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Shirley Cotwell (Smith)
Candidate

Psychology
Department

This thesis is approved, and it is acceptable in quality and form for publication:

Approved by the Thesis Committee:

,Chairperson

THE RELATION OF BDNF VAL66MET POLYMORPHISM TO NEURAL VOLUME AND DRINKING BEHAVIOR AMONG ADOLESCENTS

BY

SHIRLEY MAE CROTWELL

BACHELORS OF SCIENCE, PSYCHOLOGY
BACHELORS OF SCIENCE, BIOLOGY
UNIVERSITY OF NEW MEXICO, MAY 2008

THESIS

Submitted in Partial Fulfillment of the
Requirements for the Degree of
Master of Science

Masters of Science, Psychology

The University of New Mexico
Albuquerque, New Mexico

May, Spring, 2011

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Shirley Mae Crotwell

B.S., Biology, University of New Mexico, 2008
B.S., Psychology, University of New Mexico, 2008
M.S., Psychology, University of New Mexico, 2011

ABSTRACT

Brain-Derived Neurotrophic Factor (BDNF) plays an important role in neural plasticity, learning and memory. Due to its relation to neurogenesis, BDNF has been studied with respect to its protective effects against various cognitive dysregulations, including those related to alcohol dependence. Recent evidence suggests that alcohol dependent individuals, in addition to those with a family history of alcoholism, have lower basal levels of BDNF as compared to controls. In adult populations, the Met variant of the Val66Met polymorphism, which results in decreased release of BDNF, has been associated with decreased neural volume as well as increased risk for alcohol problems. Using samples with prolonged alcohol exposure makes it difficult to determine the direction of the relationship between neural volume and drinking behavior. Therefore the current study set out to determine whether a relationship between BDNF, neural volume and drinking behavior could be detected using a sample of adolescents. Since this relationship is well demonstrated using adult samples, it was hypothesized that BDNF

genotype would be correlated with drinking behavior among adolescents, and this relationship would be mediated by differences in neural volume. 220 adolescents gave saliva samples, participated in magnetic resonance imaging (MRI), and completed behavioral measures designed to assess drinking behavior. Although BDNF was not correlated with drinking behavior, other interesting relationships were discovered including an inverse relationship between age and cerebral cortex volume, a positive relationship between accumbens volume and drinking, and distinct findings based on gender.

Keywords: BDNF, Val66Met polymorphism, MRI, adolescents

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Chapter 1

Introduction

The prevalence of alcohol abuse and dependence is exponentially growing worldwide. According to a national epidemiological study, over 12% of Americans will suffer with alcohol dependence during their lifetime and only 24% of these individuals will receive treatment (Hasin, Stinson, Oqburn, & Grant, 2007). Alcohol consumption is of such concern that the World Health Organization has described alcohol as a primary cause of global disease (Spanagel, 2009).

In 2006, the National Institute on Alcohol Abuse and Alcoholism (NIAAA) released a report on adolescent drinking which concluded that alcohol is the primary drug of choice among adolescents. Among 15-18 year olds, 13.5% meet criteria for a lifetime alcohol use disorder (Warner, Canino, & Colón, 2001). Underage drinking results in both short and long-term consequences ranging from decreased performance, negative impacts on heath, risky decision-making and death. According to NIAAA (2006) approximately 5,000 young people under the age of 21 die each year as a result of underage drinking. Among other long-term consequences, adolescent drinking increases the chances of developing alcohol problems later in life. Grant and Dawson (1998) found that people who report drinking before the age of 15 are four times more likely to meet criteria for alcohol dependence at some point in their lives. Adolescence is a period in human development associated with a multitude of biological and psychological developments and numerous risk factors have been identified as predictive of adolescent drinking including risk-taking (as frontal cortex continues to develop), alcohol

expectancies (which switch from negative to positive around the age of 13), heredity factors (personality, tolerance and sensitivity to alcohol) and environmental influence (availability, exposure, and peer influence) (NIAAA, 2006).

Etiological research on alcoholism has found that alcohol dependence is the result of both genetic and environmental influence, with genes accounting for 50% of the inter-individual variance for both men and women (Ducci & Goldman, 2008). Since genetic information makes such a considerable contribution to the development of alcohol dependence, it is important to better understand the pathways by which genes influence neurobiology and neurobiology in turn influences behavior.

In light of alcohol's profound effects on behavior, it is no wonder that extensive and long-term alcohol use is associated with altered brain structure and diminished cognitive function. Addiction research has focused primarily on neural structures responsible for reinforcement, decision-making and impulse control, such as the amygdala, nucleus accumbens, dorsal striatum and orbitofrontal cortex (Spanagel, 2009). Alternating mechanisms and protein expression are likely to cause changes in brain structure, such as atrophy, not localized in one specific area but throughout the whole cortex (Pfefferbaum et al., 1992). These alterations in neural structure result in diminished neurocognitive abilities. For example, as frontal synapses are damaged by the toxic effects of alcohol, behavioral measures of executive functioning (e.g. decision making) show a decline (Bates et al., 2002). It is critically important to develop a more sophisticated understanding of the mechanisms that are involved in the effects of alcohol

on the structure and function of the brain. One protein that has been previously related to changes in the brain is brain-derived neurotrophic factor (BDNF).

