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Relationship between BDNF genotype, hippocampus volume, and anxiety

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Senior Honors Thesis

April 4, 2013

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Abstract

Anxiety is one of the most prevalent mood disturbances in the United States. The underlying genetic and neural bases of anxiety are complex, but previous research suggests that anxiety may be associated with alterations in brain structures, such as the hippocampus, and with genetic variations within genes like that of the Brain Derived Neurotrophic Factor (BDNF). To try to better understand these factors, we examined hippocampal volume, BDNF genotype, and self-report measures of anxiety together in a single study. We hypothesized that individuals with the Met allele of the BDNF gene would have reduced hippocampal volumes and elevated anxiety scores relative to individuals with the Val/Val genotype. This hypothesis was not well supported by the data. Implications for understanding the genetic and neural bases of anxiety are discussed.

Introduction

Anxiety disorders are among the most common mood disorders in the United States, affecting approximately 18% of adults aged 18 and older annually, yet the origins of anxiety are still largely a mystery (National Institute of Mental Health, 2002). The Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) recognizes over ten different types of clinically relevant anxiety disorders, the most common of which include generalized anxiety disorder (GAD), simple phobia disorder, obsessive-compulsive disorder (OCD), panic disorder, and post-traumatic stress disorder (APA, 2000). These conditions can differ extensively in their presentation of symptoms, but at the most general level, anxiety is characterized by marked uncertainty, fear and uneasiness, as well as uncontrollable repetitive thoughts that activate feelings of panic and inhibit the ability to stay calm. When left untreated, anxiety disorders can often develop into more persistent conditions with poorer treatment outcomes, or may become comorbid with other mood disorders including major depressive disorder (National Institute of Mental Health, 2002). Additionally, many of the symptoms of anxiety can be experienced in a more mild range within otherwise psychologically healthy individuals, which is classified as subclinical anxiety, but nevertheless can have substantial effects on cognition, sleep and mood (Ng, Chan, & Schlaghecken, 2012; Spira et al., 2008).

Although there is still considerable debate as to the specific mechanisms underlying anxiety disorders, a genetic link has been identified that may predispose individuals to higher rates of anxiety. Specifically, many studies have looked at the brain-derived neurotrophic factor protein, which is more commonly referred to as BDNF, and is coded for by the BDNF gene. Within the brain, BDNF is a prominent neurotrophin-

signaling molecule that is vital to the differentiation, growth and survival of neurons. It is found throughout the cortex but is abundant in areas of the limbic system such as the hippocampus and amygdala, which are both involved in executive functions, including memory formation and emotion control (Huang & Reichardt, 2001). BDNF displays a single nucleotide polymorphism at codon 66 that results in a Valine to Methionine substitution known as the Val66Met polymorphism (Appendix A).

The Val66Met polymorphism has been shown to influence several attributes in humans including hippocampal volume, memory performance and anxiety-related traits (Hajek, Kopecek, & Höschl, 2012; Jiang et al., 2005; Szeszko et al., 2005). Pertinent to this investigation, individuals with the Met allele of the BDNF gene show a reduced volume of the hippocampal formation compared to Val/Val individuals (Bueller et al., 2006; Hajek et al., 2012; Montag, Weber, Fliessbach, Elger, & Reuter, 2009; Pezawas et al., 2004; Szeszko et al., 2005; Yang et al., 2012). Additionally, the Val66Met polymorphism may be a risk allele for the development of anxiety as some argue that the Met allele is implicated in increased anxiety-related traits (Chen et al., 2006; Hashimoto, 2007; Jiang et al., 2005). However, others have argued the opposite, that the Val allele is more of a risk allele for increased anxiety (Lang et al., 2005)

The volume of the hippocampus is an important neurological element of anxiety to investigate because the hippocampus is a major component of the limbic system, and is most well known for its role in emotion and the formation of memory (Appendix B). It is also more subject to stress-related changes than any other area of the brain (Woon, Sood, & Hedges, 2010). This is due to the fact that the hippocampus has a high level of glucocorticoid receptors to which stress hormones, such as cortisol, bind. With abnormal

levels of stress or longer durations of stress exposure, the excitability of some hippocampal neurons may be decreased, which can lead to increased cell death and eventual atrophy of the brain region itself (Joëls, 2009). For this reason, patients with mood disorders, such as anxiety and depression, tend to have reduced hippocampal grey matter volume (Bremner & Narayan, 2000; Campbell & Marriott, 2004; Sheline, Gado, & Kraemer, 2004).

Some investigations have explored the relationship between hippocampal grey matter volume and anxiety-related traits, with conflicting results. That is, some studies have found a positive correlation between hippocampal volume and anxiety, with higher anxiety being associated with larger hippocampi (Baur, Hänggi, & Jäncke, 2012; Rusch, Abercrombie, Oakes, Schaefer, & Davidson, 2001). Others have found the opposite, with higher anxiety and depression associated with smaller hippocampal structures (Campbell & Marriott, 2004; Kalisch et al., 2006; Kühn, Schubert, & Gallinat, 2011).

The evidence of the distinct associations between genes and anxiety, genes and hippocampal volume, and hippocampal volume and anxiety, naturally leads to the investigation of how all three variables interact together. Previous studies have looked at the relationship between all three variables (Montag et al., 2009), but none have focused solely on healthy participants and their subjective experience of anxiety symptoms as they relate to BDNF genotype and hippocampal volume. Such an investigation is essential to understanding the relationship between the genetic, structural, and behavioral components of sub-clinical levels of anxiety. The purpose of this study is to investigate how a genetic polymorphism within the BDNF gene may lead to differences in hippocampal volume and vulnerability to anxiety.

