Studies in the visual cortex have shown the involvement of BDNF in LTD (Park & Poo, 2012). However, this is a theoretically difficult point to address, as it is conceptually difficult to explain how the same molecule can be involved in both the increase and decrease of synaptic strength (Zagrebelsky & Korte, 2014).

Other studies have also suggested that variations on the *BDNF* val^{66}met polymorphism affect apparent synaptic plasticity, although whether it is LTP that is being assessed in these studies or not is unclear (Cheeran et al., 2008; Kleim et al., 2006). Kleim et al (2006) showed using TMS that different expression on the *BDNF* val^{66}met polymorphism had different degrees of experience-dependent plasticity in the motor cortex. Using TMS, baseline MEPs were taken of the first dorsal interosseous (FDI) of the right hand. The cortical representation area of the FDI was also calculated, as well as the centre of gravity. Following training on three tasks (pinch grip, finger tapping, and nine hole peg board) Val/Val individuals showed increase MEP amplitude as well as increased FDI map area and greater shift in centre of gravity compared to individuals with Met versions of the val^{66}met polymorphism. Cheeran et al (2008) used theta burst stimulation, transcranial direct current stimulation, and paired associative stimulation to investigate whether the presence of a Met allele affected motor models of human plasticity. Individuals carrying the Met versions of the val^{66}met polymorphism showed significantly

less plasticity as indexed by the three motor paradigms when compared to individuals who did not carry a Met allele. It should be noted that the plastic processes that are induced in these two studies use TMS, which is a technique which differs significantly to that used in this research.

In the current work, late LTP was found to be a strong and significant predictor of immediate memory performance, while early LTP was not a significant predictor of immediate memory performance. We also found that late LTP was the best predictor of delayed memory performance. The results of the regressions confirm the original hypothesis that individuals with more LTP will perform significantly better on memory performances than individuals with less LTP. LTP has long thought to be the mechanism of long term memory storage (Cooke & Bliss, 2006), and the result of this study reflect this by showing significant predictive value of LTP for immediate and delayed memory. However, visual inspection of trend lines show this relationship to be primarily driven by Val/Val individuals.

Animal studies have supported the view that LTP is a key process in learning and memory. Disrupting hippocampal LTP has been shown to disrupt hippocampal-dependent memory tasks (Abraham & Mason, 1988; Morris et al., 1986; Tsien et al., 1996). Studies also show that by affecting the natural mechanisms of post-synaptic second messenger processes that are triggered by LTP (e.g. CaMKII), memory is subsequently affected (Giese et al., 1998). However, no studies to date have demonstrated the functional significance of LTP in humans. Research attempting to link memory to LTP have been hampered by the absence of an *in vivo* demonstration of LTP in humans. The paradigm developed by Teyler et al (2005) and refined by subsequent research (Clapp et al., 2006; McNair et al., 2006; Ross et al., 2008) has allowed a previously untouched area of research

to be investigated.

However, how does LTP induced at the primary visual cortex relate to long term memory, a process where the underlying neural substrates are primarily concentrated in the medial temporal lobe? The work of Clapp et al (2006) with animals, and Cavus et al (2009) with humans, shows that this paradigm for eliciting and measuring LTP is NMDA dependent, a key requirement for the most common form of LTP. Also, McNair et al (2006) and Ross et al (2008) show that the paradigm shows input-specificity, another key process defining LTP. The results of this study also show that this effect is modulated to an extent by levels of BDNF, which is a key protein in orchestrating synaptic plasticity. Taken together, these results suggest that it is very probable that this paradigm is eliciting LTP. However, visually-evoked LTP is not typically considered to be involved in a memory storage function. Plasticity in the visual cortex has been linked to other functional changes such as enhanced contrast sensitivity and the occurrence of visual after-effects (Adini et al., 2002; Humphrey et al., 1999). These observations do not necessarily represent long-term memory storage, and the question of how visually-evoked LTP is linked to long-term memory remains unanswered. There are two possible explanations for the link between visually induced LTP and memory performance.