Brain-Derived Neurotrophic Factor (BDNF)

Neurotrophic factors have long been recognized for their role in synaptogenesis and neural plasticity. BDNF, specifically, plays a central role in learning, memory and higher cognitive functioning. Neurotrophic factors consist of a family of proteins that are necessary for the survival, development and functioning of neurons through a modulation of neurotransmitters and enhancement of cellular growth (Joe et al., 2007). A bidirectional communication between neurons and glia is demonstrated through a secretion of BDNF in its precursor form (pro-BDNF) and later reuptake by nearby astrocytes (Bergami, 2008). A chemical gradient of BDNF allows for the chemotaxis of neuronal cell precursors (Zhou, 2007). As BDNF binds to its receptor, TrkB, BDNF is released, amplifying the gradient (Zhou, 2007).

Due to its relation to neurogenesis, BDNF has been studied in respect to its protective effects against various cognitive dysregulations, including those related to alcohol dependence. Recent evidence suggests that alcohol dependent individuals, in addition to those with a family history of alcoholism, have lower basal levels of BDNF as compared to controls (Joe et al., 2007). Long-term neuronal adaptations are thought to maintain the disease of alcoholism (Logrip, Janak & Ron, 2009) and therefore it can be hypothesized that BDNF plays a protective role against the negative effects of alcohol consumption. It also stands to reason that the effects of BDNF on both alcohol-related and age-related cognitive decline become more pronounced with greater age and greater

levels of exposure to alcohol. This may be due to the fact that BDNF levels show an inverse relationship with age among healthy individuals (Sublette et al., 2008). Regulation of BDNF levels across the lifespan is related to differential use of alternative promoter regions in the BDNF gene (Wong, 2009). However, changes in BDNF levels across the lifespan are also dependent on the brain region studied. In some studies, for example, BDNF levels in the hippocampus do not change over time (Webster, Herman, Kleinman, & Shannon, 2006). However, Webster et al. (2006) found that TrkB receptor levels did change over time. In the temporal cortex, BDNF is highest among neonates and decreases with age and the same changes in TrkB are not observed across the lifespan in the temporal cortex. BDNF mRNA levels peak in the frontal cortex during young adulthood.

Therefore, it is important to understand when the first sign of this proposed protective effect of BDNF can be seen in the human lifespan. Whether BDNF can be protective at both the initial onset and after prolonged exposure to alcohol is still to be empirically determined. An investigation into the effects of BDNF in adolescence, in particular, can lend insight into whether BDNF can be protective early in life, during a time that is characterized by high risk for the development of substance use problems. Adolescents are a prime target for an investigation into the protective effects of BDNF because in adult samples it is difficult to determine the direction of the relationship between neural structure and behavior. Among adolescents, BDNF levels are high (Webster, 2006) and it is possible to collect data on neural structure before the brain has been exposed to long-term alcohol use. Studying adolescents is also important because

the cortical developments characteristic of adolescence makes this period in the lifespan especially vulnerable to addiction (Crews, He & Hodge, 2007). Understanding the effects of BDNF in adolescent populations has been limited thus far but numerous studies in the animal literature give insight into the effects of BDNF.

Animal studies have allowed for the exploration of the effects of BDNF on brain structure and function *in vivo*. BDNF is expressed throughout the brain, with the greatest concentration seen in hippocampus and cortex (Hofer, 1990). Within a single neuron, BNDF mRNA can be found floating around the area of the dendrite to the cell body and needs to be concentrated at the synaptic end in order to be released. In adult mice, BDNF is required for the terminal differentiation of new neurons in the hippocampus (Chan, 2008). Also, the Met variant of the Val66Met polymorphism (rs6265), which is located in a major BDNF protein coding exon on chromosome 11, results in decreased BNDF secretion and decreased cell survival through impairments in TrkB signaling (Bath, 2008). With respect to alcohol dependence, animal studies have shown that increased BDNF can attenuate responses to alcohol and acute alcohol exposure leads to an increase in BDNF levels (McGough, 2004). The protective mechanism of BDNF breaks down after about 6 weeks of ethanol exposure and the level of BDNF drops (Logrip, Janak & Ron, 2009).

With human populations, BDNF has been studied extensively with respect to the hippocampus, due to BDNF's role in long-term potentiation and formation of memories (Yu, 2009). Not only are higher levels of BDNF related to larger hippocampus, frontal lobe and cortex volume (Toro et al., 2009), but hippocampal neurons additionally recycle

BDNF for activity-dependent secretion (Santi, 2006). The Val66Met polymorphism results in differences in basal BDNF level and differences in the anatomy of the hippocampus and frontal cortex (Pezawas, 2004). In addition, polymorphisms within the BDNF gene (e.g. Val66Met) have been associated with alcohol abuse (Matsushita et al., 2004). More precisely, carriers of the Met allele in the Val66Met polymorphism are more likely to have lower levels of secreted BDNF and decreased amygdala and hippocampus volume (Montag et al., 2009). The polymorphism of Val to Met at Codon 66 results in decreased BDNF distribution at dendrites and the Met allele is also associated with lower activity-dependent secretion (Sublette, 2009). The Met allele is responsible for lower activity-dependent BDNF secretion due to improper trafficking of BNDF to vesicles in cortical neurons (Chen et al., 2004). Behaviorally, carriers of the Met allele exhibit poorer episodic memory (Egan et al., 2003). Interestingly, in both the rodent and human brain model, the BDNF polymorphism is related to an impaired ability to learn and respond to cues (Soliman et al., 2010). The Met allele in the BNDF polymorphism is relatively common in the human population with a prevalence for heterozygotes between 20 and 30 percent and the prevalence for homozygotes at about four percent (Chen et al., 2004).