Therefore, the present study examines the nature of the relationship between three variables: BDNF polymorphism, subjective anxiety scores, and regional hippocampus volumes, to obtain a more comprehensive understanding of how all three variables may interact and influence one another. This study will address three distinct hypotheses: 1. The Met allele will be associated with a reduction in hippocampal volume, 2. Individuals with this reduction in hippocampal volume will show an increase in anxiety symptoms, and 3. Carriers of the Met allele will have higher anxiety.

Methods

Participants

Sixty-two healthy University of Colorado undergraduate students (33 males; 53.2%) from the department of Psychology and Neuroscience participated for course credit or payment. All participants had no history of previous psychiatric diagnoses or medication. Informed consent was acquired according to the guidelines of the Institutional Review Board of the University of Colorado at Boulder. The final sample included two groups, Met+ (N=21 (Met/Val N=20, Met/Met=1); 52% male; Age: M=19.43; SD=1.5) and Val/Val (N=41; 54% male; Age: M=20.29; SD=6.31). The two groups did not differ in age ($p>.5$) or gender ($p>.9$).

Procedure

Participants first came to the Cognitive Development Center to complete a self-report questionnaire measuring symptoms related to anxiety, and to provide a saliva sample used to identify their individual genotype. After this data was collected and

analyzed, subjects were invited to participate in the second phase of the study in which an fMRI brain scan was used to obtain brain-imaging data for VBM analysis of hippocampal volumes.

Genotyping

All subjects provided a 3mL saliva sample, which was analyzed for BDNF alleles at The Children's National Medical Center in Washington D.C. The Met and Val alleles were identified at codon 66 of chromosome 11. Genotype frequencies were in Hardy-Weinberg equilibrium (X^2= .688, df= 2, P>.7).

Self-Report Anxiety Measurement

The State-Trait Anxiety Inventory for Adults (STAI) was used to obtain a measurement of self-reported anxiety (Spielberger, 1970). The inventory consists of two scales with 20 questions each and is intended to measure current (state) anxiety and longer-term (trait) anxiety in adults (Spielberger, 2005). The STAI is used to evaluate feelings such as worry, apprehension, and nervousness. For the "state" score, participants were asked to respond to a particular sentiment in terms of how strongly they feel that emotion in that moment. For example, one statement is "I feel calm" and participants can respond with 1indicating "not at all", 2 being "somewhat", 3 being "moderately so", or 4 being "very much so". For the "trait" score, they are asked to respond to a statement with how often they feel that statement in general. For example, the statement "I feel pleasant" can have a response of 1 indicating "almost never", 2 being "sometimes", 3 being

"often", and 4 being "almost always". Scores range from 20 to 80 on each scale, with a higher score indicating elevated levels of state or trait anxiety.

The raw score on both the state and trait inventories were translated into a percentile score using the guidelines of the STAI manual (Spielberger & Vagg, 1984). Percentile scores indicate the standardized rank of anxiety traits among undergraduate college students as a whole. The percentile scores for both state and trait anxiety were thus the primary source of data used in this study to measure and represent relative anxiety within the undergraduate population. Overall, we were most interested in the trait measure of anxiety as it best represents an individual's stable anxiety level as well as differences in response intensity to psychological stress among people. Due to copyright laws, we are unable to provide the STAI in an appendix.

MRI and VBM analysis

Voxel Based Morphometry (VBM) analyses were performed using FSL software and the processing procedure followed that established by Ashburner et al. (2000) and Good et al. (2001). VBM is a technique of neuroimaging in which the volumes of different brain areas are calculated and can then be compared, both within and between groups. This technique relies on structural brain images, which are obtained through 3D-MPRAGE scans conducted within a functional MRI scan. After a brain scan from all participants was collected, the images were normalized, and the grey matter was extracted using the FSL default BET brain extraction process, to strip the skull and remove non-brain tissue from the image using the FAST4 tool. This results in grey matter images that are then aligned to MNI152 standard space using the affine registration tool

FLIRT (FMRIB's Linear Image Registration Tool) and then the nonlinear registration tool using FNIRT (FMRIB's Nonlinear Image Registration Tool). A study-specific template is then made from averaging all of these images and then non-linearly re-registering the original grey matter images using FNIRT. To correct for possible local expansion and contraction, the registered partial volume images were modulated by dividing the Jacobian of the warp field. This results in modulated segmented images that are then smoothed by an isotropic Gaussian kernel with a sigma of 3, resulting in full-width half-maximum (FWHM) of 3x2.3mm=6.9mm. Using permutation-based, non-parametric testing with Monte Carlo simulations, voxelwise thresholding is applied which corrects for multiple comparisons. Finally, clusterwise correction is applied using the built-in cluster-based thresholding technique in FSL's VBM toolbox. (Ashburner & Friston, 2000; Good et al., 2001; Smolker, Depue, Reineberg, & Banich, in progess).