The first is the notion that the process of LTP is similar in mechanism over various parts of the brain. Thus, potential levels of LTP in sensory cortical areas likely reflects potential levels of LTP in other brain regions – specifically those regions involved in long-term memory storage. LTP has been studied extensively at the cellular and molecular level where it is most commonly studied in laboratory animals and hippocampal preparations (Abraham et al., 2002; Bliss & Collingridge., 1993; Cooke & Bliss, 2006). LTP has also been demonstrated in human brain tissue, in both the dentate gyrus and temporal regions

where it shows similar properties to non-human preparations (Beck et al., 2000; Chen et al., 1996). LTP is not limited to the hippocampus, as it has been demonstrated in neocortical tissue (Fox, 2002). Visual cortical areas are amongst the neocortical areas that have been shown to support LTP (Heynen & Bear, 2001; Kirkwood & Bear, 1994; Komatsu, 1994).

By applying theta-bust stimulation to the dorsal lateral geniculate nucleus, Heynen & Bear (2001) discovered that they were able to induce LTP field potentials in the rat primary visual cortex *in vivo*. The induced LTP shared many key characteristics of LTP previously studied in the hippocampus. There was a limit to the amount of potentiation, it was dependent on NMDA receptors, and it was stable and long lasting (Abraham & Mason, 1988; Abraham et al., 2002; Barnes et al., 1994; Bliss & Lomo, 1973). This LTP could be saturated after one episode of TBS, and was extremely enduring, lasting upwards of ten hours with no visible decrease in strength. With the application of the NMDA antagonist CPP, the induction of LTP in was also showed to be NMDA receptor dependent. The induced LTP was also shown to have functional effects, as there was a noticeable increase in VEP amplitude to sine gratings of various orientations following the induction of LTP. Also, as these changes were conducted in adult animals *in vivo*, it suggests that plastic changes in the visual cortex are not limited to the developing brain and that the adult brain is able to undergo synaptic modification (Heynen & Bear, 2001). The results of this study indicate that not only can LTP be induced in the primary visual cortex, but it is structurally similar to LTP induced in other areas of the brain. It is likely that levels of LTP in the visual cortex represent levels of LTP in the hippocampus. The fact that LTP has been shown in humans and that it is similar to non-human preparations (Chen et al., 1996), as well as the fact that LTP in the primary visual cortex is similar to that in the hippocampus,

leads to the conclusion that visually-induced LTP in humans reflects a global LTP property indicating potential levels of LTP in the hippocampus and other extra hippocampal structures important for memory. Therefore, the link seen between visually evoked LTP and memory performance represents a link between LTP of the underlying neural substrates of memory and memory performance.

The second possible explanation for the correlation between visual cortical plasticity and memory performance is in the possible links between visual circuitry to neural circuitry subserving memory formation - specifically between the perirhinal cortex and the hippocampus. Although contentious, it has been suggested that the visual cortex plays an active role in facilitating long term memory formation in the hippocampus and other medial temporal lobe structures (Tsanov & Manahan-Vaughan, 2008). The visual system is able to influence memory formation due to underlying neural circuitry. This is known as the ventral visual stream. The visual ventral stream involves projections of multiple cortical areas starting at the primary visual cortex and ending up in the hippocampus (Goodale & Milner, 1992). After visual information reaches the inferior temporal cortex, it reaches the hippocampus via the perirhinal cortex (Suzuki & Amaral, 1994). Aggleton and Brown (1999) showed that recalling events or facts is reliant on a network which includes the hippocampus, and the anterior thalamic nuclei. In contrast, tasks that are based on familiarity judgements, which are a form of recognition memory, involve the perirhinal cortex and the mediodorsal nucleus of the thalamus. It is possible then that input from the visual cortex could affect subsequent recognition memory processing involving either perirhinal cortex or hippocampus, and that induction of LTP in the primary visual cortex affects plasticity in other neural substrates in the visual ventral stream. Tsanov & Manahan-Vaughan (2006a) found that by applying TBS to the

