The BDNF val66met polymorphism predicts human spatial memory behaviour, fMRI activity and brain morphology

Harrison Banner

Department of Neurology and Neurosurgery, Faculty of Medicine

McGill University, Montreal

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Abstract

Multiple memory systems are involved in processing information during navigation. A hippocampus-dependent "spatial" navigation system requires learning the relationships between environmental landmarks to build a cognitive map, while habit-based "response" learning involves the automatization of patterns of behavioral responses and is mediated by the caudate nucleus. Studies have demonstrated that people will spontaneously use one or the other of these two alternative strategies with almost equal frequency to solve a given navigation task and that strategy selection correlates with grey matter density . While there is evidence for experience modulating grey matter in the hippocampus (Maguire et al., 2000), genetic contributions may also play an important role in the hippocampus and caudate nucleus. Recently, the Brain-derived neurotrophic factor (BDNF) val66met polymorphism has emerged as a possible inhibitor of hippocampal function. Here we investigate a role for this polymorphism in strategy selection and functional magnetic resonance imaging (fMRI) activity during human virtual navigation tasks as well as an effect of this genotype on human brain morphology.

Résumé

Plusieurs systèmes de mémoire sont impliqués dans le traitement des informations durant la navigation. Un système de navigation « spatiale », qui dépend de l'hippocampe, exploite les liens entre les points de repère pour construire une carte cognitive, tandis que l'apprentissage par réponse, basé sur la répétition et le renforcement est dirigé par le noyau caudé. Des études ont démontré qu'une proportion quasi égale de gens utilisent spontanément l'une ou l'autre de ces stratégies pour résoudre une tâche de navigation donnée , et que la sélection d'une stratégie est corrélée avec la densité de la matière grise l'hippocampe et aux noyaux caudés . Bien qu'il ait été démontré que l'expérience en navigation module la matière grise a l'hippocampe (Maguire et al., 2000), des contribution génétiques peuvent aussi y jouer un rôle. Plus récemment, le polymorphisme val66met du FNDC se fait candidat comme inhibiteur potentiel de la fonction hippocampique. Nous avons examiné le rôle de ce polymorphisme dans la sélection d'une stratégie et dans l'activité neuronale par IRMf pendant des tâches de navigation virtuelle, ainsi que l'effet du génotype sur la morphologie du cerveau humain.

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Introduction

1.1 Rationale

The existence of multiple memory systems driven by different brain structures, each responsible for the processing of different types of information during learning, has been demonstrated in both non-humans and humans . The sum of this work is that the hippocampus plays a role in episodic, relational, and "spatial" memory, while the striatum, or human caudate nucleus, is implicated in procedural learning and the formation of habits. Though they may interact with one another to some extent, most evidence suggests that these two memory systems function largely independently of one another .

Spatial memory studies have taken advantage of the fact that navigation in one's environment can be achieved using either of these two memory systems, each leading to the use of a different strategy. A hippocampus-based "spatial" strategy will allow the subject to build stimulus-stimulus type relationships between landmarks in the environment in order to develop a cognitive map. This is contrasted with a caudate nucleus-dependent "response" strategy, which involves the learning of stimulus-response relationships such as a pattern or series of turns in the environment. Response learning does not allow for the understanding of the relationships between landmarks in the environment and will not allow the subject to develop a cognitive map.

Recently, human spatial navigation tasks have been developed which can be solved using either of these two independent strategies. Studies using these tasks have found that approximately half of all tested individuals spontaneously select a hippocampus-dependent spatial strategy while the other half makes use of a caudate

nucleus-mediated response strategy when navigating in an environment . Brain imaging studies have demonstrated that subjects using a spatial strategy preferentially activated their hippocampus while those using a response strategy activated their caudate nucleus on the same task . Further studies have shown that strategy selection also correlates with brain grey matter density, with spatial learners having greater grey matter density in the hippocampus relative to response learners who have greater grey matter density in the caudate .

A question remains as to the factors which contribute to this seemingly spontaneous strategy selection. Why do some people spontaneously use a spatial strategy while others use a response strategy? Further, what forms the basis for morphological differences in grey matter density between spatial and response learners? Is strategy selection a product of experience or is it possible that there could be a genetic component which predisposes individuals to one or the other strategy?

Recent work provides evidence that a valine (val) to methionine (met) single nucleotide substitution at codon 66 of the Brain-derived neurotrophic factor (BDNF) gene may lead to an impairment of behaviours which depend on the hippocampus, such as episodic and spatial recognition memory . People with a mutated met allele have decreased hippocampal function as measured by functional magnetic resonance imaging (fMRI) and have been shown to have less hippocampal grey matter compared to people with two dominant val alleles . In the presence of this polymorphism individuals may spontaneously use a caudate nucleus-dependent response strategy at the expense of a hippocampus-dependent spatial strategy.

1.2 Objectives

The purpose of this thesis is to investigate the behavioural, functional and structural correlates of the BDNF val66met polymorphism as they relate to multiple memory systems theory. It is hoped that this work will elucidate a genetic contribution to spatial memory and navigation behaviour.

This study has been divided into three components: a behavioural study, an fMRI study and a morphological study. The behavioural study investigates a potential contribution of the val66met polymorphism to spontaneous strategy selection on the 4/8 Virtual Maze (4/8VM), a task which has previously been used to classify people's preferred learning strategy as either spatial or response .

The fMRI study is an attempt to replicate previous findings showing specific effects of val66met genotype on hippocampal activity during episodic memory tasks , and to extend these findings using a task specific to spatial memory. Because our fMRI task allows the subject to utilize either a spatial or a response strategy, we will investigate potential genotypic effects not only on hippocampal activity but on the corresponding activity of the caudate nucleus as well. If as shown previously hippocampal activity is lower in the presence of a met allele , then these subjects will be more likely to spontaneously employ a caudate nucleus-dependent response strategy and will thus have increased activity in this brain area relative to homozygous val individuals.

The morphological study is an attempt to replicate previous findings which demonstrated a difference in hippocampal grey matter between Val and Met individuals and to extend these findings to our own predictions about the

consequences of this polymorphism on the caudate nucleus. Previous studies have had no a priori predictions about grey matter differences in the caudate nucleus based on val66met genotype. Findings in the Bohbot laboratory showed correlations between the use of spatial strategies and hippocampal grey matter density and between the use of response strategies and caudate nucleus grey matter density . Additionally, there was an inverse correlation between grey matter in the hippocampus and caudate nucleus (Bohbot et al., 2007). We therefore predict that differences in hippocampal grey matter could to some extent be a result of the BDNF val66met polymorphism's conferring preferences for strategy use. The morphological component of the study seeks to investigate an effect of genotype on grey matter in both the hippocampus and the caudate nucleus.

1.3 Hypotheses

Due to BDNF's demonstrated role in normal hippocampal function, we expected that a polymorphism on this gene (met substituted for val at codon 66) would result in the following:

1) Met carriers will preferentially select a striatum-based "response" strategy rather than a hippocampus-dependent "spatial" strategy on the 4-on-8 virtual maze (4/8VM), a virtual navigation paradigm designed to differentiate between spatial and response strategy selection.
2) Hippocampal fMRI activity will be reduced in Met subjects relative to Val subjects on the fMRI Pairs virtual navigation task, and caudate nucleus activity will be higher in Met than Val subjects due to the spontaneous use

of response navigation strategies which rely on the caudate but not the hippocampus.

3) Met carriers will have reduced grey matter density in the hippocampus relative to subjects homozygous for the val allele due to the former's decreased use of hippocampal-dependent learning strategies in day-to-day life. Additionally, decreased use of the affected hippocampus in met carriers will make them more likely to use response strategies in their day-to-day-lives, leading to increases in caudate nucleus grey matter density relative to homozygous val subjects.

2. Literature Review

2.1 Multiple memory systems rely on distinct brain structures in non-humans and humans

Several studies have demonstrated the existence of multiple substrate-specific memory systems existing in the brain, operating in parallel to acquire different types of information . Early work with rats by Packard et al., (1989) demonstrated the clear dissociation between the functions of the hippocampus and dorsal striatum using two different versions of an 8-arm radial maze task, in which the training conditions were altered to require the use of one type of strategy over the other. In their "win-shift" task, rats were trained to retrieve a food reward from one arm of the maze and then move on to collect food rewards from other arms without re-entering arms already visited. This task necessitates the learning of relationships between maze arms and distal cues which would allow the rat to self-orient in the maze. Conversely, the "win-stay" task requires only that the rats learn to associate a proximal cue-an illuminated lightbulb placed at the entrance to the baited arm-with a reward, without the need to know anything about the relationship between landmarks in the environment. Fornix lesions (interrupting hippocampal activity) caused performance impairment on the win-shift but not the win-stay task while dorsal striatal lesions caused impairment on the win-stay but not the win-shift task . The hippocampus is therefore required for rats using distal landmarks to navigate, though hippocampal-lesioned rats could still retrieve rewards if they were using a stimulus-response strategy. In a subsequent study, McDonald and White used two different versions of a water maze task−one in which the rat simply has to swim to a visible escape platform and another in which the rat must use distal cues

to learn the location of a hidden platform−to once again discriminate between striatal and hippocampal learning. It was found that rats with lesions to their dorsal striatum could learn both versions of the task, but when faced with both a visible platform in a new location and a hidden platform in the old learned location they preferentially swam to the old location. This is explained by the fact that the intact hippocampus had encoded the old location of the platform and led the rat to that location first instead of going directly to the visible platform. Conversely, rats with fornix lesions could acquire the visible version of the task but failed to learn the location of a hidden platform based on the relationship between distal cues . These demonstrations of structural damage leading to specific impairments in rodents formed the basis for the study of multiple memory systems and navigation.

The dissociation between hippocampus and striatum has since been replicated in humans . Using the 4/8VM, an 8-arm virtual maze task, Iaria et al. (2003) differentiated between two different strategies that subjects spontaneously use to recall which arms are baited on a given trial. “Spatial” learners are people who make use of distal visual landmarks in the environment and develop a cognitive map of the testing arena. “Response” learners number the arms or memorize a pattern of “open” vs. “closed” arms in order to recall which arms are baited. Using fMRI technology, it was demonstrated that spatial learners show greater activation of the hippocampus than response learners while navigating and that with practice, the response learners have greater activation of the caudate nucleus−the human equivalent of the rodent dorsal striatum−than spatial learners . A follow-up study has since shown that spatial learners have significantly more grey matter in the hippocampus and less grey matter in the caudate nucleus relative

to response learners . It is certainly possible that preferential use of one memory system over the other in day-to-day life could have a reinforcing effect on its function, at the expense of its counterpart, and that this could lead to long-term differences in brain morphology.

That people spontaneously adopt one navigational strategy over the other on navigation tasks seems clear. What remains unknown however, are the underlying mechanisms that could explain differences in strategy selection. Why does one person choose a spatial strategy while another uses a response strategy? What forms the basis for this difference? The answer is likely multifactorial, with environmental as well as biological contributions forming the blueprint of our learning. One possible factor involved could be a genetic polymorphism on the BDNF gene which is known to have its greatest effects on the hippocampus. If this polymorphism indeed has an adverse impact on hippocampal function, then afflicted individuals may adopt a caudate-based navigational strategy rather than one dependent on the affected hippocampus.

2.2 Brain-Derived Neurotrophic Factor is necessary for hippocampal-dependent behaviour

Brain-derived neurotrophic factor (BDNF) is a polypeptide growth factor of the neurotrophin family, a group of proteins which are known to play a role in neuronal cell survival and differentiation during development . BDNF signaling through its receptor TrkB has also recently been implicated in the regulation of synaptic plasticity through activity-dependent modulation of long-term potentiation and long-term depression (LTP and LTD) . Studies have demonstrated that a critical amount of BDNF is necessary for LTP in CA1 synapses of the hippocampus and that BDNF knock-out ($BDNF^{KO}$) animals have disrupted LTP induction at their CA1 synapses . Subsequent studies have revealed that BDNF acts to enhance the frequency of synaptic transmission by facilitating the docking of vesicles pre-synaptically .

BDNF is present throughout the central nervous system with high levels of mRNA expression having been observed in the rodent hippocampus, cerebellum, and cerebral cortex . Though BDNF expression has been demonstrated in the striatum , BDNF expression levels in the hippocampus have been shown to be about five times those of the striatum, with peak levels in the granule cells of the dentate gyrus and pyramidal cell layer of the hippocampus . Human studies have confirmed that BDNF expression is highest in the hippocampus and pre-frontal cortex , a finding which is consistent with studies showing specific effects of BDNF secretion on hippocampus-dependent processes.

Several studies have demonstrated BDNF's critical role in hippocampus-dependent behaviour. Spatial memory studies in animals represent a common

method for testing hippocampal function due to the demonstrated necessity of proper hippocampal function for performance on the Morris water maze and radial maze tasks . Studies have demonstrated that mice with a mutated-form of the BDNF gene are impaired in learning the hidden platform version of the Morris water maze and that rats who received an anti-BDNF antibody treatment were impaired on an 8-arm radial maze task . Others have demonstrated that training for as little as three days in the water maze leads to elevated levels of BDNF mRNA in the hippocampus but not the striatum and that BDNF gene expression is increased in CA1 during contextual learning in rats .

Recent work by Mizuno and colleagues (2000) has further investigated the direction of the causal relationship between BDNF function and spatial memory performance. Rats trained for 28 days on a radial-arm maze task showed an increase in BDNF mRNA expression in the hippocampus but not the frontal cortex relative to untrained controls. When spatial learning was chemically inhibited, this increase in hippocampal BDNF mRNA expression did not occur . BDNF thus acts as a marker for spatial learning, as blocking the latter shuts down expression of the former. In a second experiment, BDNF expression was blocked using an antisense BDNF oligonucleotide treatment, which specifically inhibits expression of the BDNF gene. Not only were BDNF mRNA and protein levels reduced in the hippocampus following this treatment, but there was an observed impairment of spatial learning, both during task acquisition and retrieval, on the 8-arm radial maze, demonstrating that BDNF expression itself is necessary for spatial learning .

Gene knock-out studies have also demonstrated the important role that BDNF plays in hippocampus-dependent spatial learning. Gorski (2003a) showed

that BDNF-restricted mice are unable to learn the spatial version of the Morris water maze task and show generalized rather than context-dependent freezing on the cued-contextual fear conditioning task. During a response learning version of the Morris water maze, $BDNF^{KO}$ mice initially had longer escape latencies but were able to catch up to control-level performance by the third day of training. On the spatial learning version of this task however, significant differences were seen in terms of both latency and distance to reach the escape platform between $BDNF^{KO}$ mice and controls even after extended training. In fact, total swim distance did not significantly differ for $BDNF^{KO}$ mice between the first and last trial, an indication that they were never able to learn the position of the hidden platform .