Through this process, every brain is compiled and normalized onto a template, which represents an average of all the volumes of each brain while also getting rid of some of the bigger differences in brain anatomy among people. When the brain images are smoothed, each voxel (or 3-D pixel) represents a volumetric average of itself and all of the voxels that surround it. From these measurements, volume can then be compared across brains at each voxel in order to determine small areas where volumes differ between groups. Total volume measurements can also be obtained, but in identifying an overall measure of an area, smaller more specific differences can often be overlooked. Masks may be drawn to narrow down analysis on one specific area of the brain, in this case the hippocampus. In this study, measures of both total hippocampus volume as well as voxel-by-voxel analyses were used to help identify differences in hippocampus

volume; however, an emphasis was placed on variations in total hippocampus volume to investigate the more broad-based hypotheses as well as gain a more comprehensive generalization of hippocampus differences among people in relation to anxiety traits.

Results

1. BDNF genotype and hippocampus volume

There was no significant relationship between BDNF genotype and total hippocampus volume (t62)=1.470, p=0.147; Figure 1). However, in a voxel-by-voxel measurement, the Val/Val group showed significantly larger hippocampal grey matter volumes than the Met+ group in small regions of both right posterior hippocampus (t(62)=3.824, p=0.013; Figure 2) and left anterior hippocampus (t(62)=3.287, p=0.036; Figure 3).

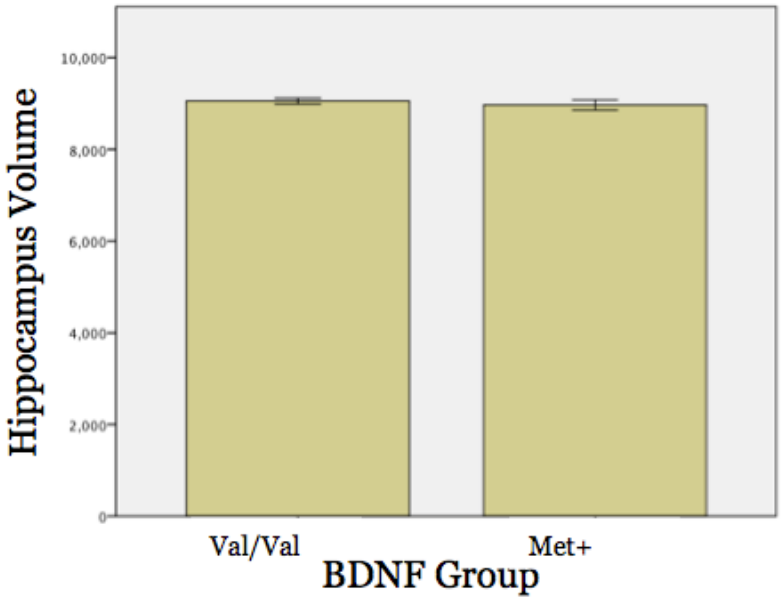

Figure 1. **Effect of BDNF group on total hippocampus volume: Val/Val participants did not differ when compared to Met+ individuals in mean total hippocampal volumes. Error bars represent standard error.**

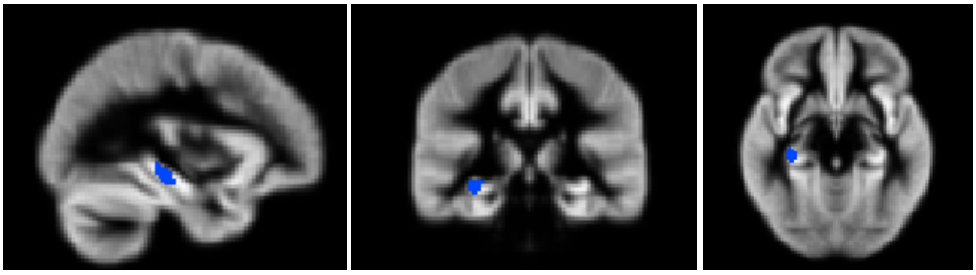

Figure 2. **Voxel–wise analysis of grey matter volume of right posterior hippocampus: The highlighted area represents the region that is significantly larger in Val/Val groups than Met+ groups.**

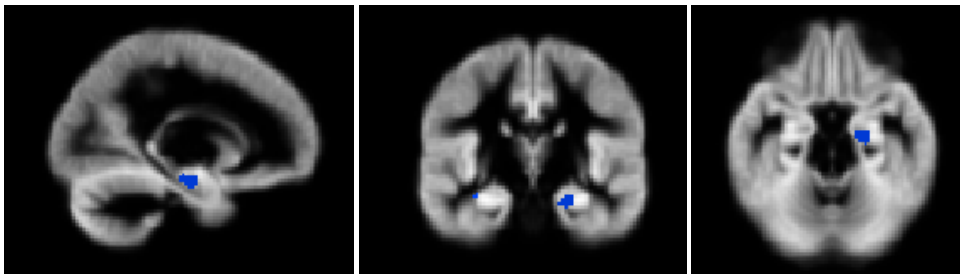

Figure 3. **Voxel–wise analysis of grey matter volume of left anterior hippocampus: The highlighted area represents the region that is significantly larger in Val/Val groups than Met+ groups.**

2. Hippocampus volume and anxiety

No significant correlation was found between total hippocampus volume and trait anxiety ($r(62)=.120$, $p=0.353$; Figure 4) or state anxiety ($r(62)=.104$, $p=.421$). Additionally, there was no significance between the right posterior hippocampal region and trait anxiety ($r(62)=.159$, $p=.216$, Figure 5) or the left anterior region and trait anxiety ($r(62)=.132$, $p=.306$ Figure 6).

