Published in final edited form as:
Pharmacol Ther. 2008 April ; 118(1): 36–57. doi:10.1016/j.pharmthera.2008.01.003.

Ethanol-BDNF interactions: Still More Questions than Answers

Margaret I. Davis, Ph.D.*
Section on Synaptic Pharmacology, Laboratory for Integrative Neuroscience, National Institute on Alcohol Abuse and Alcoholism, National Institutes of Health, Bethesda, MD 20892

Abstract

Brain Derived Neurotrophic Factor (BDNF) has emerged as a regulator of development, plasticity and, recently, addiction. Decreased neurotrophic activity may be involved in ethanol-induced neurodegeneration in the adult brain and in the etiology of alcohol-related neurodevelopmental disorders. This can occur through decreased expression of BDNF or through inability of the receptor to transduce signals in the presence of ethanol. In contrast, recent studies implicate region-specific up-regulation of BDNF and associated signaling pathways in anxiety, addiction and homeostasis after ethanol exposure. Anxiety and depression are precipitating factors for substance abuse and these disorders also involve region-specific changes in BDNF in both pathogenesis and response to pharmacotherapy. Polymorphisms in the genes coding for BDNF and its receptor TrkB are linked to affective, substance abuse and appetitive disorders and therefore may play a role in the development of alcoholism. This review summarizes historical and pre-clinical data on BDNF and TrkB as it relates to ethanol toxicity and addiction. Many unresolved questions about region-specific changes in BDNF expression and the precise role of BDNF in neuropsychiatric disorders and addiction remain to be elucidated. Resolution of these questions will require significant integration of the literature on addiction and comorbid psychiatric disorders that contribute to the development of alcoholism.

Keywords

addiction; alcoholism; fetal alcohol spectrum disorders; TrkB; neurodegeneration

1. Introduction

Alcoholism is a world-wide public health problem and it is estimated that over 14 million Americans suffer from some form of alcohol-related disorder (McGinnis and Foege, 1999). Long-term alcohol exposure in the adult causes neurodegeneration (atrophy of both grey and white matter), Wernicke-Korsakoff syndrome, tremors, alcoholic psychosis, delirium tremens, and withdrawal seizures (reviewed by Harper and Matsumoto, 2005). Alcohol-Related Neurodevelopmental Disorder/Fetal Alcohol Spectrum Disorder (ARND/FASD) is a leading cause of birth defects in the Western world, completely preventable, and irreversible. Exposure to ethanol during brain development induces apoptosis and impairs neuronal migration, resulting in hyperactivity, increased impulsivity, cognitive and motor deficits (Mukherjee *et al.*, 2006).

* Laboratory for Integrative Neuroscience, NIAAA/NIH, 5625 Fisher's Lane MSC 9411, Bethesda, MD 20892, 301-443-4106, midavis@mail.nih.gov.

Alcoholism is difficult to treat, with high relapse rates. Ethanol affects multiple neurotransmitter systems in the brain, including opiates, GABA, glutamate, serotonin, and dopamine (reviewed by Diamond and Gordon, 1997). Prolonged exposure leads to pharmacodynamic changes in these systems resulting in withdrawal-induced anxiety and seizures. Acute withdrawal is generally managed pharmacologically with benzodiazepines, anticonvulsants and dopamine antagonists but craving leading to relapse is more difficult to treat. Several different classes of neuroactive agents are in use therapeutically for preventing relapse. For example, acamprosate probably targets the glutamatergic system, naltrexone and nalmefene block opiate receptors and ondansetron targets 5-HT_3 receptors. In addition, the anticonvulsant topiramate has recently been shown to be effective in increasing abstinence rates among alcoholic patients (Johnson *et al.*, 2007); the pharmacological mechanisms underlying this response are presumably related enhanced activity of GABA receptors and antagonism of glutamate receptors. This dual mechanism of action at both excitatory and inhibitory synapses is believed to mitigate the effects of chronic ethanol exposure on glutamatergic and GABAergic receptors in the mesocorticolimbic circuit (Johnson 2008). Interestingly, topiramate has also been investigated as a treatment for binge eating (Shapira *et al.*, 2000, McElroy *et al.*, 2003) suggesting that this compound may be effective in other appetitive disorders. None of these agents are particularly effective in alcoholism, however, only modestly increasing abstinence rates (reviewed by Mann 2004; Johnson 2008).