thalamocortical pathway, a long-term enhancement of cell excitability was induced in the hippocampus. The change in excitability in the granule cells of the hippocampus was preceded by potentiation of the V1 response. It is possible that the preceding potentiation of the V1 facilitates plasticity in the hippocampus due to the strong relationship between the two (Tsanov & Manahan-Vaughan, 2008). Tsanov & Manahan (2007) suggest that experience-dependent synaptic plasticity in the adult rat primary visual cortex influences later stages of information processing leading to long-term memory, and Ji & Wilson (2007) propose that primary visual cortical synaptic plasticity in adults influences the hippocampus' ability to integrate spatial episodes in time. Therefore, it is possible that the visual LTP measured in this study subserves memory formation, as the memory tasks involved visual perception. It may be the case that this study is tapping into a possible ventral visual stream, and thus LTP induced at the primary visual cortex will affect LTP in perirhinal and hippocampal areas. Indeed, as the visual cortex is reciprocally connected to the perirhinal cortex (Clavagnier, Falchier, & Kennedy, 2004), activity in these recurrent networks may actually be necessary for the visual LTP employed in the current study and possibly LTP in other parts of the network. In any case, studies of this sort provide a more direct explanation of the link between visual cortical LTP and performance on visual recognition and recall memory tasks.

Met/Met individuals performed significantly worse than Val/Val individuals visual long term memory tasks from the WMS-III. There were no significant differences between Val/Met individuals and either group, but the trend suggests Val/Met individuals represent an intermediary stage between high performing Val/Val individuals and low performing Met/Met individuals. Previous studies have shown the link between the polymorphism and memory (Egan et al., 2003; Goldberg et al., 2008; Hariri et al., 2003). In particular, Egan et

al (2003) used measures from the WMS-R to demonstrate the discrepancies between the two groups. Therefore, this result was expected and further confirms the role of BDNF in long term memory, whilst extending it to suggest the effect of having a two copies of the Met allele leading to a worse memory impairment. Although we found a significant main effect of genotype on memory scores, no interaction effect was found. Therefore, there is a significant difference between the two groups at both immediate memory and delayed memory. It is not possible to say therefore that the difference in memory between the two groups was more pronounced for immediate memory or delayed memory. Immediate and delayed memory scores were strongly correlated. This suggests that the memory effects may be occurring earlier on in the consolidation process of memory.

The measure of memory used by Egan et al (2003) involves the free recall of elements from two stories following a 30 minute delay. Also, Hariri et al (2003) used a paradigm which involved the encoding and retrieval of novel complex scenes. Finally, Goldberg et al (2008) used verbal recognition memory as their objective measure of memory. The encoding and retrieval of novel complex scenes involved in the study by Hariri et al (2003) were shown to be dependent on the hippocampal complex (hippocampus and parahippocampal gyrus), with individuals carrying a Met allele showing decreased hippocampal activation compared to those individuals without a Met allele. There is also evidence to suggest that the recall of events or facts is reliant on hippocampus as opposed to familiarity judgements (Aggleton & Brown, 1999; Kirk et al., 2004), which would suggest the task used in the Egan et al (2003) study is dependent on the hippocampus. Bird & Burgess (2008) also showed that the recognition of familiar words but not the recognition of unfamiliar faces is dependent on the hippocampus, which suggests the task used by Goldberg et al (2008) activates hippocampal areas. These results taken together

suggest that the primary role of BDNF is in hippocampal dependent memory, and this is expected as this is consistent with the pattern of distribution of BDNF in the brain (Murer, Yan, & Raisman-Vozari, 2001).