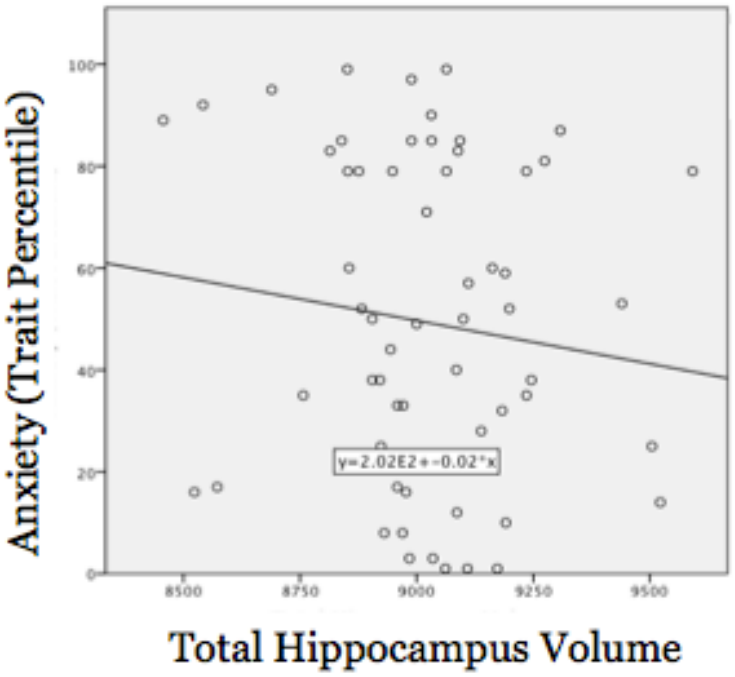

***Figure 4*. Relationship between total hippocampus volume and trait anxiety: Hippocampus volume does not significantly predict anxiety score.**

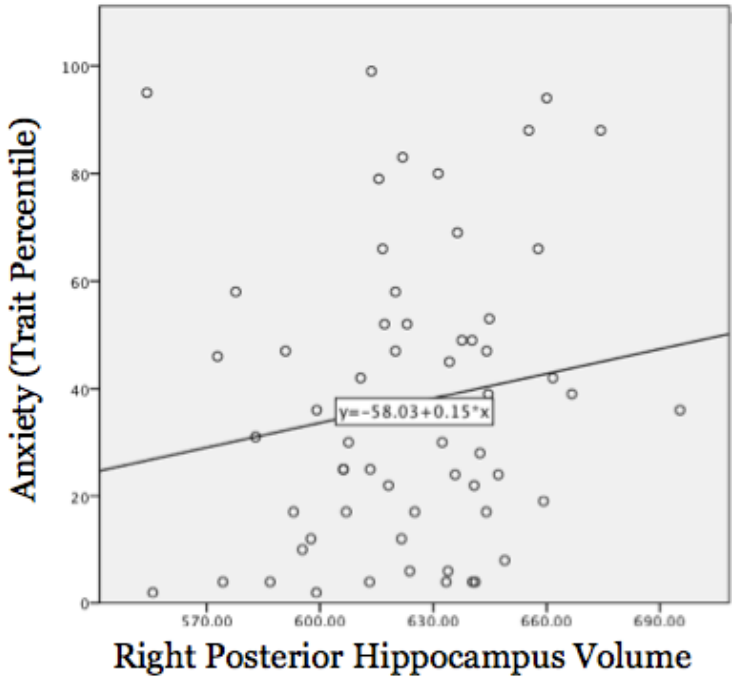

***Figure 5.* Relationship between volume of right posterior hippocampus region and trait anxiety: Volume of right posterior hippocampus volume does not significantly predict anxiety.**

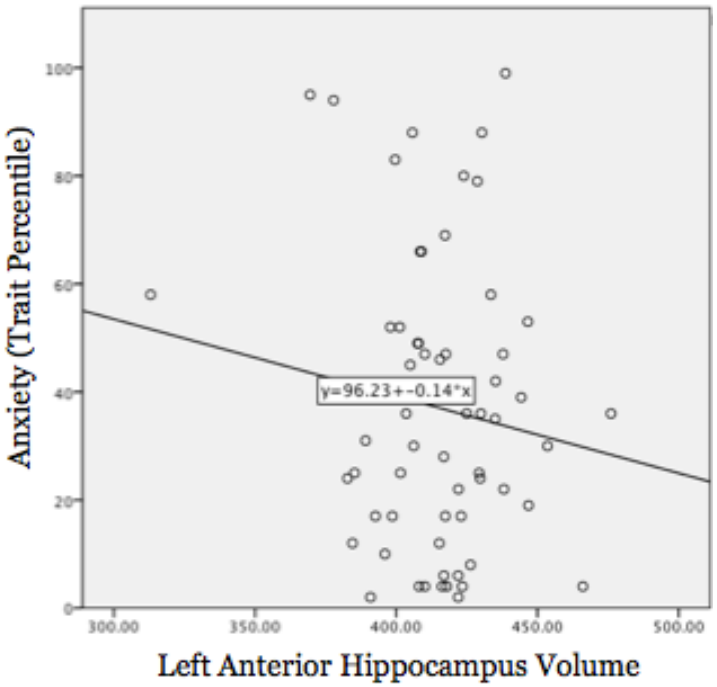

***Figure 6*. Relationship between volume of left anterior hippocampus region and trait anxiety: Volume of left anterior hippocampus region does not significantly predict anxiety.**

3. BDNF genotype and anxiety

No significance was found between BDNF genotype and anxiety during the first phase of the study (Trait: t(62)=-1.353, p=0.181, Figure 7; State: t(62)=-.974, p=.334). However, during the second phase of the study at the time of fMRI scanning, the Met+ group showed lower state anxiety than the Val/Val group (t(52)=2.475, p=0.017; Figure 8).