Study Model

The conceptualization of this study's general model is depicted in Figure 1. Human behavior is the product of many factors including an interaction between genetic information and environmental input. Our model focuses on the link between genotype and behavior. Because there are so many additional factors that influence behavior, the

relationship between genotype and behavior is an important piece of a much larger puzzle. The relationship between genotype and behavior is thought to be mediated by changes in neural structure.

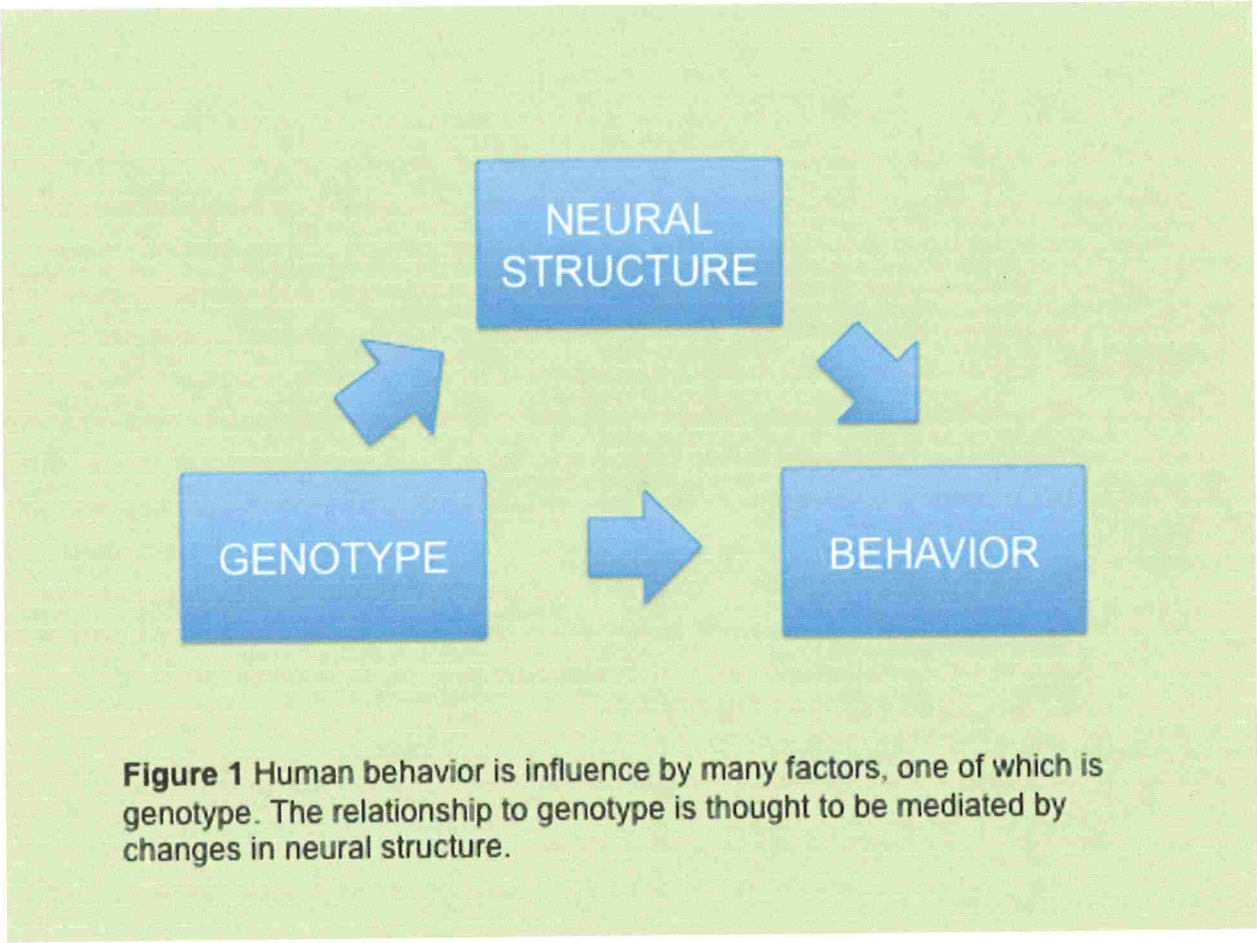

Figure 1 Human behavior is influence by many factors, one of which is genotype. The relationship to genotype is thought to be mediated by changes in neural structure.

To investigate the relationship between genotype and behavior more closely, a more specific model has been designed for this study (Figure 2). In this model, BDNF genotype is thought to influence drinking behavior through changes in brain volume. Pathway 1 (Figure 2), the relationship between BDNF and neural volume, is a complicated piece of the model because BDNF influences neural structure in many ways. The three most important functions of BNDF that lead to changes in brain volume are 1.

directing precursor cells to the areas of new growth, 2. maintaining healthy communication with neurons to ensure health and 3. aiding in final cell differentiation. BDNF guides precursor cells through a gradient in the concentration of BDNF, a process called chemotaxis. As BDNF binds to its receptor, TrkB, BDNF is released and amplifies the gradient (Zhou, 2007). Secondly, BDNF is important in maintaining the health of neurons because of its role in synaptic transmission/communication. When an action potential is propagated to the end of the axon, BDNF is released from the presynaptic terminal and the protein binds to TrkB, a homodimer receptor on the postsynaptic neuron. This perpetuates communication from one neuron to another. When BDNF is released in great quantity into the synaptic cleft, some of the protein binds to TrkB receptors on the presynaptic terminal. Through the activation of a cascade involving NFkB and cFOS, more BDNF protein is produced in the neuron. Routine communication between neurons is necessary for them to be considered valuable and without communication neurons are removed. Finally, BDNF has been shown to be a necessary component in allowing cells to reach their terminal differentiation (Chan, 2008).

