NOTE TO USERS

Molecular Genetic Investigation of Brain-Derived Neurotrophic Factor in Childhood-Onset Mood Disorder

by

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A thesis submitted in conformity with the requirements for
the degree of Masters of Science
Institute of Medical Science, School of Graduate Studies
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ABSTRACT

Childhood-onset mood disorder (COMD) is a condition with pronounced morbidity. Brain-derived neurotrophic factor (BDNF) has antidepressant-like effects at cellular and molecular levels in animals; variants of the BDNF gene have been associated with bipolar disorder. Two BDNF polymorphisms, a dinucleotide repeat, $(GT)_n$, and a single nucleotide polymorphism (SNP), $Val^{66}Met$, were genotyped in 99 adults with COMD and matched controls. The BDNF $(GT)_n$ marker was associated with COMD (168 bp OR = 3.94, CI = 1.72 to 9.04). Alleles of $Val^{66}Met$ were not associated with COMD. The haplotype val/short contributes to risk.

Studies in several species have implicated BDNF and catechol-O-methyltransferase (COMT) in memory. In 63 young adults with a history of COMD, we genotyped three BDNF polymorphisms and the COMT $Val^{108/158}$ Met SNP. Multivariate analysis of variance (MANOVA) was employed to test hypotheses of association. We found no evidence of association between the two loci and declarative memory phenotypes.

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hours on a bike is a wonderful balm and that balance is important. Copyright permissions have been obtained from Elsevier (for the reproductions from Coyle et al. 2003 and Nestler et al. 2002), LWW (Duman 2002), Wiley (Strauss et al. 2004a) and from Humana Press (Strauss et al. 2004b).

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ABBREVIATIONS

η^2	Eta squared
5-HT	serotonin
5-HT1A	serotonin 1A receptor
5-HT1Dβ	serotonin 1B receptor
5-HT2A	serotonin 2A receptor
5-HTT	serotonin transporter
5-HTTLPR	44 bp insertion/deletion polymorphism in the serotonin transporter promoter
A	adenosine
AC	adenylate cyclase
Akt	protein kinase A, downstream from PI 3-kinase
ANOVA	analysis of variance
bcl-2	B cell lymphoma associated protein 2
BDI	Beck Depression Inventory
BDNF	brain-derived neurotrophic factor
bp	base pairs
BP	bipolar disorder
BP I	bipolar I disorder
BP II	bipolar I disorder
C	cytosine
C270T	a polymorphism in the BDNF gene
CA1	a subregion of the hippocampus
CA3	a subregion of the hippocampus
CAMK	Ca^{2+}-calmodulin dependent protein kinase
cAMP	cyclic adenosine monophosphate
CAPA	Child and Adolescent Psychiatric Assessment
CBCL	Childhood Behaviour Checklist
CDI	Children's Depression Inventory
C/EBPβ	CCAAT enhancer-binding protein-beta
COD	childhood-onset depression
COMD	childhood-onset mood disorder
COMT	catechol-O-methyltransferase
CREB	cAMP response element binding protein
D'	Lewontin's linkage disequilibrium coefficient
D3	dopamine receptor D3
DAG	diacylglycerol
DD	dysthymic disorder
DNA	deoxyribonucleic acid
DSM-III	Diagnostic and Statistical Manual of Mental Disorders, 3rd Edition
DSM-IV	Diagnostic and Statistical Manual of Mental Disorders, 4th Edition
*Eco*721	a restriction endonuclease
ECS	electroconvulsive seizures

eHap	Evolutionary-Based Haplotype Analysis Package
ERK 1/ 2	extracellular-regulated protein kinase 1/ 2
G	guanosine
GC	genomic controls
GRIN2B	glutamate receptor, ionotropic, NMDA, subtype 2B
GRR	genotype relative risk
GSK-3β	glycogen synthase kinase-3 beta
$(GT)_n$	microsatellite GT repeat
HC	hippocampus
*Hinf*I	a restriction endonuclease
HWE	Hardy-Weinberg Equilibrium
IP_3	inositol triphosphate
IQ	intelligence quotient
ISCA	Interview Schedule for Children and Adolescents
Li	lithium carbonate
LM	logical memory subtest of the WMS
LTP	long term potentiation
MANOVA	multivariate analysis of variance
MAP kinase	mitogen-activated protein kinase
MARCKS	myristoylated alanine-rich C kinase substrate
MDD	major depressive disorder
MEK	MAP kinase kinase
mf	mossy fibre pathway in the hippocampus
MFQ	Mood and Feelings Questionnaire
NaOH	sodium hydroxide
NE	norepinephrine
NED®	a yellow fluorescent dye
*Nla*III	restriction endonuclease
NMDA	N-methyl-D-aspartate
p75	low affinity neurotrophin receptor
PA	Verbal Paired Associates subtest of the WMS-R
PCR	polymerase chain reaction
PI3K	phosphoinositide 3-kinase
PKA	protein kinase A
PKC	phosphokinase C
Raf	molecule in the MAP kinase pathway
Ras	molecule in the MAP kinase pathway
RCF	Rey-Osterrieth Complex Figure
Rsk	ribosomal S6-kinase
sc	Schaffer collateral neurons
SCID	Structured Clinical Interview for DSM-IV
SNP	single nucleotide polymorphism
T	thymidine
TDT	transmission disequilibrium test
TNFα	tumor necrosis factor alpha
trkB	tyrosine kinase B receptor
$Val^{66}Met$	SNP causing a non-synonomous amino acid change from valine to methionine at codon 66

val66met	as immediately above
$Val^{108/158}Met$	a functional polymorphism of the COMT gene
VPA	Verbal Paired Associates subtest of WMS-R
VPA	valproic acid
WMS	Wechsler Memory Scale
WMS-R	Wechsler Memory Scale – Revised
WVS	Wechsler Intelligence Scale Verbal subtest score

CHAPTER 1.0

INTRODUCTION

1.0 Outline

Several recent investigations in the neurobiology of mood disorders have stirred interest in brain-derived neurotrophic factor (BDNF) as a biomarker and potential genetic risk factor. The research described in the present thesis is a logical route to pursue, as should be clear by the end of the introduction.

In the Introduction, I will first outline the neurobiology of depression with an emphasis on BDNF to present neurotrophic hypotheses of depression and memory dysfunction. The complex phenotype of interest, namely childhood-onset mood disorder, will be reviewed, including descriptions of phenotypic heterogeneity and comorbidity. The reader will be provided with a rationale for use of memory measures as endophenotypes, based on the memory deficits described in depression and the involvement of the hippocampus in both mood disorders and memory.

Genetic studies of childhood-onset mood symptoms, including family, twin and molecular studies will be reviewed. Evidence of the involvement of genetic factors in memory will also receive attention. Following this, previous genetic association studies in COMD will be reviewed, as will earlier studies of BDNF in mood disorder and of BDNF and catehol-O-methyltransferase (COMT) in memory. The evidence to date will be used to further construct candidate gene hypotheses. A brief summary of the statistical design will be next, covering both case-control and multivariate designs. Finally, specific hypotheses will be stated regarding BDNF as a candidate gene for

COMD. Both BDNF and COMD will be given consideration as candidate genes for memory phenotypes.

In the Second Chapter, the case-control study which is in press will be presented in full. The Third Chapter contains the investigation on BDNF, COMT and memory, also in press.

In the Fourth Chapter, Discussion and Conclusions, I will summarize the rationale and results of the investigations, convey some of the principal limitations and interpret the results. Lastly, the Fifth Chapter, Future Directions, I address some of the environmental factors involved in depression and BDNF regulation and offer possible hypotheses for further experiments.

1.1 Neurobiology of MDD

1.1.1 Brain-Derived Neurotrophic Factor and The Neurotrophic Hypothesis of Depression

Brain-derived neurotrophic factor (BDNF) is a nerve growth factor expressed in the several brain regions including neocortex, hippocampus, and amygdala (Lindvall et al. 1994), where it influences neuronal survival, synaptic activity, plasticity and neurotransmitter synthesis (Lang et al. 2004). The BDNF gene is found on chromosome 11p14.1 (Fang et al. 2003) and has several polymorphic markers. One BDNF polymorphism is a microsatellite $(GT)_n$ dinucleotide repeat initially reported by Proschel et al. (1992). Another polymorphism is a functional coding region single nucleotide polymorphism (SNP) at nucleotide position 196/758, which results in an amino acid

change at codon 66 val→met ($Val^{66}Met$) (NCBI SNP Cluster ID: rs6265) of the proBDNF molecule. The $Val^{66}Met$ SNP is located in a section of the BDNF precursor protein that is cleaved away, rendering the amino acid change absent from mature BDNF. Substantial evidence implicating a role for BDNF in depression at a molecular and cellular level has been reviewed in detail (Lang et al. 2004; Coyle and Duman 2003; Nestler 2002; Altar 1999; Duman et al. 1999; Duman, 1998; Duman et al., 1997). The hypothesis is that BDNF may be important to stress-related depression. Experiments supporting the hypothesis demonstrate changes in BDNF expression, neural morphology and neurogenesis that occur under stress. They are complemented by studies that show reversal of such changes with antidepressant administration. The neural substrate affected by BDNF appears to be specific cell types in the hippocampus.

In animal models, stress causes a quick and long-lasting decrement in hippocampal BDNF. Down-regulation of BDNF has been noted in immobilization stress and glucocorticoid treatment paradigms. The decrease in BDNF has been localized to the dentate gyrus granule cell layer and CA1 and CA3 pyramidal cell layers of the hippocampus (Smith et al. 1995; Nibuya et al. 1995). Persistent exposure to physical stress in rodents or to psychosocial stress in nonhuman primates is associated with atrophy of CA3 neurons in the hippocampus, by decreasing the length and number of CA3 apical dendritic arbourizations (Sapolsky et al. 1985; Sapolsky et al. 1989; Magarinos et al. 1996; Uno et al. 1999). Similar atrophy of CA3 pyramidal neurons in the hippocampus has been seen in chronic glucocorticoid treatment (Woolley et al. 1990). See Figure 1.

Figure 1. Neurotrophic Effects of Stress and Antidepressant Treatment

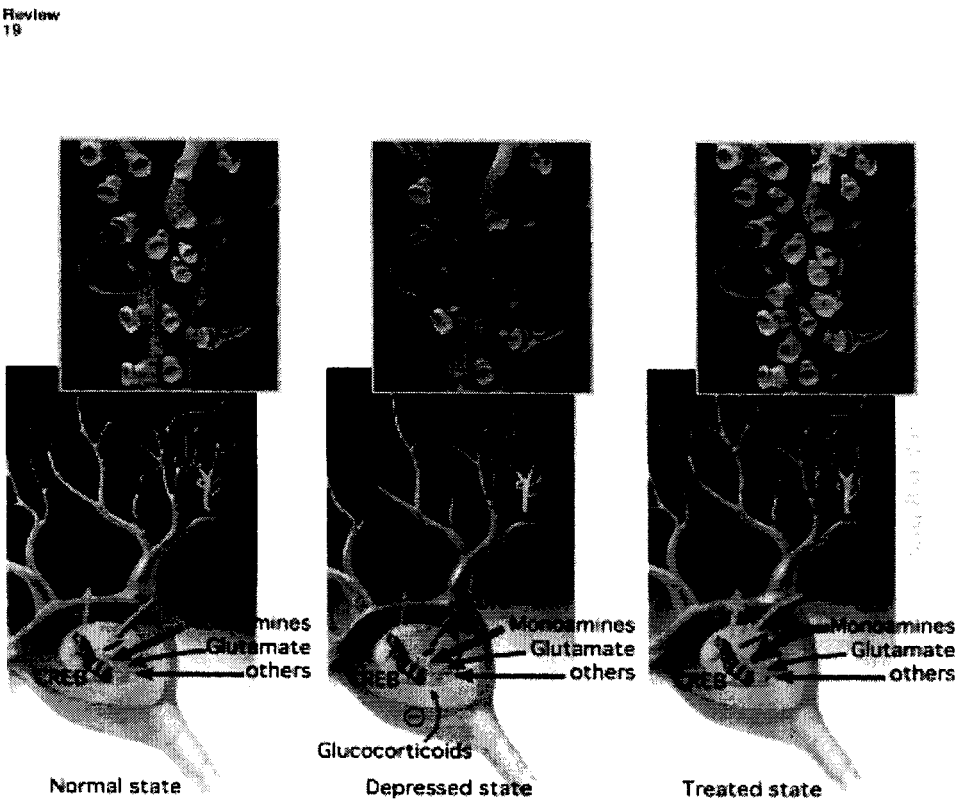

Figure 3. Neurotrophic Mechanisms in Depression

Nestler 2002

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Reduced arbourization and synaptic density along with reduced BDNF expression are observed in the stress/depressed state in the middle panel. In the right panel, monoamines (i.e. antidepressants) are associated with increased BDNF expression, improved arbourization and more synaptic connections.

Antidepressant treatments down-regulate multiple serotonergic and adrenergic post-synaptic receptors. However, they up-regulate post receptor levels of adenylyl cyclase, cAMP-dependent protein kinase (PKA), cAMP response element binding protein (CREB), BDNF and TrkB (Duman 1998; Duman et al. 1997). Long term antidepressant treatment increases levels of adenylyl cyclase and PKA (Menkes et al. 1983; Ozawa and Rasenick 1991; Colin et al. 1991; Nestler et al. 1989). See Figure 2.

Levels of BDNF increase in the dentate gyrus granule and CA1 and CA3 pyramidal cell layers in rat hippocampus with chronic antidepressant treatment. The effect is specific to a number of antidepressants with different mechanisms of action, including 5-HT and NE reuptake inhibitors and electroconvulsive shock. It is not found with non-antidepressant psychoactives such as opioids, cocaine or haloperidol. The temporal course and sites of BDNF elevation coincide with CREB activation by antidepressant. Up-regulation of BDNF occurs in cultured cells when cAMP or Ca^{2+}-dependent pathways are activated (Nibuya et al. 1995; Nibuya et al. 1996). The evidence above supports the hypothesis that antidepressants increase BDNF via the second messenger post-receptor cascade, and that CREB may be a common intracellular target for 5-HT and NE (Duman et al. 1999).

Chronic electroconvulsive shocks (ECS) induce BDNF and TrkB upregulation (Duman and Vaidya 1998) and also increase CREB expression in hippocampus. The temporal

Figure 2. Antidepressants upregulate BDNF via Post-Receptor Second Messenger Cascade

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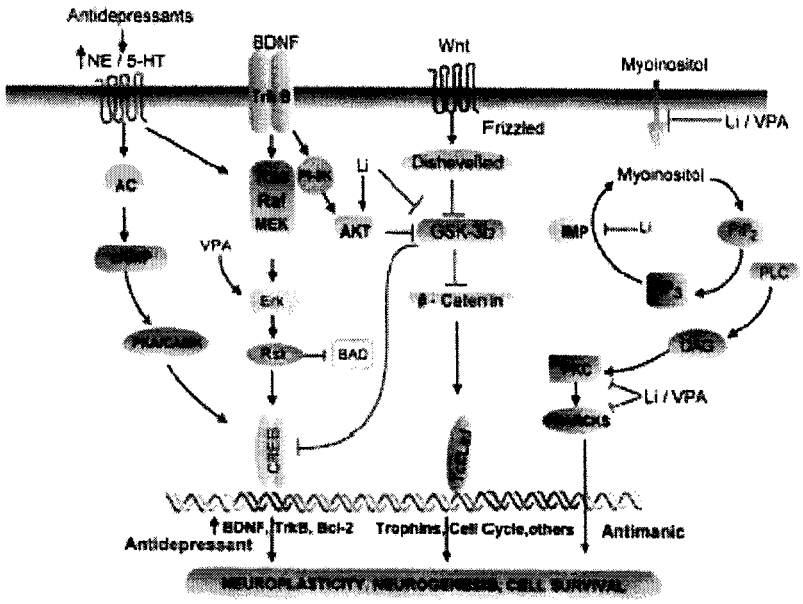

Figure 1. Schematic Representation of Intracellular Pathways Affected by Mood Stabilizers and Antidepressants

Activation →; inhibition ⊣.

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AC	adenylate cyclase
Akt	protein kinase A, downstream from PI 3-kinase
bcl-2	B-cell lymphoma associated protein 2
BDNF	brain-derived neurotrophic factor
CAMK	Ca^{2+}-calmodulin dependent protein kinase
cAMP	cyclic adenosine monophosphate
CREB	cAMP response element binding protein
DAG	diacylglycerol
ERK	extracellular-regulated protein kinase 1/ 2
GSK-3β	glycogen synthase kinase-3 beta
IP_3	inositol triphosphate
Li	lithium carbonate
MAP kinase	mitogen-activated protein kinase
MARCKS	myristoylated alanine-rich C kinase substrate
MEK	MAP kinase kinase
PI3K	phosphoinositide 3-kinase
PKA	protein kinase A
PKC	phosphokinase C
Raf	molecule in the MAP kinase pathway
Ras	molecule in the MAP kinase pathway
Rsk	ribosomal S6-kinase
trkB	tyrosine kinase B receptor
VPA	valproic acid

The two main pathways on the left are relevant to stress-related depression – the cAMP-CREB pathway and the TrkB - ERK/MAP kinase pathway. Stimulation of the two pathways with antidepressants causes increases in BDNF expression which results in increased Bcl-2 expression and increases in neuronal plasticity, neurogenesis and cell survival. The two pathways on the right are more relevant to bipolar disorder and are beyond the scope of this project.

course and sites of ECS regulated CREB expression are similar to those reported for antidepressants (Nibuya et al. 1995; Nibuya et al. 1996).

Enhanced survival and arbourization occurs with BDNF and antidepressant treatment. BDNF promotes growth and survival of cortical neurons (Ghosh et al. 1994) and 5-HT and NE neurons (Mamounas et al. 1995; Sklair-Tavron and Nestler 1995). A decrement in BDNF could adversely influence such neurons. The regeneration of cortical catecholamine neurons in cerebral cortex is increased by antidepressant (Nakamura, 1990). Long-term ECS can increase sprouting of dentate gyrus granule neurons (Vaidya and Duman 1996). Evoked potentials involving the dentate gyrus are increased in rats following ECS treatment (Stewart and Reid 1994). Decreased neurogenesis of dentate gyrus granule neurons has been found in adult animals subjected to acute stress and high levels of glucocorticoids (Gould et al. 1997; Gould et al. 1998). In contrast, increased neurogenesis of granule cells has been reported in adult mice exposed to an enriched environment (Kemperman et al. 1997; van Praag et al. 1999). Neurogenesis of hippocampal granule cells is increased by chronic antidepressant treatment (including electroconvulsive seizures) (Madsen et al. 2000; Malberg et al. 2000; Manev et al. 2000; Santarelli et al. 2003). See Figure 3.

Evidence indicates that BDNF causes similar behavioural effects to antidepressants in the forced swim and learned helplessness models. BDNF was infused into rat midbrain seven days before testing and completely reversed increased escape latency in the learned helplessness model and improved performance by 70% in the forced swim test (Siuciak et al. 1997). Midbrain BDNF infusion can to increase levels of 5-HT and metabolites in

several forebrain sites including cortex, hippocampus, striatum and nucleus accumbens (Siuciak et al. 1994, Siuciak et al. 1996).

Considering the evidence discussed pertaining to stress and antidepressant response, BDNF may be linked to depression. Glucocorticoids and stress causes decreased levels of BDNF in the CA1 and CA3 granule cells in the hippocampus, which are associated with atrophy, cell death and decreased neurogenesis. Antidepressants may act to reverse or block hippocampal changes via increased BDNF expression brought about by serotonergic and noradrenergic signals (Duman et al. 1999). Bilateral dentate gyrus BDNF infusion has produced antidepressant-like behaviour in the forced swim test and learned helplessness paradigms (Shirayama et al 2002). The bulk of preclinical evidence points to BDNF being a downstream element that mediates antidepressant effects at molecular and behavioural levels.

Human investigations corroborate results in the preclinical BDNF literature. Neuroimaging studies in depressed adults indicate hippocampal atrophy (Bremner et al. 2000; Sheline et al. 1996, 1999), which is compatible with BDNF downregulation. Postmortem data from humans are convergent with a neurotrophic hypothesis of depression: antidepressant therapy is associated with increased temporal cortex cyclic AMP response element binding protein (CREB) concentration (Dowlatshahi et al. 1998) and increased hippocampal BDNF immunoreactivity (Chen et al. 2001). Furthermore, there is evidence of lowered serum BDNF concentrations in depressed human subjects compared to controls (Karege et al. 2002) as well as elevated serum BDNF levels among

Figure 3. Hippocampal Neurogenesis with Stress and with Antidepressants

Duman 2002

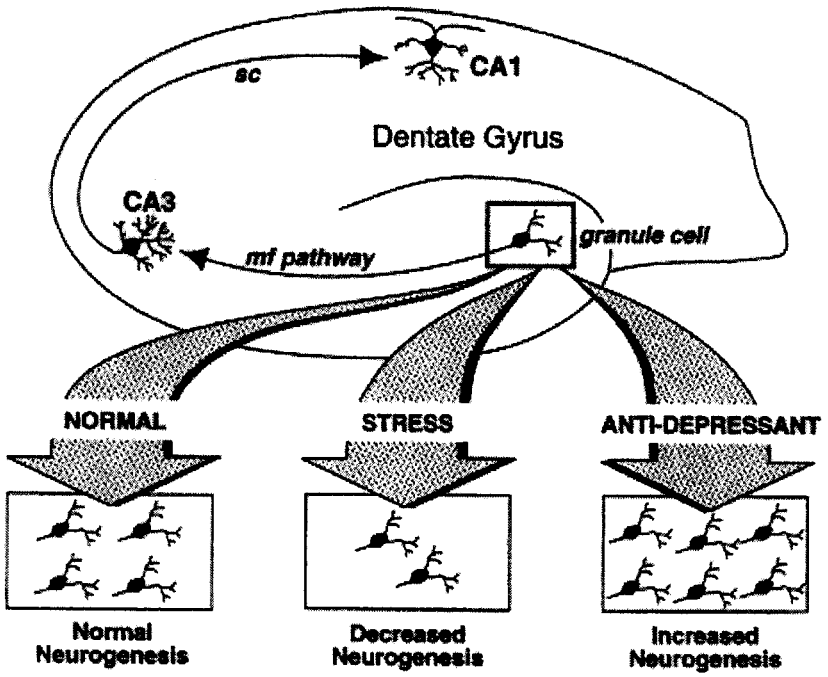

Fig. 1 Schematic model of hippocampus and regulation of adult neurogenesis. The major circuit in the hippocampus consists of granule cells in the dentate gyrus sending projections to the CA3 pyramidal neurons via the mossy fiber (mf) pathway, and CA3 neurons projecting to CA1 neurons via Schaffer collaterals (sc). Stress is reported to influence the cellular components of this pathway in the hippocampus of adult rodent or nonhuman primate. This includes down-regulation of adult neurogenesis by exposure to social or psychological stress. In addition, stress decreases the dendritic arborization of CA3 pyramidal neurons (not shown). These results demonstrate that stress can lead to structural alterations in the adult brain that could contribute to atrophy of hippocampus observed in clinical studies of patients with mood disorders. In contrast to the effects of stress, antidepressant administration increases neurogenesis in the hippocampus of the adult rodent. This effect could block or reverse the actions of stress on hippocampal atrophy.

