THE PENNSYLVANIA STATE UNIVERSITY
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DEPARTMENT OF BIOLOGY

# ANTIDEPRESSANT ACTION: THE ROLES OF BRAIN-DERIVED NEUROTROPHIC FACTOR, WNT SIGNALING, AND CYCLIC AMP RESPONSE ELEMENT BINDING

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# Abstract

Major depressive disorder (MDD) is a debilitating mood disorder with significant lifetime risk and high social costs. In the United States, the lifetime prevalence of MDD is almost 17%. Antidepressants are effective in treating symptoms of MDD, but the precise mechanisms by which they work are still largely unknown. The neurotrophin brain-derived neurotrophic factor (BDNF) is of special interest as it is required the therapeutic action of antidepressant drugs. BDNF itself exhibits antidepressant-like activity in animal models of MDD.

Chronic antidepressant treatment increases total *BDNF* expression levels in the hippocampus and frontal cortex. Transcriptional regulation of the *BDNF* gene is extremely complex. Alternative splicing of human *BDNF* transcripts can theoretically result over thirty unique mRNAs. Chronic treatment with the antidepressants fluoxetine (SSRI), duloxetine (NRI), and desipramine (TCA) results in increased relative expression of *BDNF* transcripts in the hippocampus. Duloxetine and desipramine increase relative expression of *BDNF* transcripts in the frontal cortex as well.

*BDNF* Exon IV expression increases in both the hippocampus and frontal cortex after chronic treatment with the largest variety of antidepressants. Exon IV is hypothesized to be highly targeted by the mechanisms of antidepressant function. Inhibiting cyclic AMP response element binding protein (CREB) binding prevents expression of *BDNF* Exon IV. Enhanced CREB binding from chronic treatment with antidepressants is likely involved in the relative increase in *BDNF* Exon IV expression.

Chronic antidepressant administration activates canonical Wnt signaling. Additionally, overexpression of *Wnt* genes reduces depressive-like behaviors. There is a significant interaction in retinal cells where Wnt signaling activates *BDNF* expression. Wnt signaling also increases activity of calcium/calmodulin-dependent kinase IV (CaMK-IV) activity and levels of phosphorylated CREB (pCREB).

This review proposes a mechanism for the increase in *BDNF* expression in response to chronic antidepressant treatment. Chronic antidepressant treatment activates the canonical Wnt pathway. Wnt signaling increases CaMK-IV activity, and increased CaMK-IV activity results in increased pCREB. CREB acts as a sequence-specific DNA binding protein and, in its phosphorylated form, activates transcription of target genes linked to CREB binding sites. CREB binding activates transcription, and *BDNF* expression increases. This mechanism is most thoroughly supported by observed effects of the TCA desipramine on these components. A connection needs to be established between chronic desipramine treatment and activation of Wnt signaling before this mechanism can be further explored and validated.

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# Chapter 1: Introduction

Major depressive disorder (MDD) is a debilitating mood disorder with significant lifetime risk and high social costs (Millan 2006). Symptoms of MDD are physiological, behavioral, and psychological. The World Health Organization (2001) states that depression, along with cardiovascular disease, is one of the leading causes of disability on a global scale. In the United States, the lifetime prevalence of MDD is almost 17% (Kessler et al. 2005). There is a higher prevalence of MDD diagnosis among those aged 30 to 44 as well as among women. MDD, however, is a treatable disorder. When taken chronically, antidepressants can attenuate and even eliminate the symptoms of MDD (Morilak et al. 2004). However, only approximately half of patients respond to antidepressants (Rush et al. 2006). Of those that do respond, about two thirds ever go into remission, or experience complete absence of depressive symptoms. Much of the emphasis in antidepressant research, therefore, is placed on increasing antidepressant efficacy.

Tricyclic antidepressants (TCAs) are one major class of antidepressants currently available on the market. Most TCAs block the re-uptake sites for both serotonin and noradrenaline (Millan 2006). By contrast, the selective serotonin reuptake inhibitors (SSRIs) selectively interact with the serotonin receptors to block re-uptake of serotonin but not noradrenaline. The serotonin noradrenaline re-uptake inhibitors (SNRIs) represent a third major class of antidepressants. Like most TCAs, SNRIs block both serotonin and noradrenaline re-uptake. TCAs have a varied binding profile and can act as antagonists at NMDA, muscarinic acetylcholine, and histamine receptors, which results in many negative side effects from chronic treatment. Since SNRIs are selective for serotonin and noradrenaline, they have diminished side effects.

Antidepressant drugs have been found to increase monoamine neurotransmitters, leading to the development of the Monoamine Hypothesis of Depression (Hirschfeld 2000). According to the Monoamine Hypothesis, depression results from decreased activity of noradrenergic and/or serotonergic systems. In terms of antidepressant efficacy, clinical trials indicate that SNRIs treat certain symptoms of MDD fastest (Hirschfeld et al. 2005). Although the generic mechanisms of these antidepressants are understood, the specific mechanism by which they reduce depressive symptoms remains largely unknown.

The Monoamine Hypothesis is, however, almost thirty years old. The Neurotrophin Hypothesis of Depression is a slightly newer hypothesis based mostly on the correlation of decreased levels of brain-derived neurotrophic factor (BDNF) in the hippocampus and depressive-like behaviors (Martinowich et al. 2007). Antidepressant treatment also increases expression of *BDNF*, the gene encoding this neurotrophin, leading to the belief that neurotrophin levels are implicated in depressive disorders and antidepressant function (Duman et al. 2006).

BDNF is a neurotrophin present throughout the central nervous system and the periphery that functions via tropomyosin receptor kinase B (TrkB) (Klein et al. 1991). Neurogenesis, neuronal survival, and neuron outgrowth are directly affected by BDNF from development through adulthood (Huang et al. 2001). BDNF also plays a role in synaptic plasticity in the hippocampus, specifically in learning and memory (Lu et al. 2008).

The *BDNF* gene is highly conserved (Pruunsild et al. 2007). Expression of *BDNF* is highest in the hippocampus and lowest in the striatum (Liu et al. 2006). Chronic antidepressant treatment has been shown to increase *BDNF* mRNA and protein levels in the brain, most noticeably in the hippocampus and frontal cortex (Chen et al. 2001; Dwivedi et al. 2006; Zhang

et al. 2010). Additionally, infusion of BDNF protein into the rat hippocampus results in decreased depressive-like behaviors (Sirianni et al. 2010).

In addition to *BDNF* expression, chronic treatment with diverse classes of antidepressants activates canonical Wnt signaling pathway (Okamoto et al. 2010). The Wnt family of proteins have critically important roles in development, comparable to the Hedgehog gene family (Logan et al. 2004). The non-acronymic term Wnt represents a fusion of the names of two genes: Wg (*wingless*) and Int (Rijsewijk et al. 1987). The *wingless* gene was originally identified in *Drosophila melanogaster*, first as a recessive mutation affecting wing development and then as a segment polarity gene for the formation of limbs. The *Int*-1 gene is the vertebrate homologue of *wingless*. The Wnt signaling pathway, therefore, controls many of the same processes as *wingless*, such as cell proliferation, cell fate, migration, and polarity.

Wnt proteins work through a variety of pathways, the most common of which is known as the canonical Wnt signaling pathway. On activation of canonical Wnt signaling, Wnt proteins bind to Frizzled (Fzd)/ low-density lipoprotein-related protein (LRP) co-receptors in the cell membrane (Pinson et al. 2000). In the absence of a Wnt ligand, glycogen synthase kinase-3β (GSK-3β) forms a complex with Axin and other factors to phosphorylate the nuclear transcription factor β-catenin and target it for degradation [**Figure 1-1a**]. When Wnt proteins bind, Fzd/LRP receptors bind to Axin, preventing that complex from being formed [**Figure 1-1b**]. Wnt binding acts to inhibit GSK-3β, thereby raising β-catenin levels in the nucleus of the cell. β-catenin then activates transcription of certain target genes. The ultimate goal of the canonical Wnt signaling pathway is to increase transcription of a set of Wnt target genes characterized by TCF/LEF DNA binding sites (MacDonald et al. 2009).

Finally, chronic treatment with the SSRI fluoxetine has been shown to increase expression of *CREB*, the gene encoding the cyclic AMP response element binding (CREB) protein (Tiraboschi et al. 2004). CREB is a transcription factor that works via binding to a cyclic AMP response element (CRE) in a gene (Lonze et al. 2002). CREB typically aids in transcription of genes with a role in cell survival. In particular, neural activity-induced transcription of *BDNF* and *Wnt2* genes is CREB-dependent (Wayman et al. 2006; Balkowiec-Iskra et al. 2011; Pruunsild et al. 2011).