In conceptualizing Pathway 2 (Figure 2), the relationship between neural volume and drinking behavior, it is important to remember that this study is attempting to investigate correlations, and the inference of causation is a jump that requires caution and further investigation.

Pathway 3 (Figure 2), illustrates the primary aim of the current study, which is to investigate the relationship between BDNF Val66Met polymorphism and drinking behavior among adolescents. Although this relationship has been demonstrated in adults,

with the Met allele considered risky, it is yet to be determined using an adolescent sample.

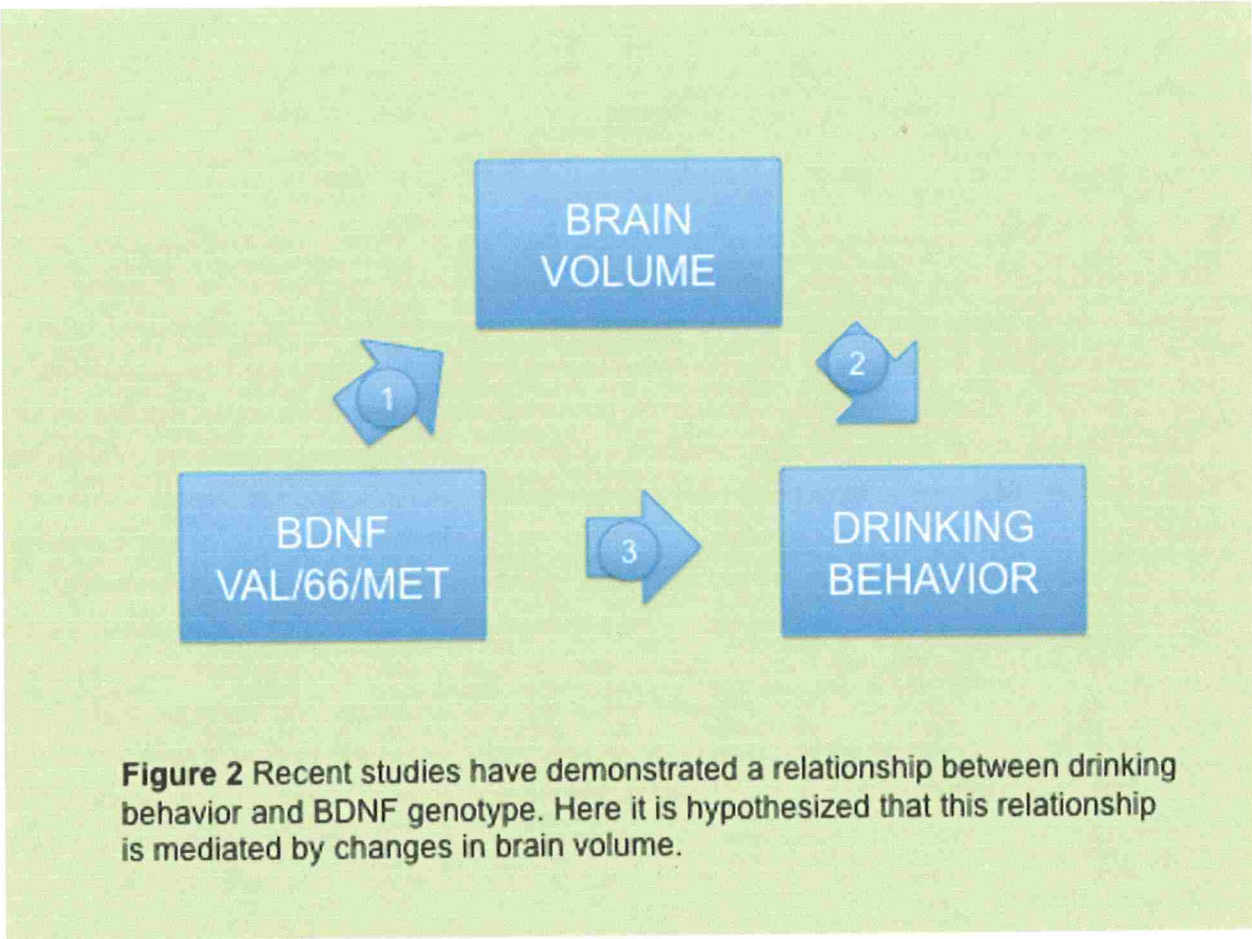

Figure 2 Recent studies have demonstrated a relationship between drinking behavior and BDNF genotype. Here it is hypothesized that this relationship is mediated by changes in brain volume.