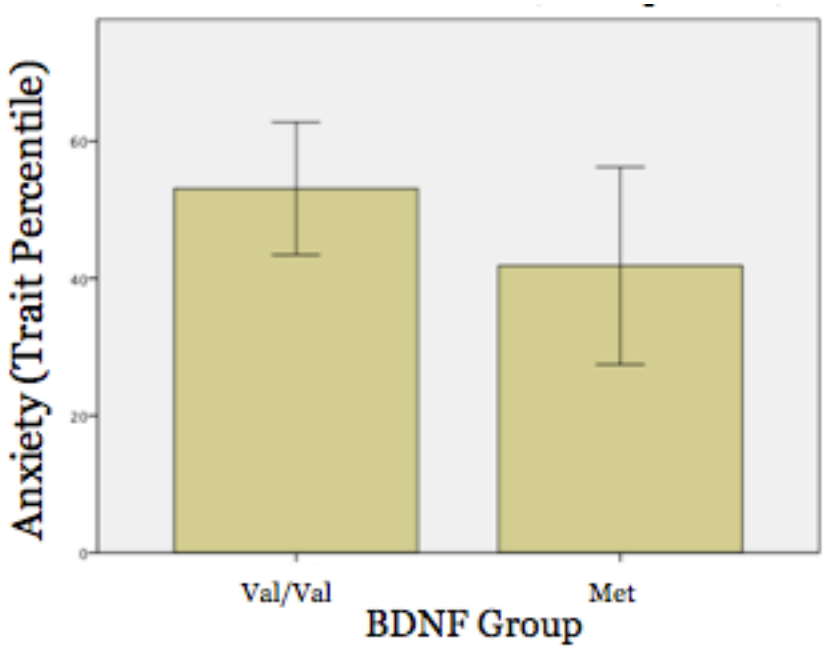

Figure 7. **Effect of BDNF genotype on trait anxiety: Genotype group does not significantly predict anxiety. Error bars represent standard error.**

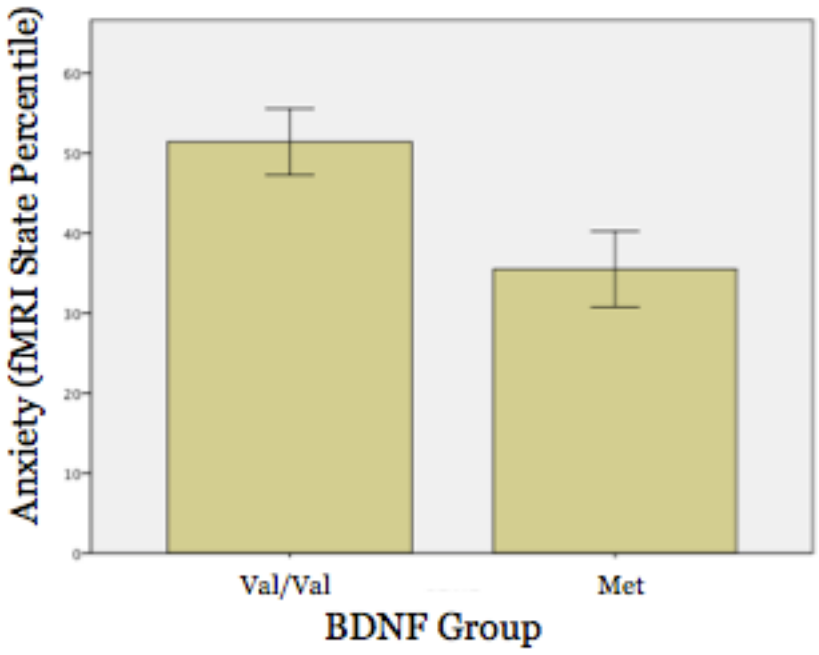

Figure 8. **Effect of BDNF genotype on anxiety: The Met+ group showed significantly lower *state* anxiety than the Val/Val group at the time of fMRI scanning. Error bars represent standard error.**

4. BDNF genotype by total hippocampus volume interaction

Overall, the interaction of BDNF genotype and total hippocampus volume was not significantly related to trait anxiety (r(62)=0.035, p=0.785). Additionally, no significant relationship was found within individual BDNF genotype groups of total hippocampus volume on trait anxiety (Val/Val: r(62)=0.720, p=0.580; Met+: r(62)=0.089, p=.235, Figure 9).