The WMS-III is designed in a way that to attain an index score of visual memory, two subtests have to be completed to give this score. The two tests that were used in this experiment were the Faces and Family Pictures subtests. Both of these subtests were described earlier, and it was suggested that the two different subtests could be tapping into different neural substrates of memory. The Faces subtest could possibly be testing 'familiarity' judgements, whereas the Family Pictures testing the recall of facts. As described in the introduction 'familiarity' judgements are independent of the hippocampus. More specifically, Bird & Burgess (2008) showed that the recognition of unfamiliar faces is independent of the hippocampus. Taken together, this suggests that the Faces subtest of the WMS-III is reliant on extra-hippocampal structures. The Family Pictures subtest involves memorising visual information and then recalling facts without cues. This type of memory has been suggested to be dependent on the hippocampus (Aggleton & Brown, 1999). The studies carried out by Egan et al (2003) and Goldberg et al (2008) do not use imaging to confirm the neural substrates underlying memory processing in their studies, and thus it is not known the degree to which that these two particular tasks are dependent on the hippocampus relative to extrahippocampal areas. This provides a good backdrop and rationale to focus on familiarity vs recollection forms of memory and BDNF conducted in Chapters 3 and 4.

5.2 *BDNF* Val^{66}Met Recognition memory: ERP Evidence

Chapter 3 showed that individuals with a copy of the Met allele had a significantly lower increase in the amplitude of their LPC component when successfully recognizing an 'old' face that had been studied a day earlier compared to Val/Val individuals. However, when looking at the FN400 for successful recognition of faces immediately after presentation, there were no differences between genotype. Interestingly, there were no behavioural differences between the two groups on either familiarity or recollection tasks. The FN400 was not seen in the experiment where faces were learnt and then recalled a day later, whilst no LPC was seen in the experiment where faces were recalled immediately after learning. Previous research has shown that the frontally distributed FN400 is associated with Familiarity, whilst the later parietal distributed LPC is associated with Recollection (Addante, Ranganath & Yonelinas, 2012; Curran, 2000; Duzel, Vargha-Khadem, Heinze & Mishkin, 2001; Rugg & Curran. 2007). The results of Chapter 3 therefore suggest that the BDNF val^{66}met polymorphism plays a role in Recollection forms of recognition memory, but not familiarity.

Recognition memory is split into two functionally dissociable systems, known as Familiarity and Recollection (Rugg & Yonelinas, 2003; Yonelinas, 2001, 2002). As described in the introduction, Familiarity describes a feeling that a particular stimulus may have been experienced before but is devoid of contextual information, whereas Recollection is the recognition of a stimulus as well as the contextual details of the environment accompanying the original presentation of the stimulus (Gardiner, 1988; Jacoby, 1991; Mandler, 1980; Tulving, 1985; Yonelinas, 1999, 2001, 2002; Yonelinas et al., 1996; Yonelinas & Jacoby, 1996). Recollection is dependent on the hippocampus, whilst Familiarity is not (Vargha-Khadem, Gadian, Watkins, Connelly, Van Paesschen, & Mishkin, 1997; Aggleton & Brown, 1999; Aggleton & Brown, 2006; Yonelinas et al.,

2002; Davachi, Mitchell and Wagner, 2003). As BDNF and its associated receptor is most strongly concentrated in the hippocampus (Pezawas et al., 2005), it is possible that the BDNF polymorphism affects Recollection forms of memory, but not Familiarity. Tying these results together with the results from Chapter 2 paints an interesting picture. . Research has shown an involvement of LTP and LTD in recognition memory and how in the perirhinal cortex (the main area associated with familiarity) deficits in these processes can lead to recognition memory failure (Banks, Bashir, & Brown, 2012; Banks, Warbuton, Brown, & Bashir, 2014). Critically, Banks, Bashir & Brown (2012) describe evidence suggesting the importance of activation and removal of AMPA receptors, a process involved in early LTP. It is possible and plausible that early forms of LTP may be enough to subserve familiarity forms of memory, or memory that does not necessarily involve the hippocampus. Thus, increased endogenous levels of BDNF seen in Met carriers could supplement this form of memory and LTP, which would explain why there are no differences between genotypes in FN400 amplitude. However, late LTP is strongly associated with hippocampal memory, or the types of memory that require stronger consolidation and protein synthesis (Reymann & Frey, 2007). As BDNF is activity-dependent, and its activity-dependent release is required for late LTP to occur, this could lead to impairments in LTP in Met carriers which as a result would affect recollection forms of memory. This could explain the differences seen in LPC amplitude between the two groups.