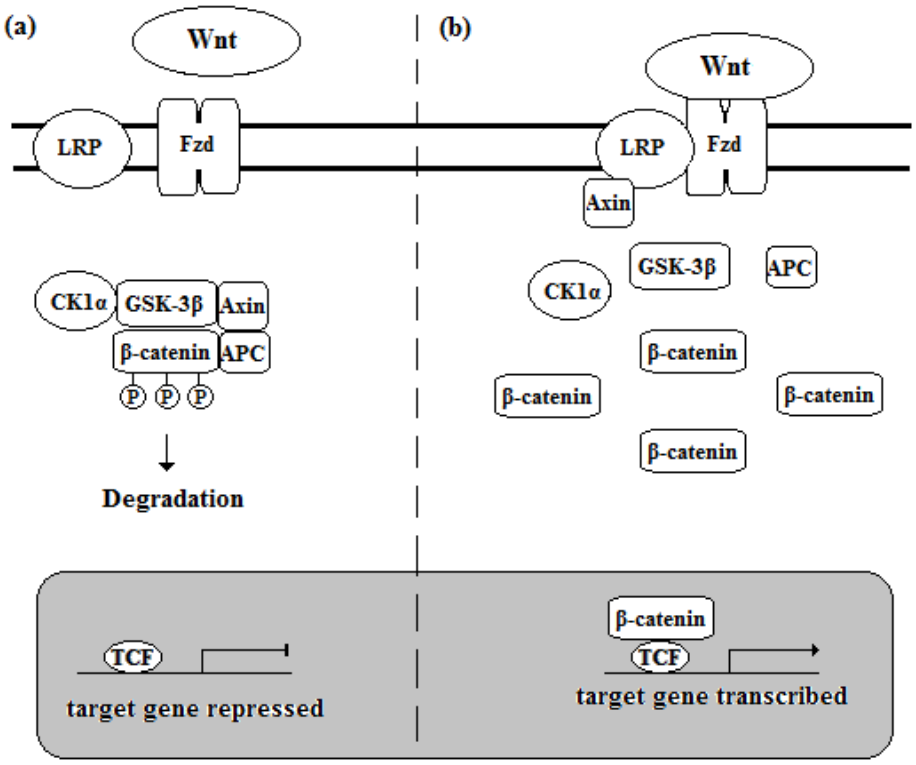


**Figure 1-1**. Simplified schematic representation of the canonical Wnt signaling pathway (a) in the absence of Wnt ligand biding and (b) after activation via Wnt ligand binding. In the absence of a Wnt ligand, glycogen synthase kinase-3β (GSK-3β) forms a complex with Axin and other factors to phosphorylate β-catenin and target it for degradation. When Wnt proteins bind, Fzd/LRP receptors bind to Axin, preventing that complex from being formed. Wnt binding acts to inhibit GSK-3β, thereby raising β-catenin levels in the nucleus of the cell. β-catenin then activates transcription of certain target genes. The ultimate goal of the canonical Wnt signaling pathway is to increase transcription of a set of Wnt target genes characterized by TCF/LEF DNA binding sites. This figure was modified from Macdonald et al. 2009.

Chronic antidepressant administration is proven to affect *BDNF* expression, Wnt signaling, and CREB. This review will examine the interaction of these three factors in antidepressant function. The ultimate objective is to propose a specific mechanism explaining how chronic antidepressant administration increases *BDNF* expression in the brain via Wnt signaling and CREB.

# Chapter 2: Experimental Thesis Work

My original intention was to complete my thesis research doing an experimental project in the Andrews Research Group at The Pennsylvania State University. The objective of the study was to utilize TOPO cloning protocols to create a cDNA library of alternate splice variants of *BDNF*, the gene coding for brain-derived neurotrophic factor. This cDNA library could then be used to create radioactive probes for *in situ* hybridization of *BDNF* transcript mRNA in different brain regions, specifically the hippocampus and frontal cortex. The results from hybridization could then be used to generate standard curves for absolute quantification of *BDNF* mRNA expression.

First, *BDNF* mRNA was isolated from the hippocampus, frontal cortex, striatum, and brainstem of specific mouse models. Then, non-cutting restriction enzymes were chosen for each of the *BDNF* splice variants. New *BDNF* primers derived from publicly available mouse DNA sequence were used in RT-PCR reactions for all nine mouse *BDNF* transcripts. Nine cDNA products of the expected length were successfully created.

*BDNF* Exon I, Exon IV, and Exon V were chosen as the first transcripts to be cloned into a plasmid vector because they were well-characterized and expressed at relatively high levels. The TOPO cloning process consists of ligating a PCR product into specified plasmid vectors containing an antibiotic resistance gene. Using TOPO cloning, Exons I, IV, and V were ligated into an Invitrogen pCR4-TOPO vector, transformed into competent *E. coli*, and analyzed by colony PCR. Overnight cultures were grown up for isolation of plasmids containing Exons I, IV, and V. The plasmids were isolated using a Qiagen mini-prep protocol. DNA sequencing confirmed successful insertion of *BDNF* Exons I, IV, and V into the plasmids as depicted in Figure 4-1.

The experimental research I began in the Andrews Research Group was not completed because the group moved to UCLA before I was able to complete my project.

# Chapter 3: The Role of BDNF in Depressive Disorders

*1. Decreased BDNF and depression*

For years, it was accepted that stress-induced decreases in BDNF levels, specifically in the hippocampus, were positively correlated with an increased risk for depression (Murakami et al. 2005). In humans, a significant decrease in *BDNF* and TrkB expression in the hippocampus and prefrontal cortex is observed in postmortem analysis of suicide subjects (Dwivedi et al. 2003). There is also a marked reduction of BDNF serum levels in depressed patients (Sen et al. 2008). Genetic polymorphisms in *BDNF* result in greater vulnerability to stress (Gatt et al. 2009). The trend is observed in rodents as well; *BDNF* knockdown mice with decreased *BDNF* expression in the dentate gyrus exhibit depressive-like behavior (Taliaz et al. 2010). Although numerous other studies associate decreases in BDNF levels with depressive-like behavior, questions about the behavior tests used to determine these conclusions have arisen. Standard behavior for investigating depressive and anxious behavior in rodents were actually designed to test rodents' responsiveness to antidepressant drugs not actual depressive behavior (Wong et al. 2004).

Currently, it is proposed that *BDNF* expression levels do not affect susceptibility to depression but, rather, antidepressant efficacy. One group has shown that BDNF deficits do not increase susceptibility to depressive-like behaviors under a chronic stress paradigm (Ibarguen-Vargas et al. 2009). *BDNF* +/- mice have approximately half the amount of BDNF of *BDNF*+/+ mice. However, after stress, the heterozygous mice showed no significant difference in depressive-like behaviors from wild-type mice. This supports the first portion of the hypothesis: BDNF deficits resulting from stress do not increase the likelihood of developing depression.

The same group also showed that BDNF levels do affect the effectiveness of the TCA imipramine (Ibarguen-Vargas et al. 2009). Chronic imipramine treatment doubles *BDNF* expression in the dentate gyrus in both *BDNF* +/+ mice and *BDNF* +/- mice. However, significant decreases in depressive-like behavior following imipramine (SSRI) administration are only observed in the wild-type mice. These results support the second part of the hypothesis: decreased BDNF levels in the dentate gyrus reduce the effectiveness of imipramine at producing an antidepressant effect.

Gender is also hypothesized to confound the correlation between reduced *BDNF* expression, stress, and depression. A gender-dependent increase in susceptibility to depressive-like and anxious behaviors is also observed in conditional *BDNF* knockout (KO) mice (Autry et al. 2009). Loss of *BDNF* in the forebrain of female mice results in increased sensitivity to depression and anxiety when coupled with a chronic stress paradigm. Contrarily, male *BDNF* KO mice do not exhibit stress-induced depressive-like behavior, suggesting that *BDNF* expression in the forebrain only increases risk for depression in females. Although the lifetime prevalence of MDD is significantly higher in women (Kessler et al. 2005), there are no patient studies in existence to support that differential *BDNF* expression is why.

*2. Effects of antidepressants on BDNF*

SSRIs have been shown to increase *BDNF* expression [**Table 3-1**]. Chronic paroxetine treatment dramatically increases *BDNF* expression in CA1, CA3, and the dentate gyrus of the rat hippocampus (Martinez-Turrillas et al. 2005). Chronic treatment with the prototypical SSRI fluoxetine also increases hippocampal *BDNF* expression but to a much lesser extent than

paroxetine (Dwivedi et al. 2006). Chronic administration of citalopram alone has no observed effect on total *BDNF* expression in the hippocampus or the cortex (Russo-Neustadt et al. 2004). However, when combined with physical exercise, citalopram increases total *BDNF* in the dentate gyrus, CA1, and CA3 regions as well as the prefrontal cortex. Fluoxetine has no effect on *BDNF* expression in the cortex unless coupled with stress (Zhang et al. 2010).