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In adult rodents and nonhuman primates, social or psychological stress is associated with down-regulation of neurogenesis in the HC which may accompany hippocampal changes observed by neuroimaging in human depression. Antidepressant administration causes increased neurogenesis which may help to reverse the effects of stress on the HC.

depressed patients treated with antidepressants compared to those remaining untreated (Shimizu et al. 2003).

Evidence for the involvement of BDNF in the pathophysiology of mood disorders is compelling. Furthermore, as will be described in Chapter 1.4.2, linkage and association data from adult samples have increased the attention given to BDNF is a potential genetic risk factor for mood disorder phenotypes.

1.1.2 A Neurotrophic Hypothesis of Memory Dysfunction

Several lines of evidence point to the involvement of BDNF in memory. Synaptic strength is increased by BDNF. This is measured by long term potentiation (LTP), a cellular model of learning and memory. Synaptic strength of hippocampal neurons is increased following incubation with BDNF. Lower levels of BDNF in knockout mice result in reduced LTP (Kang and Schuman 1995; Levine et al. 1995). Studies involving antidepressants have not provided definitive results, showing both increased and reduced LTP in the hippocampus (Birnstiel and Hass 1991; Massicotte et al. 1993). One study exists showing improved spatial learning in rats treated with antidepressants (Yau et al. 1995).

BDNF is important to neuronal transmission and plasticity (Lu and Gottschalk 2000; Tyler et al. 2002; Vicario-Abejon et al. 2002) and it is important to in vivo memory formation in the hippocampus (HC) (Alonso et al. 2002a). Several studies suggest that BDNF has a role in learning and memory performance in differing mammalian and avian species, including rats (Alonso et al. 2002b; Mu et al. 1999), chicks (Johnston and Rose 2001), and monkeys (Tokuyama et al. 2000). Thus, in vitro, animal and human experiments all suggest BDNF is important to memory function.

1.2 Description of COMD Phenotype

The broad diagnostic category of mood or affective disorders includes conditions such as major depressive, dysthymic, and bipolar disorder. The various mood disorders differ from one another in several regards including overall clinical phenomenology, duration, and course. However, the common feature of all mood disorders is pathologically dysregulated mood which is accompanied by a cluster of characteristic cognitive, neurovegetative, and behavioral symptoms and signs. Major depressive disorder is the most prevalent of the various mood disorders in the juvenile years and has been studied the most extensively.

Depression is a syndrome characterized by certain emotional, cognitive, and somatic symptoms and physical signs, which may meet criteria for a disorder if the syndrome persists, interferes with functioning, and is not secondary to drugs or medical conditions. According to the Diagnostic and Statistical Manual of the American Psychiatric Association, Fourth Revision (DSM-IV), major depressive disorder (MDD) is defined by the presence of a minimum of five criterion symptoms nearly every day for at least two weeks (Amercian Psychiatric Association, 1994). The diagnosis requires the presence of depressed mood (or irritable mood in youngsters) or anhedonia. The other four symptoms can be any combination of the following: appetite or weight change, insomnia or hypersomnia, psychomotor agitation or retardation, fatigue or anergia, feelings of worthlessness or guilt, poor concentration or indecision, and recurrent thoughts of death or suicidal ideation or suicide attempt. The symptoms must cause significant distress to

the sufferer or interfere with daily functioning and not be a direct physiologic consequence of medical illness, substance abuse (legal or illegal) or certain other specific psychiatric disorders (e.g., schizophrenia). Additionally, the symptoms and signs must represent a change in the person's functioning.

1.2.1 Comorbidity

COMD is not a single disorder, but one with different psychiatric comorbidities, social morbidities and outcomes. Comorbid psychiatric disorders are extremely common in children with MDD. According to most clinical and epidemiological studies, from 40% to 70% of depressed youngsters have one or more additional psychiatric diagnoses (Birmaher et al. 1996; Angold and Costello. 1993). In one sample of depressed psychiatrically referred youths, 90% were reported to have comorbid diagnoses (Biederman et al. 1995). The presence of multiple psychiatric disorders may affect risk of depression recurrence and be associated with increased suicidality, treatment resistance, and mental health service utilization (Birmaher et al. 1996). Psychiatric comorbidity also may possibly characterize various subtypes of childhood depression which may differ in course and outcome (Lewinsohn et al. 2000; Angold and Costello 1993; Goodyer et al. 1997).

Anxiety disorders are the most frequent comorbid psychiatric diagnoses among clinically referred depressed youngsters and have been detected in up to 50% of such samples (Birmaher et al. 1996; Kovacs 1996). Anxiety disorders include conditions characterized by phobias, excessive worry and fearfulness, panic attacks, impairing obsessions and compulsions, and various associated symptoms (APA, 1994). It is notable that when

anxiety and depressive disorders coexist in youngsters, the anxiety disorders typically precede the onset of MDD; this observation has been reported both in clinical (Kovacs et al. 1989) and nonclinical but high-risk samples (Avenevoli et al. 2001).

A chronic and symptomatically less severe form of depression called dysthymic disorder (DD) also is frequent among children with MDD and is present among up to about one-third of them (Kovacs et al. 1997; Rao et al. 1995; Biederman et al. 1995). Dysthymic disorder has been defined for children as at least a one-year period of depressed mood with other associated depressive symptoms that fail to reach the threshold for MDD (APA 1994). The nosologic boundary between MDD and dysthymia however is not entirely clear partly because dysthymic disorder is a risk factor for major depression (Kovacs et al. 1997). According to DSM-IV, no major depressive episode can occur in the first 2 years (one year for children and adolescents) of DD or the diagnosis becomes MDD and not DD.

Conduct disorder is another fairly common concurrent diagnosis that characterizes 7% to 24% of clinically referred depressed youngsters (Kovacs 1996). Conduct disorder comorbid with depressive disorder has been documented in community samples as well (Angold and Costello 1993). Conduct disorder is a persistent pattern of misbehavior in which the rights of others or important age-appropriate societal norms or rules are repeatedly violated and may result in involvement with the juvenile justice system (APA 1994). This behavior pattern may develop as a complication of depression and may persist even after the depression remits (Kovacs et al. 1988).

There is no question that major depression in children and adolescents is associated with substantial morbidity (Birmaher et al. 1996; Kovacs 1997). Interpersonal relationships and functioning of depressed youngsters are more impaired than of youths with nonaffective psychiatric disorders (Puig-Antich et al. 1985a, 1985b). Clinical depression in the school-age years is associated with difficulties in academic achievement and school performance (Kovacs and Goldston 1991). Depressed youngsters also are at high risk for attempted (Kovacs et al. 1993) and completed suicide (Brent et al. 1993) particularly as they reach early to mid-adolescence, and their rates of suicidal behaviour exceed the rates among peers with other psychiatric disorders (deWilde et al. 2001; Pfeffer 2001). Childhood depression also is a harbinger of subsequent psychiatric morbidity. In particular, while in their teens, these youngsters are at high risk for bipolar disorder (BP). Rates of "switch" from unipolar to bipolar disorder have been reported as ranging from 9% to 20% or higher (Kovacs et al. 1994; Rao et al. 1995; Strober and Carlson 1982; Strober et al. 1993; Geller et al. 1994). For example, a seven-year follow-up of adolescents with MDD revealed that 19% had converted to bipolar disorder compared to 0% of controls (Rao et al. 1995). Manic episodes are the sine qua non of BP and are characterized by a constellation of symptoms including unnaturally euphoric, expansive, or irritable mood and abnormal states of mental and physical excitement and agitation (APA 1994). Bipolar disorder usually is manifested by alternating or mixed episodes of depression and mania, can present at various levels of severity, and requires lifelong management.

In addition to the emergence of bipolar illness in adolescence, psychiatric morbidity of childhood MDD continues into adulthood for many cases as well. Controlled follow-up

studies of clinically referred as well as community-based but carefully diagnosed samples have found that, as depressed youths grow up, they are at risk for adult depression, substance abuse, as well as personality disorder (Rao et al. 1995; Weissman et al. 1999a, 1999b; Lewinsohn et al. 1999; Harrington et al. 1990; Kasen et al. 2001).

As indicated above, COMD is a heterogeneous phenotype with a variety of different comorbidities, functional impairments and outcomes.

1.2.2 Memory subphenotypes

Memory dysfunction is frequently part of the clinical presentation of depression and may be related to changes in the hippocampus, a region known to have a role in memory (Squire and Zola-Morgan, 1991). Diminished hippocampal volume is repeatedly observed in patients with major depression (Sheline et al. 1996, 1999). Although volumetric findings are not completely consistent, hippocampal shape also appears to be affected (Posener et al. 2003). Declarative memory is affected in individuals with depression (Zakzanis et al. 1998). This is evident during an acute episode Calev et al. 1998; Austin et al. 1992) as well as following remission (Marcos et al. 1992). Postpubertal major depression adversely affects performance on hippocampal-dependent memory tasks in both antidepressant-naïve first-episode individuals and in those with multiple-episodes. MacQueen et al. (2003) found hippocampal volume to be low, but only in those with multiple episodes, suggesting that memory deficits may precede anatomic change. Other lines of evidence for relationships between memory function and depression include studies demonstrating that antidepressants improve memory performance in rats (Yau et al. 2002; Barros et al. 2002) and in depressed (Levkovitz et al. 2002) and non-depressed (Harmer et al. 2002) humans. The use of a memory

phenotype in the study of depressive disorder may reduce clinical heterogeneity by dissecting out a single cognitive aspect of a complex phenotype which affects emotion, neurovegetative function and cognition.

1.3 Family and Twin Studies

1.3.1 Family and Twin Studies of COMD and Depressive Symptoms

Multiple family studies have demonstrated that COD is familial. "Bottom-up" reports consider juvenile-onset probands and consider rates in their first-degree relatives. Between 40 and 70% of first degree relatives of depressed children have a history of mood disorder – these rates are significantly greater than rates in relatives of psychiatric or normal juvenile controls (Harrington et al. 1993, Kovacs et al. 1997; Todd et al. 1993; Puig-Antich et al. 1989; Weller et al. 1994). As an example, 94% of depressed children had affected pedigrees, with families of probands having a five-fold greater odds of depression compared to psychiatric controls (Kovacs et al. 1997). "Top-down" studies of the prevalence of mood disorders in offspring of depressed adults are convergent with the data on the parents of depressed offspring. Investigators have noted high rates of affective disorder among prepubertal, adolescent and young adult children of adults with mood disorder (Hammen and Brennan 2003; Orvaschel 1990; Weissman et al. 1984a, b). There is evidence that prepubertal-onset depressive probands yield families that have twice the prevalence of mood disorder compared to adult-onset families, which may suggest that families with prepurbertal-onset depression have a greater familial loading (Neuman et al. 1997).

Most complex traits are influenced by genetic factors to a degree – including even traits such as age related hearing impairment (Fransen et al. 2003), adaptation to high altitude (Rupert et al. 2001), hemoglobin A(1c) levels in healthy individuals and those with Type I diabetes (Snieder et al. 2001), and educational achievement in 12-year-olds (Bartels et al. 2002). It is therefore not surprising that similar results have been found in twin studies of depressive symptoms with onset in childhood and adolescence. Heritability estimates of parent-rated depressive symptoms ranged from 30-80% in several studies (Hewitt et al. 1992; Deater-Deckard et al. 1997; Thapar and McGuffin et al. 1994; Edelbrock et al. 1995; Eaves et al. 1997; Gjone and Stevenson 1997; Hudziak et al. 2000). For instance, in 492 twin pairs, the additive genetic influence on the parent-rated depressive symptoms was estimated to be 0.65 [95% CI=0.55-0.73] for boys and 0.61 [95%CI=0.48-0.71] for girls, while nonshared environment was estimated to account for 0.35 and 0.39 of the variance, respectively for boys and girls (Hudziak et al. 2000). Heritability of self-reported depressive symptoms in youth has ranged from 15-80% across several twin studies (Rende et al. 1993; Thapar and McGuffin 1994; Eley 1997; Silberg et al. 1999; Eaves et al. 1997; Boomsma et al. 2000). As an example, the heritability estimate for Childhood Depression Inventory (CDI) scores was 0.34 in a sample of 707 twin and sib pairs, with a majority of the remaining variance accounted for by nonshared environmental factors (Rende et al. 1993). While the heritability of juvenile depressive symptoms is less than that for ADHD (heritability about 0.80) (Biederman and Faraone 2002), it is comparable to adult disorders that have had ample molecular investigation such as alcoholism (heritability of about 0.5-0.6) (Enoch and Goldman 2001). See Figures 4 and 5. Overall, family and twin studies suggest

molecular investigation of a depressive phenotype in youth, such as COMD, is a reasonable scientific task.

Figure 4.

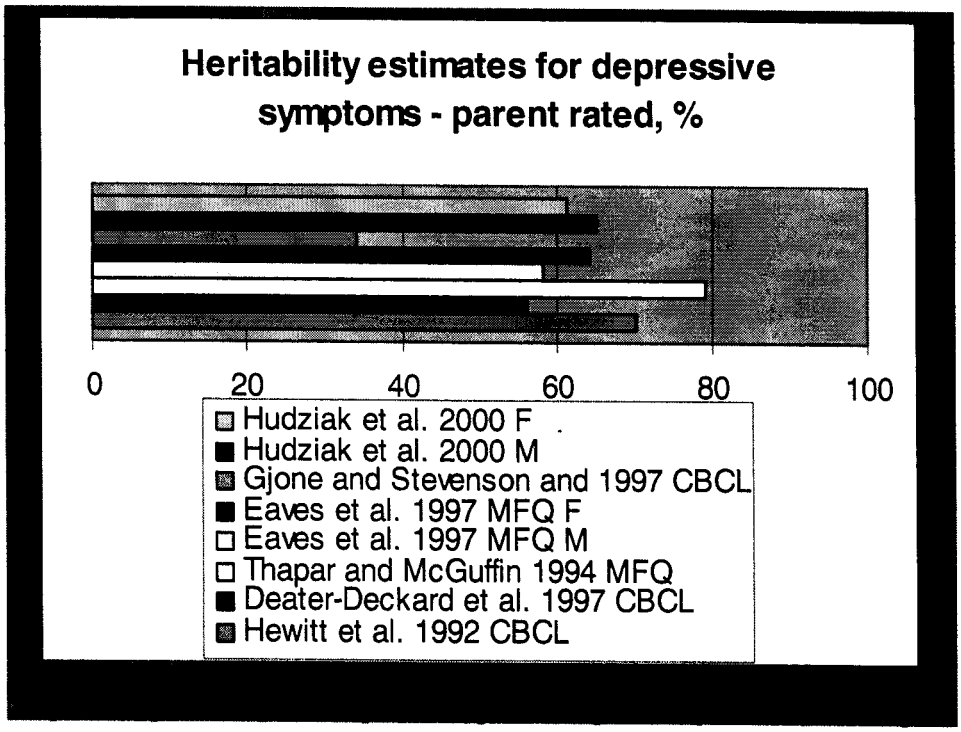
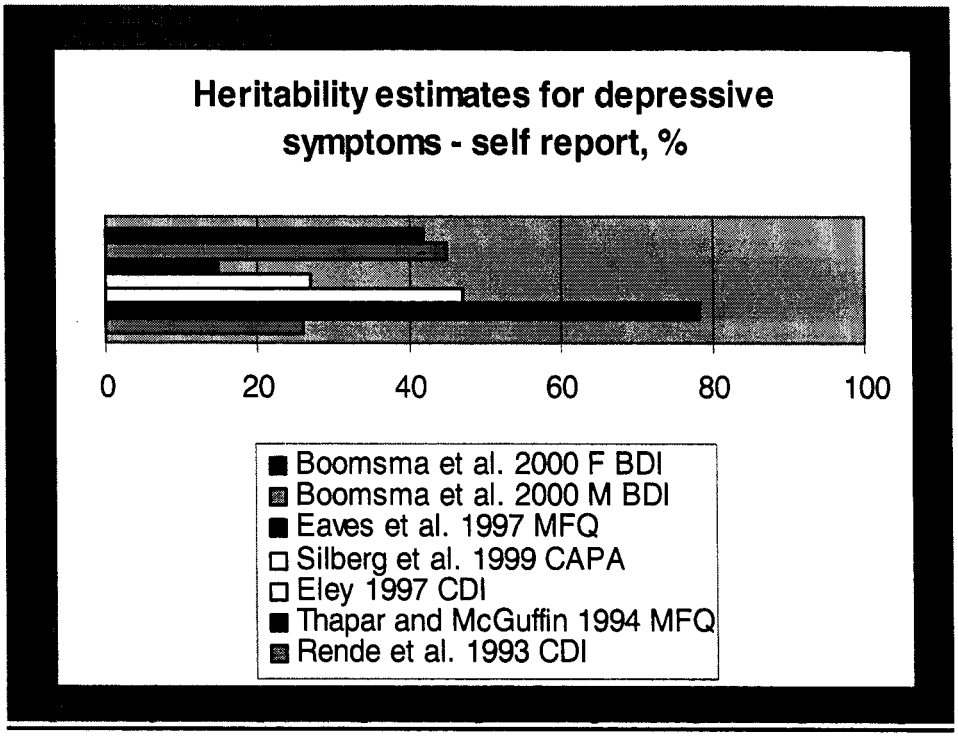


Figure 5.



Figures 4 and 5 adapted from Rice et al 2002.

1.3.2 Heritability of Memory

As a subphenotype or endophenotype of depression, it is necessary to demonstrate heritability of the proposed characteristic (Gottesman and Gould 2003). Memory phenotypes have been shown to be influenced by genetic factors and by environmental or stochastic factors. In a twin study of memory in elderly subjects, the maximum likelihood estimate of memory heritability was 0.52. Another investigation remarked a heritability for verbal memory of 0.21 and a heritability for visuo-spatial memory of 0.36 (Tuulio-Henrikssen et al. 2002). With documented heritability, it is easy to argue that memory phenotypes merit molecular genetic study.

1.4 Molecular Genetic Investigations

1.4.1 Molecular Studies in COMD

A next step after establishing heritability for COMD is to do molecular investigations. A small number of studies exploring genetic polymorphisms and mood symptoms in children have been published, mainly involving genes involved in serotonin and dopamine metabolism. The serotonin tranporter (SLC6A4) is the site of action of commonly used antidepressants and is commonly studied in mood disorders. COMT affects dopamine metabolism; dopaminergic drugs are often associated with symptoms similar to those observed with mania. A longitudinal twin study of emotional development indicated no association between internalizing symptoms and a functional polymorphism in the serotonin transporter (5-HTTLPR) (Young et al. 2003). In another report, the *l/l* genotype of the 5-HTTLPR was associated with decreased platelet serotonin uptake rate in depressed subjects compared to controls (Nobile et al. 1999). Two investigations of juvenile BP indicated no association between the 5-HTTLPR variant and juvenile BP (Geller and Cook 1999) nor between bipolar ultradian cycling and the COMT $Val^{108/158}$ Met polymorphism (Geller and Cook 2000). Until this year, there has been nothing published on BDNF as a candidate gene for juvenile mood disorders.

1.4.2 Genetic Studies Relevant to BDNF and COMD

Genetic studies of linkage, function and association have piqued interest in BDNF is a possible risk factor for mood disorder phenotypes. Linkage studies suggest that the 11p13-14 region may contain genetic variants affecting vulnerability to mood disorder (Detera-Wadleigh et al. 1999; McInnes et al. 1996). Data specifically focused on BDNF

polymorphisms show association with hippocampal function and affective disorders. Egan et al. (2003) have examined the Val^{66}Met SNP and indicated that in transfected neurons, the methionine (met) allele reduced activity-dependent BDNF secretion, and that in humans the met allele was associated with impaired hippocampal synaptic activity and deficits in episodic memory. A family-based association study of bipolar disorder using 283 trios indicated the 170 bp allele of the $(GT)_n$ marker and the val allele of the Val^{66}Met were associated with bipolar disorder (Neves-Pereira et al. 2002). Another study of 470 bipolar disorder trios from three samples showed statistically significant overtransmission of the same Val^{66}Met val allele to probands in one sample and trends towards significance in the other two samples (Sklar et al., 2002). The association between the BDNF Val^{66}Met SNP and bipolar disorder failed to be replicated in a Japanese case-control paper (n=132 cases, n=190 controls) (Nakata et al. 2003). Overall, previous investigations offer strong empirical support for more study of the BDNF locus in mood disorders.

1.4.3 Genetic Investigation of BDNF in Declarative Memory Function

Recent reports indicate that the BDNF Val^{66}Met SNP has effects on BDNF distribution, hippocampal function and performance on a declarative memory task in humans (Egan et al. 2003; Hariri et al. 2003). *In vitro* experiments noted that the met-BDNF had less dendritic expression and depolarization-induced secretion than val-BDNF. *In vivo* data remarked an allele dose effect, with more met alleles being associated with a diminished hippocampal synaptic activity gauged by N-acetyl-aspartate levels. Data were also discussed concerning human memory indicating that BDNF genotype affected

participants' facility to recall brief narratives as manifested by their score on the Logical Memory subtest of the Wechsler Memory Scale, Revised (WMS-R). Individuals homozygous for the met allele recalled less information than others (Egan et al. 2003). Synthesizing the results of the aspects of the study, the authors conclude that the BDNF met allele may influence declarative memory by affecting the intracellular movement of BDNF, thereby modifying synaptic activity in the hippocampus. Lastly, findings in Alzheimer's disease suggest BDNF and its gene may play a role in human memory function (Garzon et al. 2002; Riemenschneider et al. 2002).

1.4.4 Catechol-O-Methyltransferase and Memory

Aside from BDNF, the catechol-O-methyltransferase (COMT) locus has been studied in cognitive phenotypes. Strong evidence supports a rationale for investigating the COMT gene in memory abilities. COMT degrades dopamine by methylation and can impact prefrontal function. The methionine (met) variant of a functional SNP at codon 108/158 (COMT $Val^{108/158}$ Met) (Genbank accession no. Z26491) causes lessened dopamine catabolism in the prefrontal cortex. Accordingly, the $Val^{108/158}$ Met met allele has been associated with more optimal executive function and, to a lesser extent, better working memory in adults (Egan at al. 2001). Another report on the COMT $Val^{108/158}$ Met SNP in adults with schizophrenia found the met variant was associated with processing speed and attention, but not with measures of executive function or verbal declarative memory (Bilder et al. 2002). Although the strongest evidence finds effects of COMT on prefrontal function and working memory (Egan et al. 2001; Bilder et al. 2002), animal and human studies also suggest that COMT also has effects on spatial and verbal declarative memory abilities. Tolcapone, which is a COMT antagonist, improved ability

on a spatial memory test (Liljequist et al. 1999) and also partly repaired memory dysfunction caused by cholinotoxin damage (Khromova et al. 1997). Augmentation of verbal and visuospatial memory has also been observed in human studies of tolcapone treatment (Gasparini et al. 1997). Because of the associations between mood disorder and memory and between memory and COMT, the locus warrants investigation as a potential genetic influence on memory abilities in mood disorders. At present, there appear to be no reports that examine for possible association between COMT genotypes and memory in human subjects with affective disorders.