Aims of the Study

The goal of the present study is to explore the protective effects of BDNF genetic variation in the context of early alcohol use. Although polymorphisms in the BDNF gene have been extensively studied in terms of molecular mechanisms, the link to behavioral outcomes remains unclear. The proposed study will examine the relationship of a specific BDNF SNP (rs6265, A/G) to alcohol-related behaviors and deleterious neurological effects. The specific aims are as follows:

The first aim is to evaluate whether a relationship can be detected among adolescents between BDNF genotype and brain volume in specific regions, specifically the hippocampus, amygdala, and nucleus accumbens. The hippocampus was selected because of previous work showing a relationship between BDNF and hippocampus function (see intro). The amygdala was selected as a region of comparison because previous work with BDNF has not focused on this region. Finally the nucleus accumbens, an important player in reward neural circuitry, was studied because of its key role in pleasure seeking and addictive behavior (Gardner, 1999; Addolrato, Leggio, Abenavoli, Gasbarrini, 2005).

Hypothesis 1: Differences in brain volume will exist across genotype groups, with Met individuals showing significantly less volume in aforementioned regions.

The second aim is investigate the relationship between BNDF genotype and behavioral outcome measures related to severity of alcohol use.

Hypothesis 2: Adolescents with the Met allele will show an increased level of drinking behaviors, indicated by measures of frequency, quantity, and duration.

The third aim is to determine if a relationship exists between neural structure (particularly volume of the hippocampus, amygdala and accumbens) and adolescent drinking behaviors.

Hypothesis 3: Neural volume will be correlated with drinking behavior.

The fourth and final aim is to determine if brain volume mediates the relationship between genotype and drinking behavior.

Hypothesis 4: Grey matter volume will mediate the relationship between BDNF genotype (Val66Met) and drinking behavior.

Chapter 2

Methodology

Participants

In order to test these hypotheses, data from a previous study was analyzed in which 220 adolescents were given a battery of questionnaires regarding drinking behavior and participated in a MRI scan (Dr. Angela Bryan's SHARP project: RO1 AA017390). All adolescents also contributed a saliva sample for appropriate genotyping. In order to address the aims of the study, three constructs were quantified: genotype, brain volume and drinking behavior.

The adolescents used in the study (Ages 14-18 years, M = 16.13) are ethnically diverse (64% Hispanic/Latino, 16% Caucasian, 5% African American, 3% American Indian, 1% Asian/Pacific Islander, and 11% Mixed) and were recruited from the Youth Reporting Center (YRC), a day program offered by Bernalillo County Juvenile Justice services. The Met allele frequencies in this sample were similar to the general population with the Met allele occurring in 32 % of the sample and Val/Val for the remaining 68%. Both parental consent and adolescent assent were obtained prior to involvement in the study. Additionally all aspects of the study were approved by the institutional review board at the University of New Mexico.

DNA Collection, Extraction, and Storage

Participants were instructed to generate and deliver 5 ml of saliva into a sterile 50 ml conical centrifuge tube. The saliva sample was then placed in the refrigerator and lysis buffer was added within 24 hours. Tris-HCl, pH 8; EDTA, pH 8; SDS and NaCl were added at 100 mM, 20 mM, 0.5% and 125 mM final concentrations; respectively. The

tubes are refrigerated until the DNA is extracted, usually within 48 hours. Proteinase K (0.2 mg/ml) is added and the tubes are incubated at 65°C for 60 minutes. An equal volume of isopropyl alcohol is then added to each tube, the contents are mixed, and the DNA is collected by centrifugation at 3,500 x g for 10 minutes. The DNA pellet is rinsed once with one ml of 50% isopropyl alcohol and allowed to air dry. For RNase treatment, 20 ug/ml RNAse A and 50 U/ml RNase T1 were added and incubated at 37°C for 30 minutes. To precipitate the DNA, two volumes of 95% ethanol was added and mixed by gentle inversion then collected by centrifugation at 3,500 xg for 15 minutes. The samples were allowed to air dry followed by re-suspension in 1 ml of 10 mM Tris-HCl, 10 mM EDTA buffer, pH 8.0, and placed in a 1.8 ml cryovial. The concentration of DNA is calculated from the absorbance at 260 nm analysis and then adjusted to a concentration of 10 ng/μL.

Genotyping

Samples were genotyped using TaqMan® primer and probe pairs; the probes are conjugated to two different dyes (one for each allelic variant). Taqman assays are designed and selected using the SNPBrowser™ program (Applied Biosystems) and ordered directly from this company. The PCR reaction mixture consists of 20 ng of genomic DNA, 1× Universal PCR Master Mix, 900 nM of each primer and 200 nM of each probe in a 15 μL reaction volume. Amplification is performed using the TaqMan® Universal Thermal Cycling Protocol and fluorescence intensity will be measured using the ABI Prism 7500 Real-Time PCR System. Genotypes were acquired using the 7500 system's allelic discrimination software (SDS version 1.2.3).

Volumetric Data

For volumetric calculation of grey matter, T1 images were produced by a 3.0 Tesla Magnetic Resonance Imaging (MRI) Siemens machine and analyzed using FreeSurfer. FreeSurfer is a software package that uses the images of sequential slices of the brain produced by the MRI and aligns these images based on patterns of cortical folds (Van der Kouwe, Benner, Salat and Fischl, 2008). After a 3D image of the brain has been reconstructed, an automatic segmentation parcels the brain into distinct regions for volumetric calculations.

Self-Report Measures

The two drinking questionnaires analyzed for this study were the Time-Line Follow Back (TLFB) and the Alcohol Use questionnaire.