SNRIs also affect *BDNF* expression levels in the brain. Chronic duloxetine treatment increases *BDNF* mRNA levels in the frontal cortex (Mannari et al. 2008). The same effect is observed in the frontal cortex following chronic venlafaxine treatment (Cooke et al. 2009; Zhang et al. 2010). The prototypical SNRI venlafaxine and the NRI reboxetine increase *BDNF* mRNA levels in the hippocampus enough to attenuate the effects of chronic stress (Chen et al. 2009; Zhang et al. 2010). Most of the literature concludes that SNRIs have a greater effect on BDNF expression in the frontal cortex than SSRIs.

Finally, chronic treatment with the TCA desipramine dramatically increases *BDNF* expression in the dentate gyrus, CA1, and CA3 of the hippocampus similarly to the SSRI paroxetine (Martinez-Turrillas et al. 2005). In this case, chronic paroxetine and desipramine administration produce increases of relatively the same magnitude despite their different mechanisms of action. Cortical *BDNF* expression also increases as a result of chronic desipramine administration (Dwivedi et al. 2006). In *BDNF* knockout mice, however, cortical *BDNF* expression does not respond to chronic desipramine treatment (Monteggia et al. 2004), which provides further support for hypothesis that BDNF is required for efficient antidepressant action in the frontal cortex.

**Table 3-1**. Effect of chronic antidepressant treatments on relative expression of total *BDNF* in different brain regions of rodents.

| Antidepressant | | Total *BDNF* | |
|---|---|---|---|
| | | HP | FC |
| *SSRI* | Citalopram[1] | ↑** | ↑** |
| | Fluoxetine[2,3] | ↑ | ↑* |
| | Paroxetine[4] | ↑↑↑ | |
| *SNRI* | Duloxetine[5] | | ↑ |
| | Venlafaxine[3,6] | ↑ | ↑ |
| | Desipramine[2,4] | ↑↑↑ | ↑ |
| *NRI* | Reboxetine[7] | ↑ | |

This table shows the effects of chronic treatment with different classes of antidepressants on relative expression of BDNF in the hippocampus (HP) and frontal cortex (FC). ↑ indicates an increase in expression. A hatched bar indicates there is no current literature on this data point. All antidepressants elicited an increase in total BDNF expression. *Change is only observed when fluoxetine is coupled with a chronic stress paradigm. **Change is only observed when citalopram is paired with exercise. Rat data: (1) Russo-Neustadt et al 2004 (2) Dwivedi et al 2006 (3) Zhang et al. 2010 (4) Martinez-Turrillas et al. 2005 (5) Mannari et al. 2008 (6) Cooke et al. 2009 ;
Mouse data : (7) Chen et al. 2009

Despite extensive research on *BDNF* mRNA expression, few studies have reported effects of antidepressants on BDNF protein levels. This is of concern because, in the majority of cases, a positive correlation cannot be found between antidepressant-induced *BDNF* mRNA and protein expression (De Foubert et al. 2004; Jacobsen et al. 2004).

In rats, administration of the selective noradrenaline re-uptake inhibitors desipramine and reboxetine increases hippocampal expression BDNF protein more rapidly than the corresponding mRNA expression (Musazzi et al. 2009). Reboxetine treatment increases BDNF protein in the prefrontal cortex as well. This group hypothesizes that antidepressants function through

posttranscriptional modifications to *BDNF* mRNA, but no subsequent research has been conducted to support this conclusions.

*3. Effects of BDNF administration*

Administration of BDNF has varying effects based on where and how it is administered. When administered to the hippocampus of rodents, BDNF has dose-dependent antidepressant-like effects as shown in the forced swim and learned helplessness test (Shirayama et al. 2002; Sirianni et al. 2010). Acute and chronic hippocampal infusion of BDNF significantly decreases immobility and increases swimming time in the forced swim test and decreases latency in the learned helplessness paradigm. By contrast, infusion of BDNF into the ventral tegmental area (VTA) of the midbrain, results in a depressive-like phenotype in rats (Eisch et al. 2003).

BDNF administration maintains its antidepressant effects for a longer period of time than traditional antidepressants (Hoshaw et al. 2005), making it a promising candidate to be used as an antidepressant. Unfortunately, infusion directly into the hippocampus is not a feasible option for treating depression in humans. However, one group has found that peripheral, or humoral, BDNF administration via osmotic mini-pumps mimics chronic antidepressant treatment in mice (Schmidt et al. 2010). Peripheral BDNF administration in humans might be worth exploring as an experimental antidepressant therapy.

# Chapter 4: *BDNF* Transcript Expression

*1. BDNF gene transcription in rodent and human models*

The structure of the *BDNF* gene is highly complex due to multiple promoter regions upstream of a single protein coding exon that result in at least 11 distinct mRNA transcripts in both rodents and humans (Timmusk et al. 1993; Aoyama et al. 2001; Liu et al. 2005; Liu et al. 2006). According to the most recent data, the mouse and rat *BDNF* genes each have eight alternative 5' noncoding exons and one common 3' coding exon and (Exons I-VIII) (Aid et al. 2007) [**Figure 4-1**]. In the majority of transcripts, a single noncoding exon is spliced to the unique coding exon to create a mature mRNA transcript. In addition, Aid et al. discovered a novel unspliced *BDNF* mRNA transcript (Exon IXA) that represents an extension of the 5' protein coding region. A unique promoter controls transcription of each 5' exon. Two additional transcripts result from internal alternative splice sites within Exon II. In summary, rodent *BDNF* has nine promoter regions controlling transcription of 11 distinct mRNA transcripts.

Compared to the rodent *BDNF* gene, the human gene transcription shows additional complexity. First, the human gene has 11 exons: ten 5' noncoding exons and one 3' coding exon (Pruunsild et al. 2007) [**Figure 4-2**]. As in rodents, most human *BDNF* mRNA transcripts are composed of a single noncoding exon spliced to the common coding exon. However, three BDNF transcripts contain more than one 5' noncoding exon. The noncoding exon combinations shown to exist in these transcripts include: Exon VIb-IXbd, Exon V-VIII, and Exon V-VIII-VIIIh. Second, human *BDNF* gene has a total of nine promoters, meaning that, dissimilarly to

rodents, each exon does not have a unique promoter. The promoter of Exon V also controls transcription of Exon V-VIII and Exon V-VIII-VIIIh. Internal alternative splice sites in Exon II, Exon VI, Exon VII and Exon IXa result in five additional transcripts: Exon IIa, Exon IIb, Exon VIa, Exon VIIa, and Exon IXabd. Ultimately, human *BDNF* gene has seventeen distinct mRNA transcripts under the control of nine promoters.