Previous studies have shown that context-dependent associations in the cued-contextual fear conditioning task depend on the hippocampus . Gorski and colleagues (2003a) found that $BDNF^{KO}$ mice learned to associate the training context with an aversive stimulus, but generalized this learning to contexts other than the one in which the animals were trained, an indication of improper hippocampal function . The authors also found that discrimination learning was impaired on a Y-maze task, in which mice had to learn which of two differently patterned arms contained a reward. Though $BDNF^{KO}$ mice performed at the same level as controls on a simple colour discrimination version, they were impaired on a more complicated discrimination task which required that they differentiate between horizontally- and vertically-oriented bars. Here the authors suggest that in the absence of BDNF, simple associations can form but complex associations are not reinforced, resulting in a learning impairment . It is important to realize however that the KO animals were able to learn to discriminate between two

different pathways based on non-spatial information on the easier version of the task, despite their lack of hippocampal BDNF. This demonstrates that a lack of BDNF leads to substrate-specific deficits that can be overcome by relying on brain areas other than the hippocampus.

2.3 The BDNF val66met polymorphism interferes with normal BDNF Secretion

BDNF's role in proper hippocampal function has been clearly demonstrated using animal models. A naturally occurring mutation, the val66met polymorphism, has recently been identified in humans and has been shown to affect 20-30% of the U.S. population , and 51% of the Japanese population .

The val66met polymorphism is a single amino acid substitution (methionine for valine) occurring at codon 66 in the 5' pro-region of the BDNF gene. Normally, the precursor peptide pro-BDNF is cleaved to form the mature protein BDNF. The pro region of pro-BDNF contains the sequence required for the trafficking of this precursor through the cellular machinery, a process which is required in order for the BDNF protein to be cleaved into its active form. Though the polymorphism has no direct effect on mature BDNF protein function, it is the intracellular packaging and downstream secretion of BDNF that is altered by this single amino acid change in the pro region .

Using a green fluorescent protein (GFP) co-localization technique, Egan et al. (2003) found that both the val BDNF ($BDNF_{val}$) and met BDNF ($BDNF_{met}$) signals co-localized well with a Golgi marker but that only the $BDNF_{val}$ and not the $BDNF_{met}$ co-localized with a secretory vesicle marker, implying that met BDNF is

not being sorted from Golgi to the secretory granules. $BDNF_{met}$ also did not show co-localization with synaptophysin, a synapse marker, indicating a failure of $BDNF_{met}$ to localize to the synapse, a finding which could help explain the observed phenotypes associated with the val66met polymorphism .

It has been shown that in heterozygotes, individuals expressing both the val and the met alleles in the same cell, $BDNF_{met}$ alters the trafficking of $BDNF_{val}$ through the formation of heterodimers that are less efficiently sorted from the Golgi apparatus to secretory granules destined for the regulated secretory pathway . The total amount of mature BDNF secreted is thus reduced by this polymorphism. A dose response relationship has been found between the number of met alleles and the observed decrease in N-acetyl-aspartate (NAA) activity−an in vivo indicator of neuronal and synaptic activity−with met homozygotes having less NAA activity than heterozygotes or val homozygotes . The effects of the val66met polymorphism appear to be attributable to the amount rather than the form of activity-dependent BDNF that is secreted.

2.4 The BDNF val66met polymorphism affects normal human behaviour and corresponding fMRI activity

The val66met polymorphism appears to be linked to behavioural deficits, as the polymorphism has been shown to predict variations in human memory. Egan et al. (2003) tested subjects on the story recall component of the Wechsler Memory Scale, a test of verbal episodic memory, and demonstrated diminished performance in met/met individuals relative to those with at least one val allele . No correlation was found between BDNF genotype and performance on tests of semantic memory

or executive function, as tested with the Wisconsin Card Sorting Task, suggesting that the polymorphism's effects are specific to hippocampus-dependent processes. In a replication study, Dempster et al. (2005) also found decreased Wechsler Memory Scale scores in healthy met carriers compared to healthy val homozygotes.

In another experiment, Egan et al. (2003) used the N-back working memory fMRI task, in which subjects must recall a number seen two stimuli previously, to investigate differences in hippocampal function between val and met groups. This task has previously been shown to produce a reliable disengagement of the hippocampus which has been reported to be disrupted in clinical pathological states . Whereas val/val individuals demonstrated the expected hippocampal deactivation on this task, Egan et al. (2003) found an abnormal activation of the caudal hippocampus bilaterally in met carriers, which was shown to be statistically different when compared with val/val subjects . This finding emphasizes the important role played by BDNF in the hippocampus and the effect that the val66met polymorphism can have on the normal regulation of this structure.

The val66met polymorphism's detrimental effects on hippocampal function have been further demonstrated using an fMRI declarative memory task, on which performance was previously shown to be dependent on the hippocampal formation . Using a picture scene recognition paradigm, in which subjects had to classify pictures as "new" or "already seen", Hariri et al. (2003) showed that val/val individuals had significantly greater hippocampal activation during both the encoding and retrieval stages of the task relative to val/met subjects, though both groups did show significant bilateral activation of the posterior hippocampus and parahippocampal gyrus. Along with their decreased fMRI activity during the

encoding and retrieval of spatial material compared to homozygous val individuals, met carriers also showed decreased performance on the retrieval portion of this task . Though the task did not test spatial memory directly, the subjects were required to use their hippocampal formation (hippocampus and parahippocampal gyrus) to perceive and remember scenes . The diminished performance of met carriers on this task is further evidence for a deleterious effect of the val66met polymorphism on hippocampus-related functions and suggests that future work using a spatial memory task could show the same disparity in hippocampal activity between met carriers and homozygous val individuals.

2.5 The BDNF met66val polymorphism affects human brain morphology

Expression of the val66met polymorphism has previously been correlated with variations in brain morphology. Pezawas et al. (2004) performed structural MRI and voxel-based morphometry (VBM) on 111 healthy volunteer subjects and demonstrated reductions in grey matter volumes in the hippocampus and prefrontal cortex in val/met individuals compared to those expressing two val alleles. The authors suggest that the observed grey matter reductions are the result of abnormal secretion of the BDNF protein leading to altered cortical development and reduced synaptic plasticity . It has also been proposed that differences in hippocampal volume could be attributable to decreased dendritic complexity, fewer neuronal and supporting cells, or increased cell death during development or across the lifespan, all of which are processes that are mediated by BDNF and its receptors . Other studies have also found reduced hippocampal volume in met carriers using anatomical manual segmentation techniques . Interestingly, a VBM study in a

Japanese population found volume reductions in the caudate nucleus but not the hippocampus in subjects with the val66met polymorphism . While the evidence implicating BDNF with hippocampal function, plasticity, and grey matter is strong, there are clearly other factors that play a role in determining morphological changes in the hippocampus. There is also some evidence to suggest that the repeated use of one memory system over the other, perhaps due to spontaneous preferences for one type of learning, can have long-term morphological consequences on the hippocampus and caudate nucleus . The current project seeks to investigate a potential genetic contribution to human behavior, brain function and brain morphology.

3. Materials and Methods

3.1 Recruitment

One hundred and six volunteer subjects (53 women) aged 18-35 were recruited to the Douglas Mental Health University Institute at McGill University (Val group: mean age = 23.385 SEM = 1.095; 32 male/35 female) (Met group mean age = 22.667 SEM = 2.305; 21 male/18 female). Potential subjects were given a screening questionnaire to determine eligibility. Subjects had no personal or immediate family history of primary degenerative neurological disorders, no personal history of neurological or psychiatric diagnosis, alcohol or drug abuse. No subjects were taking any medication for or had known conditions which could affect cerebral blood flow (e.g. anti-cholesterol medication). The study was approved by the institutional review boards at McGill University, the Douglas Mental Health University Institute, and the Montreal Neurological Institute. All

subjects who participated in the brain imaging components of the study were right-handed, had normal or corrected-to-normal vision, and were screened for claustrophobia and metallic implements in their body. Informed consent was obtained from each subject prior to commencement of behavioural testing and again before brain imaging.

3.2 Genotyping

Blood samples were collected at the Douglas Mental Health University Institute and BDNF val66met genotyping was performed in the laboratory of Dr. Ridha Joober and at Génome Québec. DNA was extracted from the blood samples using standard methods and the val66met genotype was determined using the ABI PRISM SNaPshot Multiplex Kit assay.

3.3 Behavioural Study

One-hundred and six subjects were tested on the 4/8VM, a task specifically designed to differentiate between hippocampus-dependent spatial and caudate nucleus-dependent response strategies . These subjects were also tested on a battery of neuropsychological tasks, including the Shipley Institute of Living Scale, the Rey Auditory Verbal Learning Task, and the Rey-Osterrieth Complex Figure Task, to assess general cognitive function. DNA was collected from these subjects via a blood sample and genetic analysis was performed in order to determine BDNF val66met genotype.

3.4 fMRI Study

A subsample of twenty-one subjects was tested on the Concurrent Spatial Discrimination Task (CSDT) using functional magnetic resonance imaging (fMRI) and had their genotypes analyzed to determine if BDNF val66met genotype predicts differences in brain activation during this test of spatial memory.

It has been suggested that fMRI may be a more powerful tool to investigate genotypic effects on cognitive processing in the brain than traditional behavioural methods . fMRI may better estimate neurophysiological consequences of a neurobiological cause−such as a genetic variation−than do traditional behavioural methods due to the fact that behavioural performance can be compensated for by increasing the use of other related brain areas. fMRI investigation techniques make use of the blood-oxygen-level-dependent (BOLD) signal to indirectly estimate the level of neural activity occurring in a given brain region at a particular point in time . Studies measuring sex effects on behaviour, for example, have demonstrated different patterns of brain activation between groups even when task performance is equal . Additionally, the time series statistical analysis nature of fMRI data increases statistical power, greatly enhancing our ability to measure small effect size differences . We expect that fMRI techniques will allow us to observe differences between genotype groups which are more difficult to measure behaviourally.

3.5 Morphological Study

A subsample of thirty-seven subjects received anatomical MRI scans. Voxel-based morphometry (VBM) was performed on this sample in order to further investigate the morphological effects of the val66met genotype on the hippocampus

and caudate nucleus. This technique has been used by previous groups to measure grey matter differences based on the val66met polymorphism .

VBM is a computational approach to neuroanatomical analysis which utilizes a voxel-wise comparison of multiple brain images to measure differences in local concentrations of brain tissue . While VBM does not offer the same degree of statistical power and sensitivity as other neuroanatomical analysis techniques such as manual segmentation, it does offer greater intra- and inter-rater reliability and decreased operator time consumption compared to these other methods . The true value of VBM in this investigation is its ability to simultaneously detect grey matter differences throughout the brain and not just in specific structures as a whole, an advantage considering the fact that our stated objectives included the investigation of genotypic effects on both hippocampus and caudate nucleus morphology.

3.6 Methodological Details

3.6.1 Details of the Behavioral Assessment Procedure

The behavioral assessment consisted of the administration of three standard Neuropsychological tests of cognitive function. The Rey Auditory Verbal Learning Test (RAVLT) consists of word list learning, spontaneous retrieval, and recognition-type retrieval with the aid of written word lists. In this task, the experimenter reads a list of 15 unrelated words (List A) to the subject who is then asked to repeat as many words as possible. The process is repeated five times before an interference word list (List B) is read by the experimenter and the subject is asked to repeat as many words as they can remember from this second list.

Following the interference trial, the subject is asked to spontaneously recall as many words as they can remember from List A. The number of words recalled here provides the Recall after Interference score. The subject is then asked to spontaneously recall this list again after a delay of thirty minutes, giving the Delayed Recall Score. Subjects are also given a Total Recall Score based on the total number of words they were able to remember (the sum of the initial five learning trials, the interference trial, the after interference trial and the delayed recall trial). Finally, the subject is presented with a written list of words containing the contents of lists A and B as well as twenty semantically associated or phonetically similar distracter words. The subject is told to identify which, if any, list each word belongs to. We calculated a Recognition Score based on the sum of the number of words correctly attributed to list A and list B minus the sum of incorrectly attributed words and incorrectly unattributed words. This technique of calculating a Recognition Score differs from standard methods which ask subjects only to check off List A words . Our method prevents the ceiling effect of having subjects scoring highly due to a positive response bias.

This task provides an assessment of immediate memory, efficiency of learning, effects of interference, and recall following short and long delay periods (30 seconds and 30 minutes) . This task is sensitive to hippocampal function (Bohbot et al., 1998), though several different strategies exist which can be used to allow subjects to learn and retrieve the word lists. Possible strategies include but are not limited to mental rehearsal and building associations between unrelated words into a story (unpublished observation). In fact, previous studies have demonstrated impairments on the RAVLT task in both hippocampus-compromised early

Alzheimer's patients and caudate nucleus-compromised advanced Huntington's Disease patients .

The Rey-Osterrieth Complex Figure Test is a widely used assessment of visuospatial processing, memory and executive function . In this task, the subject is presented with a complex abstract figure and is asked to simply reproduce the figure on another sheet of paper. Following a 30 minute delay, the subject is asked to once again reproduce the figure on a blank sheet, this time from memory. Subjects are not forewarned that they will be asked to recall what they had to copy initially. Scoring of the task is done by assigning a mark out of two for each of 18 different components of the figure for a total score out of 36. For each component, one mark is awarded for a correct placement in space and another for accuracy of drawing. The copy and recall sections of the task are scored separately, giving each subject a Copy Score and a Recall Score. Impairments on performance of this visuospatial task have been observed in patients with lesions to their temporal lobes and hippocampus specifically (Bohbot et al., 1998), though this task does not involve "spatial memory" defined as constructing a cognitive map.

The Shipley Institute of Living Scale assesses general intellectual functioning and aids in the detection of cognitive impairment . The scale consists of two subtests, a 40-item vocabulary test and a 20-item abstract thinking test. The tests are self-administered and the subject is given a 20 minute time period in which to complete both subtests. The total number of correct multiple choice selections on the vocabulary test provides the Shipley Vocabulary Score. An estimated WAIS-R IQ is calculated by combining performance scores on the two subtests and

converting this score to an estimated WAIS-R IQ Score using a conversion table stratified by subject age .