Time-Line Follow-Back (TLFB; Sobell & Sobell, 1992). This measure collected information regarding participants' substance use during the one month prior to the experiment. As with other studies evaluating the progression of adolescent substance use (i.e., Chung, Maisto et al., 2004) during each assessment period, this instrument will be used to collect information regarding alcohol, cannabis, and tobacco use. This measure has been found to have good reliability and validity in collecting alcohol and cannabis use in general adolescent (Donohue et al., 2004) as well as with Hispanic adolescent samples (Dillon et al., 2005), yielding high coefficient alphas (alpha = .99) for past 90 day assessments (Waldron et al., 2001). This instrument yields counts for drinks per drinking day, days of consecutive abstinence, and days of alcohol use.

Alcohol use. Alcohol use was evaluated with a variation of the measure used by White

and Labouvie (1989). First, adolescents were asked if they have ever had an alcoholic drink (with instructions that define one alcoholic drink as "one beer, one glass of wine, or one serving of hard liquor either by itself or in a mixed drink"). Those (well over 90%) who answered that they had consumed alcohol before were asked to rate: (1) their frequency of use in the last three months on a 9-point scale ranging from "never" to "every day", (2) their typical quantity of drinks in one sitting on a 10-point scale ranging from "no drinks" to "more than 20 drinks", and (3) their frequency of getting drunk when drinking in the past three months on a 5-point scale ranging from "never" to "always".

Data Analysis

In order to determine the existence of a relationship between neural volume variables, drinking variables, and BDNF genotype, Pearson's correlations were computed. When either age or gender had a significant effect on the relationship, the effects were controlled using a partial correlation. In all analyses, pairwise exclusion was used for missing data.

The final aim of this study was to explore whether changes in neural structure (specifically grey matter volume) mediates the hypothesized relationship between BNDF genotype and drinking behavior. Gathering from Figure 2, Pathway 3 would represent the direct effect while the indirect effect consists of Pathways 1 and 2. To see if grey matter volume is mediating the relationship between genotype and behavior, it will be necessary to reevaluate the significance of pathway 3 after the mediator variable is fixed. According to Baron and Kenny (1986), neural volume will be considered a mediator if (a) variations in levels of genotype significantly account for variations in volume (b) variation in

volume significantly account for variation in drinking behavior and (c) when Pathway 1 and 2 are controlled, the relationship between genotype and drinking behavior will no longer be significant. The three regression equations and coefficients required will be (1) regressing the mediator (volume) on the independent variable (genotype) (2) regressing the dependent variable (behavior) on the independent variable (genotype) and (3) regressing the dependent variable (behavior) on both the independent variable (genotype) and on the mediator (volume) (Baron & Kenney, 1986).

Chapter 3

Results

Genotype and Neural Volume

The first aim was to investigate the relationship between BDNF genotype and hippocampus, amygdala and accumbens volume. Since males had significantly larger volume than females in each of these three areas ($t(218) = 4.67, p < .01$, $t(218) = 4.55, p < .01$, $t(218) = 3.54, p < .01$, respectively) (Table 2), the effects of gender were controlled in the analyses. The Val66Met polymorphism was not significantly related to the three brain regions of interest (hippocampus, amygdala and accumbens) or total cerebral cortex volume (Table 1).

Table 1.

Comparison of Met vs. Val

Variable	Met: Mean (SD)	Val: Mean (SD)	T Test
Age (yrs)	16.09 (1.03)	16.10 (1.12)	n.s.
Cerebral Cortex Volume (mm^3)	556079.76 (54038.01)	559194.15 (53951.22)	n.s.
Hippocampus Volume (mm^3)	8683.18 (923.41)	8729.82 (846.42)	n.s.
Amygdala Volume (mm^3)	3326.54 (379.15)	3416.51 (316.14)	n.s.
Accumbens Volume (mm^3)	1262.24 (172.29)	1264.85 (161.55)	n.s.
Average Drinks per Drinking Day	4.60 (6.21)	4.60 (5.11)	n.s.
Alc1	3.77 (2.43)	3.86 (2.45)	n.s.
Alc2	3.57 (2.29)	3.87 (2.27)	n.s.
Alc3	2.51 (1.30)	2.88 (1.26)	n.s.
Age of first drink	12.40 (2.53)	12.46 (2.34)	n.s.
Years of alcohol use	3.47 (2.55)	3.43 (2.61)	n.s.

Note:

Alc1 quantifies frequency of alcohol consumption in the last three months. Frequency was coded as never (1), occasionally (2), once a month (3), 2-3 times a month (4), 4-5 times a month (5), once a week (6), 2-3 times a week (7), 4-5 times a week (8), and everyday (9)
Alc2 quantifies quantity of alcohol consumed in last three months. Quantity was coded as none (1), 1 drink (2), 2-3 drinks (3), 4-6 drinks (4), 7-9 drinks (5), 10-12 drinks (6), 13-15 drinks (7), 16-18 drinks (8), 19-20 drinks (9), and more than 20 drinks (10).
Alc3 quantifies how often the adolescent got drunk if they were drinking. Frequency was coded as never (1), almost never (2), sometimes (3), almost always (4), and always (5).

Genotype and Drinking Behavior

The second aim of the current study was to examine the relationship between BDNF genotype and drinking behavior among adolescents. The Val66Met polymorphism was not significantly related to measures of drinking behavior including: average drinks per drinking day, frequency of consumption in the last three months, quantity consumed in last three months, rate of intoxication in the last three months, age of first alcoholic drink or years of alcohol consumption (Table 1).