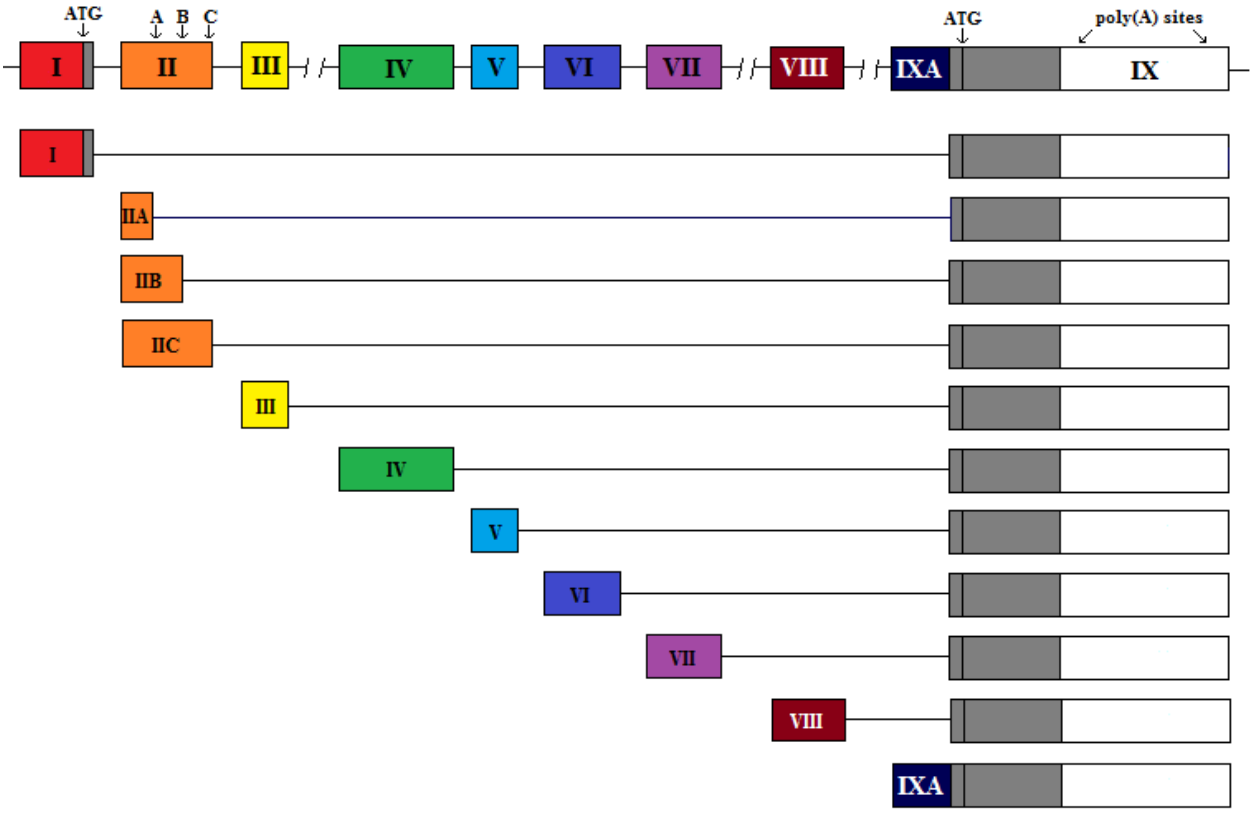


**Fig. 4-1.** Intron-exon structure and alternative transcripts of rodent *BDNF* genes.
The colored boxes represent the noncoding exons, and the lines represent introns. The gray shaded areas represent the coding region of the gene. Arrows indicate transcription start sites (ATG). In Exon II, "A," "B," and "C" are internal alternative splice sites. This figure was modified from a figure in Aid et al. 2007.

Rodent and human *BDNF* genes contain two different polyadenylation sites in Exon IX (Aid et al. 2007; Pruunsild et al. 2007). Polyadenylation is the post-transcriptional process of

adding a poly(A) tail, or a stretch of adenosine monophosphates, to the mRNA. Having two different polyadenylation sites means that each transcript can be expressed in two different forms. Therefore, the rodent *BDNF* gene can, in theory, produce twenty-two distinct mRNA transcripts, and the human gene can produce even more.

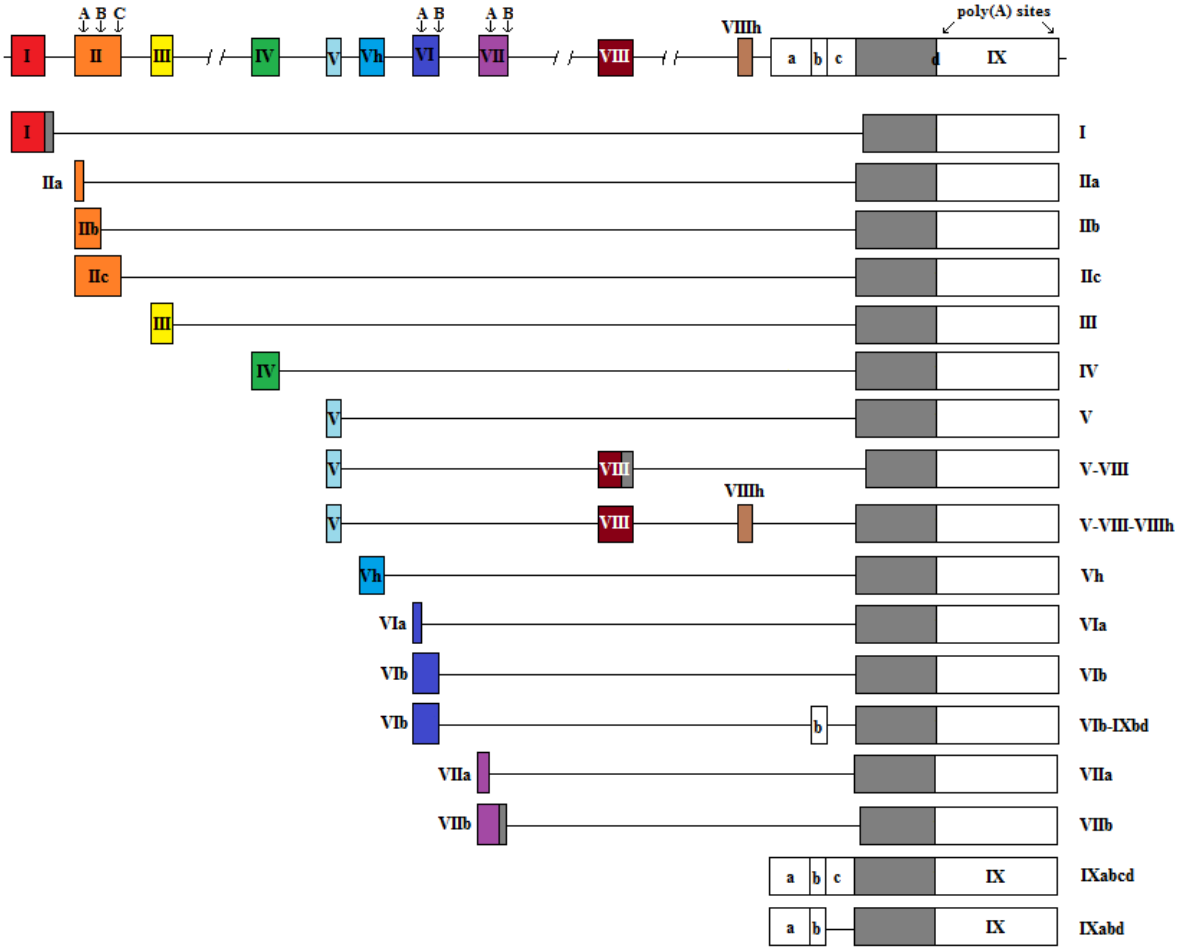


**Fig. 4-2.** Intron-exon structure and alternative transcripts of human *BDNF* gene.
The colored boxes represent the noncoding exons, and the lines represent introns. The gray shaded areas represent the coding region of the gene. Arrows with A, B, C in Exons II, VI, and VII are internal alternative splice sites. Exon IX is divided into regions called a, b, c, d, as can be seen written in the boxes. This figure was modified from a figure in Pruunsild et al. 2007.

*2. Differential expression of BDNF transcripts*

Most *BDNF* transcripts are expressed in all regions of the rodent brain but at different levels [**Table 4-1**] (Liu et al. 2006). All eleven *BDNF* transcripts have the highest relative expression level in the hippocampus and the lowest in the striatum. The frontal cortex also has high expression of most transcripts. In the hippocampus, Exon VI is the most highly expressed transcript, followed by Exon IV, I, II, III, and finally VII. The same group also measured transcript expression levels in other organs besides the brain and found high expression of most *BDNF* transcripts in the heart and spleen. Exons IV and VI were present in high levels in all CNS and peripheral tissues and, therefore, could be the most abundant noncoding *BDNF* exons in rodents.

**Table 4-1**. Relative expression of *BDNF* transcripts in the adult rat brain.

| **Brain Region** | ***BDNF* Transcript** | | | | | |
|---|---|---|---|---|---|---|
| | **Exon I** | **Exon II** | **Exon III** | **Exon IV** | **Exon VI** | **Exon VII** |
| Hippocampus | ***** | ***** | ***** | ***** | ******* | *** |
| Frontal Cortex | *** | *** | ***** | ***** | ***** | - |
| Striatum | * | * | * | *** | *** | * |
| Brain Stem | * | *** | *** | *** | *** | - |

This table shows the relative expression of specific *BDNF* transcripts in the brain of an adult rat. ******* = highest expression; ***** = high expression; *** = moderate expression; * = low expression; - = no expression. All exons are expressed at high levels in the hippocampus. Exon IV is the most highly expressed BDNF transcript in any brain region. Exon V had not yet been discovered at the time of this study and, therefore, is not included. Liu et al. 2006

Unfortunately, this study was conducted before the discovery of Exons V, VIII, and IXa in 2007, so the relative expression levels of these more recently discovered transcripts are yet to be determined. Additionally, relative expression of *BDNF* transcripts in rodent models of depression should be analyzed to further support or refute the Neurotrophin Hypothesis of Depression.

Differential expression of BDNF transcripts in different brain areas can be linked to the known function of these brain regions. For example, relative expression of Exons III, IV, and VI is high in the frontal cortex. Since the cortex plays a critically important role in working memory, it is possible that deficits in BDNF and corresponding cellular deficits contribute to deficits in working memory associated with major depression. High expression of all exons in the hippocampus supports their importance in ventral hippocampal functions, like emotional memory. Synaptic plasticity is linked to learning. Therefore, it is hypothesized that, in the hippocampus, Exons III, IV, and VI retain their role in synapse formation, while Exons I and II increase synaptic plasticity.