These tasks were used to assess general cognition and to ensure that the groups are equally balanced. No significant differences were observed between genotype groups on any of these tasks (Table 2). None of the subjects tested performed at below-normal (cognitively impaired) levels on any of these tasks and thus no subjects were excluded from further testing on this basis.

3.6.2 Details of the Behavioural Study Procedure

The 4/8VM was created using software from a commercially available computer game (Unreal; Epic Games, Raleigh, NC). A visual representation of the environment is presented in Figure 1. The virtual environment consists of an eight-arm radial maze with a central starting location. The maze is surrounded by a landscape and distal landmarks, namely two trees, a sunset and mountains. At the end of each of the eight arms is a set of stairs leading down to a location where an object can be picked up. Prior to testing, subjects are trained on the use of a keypad which they use to move within the environment during the task.

Subjects perform five trials, each of which consists of two separate parts. In Part 1, four of the eight arms are accessible with objects at the end of each arm. The other arms are blocked by a barrier. In Part 2, all arms are accessible and objects are present only in the four arms which were blocked in Part 1. Subjects are told to retrieve all four objects from the accessible arms in Part 1 and to remember which arms have been visited so that they can avoid these arms in Part 2. A “reference memory error” consists of an entry into an arm that does not contain an object, while a “working memory error” consists of re-entry into an arm from which an

object has already been collected. During the second part of the fourth trial, a wall is erected around the radial maze blocking the subject's view of the distal environment. This fourth trial serves as a probe trial to differentiate between subjects using a "spatial" strategy dependent on the relationships between objects in the environment and a "response" strategy in which the series of arms to be visited is memorized using a pattern or numbering system; the rationale for the probe being that if subjects were using a spatial strategy, this change in the environment should result in an increase in errors, whereas the use of a response strategy would not result in the same increase in errors .

At the end of the experiment, the subject is debriefed and asked to report how they solved the task from the beginning to the end of the experiment. As in previous studies , subjects are categorized as "response" learners when they report associating the arms with numbers or letters or counting the arms from a single starting point. Subjects are considered to be "spatial" learners if they mention using at least two landmarks and do not mention a response strategy. For the purposes of this study we assessed spontaneous strategy preferences by classifying subjects according to their "Initial Strategy". Initial strategy was defined as the first strategy used if the subject reported having started with one strategy and then "shifting" to the other.

3.6.3 Details of the fMRI Experimental Procedure

Concurrent Spatial Discrimination Task (CSDT): Subjects were tested using the CSDT, a 12-arm radial maze task designed using software from a commercially available computer game (Unreal; Epic Games, Raleigh, NC) and previously shown to differentially activate the hippocampus and caudate nucleus in subjects using spatial and response strategies respectively . A visual representation of the virtual environment is presented in Figures 2 and 3. In this task, subjects navigate through a virtual environment consisting of a radial maze with a central starting location. The maze is surrounded by an enriched landscape and landmarks. At the end of each arm, there is a staircase leading to the location where, in some of the arms, an object can be picked up. The 12 arms of the maze are divided into six pairs of adjacent arms, with each pair of arms differentiable based on its proximity to a visible landmark. Within each pair of arms, one arm contains an object while the other arm is always empty. The task consists of two separate parts, an encoding phase and a test phase. During the encoding phase (Stage 1), the six pairs of arms are repeatedly presented in turn to the subject according to a pseudo-random sequence and the subject is explicitly asked to learn progressively within each pair, which path contains an objects and which does not. In the encoding phase, a trial is defined as the presentation of all six pairs of arms. Choice accuracy is measured by the percentage of correct arms chosen. Training continues until the participant reaches a criterion of choice accuracy of at least 91% across two consecutive trials (11 correct arms selected with a maximum of one error) with a minimum of six trials performed. This minimum number of trials ensures that each subject has had

the opportunity to properly learn the location of the objects before moving on to the test phase.

Following the encoding phase, the subject completes a test phase. The first part of the test phase (Stage 2) consists of the "recombined pairs" condition. During the recombined pairs condition, the reward contingency among the arms remains the same but their presentation is modified, such that the arms are rearranged into novel pairs and these pairs are presented in a pseudo-random sequence (see Figure 3). Success on this phase requires that the subject used a spatial strategy to form a memory for the rewarded arms based on spatial relationships and not on stimulus-response relationships. In this way, the test phase acts as a probe for navigational strategy selection. Each subject completes two trials of four pairs each on the recombined pairs condition.

The second part of the test phase (Stage 3) which follows the recombined pairs condition is the "All-Open" condition. In this condition pathways are no longer presented as pairs. Instead, all pathways are simultaneously visible and accessible and the subject is asked to simply visit all six pathways containing objects while avoiding those that are empty. A schematic diagram showing the different phases of the CSDT task is shown in Figure 3.

Visuo-motor Control Task: The fMRI control task takes place in a different environment than the experimental task but includes a similar radial maze. For this task, the position of the rewarded arms is completely randomized and changed from one trial to another. The participant is asked to randomly visit arms in order to pick up objects. The experimenter specifies that the locations of the objects are totally randomized and varied across trials, that no rule predicts their positions, and that

the subject has nothing to learn. At the same time, the participant is asked to count backwards by increments of 3, from 1000 to discourage rehearsal of learned information during the experimental task. A control equivalent to the All-Open condition (Stage 3) is presented during the same scan as the experimental All-Open condition. There is no memory component to this control task.

fMRI Data Acquisition: The scanning session consisted of several scans of a duration of ten minutes each. The number of scans recorded varies across participants as it is a function of the number of trials needed to attain the criterion performance of the encoding phase. In each scan, participants performed alternate blocks of experimental and visuo-motor control tasks. This was repeated until participants reached the predetermined criterion performance. The encoding phase was followed by the testing phase, which was also interleaved with sequences of the visuo-motor control task. Because of the variability between participants in the time taken to perform the task, homemade software was used to record frame times, every keystroke made by the participant as well as the keystrokes, made by the experimenter, which mark the transition from one task to another . Recording the keystrokes of the experimenter allows us to exclude frames acquired during the transitions between tasks, from the analysis.

fMRI scanning protocol: MRI scans were obtained at the Montreal Neurological Institute with a 1.5T Siemens Trio scanner. A vacuum cushion was used to stabilize the participant's head and the task was presented on a projector outside of the scanner, viewable to the participant through a mirror attached to the head coil. The fMRI scanning session followed the structural scan. It consisted of a sagittal localizer followed by a series of test blood oxygen level-dependent (BOLD) scans.

Functional images were acquired using a single shot T2*-weighted gradient echo EPI pulse sequence (TR = 2000 msec, TE = 50 msec, FOV = 224 mm^2, matrix size = 64 X 64, in-plane resolution = 2 X 2 mm, 300 whole brain acquisitions/run). Each whole brain acquisition consisted of 26 oblique slices of 3.5 mm thickness, 0.5 mm slice gap, positioned parallel to the hippocampus and covering the entire brain. The structural scans were then co-registered with the fMRI scans. Each subject's fMRI session was divided into 3-8 runs of no more than ten minutes each with each run consisting of both experimental and control trials.

3.6.4 Details of the Structural MRI Scanning Procedure

Structural MRI scans were collected prior to fMRI testing using the International Consortium for Brain Mapping (ICBM) protocol . This protocol generates T1-weighted image volumes with a 1 mm isotropic resolution. The volumes are acquired with a 3-D spoiled gradient echo acquisition with sagittal volume excitation (TR = 22, TE = 10, flip angle = 30°, 160 1mm sagittal slices). The rectangular field of view (FOV) for the sagittal images is 256 mm (SI) by 224 mm (AP).

3.7 Data Analysis

For the purposes of the behavioural analyses, subjects were divided into three distinct genotype groups based on the number of met alleles (0, 1 or 2). The "0" group had genotype val/val, the "1" group had genotype val/met, and the "2" group had genotype met/met. This method of dividing subjects has been used in previous behavioural studies with large sample sizes as some evidence exists that

the val66met polymorphism may have a dose-dependent effect on hippocampus-related processes .

For the purposes of the fMRI and structural MRI analyses, subjects were divided into two distinct genotype groups. Due to the small sample size in the imaging components of the study and the infrequency of met/met individuals in the general population (i.e. <5%) , subjects were grouped into either the "Val" group consisting of individuals homozygous for the val allele of the val66met polymorphism or the "Met" group which was comprised of both homozygous met individuals and heterozygous individuals who have both a val and a met allele. This method of dividing subjects has been used in previous imaging studies involving the BDNF val66met polymorphism .

3.7.1 Behavioral Data Analysis

Dependent variables included the latencies and errors participants made on each trial of the 4/8VM (excluding probe trials that are used for strategy identification), the total number of both working memory "revisit", and reference memory "incorrect selection" errors, Shipley Vocabulary Score, Shipley estimated WAIS-R IQ, RAVLT delayed recall, RAVLT total recall, RAVLT recognition score, and Rey-Osterrieth Recall score. SPSS for Windows (version 11.01) was used to conduct analysis of variance (ANOVA) and chi-squared analysis on these variables, with navigational strategy and genotype group (Val or Met) as main effects.

3.7.2 Functional MRI Data Analysis

FMRISTAT, developed by Keith Worsley and provided by the MNI, was used for the statistical analysis of fMRI data. BOLD signal images were spatially smoothed with an 8 mm FWHM Gaussian kernel, corrected for motion and linearly transformed into stereotaxic space using in-house software . The output of the analysis was displayed as a statistical map, overlaid on an image of an MRI scan, which shows regions of significant difference in grey matter. The resulting t-statistic images were thresholded using the minimum given by a Bonferroni correction for multiple comparisons and Gaussian Random Field Theory . Restricted search (ROI) analysis using an uncorrected *p* value of 0.001 (t = 3.55) was used for voxels in the hippocampus and caudate nucleus based on our strong a priori hypothesis that we would observe significant activations in these brain areas. A Bonferroni correction for multiple comparisons was used for all brain areas outside of the hippocampus and caudate nucleus based on one million voxel comparisons (t = 6.92).

fMRI Main Comparisons: Experimental trials were contrasted against control trials of equal durations (Experimental Trial-Control Trial) for every participant. fMRI scans of the Val and Met groups were contrasted separately. A group analysis was then performed contrasting total activity for the Val group with that of the Met group for each of our selected contrasts of interest.

Correlational analyses: FMRISTAT was used for correlations between the fMRI signal and other variables. The fMRI signal was covaried with errors, latencies for performance of the task and grey matter density.

3.7.3 Structural MRI Data Analysis

MRI scans were spatially normalized by linear transformation into a stereotaxic coordinate system based on the Talairach Atlas . They were then corrected for intensity non-uniformity (shading artifact) using the N3 software package . Each voxel was then automatically labeled as white matter, grey matter, cerebrospinal fluid, or background using INSECT (Intensity Normalized Stereotaxic Environment for the Classification of Tissues), a method relying on an artificial neural network classifier . The grey matter was smoothed using an 8 mm FWHM (full-width at half-maximum) Gaussian kernel. Generalized linear models were used to investigate the predicted correlation between BDNF val66met genotype and grey matter in the brain . The resulting t-statistic images were thresholded using the same methods that were used for the fMRI analysis, including the restricted search region of interest analysis ($p < 0.001$) for the hippocampus and caudate nucleus.

4. Results

The BDNF val66met allele and genotype frequencies are summarized in Table 1. In the behavioural sample, the frequency of the val allele was 0.77, with 36.8% of participants carrying the met allele. These numbers are consistent with the expected range for a North American populations based on previous studies . Genotype distributions deviated from Hardy-Weinberg expectations for the behavioural sample ($\chi^2 = 5.62$, $p < 0.05$) but not the fMRI sample ($\chi^2 = 0.38$, $p > 0.05$), or the VBM sample ($\chi^2 = 0.03$, $p > 0.05$).

4.1 Behavioural Study:

As expected, no relationship was observed between val66met genotype and any measure of performance on the RAVLT, Rey-Osterrieth Complex Figure Task, Shipley Institute of Living Scale, or 4/8VM using an analysis of variance test with both genotype group (Val or Met) and number of met alleles (0, 1, or 2) as grouping variables at a significance level of $p < 0.05$ (see Table 2). When groups were split based on their self-reported initial strategy on the 4/8VM task, no effect of strategy selection on performance was observed for any measure of performance on our behavioral tasks (RAVLT, Rey-Osterrieth Complex Figure, Shipley Institute of Living Scale, 4/8VM), except for total latency on the 4/8VM with the spatial group taking significantly longer than the non-spatial group to complete the task (F_1=12.987, $p < 0.01$).

A chi-squared analysis of Genotype Group (Val or Met) vs. Initial Strategy revealed a strong trend towards the increased use of response strategies in met carriers compared to the Val group ($\chi_1^2 = 3.26$, $p = 0.054$) (Figure 1). Dividing subjects into groups based on the number of met alleles (0 = Val/Val, 1 = Val/Met, 2 = Met/Met) and performing a linear-by-linear association chi-squared analysis of genotype vs. initial strategy, revealed a significant relationship between genotype and strategy selection (linear-by-linear association $\chi_1^2 = 4.203$, $p = 0.04$) (Figure 2).

4.2 fMRI Study

For the purposes of fMRI analysis, we used a threshold *t* value of 3.55 in the hippocampus and caudate nucleus which was calculated using an uncorrected *p* value of < 0.001. This was based on our strong a priori predictions of activation in these brain regions for our navigation task. For all voxels located outside of these structures, a *t* threshold of 6.92 was used based on the Bonferroni correction for multiple comparisons. No voxel outside of the hippocampus and caudate nucleus crossed this threshold for any of our measured contrasts.

Consistent with our hypothesis, we found differences in memory system recruitment between met carriers and the homozygous Val group across both the encoding and test phases of the Concurrent Spatial Discrimination fMRI task (CSDT). Breaking the patterns of brain activation down into early learning, late learning, and test phase activity, revealed significant activations in the hippocampus early in learning in the Val but not the Met group, while significant activation of the caudate nucleus was observed in the Met but not the Val group later in the encoding phase and during the test phase.