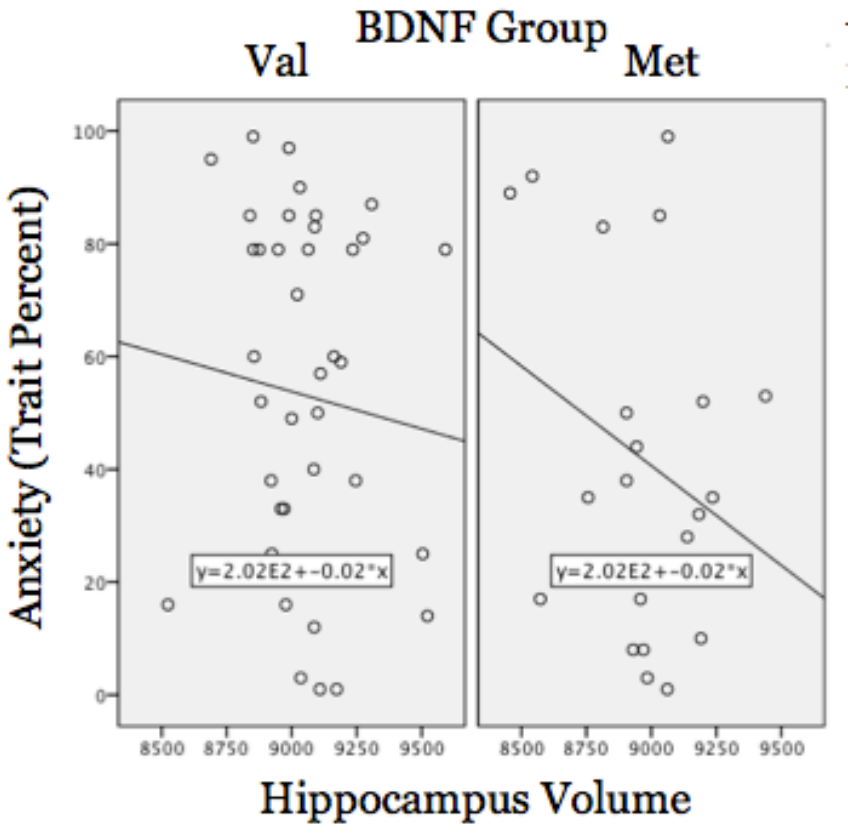

Figure 9. **Effect of Hippocampus Volume X BDNF interaction on trait anxiety: Interaction of hippocampus volume and BDNF genotype does not significantly predict anxiety.**

5. BDNF genotype by total hippocampus volume group interaction

To further explore this interaction, one other approach was taken whereby each participant's total hippocampus volume was categorized into either "high" or "low" volume categories depending on if it was above or below the average volume for all participants. The hippocampus volume and genotype classifications were then combined and the effect of group on anxiety was noted. The interaction was not significant for trait anxiety ($t(62)= -1.498$, $p=0.139$, Figure 10), although the groups show an interesting, opposing pattern depending on genotype. That is, individuals with the Val/Val genotype and a low hippocampus volume classification exhibited the most anxiety whereas the Met+ carriers with a low hippocampus volume classification exhibited the lowest levels of anxiety.

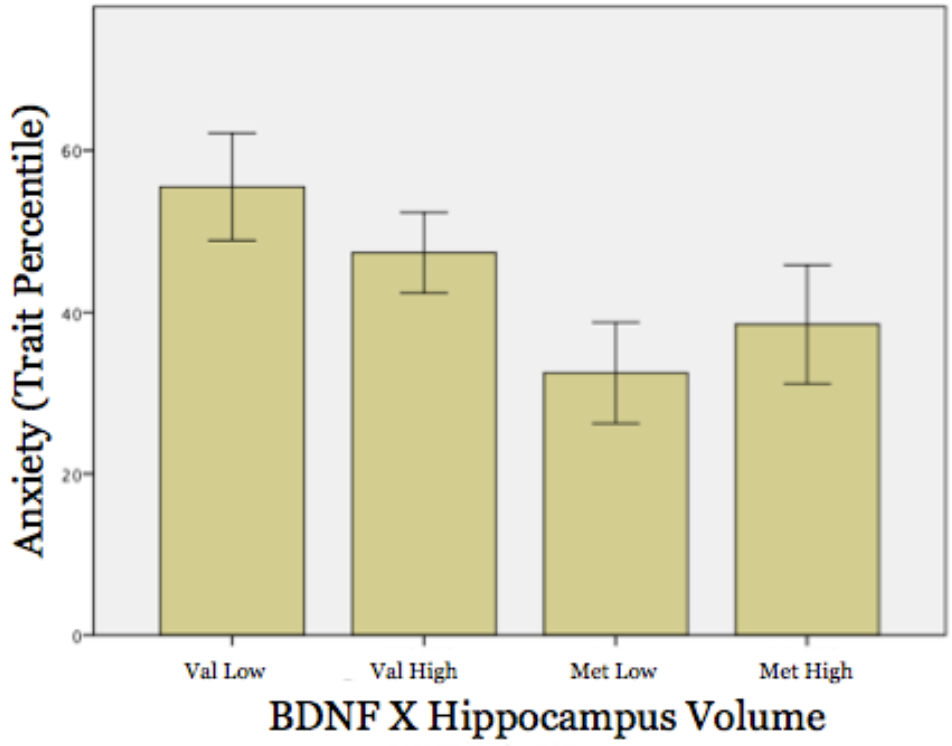

Figure 10. **Effect of BDNF genotype X hippocampus volume group designations: BDNF by hippocampus group interaction does not significantly predict anxiety though relationships are opposing depending on genotype group.**

Discussion

Overall, the hypothesized correlations between BDNF genotype and hippocampal volume, hippocampal volume and anxiety, and BDNF and anxiety were not strongly supported by the current study's data. BDNF genotype is not a significant predictor of total hippocampus volume, however, there are significant voxel-wise differences between the two groups with the Met+ group showing smaller areas of bilateral hippocampi than Val/Val. BDNF genotype is also not a significant predictor of anxiety, but there is a general tendency of the Met+ group showing lower anxiety than the Val/Val group. This difference is only significant in state measures of anxiety at the time of fMRI scanning. Finally, total hippocampus volume is not a significant predictor of anxiety score in either measure of anxiety.