Alcoholism represents a heterogeneous disease and different subtypes of alcoholics are likely to respond to different therapeutic interventions. Cloninger and colleagues (1981) attempted to categorize alcoholics in a cross-fostering study of Swedish adoptees and identified 2 subtypes. Type 1 alcoholics had a later age of onset, harm-avoidant personalities and low criminality. Type 2 alcoholics had a family history of alcohol abuse, violent behavior, novelty seeking and an earlier age of onset. Cardoso *et al.* (2006) subsequently identified 5 subtypes of alcoholics: anxiopathic (anxious), heredopathic (family history), thimopathic (affective symptomology), sociopathic and ad(d)ictopathic (polysubstance abusing). As these classifications suggest, alcoholics frequently suffer from comorbid psychiatric disorders as well, making elucidation of the underlying motivation to drink and selection of appropriate therapeutic interventions difficult. Anxiety, depression, post-traumatic stress disorder, bipolar disorder and schizophrenia all predispose patients to alcohol abuse (reviewed by Goldstein *et al.*, 2006). The genetic and environmental factors that contribute to mental illness therefore contribute to the development of alcoholism and substance abuse in a large percentage of patients.

The neurotrophin BDNF is an attractive candidate molecule for mediating many of the processes mentioned above. Evidence has accumulated that implicates BDNF as a regulator of development (Klein, 1994), modulator of appetite (Lebrun *et al.*, 2006), facilitator of synaptic plasticity (Pang and Lu, 2004), potential therapy for neurodegenerative disease (Pezet and Malcangio, 2004) and mediator of addiction (Bolanos and Nestler, 2004). In addition to numerous earlier studies on BDNF in the context of FASD, recent biochemical and genetic data implicate BDNF and its associated signaling intermediates in ethanol-induced neurotoxicity, acute tolerance and addiction. There are fascinating possibilities for integrating the extensive literature on BDNF in neurodevelopmental and neuropsyciatric disorders with the recent observations of the effects of ethanol on BDNF expression and signaling. However there still remain unresolved questions, particularly in terms of region-specific effects, pharmacology and potential for pharmacotherapy.

2. A Brief History of BDNF

Neurotrophins were originally characterized as target derived substances that support the innervating axon to a level that is proportional to the size of the target tissue (reviewed by

Levi-Montalcini, 1987). Mid-twentith century observations, combined with the earlier observations of Ramon y Cajal in the late 1800's, cemented the neurotrophic hypothesis which prevails, with modifications, to this day in developmental neuroscience. BDNF is a member of a family of cysteine knot, dimeric neurotrophic substances that also includes NGF, Neurotrophin (NT)-3, and NT-4/5 in mammals. NGF has served as a model for neurotrophins since its relatively early discovery by Cohen and Levi-Montalcini in the mid 20th century (Levi-Montalcini and Cohen, 1960). BDNF was purified over 20 years later (Barde *et al.*, 1982) and this discovery was rapidly followed by homology cloning of a family of neurotrophins with both distinct and overlapping actions (reviewed by Barde 1994; Reichardt 2006). NGF is the survival factor for sympathetic neurons and cholinergic neurons in the CNS. BDNF and NT-4 are more widely expressed, with the highest levels of both neurotrophins in the hippocampus, cerebellum and cortex. BDNF can be produced by astrocytes under pathological conditions and after monoaminergic receptor stimulation (Zafra *et al.*, 1992; Juric *et al*., 2006). BDNF is also produced by platelets (Karege *et al*., 2002), lymphocytes (Kruse *et al*., 2007), and the vascular endothelium (Wang *et al.*, 2006; Kermani and Hempstead, 2007). BDNF is an angiogenic factor, and therefore may be involved in vascular and hemodynamic responses to ethanol as well.