Neural Volume and Drinking Behavior

The third aim was to explore the relationship between neural volume and drinking behavior (controlling for gender and age). Neither hippocampus nor amygdala volume were significantly correlated with measures of drinking behavior when partially out the effects of gender and age. Accumbens volume, however, was positively correlated with frequency of alcohol consumption in the last three months ($r(189) = .236$, $p = .001$) and quantity of alcohol consumed in the last three months ($r(189) = .190$, $p = .009$). After controlling for the effects of not only gender and age but also total cerebral cortex volume, the relationship between accumbens volume and both frequency and quantity in the last three months remained significant ($r(189) = .247$, $p = .001$, $r(189) = .182$, $p =$

.013, respectively) and there was also a significant relationship with average drinks per drinking day ($r(219) = .149$, $p = .029$). The relationship between drinking behavior and accumbens volume was further explored by creating an accumbens ratio (accumbens volume divided by total cerebral cortex volume). Controlling the effects of gender and age, the accumbens ratio was positively correlated with average drinks per drinking day ($r(189) = .157$, $p = .021$), frequency of alcohol consumption in the last three months ($r(189) = .211$, $p = .004$), and quantity of alcohol consumed in the last three months ($r(189) = .148$, $p = .043$). Interestingly, the positive relationship between accumbens volume and behavior was specific to alcohol, in that similar marijuana and tobacco use variables were nonsignificant.

Mediation

Since there was not a significant direct relationship between genotype and drinking behavior (fourth and final aim of the study), traditional Baron and Kenny (1986) statistical methods would forego further meditational analyses. It has been argued, however, that a significant direct relationship is not necessarily required in order to justify exploration of mediation because real world effects are often small and behavior is influenced by a multitude of variables. However, for this particular study, the relationship between genotype and neural volume (mediator) was not significant and therefore meditational analyses were not pursued.

Additional findings of interest

Gender was associated with the age at which the adolescent tried alcohol with males trying alcohol earlier than females ($t(257) = -2.27$, $p = .02$). Males also consumed more drinks on an average drinking day ($t(299) = 2.23$, $p = .03$) (Table 2).

Table 2.

Comparison of Demographics for Males and Females

Variable	**Males: Mean (SD)**	**Females: Mean (SD)**	**T Test**
Age (yrs)	16.13 (1.10)	16.13 (1.16)	n.s.
Cerebral Cortex Volume (mm^3) **	568834.21 (51479.57)	533566.34 (50729.45)	t(218)=4.27 **
Hippocampus Volume (mm^3) **	8897.33 (858.72)	8274.08 (720.43)	t(218)=4.67 **
Amygdala Volume (mm^3) **	3454.57 (320.69)	3223.78 (295.30)	t(218)=4.55 **
Accumbens Volume (mm^3) **	1283.60 (165.77)	1191.58 (147.28)	t(218)=3.54 **
Average Drinks per Drinking Day *	5.34 (6.14)	3.63 (4.50)	t(299)=2.23 *
Alc1	3.99 (2.39)	3.66 (2.44)	n.s.
Alc2	3.94 (2.38)	3.83 (2.33)	n.s.
Alc3	2.81 (1.25)	2.71 (1.44)	n.s.
Age of first drink *	12.42 (2.21)	13.13 (2.28)	t(256)=-2.27 *
Years of alcohol use	3.40 (2.46)	2.96 (2.30)	n.s.

Note: ** indicates $p<.01$, * indicates $p<.05$
Alc1 quantifies frequency of alcohol consumption in the last three months. Frequency was coded as never (1), occasionally (2), once a month (3), 2-3 times a month (4), 4-5 times a month (5), once a week (6), 2-3 times a week (7), 4-5 times a week (8), and everyday (9)
Alc2 quantifies quantity of alcohol consumed in last three months. Quantity was coded as none (1), 1 drink (2), 2-3 drinks (3), 4-6 drinks (4), 7-9 drinks (5), 10-12 drinks (6), 13-15 drinks (7), 16-18 drinks (8), 19-20 drinks (9), and more than 20 drinks (10).

Alc3 quantifies how often the adolescent got drunk if they were drinking. Frequency was coded as never (1), almost never (2), sometimes (3), almost always (4), and always (5).

Although age was not correlated with hippocampus or amygdala volume, there was a highly significant negative relationship between age (years) and cerebral cortex