In addition to differential expression in different adult brain regions, rodent *BDNF* transcripts are also differentially expressed during development [**Table 4-2**]. For example, Exon II expression in the CA1 region of the hippocampus peaks during the P4 stage in rats and decreases in adulthood (Sathanoori et al. 2004). Exon I expression in CA1, however, decreases during the postnatal stages and then rapidly peaks in adulthood. This shows the possibility of differential expression in the same brain region during different developmental stages. Relative transcript expression levels in different brain regions can peak at similar postnatal ages. *BDNF* Exon I peaks during adulthood in CA1, CA3 and the dentate gyrus (DG). Exon II peaks at P14 in CA3 and DG.

**Table 4-2**. Relative expression of specific *BDNF* exons during postnatal development in the hippocampus of rats.

| Developmental Stage | | BDNF Transcript | | | | | |
|---|---|---|---|---|---|---|---|
| | | CA1 | | CA3 | | DG | |
| | | Exon I | Exon II | Exon I | Exon II | Exon I | Exon II |
| *Postnatal* | P0 | *** | *** | * | ** | ** | * |
| | P4 | ** | **** | ** | *** | * | *** |
| | P14 | * | ** | *** | **** | *** | **** |
| *Adult* | | **** | * | **** | * | **** | ** |

This table shows the differential relative expression of specific *BDNF* transcripts in the rat CA1, CA3, and dentate gyrus (DG) during development. The values range from highest relative expression (****) to lowest relative expression (*). Exon I is most highly expressed in all three hippocampal regions in adulthood. Exon II is most highly expressed during the P14 stage of development. Sathanoori et al. 2004

Similar to the rodent gene, the human *BDNF* gene also exhibits differential transcript expression during different stages of development [**Table 4-3**]. Expression of *BDNF* Exons I, II, IV, and VI in the dorsolateral prefrontal cortex (DLPFC) peaks during postnatal development (Wong et al. 2009). Specifically, the relative expression of Exons I, IV, and VI is highest during infancy. Expression of Exon II peaks slightly after, in the toddler stage. There is also a trend of decreased expression in the DLPFC of adults for all four of these exons. BDNF plays a role in synapse formation and synaptic plasticity, which occur at higher rates in postnatal development than in adults (Lu et al. 2008). This explains the increased expression of *BDNF* during the infant and toddler stages.

**Table 4-3**. Relative expression of specific *BDNF* exons during cortical development in humans.

| **Developmental Stage** | ***BDNF* Transcript** | | | |
|---|---|---|---|---|
| | Exon I | Exon II | Exon IV | Exon VI |
| Neonate | ****** | * | * | ****** |
| Infant | ******** | **** | ******** | ******** |
| Toddler | ***** | ******** | ****** | ***** |
| School Age | *** | *** | ***** | *** |
| Teenager | ** | ***** | ** | **** |
| Young Adult | **** | ****** | **** | * |
| Adult | * | ** | *** | ** |

This table shows the differential relative expression of specific *BDNF* transcripts in the dorsolateral prefrontal cortex (DLPFC) of humans during development. The values range from highest relative expression (********) to lowest relative expression (*). Expression of exons I, IV, and VI is at its highest during the infant stage. Exon II expression peaks slightly later during the toddler stage. Wong et al. 2009

*3. Effects of depression risk factors*

The serotonin transporter (SERT) is an integral membrane protein responsible for reuptake of the monoamine neurotransmitter serotonin from the synapse into the presynaptic cell (Rudnick et al. 1993). *SERT* knockout (*SERT*-KO) mice have been proposed to serve as a model for major depression (Olivier et al. 2008), however, with conflicting results (Perona et al. 2008). This has spawned hypotheses about an interaction between BDNF and the serotonergic pathway in the pathology of depressive disorders. A recent study has shown that *SERT*-KO mice have differential expression of distinct *BDNF* exons in the hippocampus and prefrontal cortex when compared with wild-type mice (Molteni et al. 2010). *SERT*-KO mice show significantly decreased expression of Exons IIb, III, IV, VI, and IXA in the hippocampus and Exons I, IIa, IIb,

IIc, III, IV, VI, and IXA in the prefrontal cortex. Exon IV and Exon VI expression experienced the most dramatic decrease in expression in the hippocampus and prefrontal cortex. The differential BDNF transcript expression observed between *SERT*-KO and wild-type mice in this study provides evidence supporting of the interaction between *SERT* and *BDNF*.

**Table 4-4**. Relative changes in hippocampal expression of specific *BDNF* exons in different stages of postnatal development in rats in response to chronic stress.

| **BDNF Transcript** | **Developmental Stage** | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | P14 | | | P21 | | | Adult | | |
| | DG | CA1 | CA3 | DG | CA1 | CA3 | DG | CA1 | CA3 |
| Exon I | - | - | - | - | - | - | ↑ | - | ↑ |
| Exon II | ↑ | ↑ | ↑ | - | - | - | ↑ | ↑ | ↑ |
| Exon III | - | - | - | - | - | - | ↓ | ↓ | ↓ |
| Exon IV | - | - | - | ↑ | ↑ | - | ↓ | ↓ | ↓ |
| Exon V | - | - | - | ↑ | ↑ | ↑ | ↓ | - | - |

This table shows the brain region-specific effect of stress on specific *BDNF* transcripts in the rat hippocampus. ↑ indicates an increase, ↓ indicates a decrease, and – indicates no change. Stress increases hippocampal expression of Exon II at the P14 and adult stages, and Exons IV and V during P21. The greatest effects of chronic stress are seen in adult rats. Decreased hippocampal expression of exons III, IV and V in adult rats exposed to chronic stress. Nair et al. 2007

Chronic stress induces hippocampal damage and increases the risk of depression, and changes in *BDNF* expression have been implicated to be responsible (Duman 2004). One group compared the effects chronic stress exposures on differential *BDNF* transcript expression in the hippocampus of rats during different stages of development (Nair et al. 2007). Chronic stress regulates *BDNF* transcription based on developmental stage [**Table 4-4**]. At P14, hippocampal

Exon II expression increases, while at P21, expression of Exons IV and V increases. In adult rats, however, relative expression of Exons IV and V significantly decreases.

*4. Effects of antidepressants*

Chronic treatment with antidepressants attenuates chronic stress-induced decreases in total *BDNF* expression in the hippocampus and frontal cortex (Dwivedi et al. 2006; Chen et al. 2009; Zhang et al. 2010). Investigation of the effects of antidepressants on individual *BDNF* transcript expression increases understanding of which transcripts are affected by different classes of antidepressants. The three main classes of antidepressants according to re-uptake behavior are SSRIs, SNRIs, and NRIs. The ultimate goal is to determine the specific mechanism by which each class of antidepressant affects BDNF and increase their efficacy in treating depression.

Chronic antidepressant administration has differing effects on relative expression of specific *BDNF* transcripts [**Table 4-5**]. SSRIs are shown to affect *BDNF* transcript expression in the hippocampus more significantly than in the frontal cortex. The SNRI duloxetine and the NRI desipramine, however, significantly increase hippocampal and cortical *BDNF* transcript expression in rats. These results argue that NE uptake inhibition utilizes a different mechanism that is more effective at increasing *BDNF* levels throughout the brain. In addition, chronic administration of the SSRI escitalopram decreases hippocampal expression of all *BDNF* exons in female rats (Hansson et al. 2011). It is, therefore, proposed that gender could affect SSRI efficacy. Further studies are necessary to determine if chronic SNRI administration results in the same effect in female rats.

Fluoxetine has been established as a prototypical SSRI. While chronic fluoxetine treatment reduces anxious behaviors, acute fluoxetine actually increases anxiety in mouse models (Handley et al. 1993). Total *BDNF* mRNA expression also differs between acute and chronic fluoxetine treatment; expression decreases in response to acute fluoxetine administration and increases in response to chronic administration (De Foubert et al. 2004). A proposed mechanism for the biphasic effect of fluoxetine treatment is differential transcription of *BDNF* exons. Acute fluoxetine administration significantly decreases expression of *BDNF* Exons IV and VI in the dentate gyrus (Khundakar et al. 2006). Chronic fluoxetine administration, however, has the opposing effect, significantly increasing hippocampal levels of *BDNF* Exon I, II, and VI (Dwivedi et al. 2006; Khundakar et al. 2006). These results support the hypothesis that acute and chronic treatments differentially affect expression of *BDNF* exons to result in the biphasic effects of fluoxetine.