There is considerable complexity within the BDNF gene itself. BDNF mRNA is encoded by up to 9 potential exons in humans (Pruunsild *et al.*, 2007) and 8 in rodents (Aid *et al.*, 2007) but was initially characterized as having 4 exons (Timmusk *et al.*, 1993). Anti-BDNF transcripts are also present in humans (Pruunsild *et al*., 2007). The first 4 (7–8, according to the recent nomenclature) exons are encoded by unique promoters and are differentially regulated by neuronal activity and during pathological states. The final exon contains the BDNF coding sequence. Exons I–III (the original nomenclature will be used since most studies still use this scheme) are expressed in brain while exon IV is active in lung and heart (Timmusk *et al*., 1993). BDNF exons I–III are induced by multiple forms of plasticity and during seizures. Transcription of exons I and II is dependent on protein synthesis, while exons III and IV are not. This property gives exon III (in brain) the ability to be readily inducible and, not surprisingly, this promoter is regulated by calcium, mitogen-activated protein kinases and cAMP (Shieh *et al*., 1998; Tao *et al.*, 1998; Chen *et al.*, 2003a). In addition, Marini and colleagues identified an NK-κB binding site within promoter III that is regulated by NMDA receptors and contributes to NMDA-mediated neuroprotection (Marini *et al*., 2004). The BDNF promoter is also regulated by epigenetic methylation. Calcium-dependent phosphorylation of Methyl CpG binding protein (MeCP2) leads to derepression of the promoter (Chen *et al.*, 2003b). Interestingly, BDNF mRNA is also targeted to different regions within the cell for translation, depending on the promoter used (reviewed by Tongiorgi *et al*., 2006).

Neurotrophins signal through high affinity tropomyosin-related kinases (Trks) which were originally named for a translocation of a portion of the TrkA gene to the smooth muscle tropomyosin locus in a colon carcinoma (Mitra *et al*., 1987). TrkB and the truncated receptors were subsequently characterized (Middlemas *et al.*, 1991). Neurotrophins bind with high affinity to Trk receptors (10–100 pM). TrkA is the preferred receptor for NGF, TrkB binds NT-4 and BDNF, while TrkC is the high affinity receptor for NT-3 (reviewed by Chao and Hempstead, 1995). Trks are not completely selective and will bind other neurotrophins, although with reduced affinity. This is particularly true for NT-3, which can bind TrkA and TrkB with an affinity of 300–500 pM (Barbacid, 1995; Ip *et al.*, 1993a,b). Traditionally neurotrophin concentrations have been expressed as ng/mL. The affinity of BDNF in these units is less than 2.8 ng/mL for high affinity binding and 28 ng/mL for low affinity binding and approximately 10 ng/mL for non-specific activation of TrkB by NT3. Affinity is also regulated by alternative splicing of transcripts that leads to an insertion in the juxtamambrane region of all Trks and relaxes ligand preference (Clary and Reichardt 1994; Shelton *et al.*, 1995). Both TrkB and TrkC can be expressed as truncated receptors that lack the tyrosine kinase

domain which can modulate the activity of the full length receptor. In addition, neurotrophins bind a low affinity (~1 nM) receptor as well, p75, which is a member of the tumor necrosis factor receptor superfamily and has been linked to both cell death and plasticity. The p75 receptor complex is beyond the scope of this review but is the subject of several recent reviews (Dechant and Barde, 2002; Woo *et al.*, 2005). BDNF is produced as a pro-peptide and is cleaved by multiple proteolytic pathways for both activity-dependent and constitutive secretion. Recent evidence suggests that p75 is the preferred receptor complex for pro-BDNF while Trks bind the mature neurotrophins (Reviewed by Lu, 2003).