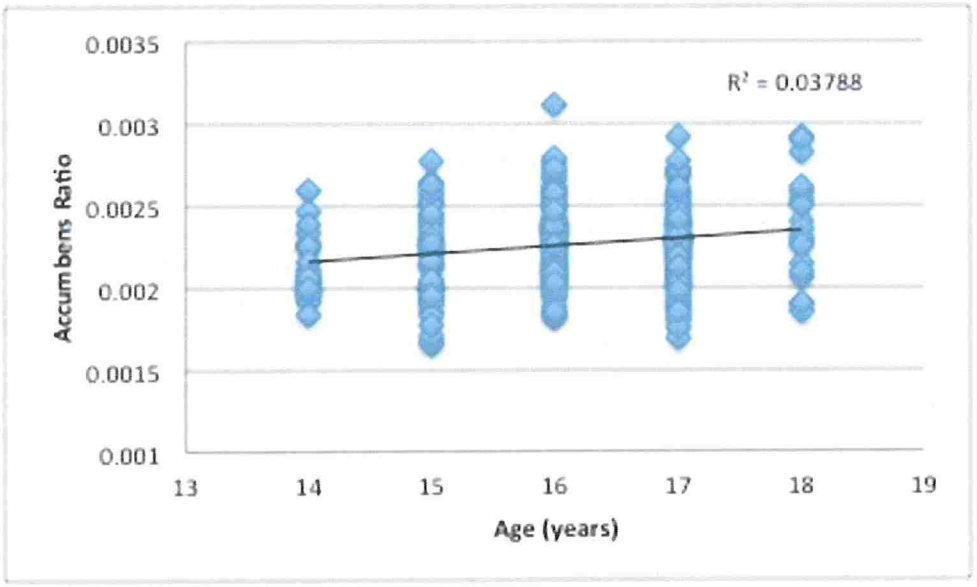

Figure 4. There was a significant relationship between accumbens ratio and age ($r(220) = .195$, $p < .01$).

Chapter 4

Discussion

The key finding in the current study was the positive relationship between nucleus accumbens volume and drinking behavior. Larger accumbens volume was correlated with increased drinking behavior in these adolescents. The nature of correlation work prevents us from making causal attributions. However, this finding supports a theory of bottom-up motivation versus top-down control. Adolescence is a period of significant neural development. While white matter increases linearly through adolescence, grey matter changes over time in an inverted U-shaped pattern (Casey, Jones, & Hare, 2008). During this period of development, bottom-up (pleasure-seeking/emotion-motivated) processes have a significant influence on behavior through differential development of bottom-up limbic systems, to top-down executive control systems (Casey et al., 2008). Hare et al. (2008) found that adolescents produce exaggerated neural response in regions associated with emotional reactivity, more so than either children or adults, leading to the inference that adolescent behavior is highly motivated by emotional processes. Additionally the study found failure to habituate emotional reactivity was associated with decreased connectivity between frontal cortex and subcortical regions (Hare et al., 2008) which suggests that lack of frontal lobe development plays a role in decreased executive control over emotion-guided behavior. The findings from the current study are important because they give us additional insight regarding the differences between white vs. grey matter developments during the period of adolescence. Galvan et al. (2006) found that subcortical regions (like the accumbens) are developed earlier in adolescence while top-

down control systems mature later, biasing the adolescent's judgment and decision-making. In the current study, the finding of a relationship between increased accumbens volume and increased drinking behavior highlights the period of adolescence as a time of vulnerability to addiction.

If adolescent behavior is largely motivated by pleasure-seeking and reward rather than complex cognitive control, then there are significant implications for the direction of future intervention strategies. If adolescent neurodevelopment is biased toward subcortical (limbic) regions rather than cortical (executive functioning) regions, it seems intuitive to utilize the adolescent's strengths by focusing on reward saliency rather than working from a purely cognitive model. One example of such treatments is the Adolescent Community Reinforcement Approach (A-CRA) (Godley, Smith, Meyers, & Godley, 2009), in which adolescents learn to find alternative reinforcements/rewards, as an alternative to the perceived rewards of alcohol use.

Despite previous studies suggesting an association between BDNF genotype and drinking behavior among adults, the current study did not find such a relationship in a sample of adolescents. Various speculations could be made in an attempt to understand these findings. Because BDNF levels change over the lifespan, in that BDNF levels show an inverse relationship with age (Sublette et al., 2008), the period of adolescence may be too early to detect a relationship. Because adolescents have not experienced long-term exposure to alcohol, it is imperative that future research continues to focus on this population in order to better understand the direction of the relationship between drinking behavior and biological measures. The Val66Met polymorphism did not have a

significant effect on drinking in the current study and supports the possibility that premorbid differences cannot fully account for subsequent drinking behavior.

The negative relationship between age and cerebral cortex volume became even more interesting when each gender was analyzed separately. The relationship was only found in male participants. Interestingly, other studies have also found differences in male and female neural development trajectories across adolescence (Giedd et al., 1996). De Bellis et al. (2001) took these findings one step further and found that males and females not only show differences in maturation, but they also found differences exist between grey and white matter changes. Consistent with prior work, males show more prominent age-related gray matter decreases in comparison with females, particularly during adolescence.

The use of a high-risk sample of adolescents may limit the generalizability of the findings to adolescents in general but research on this particular population has thus far been limited. Additionally, the increased need for intervention in this particular population offsets the limits of generalizability and warrants research using this group. One particular limitation is the use of self-report in collecting behavioral information on alcohol use. However, the instruments selected for this study (TLFB, AUQ) have demonstrated reliability and validity and other studies have shown that adolescents can accurately report their substance use (Lintonen, Ahlstrom, & Metso, 2004).

Future studies should continue to explore the hypothesized relationship between the Val66Met polymorphism of BDNF and drinking behavior among individuals who have not been exposed to long-term alcohol use. This information is imperative in

understanding the direction of the relationship between neural structure and drinking behavior later in life. Also, researchers should carefully consider neurobiological differences between male and female participants, in that the current study found differences in maturation of grey matter. As previously mentioned, other work has demonstrated that males and females have different developmental trajectories that should be taken into consideration. Finally, it is now believed that changes in adolescent behavior and neurodevelopment are not linear (Casey et al., 2008) so future studies should evaluate and compare adolescents at each year in development rather than combining a large range, such as 13-18 year olds.

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