Additionally, the interaction of BDNF genotype and hippocampus volume does not significantly predict anxiety. However, the interaction of BDNF genotype group and hippocampus volume group shows an opposing pattern between genotypes whereby a low hippocampus volume in Met+ individuals may be protective against higher levels of anxiety, while a low hippocampus volume in the Val/Val category may be unfavorable and increase vulnerability to higher levels of anxiety.

Many limitations were present that could have had an effect on significance between variables. For one, the sample size may have been too small to see notable differences between groups, especially in the independent sizes of genotype groups. Regardless of population size, this investigation is still limited in the sense that the difference in BDNF processing between Met and Val alleles may not yet be well understood. That is to say, our interpretation of the dampened BDNF processing of Met+

alleles may not be as unfavorable in terms of hippocampus volume or anxiety as we suspect it is. Perhaps the decrease in activity-dependent BDNF secretion seen in the Met+ group is not an entirely defective molecular process but rather a more fine-tuned one, resulting in a reduced need for neurogenesis, as shown by the trend of the Met+ group showing smaller hippocampus structures.

Similarly, if a more efficient process such as this were actually the case, then it would seem intuitive that the Met+ group displays less anxiety than the Val/Val group as suggested by some investigations (Lang et al., 2005; Sen et al., 2003). One study in particular investigated serum BDNF concentrations in humans and found that in Val/Val participants, BDNF serum concentrations were lower but risk of having an episode of major depression was increased (Lang, Hellweg, Sander, & Gallinat, 2009). Additionally, studies have looked into the phenomenon of the Val allele being preferentially transmitted more so in families with histories of depression or bipolar disorder, thereby possibly increasing the co-occurrence of Val/Val genotypes and difficult mood disorders (Neves-Pereira et al., 2002; Sklar et al., 2002). Both of these ideas give further support to the notion that what we know about the individual Met and Val allele processes may not encapsulate the entirety of the effect they have on anxiety.

Furthermore, there is a possibility that teasing apart clinical and subclinical anxiety more finely would aid in understanding the cellular and molecular differences at work. For instance, some postulate that higher state anxiety is not necessarily an indication of higher situational subclinical anxiety traits, but rather a risk factor for major depressive disorder, being that depressive disorders are typically state dependent (Sklar et al., 2002). In a similar way, others suspect that trait anxiety may not be an accurate

reflection of anxiety-related traits but also depression-related traits since depression and sub-threshold anxiety symptoms are often comorbid (Silverstone & Von Studnitz, 2003). In either case, the takeaway is that subclinical anxiety is difficult to accurately measure on its own without further confounds from clinical symptoms. In addition, the molecular and physiological underpinnings of anxiety may operate differently when symptoms do not meet criteria for clinical diagnosis. For instance, recent evidence suggests that hyperactivation of the hippocampal-amygdala network may be more of an indication of elevated subclinical anxiety symptoms which cannot be deduced by just the size of either area on its own (Blackmon, Barr, & Carlson, 2011; Laeger et al., 2012).

In line with this idea of other brain mechanisms at work in subclinical anxiety traits, is the question of what mechanisms can actually be inferred by the grey matter volume of the area in question, and what the size of the hippocampus actually tell us. The implicit assumption behind many past morphological studies, as well as this one, is that a decrease in size must indicate and decrease in function, however, that may be far from the case. Especially when trying to associate a complex behavior such as anxiety with a brain region that carries out multiple functions, relative brain volume may not have the answers that we expect it to and, even further, any significant correlations must be approached delicately as they may not imply any degree of causation (Healy & Rowe, 2007). In addition, the hippocampus is one of the few structures of the brain that has the ability to vary in size throughout life and, moreover, gradually decreases in size with age (Erickson et al., 2011). This means that, at any point during the lifespan, the grey matter volume of the hippocampus has the potential to be implicated in a number of behavioral relationships, all of which may not be entirely conclusive.

In general, smaller hippocampus size has been linked to elevated stress levels, but as our data demonstrates, this might not always be the case being that the group that had higher levels of anxiety in our sample (Val/Val) also showed larger hippocampal structures. As exemplified by the interaction of BDNF genotype and total hippocampal volume, the Met+ and Val/Val groups appear to work in different ways. Continuation of morphological and genetic studies in association with complex behaviors will be necessary to broaden the current understanding of the insight truly gained from these factors. Not only will it be imperative in understanding the meaning of grey matter volume and molecular genetic processes, but also of maladaptive behavior in order to better treat, control and manage complex behavior traits such as anxiety.

The limitations of the current study could potentially be minimized by continued research in several subject areas. The first would be to understand and maintain a consistent difference in symptomology between subclinical anxiety and clinically significant anxiety disorders such that continued research into the genetic, molecular, cellular, and structural foundations of symptoms will help distinguish clinical treatment plans from everyday management of anxiety characteristics. In this way, more research into the difference in function of BDNF alleles may help to better individualize stress-management strategies. Furthermore, the understanding of variation in hippocampal grey matter volumes could likely help distinguish between differing levels of anxiety both on a large scale as well as within separate BDNF polymorphism groups. Beyond these necessary areas of further exploration, critical information could likely be gained from examining other genes and/or brain areas that may be influential in the augmentation of anxiety features as well as interactions between and within these areas.