1.5 Justification for Statistical Design

1.5.1 COMD phenotype and Case-Control Analysis

Case-control association studies have been popular in the genetic investigation of neuropsychiatric disorders. Their appeal in part stems from successes in complex disorders – genetic case-control association methods have yielded significant positive results in Alzheimer's disease (Farrer et al. 1997) and Type I diabetes (Cudworth and Wolfe 1982). Nonetheless case-control association studies have been surrounded by controversy (Sullivan et al. 2001) and have caused confusion as initially positive studies often are followed by multiple nonreplications. False positives in case-control studies may result from population stratification, which occurs when a sample is composed of two or more subgroups with differing genetic histories. False positives can occur when a trait is more prevalent in one subgroup and if the prevalence of the genetic marker also differs by subgroup. Several issues related to case and control selection may help to reduce bias contributing to spurious positive results. Participants should not be related; lifetime phenotype should be considered; comparable periods of risk should exist

between cases and controls; cases and controls should be matched by ethnicity. The above measures serve increase homogeneity and comparability of cases and controls. It may also be useful to study subphenotypes where genetic factors may be more important – e.g. early onset MDD (Sullivan et al. 2001). Subphenotypes reduce clinical heterogeneity by focusing on a single dimension of a complex phenotype. Genomic control strategies can be used to evaluate the degree of stratification and to adjust for population substructure (Devlin and Roeder 1999; Bacanu et al. 2000). In this design, multiple markers across the genome are used to determine the correction for case-control (i.e. population-based) tests, - a variance inflation (λ) is calculated using Bayesian methods.

1.5.2 Memory phenotypes and MANOVA for Correlated Variables

Analysis of variance discerns whether variation associated with independent or predictor variables is sufficiently large when compared to variation within experimental subjects, to conclude that the predictor variables differ in their effects (Glantz and Slinker 2001 p274). Multivariate analysis of variance (MANOVA) is used to test the hypothesis that two or more dependent variables which are correlated are together affected by independent variables. The correlation between dependent variables is taken into account in the MANOVA (http://www.statsoftinc.com/textbook/stathome.html; Statsoft, Inc. 2004). MANOVA is useful for memory subtests which are often not independent.

1.6 Hypotheses and Purpose of Study

Emerging findings implicating the BDNF gene in mood disorders suggest that further investigation of this locus is important. BDNF is associated with biochemical,

microscopic and behavioural antidepressant-like effects in animal stress paradigms. Human physiologic, neuroimaging and genetic studies lend support to its investigation as a candidate locus for affective disorders. Moreover, BDNF has demonstrated hippocampal expression and been implicated in memory. Both preclinical and human studies also suggest COMT has relevance to memory phenotypes.

The first goal of the study was to evaluate two BDNF polymorphisms, $(GT)_n$ and $Val^{66}Met$ for association with mood disorder in our sample of cases with onset in childhood and early adolescence. The second goal of this study was to explore the relationships between childhood-onset mood disorder, the presence of BDNF and COMT polymorphisms and declarative memory abilities. Based on our review of the extant literature, we hypothesized that in a sample of young adults with onset of mood disorder before the age of 14, relatively poorer declarative memory would be found in those with the met allele of the $Val^{66}Met$ marker of BDNF. While one previous study in schizophrenia was negative (Bilder et al. 2002), the COMT $Val^{108/158}$ Met marker is associated with reduced dopamine breakdown; reduced dopamine catabolism has been associated with improved verbal and spatial memory performance (Gasparini et al. 1997; Liljequist et al. 1999). Our secondary hypothesis was that better verbal/declarative or visuospatial memory performance would be associated with the presence of the met allele of the COMT $Val^{108/158}$ Met marker in the current sample.

CHAPTER 2.0

ASSOCIATION STUDY OF BRAIN-DERIVED NEUROTROPHIC FACTOR IN ADULTS WITH A HISTORY OF CHILDHOOD-ONSET MOOD DISORDER

2.1

Association Study of Brain-Derived Neurotrophic Factor in Adults with A History of Childhood Onset Mood Disorder

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ABSTRACT

Background: Brain-derived neurotrophic factor (BDNF) is a nerve growth factor that has antidepressant-like effects in animals. BDNF gene polymorphisms have been associated with bipolar disorder. We tested two genetic polymorphisms of BDNF for their association with childhood-onset mood disorders (COMD) within the context of a case-control design.

Methods: Two BDNF polymorphisms, a dinucleotide repeat, $(GT)_n$, and a single nucleotide polymorphism (SNP) in the coding region, val66met, were genotyped in 99 adults with a history of COMD and matched psychiatrically healthy controls. A Genomic Control (GC) method was used to evaluate population substructure.

Results: Alleles at $(GT)_n$ were highly associated with COMD in this sample (χ^2= 17.8; d.f.=5; p= 0.0032). The odds of carrying the 168 bp allele were 3.94 times greater for cases than controls (CI = 1.72 to 9.04). Alleles of val66met were not significantly associated with COMD. GC analysis suggested population substructure was not a confounder of association. Analysis of haplotypes, in which $(GT)_n$ was treated as a binary variable (long versus short alleles), provided significant evidence that the haplotype val/short contributes to liability to COMD.

Conclusions: The BDNF $(GT)_n$ marker and the val/short haplotype are associated with COMD in this sample, in accord with the previously described neurotrophic hypothesis of depression and some previous studies of association for bipolar disorder and neuroticism.

2.1.1 Introduction

The role of hereditary factors in vulnerability to depressive symptoms has empirical support in both youth and adult populations [Englund and Klein, 1990; Kendler et al., 1992; Hudziak et al., 2000]. To disentangle genetic contributors, strategies have included traditional genetic linkage analysis and association studies of candidate genes. Candidate genes are hypothesized to contribute to a disease based on being part of a metabolic pathway important to the phenotype. One potential candidate gene for mood disorder phenotypes is brain-derived neurotrophic factor (BDNF). BDNF is a nerve growth factor that influences neuronal survival and plasticity [Thoenen, 1995]. The BDNF gene is localized to chromosome 11p14.1 [Fang et al., 2003] and has several polymorphic markers. One BDNF polymorphism is an intronic microsatellite $(GT)_n$ dinucleotide repeat [Proschel et al., 1992]. Another marker is a functional coding region single nucleotide polymorphism (SNP) at nucleotide position 196/758, which results in an amino acid change at codon 66 val→met (val66met) (NCBI SNP Cluster ID: rs6265) of the proBDNF molecule. The SNP is located in a section of the BDNF precursor protein that is cleaved away, rendering the amino acid change absent from mature BDNF.

A role for BDNF in stress-related depression via cellular signaling has been described in animal models [Duman, 2002]. Repeated antidepressant administration, including electroconvulsive seizures, increases hippocampal BDNF expression [Nibuya et al., 1995; Duman and Vaidya, 1998] and neurogenesis [Madsen et al., 2000; Malberg et al., 2000; Santarelli et al., 2003].

Human experiments, including neuroimaging [Sheline et al., 1996; Sheline et al., 2003], postmortem [Chen et al., 2001] and clinical [Karege et al., 2002, Shimizu et al., 2003] investigations support findings in the preclinical BDNF literature. Previous human

linkage studies suggest that the 11p13-14 region could harbor alleles affecting susceptibility to mood disorder [Detera-Wadleigh et al., 1999; McInnes et al., 1996]. Emerging data specifically focused on BDNF polymorphisms show association with hippocampal dysfunction and mood disorders. Recently Egan et al. [2003] studied the val66met SNP and demonstrated that in transfected neuronal cells, the methionine (met) variant impaired activity-dependent BDNF secretion, and that in humans the met allele was associated with reduced hippocampal synaptic activity and poorer episodic memory. An association study of bipolar disorder using 283 triad families indicated the 170 bp allele of the $(GT)_n$ marker and the val allele of the val66met were associated with illness, suggesting the site conferring risk was in linkage disequilibrium with both markers [Neves-Pereira et al., 2002]. A larger study involving 470 bipolar trios from three separate samples showed significant overtransmission of the same val66met val allele to probands in one sample and trends towards overtransmission in the other two samples [Sklar et al., 2002]. However, association between the BDNF val66met polymorphism and bipolar disorder was not replicated in a Japanese study of 132 cases and 190 controls [Nakata et al., 2003]. Emerging findings implicating the BDNF gene in mood disorders suggest that further investigation of this locus is important. We evaluated two BDNF polymorphisms for association with mood disorder in our sample of cases with onset in childhood and early adolescence. This genetic study occurs in the context of a larger multidisciplinary research project on risk factors for and correlates of juvenile-onset mood disorder, whose overarching theme concerns the characteristics, development, and utilization of emotion regulatory strategies.

2.1.2 Methods and Materials

Subjects

Sixty-one cases in our sample were originally ascertained as clinic-referred children between the ages of 8 and 13 in Pittsburgh, PA and were followed longitudinally [Kovacs et al., 1984, 1994]. Diagnoses were ascertained at initial and repeated follow-up assessments through a standardized semi-structured interview, the Interview Schedule for Children and Adolescents or its version for young adults [Sherril and Kovacs, 2000], as age appropriate. Another 42 cases were ascertained in Pittsburgh as adults through a variety of means (e.g. prior research study participants, self-referred in response to advertisements). Diagnoses in these subjects were determined based on the Structured Clinical Interview for DSM-IV [First et al., 1995] with the subject about him/herself, a separate interview about the subject with a second informant, and research and medical records. Consent (or assent) were obtained from all subjects and conformed to IRB guidelines. All cases met DSM-III [APA, 1980] or DSM-IV [APA, 1994] criteria for affective disorder (MDD, n=66; DD, n=14; (bipolar I/ II disorder) BP, n=23) with onset in childhood or adolescence. Participants provided blood samples and sometimes cheek swabs for DNA extraction at a mean age of 25.7 (SD=3.5) years. The ethnic distribution of the sample was 81% Caucasian, 16% African American, and 3% mixed ethnicity, and there were altogether 60 (58%) females.

Controls

COMD subjects were individually matched to controls for sex and ethnicity. Control subjects were healthy adults who had no history of mental illness based on screening questions from the SCID interview. Controls were ascertained from a variety of sources such as undergraduate university students, newspaper advertisements, and surgical

clinics, both in Toronto and through collaborators. The mean age of controls was 32.2 (SD=9.7) years.

Genotyping

DNA was extracted from lymphocytes using a high salt method [Lahiri and Nurnberger, 1991] and from buccal swabs using NaOH and Proteinase K (details available upon request). The $(GT)_n$ variant was genotyped using PCR conditions previously described [Proschel et al., 1992]. The products were resolved on a 6% polyacrylamide gel and visualized by autoradiography. Results were confirmed using the ABI PRISM® 3100-Avant Genetic Analyzer (Applied Biosystems, Foster City CA). The val66met SNP was selected from the NCBI SNP database (SNP Id: rs6265). PCR amplification used the following primers: 5'-GAGGCTTGACATCATTGGCT-3' and 5'-CGTGTACAAGTCTGCGTCCT-3'. The PCR products were digested with *Eco*72I (MBI Fermentas) and the fragments were separated on 3.5% agarose gel and visualized with ethidium bromide. Genomic control testing was performed in the cases and controls with 22 informative markers randomly distributed across the genome.

Statistical analysis

Chi-square, genomic control analyses and regression models utilised SAS V8 [SAS Institute, 1999]. The HWE program of the LINKUTIL package was used to test Hardy-Weinberg assumptions [Ott, 1991]. Genomic control analysis provides a factor by which one can correct for potential stratification [Devlin and Roeder, 1999]. There were no corrections for multiple testing. Haplotype analysis made use of eHap, a freeware package that makes use of evolutionary relationships among haplotypes to examine whether certain haplotypes are associated with illness vulnerability [Seltman et al., 2003].

2.1.3 Results

There were no detectable deviations from Hardy-Weinberg equilibrium (HWE) for the val66met SNP for either COMD or control groups; there were no detectable deviations from HWE when COMD and control groups were split and examined according to ethnicity. For the $(GT)_n$ marker, no differences between observed and expected homozygosities were observed in cases or controls. Based on the distribution of val66met alleles and genotypes (Table 1.), neither the allele nor the genotype frequencies were significantly different between cases and controls. [Table 1 about here.] By contrast, the allele distribution of $(GT)_n$ for COMD individuals (Table 2.) differed significantly from control individuals (χ^2=17.8; d.f.=5; p= 0.0032). In particular, there was a significant excess of the 168 bp allele (allele 4; odds ratio=3.94, 95%CI=1.72 to 9.04) in cases. [Table 2 about here.]

Genomic control (GC) analysis [Devlin and Roeder, 1999] revealed a robust correction factor of 0.656 across the 22 biallelic markers (using the mean chi-square yields a similar correction of 1.36). Because the distribution of the χ^2 values for the 22 case-control

comparisons showed variance close to that expected by chance – expected correction for a population without substructure is 1.0 - we did not use a GC correction for our analyses. Haplotype analysis was done by dividing the $(GT)_n$ alleles into two categories, namely short (166 & 168 bp) and long (≥170 bp), to reduce the number of haplotypes created by combining microsatellite and SNP markers. The four haplotypes were val/long, val/short, met/long and met/short. Maximum likelihood estimation of the haplotype frequencies revealed substantial differences in their distribution by case/control sample (See Table 3.). We performed a likelihood ratio test after eliminating the one individual carrying the met/long haplotype. This analysis, which used eHap [Seltman et al., 2003], shows the distributions to be significantly differentiated (χ^2 = 13.8, d.f.=2, p=0.0032). Using eHap we also performed a test based on cladistic relationships among haplotypes for this sample. For these two loci, the one-step-mutation cladogram was quite simple, namely met/long connected to val/long, which is then connected to val/short. Nested tests described in Seltman et al. [2003] group met/long and val/long as having a similar effect on affection status, and highlight the increased risk for COMD due to the val/short haplotype (roughly 2.6 fold) relative to the met/long and val/long haplotypes (contrast: χ^2 = 13.1, d.f.=1, p=0.0003). It is worth noting that the haplotype-based results might be driven solely by the marginal effect of the $(GT)_n$ locus. [Table 3 about here.]

Linkage disequilibrium between the val66met and $(GT)_n$ markers calculated for the COMD and control samples using the data in Table 3 yields an estimate of Lewontin's [1964] D' of 0.422 and 1.0, respectively. The analyses are based on the frequency of the val/short haplotype.

2.1.4 Discussion

While our data indicate an association between the 168 bp allele of the BDNF $(GT)_n$ dinucleotide repeat and COMD, this finding should be interpreted with caution. Limitations to the present report include the diagnostic heterogeneity of mood disorders within the sample and the relatively small sample size. It is also conceivable that our results may be confounded by population substructure [Devlin et al., 2001a; 2001b]. The GC results provide evidence against confounding due to substructure, suggesting that population stratification due to ethnicity is not likely to affect our results. Under the assumption that the risk allele is the $(GT)_n$ 168 bp allele, and for the results given in Table 2, we estimate that a new sample size of n=102 case-control pairs will be required to replicate our results with 80% power (α=0.05).

Results of Egan and colleagues support a role for BDNF in hippocampal function and a functional role for the val66met SNP, which could be germane to mood disorders [e.g. Sheline et al., 2003; Egan et al., 2003]. As noted previously, among adults with bipolar disorder, associations with both the BDNF $(GT)_n$ repeat polymorphism and the BDNF val66met SNP support the hypothesis that BDNF variants are risk factors for affective disorder [Neves-Perieira et al., 2002; Sklar et al., 2002]. Alternately, the $(GT)_n$ polymorphism may be in linkage disquilibrium with a true risk marker within or near this locus.

Other genetic investigations of BDNF in human psychiatric disorders are of note. Sen and colleagues reported that the val allele of the BDNF val66met polymorphism is associated with neuroticism, a heritable risk factor for depression [Sen et al., 2003]. Two large published studies have undertaken analysis of BDNF markers in adult bipolar disorder. The haplotype analysis of Sklar et al. [2002] indicated a haplotype containing

the a39 (our val66met) minor met allele was undertransmitted. Neves-Pereira et al.[2002] found the 170 bp/G (i.e. 170 bp/val) haplotype overtransmitted to cases. In our data, a small number of cases (23%) were diagnosed as having bipolar disorder. Predictably, because the small sample has low power, analysis of that subsample did not yield significant association to val66met alleles.

Our results and related reports provide encouraging evidence for BDNF as a genetic risk factor in mood disorders. This body of literature implicates BDNF also as a potential genetic influence on the development of emotion regulation. Specifically, our results suggest the $(GT)_n$ polymorphism in the BDNF locus could be a risk factor for COMD, especially the 168 bp allele, although other loci in the gene or the surrounding region cannot be ruled out. Studies of its functional significance and follow-up studies of association of BDNF polymorphisms will be required to clarify the role genetic variation in BDNF plays in risk to mood disorders.

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2.1.5 References

American Psychiatric Association. 1980. Diagnostic and Statistical Manual of Mental Disorders, 3rd ed. Washington DC: American Psychiatric Press.

American Psychiatric Association. 1994. Diagnostic and Statistical Manual of Mental Disorders, 4th ed. Washington DC: American Psychiatric Press.

Chen B, Dowlatshahi D, MacQueen G, Wang J, Young LT. 2001. Increased hippocampal BDNF immunoreactivity in subjects treated with antidepressant medication. Biol Psychiatry 50: 260-265.

Detera-Wadleigh SD, Badner JA, Berrettini WH, Yoshikawa T, Goldin LR, Turner G et al. 1999. A high-density genome scan detects evidence for a bipolar-disorder susceptibility locus on 13q32 and other potential loci on 1q32 and 18p11.2. Proc Natl Acad Sci USA 96: 5604-9.

Devlin B and Roeder K. 1999. Genomic Control for Association Studies. Biometrics 55: 997-1004.

Devlin B, Roeder K, Bacanu S-A. 2001a. Unbiased methods for population-based association studies. Genet Epidemiol 21: 273-284.

Devlin B, Roeder K, Wasserman L. 2001b. Genomic control, a new approach to genetic-based association studies. Theor Pop Biol 60: 156-166.

Duman RS. 2002. Synaptic plasticity and mood disorders. Mol Psychiatry 7: S29-S34.

Duman RS, Vaidya VA. 1998. Molecular and cellular actions of chronic electroconvulsive seizures. J ECT 14: 181-93.

Egan MF, Kojima M, Callicott JH, Goldberg TE, Kolachana BS, Bertolino A et al. 2003. The BDNF val66met polymorphism affects activity-dependent secretion of BDNF and human memory and hippocampal function. Cell 112: 257-269.

Englund SA, Klein DN. 1990. The genetics of neurotic-reactive depression: a reanalysis of Shapiro's twin study using diagnostic criteria. J Affect Disord 18: 247-252.

Fang H, Chartier J, Sodja C, Desbois A, Ribecco-Lutkiewicz M, Walker PR et al. 2003. Transcriptional activation of the human BDNF gene promoter III by dopamine signaling in NT2/N neurons. J Biol Chem 278: 26401-9.

First MB, Spitzer RL, Gibbon M, Williams JBW. 1995. Structured Clinical Interview for DSM-IV Axis I Disorders - Patient Edition (SCID-I/P, Version 2.0). New York: Biometrics Research Department, New York State Psychiatric Institute.

Hudziak JJ, Rudiger LP, Neale MC, Heath AC, Todd RD. 2000. A Twin Study of Inattentive, Aggressive and Anxious/Depressed Behaviors. J Am Acad Child Adolesc Psychiatry 39: 469-476.

Karege F, Perret G, Bondolfi G, Schwald M, Bertschy G, Aubry J. 2002. Decreased serum brain-derived neurotrophic factor levels in major depressed patients. Psychiatry Research 109: 143-148.

Kendler KS, Neale MC, Kessler RC, Heath AC, Eaves LJ. 1992. A population-based twin study of major depression in women. Arch Gen Psychiatry 49: 273-281.

Kovacs M, Feinberg TL, Crouse-Novak MA, Paulauskas SL, Finkelstein R. 1984. Depressive disorders in childhood. I. A longitudinal prospective study of characteristics and recovery. Arch Gen Psychiatry 41: 229-237.

Kovacs M, Akiskal HS, Gatsonis C, Parrone, P. 1994. Childhood-onset dysthymic disorder. Clinical features and prospective naturalistic outcome. Arch Gen Psychiatry 51: 365-374.

Lahiri DK, Nurnberger JI Jr. 1991. A rapid non-enzymatic method for the preparation of HMW DNA from blood for RFLP analysis. Nucleic Acids Research 19: 5444.

Lewontin RC. 1964. The interaction of selection and linkage. I. General considerations; heterotic models. Genetics 49: 49-67.

Madsen TM, Treschow A, Bengzon J, Bolwig TG, Lindvall O, Tingstrom A. 2000. Increased neurogenesis in a model of electroconvulsive therapy. Biol Psychiatry 47: 1043-1049.

Malberg JE, Eisch AJ, Nestler EJ, Duman RS. 2000. Chronic antidepressant treatment increases neurogenesis in adult rat hippocampus. J Neurosci 20: 9104-10.

McInnes LA, Escamilla MA, Service SK, Reus VI, Leon P, Silva S et al. 1996. A complete genome screen for genes predisposing to severe bipolar disorder in two Costa Rican pedigrees. Proc Natl Acad Sci USA 93: 13060-5.

Nakata K, Ujike, H, Sakai A, Uchida N, Nomura A, Imamura T et al. 2003. Association study of the brain-derived neurotrophic factor (BDNF) gene with bipolar disorder. Neurosci Lett 337: 17-20.

Neves-Pereira M, Mundo E, Muglia P, Kennedy JL, Macciardi F. 2002. The brain derived neurotrophic factor gene confers susceptibility to bipolar mood disorder: evidence from a family-based association study. Am J Hum Genet 71: 651-655.

Nibuya M, Morinobu S, Duman RS. 1995. Regulation of BDNF and trkB mRNA in rat brain by

chronic electroconvulsive seizure and antidepressant drug treatments. J Neurosci; 15: 7539-7547.

Ott J. 1991. Analysis of Human Genetic Linkage. Baltimore, MD: John Hopkins University Press.

Proschel M, Saunders A, Roses AD, Muller CR. 1992. Dinucleotide repeat polymorphisms at the human gene for brain-derived neurotrophic factor (BDNF). Hum Mol Genet 1: 353.

Santarelli L, Saxe M, Gross C, Surget A, Battaglia F, Dulawa S et al. 2003. Requirement of Hippocampal Neurogenesis for the Behavioral Effects of Antidepressants. Science 301: 805-809.

SAS Institute Inc. 1999. SAS OnlineDoc®, Version 8. Cary NC: SAS Institute Inc.

Seltman H, Roeder K, Devlin B. 2003. Evolutionary-based association analysis using haplotype data. Genet Epidemiol 25: 48-58.

Sen S, Nesse RM, Stoltenberg SF, Li S, Gleiberman L, Chakravarti A et al. 2003. A BDNF coding variant is associated with the NEO personality inventory domain neuroticism, a risk factor for depression. Neuropsychopharmacology 28: 397-401.

Sheline YI, Wany P, Gado MH, Csernansky JG, Vannier MW. 1996. Hippocampal atrophy in recurrent major depression. Proc Natl Acad Sci USA 93: 2897-2902.

Sheline YI, Gado MH, Kraemer HC. 2003. Untreated depression and hippocampal volume loss. Am J Psychiatry 160: 1516-1518.

Sherril JT, Kovacs M. 2000. Interview Schedule for Children and Adolescents (ISCA). J Am Acad Child Adolesc Psychiatry 39: 67-75.