Both full length and truncated TrkB receptors are expressed throughout the brain, however the full length receptor is mostly associated with neurons while the truncated receptor is also expressed on glia (Altar, *et al.*, 1994; www.brainatlas.org). The highest levels of TrkB are in hippocampus, cerebellum and cortex but target areas for dopamine (DA) neurons also show diffuse TrkB expression. Diencephalic neurons, hypothalamic and midbrain monoaminergic neurons (raphe, locus coeruleus, Substantia Nigra (SN), Ventral Tegmental area (VTA)) express TrkB and respond to BDNF with increased neurotransmitter synthesis and increased survival (Akbarian *et al.*, 2002; Altar *et al.*, 1992; Altar *et al.*, 1994; Altar *et al.*, 1999; Baquet *et al.*, 2005; Madhav *et al.*, 2001). TrkB is expressed on the majority of mesencephalic DA neurons while a subpopulation also produce BDNF (Numan and Seroogy, 1999). BDNF, NT-4 and TrkB are often expressed in the same cell populations within the CNS but there are subtle differences between mRNA expression and localization by immunohistochemistry (Altar *et al.*, 1997; Conner *et al.*, 1997; Krause *et al.*, 2008). For example, BDNF mRNA is expressed in the Basolateral Amygdala (BLA) and Lateral Amygdala (LA), CA3 and dentate gyrus with less expression in CA1. BDNF mRNA is generally below detection in the Nucleus Accumbens (NAc), an area intimately involved in addiction, but high in cortical areas and in dopaminergic neurons in the VTA and SN (Altar *et al.*, 1997; Conner *et al.*, 1997). Neurotrophins are often synthesized in one area and retrogradely or anterogradely transported. Areas such as the striatum, NAc, Central Amygdala (CeA) and Bed Nucleus of the Stria Terminalis (BNST) are devoid of BDNF mRNA detected by in situ hybridization but rich in BDNF-containing fibers detected by enzymatically amplified immunohistochemistry (Altar *et al.*, 1997; Baquet *et al.*, 2004; Conner *et al.*, 1997; Guillin *et al.*, 2001; Agassandian *et al.*, 2006; Krause *et al.*, 2008). Recent studies on alcoholism using commercially available antibodies coupled to immunogold, *in situ* PCR and RT-PCR have challenged these initial observations, however (for examples see Pandey *et al.*, 2006 and McGough *et al.*, 2004).

Deletion of TrkB developmentally is lethal shortly after birth, probably because the animals fail to feed (reviewed by Klein, 1994). BDNF deletion produces a more subtle phenotype with bobbing, head turning and spinning by 2 weeks of age (Ernfors *et al.*, 1994; Jones *et al.*, 1994). There is near complete loss of neurons in the vestibular ganglia of homozygotes and partial, progressive loss of these neurons in heterozygotes which likely contributes to this phenotype. There is a loss of motor neurons in TrkB knock outs (Klein, 1994) but no loss with BDNF deletion (Ernfors *et al.*, 1994; Jones *et al.*, 1994). Loss of BDNF also produces a selective depletion of Neuropeptide Y (NPY) and parvalbumin expression in the cortex with no change in the total number of interneurons, implicating BDNF in GABAergic interneuron function and peptide expression (Jones *et al.*, 1994). Interestingly, NT-4 deficient mice show no gross neurological abnormalities (Conover *et al.*, 1995) but, like BDNF deficient mice, show deficiencies in gustatory papillae and taste bud formation (Liebl *et al.*, 1999). Impaired development of gustatory systems may in turn regulate appetitive behaviors in BNDF deficient mice (reviewed by Krimm, 2007). The more subtle phenotype of the $BDNF^{+/-}$ mice is discussed later as it relates to anxiety, depression and ethanol consumption.