During the first experimental trial of the encoding phase (Stage 1), when subjects are first exposed to the pathways and are asked to actively memorize which of each pair of pathways contains an object, activation of the hippocampus was observed in the Val group (x = 30, y = -9, z = -32; *t* = 4.166) but not the Met group (maximum peak: x = 21, y = -17, z = -20; t =1.904) (Figure 6).. During the first experimental trial, no significant caudate nucleus activations were observed for either the Val group (maximum peak: x = 10, y = 16, z = 6; t = 2.645) or the Met group (maximum peak: x = -8, y = 14, z = 8; t= 2.789). Hippocampal activity early

in learning is consistent with previous rodent studies which demonstrated that animals began the learning of a strategy-neutral navigation task using a spatial strategy and only later shifted to a response strategy if the hippocampus was impaired .

By the end of the encoding phase (the last two experimental trials before moving to the test phase), when subjects had learned the location of the objects to criteria, we observed significant caudate nucleus activity in the Met group (x = 22, y = -18, z = 26; t = 4.23) but not the Val group (maximum peak: x = 14, y = 10, z = 4; t = 2.00) (Figure 7). During these trials, hippocampal activity was below threshold for significance in both the Val (maximum peak: x = -24, y = -14, z = -16; t = 2.09) and the Met group (maximum peak: x = 20, y = -8, z = -26; t = 2.89). Contrasting the Val Group and the Met Group directly, we observed a sub-threshold negative activation in the caudate nucleus (x = 20, y = -19, z = 24; *t* = -3.14) (Figure 7), suggesting that the Met group activated this structure to a greater extent than did the Val group during late encoding.

During the Recombined condition (Stage 2), no voxels in the hippocampus or caudate nucleus crossed our threshold for significance for either the Val or the Met group. This could be a result of both groups making use of both the hippocampus and caudate nucleus to some extent during this condition.

Differential recruitment of memory systems between genotype groups was conserved during the "All-Open" post-learning test phase. During the "All-Open" condition (Stage 3), the Met group continued to demonstrate significant activation of the caudate nucleus (x = -12, y = -4, z = 20 *t* = 4.09) (Figure 8), while the Val group did not activate the caudate (maximum peak: x = 41, y = -24, z = -12; *t* =

2.14). No significant activity was found in the hippocampus for the Val group (maximum peak: x = 41, y = -24, z = -12; t = 2.14) or the Met group (maximum peak: x = -18, y = -21, z = -22; t = 3.42), though the Met hippocampal activity did approach significance. A group contrast of Val vs. Met revealed a strong negative activation in the caudate nucleus, the same brain region that was found to differ between groups late in the encoding phase (x = -16, y = 8, z = 16; t = -3.3) (Figure 8). This suggests that the Met group continues to preferentially recruit the caudate nucleus much more than the Val group during the test phase and that the Val group is likely recruiting both the hippocampus and the caudate nucleus to some extent during retrieval.

Importantly, no performance differences were observed on the CSDT fMRI task between genotype groups as measured by trials to criteria during the Encoding Phase (Stage 1) or total number of errors made during the All-Open condition (Stage 3) (Table 3). This demonstrates that strategy selection is not related to performance on these parts of the task and that individuals with lower functioning hippocampi due to the presence of the polymorphism can compensate for this impairment by making use of the alternative response strategy.

Performance on the Recombined Condition of the Test Phase (Stage 2) did differ between those who spontaneously use spatial and response strategies on the 4/8VM. An independent samples t-test showed that,when pairs were recombined during Stage 2, those who had used a spatial strategy on the 4/8VM task had a higher percentage of correct pathway choices (81.25% correct, SEM=7.087) than those who had used a response strategy (46.10% correct, SEM=7.741 (t = 3.092, p = 0.006). The fact that there was a performance difference not only validates the

use of our CSDT recombined pairs probe as a means of classifying strategy but it also demonstrates that spontaneous strategy selection is consistent between our two virtual navigation tasks. In other words, our phenotype measure is highly reliable across tasks. Moreover, the fact that previous studies showed a correlation between our phenotype and brain function and morphology in a relatively small number of subjects indicates that the phenotype has high specificity relative to other Neuropsychological tests.

Interestingly, the spatial group also learned the location of the correct pathways more quickly than the response group as measured by the number of trials to criteria, although this difference did not reach statistical significance (spatial group mean TTC = 5.125, SEM = 0.515; response group mean TTC = 7.154, SEM = 0.815; $t = 1.807$, $p = 0.087$).

Though the Val group tended to perform better than the Met group on the first trial of the Recombined Pairs (Stage 2) (Val group mean probe performance = 63.0% correct, SEM = 6.538; Met group mean probe performance = 36.6% correct, SEM = 17.946), this difference did not reach statistical significance ($t = 1.738$, $p = 0.098$).

4.3 Morphological Study

For the purposes of our morphological study, we set a threshold t value of 5.73 based on a Bonferroni correction for the million voxels in the brain. No voxel outside of the hippocampus and caudate nucleus crossed this significance threshold and we thus conclude that for our sample, there were no differences in grey matter density between genotype groups outside of the hippocampus and caudate nucleus.

Because of our a priori predictions about grey matter differences in the hippocampus and caudate nucleus, a threshold *t* value of 3.34 was used for voxels residing within these two structures based on an uncorrected *p* value of 0.001. We did not observe any significant differences between the Val and Met groups in either the hippocampus or the caudate nucleus.

Our null result is not consistent with previous BDNF volumetry studies such as that of Pezawas et al. (2004), who had found significant hippocampal grey matter density differences between the Val and Met genotype groups . The fact that we found no significant difference in hippocampal grey matter density could be a result of our low sample size (N = 37). Previous studies which have used VBM to demonstrate differences in grey matter density between the Val and Met groups had minimum sample sizes of 109 subjects .

5. Discussion

5.1 Effects of the BDNF val66met on Multiple Memory Systems

Our data suggest that the BDNF val66met polymorphism shifts the balance between multiple memory systems, biasing individuals towards the use of a caudate nucleus-dependent system at the expense of a hippocampus-dependent system on our virtual navigation memory tasks. This biasing effect was seen in the breakdown of strategy selection on the 4/8 Virtual Maze and in fMRI activation differences during the Concurrent Spatial Discrimination Task, two tasks which can be solved using either a hippocampus-based spatial or a caudate-based response strategy. These findings support the conclusions of previous studies which have suggested an impairing effect of the valine to methionine mutation on performance and fMRI

activity during tasks that require the use of hippocampus-dependent episodic memory . That we did not observe significant grey matter density differences between the Val and Met genotype groups is not surprising given our small sample size. Previous volumetric studies have found decreased hippocampal grey matter in met relative to val individuals .

The availability of two potential parallel strategies in our tasks differs from the experimental design of previous val66met studies . The use of the 4/8VM and CSDT allowed us to study the effects of the val66met polymorphism on the interaction between multiple memory systems. The spontaneous quantifiable variability in our phenotype increases the sensitivity to differences in genetic variability. Whereas the use of hippocampus-dependent episodic memory tasks have allowed others to demonstrate impairments in met carriers, we have shown that the impairing effect of the met allele on the hippocampus simply makes a subject less likely to select a strategy that depends on that structure when another option is available to them. That 4/8VM and CSDT performance measures did not differ between genotype groups (Tables 2 and 3) is further evidence for a compensatory effect of selecting another strategy when spatial memory is at a disadvantage.

The differences we observed in strategy selection on the 4/8VM suggest that the val66met genotype does have an effect on the spontaneous recruitment of memory systems. The presence of at least one met allele leads to an increased likelihood of not selecting a strategy that requires use of the affected hippocampus. Homozygous val individuals showed a greater propensity towards the selection of a spatial strategy over a response strategy relative to the met carriers (40.3% spatial

for Val group relative to only 23.1% spatial in met carriers) (Figure 4). When met carriers were further broken down into heterozygous (val/met: 72.4% response vs. 27.6% spatial) and homozygous (met/met: 90.0% response vs. 10.0% spatial), the met allele effect on strategy selection was even more striking. Dividing subjects into only two genotype groups, as either Val (Val/Val only) or Met (Val/Met or Met/Met) based on the presence of at least one Met allele revealed a strong trend toward decreased use of spatial strategies in met carriers (X^2=3.26, p=0.054). When subjects were divided into one of three groups based on their exact number of met alleles however, a linear-by-linear association taking into account the ordinality of the data, revealed a statistically significant relationship (X^2=4.203, p=0.04) (Figure 5). This supports the findings of Hashimoto et al. (2008), who have suggested that the effect of the met allele on hippocampus-dependent memory is dose dependent. Similarly, Egan et al. (2003) found that met/met homozygotes scored much worse on their measures of episodic memory than either their val/val or val/met groups. Because Dempster et al. (2005) grouped their met/met and val/met subjects together and made separate analyses, it is impossible to know whether their data would also support a compounding impairment on hippocampal tasks when more than one BDNF met allele is present. The pattern that emerges in our data and that of others just described suggests that while the val/met genotype is worse for hippocampal function than the val/val genotype, the presence of at least one val allele rescues some function when compared to met/met individuals. Due to the relative scarcity of met/met individuals (less than 5% in the normal population), most studies do group val/met and met/met individuals into a single met carrier genotype, as we did for the imaging components of our study. Future studies with much larger sample

sizes should further examine the possibility of a dose-dependent effect of the met allele on behaviour.

In addition to the 4/8VM, the participants in the behavioural component of this study were also tested on a behavioral battery that included standard Neuropsychological tests such as the Rey Auditory Verbal Learning Task (RAVLT), the Rey-Osterrieth Complex Figure Task, and the Shipley Institute of Living Scale (SILS). That no performance difference was seen on any measure of these tasks is not surprising. Previous studies that have linked performance on the RAVLT and Complex Figure tasks to hippocampal function have often been carried out in patients with severe brain pathology. For example, Bohbot et al. (1998) showed that patients with right hippocampal lesions were impaired on several aspects of these tasks. In the present study, subjects who could have been expected to have a hippocampal impairment based on their BDNFmet status were still able to use their hippocampus. The val66met polymorphism only confers a small impairment to hippocampal function compared to a serious brain lesion. While these neuropsychological tasks are well suited at detecting large-scale brain impairments, they are not sensitive enough to detect differences based on a single polymorphism, although one study did find a significant effect of val66met genotype on verbal memory performance using the RAVLT in patients with schizophrenia and control subjects, and on visuospatial processing using the Rey-Osterrieth Complex Figure . However, the effect size in that study was extremely small and the sample size much larger than the one used in our study (N = 437 for Ho et al.'s study vs. N = 106 for our study). Though it is likely that hippocampal function is important for performance on these tasks, the hippocampal impairment

resulting from the presence of the val66met polymorphism is not severe enough for us to observe significant effects on performance with our sample.

Estimated WAIS-R IQ calculated from scores on the SILS was found not to differ between genotype groups ($t = 0.188$ $p = 0.830$). This finding supports other studies which also found no effect of val66met genotype on general intelligence . The only performance differences between homozygous val individuals and met carriers observed to this point have been on specific episodic memory-related subtests such as the story recall and logical memory subtests of the Wechsler Memory Scale and episodic memory recognition for scenes . It seems unlikely that the specific effects of this polymorphism on the hippocampus would translate into a generalized impairment in intelligence.

The results of the fMRI component of this study further support a specific effect of the val66met polymorphism on the balance between memory systems. While the Val group showed significant hippocampal activity early in learning with no significant hippocamal activity in the Met group(Figure 6), with practice, the Met group demonstrated significant activation of the caudate nucleus while the Val group did not (Figure 7). These activation differences between groups were consistent with previous studies demonstrating increased hippocampal activity in the Val group compared to the Met group on an episodic memory task and with our hypothesis that differences in hippocampal activity would translate to our multiple memory systems spatial navigation task.

Consistent with Hariri et al.'s study (2003), we observed robust fMRI activity in the parahippocampus during both the learning and test phases of the CSDT. While the maximum peaks we observed during learning (Val group

maximum parahippocampus peak: x = 28, y = -46, z = -9; t = 4.72), (Met group maximum parahippocampus peak: x = 15, y = -35, z = -4; t = 3.66) and during the All-Open test condition (Val group maximum parahippocampus peak: x = 38, y = -48, z = -5; t = 4.18), (Met group maximum parahippocampus peak: x = 18, y = -38, z = -2; t = 3.96) surpassed our region of interest t threshold using an uncorrected p value of 0.001 (t = 3.55), they did not surpass the whole brain-corrected threshold (t = 6.92). Since we had made no a priori predictions about the parahippocampal gyrus, we used the whole brain-corrected t threshold and found these voxels to not be significant. Many of the fMRI activation differences which Hariri et al. attributed to the "hippocampal formation" were actually not in the hippocampus itself but in the posterior parahippocampal gyrus, the same region in which we found differences during the Test Phase. While no distinction was made between voxels in the hippocampus proper and those in the hippocampal formation (hippocampus + parahippocampal gyrus) in their study, we feel that it is imperative to make one. These two brain regions are close to one another in space and functionally related to one another, but they are not analogous. While the hippocampus is thought to play an important role in processing and remembering spatial and contextual information , the formation of a cognitive map and building complex relationships in space , the parahippocampal cortex has been implicated in passively viewing and recognizing scenes , a considerably less cognitively complex task. That Hariri and colleagues found parahippocampal activation on their task is not surprising considering that the task involved passively viewing and classifying scenes. It seems plausible that the val66met polymorphism could play a role in the activity of the posterior parahippocampal gyrus as well as the hippocampus, as

these two regions are so closely related to one another functionally.

The results of the morphological component of our study did not reach the threshold for significance. Our VBM results were not consistent with previous studies which found significant grey matter differences in the hippocampus . The fact that no voxels in our VBM analysis reached significance is very likely a result of the small sample size (N = 37) for this type of analysis. Previous studies which found significant effects of the val66met polymorphism on grey matter using VBM had samples of 111 ,109 subjects, and studies using manual segmentation techniques had sample sizes of 36 and 44 subjects. We decided to use a VBM analysis because it allows for voxel-by-voxel analysis of the entire MRI volume rather than obtaining volumes for a single brain structure and more importantly because it has previously been proven to be sensitive to grey matter differences in the hippocampus and caudate nucleus of spatial and response learners. This was important because of our a priori predictions about genotype effects on both the hippocampus and caudate nucleus.