Shimuzu E, Hashimoto K, Okamura N, Koike K, Komatsu N, Kumakiri C et al. 2003. Alterations of serum levels of brain-derived neurotrophic factor (BDNF) in depressed patients with or without antidepressants. Biol Psychiatry 54: 70-75.

Sklar P, Bolk S, Mc Innis MG, Bennett P, Lim Y-M, Schaffner S et al. 2002. Family-based association study of 76 candidate genes in bipolar disorder: BDNF is a potential risk locus. Mol Psychiatry. 7: 579-593.

Thoenen H. 1995. Neurotrophins and neuronal plasticity. Science 270: 593-598.

Tables

Table 1: BDNF val66met allele and genotype frequencies

	BDNF val66met allele			BDNF val66met genotype			
	met	val	Total	met/met	val/met	val/val	Total
COMD	42	156	198	5	32	62	99
Controls	34	164	198	3	28	68	99
A.A.:							
COMD	4	36	40	0	4	16	20
Control	5	35	40	1	3	16	20
Caucasian:							
COMD	38	120	158	5	28	46	79
Control	29	129	158	2	25	52	79

Ailele χ^2=1.0; d.f.=1; p=0.31 Genotype χ^2=1.0; d.f.=2; p=0.59

(A.A.= African American)

Table 2: BDNF GT(n) polymorphism allele frequencies

BDNF GT(n) allele n=93

	Allele 0 176 bp	Allele 1 174 bp	Allele 2 172 bp	Allele 3 170 bp	Allele 4 168 bp	Allele 5 166 bp	Total
COMD	1	39	7	107	27	5	186
Controls	2	35	15	125	8	1	186
A.A.:							
COMD	1	2	3	16	10	0	32
Controls	1	1	5	24	1	0	32
Caucasian:							
COMD	0	37	4	91	17	5	154
Controls	1	34	10	101	7	1	154

For total sample Table 2 (African American (A.A.) + Caucasians):
χ^2= 17.8; d.f.=5; p= 0.0032 ;
Allele 2 (172 bp): OR=0.55; 95%CI=[0.21-1.39];
Allele 4 (168 bp): OR= 3.94; 95%CI=[1.72 to 9.04]

Table 3: Maximum-likelihood estimates of haplotype frequencies for COMD+Controls, COMD only and Controls only.

Sample	**Haplotype**			
	Val/Long	Val/Short	Met/Long	Met/Short
COMD+Controls	0.7058	0.1027	0.1837	0.0078
COMD	0.6299	0.1590	0.1970	0.0140
Controls	0.7798	0.0483	0.1719	0.0000

See Results for test statistics.

Supplemental Material:

Association Study of Brain-Derived Neurotrophic Factor in Adults with A History of Childhood Onset Mood Disorder

Strauss et al.

Introduction

Several sources of evidence support our use of a phenotype that includes major depressive disorder (MDD), dysthymic disorder (DD) and bipolar disorder (BP). Regarding symptoms and outcome, there is great overlap between MDD and DD [Kovacs et al., 1994]. Considering natural history, BP develops in about 30% of prepubertal children with MDD [Geller et al., 1994] and in about 20% of adolescents with MDD [Strober and Carlson, 1982], rates that are an order of magnitude higher than in the general population. Family studies also indicate a much higher prevalence of BP in first- and second-degree relatives of childhood MDD probands than in the general population [Todd et al., 1993] and a much higher prevalence than in adult-onset MDD [Neuman et al., 1997]. Neuman et al. [1997] also found that first-degree relatives of childhood-onset mood disorder probands have higher rates of affective disorder than adult-onset mood disorder probands. It has been proposed that genetic contributions to liability to mood disorder may be more readily identified among very early onset probands [Todd et al., 1993]. To summarize, the findings suggest that there is sufficient phenomenological continuity and familiality across the three diagnoses to consider their use as a single phenotype potentially more homogeneous than adult-onset MDD.

Methods and Materials

Each subject with COMD was individually matched to a control with the same ethnic background. The ethnic matching was based on detailed descriptions of ancestry. For

example, a randomly selected pair from the data set is a COMD case of Scottish, Irish, Polish and German descent matched to a control of British and German origins. Subjects were also matched for sex in 99 of the 103 cases (96%). Of the samples available, 99 case-control pairs were successfully genotyped for the val66met SNP; and 93 pairs were genotyped for the $(GT)_n$ microsatellite.

Results

The HWE val66met test statistics were χ^2=0.11, d.f.=1, p=0.74 and χ^2=0.01, d.f.=1, p=0.95 for case and control samples respectively. Because the relative frequencies of several $(GT)_n$ alleles were small compared to the group sizes, we tested HWE by contrasting observed versus expected homozygosity. For the control group, observed versus expected homozygosities were 55.9% versus 50.0% (χ^2=1.50, d.f.=1, p=0.22); for the cases, observed versus expected homozygosities were 49.5% versus 39.8% (χ^2=3.62, d.f.=1, p=0.06).

An analysis of the GT repeat by ethnicity showed similar associations in both African Americans and Caucasians (not shown). To examine the potential confounding by ethnicity in more detail, we constructed a logit model incorporating terms for self-identified ethnicity (E), presence/absence of one or more 168 bp alleles (A), and their interaction (A*E). In the logit model, only the presence of 168 bp alleles significantly affected the outcome (A: χ^2=9.32, d.f.=1, p=0.002; E: χ^2=0.94, d.f.=1, p=0.33; A*E: χ^2=2.04, d.f.=1, p=0.15). It should be recognized that small sample size limited our power to distinguish subtle differences in association between ethnic subgroups. Finally, because we matched cases and controls based on ethnicity and gender, association between candidate loci and COMD was also evaluated using conditional logistic regression to account for the matching [Breslow and Day, 1980]. The conditional logistic

regression analysis, using the presence/absence of one or more 168 bp alleles as the genetic variable, again showed a significant association between the 168 bp allele and COMD status (Likelihood Ratio χ^2 =18.82, d.f.=5, p=0.002; Odds Ratio (168 bp heterozygotes vs. 170 bp homozygotes)=5.60, 95% CI=[1.85-16.98]). Maximum likelihood estimate analysis of clinical subgroups (BP, MDD, DD) yielded no significant results.

Discussion

When we used regression methods to test for different associations attributable to ethnicity, none could be detected.

Although the functional relevance of the $(GT)_n$ polymorphism is unknown, other dinucleotide repeat polymorphisms that alter gene expression have been noted by schizophrenia researchers - variation in expression has been associated with number of repeats, offering additional rationale for our dichotomizing the $(GT)_n$ into long and short repeats [Miyatake et al,. 2002; Itokawa et al., 2003].

In the Michigan sample, the A (met) allele was associated with lower neuroticism scores. Val66met genotype was significantly associated with four of the six neuroticism facets [Sen et al., 2003].

Recent results from Taiwan found no association between val66met genotype and adult MDD [Tsai et al., 2003]. The $(GT)_n$ marker was not evaluated in either paper. While our single marker analysis did not show the val allele to be significant, the val/short haplotype was significant, though this may be due to the $(GT)_n$ 168 bp allele association. The BDNF val66met polymorphism has also been associated with obsessive-compulsive disorder - notably the minor met allele was undertransmitted, especially in the subgroup with child- or adolescent-onset. The association was independent of mood disorder [Hall

et al., 2003]. BDNF may be relevant to phenotypes that have significant [Sen et al., 2003] or no [Hall et al., 2003] overlap with mood disorders.

Related to BDNF by being in the same molecular cascade [Duman, 2002] is cAMP response element binding protein (CREB). Chronic antidepressants typically upregulate hippocampal CREB [Nibuya et al., 1996] and the CREB1 gene has been associated with major depressive disorder in women with age of onset less than twenty-five [Zubenko et al., 2002]. Evidence supports the hypothesis that antidepressants exert neuroprotective effects by augmenting CREB and BDNF expression and increasing neurogenesis in the hippocampus. The post-receptor cAMP-CREB-BDNF metabolic pathway appears to have increasing pertinence to mood disorders.

References

Breslow NE, Day NE. 1980. Statistical methods in cancer research. Lyon: International Agency for Research on Cancer.

Geller B, Fox LW, Clark KA. 1994. Rate and predictors of prepubertal bipolarity during follow-up of 6- to 12-year-old depressed children. J Am Acad Child Adolesc Psychiatry 33:461-468.

Hall D, Dhilla A, Charalambous, Gogos JA, Karayiorgou M. 2003. Sequence variants of the brain-derived neurotrophic factor (BDNF) gene are strongly associated with obsessive-compulsive disorder. Am J Hum Genet 73: 370-376.

Itokawa M, Yamada K, Yoshitsugu K, Toyota T, Suga T, Ohba H et al. 2003. A microsatellite repeat in the promoter of the N-methyl-D-aspartate receptor 2A subunit (GRIN2A) gene suppresses transcriptional activity and correlates with chronic outcome in schizophrenia. Pharmacogenetics 13: 271-278.

Miyatake R, Furukawa A, Suwaki H. 2002. Identification of a novel variant of the human NR2B gene promoter region and its possible association with schizophrenia. Mol Psychiatry; 7: 1101-1106.

Neuman RJ, Geller B, Rice JP, Todd RD. 1997. Increased prevalence and earlier onset of mood disorders among relatives of prepubertal versus adult probands. J Am Acad Child Adolesc Psychiatry 36: 466-73.

Nibuya M, Nestler EJ, Duman RS. 1996. Chronic antidepressant administration increases the expression of cAMP response element binding protein (CREB) in rat hippocampus. J Neurosci 16: 2365-2372.

Sen S, Nesse RM, Stoltenberg SF, Li S, Gleiberman L, Chakravarti A et al. 2003. A BDNF coding variant is associated with the NEO personality inventory domain neuroticism, a risk factor for depression. Neuropsychopharmacology 28: 397-401.

Strober M, Carlson G. 1982. Bipolar illness in adolescents with major depression: clinical, genetic, and psychopharmacologic predictors in a three- to four-year prospective follow-up investigation. Arch Gen Psychiatry 39:549-55.

Todd RD, Neuman R, Geller B, Fox LW, Hickok J. 1993. Genetic studies of affective disorders: should we be starting with childhood onset probands? J Am Acad Child Adolesc Psychiatry 32: 1164-1167.

Tsai, SJ; Cheng, CY; Yu, YWY; Chen, TJ; Hong, CJ. 2003. Association study of a brain-derived neurotrophic-factor genetic polymorphism and major depressive disorders, symptomatology, and antidepressant response. Am J Med Genet 123: 19 - 22.

Zubenko GS, Hughes III HB, Maher BS, Stiffler JS, Zubenko WN, Marazita ML. 2002. Genetic linkage of region containing the CREB1 gene to depressive disorders in women from families with recurrent, early-onset major depression. Am J Med Genet 114: 980-987.

Table 4: Genomic control results for twenty-two markers, n ≥ 79.

Marker & Chromosome		Allele		χ^2 (d.f.=1)	P
		1	2		
Rs 1035712	cases	36	136	0.004	0.947
	controls	38	146		
rs 10590 4	cases	118	58	0.16	0.689
	controls	128	56		
rs 11240 4	cases	121	53	0.104	0.747
	controls	129	51		
rs 11608 4	cases	59	123	0.08	0.777
	controls	72	138		
rs 11796 6	cases	131	65	0.112	0.738
	controls	131	59		
rs 11801 16	cases	115	77	2.368	0.124
	controls	141	67		
rs 13646 7	cases	119	81	0.11	0.74
	controls	133	83		
rs 13651 20	cases	83	111	0.007	0.932
	controls	90	129		
rs 13735 6	cases	79	101	0.462	0.497
	controls	70	106		
rs 13873 6	cases	128	40	0.141	0.707
	controls	127	45		
rs 3937 3	cases	105	105	12.0	0.0005
	controls	126	60		
rs 4453 22	cases	57	131	1.958	0.162
	controls	78	130		
rs 4457 22	cases	112	88	1.051	0.305
	controls	107	105		
rs 4677 14	cases	45	151	5.329	0.021
	controls	71	139		
rs 474 7	cases	65	129	0.002	0.966
	controls	73	143		
rs 5096 11	cases	99	59	0.383	0.536
	controls	101	71		
rs8610 17	cases	97	75	0.19	0.663
	controls	109	75		
5-HT1a 450T/C 1	cases	201	21	0.157	0.692
	controls	176	22		
5-HT1Dβ G861C 6	cases	167	57	0.029	0.865
	controls	156	50		
5-HT2A 102T/C 13	cases	81	135	4.183	0.041
	controls	94	102		
GRIN2B 12	cases	110	104	0.008	0.927
	controls	87	79		
TPH A218C 11	cases	76	144	1.00	0.317
	controls	68	102		

CHAPTER 3.0

BDNF AND COMT POLYMORPHISMS: RELATION TO MEMORY PHENOTYPES IN YOUNG ADULTS WITH CHILDHOOD-ONSET MOOD DISORDER

3.1

BDNF and COMT polymorphisms: relation to memory phenotypes in young adults with childhood-onset mood disorder

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Key Words: brain derived neurotrophic factor, mood disorder, childhood, memory
Running Title: BDNF, COMT polymorphisms and memory phenotypes

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ABSTRACT

Recent investigations in several species have suggested a role for brain-derived neurotrophic factor (BDNF) in memory, which may be mediated by the influence of BDNF on neuronal plasticity in the hippocampus. BDNF polymorphisms have also been associated with mood disorders. Catechol-O-methyltransferase (COMT) metabolizes dopamine and has been implicated in prefrontal function, another area of the brain relevant for memory. In a sample of sixty-three young adults with a history of childhood-onset mood disorder, we typed three BDNF polymorphisms, including the BDNF $Val^{66}Met$ single nucleotide polymorphism (SNP), and the COMT $Val^{108/158}$ Met SNP. Multivariate analysis of variance (MANOVA) was used to test for association between BDNF and COMT markers and measures of declarative memory. Variants at the three BDNF markers and one COMT marker were not associated with declarative memory function - p-values ranged from 0.25 to 0.98. Higher IQ ($F=6.18$, d.f.=4,58, $p=0.0003$) and female gender ($F=4.41$, d.f.=4,58, $p=0.0035$) were associated with more optimal performance on the memory tasks. This study did not provide evidence supporting an association between BDNF and COMT genes and declarative memory phenotypes.

3.1.1 Background

Brain derived neurotrophic factor (BDNF) is known to be important to neuronal transmission and plasticity *(1-3)* and influences in vivo memory formation in the hippocampus (HC) *(4)*. Multiple investigations provide evidence that BDNF is implicated in learning and memory performance in several species, including rats *(5,6)*, chicks *(7)*, and monkeys *(8)*.

A single nucleotide polymorphisim (SNP) causing an amino acid change from valine (val) to methionine (met) at position 66 of the BDNF precursor protein (BDNF $Val^{66}Met$) has recently been reported to have effects on BDNF distribution, hippocampal function and declarative memory encoding and retrieval in human subjects *(9,10)*. *In vitro* assays showed that the met-BDNF had significantly less dendritic expression and depolarization-induced secretion than val-BDNF. *In vivo* data from the same report indicated a possible allele dose effect, with an increasing number of met alleles being associated with a significant linear reduction in hippocampal synaptic activity as measured by N-acetyl-aspartate levels. An accompanying analysis of human memory showed that the BDNF genotype influenced subjects' ability to remember stories over a delay period, as indexed by their performance on the Logical Memory (subtest of the Wechsler Memory Scale, Revised (WMS-R). Individuals homozygous for the met allele recalled fewer story elements than individuals with other genotypes *(9)*. This work suggests that the BDNF met allele may affect declarative memory by altering the release and the intracellular distribution of BDNF, thereby influencing hippocampal synaptic activity. BDNF and its gene have also been implicated in Alzheimer's disease *(11,12)*, further suggesting a role for this gene in memory ability.

Increasing evidence suggests that the effects of BDNF in the hippocampus may not be limited to memory, but may also be associated with mood disorders. BDNF is upregulated in the hippocampus by antidepressants of different classes and may be relevant to human depressive disorders *(13,14)*. Not only are reduced serum BDNF concentrations present in depressed patients, as compared to controls *(15)*, but reduced hippocampal volume is repeatedly observed in patients with major depression *(16,17)*. Though volumetric findings are not completely consistent, hippocampal shape also appears to be affected *(18)*. From a neurocognitive perspective, declarative memory is impaired in subjects with depression *(19)*, and this is evident during an acute episode *(20, 21)* as well as following remission *(22)*. Postpubertal major depression affects performance on hippocampal-dependent memory tasks in both antidepressant-naïve first-episode subjects and in multiple-episode subjects *(23)*. MacQueen et al. *(23)* also find hippocampal volume to be reduced, but only in the multiple-episode depressed subjects, suggesting that memory dysfunction may precede detectable neuroanatomic changes. Other support for interrelationships among BDNF, memory function, and depression include studies demonstrating that antidepressants improve memory performance in rats *(24,25)* and in depressed *(26)* and non-depressed *(27)* patients. Also consistent are data linking BDNF polymorphisms with bipolar disorder *(28-30)*, childhood onset mood disorder *(31)* and with neuroticism *(32)*.

Although the data are less compelling, another candidate gene for neurocognitive phenotypes has been the catechol-O-methyltransferase (COMT) locus. COMT metabolizes dopamine and has been implicated in prefrontal function, insofar as the

methionine (met) variant of the functional SNP at codon 108/158 (COMT $Val^{108/158}$ Met) (Genbank accession no. Z26491) results in reduced dopamine catabolism in the prefrontal cortex. The met variant was associated with better performance on a measure of planning and deductive reasoning ("executive function") and to a lesser extent, on measures of working memory *(33)*. Another study of the COMT $Val^{108/158}$ Met SNP in patients with schizophrenia indicated the met variant was associated with more optimal processing speed and attention, but not with measures of executive function or verbal declarative memory *(34)*. While a significant body of evidence demonstrates a role for COMT in prefrontal function and working memory *(33,34)*, some animal and human studies suggest that COMT is associated with spatial and verbal declarative memory functions. Tolcapone, a COMT inhibitor, improved performance on a spatial memory task *(35)* and also partially restored memory deficits induced by cholinotoxin lesions *(36)*. Improvements in verbal and visuospatial memory have also been reported in humans treated with tolcapone *(37)*. To date, we know of no studies that investigate the association between COMT genotypes and memory in humans with mood disorders.

The present report is part of a broader research program examining biological, social, cognitive and behavioural aspects of emotion regulation in childhood-onset mood disorder. The goal of this study was to explore the relationships between childhood-onset mood disorder, the presence of BDNF and COMT polymorphisms and declarative memory abilities. Based on our review of the extant literature, we hypothesized that in a sample of young adults with onset of mood disorder before the age of 14, relatively poorer declarative memory would be found in those with the met allele of the Val^{66} Met marker of BDNF. While one previous study in schizophrenia was negative *(34)*, the

COMT $Val^{108/158}$ Met marker is associated with reduced dopamine breakdown; reduced dopamine catabolism has been associated with improved verbal *(37)* and spatial *(35,37)* memory performance. Although it had less empirical support than the first hypothesis involving BDNF, our secondary hypothesis was that better verbal/declarative or visuospatial memory performance would be associated with the presence of the met allele of the COMT $Val^{108/158}$ Met marker in the current sample.

3.1.2 Methods

Subjects The mean age of participants at memory assessment was 18.4 years (S.D. 2.5). Sixty-three young adults with onset of major depression (MDD) or dysthymic disorder (DD) were initially recruited between the ages of 8 to 13 years in Pittsburgh, PA as part of a systematic investigation of risk factors and correlates of pediatric depression *(38,39)*. Psychiatric diagnoses were ascertained at initial and repeated follow-up assessments through a standardized semi-structured interview, the Interview Schedule for Children and Adolescents or its version for young adults *(40)*, as age appropriate. All cases met DSM-III or -IV criteria *(41,42)* for affective disorder (MDD with or without DD (n=39), pure DD (n=5), or Bipolar I or II Disorder (BP) (n= 19)). The mean age of onset was 10.3 (S.D. 2.3) years. The ethnic distribution of the sample was 65 % European American, 29 % African American, and 6 % mixed ethnicity, and 48% of the entire sample was female. Subjects taking antidepressants (selective serotonin reuptake inhibitors or tricyclic antidepressants) at the time of testing were included in the present study. Experimental methods met with IRB approval at the University of Pittsburgh and the Centre for Addiction and Mental Health. Written informed consents were obtained from the parent(s) and child.

Measures Verbal declarative memory was assessed with three measures: the immediate and delayed recall Logical Memory (LM) subtests from the Wechsler Memory Scale (WMS) *(43)*, and the delayed recall subtest from the Verbal Paired Associate Learning Test *(44)*. The LM task assesses the ability to recall connected discourse. A brief story consisting of 25 scored units is read to the subject, who has to retell it immediately thereafter, and again, after a 30 minute delay. The Verbal Paired-Associate Learning Test examines learning efficiency by presenting the subject with 10 pairs of unrelated nouns (e.g., neck/salt); after all 10 pairs are presented, learning is assessed by presenting the first word of each pair as a clue or cue. Four such study/test trials are presented. Delayed recall of the pairs is evaluated 30 minutes later by again presenting the cues. A large body of literature suggests that optimal performance on these tasks reflects hippocampal function *(45,46)*. The Rey-Osterrieth Complex Figure (RCF) *(47, 48)* is a measure of visuospatial memory skills, which are affected by BDNF (*2, 6*) and COMT (*35*). In the RCF, the subject copies a complex geometric design and then, without warning, is asked to reproduce it from memory 30 minutes later. A detailed scoring system has been developed *(49)*. Because performance on cognitive tests is associated with overall level of intelligence, the Vocabulary subtest from the Wechsler Adult Intelligence Scale *(50)* or the Wechsler Intelligence Scale for Children *(51)* was administered. Vocabulary is highly correlated with Full Scale IQ, is widely considered to be the best estimate of global intellectual ability, is quite stable over time, and is relatively insensitive to either neurological or psychological disturbances *(52)*.

Laboratory

Twenty millilitres of blood were drawn from each subject and DNA was extracted with a high salt method *(53)*. Subjects were genotyped for an intronic BDNF dinucleotide repeat

polymorphism, $(GT)_n$, previously thought to be upstream from the transcription start site *(54)*; it is now thought to be located in the first intron because of the finding of another exon in the 5' direction. Polymerase chain reaction (PCR) was performed on 50 ng of template DNA to amplify a fragment containing the $(GT)_n$ dinucleotide repeat polymorphism. The forward primer was labelled with a yellow fluorescent dye (NED™). PCR products were separated using the ABI Prism® 3100-Avant Automated Genetic Analyzer. Allele numbers were assigned according to their size (allele 1 = 174 bp; allele 2 = 172 bp; allele 3 = 170 bp; allele 4 = 168 bp; allele 5 = 166 bp), using GENOTYPER® software, version 3.7.

The BDNF Val^{66} Met SNP (NCBI SNP Id rs6265) was typed using primers, probes and PCR conditions developed by Applied Biosystems Inc. (ABI Assay ID C__11592758_10). Amplification and detection of the PCR product were performed with an ABI Prism® 7000 sequence detection instrument (Applied Biosystems Inc.), as suggested by the manufacturer, by use of all default program settings. The PCR product was detected as an increase in reporter dye fluorescence during the PCR extension phase when the probe was cleaved by the 5' exonuclease activity of the *Taq* DNA polymerase. The BDNF $(GT)_n$ and Val^{66} Met polymorphisms are in strong linkage disequilibrium *(28)*, thus the BDNF markers we examined were not independent.