Whilst this is an attractive explanation, there are some other points of consideration. Whilst Val/Val have a more positive LPC for successfully identifying an ‘old’ face compared to Met carriers, examination of the results show that it is more complex than that. Specifically, Met carriers actually show a trend towards a positive LPC for ‘new’

faces (however, this is not significant). Addante et al (2012) showed that the LPC is actually modulated by response confidence. Individuals in this study had to make a source memory judgment (when they learned the memory) and then make a response indicating how confident they were in their response. Enhancement in LPC amplitudes was only observed with the highest rating of item recognition confidence. What this suggests is that Met carriers are more confident when presented with 'new' faces, (correct rejections) rather than necessarily remembering 'old' faces. This potentially suggests that Met carriers actually haven't developed as strong as an association with 'old' faces. One way to identify this would have been through the use of a Remember/Know paradigm. Participants could be asked whether they actually 'remember' the face or whether they just 'know' the face was one they learnt. EEG responses could then be looked at to see whether there were differences between genotypes in 'remember' and 'know' responses. Research has shown that when participants 'remember' a stimulus versus 'know' a stimulus, an LPC is elicited (Duzel, Yonelinas, Mangun, Heinze, & Tulving, 1997). However there are concerns about the subjectivity of R/K tasks, as they can often depend on how the memory is tested (Hicks & Marsh, 1999). Regardless, these results are interesting as the fact that there is no behavioural differences could potentially explain the relative high prevalence of the BDNF val66met polymorphism in the population, as Met carriers could potentially adopt different memory strategies to achieve a similar result and thus have a similar behavioural performance. Further examination of the results show that Met carriers have a high standard deviation in their results. This suggests two potential different groups that differ in LPC amplitude. As seen in the results of Chapter 2, there are quantitative differences in memory and LTP between Val/Mets and Met/Mets. Due to a lack of sample size, Val/Met and Met/Met participants are combined into a single group. The high standard deviations

could suggest that Val/Mets are responding in a different way (and thus having different LPC amplitudes) to the Met/Met participants. This further emphasizes the importance and the need for research to try to analyze these two groups separately. It is likely that the study lacks statistical power due to the smaller sample size present. An increase to the sample size could see an increase in statistical significance.

5.3 *BDNF* Val^{66}Met and Recognition memory: fMRI Evidence

Lastly, the last experiment in this thesis examined whether the differences seen between genotype electrically could be replicated haemodynamically. Also, as EEG has poor spatial resolution, fMRI was utilized to examine whether there were differences in BOLD response in hippocampal and extra hippocampal structures. The successful identification of a famous face over a non-famous face showed increased hippocampal activation, replicating earlier results (Bird & Burgess, 2008, Trinkler et al., 2009). This difference was far stronger for Val/Val individuals when observing a famous face (regardless of if it's an 'old' face or a 'new' face) than individuals with a Met allele. There were no differences seen between genotype when successfully recalling an 'old' non famous face. Whilst there was increased hippocampal activation for Val/Val individuals compared to Met carriers for successfully remembering a famous face, this was not significant. There were also no behavioural differences between genotype.

The hippocampus is involved in the successful recollection of familiar faces, but not unfamiliar faces (Bird & Burgess, 2008, Trinkler et al., 2009). Bird & Burgess (2008) showed damage to the hippocampus produced performance impairment on tests of verbal but not facial recognition memory. Performance on the Recognition Memory Test (RMT: Warrington, 1984) Trinkler et al (2009) resulted in an increased hippocampal BOLD