The SNRI duloxetine causes differential expression of *BDNF* exons. Chronic duloxetine treatment up-regulates expression of *BDNF* Exons I, IIc, and IV and down-regulates Exon VI expression in the frontal cortex (Calabrese et al. 2007). This result implicates Exons I, IIc, and IV in the increase in total *BDNF* mRNA observed the frontal cortex after chronic duloxetine treatment.

Further experimentation following Aid et al.'s discovery of rodent *BDNF* gene structure examined the effects of duloxetine on other mRNA transcripts. Chronic duloxetine also increases mRNA levels of *BDNF* Exons III and IXA in the hippocampus (Molteni et al. 2009). Interestingly, chronic stress in combination with duloxetine administration increased expression of Exons IV and VI but no others. Although duloxetine is widely used, studies on the effects of

chronic treatment with venlafaxine, the prototypical SNRI, on differential expression of *BDNF* transcripts are necessary.

**Table 4-5.** Relative changes in expression of specific *BDNF* exons in the adult rat brain following chronic antidepressant treatment.

| | Antidepressant | Exon I | | Exon II | | Exon III | | Exon IV | | Exon VI | | Exon IX a | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | HP | FC | HP | FC | HP | FC | HP | FC | HP | FC | HP | FC |
| *SSRI* | Citalopram[1] | - | | - | | | | - | | ↑ | | | |
| | Escitalopram[2] | ↓* | - | ↓* | - | ↓* | - | ↓* | ↓ | ↓* | - | ↓* | - |
| | Fluoxetine[3,4] | ↑* | - | ↑ | - | | - | ↑ | - | | | | |
| *SNRI* | Duloxetine[5,6] | | ↑ | ↑ | ↑ | ↑ | | ↑** | ↑ | ↑** | ↓ | ↑ | |
| | Desipramine[3,4,7,8] | ↑ | ↑ | - | - | | | ↑ | ↑ | ↑* | - | | |
| *NRI* | Reboxetine[1] | - | | - | | - | | ↑ | | | | | |

This table illustrates the change in expression of specific *BDNF* transcripts in rat after chronic treatment with different classes of antidepressants. Expression changes were analyzed in the hippocampus (HP) and frontal cortex (FC). ↑ indicates an increase, ↓ indicates a decrease, and – indicates no change. A hatched bar indicates that there is no data available. Most treatments result in an increase in hippocampal transcript levels. The SNRI duloxetine and the NRI desipramine result in increases in *BDNF* expression in the FC. Exon IV is the transcript that is most affected by antidepressant treatment. * These results are observed only in female rats. ** These results represent chronic antidepressant treatment in a chronic stress rat model of depression.
(1) Russo-Neustadt et al. 2004 (2) Hansson et al. 2011 (3) Khundakar et al. 2006 (4) Dwivedi et al. 2006 (5) Molteni et al. 2009 (6) Calabrese et al. 2007 (7) Martinez-Turrillas et al. 2005 (8) Dias et al. 2003

Chronic treatment with all classes of antidepressants primarily affects expression of *BDNF* Exon IV. Five out of the six antidepressants in three different classes resulted in a relative expression change in Exon IV. Additionally, frontal cortex expression of Exon IV

changed more than that of any other exon. Exon IV is, therefore, proposed to be an important target in the mechanism of antidepressant action, specifically for fluoxetine, duloxetine, reboxetine, and desipramine. Interestingly, CREB binding inhibition results in decreased activity at promoter IV, the promoter responsible for transcription of Exon IV (Balkowiec-Iskra et al. 2011; Pruunsild et al. 2011). An increase in CREB binding, therefore, could be the mechanism that increases *BDNF* Exon IV expression in response to chronic antidepressant treatment.

# Chapter 5: Wnt Signaling

*1. Wnt signaling in antidepressant action*

GSK-3β inhibition, which results from activation of the canonical Wnt pathway, has been found to play a role in treating mood disorders. Chronic stress up-regulates *GSK-3β* expression in mice (Silva et al. 2008). Lithium salt administration, a common treatment for mood disorders, counteracts this up-regulation of the GSK-3β (Silva et al. 2008). Lithium also inhibits GSK-3β function in mice. The inhibitory effect of lithium on GSK-3β is observed in humans as well (Adli et al. 2007). The antidepressant effect and up-regulation of the *GSK-3β* gene can both be explained by activation of Wnt signaling.

These results implicate the canonical Wnt signaling pathway as a likely mechanism for antidepressant action. An elaborate study was recently conducted to examine the effects of different antidepressant treatments on hippocampal expression of a variety of factors in the Wnt signaling pathway (Okamoto et al. 2010). Their study found that chronic administration of the prototypical SSRI fluoxetine, the prototypical SNRI venlafaxine, and electroconvulsive shock treatment result in increased expression of the *Wnt2* gene. Chronic fluoxetine treatment has shown the same effect on Wnt3a expression in the dentate gyrus (Pinnock et al. 2010). *Wnt* genes are, therefore, a target of both SSRI and SNRI antidepressants.

The SSRI citalopram and the SNRI venlafaxine also increase β-catenin mRNA levels in the hippocampus (Okamoto et al. 2010). These results are consistent with previous studies concluding that chronic venlafaxine administration increases cell proliferation and β-catenin levels in the dentate gyrus (Mostany et al. 2008). β-catenin is a downstream effector of the Wnt signaling, which supports the hypothesis that antidepressants activate the canonical Wnt pathway in the hippocampus.

The same group investigated the effects of *Wnt2* overexpression on behavior in mice (Okamoto et al. 2010). According to their findings, injection of a *Wnt2* expressing vector into the dentate gyrus results in decreased escape failures in the learned helplessness paradigm and increased sucrose consumption in the sucrose preference test. Overexpression of *Wnt2*, therefore, results in reduced depressive-like behaviors, or an antidepressant action. Ultimately, Okamoto et al. determined that *Wnt* genes are a target of SSRIs and SNRIs and that increased *Wnt* expression has an antidepressant effect. These results strongly implicate the canonical Wnt pathway as a mechanism in antidepressant action and verify the need for further research into this area.

*2. Interaction of Wnt signaling and BDNF*

The exploration of a connection between the canonical Wnt pathway and *BDNF* expression has only begun recently. There is an established interaction between *BDNF* expression and Wnt signaling in neural tissues outside of the brain. Norrin is a protein that binds to the frizzled 4 receptor (Fzd4) and activates the canonical Wnt pathway (Xu et al. 2004; Smallwood et al. 2007). Norrin has been found to mediate the interaction between Wnt signaling and BDNF in the retina (Seitz et al. 2010) [**Figure 5-1**]. Norrin increases β-catenin production in normal and injured retinal cells. β-catenin is a downstream effector of the active canonical Wnt signaling, which indicates that Norrin activates the Wnt pathway.

Additionally, administration of Norrin increases *BDNF* expression in retinal cells (Seitz et al. 2010). However, when combined with the Wnt signaling inhibitor dickkopf-1 (DKK-1), Norrin decreases *BDNF* expression in retinal cells. The increase in *BDNF* expression in response to Norrin administration can, therefore, be linked to the activation of Wnt signaling. This is the first study to find a positive relationship between Wnt signaling and *BDNF* expression.

To increase understanding, the relationship should next be examined in the brain, specifically the hippocampus. It is also necessary to investigate if the relationship extends from Wnt signaling affecting *BDNF* expression to also affecting BDNF protein levels.

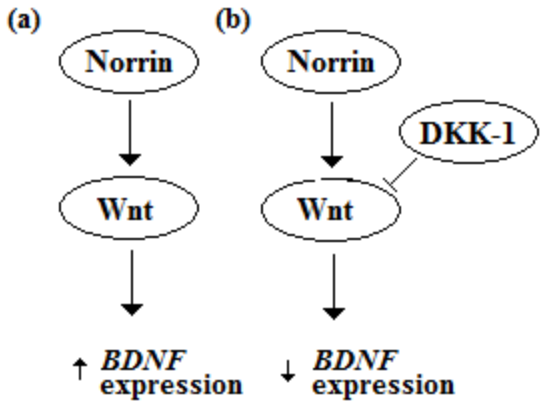


**Figure 5-1.** Relationship between Wnt signaling and *BDNF* expression in retinal cells. (a) Norrin activates Wnt signaling, which results in an increase in *BDNF* expression. (b) Norrin activates Wnt signaling, which is then inhibited by dickkopf-1 (DKK-1). The inhibition of Wnt signaling by DKK-1 results in a decrease in *BDNF* expression. Seitz et al. 2010

Evidence arguing against a connection between *Wnt* gene and *BDNF* gene expression does exist. One group reported on associations in healthy subjects between three SNPs in the *BDNF*, *GSK-3β*, and *Wnt* genes and depressive temperament (Tsutsumi et al. 2010). None of the SNP genotypes are significantly related to depressive temperament, suggesting that these three polymorphisms are not related to the development of this personality trait. Polymorphisms in these genes may not be individually related to depression, but evidence shows that, in combination, SNPs in *BDNF* and *GSK-3β* have significant gene-gene interaction effects, especially when coupled with negative life events (Yang et al. 2010).