A third BDNF polymorphism was genotyped using restriction enzyme analysis – a SNP in the 5'-noncoding region – C270T, using PCR primers described by Kunugi et al. *(55)*. Restriction digest was with *Hinf*I. Products were separated on 3.5% high-resolution agarose gel and visualized using ethidium bromide.

The COMT $Val^{108/158}$ Met SNP was genotyped using modifications to the PCR conditions and primers used by Kunugi et al. *(56)*. Restriction digest was with *Nla*III and products were visualized with ethidium bromide on 3.5% high-resolution agarose gel. Further information about primers and PCR conditions for any of the polymorphisms discussed is available upon request. Hardy-Weinberg equilibrium was calculated for each of the SNP's. The Hardy Weinberg statistics were – BDNF Val^{66} Met χ^2 =2.0, d.f.=1, p=0.15; BDNF C270T χ^2 =0.97, d.f.=1, p=0.32; and COMT $Val^{108/158}$ Met χ^2 =0.009, d.f.=1, p=0.92. For the BDNF $(GT)_n$ marker, observed and expected homozygosities were compared. The number of observed homozygotes was 30 (49%); and expected, 25.2 (41%). The observed homozygosity in this sample did not differ statistically from expected (χ^2 =1.5, d.f.=1, p=0.22). Heterozygosities were 51% for the BDNF $(GT)_n$ variant, 31% for the BDNF Val^{66}Met SNP, 13% for the BDNF C270T SNP and 49% for the COMT $Val^{108/158}$Met SNP. Minor allele frequencies were 0.15 for the BDNF Val^{66}Met SNP, 0.083 for the BDNF C270T SNP and 0.49 for the COMT $Val^{108/158}$ Met SNP. Allele frequencies for BDNF $(GT)_n$ and genotype frequencies for the SNP's are reported in Table 1.

Statistical Methods Analyses were completed with SAS v8 *(57)*. Pearson correlation coefficients were calculated to test for correlation among the four memory measures. To account for multiple testing of four different dependent variables (i.e. the memory test scores) of interest, we employed multivariate analysis of variance (MANOVA). The PA delayed, LM immediate and delayed and the RCF delayed rendered a global impression of memory. This enabled us to make the simultaneous inference on the null hypothesis

that the independent variables had no effect on any of the dependent variables. Conversely, the statistical significance of a given independent variable would have led us to conclude that that factor influenced at least one of the dependent variables. Independent variables included the presence of alleles of the four genetic markers discussed above. Out of necessity, we made the assumption that the allele effects were additive. If the true effect of heterozygosity was more (or less) than additive, we would have needed interaction terms in the model and we lacked statistical power for such purposes. Covariates included age, gender, ethnicity, Wechsler Intelligence Scale Vocabulary subtest score (WVS), current mood episode, current substance abuse and current antidepressant treatment. To adjust for covariates in a parsimonious fashion, the following steps were used before testing the effects of the independent variables: i) covariates were added to the model in order of decreasing statistical significance if, according to Pillai's Trace statistic, they were independently associated with memory scores at $p<0.10$, ii) if, after adjusting for other covariates, a covariate was not significant at $p<0.10$, it was removed from the model, iii) each covariate was entered only once. Once the potentially influential covariates were identified, a model for each locus was generated by including indicator variables for the presence or absence of each allele associated with that locus.

3.1.3 Results

Memory subtest scores were highly intercorrelated (r values ranged from 0.32 to 0.87; p values ranged from < 0.05 to < .0001), thereby meeting statistical criteria for the use of MANOVA. Covariates, when tested one-at-a-time, indicated gender ($p=0.0035$) and

WVS (p=0.0003) as significant, with the other covariates not being significant on their own. After controlling for IQ, of the remaining covariates, only gender remained significant (p= 0.0016). A total of five subjects were being treated with antidepressants, including imipramine, nortriptyline and fluoxetine at the time of testing. Antidepressant at the time of testing was associated with lower overall memory test scores (F=2.31, d.f.=4,56, p=0.07) after controlling for gender and WVS, meeting our criteria for being included in the final model (p=0.07), which is presented below. Current mood episode did not affect memory: current MDD (n=5) (F=0.35, d.f.=4,55, p=0.84), DD (n=6) (F=1.0, d.f.=4,55, p=0.41) or BP (n=7) (F=0.61, d.f.=4,55, p=0.66) did not influence memory test scores. Age, ethnicity, and current substance use also did not impact memory scores according to the selection rule outlined above and were not included as covariates.

Analyses of candidate gene alleles and memory scores were done controlling for gender, WVS and current antidepressant use. See Table 3. Alleles of the BDNF $(GT)_n$ polymorphism did not significantly impact upon memory scores (174 bp: F=1.37, p=0.26; 172 bp: F=0.39, p=0.82; 170 bp: F=0.16, p=0.96; 168 bp: F=0.56, p=0.69; 166 bp: F=2.48, p=0.06; d.f.=4,51 for each allele). There were only two 168/166 bp heterozygotes contributing to the result noted with the 166 bp allele. Similarly, there was no effect for the BDNF Val^{66} Met variant (F=1.40, d.f.=4,54, p= 0.25 for the Met allele, N/A for the Val allele because all cases had at least one Val at this position). Neither were BDNF C270T alleles (F=0.11, d.f.=4,51, p=0.98; F=0.34, d.f.=4,50, p=0.85, for C and T variants respectively) nor COMT $Val^{108/158}$ Met alleles (F=0.49, d.f.=4,54, p=0.75; F=0.75, d.f.=4,54, p=0.56; for Val and Met alleles respectively) associated with memory

function. Allele effects and confidence intervals around the allele effects are found in Table 3. Data are the adjusted effects rather than the unadjusted means. So, for example, the BDNF $(GT)_n$ 174 bp allele accounts for a 0.26 point increment in LM Immediate. Raw means for the sample are presented in a note under the table.

Effect sizes were calculated for individual alleles *(58)*. For BDNF $(GT)_n$, the Eta Squared (η^2) values range from 0.01 to 0.16, all but the 166 bp allele had η^2 values less than 0.10. Similarly, for C270T, η^2 values were 0.01 and 0.03; for val66met, the η^2 was 0.09; and for COMT, the η^2 values were 0.03 and 0.05.

3.1.4 Discussion

The present investigation does not support an association between the proposed BDNF and COMT candidate polymorphisms and selected memory measures in young adults with COMD. Specifically, three BDNF variants, including the Val^{66} Met SNP, were not associated with declarative memory function. The COMT $Val^{108/158}$ Met marker also had no effect on the memory measures. Higher WVS, and female gender were associated with more optimal performance on the memory tasks. Current mood episode was not associated with altered declarative memory capacity, while a trend was observed for antidepressant use to be associated with less robust declarative memory. While we cannot exclude the possibility of Type II error as a result of the ethnic structure of the sample, our analysis did indicate that ethnicity was not associated with memory scores.

Our results should be viewed in light of certain limitations. The absence family members or other controls precluded the use of other statistical genetic methods, such as those using quantitative phenotypes with family-based controls e.g. quantitative TDT *(59)*. The

small sample size limited statistical power - the lack of evidence for an association between BDNF or COMT candidate polymorphisms and the memory measures may be related to our sample's low power to detect more modest effect sizes. Modeling complete genotypes or potential gene-gene interactions in this small sample was likely to introduce strata that could produce spurious results, or, worse, mask significant results by consuming degrees of freedom. Haplotype analysis of the BDNF and COMT markers would have been an alternate approach *(60)*, but would require a larger sample to produce meaningful results. Instead we focused on two *a priori* hypotheses for individual markers. In other studies, the BDNF Val^{66} Met SNP has accounted for 1.9% of the variance in the WMS-R LM delay phenotype *(9)* and the COMT $Val^{108/158}$ Met SNP has explained 4.1% of the variance for the WCST respectively *(33)*. Variances of such magnitude may be difficult to observe in our sample. Effect sizes that we calculated are further lack of evidence to reject a null hypothesis. In fact, Eta Squared (η^2) is known to be biased in more increasingly complex analyses of variance *(58)*; therefore, the η^2 figures represent an upper boundary of effect size. The true allelic effect sizes could be somewhat smaller. With this low level of phenotypic variation, a substantially larger sample would be needed to find a significant result -- at which point one would be left to wonder how meaningful such small differences are. In a growing literature of potential associations where individual reports may difficult to interpret, our results may be useful when considered as part of a meta-analysis.

Before discussing main results further, we will elabourate briefly on the results of other variables in the MANOVA model. The narrow range of ages in the present study may explain why age did not impinge upon declarative memory tests as would be expected (49). Since the measures we selected were primarily verbal declarative memory, we

expected female subjects would score higher *(61)*. Altered declarative memory in childhood-onset mood disorder might be expected based on adult literature *(62)*. We did not find an association between current episode of BP or MDD and declarative memory. If memory dysfunction progresses with manic-depressive *(63)* or unipolar illness *(23)*, our young adult subjects may not have accrued sufficient morbidity to manifest declarative or verbal memory disturbance on the measures we used. The unexpected trend with antidepressant treatment affecting memory function is made difficult to interpret by the low number of subjects taking antidepressants. An alternate possibility is that those antidepressants (largely tricyclics) had anticholinergic effects that may have impaired cognition. Antidepressant-induced BDNF upregulation depends on chronic treatment lasting weeks, consistent with therapeutic drug effects *(64,65)*. We recognized that neurological conditions and substance abuse may add to phenotypic variance. Participants were excluded from the study at intake if medical or neurological illness was present; substance abuse was considered as a covariate in the MANOVA but was not significant.

Previous reports on memory phenotypes in juvenile mood disorders are few in number. Adolescents with unipolar depression achieved lower scores on the RCF and other visual tasks compared to healthy controls *(66)* – we did not have controls. Depressive symptoms in juvenile populations have been associated with lower scores on the Block Design *(67,68)* and the Coding and Digit Span subtests *(67)* of the WISC-R *(51)*. However, there were no genetic aspects to the prior studies in youth. Previous genetic studies examining BDNF and COMT genes with memory have been exclusively in healthy adults and schizophrenia spectrum phenotypes *(9,33)*, but not COMD. Another

contrast between this study and that of Egan and colleagues *(9)* was younger age at memory measurement - participants in this study were on average at least 13 years younger than in the earlier study. We examined a smaller sample when compared to the three samples with $n \geq 143$ each. We used the same LM delay and some memory measures that were not examined in the original study by Egan et al. *(9)* that considered mainly the LM delay. A possible reason for our nonreplication of adult studies might be the observation that the first published genetic association study for a phenotype is often positive, even if subsequent investigators fail to repeat the initial finding - reasons for this include bias and between-study heterogeneity *(69)*. Our failure to replicate previous findings, if verified, would suggest 1) the influence of the BDNF Val^{66} Met on LM delay reported *(9)* may be age-related and not significant before the late twenties; 2) other molecular targets that affect memory - NMDA *(70)*, C/EBP β *(71)*, TNFα *(72)* and other proteins, such as cAMP response element binding protein (CREB) *(73)* – are likely to be relevant; and 3) environmental factors affecting BDNF mRNA expression *(74-76)* may influence memory independently of the BDNF Val^{66} Met polymorphism, perhaps via transcription factors, such as CREB. We find no evidence to suggest the COMT $Val^{108/158}$ Met SNP is related to declarative memory, in agreement with the previous study that considered the same COMT SNP and declarative memory *(34)*. The role of genetics in memory and mood disorders may be much more complex than suggested by previous molecular studies. It is curious that the met allele of the BDNF Val^{66} Met SNP is associated with reduced hippocampal function and lower memory scores *(9)*, while the same met allele has also been associated with lower neuroticism scores *(32)* and a reduced vulnerability to obsessive-compulsive disorder *(77)*. The SNP may have pleiotropic effects.

Strengths of the present study include utilization of 1) a neurobiologically based dimensional phenotype, 2) a rigorously characterized sample 3) standardized, commonly used neuropsychological measures and 4) a juvenile population where any molecular genetic investigation of declarative memory would be novel. In conclusion, we find no evidence of association between selected BDNF or COMT genetic variants and declarative or visuospatial memory phenotypes in this sample, suggesting that the BDNF and COMT markers we examined are not related to such memory phenotypes in paediatric-onset mood disorders.

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References

1. Lu B., Gottschalk W. (2000) Modulation of hippocampal synaptic transmission and plasticity by neurotrophins. *Prog. Brain Res.* **128**, 231-241.

2. Tyler W.J., Alonso M., Bramham C.R., et al. (2002) From acquisition to consolidation: on the role of brain-derived neurotrophic factor signaling in hippocampal-dependent learning. *Learn. Mem.* **9**, 224-237.

3. Vicario-Abejon C., Owens D., McKay R., Segal M. (2002) Role of neurotrophins in central synapse formation and stabilization. *Nat. Rev. Neurosci.* **3**, 965-74.

4. Alonso M., Vianna M.R., Izquierdo I., and Medina J.H. (2002) Signaling mechanisms mediating BDNF modulation of memory formation in vivo in the hippocampus *Cell Mol. Neurobiol.* **22**, 663-674.

5. Alonso M., Vianna M.R., Depino A.M., et al. (2002) BDNF-triggered events in the rat hippocampus are required for both short- and long-term memory formation. *Hippocampus* **12**, 551-560.

6. Mu J.S., Li W.P., Yao Z.B., Zhou X.F. (1999) Deprivation of endogenous brain-derived neurotrophic factor results in impairment of spatial learning and memory in adult rats. *Brain Res.* **835**, 259-265.

7. Johnston A.N., Rose S.P. (2001) Memory consolidation in day-old chicks requires BDNF but not NGF or NT-3; an antisense study. *Brain Res. Mol. Brain Res.* **88**, 26-36.

8. Tokuyama W., Okuno H., Hashimoto T., et al. (2000) BDNF upregulation during declarative memory formation in monkey inferior temporal cortex. *Nat. Neurosci.* **3**, 1134-1142.

9. Egan M.F., Kojima M., Callicott J.H., et al. (2003) The BDNF val66met polymorphism affects activity-dependent secretion of BDNF and human memory and hippocampal function. *Cell* **112**, 257-269.

10. Hariri A.R., Goldberg T.E., Mattay V.S., et al. (2003) Brain-derived neurotrophic factor val66met polymorphism affects human memory-related hippocampal activity and predicts memory performance. *J Neurosci.* **23**, 6690-4.

11. Garzon D., Yu G., Fahnestock M. (2002) A new brain-derived neurotrophic factor transcript and decrease in brain-derived neurotrophic factor transcripts 1, 2 and 3 in Alzheimer's disease parietal cortex. *J. Neurochem.* **82**, 1058-64.

12. Riemenschneider M., Schwarz S., Wagenpfeil S., et al. (2002) A polymorphism of the brain-derived neurotrophic factor (BDNF) is associated with Alzheimer's disease in patients lacking the Apolipoprotein E epsilon4 allele. *Mol. Psychiatry.* **7**, 782-5.

13. Duman R.S, Malberg J., Nakagawa S., D'Sa C. (2000) Neuronal plasticity and survival in mood disorders. *Biol. Psychiatry* **48**, 732-739.

14. Coppell A.L., Pei Q., Zetterstrom T.S. (2003) Bi-phasic change in BDNF gene expression following antidepressant drug treatment. *Neuropharmacology* **44**, 903-10.

15. Karege F., Perret G., Bondolfi G., et al. (2002) Decreased serum brain-derived neurotrophic factor levels in major depressed patients. *Psychiatry Res.* **109**, 143-148.

16. Sheline Y.I., Wany P., Gado M.H., Csernansky J.G., Vannier M.W. (1996) Hippocampal atrophy in recurrent major depression. *PNAS USA* **93**, 2897-2902.

17. Sheline Y.I., Gado M.H., Kraemer H.C. (2003) Untreated depression and hippocampal volume loss. *Am. J. Psychiatry* **160**, 1516-1518.

18. Posener J.A., Wang L., Price J.L., et al. (2003) High-dimensional mapping of the hippocampus in depression. *Am. J. Psychiatry* **160**, 83-9.

19. Zakzanis K.K., Leach L., Kaplan E. (1998) On the nature and pattern of neurocognitive function in major depressive disorder. *Neuropsychiatry Neuropsychol. Behav. Neurol.* **11**, 111-9.

20. Calev A., Korin Y., Shapira B., et al. (1986) Verbal and non-verbal recall by depressed and euthymic affective patients. *Psychol. Med.* **16**, 789-94.

21. Austin M.P., Ross M., Murray C., et al. (1992) Cognitive function in major depression. *J. Affect. Disord.* **25**, 21-29.

22. Marcos T., Salamero M., Gutierrez F., et al. (1994) Cognitive dysfunctions in recovered melancholic patients. *J. Affect. Disord.* **32**, 133-7.

23. MacQueen G.M., Campbell S., McEwen B.S. (2003) Course of illness, hippocampal function, and hippocampal volume in major depression. *PNAS USA* **100**, 1387-1392.

24. Yau J.L., Noble J., Hibberd C., et al. (2002) Chronic treatment with the antidepressant amitriptyline prevents impairments in water maze learning in aging rats. *J. Neurosci.* **22**, 1436-1442.

25. Barros D.M., Izquierdo L.A., Medina J.H., Izquierdo I. (2002) Bupropion and sertraline enhance retrieval of recent and remote long-term memory in rats. *Behav. Pharmacol.* **13**, 215-220.

26. Levkovitz Y., Caftori R., Avital A., Richter-Levin G. (2002) The SSRI drug fluoxetine, but not the noradrenergic tricyclic drug desipramine, improves memory performance during acute major depression. *Brain Res. Bull.* **58**, 345-350.

27. Harmer C.J., Bhagwagar Z., Cowen P.J., Goodwin G.M. (2002) Acute administration of citalopram facilitates memory consolidation in healthy volunteers. *Psychopharmacology (Berl)* **163**, 106-110.

28. Neves-Pereira M., Mundo E., Muglia P., et al. (2002) The brain-derived neurotrophic factor gene confers susceptibility to bipolar disorder: evidence from a family-based association study. *Am. J. Hum. Genet.* **71**, 651-655.

29. Sklar P., Gabriel S.B., McInnis M.G., et al. (2002) Family-based association study of 76 candidate genes in bipolar disorder: BDNF is a potential risk locus. *Mol. Psychiatry* **7**, 579-593.

30. Nakata K., Ujike H., Sakai A., et al. (2003) Association study of the brain-derived neurotrophic factor (BDNF) gene with bipolar disorder. *Neurosci. Lett.* **337**, 17-20.

31. Strauss J., Barr C.L., George C.J., et al. (2004) Association study of brain-derived neurotrophic factor in adults with a history of childhood-onset mood disorder. *Am. J. Med. Genet. (Neuropsychiatric Genetics)*, in press.

32. Sen S., Nesse R.M., Stoltenberg S.F., et al. (2003) A BDNF coding variant is associated with the NEO personality inventory domain neuroticism, a risk factor for depression. *Neuropsychopharmacology* **28**, 397-401.

33. Egan M.F., Goldberg T.E., Kolachana B.S., et al. (2001) Effect of COMT Val108/158 Met genotype on frontal lobe function and risk for schizophrenia. *PNAS USA* **98**, 6917-6922.

34. Bilder R.M., Volavka J., Czobor P., et al. (2002) Neurocognitive correlates of the COMT Val(158)Met polymorphism in chronic schizophrenia. *Biol. Psychiatry* **52**, 701-707.

35. Liljequist R., Haapalinna A., Ahlander M., et al. (1997) Catechol O-methyltransferase inhibitor tolcapone has minor influence on performance in experimental memory models in rats. *Behav. Brain Res.* **82**, 195-202.

36. Khromova I., Voronina T., Kraineva V.A., Zolotov N., Mannisto P.T. (1997) Effects of selective catechol-O-methyltransferase inhibitors on single-trial passive avoidance retention in male rats. *Behav Brain Res* **86**:49-57.

37. Gasparini M., Fabrizio E., Bonifati V., Meco G. (1997) Cognitive improvement during tolcapone treatment in Parkinson's disease. *J. Neural Transm.* **104**, 887-894.

38. Kovacs M., Feinberg T.L., Crouse-Novak M.A., et al. (1984) Depressive disorders in

childhood. I. A longitudinal prospective study of characteristics and recovery. *Arch. Gen. Psychiatry* **41**, 229-237.

39. Kovacs M., Akiskal H.S., Gatsonis C., Parrone, P. (1994) Childhood-onset dysthymic disorder. Clinical features and prospective naturalistic outcome. *Arch. Gen. Psychiatry* **51**, 365-374.

40. Sherril J.T., Kovacs M. (2000) Interview Schedule for Children and Adolescents (ISCA). *J. Am. Acad. Child Adolesc. Psychiatry* **39**, 67-75.

41. American Psychiatric Association. (1980) Diagnostic and Statistical Manual of Mental Disorders, 3rd ed. Washington, DC, American Psychiatric Press.

42. American Psychiatric Association. (1994) Diagnostic and Statistical Manual of Mental Disorders, 4th ed. Washington, DC, American Psychiatric Press.

43. Wechsler D. (1945) A standardized memory scale for clinical use. *J. Psychol.* **19**, 87-95.

44. Ryan C., Vega A., Longstreet C., Drash A. (1984) Neuropsychological changes in adolescents with insulin-dependent diabetes. *J. Consult. Clin. Psychol.* **52**, 335-42.

45. Miller L.A., Lai R., Munoz D.G. (1998) Contributions of the entorhinal cortex, amygdala and hippocampus to human memory. *Neuropsychologia* **36**, 1247-56.

46. Cameron K.A., Yashar S., Wilson C.L., Fried I. (2001) Human hippocampal neurons predict how well word pairs will be remembered. *Neuron* **30**, 289-298.

47. Rey A. (1941) L'examen psychologique dans les cas d'encephalopathie tramatique. *Archives de Psychologie* **28**, 286-340.

48. Osterrieth P.A. (1944) Le test de copie d'une figure complex: Contribution a l'etude de la perception et de la memoire. *Archives de Psychologie* **30**, 286-356.

49. Spreen O., Strauss E. 1998. A Compendium of Neuropsychological Tests: Administration, Norms, and Commentary (2nd ed.). New York, Oxford University Press.

50. Wechsler D. (1981) Wechsler Adult Intelligence Scale, Revised. New York, Psychological Corporation.

51. Wechsler D. (1974) Wechsler Intelligence Scale for Children, Revised. New York, Psychological Corporation.

52. Sattler J.M. (1988) Assessment of Children, 3rd ed. San Diego, J.M. Sattler.

53. Lahiri D.K., Nurnberger J.I. Jr. (1991) A rapid non-enzymatic method for the preparation of HMW DNA from blood for RFLP analysis. *Nucleic Acids Research* **19**, 5444.

54. Proschel M., Saunders A., Roses A.D., Muller C.R. (1992) Dinucleotide repeat polymorphisms at the human gene for brain-derived neurotrophic factor (BDNF). *Hum. Mol. Genet.* **1**, 353.

55. Kunugi H., Ueki A., Otsuka M., et al. (2001) A novel polymorphism of the brain-derived

neurotrophic factor (BDNF) gene associated with late-onset Alzheimer's disease. *Mol. Psychiatry* **6**, 83-86.