response in participants for previously known faces in both the study and testing phase , but not new learned faces (Trinkler et al., 2009). The results of Chapter 4 support this finding, with all participants (regardless of genotype) showing an increase in hippocampal activity when observing a famous face. Whilst there were no significant differences between genotype in the encoding condition when observing a famous face (and this may not necessarily be expected initially) there was significantly more activation seen in the hippocampus of Val/Val individuals in the retrieval task. That is, whenever Val/Val individuals observed a famous face (regardless of whether it was 'old' or 'new') there was a stronger BOLD response seen in the hippocampus compared to Met carriers. These results parallel those of Chapter 3 in that a brain signal (ERPs or BOLD) related to hippocampal dependent memory is stronger in Val/Val individuals than Met carriers. As is the case with the LPC ERP results discussed earlier, increased BDNF release, and subsequent increases to late LTP, may be the reason Val/Val individuals show an increase in BOLD activation for hippocampal dependent facial recognition. There was also no difference in activation seen between the two groups when looking at successful recognition of a non-famous face. As stated earlier this process is thought not to be dependent on the hippocampus, and indeed it was hypothesized there would be no differences between the genetic groups. Although the data support this hypothesis, there was no support for the notion that the recognition of non-famous faces relies on the perirhinal cortex or associated familiarity network (Aggleton & Brown, 1999). Once again, there were no behavioural differences between the groups.

Whilst there was an apparent increase in activity in hippocampal and parahippocampal areas seen for Val/Val individuals when successfully identifying a previously presented famous face, this activation was not significant. The reason for this is

likely related to sample issues. Not only is it a small sample, but as was the case with Chapter 3, Met/Met and Val/Mets have been combined into a single group for this study (there were 4 Met/Met participants in the Met carrier group). The differences seen in Chapter 2 may also be contributing to the lack of significant activation differences seen in the hippocampus. It could be the case that Val/Mets show an intermediary level of hippocampal activation, whilst Met/Mets show the least. The combination of these two groups possibly results in any significant difference that may have been observed between Val/Val individuals and Met/Met individuals is being buffered by Val/Met individuals. This further emphasizes the importance and the need for research to try to analyze these two groups separately.

5.4 More than LTP and Memory? Other considerations involving BDNF

While BDNF and the associated Val^{66}Met polymorphism likely play a very important role in LTP and memory, it is not its only influence. BDNF plays a critical role in early cell survival, development, and proliferation (Davies, 1994; Levi Montalcini, 1987; Lewin and Barde, 1996). BDNF has also been shown to be able to alter the properties of synapses in the mature nervous system (Gottmann et al., 2009; Park & Poo, 2012). In fact, this is likely its most important role. With respect to the Val^{66}Met polymorphism, the presence of a Met allele has linked with brain abnormalities, such as reductions to hippocampal volume, less gray matter in frontal, occiptial and temporal regions, and lower hippocampal n-acetyl aspartate (NAA) which is a putative marker of neuronal integrity and synaptic abundance (Egan et al., 2003; Hariri et al., 2003; Pezewas

et al., 2004; Ho, Andreasen, Dawson, & Wassink, 2007; Ho et al., 2006; Harrisberger et al., 2014). It is possible therefore that the results seen in this thesis with Met carriers may be due to chronic developmental adaptations in brain anatomy rather than acute changes in neural transmission.

Differing lifestyle choices also have a role to play with BDNF. Hopkins, Nitecki & Bucci (2011) compared a group of rats that did no exercise to a group that had elevated levels of voluntary wheel running during adolescence. The results showed those rats who had previously exercised could still discriminate between novel and learned environments from two or four weeks earlier, whilst the rats who did not exercise could not. Coupled with this was the fact that the rats who undertook exercise showed higher levels of BDNF. Further to this point, work done with humans show increases to hippocampal-dependent memory function following 5 weeks of aerobic exercise, as well as an increase to BDNF serum levels (Griffin et al., 2011). In fact, recent unpublished work at the University of Auckland used the LTP paradigm described in this thesis to assess whether individuals who exercised regularly showed different levels of LTP than those who did not. These data showed that individuals who exercised regularly had a greater increase in their ERP amplitudes following tetanic stimulation.