Because of the severe developmental phenotype of the complete knock out, recent work has employed region-specific gene deletion strategies, such as Lox-Cre recombinase under the

control of forebrain or cortex-specific promoters (Gorski *et al.*, 2003; Zorner *et al.*, 2003; Baquet *et al.*, 2004; Strand *et al.*, 2007). Similarly, drug-inducible promoters have been constructed that allow the gene to be deleted selectively during developmental windows or in adults (Glorioso *et al.*, 2006). Deletion of BDNF selectively during development, either by using conventional knock-out strategies (heterozygotes), the αCaMKII promoter driving Cre or a drug inducible strategy, leads to impaired hippocampal function, hyperactivity and hyperphagia (Duan *et al.* 2003; Glorioso *et al.*, 2006; Kernie *et al.*, 2000; Lyons *et al.*, 1999; Rios *et al.*, 2001; Zorner *et al.*, 2003). Cortex-specific deletion of BDNF also causes degeneration of striatal medium spiny neurons (Baquet *et al.*, 2004; Strand *et al.*, 2007). Because no BDNF expression was detected in striatum when measured in reporter mice with LacZ inserted within the BDNF gene, Baquet and colleagues attributed the degeneration of medium spiny neurons to a loss of trophic support from cortex. A subsequent study from this group found striking similarities in gene expression patterns between cortex-specific delection of BDNF in transgenic mice and human and mouse models of Huntington ’s disease (Strand et al., 2007). The severe phenotype of the $TrkB^{-/-}$ and $BDNF^{-/-}$ mice indicates an essential role in for this neurotrophin in CNS development. Region-specific and drug-inducible knock out strategies in the maturing mouse reveal an essential role for BDNF in neuronal survival, learning, locomotor activity and appetite regulation.

Signaling events initiated by TrkB activation are well-established. Binding of BDNF to TrkB results in receptor autophosphorylation and recruitment of adaptor proteins which link to Ras family proteins and Extracellular-signal Regulated Kinases (ERK), as well as to activation of phospholipase C and phosphatidyl inositol 3-OH kinase (PI 3-Kinase) pathways (reviewed by Reichardt 2006). Upon ligand binding and phosphorylation, local signaling events occur but the BDNF-TrkB signaling complex is frequently internalized and transported from the cell periphery to the nucleus (reviewed by Ginty and Segal, 2002). ERK is activated following recruitment of the adaptor protein Shc and subsequent recruitment of Grb and Sos (or a similar nucleotide exchange factor) to a phosphotyrosine residue through SH2 domain interactions. Subsequent activation of a small molecular weight GTPase of the Ras family results in Raf activation and sequential phosphorylation of MEK and then ERK. Activation of the ERK pathway links to phosphorylation of numerous other transcription factors (discussed below) thereby changing gene transcription, but TrkB also couples to translation of plasticity-associated proteins such as Arc (Yin *et al.*, 2002). In addition to the initial Ras-activated pathway, ERK can also be activated by Trks in a sustained fashion that involves a different SH2-binding partner to activate B-Raf through Rap-1 (Kao *et al.*, 2001; York *et al.*, 1998).

TrkB activation of PI-3 kinases results in the production of membrane phosphatidyl inositol 3,4,5 triphosphate and recruitment of phospholipid-dependent kinases through membrane association and subsequent phosphorylation of the pro-survival kinase Akt. Akt phosphorylates multiple substrates involved in cell survival and metabolism in an isozyme-specific fashion. Interestingly, activation of the pro-survival pathways appears to be independent of receptor internalization (Kuruvilla *et al.*, 2000; MacInnis and Campenot, 2002).