The mechanisms by which the BDNF met allele might affect grey matter brain volumes are not yet well understood. Some have suggested that these grey matter effects may be mediated neurodevelopmentally through neurotrophic effects of BDNF on the developing brain . Others have reasoned that since the met allele results in reduced BDNF synthesis and since BDNF is known to play a critical role in neuronal proliferation and survival, then this neuronal survival may in fact be threatened in a relatively BDNF-poor individual, leading to decreased volume as a result of decreased cell survival . This explanation, coupled with observations that decreases in BDNF result in not only fewer surviving neurons but also in the

surviving neurons having reduced soma size and diminished dendritic growth , leads to the conclusion that BDNF plays a critical role in several aspects of hippocampal health, not only during development but throughout life. Decreases in the amount of BDNF that is secreted in the hippocampus as a result of interrupted trafficking of the pro-BDNF protein through the cellular machinery in the presence of the met allele will have wide ranging effects on the health of hippocampal neurons. Despite our own inconclusive findings, we accept the findings of other groups who have shown a very real effect of val66met polymorphism on hippocampal grey matter.

We did not find sufficient evidence to conclude based on the present study that increased use of a response strategy as a result of this polymorphism confers any grey matter changes in the caudate nucleus, despite evidence demonstrating that greater use of this structure can lead to grey matter increases . Despite these findings, there is no known mechanism by which the caudate nucleus would increase in grey matter density or volume as a result of increased use of a response strategy. Further studies could be undertaken to look more closely for caudate grey matter differences by genotype, either using a larger sample of subjects in a VBM study or a structure-specific manual segmentation analysis in the caudate.

5.2 Interactions between Multiple Memory Systems

The results of the fMRI component of this study are instructive for understanding how the val66met polymorphism might affect the balance between competing memory systems. Several studies have characterized the relationship between the hippocampus- and caudate nucleus-dependent memory systems as

being one of competition. While it is possible for both systems to be engaged simultaneously, encoding different types of information, one or the other system is generally more active at any given time. There is a good deal of evidence to suggest that this is because one system actively inhibits the other. Indirect connections between the hippocampus and caudate nucleus running through the prefrontal cortex have been proposed as one possible pathway by which this interaction might take place . When Packard and colleagues (1989) found that rat fimbria-fornix lesions-which cut hippocampal efferents-improved performance on a task requiring stimulus-response learning, they concluded that this improved striatal performance was the result of decreased interference from the hippocampus; in other words, the lesioned hippocampus was no longer competitively inhibiting the striatum. A study of patients with Huntington's Disease, a neurodegenerative disorder affecting the caudate nucleus, showed this effect in the opposite direction. fMRI activity in the hippocampus was found to increase on a caudate-dependent response memory task with disease progression, possibly as a result of decreased inhibition by the affected caudate on the hippocampus . It seems likely that a genetic polymorphism impairing the hippocampus would have a similar effect, at least partially removing the inhibitory effect of the hippocampus on the caudate system. Our data showing increased caudate fMRI activity in met relative to val subjects with learning supports such a hypothesis.

The fMRI activation of the hippocampus in the Val group that we observed early in learning is consistent with the rodent literature on memory systems interaction. Using a navigation task which could be solved using either a spatial or a response strategy, Packard and McGaugh (1996) trained rats for a period of 16 days

to locate a food reward. On days 8 and 16, they used a probe trial in which they changed the rat's starting position in the maze to determine which of the two alternative strategies the animal was using to locate the reward. They observed that while control rats were preferentially using a hippocampus-based strategy on day 8, by day 16, most of the rats had shifted to a response strategy. They thus concluded that hippocampus-dependent spatial learning occurs faster and is more useful in early learning but that with time and experience, animals tend to rely more on their habit-based response system. Similarly, we found that both groups initially recruited the hippocampus to encode the locations of the objects in the maze in trial 1 but that by the end of the encoding phase, when retrieval of the objects had become much more of a habit, the Met group was much more likely to recruit the caudate nucleus. The difference in how long it takes each group to shift on our task is probably a product of the relative strengths of these competing memory systems between genotype groups. Had we over-trained our participants and continued to measure brain activity long after the locations of the objects had been encoded, it is likely that we would have eventually seen the Val group also preferentially recruit their caudate nucleus at the expense of the hippocampus. The presence of the met allele and a consequently weakened hippocampus probably makes this process occur more quickly in the Met group. Studies should be undertaken to further study the balance and interaction between these multiple memory systems. It would be helpful to understand exactly when and how the shift from spatial to response learning happens. The temporal relationship between these strategies is intuitive when conceptualized using a real-world example. When taking a new route for the first time from home to your new job, you are highly aware of your surroundings

and consciously engaged in understanding your own position within the environment. Your hippocampus is engaged as you use your higher-order brain areas to find your way to the novel location. Over time, as you take the same route from home to work everyday, the environmental details become less salient as you rely more and more on unconscious habit-based brain systems to find your way. What many people refer to as "the car driving itself to work" could actually be better described as "the habit-based response system driving itself to work". With time, conscious hippocampal processing is no longer required to follow the same route. Think about the relative cognitive difficulty of a situation when on the way to work, you encounter construction which forces you to take a detour. When this happens, you are effectively forced to rely on the hippocampus-based spatial system to integrate what you know about the relationships between landmarks in your environment to find your way using an alternative route. This is far more cognitively demanding than following an unconscious route and naturally some people will be better at it than others. Both the spatial and the response memory systems are important for navigation and memory. The relative use of these strategies will differ between individuals, as will our spontaneous preference for one or the other, but they are not mutually exclusive and independent. An understanding of how memory systems interact is paramount to our understanding of learning and memory differences at the individual level.

5.3 Genetic Contributions to Cognition: Limitations and Considerations

Studies which investigate the genetic contribution to cognition represent a burgeoning field of study in behavioural neuroscience. Preliminary progress is

being made in elucidating some of the genetic contributors to cognitive processes. BDNF represents one example of a gene which has been implicated in cognitive activity and whose naturally occurring polymorphic variability has been linked to differences in human behaviour. It is important to realize that cognition itself is a highly complex and often ill-defined process which in all probability depends on the interactions between a large number of genes and proteins.

Recent sequencing of the human genome has revealed the presence of approximately 35 000 distinct genes, upwards of 20 000 of which play a role in the development, plasticity, and maintenance of the central nervous system. In addition, approximately six million single nucleotide polymorphisms have been identified in humans. It is these genetic mutations which bestow genetic variability across the human population .

The picture is complicated further by the genetic phenomenon of epistasis, the process by which multiple non-allelic genes interact, with one gene variant having a modifying effect on the expression of one or more other genes. Recent evidence suggests that BDNF val66met genotype might interact with the polymorphism COMT val158met, to mediate executive processes . COMT is an enzyme which degrades dopamine. The valine to methionine substitution at codon 158 causes a decrease in enzymatic activity which results in a net increase in endogenous dopamine in the brain. COMTmet individuals had previously been shown to perform better than COMTval on the Wisconsin Card Sorting and N-Back tasks, two common tests which rely on fronto-striatal networks for executive processing . Nagel and colleagues (2008) have suggested that differences in performance between COMT genotype groups on these tasks might be related to an

interaction with BDNF met66val polymorphism . Interestingly, Rybakowski et al. (2003) have previously shown that bi-polar BDNFmet carriers perform significantly worse than bi-polar BDNFval individuals on the WCST, though COMT genotype was not assessed in that study. It is possible that their results could be explained by a modulary effect of BDNF on COMT in the prefrontal cortex.

A potential role for BDNF met66val in the prefrontal cortex demonstrates a further genetic phenomenon which also complicates the assignment of specific roles in cognition to genes. Pleiotropy is the phenomenon of a single gene influencing multiple phenotypic traits . Although the majority of BDNF research conducted to this point has focused on the hippocampus, there is no reason to assume that this gene and its polymorphism could not have other effects elsewhere in the brain or body. Even within the hippocampus, BDNF has been implicated in a diverse combination of processes including neuronal cell survival and differentiation during development , regulation of synaptic plasticity through modulation of long term potentiation and long term depression , and in many different forms of hippocampus-related memory behaviour . Volumetric studies have demonstrated that BDNFmet carriers display age and gender-independent gray matter volume reductions, not only in the hippocampus but also in the dorsolateral prefrontal cortex . Other studies have shown widespread expression of the BDNF gene throughout the brain, though expression levels are highest in the hippocampus . Further studies should look more closely at the role that BDNF val66met might play in prefrontal cortex function and in other brain areas outside of the hippocampus.

Finally, in addition to understanding a gene's interactions with other genes and its potential for multiple diverse functions, it is important to understand that genes also interact with their environment. Aspects of the environment can be in the pathway of genetic influence through epigenetics, leading to changes to the structure of DNA itself. For example, it has been demonstrated that environmental stressors during childhood can lead to specific mRNA expression changes later in life . There is evidence to suggest that methylation and chromatin remodeling may provide mechanisms by which the environment directly impacts DNA folding and subsequent expression of the BDNF gene .

In light of the complexity of variations in cognition, some estimate that the sum of all genetic effects on cognitive variation is not likely to be greater than 50% . Despite this, association studies have identified differences between different alleles of the same gene and behavioural phenotype across several modalities of human cognition. Because there are so many genes which contribute to the complex processes of cognition and because multiple genes often affect the same cognitive trait, the variance that can be explained by any one gene is relatively small . Given this, any measurable effect of a single gene or polymorphism on behaviour is remarkable.

5.4 Clinical Implications of BDNF

Consistent with the finding that normal BDNF expression is higher in the hippocampus than in other brain structures , BDNF val66met genotype has been associated with differential susceptibility to a number of neurological and psychiatric disorders which are thought to involve hippocampal pathology.

Although the mechanisms by which the polymorphism confers differences in disease risk remains to be worked out, gene association studies have suggested that met carriers are at increased risk for pathologies as diverse as Parkinson's Disease , bipolar disorder , eating disorder , and obsessive compulsive disorder . A neuroprotective effect of BDNF against Alzheimer's disease has been demonstrated in rodents and non-human primates , although a correlation study conducted with 130 Alzheimer's patients and 111 ethnically and age-matched healthy controls found that the Alzheimer's group had significantly fewer carriers of the met allele than the control group . This contradictory finding serves as a warning against oversimplifying complex pathological mechanisms into simple gene associations. Certainly, the BDNF gene is but one in a complex array of genetic and behavioural risk factors for hippocampal pathology and the val66met polymorphism represents a mutation at a single nucleotide in that gene. Nevertheless, the connection between BDNF val66met genotype and hippocampal pathology clearly calls for further investigation.

Due to its known role in promoting and maintaining hippocampal function, it has been suggested that boosting BDNF's endogenous expression and activity or even administering BDNF exogenously may prove to be an effective therapeutic avenue to encourage healthy aging of the hippocampus and to fight against age-related hippocampus-impairing pathology . Several promising models have also emerged which seek to explain the demonstrated link between BDNF val66met polymorphism and susceptibility to stress and depressive disorders.

5.4.1 BDNF and Stress

A BDNF-mediated mechanism could help explain some of the observed effects of stress on memory. The literature on stress and memory describes differences between chronic and acute stress and their differential effects on different types of learning . Spatial navigation studies provide a convenient technique for measuring these differential effects of stress on memory and allow us to suggest mechanisms by which stress differentially affects different types of memory.

The effect of stress on navigation has been the subject of contradictory findings in the literature, with both facilitating and impairing effects reported. These discrepancies could be explained by a mechanism through which stress modulates the balance between multiple memory systems.

A 2001 study by Kim et al. (2001) used a probe trial following training on the Morris water maze task to show that rats who had received a foot-shock stressor prior to training were more likely to use a non-hippocampal response strategy than rats who had not been stressed. Whereas all of the control, non-stressed rats initially swam to the old platform location based on distal landmarks in the environment (hippocampus-dependent spatial strategy), 50% of the rats who had been exposed to acute stress prior to training swam to the visible cued platform on the probe trial, indicating a preference for a non-spatial stimulus-response strategy. In a similar study, rats receiving pre-training injections of the anxiogenesis-inducing drug yohimbine displayed a preference for striatum-dependent response learning on the probe trial of a water plus maze task. Rats receiving vehicle on the other hand, predominantly made use of a hippocampus-dependent place strategy .

Behavioural studies have demonstrated that stress also modulates the balance between memory systems in humans. Schwabe et al. (2007) administered the Trier Social Stress Test (TSST) to subjects prior to conducting a behavioural experiment in which they were asked to repeatedly locate a "win" card out of four cards lying on a table in a model of a room. This location of the win card could be learned by either associating it with a proximal cue, a plant located on the table (caudate nucleus-dependent stimulus-response) or by learning the position of the win card relative to other distal cues found along the walls of the room (hippocampus-dependent spatial strategy). A probe trial, in which the proximal cue was moved to a different corner of the table, allowed subjects to be classified by strategy. Using this probe task, it was found that subjects who had been exposed to acute stress used a response strategy significantly more often than their non-stressed control counterparts. These results demonstrate that stress induced by the TSST invoked a preference for a habit-based response strategy at the expense of a spatial strategy.

A subsequent study demonstrated a response strategy preference, not only in cases of acute stress but also in individuals who endure chronic stress. Human subjects were divided into high chronic stress and low chronic stress groups based on their answers to the Trier Inventory of Chronic Stress. It was found that the high stress group used a stimulus-response strategy significantly more often than subjects in the low chronic stress group on a 2D learning task , suggesting that not only acute but also long-term chronic stress plays a role in determining the balance between multiple memory systems.

BDNF may represent one potential mechanism by which stress impacts on choice of navigation strategy. It has previously been shown that chronically stressed rats demonstrate a marked decrease in BDNF mRNA expression in the dentate gyrus and hippocampus, a reduction which could occur through a corticosterone negative feedback mechanism . Other studies have demonstrated behavioural effects of stress as well, with chronic mild stress leading to both decreased hippocampal BDNF expression and impairment on the hippocampus-dependent Morris water maze task , just as genetically modified mice with reduced BDNF expression display poor water maze performance . Direct infusion of BDNF into the hippocampus before and during chronic stress rescued water maze performance in rats who had been subjected to repeated immobilization stress , a finding which suggests that exogenous BDNF could help protect against stress effects in humans who do not produce optimal levels of mature BDNF as a result of the val66met polymorphism.