Whilst exercise is thought to play a large part in BDNF levels, other life style choices can affect BDNF also. Smoking leads to higher serum levels of BDNF, whilst high fat diets lead to a decrease in BDNF which can be potentially reversed by caffeine (Moy & McNay, 2013; Suriyaprom et al., 2013). Work with rats shows a combination of western diet (high fat diet) and sleep deprivation leads to decrease in BDNF levels and increase in oxidative stress which leads to significant memory impairments on the Morris water maze (Alzoubi, Khabour, Salah, & Rashid, 2012).

5.5 Limitations, future directions and conclusions

Limitations with this study centre on limited sample size. As was identified in Chapter 2, there are considerable differences between Val/Met and Met/Met individuals in memory ability, and capacity for LTP. While it is common practice to combine these groups, there is the potential for valuable information to be lost by doing this. Unfortunately, time scales with genotyping individuals and the nature of human research usually leads to struggles in getting an adequate sample. The results for Chapter 3 and 4 may potentially have been different, or trends significant in some instances, if the groups were split into each genotype. In addition, the widely employed memory test used in Chapter 2 is made up of two subtests which parallel to a degree two different types of memory test (faces measuring Familiarity and family pictures measuring Recollection). Subsequent research in the area employing this test might consider separately these two subtests.

The paradigm used in this study to measure LTP has been shown to be NMDA dependent, stimulus-specific, and due to the data presented in this thesis can be said to be modulated by BDNF genotype. Thus, it is possible to say with some confidence that there exists a paradigm to measure LTP in humans non-invasively. The ability to elicit LTP from non-surgical patients provides a human model system allowing the detailed examination of synaptic plasticity in normal subjects which may have future clinical applications in the assessment of cognitive disorders. Recently, there have been demonstrations of impaired LTP of visual-evoked potentials in people with schizophrenia (Cavus et al, 2009b) and in people suffering from major depressive disorder (Normann, Schmitz, Furmaier, Doing, & Bach, 2007). The current paradigm has potential in aiding diagnosis in disorders of this sort, and in assessing the efficacy and monitoring treatments. If LTP is the neural basis of

memory, then it would be expected that individuals with neurodegenerative diseases affecting memory (e.g. Alzheimer's disease) would have impaired LTP of visual evoked potentials. Ongoing research at the University of Auckland aims to assess levels of LTP in individuals potentially suffering from early Alzheimer's disease. The aim is to develop a reliable early diagnosis of these disorders, or to identify those at high risk of developing them. Early unpublished work suggests that healthy older adults exhibit LTP. Therefore, future work should look into the role of visually evoked LTP in Alzheimers disease and other dementias.

Taking the evidence from all of the three experimental chapters together, it seems that a picture about the BDNF val^{66}met polymorphism, and the role it plays in memory/brain activity is starting to emerge. Clear and significant differences due to BDNF polymorphism are seen with respect to LTP. The hypothesized concomitant differences in ERP and BOLD were less clear. It may be that the more remote or higher level the concomitant process is, the less clear a difference in genotype is likely to be. BOLD is the result of the brain needing more energy to carry out an activity, and is therefore a little less direct than EEG and ERPs. Also, the process we are looking at in fMRI is the actual recollection of the face itself, which involves more than just hippocampal activity. The ERP differences we see (FN400 and LPC) are earlier processes than those observed in fMRI. Finally, the process of LTP as indexed here is arguably more elemental in nature, and represents an aspect of neural activity, rather than increasingly complex higher order processing into which it is incorporated. Issues with group size and combining allele groups have been discussed, but to reiterate briefly, there are differences between all three groups, with individuals having 2 Met alleles being more affected than individuals with one Met allele.

It is important to note that there were often no differences in behavioural performance between the groups. This might partly explain why the polymorphism is still prevalent in the normal population. TMS protocols show different motor plasticity between genotypes, yet Met carriers clearly still have perfectly functioning motor systems. There may well be advantages to lower neural plasticity in certain environmental situations, but consideration of these issues is beyond the scope of the current thesis.

In conclusion therefore, the role of BDNF in human cognition is complex and potentially subtle, and thus using tools that are able to measure the underlying neuronal function that BDNF is associated with, are of great importance in attempting to determine the role that the BDNF Val^{66}Met polymorphism has on cognition.

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