Phospholipase C activation results in the production of 2 messengers, diacylglycerol and Ca^{2+}. Membrane 4, 5 phosphatidyl inositol is cleaved to generate diaclyglycerol and inositol 1, 4, 5 triphosphate, leading to protein kinase C activation and the release of intracellular calcium. In addition to these canonical pathways, other TrkB effectors such as ERK5 have also been characterized (Cavanaugh *et al.*, 2001). The truncated TrkB and TrkC isoforms do not activate these pathways but decrease full-length Trk receptor activation (Eide *et al.*, 1996) and signal through G-proteins to increase intracellular calcium (Rose *et al.*, 2003). TrkB.T1 increases filopodia formation (Hartmann *et al.*, 2004) and truncated TrkC can signal through PSD-95 to increase membrane ruffling (Esteban *et al.*, 2006).

Activation of signal transduction cascades by TrkB leads to expression and/or phosphorylation of multiple transcription factors. Spatial and temporal integration of these signals and induction of immediate early genes regulates gene transcription in response to BDNF in the context of concurrent synaptic activity. The best characterized of these factors are Cyclic AMP Response Element Binding protein (CREB), AP1 (Fos/Jun) and the serum response element binding/ternary complex transcription factor Elk-1. CREB is phosphorylated through multiple pathways, including calcium-dependent kinases, MAP kinase pathways and cAMP-dependent protein kinases (reviewed by Impey and Goodman, 2001). Elk-1 is phosphorylated through JNK in addition to ERKs 1, 2 and 5 in response to growth factor stimulation (reviewed by Turjanski *et al.*, 2007). In addition, TrkB activates many of the same transcription factors as PKC cascades through distinct and overlapping pathways. Since these transcription factors are regulated by multiple overlapping pathways and neuronal activity through ionotropic receptors, the repertoir of transcription factors available to activate a given promoter is complex. Phosphorylation of transcription factors is frequently used in mapping studies but this too is a dynamic process. Regulation may not occur immediately after a stimulus through phosphorylation of a transcription factor but may require multiple pathways to be simultaneously active. Moreover, synthesis of a *trans* acting factor or second growth factor can modulate the later stages of gene expression.

Receptor signaling is also a dynamic process and this is particularly true for TrkB. NGF exposure increases TrkA in a positive loop (Holtzman *et al.*, 1992) while BDNF activation of TrkB not only leads to desensitization but also reduces levels of TrkB protein but not mRNA (Frank *et al.*, 1996; Frank *et al.*, 1997). The ability of TrkB to signal in heterologous systems and in cerebellar granule cells is maximal at 10–100 pM BDNF concentrations and desensitizes at concentrations above 250 pM (Carter *et al.*, 1995; Ip *et al.*, 1993a,b). This concentration-response relationship is retained at the level of ERK activation by BDNF in cerebellar granule cells (Ohrtman *et al.*, 2006). BDNF-supported survival in cerebellar granule cell and hippocampal neurons also plateaus and declines (Ip *et al.*, 1993; Segal *et al.*, 1992), indicating that both the pro-survival and plasticity signals desensitize. The pattern of neurotrophin signaling can also determine the phenotypic response, with sustained signaling leading to differentiation and intermittent signaling leading to proliferation (Vaudry *et al.*, 2002; York *et al.*, 1998). Therefore, it is necessary to consider not only concentration but the temporal signaling pattern generated by the neurotrophin receptor. This complex pattern of regulation indicates that the effect of the neurotrophin is dependent on concentration, receptor variant expressed, place of expression, duration of exposure, internalization and cross-regulation by neurotransmitters and hormones. The regulation of these pathways is the subject of several excellent reviews (Jeanneteau and Chao, 2006; Lee *et al.*, 2002) and is only introduced here as it relates to studies on ethanol-BDNF interactions.

3. Is BDNF involved in ethanol-induced neurodegeneration?

BDNF was initially identified as a trophic factor; therefore it is not surprising that the first studies of ethanol-BDNF interactions were initiated to examine the role of BDNF in FASD and neurodegeneration with chronic exposure in adults. The effect of ethanol on BDNF expression in the VTA and NAc will be discussed below in detail in Section 6 as it relates to addiction. The hippocampus has been most extensively examined in terms of neurotoxicity because of the role of this structure in memory and the impaired cognitive function observed clinically in alcoholics (Harper and Matsumoto, 2005). The majority of the studies examining BDNF and/or TrkB in adult preparations have examined mRNA levels and are summarized in Table 1.