A recent study has demonstrated that multiple stressors may have a cumulative effect on hippocampus-dependent spatial memory performance. Choy et al. (2008) found that combining neonatal stress and young-adult glucocorticoid stimulation led to a 25-35% reduction of BDNF expression in the dentate gyrus as well as similar trends (15-20% reduction) in the CA1 and CA3 regions of the hippocampus compared to rats who underwent only one of these two stressors. In addition to BDNF expression changes, rats who had undergone both stressors exhibited a learning delay in the Morris water maze and a marked deficit in acquiring a spatial Y-maze task. Importantly, these same rats were not impaired on a working memory delayed-alternation T-maze task. These findings seem to

suggest a direct effect of stress on the hippocampus working through BDNF and sparing other memory systems. Previous studies have demonstrated however, that the effects of stress on multiple memory systems work through an amygdala-dependent mechanism , suggesting that the observed hippocampal BDNF reductions could have been a consequence of decreased hippocampal use in stressed rats due to amygdala inhibition. In this experiment, BDNF expression levels were significantly reduced in rats subjected to two as opposed to one stress treatment suggesting that multiple independent stressors lead to more dramatic molecular effects and that the effect of stress on the hippocampus is not an "all or none" phenomenon.

It is important to understand that stress, whether acute or chronic, directly affects the hippocampal memory system but not other competing systems such as the amygdala and caudate nucleus. In the Choy et al. study (2008), stressed animals with decreased BDNF expression were impaired on the water maze and Y-maze, the two spatial tasks previously shown to rely on the hippocampus. On the delayed alternation T-maze, a task which involves simple rule learning and which has previously been shown to depend on the striatum and prefrontal cortex , there were no significant performance differences observed between the stress groups. Interestingly, the group who had been subjected to both stressors acquired the task more rapidly than the other groups, suggesting that stress-induced decreases in hippocampal activity removed a degree of competition between memory systems and expedited the learning of non-hippocampal memory systems in the striatum and prefrontal cortex. This finding reinforces the idea of competition between memory systems, and demonstrates that by inhibiting one system, we are removing this

competition and in fact facilitating learning in the other system, a phenomenon which has been regularly reported in the literature . These findings reinforce the relationship between memory systems suggested by our data, in which response learning is facilitated in those whose hippocampal systems are impaired, in our case because of the presence of the BDNFmet allele.

Under circumstances where more than one strategy is possible, the presence of stress has the effect of pushing individuals towards the use of hippocampus-independent strategies. The findings of the Kim and Schwabe groups in particular show that stress pushes individuals towards the use of striatum-dependent response strategies at the expense of hippocampus-dependent spatial strategies, a trend that could be a direct result of a stress-induced reduction in BDNF production. This explanation is supported by the fact that a reduction in available activity-dependent BDNF due to the val66met polymorphism had the exact same effect of promoting use of hippocampus-independent response strategies at the expense of hippocampus-dependent spatial strategies in our experiments. Future studies should investigate potential differences in the effect of stress on spatial navigation behaviour in the two different BDNF val66met genotype groups.

Epigenetic studies have shown that decreases in BDNF expression following stress might be a result of altered methylation of BDNF promoter regions . These changes would alter gene expression by impairing the efficacy of the promoter region of the DNA through chromatin remodeling, thereby turning down expression of the BDNF gene itself. This has been proposed as a potential model for depression, in which stress leads directly to epigenetic changes that effectively silence a given promoter region. Martinowich and colleagues (2003) demonstrated

that methylation at several different promoter sites all significantly decreased BDNF promoter activity, a fact which suggests that methylation at these sites occurring as a result of environmental stressors could be a viable mechanism by which activity-dependent transcription of BDNF is suppressed. This decrease in hippocampal BDNF expression could have long-term consequences in cases of chronic stress, with BDNF expression epigenetically inhibited over time to the extent that behavioural changes favouring the use of brain areas other than the hippocampus develop. Decreased use of hippocampal strategies in favour of non-hippocampal response strategies in individuals who undergo chronic stress would seem to support this model. The effect of decreasing the extent of BDNF transcription through Martinowich's proposed stress-methylation method would have functional consequences similar to the val66met polymorphism which also effectively decreases the amount of mature BDNF protein secreted by hippocampal neurons.

It has been proposed that stress-induced decreases in BDNF expression contribute to the hippocampal damage which has been shown to occur in cases of chronic stress and which has been linked to a number of psychiatric disorders such as depression .

5.4.2 BDNF and Depression

Depression and mood disorders form a heterogeneous collection of syndromes in which genetics and environmental effects are both thought to play an important role. Estimates for the genetic contribution to risk for developing major depression have been as high as 40 to 50% but so far, the search for the specific genes which confer this risk has been largely unsuccessful. Depression is a complex

phenomenon which most likely involves the pathological expression and interaction of many different genes. As a result, the individual contribution of any single gene is probably relatively small and difficult to detect experimentally . The picture is complicated further by the fact that genetic factors alone are probably not sufficient to confer vulnerability to depression. Non-genetic factors such as stress are also generally necessary in order for depression to develop. One common theory, the so-called “Two-hit hypothesis” stipulates that in most cases depression is caused by an interaction between a genetic predisposition and environmental factors .

BDNF may represent one genetic contributor to the complex etiology of the depressive disorders. Previous studies have shown that patients with major depression who are not taking drugs for their illness display a significantly decreased serum BDNF concentration compared to healthy control subjects . Decreased BDNF levels have been shown to be more severe in subjects with greater degrees of depression severity . Even in a sample of non-clinically depressive healthy subjects, those who displayed some depressive personality traits were found to have a significantly lower serum BDNF concentration than healthy subjects without any depressive personality traits . Taken together, these findings suggest that a lower than normal ability to produce BDNF may confer an increased risk of developing depressive disorders . It is reasonable to conclude that individuals with the met form of the BDNF val66met polymorphism may be at higher risk for developing depression as a result of their decreased BDNF secretion.

It has been suggested that high circulating levels of glucocorticoids lead to an impairment of neurotrophic support, contributing to hippocampal pathology during the development of depression . This argument is particularly convincing in

light of studies showing that both acute and chronic stress decrease expression of BDNF in the dentate gyrus and pyramidal cell layer of the hippocampus in rodents, at least partly through a stress-induced glucocorticoid mechanism . It has also been shown that chronic but not acute administration of almost all classes of anti-depressant drugs, including selective serotonin (SSRI) and norepinephrine (NERI) reuptake inhibitors increase BDNF levels and are capable of preventing stress-induced BDNF decreases .

Though the mechanisms through which anti-depressant medications work in the brain remain unclear, recent work suggests that BDNF may be prominently involved. It has been proposed that SSRIs and NERIs do not work only by increasing the amount of neurotransmitter available in the synaptic cleft as previously thought. Rather, these treatments for depression could work by increasing endogenous brain levels of neurotrophins such as BDNF or NT-3, which could in turn promote monoamine-containing neuron growth and function .

Excessive glucocorticoids are thought to interfere with normal BDNF transcriptional mechanisms by interfering with cAMP response element binding protein (CREB), leading to decreased dendritic arborization . It has been suggested that anti-depressants work by increasing dendritic arborizations and BDNF expression through direct activation of CREB, reversing and preventing the actions of stress-induced glucorticoids on the hippocampus . This raises the possibility that anti-depressant induced upregulation of BDNF could both repair some of the stress-induced damage suffered by hippocampal neurons and protect other vulnerable neurons from further damage . This theory of action seems to be in line with rodent experiments. Administration of SSRIs and NERIs as well as electroconvulsive

therapy, all traditional forms of treatment for depression, have been shown to elevate BDNF mRNA levels in the rat hippocampus. Additionally, direct administration of BDNF in the dentate gyrus or hippocampus area CA3 have led to anti-depressant-like effects in both the forced swim and learned helplessness tasks, two classic animal models of depression .

One further piece of evidence for a link between BDNF and depression lies in the fact that anti-depressant treatments exert their mood-elevating effects only after prolonged administration, on the order of several weeks to months. This is far too long a time scale for clinical drug action to be the result of a simple enhancement of serotonergic or noradrenergic neurotransmission. The delay seen in anti-depressant response could very well be due to the time required for BDNF levels to rise and gradually exert their neurotrophic effects . A genetic mechanism, in which drug action works through CREB to increase transcription of BDNF, ultimately promoting hippocampal function and repairing hippocampal damage caused by stress-induced glucocortoids, seems to be much more in sync with the time scale required for SSRI and NERI efficacy . It has also been suggested that BDNF plays a neuroprotective role through activation of the MAPK/ERK cascade through Trk-B receptors leading to transcriptional regulation of CREB and subsequent synthesis of Bcl-2, an anti-apoptotic factor which could protect against the apoptosis cascade induced by excess cortisol in the hippocampus . This could provide an additional mechanism by which SSRIs and NERIs promote neuronal survival through BDNF in depressed patients who have been previously shown to be at greater risk of developing hippocampal atrophy .

Numerous studies have now linked depression and its pharmacological treatment to BDNF. The fact that SSRIs and NERIs may have been working through this previously uninvestigated mechanism for decades is promising in that it shows the potential for future drug development which even more selectively targets the stimulation of neurotrophin production. This could very well be the type of research and investigation that will lead to the development of the next generation of depression-treating pharmaceuticals.

The val66met polymorphism may provide an additional level of precision to future studies regarding the etiology and treatment of major depression. The met allele has been associated with decreases in hippocampal volume both in depressed patients and in controls , a finding which is consistent with previous studies demonstrating volume reductions in the hippocampus in depressed patients . This suggests that met individuals may be at increased risk of developing major depression compared to those who are homozygous for the val allele, a relationship which has been demonstrated in a genetic association study . An understanding of the mechanisms behind BDNF regulation, particularly as it varies in the normal population, will only help us to understand the pathological implications of reduced BDNF secretion and allow us to target pharmacological therapies accordingly.

5.5 Other Candidate Genes Involved in Multiple Memory Systems

The BDNF val66met polymorphism is the best candidate gene so far identified to play a role in hippocampus-dependent processes such as episodic and spatial memory. Its high levels of expression in the hippocampus and the demonstrated role for BDNF in hippocampus-related memory processes in rodents

made this gene an attractive candidate for study in humans. The presence of a specific polymorphism which affects the amount of BDNF protein secreted in humans allowed for a convenient method of studying this gene and its importance in hippocampus-dependent behaviour . This is not to say that the BDNF val66met polymorphism is the only genetic variant which might have an effect on navigation behaviour.

Though the val66met polymorphism is the only commonly occurring source of BDNF expression variation that has been well characterized in humans so far, there is early evidence to suggest that there may be other mechanisms by which the BDNF gene is differentially expressed. One study identified a functional polymorphism 281 base pairs upstream from the putative transcription-initiation site of exon 1 of the BDNF gene . It has been suggested that one rare variant of this polymorphism, the BDNF -281A variant, may have protective effects against anxiety and psychiatric illnesses, exerting an independent but opposite effect on pathological risk to that of the val66met polymorphism's met variant. No studies have been done so far to explore the behavioural effects of this promoter polymorphism. Due to its relative scarcity (allele frequency of 0.03 in Caucasian populations and virtually absent in other populations) , this polymorphism is unlikely to explain much of the variation in the spatial navigation phenotype under study in this thesis. Future val66met studies should at least control for the presence of this additional genotype variation however, as it might help explain some of the differences seen in the literature between val66met's effects on psychiatric populations and healthy controls .

Genetic studies have demonstrated a link between the apolipoprotein E4 (Apo-E4) allele and the most common form of Alzheimer's disease , which is linked to significant atrophy of the hippocampus . This has led researchers to investigate a potential link between apolipoproteinE genotype and performance on hippocampus-dependent tasks. Studies in mice have demonstrated that animals expressing the Apo-E4 isoform have diminished long-term potentiation and performance on spatial memory tasks compared to those expressing the Apo-E3 isoform . Human studies in elderly non-Alzheimer's subjects have demonstrated that Apo-E4 carriers have decreased hippocampal activation compared to non Apo-E4 carriers during a task which required processing and classification of novel versus familiar items . Differences in performance on object location and virtual navigation tasks have also been demonstrated in a healthy elderly population, with Apo-E4 carriers performing significantly worse than non Apo-E4 carriers on both of these tasks . This study demonstrates a clear effect of apolipoproteinE genotype on the hippocampal memory system necessary for the use of spatial strategies in elderly. To this point, no studies have been undertaken to investigate an effect on spontaneous strategy selection when two alternative strategies are possible, as in our study. A study is currently underway in the Bohbot laboratory which seeks to better understand the relationship between apolipoproteinE genotype and the interaction between multiple memory systems in a young population.

The gene for the 5-HT2A serotonin receptor (HTR2A) has also previously been implicated in variation in hippocampus-dependent behaviours such as verbal episodic memory. One study investigated the effects of this gene's H452Y polymorphism on word-list learning and Rey-15-figures test performance and found

that 5HT2A genotype exerted a significant effect on delayed free recall after five minutes and again after 24 hours . The effect of this genotype on human memory behaviour was somewhat surprising. Although the serotonin system has been implicated in learning and memory in simple organisms such as aplysia , serotonin and its receptors had not been previously linked to human memory processes. This has led some to suggest that the effect of this 5HT2A polymorphism on episodic memory may in fact be mediated by a signaling partner of serotonin within the hippocampus, such as BDNF . Though it might be too early to conclude that the serotonin system plays a role in hippocampus-dependent behaviours such as spatial memory, future genetic studies should consider characterizing the relationship between this and other serotonin-related polymorphisms with hippocampus-related processes and the interactions between multiple memory systems.

The findings of the present study demonstrate that the BDNF val66met polymorphism plays a role in hippocampus-dependent processes such as spatial memory and that the presence of the met version on the hippocampus pushes the balance between multiple memory systems in the direction of hippocampus-independent response strategies. While BDNF is not the only gene important for hippocampal function, it is probably the best characterized. Future studies should examine the role that other genes play in the hippocampus- and caudate nucleus-based memory systems. This will help us better understand the full role that genetics might play in memory systems interaction.

5.6 Experience vs. Genetic Contributions to Multiple Memory Systems

Genetic predisposition−even when the combined contributions of multiple genes and polymorphisms are considered−does not sufficiently explain the variation in strategy selection between multiple memory systems. Cognitive behaviour is far too complex and varied in the human population to be determined by genes alone. The role that environment and experience play in human behaviour and the balance between memory systems is also significant. There is a growing body of evidence which suggests that not only do one's experiences directly influence behavioural tendencies, but that experience and willful training can have a measurable effect on brain morphology.

In a study of London taxi drivers, Maguire et al. (2000) found a correlation between the number of years spent driving a taxi−an activity which the authors contend relies on hippocampus-dependent spatial memory-−and grey matter volume in the hippocampus. This was a striking finding which suggests that training and use of a brain system can lead directly to neuroplasticity in that structure.