56. Kunugi H., Vallada H.P., Sham P.C., et al. (1997) Catechol-O-methyltransferase polymorphisms and schizophrenia: a transmission disequilibrium study in multiply affected families. *Psychiatr. Genet.* **7**, 97-101.

57. SAS Institute Inc. SAS OnlineDoc®, Version 8. (1999) Cary, NC, SAS Institute Inc.

58. Levine T.R., Hullett C.R. (2002) Eta squared, partial eta squared, and misreporting of effect size in communication research. *Human Communication Research* **28**, 612-625.

59. Waldman I.D., Robinson B.F., Rowe DC. (1999) A logistic regression based extension of the TDT for continuous and categorical traits. *Ann. Hum. Genet.* **63**, 329-340.

60. DeMille M.M., Kidd J.R., Ruggeri V., et al. (2002) Population variation in linkage disequilibrium across the COMT gene considering promoter region and coding region variation. *Hum. Genet.* **111**, 521-537.

61. Kimura D., Clarke P.G. (2002) Women's advantage on verbal memory is not restricted to concrete words. *Psychol. Rep.* **91**, 1137-1142.

62. Seidman L.J., Kremen W.S., Koren D., Faraone S.V., Goldstein J.M., Tsuang M.T. (2002) A comparative profile analysis of neuropsychological functioning in patients with schizophrenia and bipolar psychoses. *Schizophr. Res.* **53**, 31-44.

63. Cavanagh J.T., Van Beck M., Muir W., Blackwood D.H. (2002) Case-control study of neurocognitive function in euthymic patients with bipolar disorder: an association with mania. *Br. J. Psychiatry* **180**, 320-326.

64. Nibuya M., Morinobu S., Duman R.S. (1995) Regulation of BDNF and trkB mRNA in rat brain by chronic electroconvulsive seizure and antidepressant drug treatments. *J Neurosci.* **15**, 7539-7547.

65. Duman R.S. (2002) Synaptic plasticity and mood disorders. *Mol. Psychiatry* **7**, S29-34.

66. Coello E., Ardila A., Rosselli M. (1990) Is there a cognitive marker in major depression? *Int. J. Neurosci.* **50**, 137-145.

67. Kaslow N.J., Rehm L.P., Siegel A.W. (1984) Social-cognitive and cognitive correlates of depression in children. *J. Abnorm. Child Psychol.* **12**, 605-620.

68. Blumberg S.H., Izard C.E. (1985) Affective and cognitive characteristics of depression in 10- and 11-year-old children. *J. Pers. Soc. Psychol.* **49**, 194-202.

69. Ioannidis J.P., Ntzani E.E., Trikalinos T.A., Contopoulos-Ioannidis D.G. (2001) Replication validity of genetic association studies. *Nat. Genet.* **29**, 306-309.

70. Grunwald T., Beck H., Lehnertz K., et al. (1999) Evidence relating human verbal memory to hippocampal N-methyl-D-aspartate receptors. *PNAS USA* **6**, 12085-9.

71. Taubenfeld S.M., Milekic M.H., Monti B., Alberini C.M. (2001) The consolidation of new but not reactivated memory requires hippocampal C/EBPbeta. *Nat. Neurosci.* **4**, 813-8.

72. Golan H., Levav T., Mendelsohn A., Huleihel M. (2004) Involvement of tumor necrosis factor alpha in hippocampal development and function. *Cereb. Cortex* **14,** 97-105.

73. Weeber E.J., Sweatt D.J. (2002) Molecular neurobiology of human cognition. *Neuron* **33**, 845-848.

74. Liu D., Diorio J., Day J.C., et al. (2000) Maternal care, hippocampal synaptogenesis and cognitive development in rats. *Nat. Neurosci.* **3**, 799-806.

75. Russo-Neustadt A., Ha T., Ramirez R., Kesslak J.P. (2001) Physical activity-antidepressant treatment combination: impact on brain-derived neurotrophic factor and behavior in an animal model. *Behav. Brain Res.* **120**, 87-95.

76. Molteni R, Wu A, Vaynman S, Ying Z, Barnard RJ, Gomez-Pinilla F. (2004) Exercise reverses the harmful effects of consumption of a high-fat diet on synaptic and behavioral plasticity associated to the action of brain-derived neurotrophic factor. *Neuroscience* **123**, 429-40.

77. Hall D., Dhilla A., Charalambous A., et al. (2003) Sequence variants of the brain-derived neurotrophic factor (BDNF) gene are strongly associated with obsessive-compulsive disorder. *Am. J. Hum. Genet.* **73**, 370-376.

Table 1a: BDNF $(GT)_n$ allele frequency distribution

BDNF $(GT)_n$	
Allele (bp)	n (%)
174	20 (18)
172	4 (3.6)
170	67 (60)
168	19 (17)
166	2 (1.8)

Table 1b: SNP genotype frequency distributions

BDNF Val^{66} Met			**BDNF C270T**			**COMT $Val^{108/158}$ Met**	
Genotype	n (%)		Genotype	n (%)		Genotype	n (%)
Val/Val	43 (69)		C/C	51 (85)		Val/Val	18 (29)
Val/Met	19 (31)		C/T	8 (13)		Val/Met	31 (49)
Met/Met	0 (0)		T/T	1 (2)		Met/Met	14 (22)

Table 2: Pearson Correlation Coefficients for memory scores

	LM Immediate	LM Delay	PA Delay	RCF Delay
LM Immediate		0.873***	0.209	0.249*
LM Delay	0.873***		0.179	0.303*
PA Delay	0.209	0.179		0.320**
RCF Delay	0.249*	0.303*	0.320**	

*p<0.05 **p<0.01 ***p<0.0001

Table 3: Effect of Selected Gene Polymorphisms on Memory Test Scores

	N of Subjects*	Allele Effect (Standard Error) [95% Confidence interval] on Memory Test Score Adjusted for IQ, Gender, and Antidepressant Use **				Test of allele main effect.**		
Polymorphism (Marker Allele)		LM Immediate	LM Delay	PA Delay	RCF Delay	F	d.f.	P
BDNF $(GT)_n$ At least one:								
174 bp Allele	18	0.26 (1.24) [-2.22, 2.74]	0.64 (1.20) [-1.76, 3.04]	1.20 (0.57) [0.06, 2.34]	2.24 (1.91) [-1.59, 6.06]	1.37	4,51	0.26
172 bp Allele	8	-0.33 (1.53) [-3.39, 2.73]	0.69 (1.48) [-2.27, 3.65]	0.00 (0.70) [-1.41, 1.42]	-0.26 (2.36) [-4.99, 4.46]	0.39	4,51	0.82
170 bp Allele	48	-0.35 (1.44) [-3.25, 2.55]	-0.76 (1.40) [-3.56, 2.04]	0.04 (0.67) [-1.30, 1.37]	0.73 (2.23) [-3.73, 5.20]	0.16	4,51	0.96
168 bp Allele	16	0.71 (1.31) [-1.91, 3.33]	0.72 (1.26) [-1.81, 3.25]	0.35 (0.60) [-0.86, 1.56]	-1.92 (2.02) [-5.96, 2.12]	0.56	4,51	0.69
166 bp Allele	2	-3.96 (2.82) [-9.62, 1.69]	-4.37 (2.72) [-9.83, 1.10]	-2.21 (1.30) [-4.82, 0.40]	5.34 (4.35) [-3.38, 14.06]	2.48	4,51	0.06
BDNF Val66 Met At least one:								
Val Allele***	62	0	0	0	0	-	-	-
Met Allele	19	0.19 (1.08) [-1.97, 2.35]	0.21 (1.06) [-1.91, 2.33]	1.13 (0.49) [0.16, 2.11]	1.77 (1.68) [-1.59, 5.14]	1.40	4,54	0.25
BDNF C270T At least one:								
C Allele	59	-1.18 (4.17) [-9.53, 7.17]	0.21 (4.07) [-7.95, 8.38]	-0.66 (1.95) [-4.58, 3.25]	-0.30 (6.45) [-13.2, 12.64]	0.11	4,51	0.98
T Allele	9	-0.60 (1.49) [-3.58, 2.38]	0.01 (1.45) [-2.91, 2.93]	-0.55 (0.70) [-1.95, 0.84]	-1.62 (2.30) [-6.24, 3.00]	0.34	4,51	0.85
COMT Val108/158 Met At least one:								
Val Allele	49	0.06 (1.24) [-2.42, 2.54]	0.15 (1.22) [-2.29, 2.58]	0.79 (0.58) [-0.39, 1.96]	1.26 (1.87) [-2.49, 5.01]	0.49	4,54	0.75
Met Allele	45	-0.17 (1.14) [-2.45, 2.12]	-0.40 (1.12) [-2.64, 1.84]	-0.34 (0.54) [-1.42, 0.73]	-2.92 (1.72) [-6.37, 0.54]	0.75	4,54	0.56

* Since subjects have two alleles for each marker, heterozygotes are represented twice in this column. The net effect of a heterozygote genotype in this model is therefore the sum of the two corresponding allele effects.

** Other potential covariates such as age, race, currently in episode, and substance abuse were not statistically significant and therefore not included in this model. The unadjusted mean LM Immediate, LM Delay, PA Delay, RCF Delay was 12.4 ±4.0, 11.0 ±4.2, 8.2 ±2.1, 18.9 ±6.3, respectively.

*** All subjects with genotypes and memory scores for Val^{66} Met had at least one Val allele.

CHAPTER 4.0

DISCUSSION AND CONCLUSIONS

4.1 Summary of Results

To summarize, the main purpose of this project was aimed towards expanding the neurotrophic hypotheses of depression and memory function by analyzing several BDNF polymorphisms as putative risk factors for the COMD phenotype. The approaches taken involved a case-control association study and a quantitative genetic analysis using MANOVA. In the first set of analyses, individually matched cases and controls were tested using standard RxC tables for BDNF polymorphism alleles and/or genotypes. Results indicated no association of $Val^{66}Met$ variant genotypes or alleles with COMD, but did find an association of the 168 bp allele of the $(GT)_n$ with caseness. Conditional logistic regression also supported these results after accounting for strata introduced by matching. The result is further supported by GC data that do not indicate any significant population heterogeneity between cases and controls. Haplotype analysis made use of evolutionary relationships to reduce the number of hypothesis tests and to make results more interpretable, indicating that the BDNF val/short haplotype was associated with COMD. We found no relationship between three BDNF or the COMT $Val^{108/158}$ Met variants and declarative memory. Gender and a proxy variable for IQ (WVS) were covariates that had statistically significant effects on declarative memory.

4.2 Synopsis of Rationale for Research

To our knowledge, the present effort is the first to molecular genetic study to implicate a neurotrophin gene, namely BDNF, as a possible risk factor for COMD. The field of psychiatric genetics of child- and adolescent-onset affective disorders is in its infancy, with only a handful of published papers. Most of the published molecular genetic studies

on COMD concern polymorphisms of the 5-HTT or COMT genes, ostensibly based on the monoaminergic hypothesis of depression (Schildkraut et al. 1965). However, the monaminergic theory arose when little was known about the importance of cellular resilience and structural plasticity in mood disorders (Manji et al. 2003). Both theories overlook the phenotypic variability of COMD, which can be unipolar or bipolar and can be comorbid with anxiety or disruptive behaviour disorders.

BDNF has been implicated in stress-related depression via cellular signaling in animal models. Stress could contribute to the hippocampal changes observed in preclinical models of depression, while antidepressants reverse such changes via second messengers (for reviews, see Duman et al. 2000; Nestler et al. 2002). Hippocampal neurotoxicity in CA3 neurons has been described in chronic stress paradigms (Sapolsky et al 1990; Magarinos et al. 1996). Stress is also associated with adverse effects in hippocampal CA3 neurons and dentate gyrus granule cells manifested by reduced BDNF (Smith et al. 1995). Chronic antidepressant administration over a period of weeks, including electroconvulsive seizures, boosts hippocampal BDNF expression and neurogenesis. Central BDNF infusion into the dentate gyrus has produced adaptive behaviours in the animal stress paradigms (Shirayama et al. 2002). Although exact replication of such findings is not possible in humans, neuroimaging and postmortem data to date support the animal research. In a complex disorder such as COMD, any mono-biomarker hypothesis is likely a gross oversimplification. New preclinical evidence supports the potential involvement of multiple genes from at least five different gene groups (Altar et al. 2004).

4.3 Limitations of Study

A first limitation of the study is the hypothesis relating to neurotrophic effects and depression. Beyond being a blatant oversimplification, there are specific elements of the neurotrophic hypothesis of depression which have been remarkable. Firstly, BDNF has been implicated in schizophrenia in several studies. For example, BDNF interacts with the dopamine D3 receptor (Guillin et al. 2001) and evidence that dopamine antagonists affect BDNF expression (Chlan-Fourney et al. 2002) exists. Secondly, the human serum neurotrophin changes that have been observed in depression are believed to reflect CNS neurotrophin support – a premature notion considering the serum neurotrophin changes may simply be epiphenomena. Thirdly, increases in BDNF expression need to be associated directly with increased neural plasticity, and improved mood and cognitive function in depressed human subjects (Lang et al. 2004). Such reports have not been forthcoming at this time. It is clear the limitations of the neurotrophic hypothesis of depression may affect the prior probability of the current project.

A second important limitation has already been alluded to above, namely the heterogeneity of the COMD phenotype. As with psychosis (Kendler et al. 1998), phenotypic heterogeneity is the rule with COMD. Heterogeneity of this nature makes the interpreting of results more cumbersome. COD likely involves symptom overlap in conditions with differing pathogenetic sequences. Examining genetic factors that may affect sub- or endophenotypes may increase the probability of finding common biologic substrates.

Third on a list of limitations would be the small number of variants genotyped in a gene with multiple polymorphisms. As expanded upon in the manuscripts, the polymorphisms studied have been found to be in near complete linkage disequilibrium, making the small number of markers less concerning, and potentially a strength by reducing the amount of redundant data and by diminishing multiple testing concerns. Again, no corrections for multiple tests were made since our examination of COMD was exploratory.

A fourth difficulty we faced was the retrospective aspect of the memory analysis. Memory data for some participants had been collected and then stopped without the entire sample having been characterized cognitively. The experimental design was retrospective and failed to capture memory data on the maximum possible number of subjects.

Which naturally leads discussion to the fifth and most obvious limitation of the present study, that of power. The small sample is offset a little by the power of case-control design. Genotype relative risk is the chance of disease for one genotype at a locus versus another genotype. For complex traits, GRR's of between two and four are often assumed, meaning and individual with a risk genotype is two to four times more likely to have the disorder. The estimated sample size required for replication of the BDNF $(GT)_n$ 168 bp allele finding in the current study was n=102 for the same power and alpha, using the genotype frequencies we observed.

The sample size for the memory analysis was smaller. For this reason we reported allele effects with their respective confidence intervals so as to not just rely on significance testing. Allele effects were small, especially for alleles possessed by larger numbers of

subjects. Effect sizes for the MANOVA we reported as Eta Squared (η^2) values: all were between 0.01 and 0.16. Eta squared values tend to be biased upwards with smaller samples, so the true effect sizes are most likely smaller than our estimates. One contributor to the small effect size is the limited variation in the sample on memory test scores. A more ideal design would have prospectively obtained memory data and DNA on a larger sample with a control group.

Case-control designs have been criticized for relying on the assumption of population homogeneity and thereby producing false positive results. Population heterogeneity tends to be the rule in large North American cities, such as Pittsburgh. Cryptic relatedness – when affected individuals are more likely to be related than controls because they share a common genetic disorder - can also contribute to spurious associations. GC methods we used help to avoid many of the difficulties of the case-control design by using multiple markers across the genome to correct for stratification.

There is no one neuropsychological test that is an absolute measure of hippocampal function. The most optimal test of hippocampal function is most likely spatial navigation (Ekstrom et al. 2003). While we did not have such data available for our sample, other measures available were reasonable proxies for hippocampal function.

Lastly, there are more general difficulties common to genetic association studies, namely problems with reproducibility. The point is well illustrated by the recent nonreplication of a finding that a polymorphism found in the 3' UTR of the interleukin-12B p40 gene was associated with Type I diabetes. Linkage data, biological plausibility, and positive

association in two samples (249 sib pairs and 235 simplex families) supported this as a candidate marker for Type I diabetes (Morahan et al. 2001). A subsequent attempt at replication examining five samples individually and with a total combined sample size of 2873 families found no association, with 99.9% power to detect a difference at $p<0.01$. The authors emphasized a need for independent replication, large datasets and small P-values to make results more reliable (Dalhman et al. 2002).

4.4 A Putative Role for BDNF in Depressive Disorders

Results from the case-control analysis provided data indicating an association between the 168 bp allele of the BDNF $(GT)_n$ polymorphism and COMD. In addition, the val/short haplotype was associated with caseness. Caution must be used in interpreting the results, in light of the diagnostic heterogeneity of the sample, the limited sample size and the absence of correcting for multiple testing. In contrast there was no association between the val66met marker and COMD, as one might expect if the LD were high between val66met and $(GT)_n$ in other samples (Neves-Pereira et al. 2002; Lanktree personal communication). We observed a D' of 0.422 for cases and 1.0 for controls. This does suggest the possibility of a spuriously differing genotype distribution in cases, or may reflect the use of a small sample size with unrelated individuals. The BDNF $Val^{66}Met$ polymorphism has been associated with adult bipolar disorder in North American (Neves-Pereira et al. 2002; Sklar et al. 2002) but not Japanese (Nakata et al. 2003) or Chinese (Hong et al. 2003) adults, suggesting that ethnic influences may be possible. The positive findings in adult bipolar disorder may be of relevance to juvenile onset mood disorders, considering the high rate of switching to bipolar disorder in this population. The present sample was too small to adequately address that question.

Hypotheses derived from several sources include 1) the small risk associated with the Val^{66}Met SNP varies with ethnicity and 2) a phenotype of dysregulated emotionality underlies the pleiotropy exhibited by BDNF polymorphisms, specifically Val^{66}Met. Looking beyond the mood disorder literature, study of disorders associated with depressive symptoms may be informative. BDNF markers, including Val^{66}Met, have been associated with other mood disorder-related phenotypes which have irritability and mood lability as prominent symptoms. Such phenotypes include attention-deficit hyperacitivity disorder (ADHD), eating disorders such as bulimia nervosa, rapid-cycling bipolar disorder and the personality trait of neuroticism – all of which frequently share depressive comorbidity. For example, recent data indicate association between ADHD and the BDNF Val^{66}Met G(valine) allele (Kent L et al. 2004; Lanktree M et al. 2004). Comorbidity involving eating disorders is common in depressive disorders - BDNF serum levels have been associated with eating disorders (Nakazato M et al. 2003) and the BDNF Val^{66}Met A (methionine) variant has been associated with eating disorders including bulimia nervosa (Ribases M et al. 2003; Koizumi H et al. 2004; Ribases M et al. 2004a). Further, evidence has implicated multiple BDNF SNPs, including the Val^{66}Met G allele in the DSM-IV-TR (American Psychiatric Association, 2000) "with rapid cycling" phenotype (Mueller DJ al. 2004). Both BDNF Val^{66}Met polymorphism and BDNF serum concentration have also been implicated in neuroticism in Western but not Asian populations (Sen S et al. 2003; Itoh K et al. 2004; Lang UE et al. 2004; Tsai SJ et al. 2004) Taken together, these studies suggest 1) that the two variant alleles at Val^{66}Met increase risk for different clinical phenotypes in different populations, as with adult bipolar disorder, and 2) that BDNF variants are associated with emotion dysregulation manifested by irritability or mood lability across different DSM

syndromes. The two hypotheses presented would be reasonable if we are considering small effects of the BDNF locus against differing genetic backgrounds involving a complex, polygenic phenotype. Hence it is not surprising that the Val^{66}Met valine allele is associated with adult bipolar disorder (Sklar et al. 2002; Neves-Pereira et al. 2002) while the Val^{66}Met methionine allele appears to be associated with less optimal declarative memory (Egan et al. 2003).

In the setting of multiple polymorphisms existing at a locus, it is beneficial to consider haplotypes to evaluate how often a set of variants are found together as a unit. The value of haplotypes lies in making more full use of the information contained in a gene. For instance, studying a single marker for association with a particular phenotype may be difficult, given that the markers used as candidates are often common on a population level. Further, multiple mutations at different points in a gene can have an important effect on the phenotype. Over the course of evolution, a unique set of markers may be in high LD and therefore be inherited as a single unit together with the etiological polymorphism(s) or mutation(s). Haplotype analysis, therefore, may be a more powerful tool to detect association than single-marker analysis.

A problem that can occur with haplotype analysis is that for increasing numbers of markers, there can be a very large number of haplotypes, many of which are not informative, and use up degrees of freedom. Templeton et al. (1987) first suggested the use of evolutionary relationships among haplotypes, as illustrated in a cladogram, to reduce the number of hypotheses tested in detecting an association between a trait and one or more clusters of haplotypes. A method involving the use of a generalized linear

model as applied to cladistic analysis that can be applied to both family- and population-based samples has been proposed (Seltman et al. 2003). We made use of this Evolutionary-Based Haplotype Analysis Package, eHap. We used eHap performed an overall test of significance and tests based on cladistic relationships. Met/long and val/long were grouped while val/short was a separate node. Met/short was discarded from the analysis as it was present in only one case. Although we suspect the association of the val/short haplotype to COMD may be driven by the association of the $(GT)_n$ 168 bp allele, it allows room to speculate that the val66met marker may have an association which our sample is not large enough to detect.

In more recent analyses we have begun, seven BDNF polymorphisms spanning 30.3 kb were genotyped in 132 pedigrees of Hungarian probands with childhood-onset DSM-IV major depressive disorder or dysthymic disorder. The BDNF polymorphisms included the $(GT)_n$, $Val^{66}Met$ and five other single nucleotide polymorphisms (SNPs) distributed across the BDNF gene. The transmission disequilibrium test (TDT) was used to test for allelic association with diagnosis. Statistically significant overtransmission involving five of six SNPs was found, with p-values ranging from 0.013 to 0.046. A sixth SNP exhibited a trend towards biased transmission (BDNF1: chisq=2.9, df=1, p=0.087). Specifically, the $Val^{66}Met$ SNP was associated with COD (chisq=4.9, df=1, p=0.027), with the val allele being overtransmitted. Alleles of the $(GT)_n$ polymorphism demonstrated no bias in transmission, with p-values for this marker ranging from 0.17 to 0.47. High D' coefficients were observed for the SNP's ($0.84<D'<1.0$). Although $Val^{66}Met$ was associated with caseness and $(GT)_n$ was not, the Hungarian results provide further evidence of a signal in the region, from one of the polymorphisms typed or

another variant in high LD with those already analyzed. Aside from $Val^{66}Met$, none of the other polymorphisms are known to be functional.