By studying subclinical anxiety, we are seeking to understand the underlying genetic mechanisms and what molecular and cellular factors play important roles in contributing to the severity of anxiety symptoms. From this information, treatment methods may be better understood and developed to help correct for anxiety before it reaches a level worthy of clinical diagnosis. For example, with a better understanding of the underlying neuropathology of anxiety and the specific role of BDNF in anxiety, therapies and/or drug treatments could be tailored to individuals depending on BDNF genotype. That is, if the neurotransmitter BDNF is found to be a major cause of anxiety, then it is possible that drug treatments may be helpful in blocking or increasing the neurotransmitter processing within the brain. Conversely, if inefficient generation of neurons (associated with reduced volume) within the hippocampus is found to be an important causal factor, therapies involving the stimulation and growth of neurons in these areas may prove to be extremely useful in treating anxiety. If both factors are found to play important roles, then treatment plans may need to find a way to integrate the two corrective approaches. Even more broadly, the information gained from this research can improve our biological and psychological understanding of levels of anxiety in healthy individuals with no diagnosis of anxiety disorders. In doing so, this data can provide a baseline of anxiety within a healthy population to then better recognize and understand abnormalities and deviations from this baseline.

To summarize, this is a complicated picture with many caveats and various possible routes of interpretation. Continuing to investigate and pull apart different underlying neurobiological components of anxiety will be of great importance to

accomplishing the goal of this study as well as that of the overall attempt to understand and minimize anxiety, as well as better individualize treatment.

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Appendix A

The BDNF gene is located on chromosome 11 in humans, and like many other genes, displays a common and functional single nucleotide polymorphism (SNP), which simply means that the gene is present in more than one operational form and has multiple alleles. This type of polymorphism is the most common type of genetic variation among people, and describes an instance in which the DNA sequence of a gene differs by a single nucleotide—A, T, C or G. Oftentimes, this small change goes unnoticed because it either occurs outside of a coding region of a gene or has no effect on the amino acid produced from a specific codon (a set of three adjacent nucleotides that correspond to a particular amino acid in a polypeptide chain during DNA protein synthesis). The genetic code consists of 64 possible codons which each code for one of 20 amino acids. The genetic code is often described as redundant because of the fact that most amino acids can be coded for by more than one codon. Thus, the change in a single nucleotide of a codon can sometimes have no effect on the amino acid produced if two codons that differ by one nucleotide still code for the same amino acid. In other cases, though, a single nucleotide change can produce a range of differences in physiology depending on the function of the protein chain as well as the importance of the specific arrangement of the amino acids. A gene is said to have different alleles when there is an observed difference between different proteins coded for by the same gene due to genetic polymorphisms.

In terms of BDNF, a common SNP occurs at codon 66, in which a single G nucleotide is replaced with an A nuclueotide and results in an amino acid substitution from Valine (Val) to Methionine (Met) more commonly known as Val66Met (Egan et al., 2003). The Met allele of the BDNF SNP, while carried by only 30% of humans, and

therefore far less common than the Val allele, carries some important implications. Most notably, the Met allele contributes to defective activity-dependent secretion of BDNF in neurons. It does not alter the total level of BDNF within the brain, but rather impedes protein binding and processing (Frielingsdorf et al., 2010). The primary protein implicated in this change is sortillin, which is expressed in the cortex and hippocampus at high levels. Sortillin functions as a receptor for various ligands, including BDNF. More specifically, sortillin helps direct BDNF to neuronal secretory pathways where it may then respond to cellular depolarizations and be released to promote cell survival and synaptic plasticity (Evans et al., 2011). Therefore, the Met allele interferes with this cellular processing and generates lower depolarization-induced secretion in neurons and produces sub-optimal BDNF processing. This may, in turn, give rise to increased cell death or defective synaptic processing. Additionally, these effects are seen regardless of zygosity. That is, carriers of both a Met and a Val allele show the same dampened BDNF processing as individuals with two Met alleles. In most studies of BDNF, as in this one, Met carriers are studied as one group (Met+) and are compared to Val/Val individuals, rather than studying heterozygotes (i.e. Met/Val individuals) as a distinct and separate third group.

Appendix B

Grey matter is an informative area of investigation in the field of neuropsychology because it is the major substantive component of the central nervous system and the brain in particular. The term ‘grey matter’ describes cerebral networks that appear grey as they are made up of specialized cells called neurons, which include neuronal cell bodies as well as their corresponding axons and dendrites. Neurons are not in direct contact with one another, so they communicate by way of chemical neurotransmitters such as BDNF. In contrast, white matter describes myelinated axon fibers that appear white, which help transmit messages to and from separate grey matter areas in the brain. While neurons are a type of cell, they do not undergo cell division but are rather generated by special types of stem cells in a process called neurogenesis. This process is most active during pre-natal and childhood development and largely ceases during adulthood, meaning that as neurons die, they are not regenerated. Recent evidence suggests, however, that the hippocampus is one of two areas of the brain within which new neurons are continually produced even throughout adulthood. Therefore, researchers have found that hippocampal neurogenesis in adulthood is one of the primary beneficial actions behind antidepressants in terms of reducing stress. That is, increased levels of neurogenesis are largely correlated with decreased levels of stress. This, in turn, means that the grey matter volume of this area may provide important information about the underlying mechanisms of stress processing and thus provide clues as to how to best manage stress at a cellular level.