The results indicate that the effects of at least two of these three SNPs are additive in increasing susceptibility to depression. Further research would need to be conducted to confirm this. Contrarily, the negative association between *BDNF* SNPs and depression provides further evidence that *BDNF* expression levels are not related to risk of depressive behavior but only to antidepressant action. Using Tsutsumi et al.'s (2010) results, the same hypothesis can be applied to *Wnt* expression. Increases in *Wnt* expression are observed in response to antidepressant treatment but SNPs in *Wnt* genes show no increases in development of depressive temperament. Therefore, Wnt signaling cannot be implicated in the pathology of depression, only in the mechanism of antidepressant action.

# Chapter 6: Cyclic AMP Response Element Binding (CREB)

*1. Effects of antidepressants on pCREB and CaMK-IV*

The SSRI fluoxetine and the NRI desipramine have been found to increase pCREB in the rat prefrontal cortex when administered chronically (Tiraboschi et al. 2004; Laifenfeld et al. 2005; Sairanen et al. 2007) [**Table 6-1**]. Chronic fluoxetine also increases pCREB levels in the rat hippocampus. pCREB binding is necessary in order to observe antidepressant-mediated increases in *BDNF* expression and protein levels (Chen et al. 2009). Specifically, chronic treatment with the NRI reboxetine is shown to increase hippocampal *BDNF* mRNA and protein in wild-type mice. However, in transgenic mice overexpressing a dominant negative CREB protein, these increases are prevented.

Calcium/calmodulin-dependent protein kinase IV (CaMK-IV) is the kinase responsible for phosphorylating CREB in response to antidepressant treatment (Tiraboschi et al. 2004). CaMK-IV enzymatic activity significantly increases in the frontal cortex following chronic treatment with reboxetine, desipramine, and fluoxetine.

*2. Interaction of Wnt signaling, BDNF, and CREB in antidepressant action*

Chronic antidepressant treatment increases *BDNF* mRNA expression in the hippocampus and frontal cortex. The mechanism for this, however, remains unknown. Chronic antidepressant administration also activates the canonical Wnt pathway (Okamoto et al. 2010). In retinal cells, activation of the canonical Wnt pathway is associated with increased *BDNF* expression (Seitz et al. 2010). The findings in this review propose that antidepressants increase hippocampal and cortical expression of *BDNF* via activation of the canonical Wnt signaling pathway.

GSK-3β prevents CREB from binding to DNA (Grimes et al. 2001). Lithium reverses this effect by inhibiting GSK-3β. Activation of the canonical Wnt signaling pathway accounts for inhibition of GSK-3β after lithium administration (Silva et al. 2008). Wnt signaling, therefore, affects CREB binding. Phosphorylation of CREB to pCREB allows the protein to bind to a cyclic AMP response element (CRE) in a gene and function as a transcription factor. In order to examine the mechanism by which the canonical Wnt pathway affects CREB binding, a mediator between Wnt and pCREB must be found.

**Table 6-1.** Summary of effects of chronic antidepressant treatment on canonical Wnt pathway activation, CaMK-IV activity, pCREB levels, and *BDNF* expression in the rodent brain.

| **Antidepressant** | | **Wnt** | | **CaMK-IV activity** | | **pCREB** | | **Total *BDNF* Expression** | |
|---|---|---|---|---|---|---|---|---|---|
| | | HP | FC | HP | FC | HP | FC | HP | FC |
| *SSRI* | Citalopram[1,2] | ↑ | | | | | | - | - |
| | Fluoxetine[1,3,4,5] | ↑ | | - | ↑ | ↑ | ↑ | ↑ | ↑ |
| *SNRI* | Venlafaxine[1,5,7] | ↑ | | | | - | | ↑ | ↑ |
| *NRI* | Desipramine[3,4,8] | | | - | ↑ | - | ↑ | ↑ | ↑ |
| | Reboxetine[3,6] | | | - | ↑ | ↑ | ↑ | ↑ | |

This table summarizes the effects of chronic antidepressant treatment on all components of the proposed mechanism of action in the hippocampus (HP) and the frontal cortex (FC). ↑ indicates an increase, ↓ indicates a decrease, and – indicates no change in protein levels. A hatched area indicates no data was available. Fluoxetine, reboxetine, and desipramine have the most complete data. Desipramine effects fit the proposed mechanism of antidepressant function best with increases in CaMK-IV activity, pCREB, and *BDNF* expression in the FC. All results are from rat models, except (6) which is from mouse. (1) Okamoto et al. 2010 (2) Russo-Neustadt et al. 2004 (3) Tiraboschi et al. 2004 (4) Dwivedi et al. 2006 (5) Zhang et al. 2010 (6) Chen et al. 2009 (7) Cooke et al. 2009 (8) Martinez-Turrillas et al. 2005

Calcium/calmodulin-dependent protein kinase IV (CaMK-IV) is a likely candidate for this mediator. Lithium administration and *Wnt3a* ligand binding activate the promoter of *CaMKIV* and up-regulate expression of *CaMK-IV* in hippocampal neurons (Arrazola et al. 2009). Both lithium and Wnt3a ligands function to activate the canonical Wnt pathway. This means that *CaMK-IV* is a target gene of Wnt signaling. For CaMK-IV to be the link between Wnt and CREB binding, it must also affect pCREB. CaMK-IV is responsible for phosphorylating CREB to pCREB in response to antidepressant treatment (Tiraboschi et al. 2004) [**Figure 6-1**]. Wnt signaling increases CaMK-IV activity, which, in turn, increases pCREB and CREB binding.

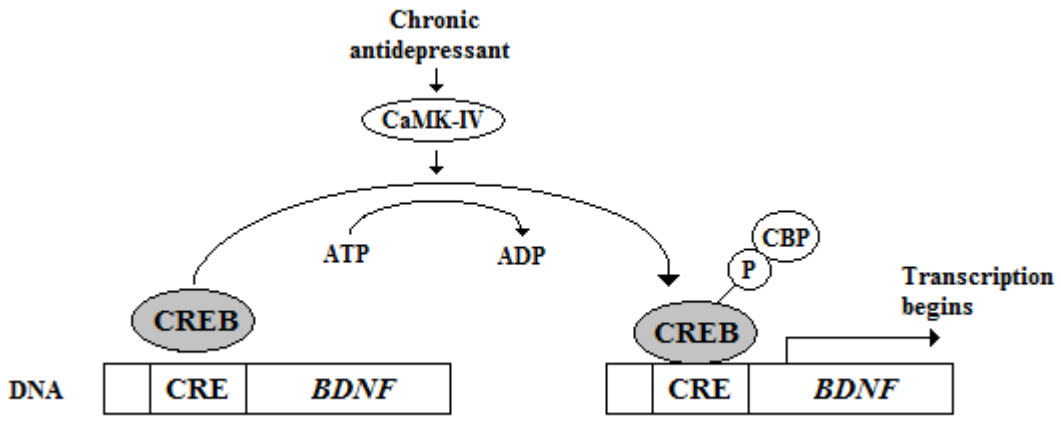


**Fig. 6-1.** Schematic representation of CREB/*BDNF* binding in response to chronic antidepressant treatment. Chronic antidepressants induce CaMK-IV to phosphorylate CREB to pCREB. pCREB can then bind to the CRE region in the DNA with help from CREB binding protein (CBP). After CREB is bound, it signals the start of transcription of the *BDNF* gene.

CaMK-IV has also been implicated in *BDNF* expression, especially at the *BDNF* promoter IV (Shieh et al. 1998). Expression of total *BDNF*, *BDNF* Exon I, and *BDNF* Exon IV and levels of pCREB are significantly decreased in *CaMK-IV* knockout mice (Kokubo et al.

2009). When CaMK-IV function is restored in these mice, *BDNF* expression and pCREB levels are also restored to a level equivalent to that in wild-type mice. This provides a link between increased CaMK-IV activity and increased *BDNF* expression.

CREB binding is necessary to activate transcription at human *BDNF* promoters IV and VI (Tao et al. 1998; Balkowiec-Iskra et al. 2011; Pruunsild et al. 2011). CaMK-IV phosphorylates CREB in response to antidepressant treatment, and pCREB facilitates CREB binding to DNA. Therefore, pCREB can be said to mediate the relationship between CaMK-IV and *BDNF* expression.