4.5 Interpretation of Memory Results

We found no association between BDNF and COMT polymorphisms and selected memory measures in young adults with COMD. Three BDNF variants and the COMT $Val^{108/158}$ Met marker had no influence on the memory test performance. For COMT, the rationale of the hypothesis was less robust. The chance of of Type II error is a realistic consideration. Without controls or family members, we were not able to take advantage of other statistical methods, such as quantitative TDT. The principal problem in interpreting the results is the limited statistical power. However, effect sizes offer no support to reject a null hypothesis. Eta Squared (η^2) is known to be biased upward in smaller samples – so the η^2 figures presented would be an upper bound, meaning the actual effect sizes would be even less than what we report. With this low variability in memory, a much bigger sample would be required to detect a statistically significant result, which would increase the difficulty of interpreting the result. Although the results of the memory analyses are cumbersome to interpret, they may be of some use in the context of a future meta-analysis. There is no one neuropsychological test that is an absolute measure of hippocampal function. The most optimal test of hippocampal function is most likely spatial navigation (Ekstrom et al. 2003). While we did not have such data available for our sample, other measures available were reasonable proxies for hippocampal function.

Genotypes of the COMT polymorphism studied are known to be difficult to read - regenotyping with automated methods indicated four genotypes discrepant with the

original genotypes done by manual methods. Reanalyzing the memory data with the more reliable automated genotypes did not significantly alter the results.

4.6 Conclusions

To conclude, the data from the current study are among the first to implicate the BDNF gene as a putative risk factor for mood disorders with onset in childhood and adolescence. The finding also suggests other elements in the cAMP-CREB and ERK/MAP kinase pathways may be worth investigating. It is imperative that the results from the current study are viewed as preliminary, until multiple replications have occurred. The observations provided by the present investigation underscore the importance of broadening the scope of molecular genetic research of depressive disorders beyond monoaminergic systems, to heed the notion that several molecular systems may be germane. Should our results be replicated, they would illustrate the value of using preclinical research to inform candidate gene selection. Haplotype analysis was a useful tool, with the involvement of the val/short haplotype converging with results from genetic association studies of adult bipolar disorder. Endophenotypes may serve to reduce the heterogeneity of the phenotype in polygenic complex disorders. Since BDNF is a highly conserved protein within and between species, coding sequence polymorphisms may not account for major functional changes. Other mechanisms may regulate expression of this protein, as outlined in Future Directions.

New hypotheses derived from several sources include 1) the small risk associated with the $Val^{66}Met$ SNP varies with ethnicity and 2) a phenotype of dysregulated emotionality underlies the pleiotropy exhibited by BDNF polymorphisms, specifically $Val^{66}Met$.

Looking beyond the mood disorder literature, study of disorders associated with depressive symptoms may be informative. BDNF markers, including Val^{66}Met, have been associated with other mood disorder-related phenotypes which have irritability and mood lability as prominent symptoms. Such phenotypes include attention-deficit hyperacitivity disorder (ADHD), eating disorders such as bulimia nervosa, rapid-cycling bipolar disorder and the personality trait of neuroticism – all of which frequently share depressive comorbidity. For example, recent data indicate association between ADHD and the BDNF Val^{66}Met G(valine) allele (Kent L et al. 2004; Lanktree M et al. 2004). Comorbidity involving eating disorders is common in depressive disorders - BDNF serum levels have been associated with eating disorders (Nakazato M et al. 2003) and the BDNF Val^{66}Met A (methionine) variant has been associated with eating disorders including bulimia nervosa (Ribases M et al. 2003; Koizumi H et al. 2004; Ribases M et al. 2004a). Further, evidence has implicated multiple BDNF SNPs, including the Val^{66}Met G allele in the DSM-IV-TR (American Psychiatric Association, 2000) "with rapid cycling" phenotype (Mueller DJ al. 2004). Both BDNF Val^{66}Met polymorphism and BDNF serum concentration have also been implicated in neuroticism in Western but not Asian populations (Sen S et al. 2003; Itoh K et al. 2004; Lang UE et al. 2004; Tsai SJ et al. 2004) Taken together, these studies suggest 1) that the two variant alleles at Val^{66}Met increase risk for different clinical phenotypes in different populations, as with adult bipolar disorder, and 2) that BDNF variants are associated with emotion dysregulation manifested by irritability or mood lability across different DSM syndromes. The two hypotheses presented would be reasonable if we are considering small effects of the BDNF locus against differing genetic backgrounds involving a complex, polygenic phenotype. Hence it is not surprising that the Val^{66}Met valine allele

is associated with adult bipolar disorder while the Val^{66}Met methionine allele appears to be associated with less optimal declarative memory (Egan et al. 2003).

CHAPTER 5.0

FUTURE DIRECTIONS

The neurobiology of affective disorders has greatly broadened since increasing attention has been given to metabolic pathways beyond traditional receptor targets. Multiple factors – molecular and environmental - have been shown to influence BDNF expression. At least two other important molecules affect BDNF expression. MeCP2 binds to a BDNF promoter and when the MeCP2 is phosphorylated, it releases from the BDNF promoter, facilitating BDNF transcription (Chen et al. 2003). Augmented synthesis of BDNF correlates with a decrease in CpG methylation in its regulatory region and is associated with dissociation of the MeCP2 repression complex from the promoter (Martinowich et al. 2003). Both studies showing interactions between BDNF and MeCP2 suggest that modulation of methylation occurs in acute gene regulation. Multiple studies have suggested interactions between estrogen and BDNF (Solum and Handa 2002; Cavus and Duman 2003; Scharfman et al. 2003) and may begin to improve the biological understanding of why unipolar depression is more common in women.

Further, several environmental factors have been found to influence BDNF expression. Exercise promotes BDNF expression in the hippocampus (Farmer et al. 2004; Adlard and Cotman 2004; Russo-Neustadt et al. 2001, 2000, 1999) and is known to have antidepressant effects (Brosse et al. 2002). Maternal deprivation is another known environmental risk factor for depression associated with diminished BDNF expression (Roceri et al. 2004, 2002; Liu et al. 2000). Obesity and sugar intake have been associated with depression humans (Westover and Marangell. 2002; Stunkard et al. 2003) while diets high in sugar and fat have been associated with reduced BDNF expression in animals (Molteni et al. 2002). Though some of the many of the above associations

remain speculative in nature, they point out some of the adverse mental health effects of current North American lifestyle. The studies cited above enhance the idea that epigenetic effects (Petronis 2001) and other social, molecular and physiological influences on BDNF expression may be relevant to depression.

With a complex, non-Mendelian disorder, simple monogenic investigations such as this one likely cannot comprehensively address the phenotype, reflecting the limitations of the investigator and the available diagnostic and laboratory methods. Epigenetic investigations and endophenotypes may have increasing relevance (Abdolmaleky et al. 2004; Gottesman and Gould 2003). Microarray studies will be valuable in addressing patterns of gene expression and identifying novel, empirical loci for future studies (Alfonso et al. 2004; Altar et al. 2004). The present area of research offers ample room to expand with future projects.

CHAPTER 6.0

REFERENCES

Abdolmaleky HM, Smith CL, Faraone SV et al (2004) Methylomics in psychiatry: Modulation of gene-environment interactions may be through DNA methylation. Am J Med Genet 127B:51-9

Adams JH, Wigg KG, King K et al. (2004) Association study of neurotrophic tyrosine kinase receptor type 2 (NTRK2) and childhood-onset mood disorders. Am J Med Genet Part B (Neuropsychiatric Genetics) In press.

Adlard PA, Cotman CW (2004) Voluntary exercise protects against stress-induced decreases in brain-derived neurotrophic factor protein expression. Neuroscience 124:985-92

American Psychiatric Association. (1980) Diagnostic and Statistical Manual of Mental Disorders, 3rd Ed. American Psychiatric Press, Washington D.C.

American Psychiatric Association (1994) Diagnostic and Statistical Manual of Mental Disorders, 4th Ed. American Psychiatric Press, Washington D.C.

Alfonso J, Pollevick GD, Van Der Hart MG, Flugge G, Fuchs E, Frasch AC. (2004) Identification of genes regulated by chronic psychosocial stress and antidepressant treatment in the hippocampus. Eur J Neurosci 19:659-66

Alonso M, Vianna MR, Izquierdo I, and Medina JH (2002) Signaling mechanisms mediating BDNF modulation of memory formation in vivo in the hippocampus. Cell Mol. Neurobiol 22:663-674

Alonso M, Vianna MR, Depino AM, et al (2002) BDNF-triggered events in the rat hippocampus are required for both short- and long-term memory formation. Hippocampus 12: 551-560

Altar CA (1999) Neurotrophins and depression. Trends Pharmacol Sci 20:59-61

Altar CA, Laeng P, Jurata LW et al (2004) Electroconvulsive seizures regulate gene expression of distinct neurotrophic signaling pathways. J Neurosci 24:2667-77

Angold A, Costello EJ (1993) Depressive comorbidity in children and adolescents: Empirical, theoretical, and methodological issues. Am J Psychiatry 150:1779-1791

Austin MP, Ross M, Murray C, et al (1992) Cognitive function in major depression. J Affect Disord 25, 21-29

Avenevoli S, Stolar M, Li J, Dierker L, Ries Merikangas KR (2001) Comorbidity of depression in children and adolescents: Models and evidence from a prospective high-risk family study. Biol Psychiatry 49:1071-1081

Bacanu SA, Devlin B, Roeder K (2000) The power of genomic control. Am J Hum Genet 66:1933-44

Barros DM, Izquierdo LA, Medina JH, Izquierdo I (2002) Bupropion and sertraline enhance retrieval of recent and remote long-term memory in rats. Behav Pharmacol 13: 215-220

Bartels M, Rietveld MJ, Van Baal GC, Boomsma DI (2002) Heritability of educational achievement in 12-year-olds and the overlap with cognitive ability. Twin Res 5:544-53

Biederman J, Faraone SV (2002) Current concepts on the neurobiology of Attention-Deficit/Hyperactivity Disorder. J Atten Disord 6:S7-S16 (Suppl 1)

Biederman J, Faraone S, Mick E, Lelon E (1995) Psychiatric comorbidity among referred juveniles with major depression: Fact or artifact? J Am Acad Child Adolesc Psychiatry 34:579-590

Bilder RM, Volavka J, Czobor P, et al (2002) Neurocognitive correlates of the COMT Val(158)Met polymorphism in chronic schizophrenia. Biol Psychiatry 52: 701-

707
Birmaher B, Ryan ND, Williamson DE, Brent DA, Kaufman J, Dahl RE, Perel J, Nelson B: Childhood and adolescent depression: A review of the past 10 years. Part I. (1996) J Am Acad Child Adolesc Psychiatry 35:1427-1439
Birnstiel S and Haas HL (1991) Acute effects of antidepressant drugs on long-term potentiation (LTP) in rat hippocampal slices. Naunyn Schmiedebergs Arch Pharmacol 344:79-83
Boomsma DI, Beem AL, van den Berg M et al (2000) Netherlands twin family study of anxious depression (NETSAD). Twin Res 2000 3:323-34
Bremner JD, Narayan M, Anderson ER, Staib LH, Miller HL, Charney DS (2000) Hippocampal volume reduction in major depression. Am J Psychiatry 157: 115-118
Brent DA, Perper JA, Moritz G, Allman C, Friend A, Roth C, Schweers J, Balach L, Baugher M (1993) Psychiatric risk factors for adolescent suicide: A case-control study. J Am Acad Child Adolesc Psychiatry 32:521-529
Brosse AL, Sheets ES, Lett HS, Blumenthal JA (2002) Exercise and the treatment of clinical depression in adults: recent findings and future directions. Sports Med 32:741-60
Calev A, Korin Y, Shapira B, et al (1986) Verbal and non-verbal recall by depressed and euthymic affective patients. Psychol Med 16: 789-94
Cavus I, Duman RS (2003) Influence of estradiol, stress, and 5-HT2A agonist treatment on brain-derived neurotrophic factor expression in female rats. Biol Psychiatry 54:59-69
Chen B, Dowlatshahi D, MacQueen G, Wang J, Young LT (2001) Increased hippocampal BDNF immunoreactivity in subjects treated with antidepressant medication. Biol Psychiatry 50: 260-265
Chen WG, Chang Q, Lin Y, Meissner A et al (2003) Derepression of BDNF Transcription involves calcium-dependent phosphorylation of MeCP2. Science 302:885-9
Chlan-Fourney J, Ashe P, Nylen K, Juorio AV, Li XM (2002) Differential regulation of hippocampal BDNF mRNA by typical and atypical antipsychotic administration. Brain Res 954:11-20
Colin SF, Chang HC, Mollner S, Pfeuffer T, Reed RR, Duman RS, Nestler EJ (1991) Chronic lithium regulates the expression of adenylate cyclase and Giα in rat cerebral cortex. PNAS USA 88:10634-10637
Coyle JT and Duman RS (2003) Finding the intracellular signaling pathways affected by mood disorder treatments. Neuron 38:157-60
Cudworth AG, Wolf E (1982) The genetic susceptibility to type I (insulin-dependent) Diabetes mellitus. Clin Endocrinol Metab 11:389-408
Dahlman I, Eaves IA, Kosoy R, Morrison VA et al (2002) Parameters for reliable results in genetic association studies in common disease. Nat Genet 30:149-50
Deater-Deckard K, Reiss D, Hetherington EM, Plomin R (1997) Dimensions and disorders of adolescent adjustment: a quantitative genetic analysis of unselected samples and selected extremes. J Child Psychol Psychiatry 38:515-25
Detera-Wadleigh SD, Badner JA, Berrettini WH, Yoshikawa T, Goldin LR, Turner G et al (1999) A high-density genome scan detects evidence for a bipolar-disorder susceptibility locus on 13q32 and other potential loci on 1q32 and 18p11.2. Proc Natl Acad Sci USA 96: 5604-9

Devlin B and Roeder K (1999) Genomic Control for Association Studies. Biometrics 55: 997-1004

de Wilde EJ, Kienhorst ICWM, Diekstra RFW (2001) Suicidal behaviour in adolescents, in Goodyer IM, (ed): The depressed child and adolescent. 2nd ed. Cambridge University Press, New York, N.Y.

Dowlatshahi D, MacQueen GM, Wang JF, Reiach JS, Young LT (1999) G Protein-coupled cyclic AMP signaling in postmortem brain of subjects with mood disorders: effects of diagnosis, suicide, and treatment at the time of death. Journal of Neurochemistry 73: 1121-1126

Duman RS (1998) Novel therapeutic approaches beyond the serotonin receptor. Biol Psychiatry 44:324-35

Duman RS (2002) Genetics of childhood disorders: XXXIX. Stem cell research, part 3: Regulation of neurogenesis by stress and antidepressant treatment. J Am Acad Child Adolesc Psychiatry 41:745-8

Duman RS, Heninger GR, Nestler EJ (1997) A molecular and cellular theory of depression. Arch Gen Psychiatry 54:597-606

Duman RS, Malberg J, Thome J (1999) Neural plasticity to stress and antidepressant treatment. Biol Psychiatry 46:1181-91

Duman RS, Vaidya VA, (1998) Molecular and cellular actions of chronic electroconvulsive seizures. Journal of ECT 14:181-93

Eaves LJ, Silberg JL, Meyer JM, et al (1997) Genetics and developmental psychopathology: 2. The main effects of genes and environment on behavioral problems in the Virginia Twin Study of Adolescent Behavioral Development. J Child Psychol Psychiatry 38:965-80

Edelbrock C, Rende R, Plomin R, Thompson LA (1995) A twin study of competence and problem behavior in childhood and early adolescence. J Child Psychol Psychiatry 36:775-85

Egan MF, Goldberg TE, Kolachana BS, et al (2001) Effect of COMT Val108/158 Met genotype on frontal lobe function and risk for schizophrenia. PNAS USA 98:6917-6922.

Egan MF, Kojima M, Callicott JH, Goldberg TE, Kolachana BS, Bertolino A et al (2003) The BDNF val66met polymorphism affects activity-dependent secretion of BDNF and human memory and hippocampal function. Cell 112:257-269

Ekstrom AD, Kahana MJ, Caplan JB, Fields TA, Isham EA, Newman EL, Fried I (2003) Cellular networks underlying human spatial navigation. Nature 425:184-8

Eley TC (1997) Depressive symptoms in children and adolescents: etiological links between normality and abnormality: a research note. J Child Psychol Psychiatry 38:861-5

Enoch MA, Goldman D (2001) The genetics of alcoholism and alcohol abuse. Curr Psychiatry Rep 3:144-51

Fang H, Chartier J, Sodja C, Desbois A, Ribecco-Lutkiewicz M, Walker PR et al (2003) Transcriptional activation of the human BDNF gene promoter III by dopamine signaling in NT2/N neurons. J Biol Chem 278:26401-9

Farmer J, Zhao X, van Praag H, Wodtke K, Gage FH, Christie BR (2004) Effects of voluntary exercise on synaptic plasticity and gene expression in the dentate gyrus of adult male Sprague-Dawley rats in vivo. Neuroscience 124:71-9

Farrer LA, Cupples LA, Haines JL et al (1997) Effects of age, sex, and ethnicity on the

association between apolipoprotein E genotype and Alzheimer disease. A meta-analysis. APOE and Alzheimer Disease Meta Analysis Consortium. JAMA 278:1349-56

Fransen E, Lemkens N, Van Laer L, Van Camp G (2003) Age-related hearing impairment (ARHI): environmental risk factors and genetic prospects. Exp Gerontol 38:353-9

Garzon D, Yu G, Fahnestock M (2002) A new brain-derived neurotrophic factor transcript and decrease in brain-derived neurotrophic factor transcripts 1, 2 and 3 in Alzheimer's disease parietal cortex. J Neurochem 82:1058-64

Gasparini M, Fabrizio E, Bonifati V, Meco G (1997) Cognitive improvement during tolcapone treatment in Parkinson's disease. J Neural Transm 104: 887-894

Geller B, Cook EH Jr (2000) Ultradian rapid cycling in prepubertal and early adolescent bipolarity is not in transmission disequilibrium with val/met COMT alleles. Biol Psychiatry 47:605-9

Geller B, Cook EH Jr (1999) Serotonin transporter gene (HTTLPR) is not in linkage disequilibrium with prepubertal and early adolescent bipolarity. Biol Psychiatry. 45:1230-3

Geller B, Fox LW, Clark KA (1994) Rate and predictors of prepubertal bipolarity during follow-up of 6- to 12-year-old depressed children. J. Am Acad Child Adolesc Psychiatry 33:461-468

Ghosh A, Carnahan J, Greenberg ME (1994) Requirement for BDNF in activity – dependent survival of cortical neurons. Science 263:1618-1623

Gjone H, Stevenson J The association between internalizing and externalizing behavior in childhood and early adolescence: genetic of environmental common influences? J Abnorm Child Psychol 25:277-86

Gjone H, Stevenson, Sundet JM, Eilertsen DE (1996) Changes in heritability across increasing levels of behavior problems in young twins. Behav Genet 26:419-26

Glantz SA and Slinker BK (2001) Primer of Applied Logistic Regression & Analysis of Variance. 2nd Ed. McGraw-Hill, New York

Goodyer IM, Herbert J, Secher SM, Pearson J (1997) Short-term outcome of major depression:I. Comorbidity and severity at presentation as predictors of persistent disorder. J Am Acad Child Adolesc Psychiatry 36:179-187

Gottesman II, Gould TD (2003)The endophenotype concept in psychiatry: etymology and strategic intentions. Am J Psychiatry 160:636-45

Gould E, McEwen BS, Tanapat P, Galea LA, Fuchs E (1997) Neurogenesis in the dentate gyrus of the adult tree shrew is regulated by psychosocial stress and NMDA receptor activation. Journal of Neuroscience 17:2492-2498

Gould E, Tanapat P, McEwen BS, Flugge G, Fuchs E (1998) Proliferation of granule cell precursors in the dentate gyrus of adult monkeys is diminished by stress. Proc Nat Acad Sci USA 95:3168-3171

Guillin O, Diaz J, Carroll P, Griffon N, Schwartz JC, Sokoloff P (2001) BDNF controls dopamine D3 receptor expression and triggers behavioural sensitization. Nature 411:86-9

Hammen C, Brennan PA (2003) Severity, chronicity, and timing of maternal depression and risk for adolescent offspring diagnoses in a community sample. Arch Gen Psychiatry 60:253-8