Rodent studies have demonstrated that training on either a spatial- or a response-dependent task can lead to very obvious morphological changes in a relatively short period of time . Mice who were trained on a spatial version of the Morris water maze for only five days showed an 11% increase in hippocampal cortical thickness, whereas mice who were trained on a response version of the same task had no change in hippocampal volume but did show a measurable increase in the volume of their striatum. Studies are ongoing which seek to replicate this training effect in humans. Preliminary work in the Bohbot laboratory suggests

that experience-mediated training could potentially lead to measurable effects on spatial memory and to potentially even lead to human brain morphology (Andersen, personal communication).

This work suggests that while genetics may play a role in predisposing individuals towards the spontaneous use of one strategy over another, it is the reinforcing effect of continually activating certain memory systems which leads to differences in function and morphology between individuals over time. The fact that the hippocampus can be trained, leading to improvements in function and even structure, is encouraging news in light of evidence linking hippocampal atrophy to psychiatric and neurological brain pathology . An understanding of genetic and environmental risk factors coupled with targeted cognitive training may eventually provide us with the tools required to selectively overcome genetic predispositions to certain types of behaviours and illnesses.

6. Conclusion:

The hippocampus-dependent spatial and caudate nucleus-dependent response memory systems are important for the acquisition of different types of information. On tasks of spatial navigation, these memory systems each allow for the use of distinct learning strategies. When given the choice, individuals will spontaneously use one or the other of these two strategies. To this point, no viable mechanism has been proposed to explain differences in spontaneous strategy selection at the individual level.

The present study demonstrates that the BDNF val66met polymorphism plays a role in spontaneous strategy selection through its detrimental effects on

hippocampal function, leading to increased use of hippocampus-independent response strategies in individuals with one or more met alleles. These strategy differences translated into differences in brain activation between Val and Met groups, with val individuals preferentially activating their hippocampus while met carriers exhibited enhanced caudate nucleus activation with increased use of response strategies over time. Though our data did not confirm a significant effect of val66met genotype on grey matter density in the hippocampus and caudate nucleus, there is sufficient evidence in the literature to conclude that the presence of the met allele leads to morphological changes in the hippocampus. While genetic factors alone do not explain the differences in behaviour between spatial and response learners, we conclude that the BDNF val66met polymorphism may be one of several factors important to determining spontaneous navigation strategy selection at the individual level.

7. References

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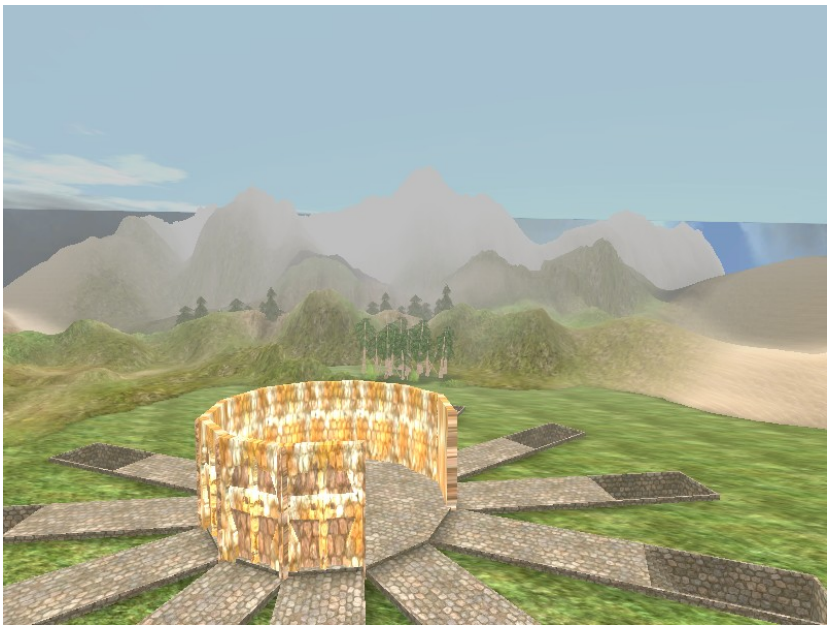
Figure 1: Visual Depiction of the 4 on 8 Virtual Maze Task Environment

Figure 2: Visual Depiction of the Concurrent Spatial Discrimination Task Environment

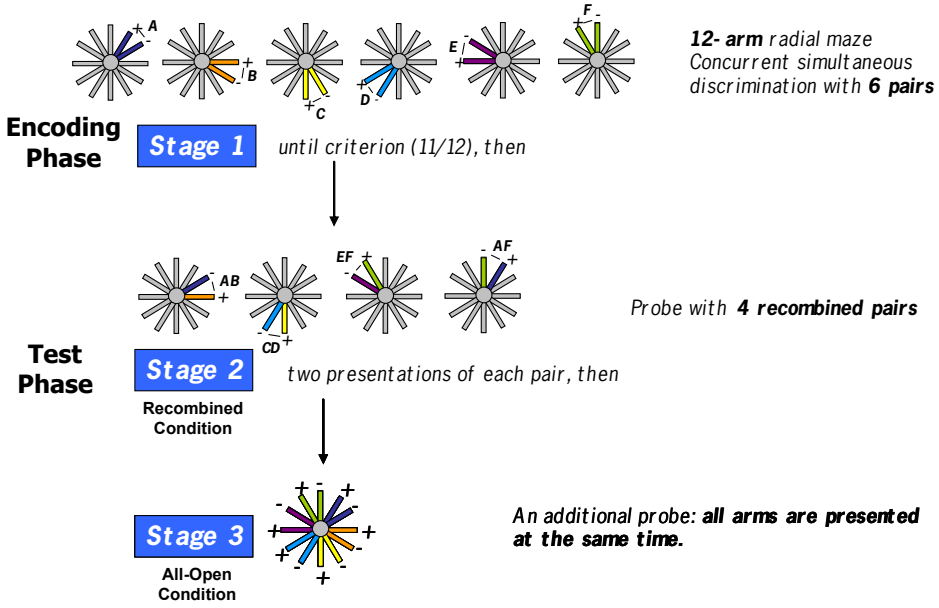
A)

B)



A) Subject's view of the environment showing one of six pairs of pathways.
B) Overhead view of the environment showing enriched distal landscape.

Figure 3: Schematic Representation of the Concurrent Spatial Discrimination Task



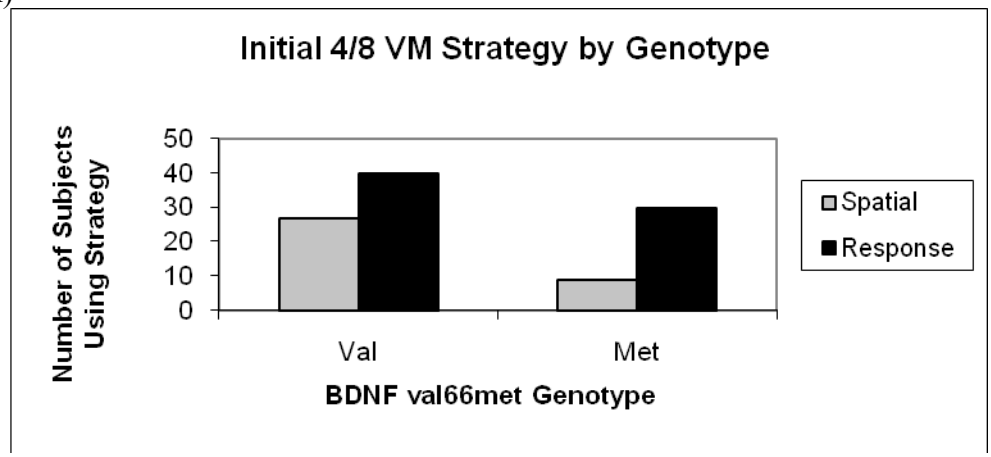
Encoding Phase: Six pairs of pathways are presented individually and repeatedly in a pseudo-random order until subject learns to a criterion of 11/12 correct choices.

Test Phase Recombined Condition: Reward contingency remains the same but pairs are recombined into new combinations. Subjects complete two trials of four recombined pairs each.

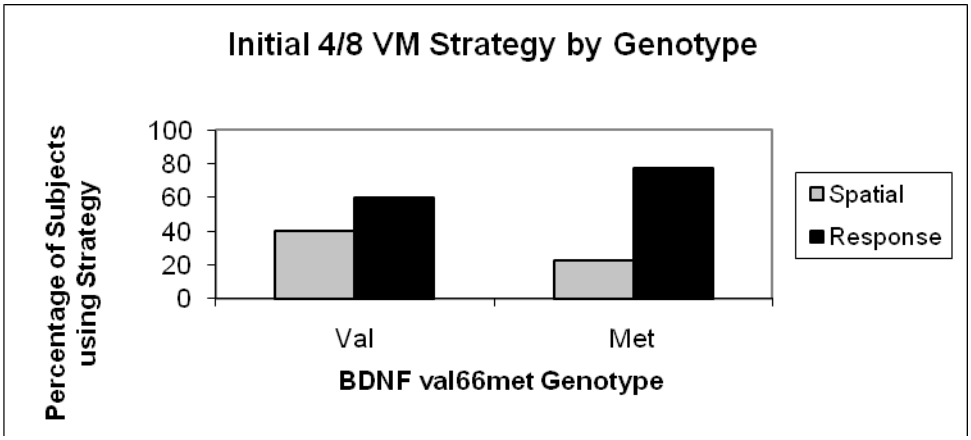
Test Phase All-Open Condition: Pathways are no longer divided into pairs and subject has access to entire visual environment. Subject is told to collect the objects from the six rewarded pathways while avoiding empty pathways.

Figure 4: Initial 4/8 Virtual Maze Strategy vs. Genotype Group

(A)



(B)

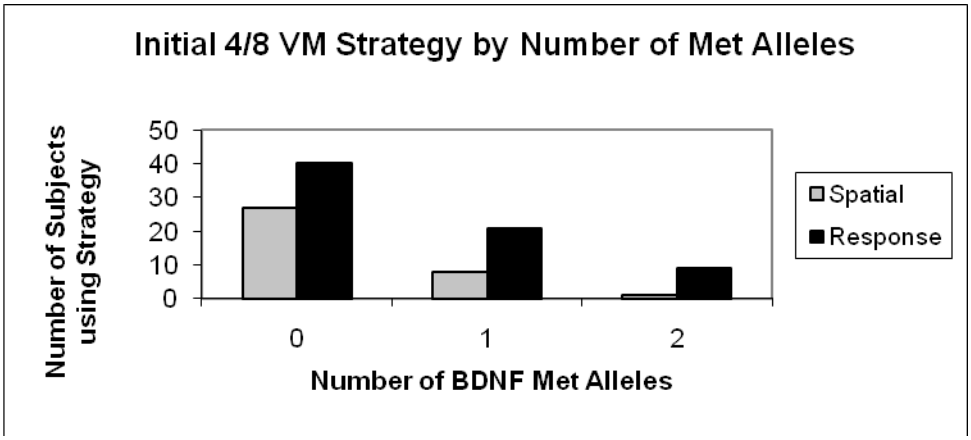


Comparison of initial strategy use on the 4/8 Virtual Maze task between homozygous val and met carrier groups. Division is shown by (A) number of subjects within given genotype group and (B) percentage of subjects within given genotype group. Error bars represent standard error.

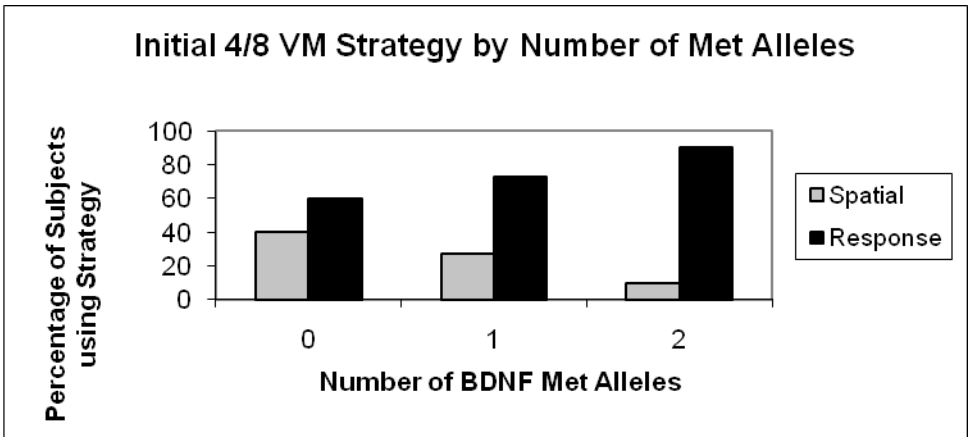
Non-significant trend observed between BDNF val66met genotype group and initial 4/8 virtual maze task strategy selection ($X_1^2 = 3.26$ $p = 0.054$) with met carriers having a greater tendency than homozygous val subjects to select a non-hippocampal response strategy.

Figure 5: Initial 4/8 Virtual Maze Strategy vs. Number of Met Alleles

(A)



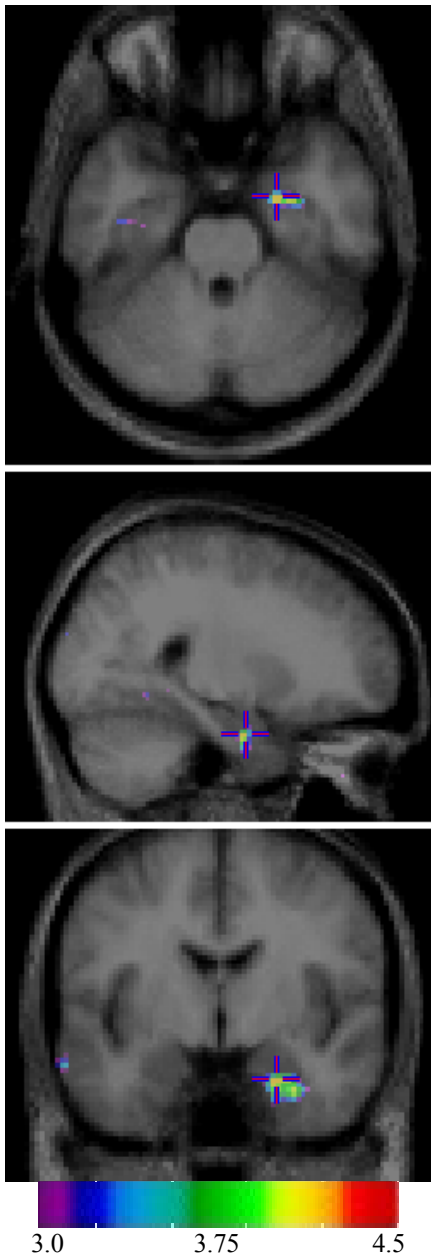
(B)



Comparison of initial strategy use on the 4/8 Virtual Maze Task grouped by number of met alleles. (0=val/val, 1=val/met, 2=met/met). Division is shown by (A) number of subjects within given genotype group and (B) percentage of subjects within given genotype group. Error bars represent standard error.