To summarize, in the proposed mechanism [**Figure 6-2**], Wnt signaling increases CaMK-IV activity, which then increases the level of pCREB present in the nucleus. Increased pCREB levels then result in increased CREB binding to activate transcription of the *BDNF* gene. All of these interactions ultimately result in the increased *BDNF* mRNA expression observed in the hippocampus and frontal cortex following chronic antidepressant treatment.

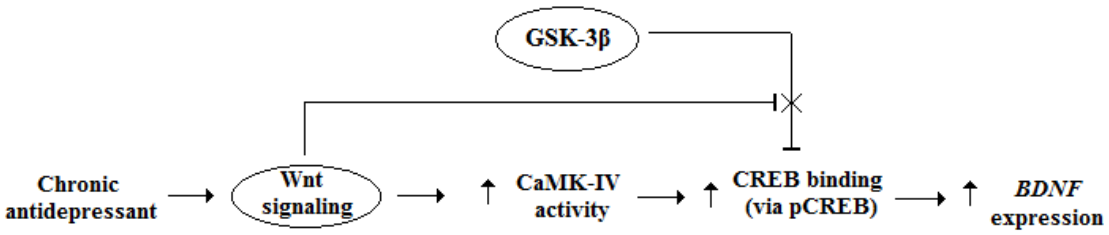


**Figure 6-2**. Schematic representation of the proposed mechanism of antidepressant-induced increases in *BDNF* expression in the hippocampus and frontal cortex. Chronic antidepressant treatment activates the canonical Wnt pathway. Wnt activation results in an increase of one of its target genes, *CaMK-IV*, and CaMK-IV activity. Wnt activation also inhibits GSK-3β inhibition of CREB binding. Increased CaMK-IV activity induced an increase in phosphorylation of CREB to pCREB. Finally, increased pCREB results in increased CREB/*BDNF* binding and, therefore, increased expression of *BDNF*.

According to the literature, the SSRI fluoxetine, the NRI reboxetine, and the NRI desipramine are the three antidepressants that fit this mechanism the most. Interestingly, they are all in different antidepressant classes. Fluoxetine increases Wnt, pCREB, and *BDNF* expression in the hippocampus (Tiraboschi et al. 2004; Dwivedi et al. 2006; Okamoto et al. 2010; Zhang et al. 2010). Increased CaMK-IV activity is observed in the frontal cortex but not the hippocampus. pCREB and *BDNF* expression are increased in the frontal cortex as well.

Chronic treatment with the NRI reboxetine also increases CaMK-IV activity and pCREB in the frontal cortex (Tiraboschi et al. 2004; Chen et al. 2009). There are no available studies, however, on the effect of reboxetine on cortical *BDNF* expression or Wnt activation. The NRI desipramine has effects similar to those of fluoxetine. Chronic desipramine increased cortical CaMK-IV activity, pCREB, and total *BDNF* expression (Tiraboschi et al. 2004; Martinez-Turrillas et al. 2005; Dwivedi et al. 2006). These results indicate that canonical Wnt signaling is responsible for the increase in *BDNF* expression in the frontal cortex following chronic antidepressant treatment. Unfortunately, none of the downstream effectors in this review can be linked to Wnt activation because there are no current studies on antidepressants and cortical Wnt signaling.

Additionally, not all of the available literature supports the proposed mechanism. One group conducted an experiment to investigate the effects of fluoxetine treatment coupled with inhibition of TrkB receptors on levels of *BDNF* expression, pCREB, and Wnt3a as well as proliferation of progenitor cells in the dentate gyrus (Pinnock et al. 2010). Chronic fluoxetine treatment increases mRNA expression of *BDNF* and *Wnt3a* as well as levels of pCREB, but TrkB inhibition reverses this effect of fluoxetine on pCREB. Decreased expression of pCREB

after inhibition of TrkB, the receptors for BDNF, suggests that pCREB is a downstream effector of BDNF in this pathway.

# Chapter 7: Conclusion

Chronic antidepressant treatment increases total *BDNF* expression levels in the hippocampus and frontal cortex ((Dwivedi et al. 2006; Chen et al. 2009; Zhang et al. 2010). Most chronic antidepressant drug treatments increase expression of most *BDNF* transcripts only in the hippocampus (Russo-Neustadt et al. 2004; Khundakar et al. 2006; Molteni et al. 2009). However, the SNRI duloxetine and the NRI desipramine increase relative expression of *BDNF* Exons I, II, and IV also in the frontal cortex. Differential expression of individual *BDNF* transcripts following chronic antidepressant administration can shed light on more specific targets of antidepressant drugs as well as which transcripts could be implicated in the pathology of depression.

This review proposes a mechanism for this antidepressant drug-induced increase in *BDNF* expression. Chronic antidepressant administration activates canonical Wnt signaling (Okamoto et al. 2010). The activation of *BDNF* expression by Wnt signaling in retinal cells (Seitz et al. 2010) supports the possible link between Wnt signaling and increased *BDNF* expression in response to antidepressant treatment.

As a possible mechanism, Wnt signaling increases *CaMK-IV* expression and kinase activity (Arrazola et al. 2009). Chronic antidepressant treatment-induced CaMK-IV phosphorylates CREB, which then activates CREB target genes including BDNF (Tiraboschi et al. 2004). Among the different BDNF gene promoters, the one responsible for transcripts initiated at Exon IV shows the highest response to antidepressant treatment of all the *BDNF* promoters. Inhibiting CREB binding prevents expression of *BDNF* Exon IV (Balkowiec-Iskra et al. 2011; Pruunsild et al. 2011). Enhanced CREB binding from chronic treatment with

antidepressants is likely involved in the relative increase in *BDNF* Exon IV expression. This verifies the link between pCREB and increased *BDNF* expression in the proposed mechanism.

Further research must first be conducted on the effects of chronic antidepressant treatment on Wnt signaling activation in the frontal cortex to determine the accuracy of this mechanism. If chronic antidepressant administration is found affect activation of the canonical Wnt pathway in the frontal cortex, this mechanism could be validated. The Neurotrophin Hypothesis of Depression stresses the importance of BDNF levels in the pathology of depressive disorders, however no antidepressants have been developed that utilize this interaction. If antidepressant efficacy is the issue, it seems that a transition in antidepressants needs to be made from the Monoamine Hypothesis to the Neurotrophin Hypothesis. Antidepressants should be designed to up-regulate *BDNF*, or even specific *BDNF* exons. In order to develop this type of antidepressant, however, the mechanism of increased *BDNF* expression needs to be fully understood. Hopefully, the mechanism proposed in this paper will increase understanding and prompt further research on the roles of Wnt signaling, *BDNF* expression, and CREB in antidepressant action.

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# Appendix A: BDNF Exon Nomenclature

**Rodent Nomenclature**

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 1993 | I | II | - | III | - | IV | - | - | V |
| 2006 | I | II | III | IV | - | V | VI | VII | VIII |
| Current | I | II | III | IV | V | VI | VII | VIII | IX |

**Human Nomenclature**

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 2004 | I | II | - | III | - | - | IV | V | - | - | - | VI |
| 2006 | I | II | III | IV | - | - | V | VI | - | - | VII | VIII |
| Current | I | II | III | IV | V | Vh | VI | VII | VIII | VIIh | IX b | IX d |

BDNF Exon nomenclature was kept consistent with the current literature throughout this review.

# Appendix B: Antidepressant Brand Names

| Generic Name | Brand Name |
|---|---|
| Citalopram | Celexa |
| Desipramine | Norpramin |
| Duloxetine | Cymbalta |
| Escitalopram | Lexapro |
| Fluoxetine | Prozac |
| Paroxetine | Paxil |
| Reboxetine | Vestra |
| Venlafaxine | Effexor |

# Academic Vita

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**Education**

**The Pennsylvania State University**, University Park, PA
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- Bachelor of Science in Biology, Vertebrate Physiology option
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**Research Experience**

**Honors Thesis, 2011**
*Antidepressant Action: The Roles of Brain-Derived Neurotrophic Factor, Wnt Signaling, and Cyclic-AMP Response Element Binding*

**Student Research Assistant**
January 2009 – August 2010, *Andrews Research Group*, University Park, PA and Los Angeles, CA

- Designed and executed protocol for TOPO cloning of three transcript variants of brain-derived neurotrophic factor.
- Created cDNA library of three BDNF transcripts for use in *in situ* hybridization.
- Isolated total mRNA and microRNA from mouse brain tissue.
- Performed real-time quantitative and colony PCR reactions.

**Undergraduate Research Assistant**
August 2008 – December 2008, *Assmann Lab*, University Park, PA

- Designed and executed the control for an experiment studying stomatal development in *Arabidopsis thaliana* mutants.
- Cloned promoter of TMM gene using the TOPO cloning method.
- Created TMM::GUS construct for use as a stomatal development marker.

**Activities**

- Mentor, Schreyer Honors College Freshman Orientation
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