Hariri AR, Goldberg TE, Mattay VS et al (2003) Brain-derived neurotrophic factor

val66met polymorphism affects human memory-related hippocampal activity and predicts memory performance. J Neurosci 23:6690-4
Harmer CJ, Bhagwagar Z, Cowen PJ, Goodwin GM (2002) Acute administration of citalopram facilitates memory consolidation in healthy volunteers. Psychopharmacology (Berl) 163:106-110
Harrington R, Fudge H, Rutter M, Pickles A, Hill J (1990) Adult outcomes of childhood and adolescent depression: I. Psychiatric status. Arch Gen Psychiatry 47:465-473
Harrington R, Fudge H, Rutter M, et al (1993) Child and adolescent depression: A test of continuities with data from a family study. Brit J Psychiatry 162:627-633
Hewitt JK, Silberg JL, Neale MC, Eaves LJ, Erickson M (1992)The analysis of parental ratings of children's behavior using LISREL. Behav Genet 22:293-317
Hong CJ, Huo SJ, Yen FC, Tung CL, Pan GM, Tsai SJ (2003) Association study of a brain-derived neurotrophic-factor genetic polymorphism and mood disorders, age of onset and suicidal behavior. Neuropsychobiology 48:186-9
Hudziak JJ, Rudiger LP, Neale MC, Heath AC, Todd RD (2000) A Twin Study of Inattentive, Aggressive and Anxious/Depressed Behaviors. J Am Acad Child Adolesc Psychiatry 39:469-476
Itoh K, Hashimoto K, Kumakiri C, Shimizu E, Iyo M (2004) Association between brain-derived neurotrophic factor 196 G/A polymorphism and personality traits in healthy subjects. Am J Med Genet B Neuropsychiatr Genet 124:61-3
Johnston AN, Rose SP (2001) Memory consolidation in day-old chicks requires BDNF but not NGF or NT-3; an antisense study. Brain Res. Mol. Brain Res. 88:26-36
Kang H and Schuman FM (1995) Long-lasting neurotrophin-induced enhancement of synaptic transmission in the adult hippocampus. Science 267:1658-62
Karege F, Perret G, Bondolfi G, Schwald M, Bertschy G, Aubry J (2002) Decreased serum brain-derived neurotrophic factor levels in major depressed patients. Psychiatry Research 109:143-148
Kasen S, Cohen P, Skodol AE, Johnson JG, Smailes E. Brook JS (2001) Childhood depression and adult personality disorder: Alternative pathways of continuity. Arch Gen Psychiatry 58:231-236
Kempermann G, Kuhn HG, Gage FH (1997) More hippocampal neurons in adult mice living in an enriched environment. Nature 386:493-495
Kendler KS, Karkowski LM, Walsh D (1998) The structure of psychosis: latent class analysis of probands from the Roscommon Family Study. Arch Gen Psychiatry 55:492-9
Kent L et al (2004) ISPG Abstracts Dublin Ireland, Am J Med Genet 130B
Khromova I, Voronina T, Kraineva VA, Zolotov N, Mannisto PT (1997) Effects of selective catechol-O-methyltransferase inhibitors on single-trial passive avoidance retention in male rats. Behav Brain Res 86:49-57
Koizumi H, Hashimoto K, Itoh K, Nakazato M, Shimizu E, Ohgake S et al (2004) Association between the brain-derived neurotrophic factor 196G/A polymorphism and eating disorders. Am J Med Genet B Neuropsychiatr Genet 127:125-7
Kovacs M (1996) Presentation and course of major depressive disorder during childhood and later years of the life span. J Am Acad Child Adolesc Psychiatry 35:705-715
Kovacs M (1997) Depressive disorders in childhood: An impressionistic landscape. J Child Psychol Psychiatry 38:287-298
Kovacs M, Akiskal HS, Gatsonis C, Parrone PL (1994) Childhood-onset dysthymic

disorder: Clinical features and prospective naturalistic outcome. Arch Gen Psychiatry 51:365-374
Kovacs M, Devlin B, Pollock M, Richards C, Mukerji P (1997) A controlled family history study of childhood-onset depressive disorder. Arch Gen Psychiatry 54:613-23
Kovacs M, Gatsonis C, Paulauskas SL, Richards C (1989) Depressive disorders in childhood. IV. A longitudinal study of comorbidity with and risk factors for anxiety disorders. Arch Gen Psychiatry 46:776-782
Kovacs M, Goldston D (1991) Cognitive and social cognitive development of depressed children and adolescents. J Am Acad Child Adolesc Psychiatry 30:388-392
Kovacs M, Goldston D, Gatsonis C (1993) Suicidal behaviors and childhood-onset depressive disorders: A longitudinal investigation. J Am Acad Child Adolesc Psychiatry 32:8-20
Kovacs M, Obrosky DS, Gatsonis C, Richards C (1997) First episode major depressive and dysthymic disorder in childhood: Clinical and sociodemographic factors in recovery. J Am Acad Child Adolesc Psychiatry 36:777-784
Kovacs M, Paulauskas S, Gatsonis C, Richards C (1988) Depressive disorders in childhood. III. A longitudinal study of comorbidity with and risk for conduct disorders. J Affective Disord 15:205-217
Lang UE, Hellweg R, Gallinat J (2004a) BDNF serum concentrations in healthy volunteers are associated with depression-related personality traits. Neuropsychopharmacology. 29:795-8
Lang UE, Jockers-Scherubl MC, Hellweg R (2004b) State of the art of the neurotrophin hypothesis in psychiatric disorders: implications and limitations. J Neural Transm 111:387-411
Lanktree M et al (2004) ISPG Abstracts Dublin Ireland, Am J Med Genet 130B
Levine ES, Dreyfus CF, Black IB, Plummer MR (1995) Brain-derived neurotrophic factor rapidly enhances synaptic transmission in hippocampal neurons via postsynaptic tyrosine kinase receptors. PNAS USA 92:8074-7
Levine TR, Hullett CR (2002) Eta squared, partial eta squared, and misreporting of effect size in communication research. Human Communication Research 28:612-625.
Levkovitz Y, Caftori R, Avital A, Richter-Levin G (2002) The SSRI drug fluoxetine, but not the noradrenergic tricyclic drug desipramine, improves memory performance during acute major depression. Brain Res Bull 58:345-350
Lewinsohn PM, Rohde P, Klein DN, Seeley JR (1999) Natural course of adolescent major depressive disorder: I. Continuity into young adulthood. J Am Acad Child Adolesc Psychiatry 38:56-63
Lewinsohn PM, Rohde P, Seeley JR, Klein DN, Gotlib IH (2000) Natural course of adolescent major depressive disorder in a community sample: Predictors of recurrence in young adults. Am J Psychiatry 157:1584-1591
Liljequist R., Haapalinna A., Ahlander M., et al (1997) Catechol O-methyltransferase inhibitor tolcapone has minor influence on performance in experimental memory models in rats. Behav Brain Res 82:195-202
Liu D, Diorio J, Day JC, Francis DD, Meaney MJ (2000) Maternal care, hippocampal synaptogenesis and cognitive development in rats. Nat Neurosci 3:799-806.
Lu B, Gottschalk W (2000) Modulation of hippocampal synaptic transmission and plasticity by neurotrophins. Prog Brain Res 128:231-241
MacQueen GM, Campbell S, McEwen BS (2003) Course of illness, hippocampal

function, and hippocampal volume in major depression. PNAS USA 100:1387-1392
Madsen TM, Treschow A, Bengzon J et al. Increased neurogenesis in a model of electroconvulsive therapy. Biol Psychiatry 47:1043-9
Magarinos AM, McEwen BS, Flugge G, Fuchs E. Chronic psychosocial stress causes apical dendritic atrophy of hippocampal CA3 pyramidal neurons in subordinate tree shrews. J Neurosci 16:3534-40
Malberg JE, Eisch AJ, Nestler EJ, Duman RS (2000) Chronic antidepressant treatment increases neurogenesis in adult rat hippocampus. J Neurosci 20:9104-10
Mamounas LA, Blue ME, Siuciak JA, Altar CA (1995) Brain-derived neurotrophic factor promotes the survival and sprouting of serotonergic axons in rat brain. J Neurosci. 15:7929-39
Manev H, Uz T, Smalheiser NR, Manev R (2001) Antidepressants alter cell proliferation in the adult brain in vivo and in neural cultures in vitro. Eur J Pharmacol 411:67-70
Marcos T, Salamero M, Gutierrez F et al (1994) Cognitive dysfunctions in recovered melancholic patients. J Affect Disord 32:133-7
Martinowich K, Hattori D, Wu H, Fouse S et al (2003) DNA methylation-related chromatin remodeling in activity-dependent BDNF gene regulation. Science 302:890-3
Massicotte G, Bernard J, Ohayon M (1993) Chronic effects of trimipramine, an antidepressant, on hippocampal synaptic plasticity. Behav Neural Biol 59:100-6
McClearn GE, Johansson B, Berg S, Pedersen NL, Ahern F, Petrill SA, Plomin R (1997) Substantial genetic influence on cognitive abilities in twins 80 or more years old. Science 276:1560-3
McInnes LA, Escamilla MA, Service SK, Reus VI, Leon P, Silva S et al (1996) A complete genome screen for genes predisposing to severe bipolar disorder in two Costa Rican pedigrees. PNAS USA 93:13060-5
Menkes DB, Rasenick MM, Wheller MA, Bitensky MW (1983) Guanosine triphosphate activation of brain adenylate cyclase: enhancement by long term antidepressant treatment. Science 129:65-67
Molteni R, Barnard RJ, Ying Z, Roberts CK, Gomez-Pinilla F (2002) A high-fat, refined sugar diet reduces hippocampal brain-derived neurotrophic factor, neuronal plasticity, and learning. Neuroscience 112:803-14
Morahan G, Huang D, Ymer SI et al (2001) Linkage disequilibrium of a type 1 diabetes susceptibility locus with a regulatory IL12B allele. Nat Genet 27:218-21
Mu JS, Li WP, Yao ZB, Zhou XF (1999) Deprivation of endogenous brain-derived neurotrophic factor results in impairment of spatial learning and memory in adult rats. Brain Res 835: 259-265
Mueller DJ et al (2004) ISPG Abstracts Dublin Ireland, Am J Med Genet 130B
Nakamura S (1990) Antidepressants induce regeneration of catecholaminergic axon terminals in the rat cerebral cortex Neurosci Lett 111:64-68
Nakata K, Ujike H, Sakai A, et al (2003) Association study of the brain-derived neurotrophic factor (BDNF) gene with bipolar disorder. Neurosci Lett 337:17-20
Nakazato M, Hashimoto K, Shimizu E, Kumakiri C, Koizumi H, Okamura N et al (2003) Decreased levels of serum brain-derived neurotrophic factor in female patients with eating disorders. Biol Psychiatry 54:485-90
Nestler EJ, Barrot M, DiLeone RJ, et al (2002) Neurobiology of depression. Neuron

34:13-25
Nestler EJ, Terwilliger RZ, Duman RS (1989) Chronic antidepressant administration alters the subcellular distribution of cyclic AMP-dependent protein kinase in rat frontal cortex. Journal of Neurochemistry 53:1644-1647
Neuman RJ, Geller B, Rice JP, Todd RD (1997) Increased prevalence and earlier onset of mood disorders among relatives of prepubertal versus adult probands. J Am Acad Child Adolesc Psychiatry 36: 466-73
Neves-Pereira M, Mundo E, Muglia P, Kennedy JL, Macciardi F (2002) The brain derived neurotrophic factor gene confers susceptibility to bipolar mood disorder: evidence from a family-based association study. Am J Hum Genet 71:651-655
Nibuya M, Morinobu S, Duman RS (1995) Regulation of BDNF and trkB mRNA in rat brain by chronic electroconvulsive seizure and antidepressant drug treatments. J Neurosci 15:7539-47
Nibuya M, Nestler EJ, Duman RS (1996) Chronic antidepressant administration increases the expression of cAMP response element binding protein (CREB) in rat hippocampus. J Neurosci 16:2365-72
Nobile M, Begni B, Giorda R, Frigerio A, Marino C, Molteni M, Ferrarese C, Battaglia M (1999) Effects of serotonin transporter promoter genotype on platelet serotonin transporter functionality in depressed children and adolescents. J Am Acad Child Adolesc Psychiatry 38:1396-402
Orvaschel H (1990) Early onset psychiatric disorder in high risk children and increased familial morbidity. J Am Acad Child Adolesc Psychiatry 29:184-8
Ozawa H, Rasenick MM (1991) Chronic electroconvulsive treatment augments coupling of the GTP-binding protein Gs to the catalytic moiety of adenylyl cyclase in a manner similar to that seen with chronic antidepressant drugs. J Neurochem 56:30-38
Pfeffer CR (2001) Diagnosis of childhood and adolescent suicidal behavior: Unmet needs for suicide prevention. Biol Psychiatry 49:1055-1061
Posener JA, Wang L, Price JL et al (2003) High-dimensional mapping of the hippocampus in depression. Am. J Psychiatry 160:83-9
Proschel M, Saunders A, Roses AD, Muller CR (1992) Dinucleotide repeat polymorphisms at the human gene for brain-derived neurotrophic factor (BDNF). Hum Mol Genet 1:353
Puig-Antich J, Goetz D, Davies M et al (1989) A controlled family history study of prepubertal major depressive disorder. Arch Gen Psychiatry 46:406-18
Puig-Antich J, Lukens E, Davies M, Goetz D, Brennan-Quattrock J, Todak G (1985a) Psychosocial functioning in prepubertal major depressive disorders. I. Interpersonal relationships during the depressive episode. Arch Gen Psychiatry 42:500-7
Puig-Antich J, Lukens E, Davies M, Goetz D, Brennan-Quattrock J, Todak G (1985b) Psychosocial functioning in prepubertal major depressive disorders. II. Interpersonal relationships after sustained recovery from affective episode. Arch Gen Psychiatry 42:511-7
Purcell S, Cherny SS, Sham PC. (2003) Genetic Power Calculator: design of linkage and association genetic mapping studies of complex traits. Bioinformatics 19:149-150
Rao U, Ryan ND, Birmaher B, Dahl RE, Williamson DE, Kaufman J, Rao R, Nelson B

(1995) Unipolar depression in adolescents: Clinical outcome in adulthood. J Am Acad Child Adolesc Psychiatry 34:566-578
Rende RD, Plomin R, Reiss D, Hetherington EM (1993) Genetic and environmental influences on depressive symptomatology in adolescence: individual differences and extreme scores. J Child Psychol Psychiatry 34:1387-98
Ribases M, Gratacos M, Armengol L, de Cid R, Badia A, Jimenez L et al (2003) Met66 in the brain-derived neurotrophic factor (BDNF) precursor is associated with anorexia nervosa restrictive type. Mol Psychiatry 8:745-51
Ribases M, Gratacos M, Fernandez-Aranda F, Bellodi L, Boni C, Anderluh M et al (2004) Association of BDNF with anorexia, bulimia and age of onset of weight loss in six European populations. Hum Mol Genet 13:1205-12
Rice F, Harold G, Thapar A (2002) The genetic aetiology of childhood depression: a review. J Child Psychol Psychiatry 43:65-79
Riemenschneider M, Schwarz S, Wagenpfeil S et al (2002) A polymorphism of the brain-derived neurotrophic factor (BDNF) is associated with Alzheimer's disease in patients lacking the Apolipoprotein E epsilon4 allele. Mol Psychiatry 7:782-5
Risch NJ (2000) Searching for genetic determinants in the new millennium. Nature 405:847-56
Roceri M, Cirulli F, Pessina C, Peretto P, Racagni G, Riva MA (2004) Postnatal repeated maternal deprivation produces age-dependent changes of brain-derived neurotrophic factor expression in selected rat brain regions. Biol Psychiatry 55:708-14
Roceri M, Hendriks W, Racagni G, Ellenbroek BA, Riva MA (2002) Early maternal deprivation reduces the expression of BDNF and NMDA receptor subunits in rat hippocampus. Mol Psychiatry 7:609-16
Rupert JL, Hochachka PW (2001) Genetic approaches to understanding human adaptation to altitude in the Andes. J Exp Biol 204:3151-60
Russo-Neustadt A, Beard RC, Cotman CW (1999) Exercise, antidepressant medications, and enhanced brain derived neurotrophic factor expression. Neuropsychopharmacology 21:679-82
Russo-Neustadt AA, Beard RC, Huang YM, Cotman CW (2000) Physical activity and antidepressant treatment potentiate the expression of specific brain-derived neurotrophic factor transcripts in the rat hippocampus. Neuroscience 101:305-12
Russo-Neustadt A, Ha T, Ramirez R, Kesslak JP (2001) Physical activity-antidepressant treatment combination: impact on brain-derived neurotrophic factor and behavior in an animal model. Behav Brain Res 120:87-95
Santarelli L, Saxe M, Gross C et al (2003) Requirement of hippocampal neurogenesis for the behavioral effects of antidepressants. Science 301:805-9
Sapolsky RM Glucocorticoid toxicity in the hippocampus: temporal aspects of neuronal vulnerability. Brain Res 359:300-5
Sapolsky RM, Uno H, Rebert CS, Finch CE (1990) Hippocampal damage associated with prolonged glucocorticoid exposure in primates. J Neurosci 10:2897-902
Scharfman HE, Mercurio TC, Goodman JH, Wilson MA, MacLusky NJ (2003) Hippocampal excitability increases during the estrous cycle in the rat: a potential role for brain-derived neurotrophic factor. J Neurosci 23:11641-52
Schildkraut JJ (1995) The catecholamine hypothesis of affective disorders: a review of supporting evidence. 1965. J Neuropsychiatry Clin Neurosci 7:524-33
Seltman H, Roeder K, Devlin B (2003) Evolutionary-based association analysis using

haplotype data. Genet Epidemiol 25: 48-58
Sen S, Nesse RM, Stoltenberg SF, Li S, Gleiberman L, Chakravarti A, Weder AB, Burmeister M. A BDNF coding variant is associated with the NEO personality inventory domain neuroticism, a risk factor for depression. Neuropsychopharmacology 28:397-401
Sheline YI, Wany P, Gado MH, Csernansky JG, Vannier MW (1996) Hippocampal atrophy in recurrent major depression. PNAS USA 93:2897-2902
Sheline YI, Sanghavi M, Mintun MA, Gado MH (1999) Depression duration but not age predicts hippocampal volume loss in medically healthy women with recurrent major depression. J Neurosci 19:5034-5043
Shimuzu E, Hashimoto K, Okamura N, Koike K, Komatsu N, Kumakiri C et al (2003) Alterations of serum levels of brain-derived neurotrophic factor (BDNF) in depressed patients with or without antidepressants. Biol Psychiatry 54:70-75
Shirayama Y, Chen AC, Nakagawa S, Russell DS, Duman RS (2002) Brain-derived neurotrophic factor produces antidepressant effects in behavioral models of depression. J Neurosci 22: 3251-61.
Silberg J, Pickles A, Rutter M (1999)The influence of genetic factors and life stress on depression among adolescent girls. Arch Gen Psychiatry 56:225-32
Siuciak JA, Altar CA, Wiegand SJ, Lindsay RM (1994) Antinociceptive effect of brain-derived neurotrophic factor and neurotrophin-3. Brain Research 633:326-30
Siuciak JA, Boylan C, Fritsche M, Altar CA, Lindsay RM (1996) BDNF increases monoaminergic activity in rat brain following intracerebroventricular or intraparenchymal administration. Brain Research 710:11-20
Siuciak JA, Lewis DR, Wiegand SJ, Lindsay RM (1997) Antidepressant-like effect of brain derived neurotrophic factor(BDNF). Pharmacology Biochemistry and Behaviour 56:131-137
Sklair-Tavron L, Nestler EJ (1995) Opposing effects of morphine and the neurotrophins, NT-3, NT-4 and BDNF, on locus coeruleus neurons in vitro. Brain Research 702:117-125
Sklar P, Gabriel SB, McInnis MG et al (2002) Family-based association study of 76 candidate genes in bipolar disorder: BDNF is a potential risk locus. Mol Psychiatry 7:579-593
Solum DT, Handa RJ (2002) Estrogen regulates the development of brain-derived neurotrophic factor mRNA and protein in the rat hippocampus. J Neurosci 22:2650-9
Smith MA, Makino S, Kvetnansky R, Post RM (1995) Stress and glucocorticoids affect the expression of brain-derived neurotrophic factor and neurotrophin-3 mRNAs in the hippocampus. J Neurosci 15:1768-77
Snieder H, Sawtell PA, Ross L, Walker J, Spector TD, Leslie RD (2001) HbA(1c) levels are genetically determined even in type 1 diabetes: evidence from healthy and diabetic twins. Diabetes 50:2858-63
Squire LR, Zola-Morgan S (1991) The medial temporal lobe memory system. Science 253:1380-6
Statsoft, Inc. (2004) Electronic Statistics Textbook http://www.statsoftinc.com/textbook/stathome.html; Statsoft, Inc.
Stewart CA, Reid IC (1994) Ketamine prevents ECS-induced synaptic enhancement in rat hippocampus. Neurosci Lett 178:11-14

Strauss J, Barr CL, George CJ, King N, Shaikh S, Devlin B et al (2004a) Association study of brain-derived neurotrophic factor in adults with a history of childhood onset mood disorder. Am J Med Genet 131B:16-9

Strauss J, Barr CL, George CJ, Ryan CM, King N, Shaikh S, et al (2004b) BDNF and COMT Polymorphisms: Relation to Memory Phenotypes in Young Adults With Childhood-Onset Mood Disorder. Neuromolecular Med 5:181-192.

Strober M, Carlson G (1982) Bipolar illness in adolescents with major depression: Clinical, genetic, and psychopharmacologic predictors in a three- to four-year prospective follow-up investigation. Arch Gen Psychiatry 39:549-555

Strober M, Lampert C, Schmidt S, Morrell W (1993) The course of major depressive disorder in adolescents: I. Recovery and risk of manic switching in a follow-up of psychotic and nonpsychotic subtypes. J Am Acad Child Adolesc Psychiatry 32:34-42

Stunkard AJ, Faith MS, Allison KC (2003) Depression and obesity. Biol Psychiatry 54:330-7

Sullivan PF, Eaves LJ, Kendler KS, Neale MC (2001) Genetic case-control association studies in neuropsychiatry. Arch Gen Psychiatry 58:1015-24

Templeton AR, Boerwinkle E, Sing CF(1987) A cladistic analysis of phenotypic associations with haplotypes inferred from restriction endonuclease mapping. I. Basic theory and an analysis of alcohol dehydrogenase activity in Drosophila. Genetics 117:343-51

Thapar A, McGuffin P (1994) A twin study of depressive symptoms in childhood. Br J Psychiatry 165: 259-65

Todd RD, Neuman R, Geller B, Fox LW, Hickok J (1993) Genetic studies of affective disorders: should we be starting with childhood onset probands? J Am Acad Child Adolesc Psychiatry 32:1164-1167

Tokuyama W, Okuno H, Hashimoto T et al (2000) BDNF upregulation during declarative memory formation in monkey inferior temporal cortex. Nat Neurosci 3:1134-1142

Tsai SJ, Hong CJ, Yu YW, Chen TJ. Association study of a brain-derived neurotrophic factor (BDNF) Val66Met polymorphism and personality trait and intelligence in healthy young females. Neuropsychobiology 49:13-6.

Tuulio-Henriksson A, Haukka J, Partonen T, Varilo T, Paunio T, Ekelund J, Cannon TD, Meyer JM, Lonnqvist J (2002) Heritability and number of quantitative trait loci of neurocognitive functions in families with schizophrenia Am J Med Genet 114:483-90

Tyler WJ, Alonso M, Bramham CR et al (2002) From acquisition to consolidation: on the role of brain-derived neurotrophic factor signaling in hippocampal-dependent learning. Learn Mem 9:224-237

Uno H, Tarara R, Else JG, Suleman MA, Sapolsky RM (1989) Hippocampal damage associated with prolonged and fatal stress in patients. Journal of Neuroscience 9:1705-1711

Vaidya VA Duman RS (1996) Chronic ECS induces mossy fiber sprouting. Society for Neuroscience Abstracts 22:181

van Praag H, Kempermann G, Gage F (1999) Running increases cell proliferation and neurogenesis in the adult mouse dentate gyrus. Nat Neurosci 2:266-70

Vicario-Abejon C, Owens D, McKay R, Segal M (2002) Role of neurotrophins in central synapse formation and stabilization. Nat Rev Neurosci 3:965-74

Weissman MM, Leckman JF, Merikangas KR (1984a) Depression and anxiety disorders

in parents and children. Results from the Yale family study. Arch Gen Psychiatry 41:845-52

Weissman MM, Wickramaratne P, Merikangas KR (1984) Onset of major depression in early adulthood. Increased familial loading and specificity. Arch Gen Psychiatry 41:1136-43

Weissman MM, Wolk S, Goldstein RB, Moreau D, Adams P, Greenwald S, Klier CM, Ryan ND, Dahl RE, Wickramaratne P (1999a) Depressed adolescents grownup. JAMA 281:1707-1713

Weissman MM, Wolk S, Wickramaratne P, Goldstein RB, Adams P, Greenwald S, Ryan ND, Dahl RE, Steinberg D (1999b): Children with prepubertal-onset major depressive disorder and anxiety grown up. Arch Gen Psychiatry 56:794-801

Weller RA, Kapadia P, Weller EB, Fristad M, Lazaroff LB, Preskorn SH (1994) Psychopathology in families of children with major depressive disorders. J Affect Disord 31:247-52

Westover AN, Marangell LB (2002) A cross-national relationship between sugar consumption and major depression? Depress Anxiety 16:118-20

Wooley CS, Gould E, McEwen BS (1990) Exposure to excess glucocorticoids alters dendritic morphology of adult hippocampal pyramidal neurons. Brain Research 531:225-231

Yau LJ, Olsson T, Morris RG, Meaney MJ, Seckl JR (1995). Glucocorticoids, hippocampal corticosteroid receptor gene expression and antidepressant treatment: relationship with spatial learning in young and aged rats. Neuroscience 66:571-581

Yau JL, Noble J, Hibberd C et al (2002) Chronic treatment with the antidepressant amitriptyline prevents impairments in water maze learning in aging rats. J Neurosci 22:1436-1442

Young SE, Smolen A, Stallings MC, Corley RP, Hewitt JK (2003) Sibling-based association analyses of the serotonin transporter polymorphism and internalizing behavior problems in children. J Child Psychol Psychiatry 44:961-7

Zakzanis KK, Leach L, Kaplan E (1998) On the nature and pattern of neurocognitive function in major depressive disorder. Neuropsychiatry Neuropsychol Behav Neurol 11:111-9