Significant difference observed between number of val66met met alleles and initial 4/8 virtual maze task strategy selection (linear-by-linear association $X_1^2 = 4.203$ $p = 0.04$) with the proportion of subjects selecting a non-hippocampal response strategy increasing with the number of BDNF val66met met alleles.

Figure 6: fMRI activity during early encoding phase (experimental trial 1)



Statistical parametric maps showing engagement of the hippocampus in the Val group during early learning (experimental trial 1). The t-statistic maps are superimposed onto the anatomical average of all participants and displayed in the axial, sagittal and coronal planes. Cross-hairs are centered on the voxel with the highest BOLD response activity in the hippocampus for the Val group (x=23, y=-6, z=-27; t=4.05). No other region of the brain crossed the threshold for significance corrected for multiple comparisons. The colour bars illustrate the range of t statistical values shown.

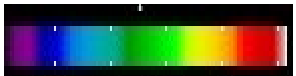
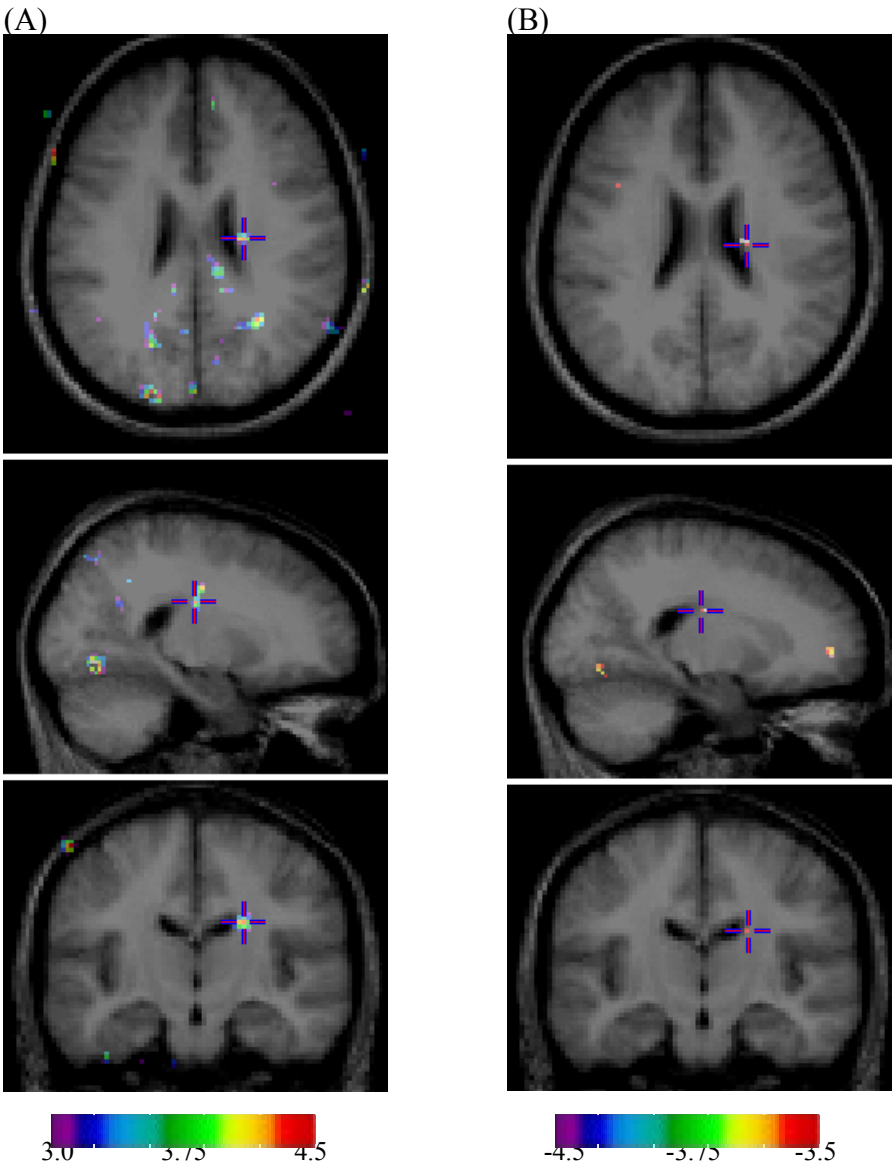
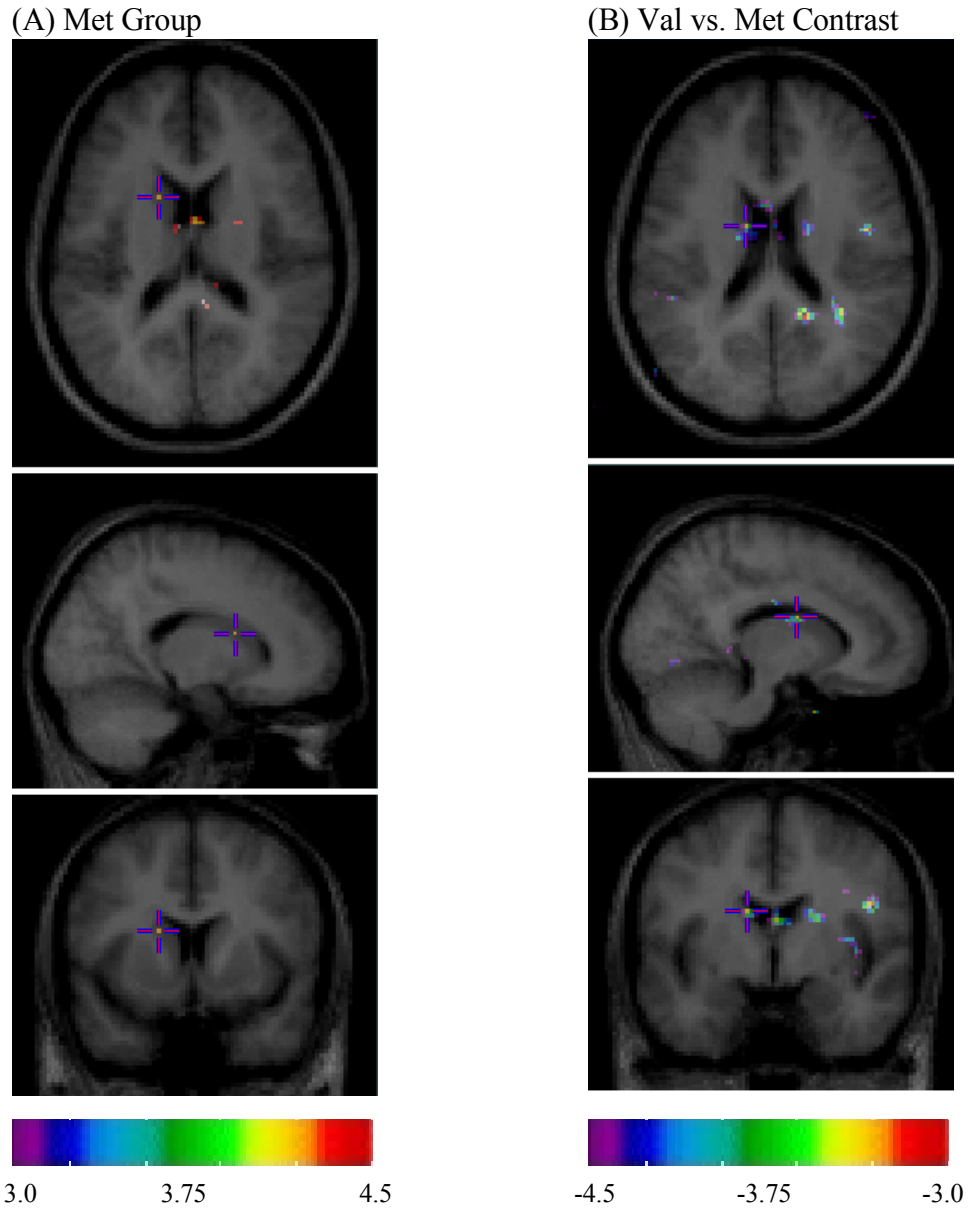


Figure 7: fMRI activity during late encoding phase (last 2 experimental trials of encoding phase)



Statistical parametric maps showing (A) engagement of the caudate nucleus in the Met group and (B) increased engagement of the caudate nucleus in the Met group compared to the Val group during late learning (last two experimental trials before recombined condition). The t-statistic maps are superimposed onto the anatomical average of all participants and displayed in the axial, sagittal and coronal planes. Cross-hairs are centered on the voxel with the highest BOLD response activity in the caudate nucleus for the Met group (x=22, y=-18, z=26; t=4.23). In the Val vs. Met contrast (B), the cross-hairs are centered on the voxel with the greatest degree of negative activation in the caudate nucleus (x=20, y=-19, z=24; t=-3.14). No other region of the brain crossed the threshold for significance corrected for multiple comparisons. The colour bars illustrate the range of t statistical values shown.

Figure 8: fMRI activity during test phase "All-Open" condition



Statistical parametric maps showing (A) engagement of the caudate nucleus in the Met group and (B) increased engagement of the caudate nucleus in the Met group compared to the Val group during during the All-Open test phase condition.. The t-statistic maps are superimposed onto the anatomical average of all participants and displayed in the axial, sagittal and coronal planes. Cross-hairs are centered on the voxel with the highest BOLD response activity in the caudate nucleus for the Met group (x=-12, y=-4, z=20; t=4.09). In the Val vs. Met contrast, the cross-hairs are centered on the voxel with the greatest degree of negative activation in the caudate nucleus (x=-6, y=8, z=16; t=-3.30). No other region of the brain crossed the threshold for significance corrected for multiple comparisons. The Val group had no activations which crossed the threshold for significance in either of our regions of interest. The colour bars illustrate the range of t statistical values shown.

Table 1: BDNF Genotype Frequencies

	Val/Val Genotype Frequency (n)	Val/Met Genotype Frequency (n)	Met/Met Genotype Frequency (n)	X^2
Behavioural Sample	67	29	10	5.62
fMRI Sample	16	5	0	0.38
VBM Sample	25	11	1	0.03

Genotype frequencies are in Hardy –Weinberg equilibrium for the fMRI and VBM samples of subjects ($p>0.05$) but not the behavioural sample ($p<0.05$).

Table 2: Effect of BDNF val66met Genotype on Task Performance (ANOVA)

Neurocognitive Measure	Initial Strategy F_1 Value	Effect of Initial 4/8VM Strategy Significance Level (p=)	Genotype (Val or Met) F_1 Value	Effect of Genotype (Val or Met) Significance Level (p=)	Genotype (number of met alleles) F_2 Value	Effect of Genotype (number of met alleles) Significance Level (p=)
4/8VM Total Latency	12.987	0.000*	3.088	0.082	2.277	0.108
4/8VM Total Errors (Working Memory Correct)	1.429	0.235	2.211	0.140	1.458	0.238
4/8VM Total Errors (Working Memory Incorrect)	1.934	0.167	0.601	0.440	0.355	0.702
4/8VM Total Errors (Reference Memory)	2.116	0.149	1.714	0.193	1.739	0.181
RAVLT Total Recall Score	1.287	0.259	0.001	0.972	0.798	0.453
RAVLT Recognition Score	0.001	0.974	0.202	0.654	0.276	0.760
RAVLT Recall after Interference	2.739	0.101	0.232	0.631	0.143	0.867
RAVLT Recall after 20 minute Delay	0.473	0.493	0.425	0.516	0.846	0.432
Rey-Osterrieth Complex Figure Copy Score	0.035	0.852	3.625	0.067	1.754	0.192
Rey-Osterrieth Complex Figure Recall Score	0.207	0.653	0.693	0.412	0.887	0.423
Shipley Institute of Living Vocabulary Score	1.481	0.233	0.014	0.905	0.011	0.989
Shipley Institute of Living Estimated WAIS-R IQ Score	1.313	0.261	0.328	0.572	0.188	0.830

Table 2 Continued

"4/8VM" = 4 on 8 Virtual Maze Task
"RAVLT" = Rey Auditory Verbal Learning Task
"WAIS-R IQ" = Wechsler Adult Intelligence Scale-Revised Intelligence Quotient

Subjects did not differ in performance across genotype groups (Val vs. Met) or number of met alleles (0,1 or 2) for any measure of behavioral performance.

Subjects did not differ in performance across strategy groups (spatial vs. response) for any measure of behavioral performance except total time taken to complete the 4/8VM task, with the spatial group requiring significantly more time on average to complete the task (F_1=12.987; $p<0.001$).

Table 3: Effect of val66met polymorphism group on performance of the fMRI Concurrent Spatial Discrimination Task (CSDT)

	Genotype Group	Mean (SEM)	Effect of Genotype Group (Val vs. Met) (t_{20}=)	Effect of Genotype Group (Val vs. Met) Significance Level (p=)
CSDT Number of Trials to Criteria	Val	6.813(0.627)	1.371	0.186
	Met	5.000 (1.265)		
CSDT Percentage Correct on Recombined Pairs Trial 1	Val	63.00 (6.538)	1.738	0.098
	Met	36.60 (17.946)		
CSDT Percentage Correct on Recombined Pairs Trials 1 and 2	Val	63.00 (6.637)	0.950	0.354
	Met	48.25 (18.546)		
CSDT Number of Errors Made on All-Open Condition	Val	2.40 (0.696)	0.627	0.539
	Met	1.50 (0.866)		

"CSDT" = Concurrent Spatial Discrimination fMRI Task

No significant performance differences were observed between val66met genotype groups on the fMRI task as measured by number of trials needed to reach criteria on the encoding phase, percentage of correct pathway choices when pairs were recombined during the first part of the test phase, and number of errors made during the All-Open condition.