Some of these studies indicate that ethanol does not change TrkB mRNA levels in the adult (Miller *et al.*, 2002; Zhang *et al.*, 2000) while others report up-regulation in vivo during

withdrawal (Baek *et al.*, 1996; Tapia-Arancibia *et al.*, 2001). This variability is not surprising since the receptor is not significantly regulated at the level of mRNA expression but shows significant down-regulation at the protein level with chronic BDNF treatment (Frank *et al.*, 1996; Frank *et al.*, 1997); one would therefore expect reciprocal regulation of BDNF and TrkB. The data on BDNF protein and mRNA levels are also inconsistent, with some studies showing no change in hippocampal BDNF levels (Miller *et al.*, 2002; Okamoto *et al.*, 2006), while others show a decrease in BDNF (MacLennan *et al.* 1995, Tapia-Arancibia *et al.*, 2001). Surprisingly, two of the studies showing disparate effects in the same brain region are from the same laboratory. The authors attribute this discrepancy to the time of analysis. In the MacLennan study, the animals were withdrawn from ethanol while animals were still on drug/diet when sacrificed in the Miller study. This time discrepancy is bridged by Tapia-Arancibia and colleagues who found a decrease in BDNF mRNA while the animals were on drug/diet and an increase after withdrawal.

Zou and Crews (2006) reported a decrease in hippocampal BDNF mRNA in hippocampal explant cultures exposed acutely to ethanol under conditions of oxidative stress combined with tumor necrosis factor treatment. This was accompanied by an increase in NF-κB and a decrease in CREB DNA binding activity. This is in contrast to the observations of McGough and colleagues (2004) who reported an increase in BDNF mRNA that was mediated through Receptor for Activated C-Kinase 1 (RACK1) in hippocampal pyramidal cells in culture, but noted a decrease in BDNF with long-term treatment. As discussed by McGough and colleagues (2004), this may represent a homeostatic pathway that negatively regulates ethanol consumption. These data are complementary to the observation that intracerebroventricular administration of BDNF maintains ethanol tolerance in C57/BL6J mice (Szabo and Hoffman, 1995). Therefore, BDNF induction may regulate pharmacodynamic adaptations to ethanol exposure.

The disparate observations may reflect differences between homeostatic changes (or pharmacodynamic tolerance) that occurs acutely and the pathological changes that lead to neurodegeneration with chronic exposure. The majority of these studies report a decrease in BDNF mRNA in the hippocampus with long-term exposure, suggesting this decrease may play a role in, or is the consequence of, neurodegeneration. However, there are still temporal and methodological issues to be resolved. Long-term ethanol exposure causes hippocampal atrophy in rats (Walker *et al.*, 1980), therefore observations made after 28 weeks of chronic ethanol exposure may occur as a result of a decrease in dendritic complexity and do not address the mechanisms that produced the degeneration. Short term exposure may cause the system to compensate in an attempt at homeostasis or repair (i.e. McGough *et al.*, 2004; Miller 2004) that eventually fails with longer exposures (McGough *et al.*, 2004; Miller and Mooney, 2004;) whereas withdrawal may cause hyperactivity due to upregulation after signal inhibition (Tapia-Arancibia *et al.*, 2001). These variable changes may occur secondarily to an inability of the receptors to transduce signal in the presence of ethanol (See Section 5). However, these studies primarily examined mRNA, therefore the changes reported may not reflect differences in secreted protein or in its activity in vivo.

Recent advances in genomics, proteomics, in situ mapping and kinase activation screening will be useful in resolving many of these differential effects. One of the most pressing questions to be addressed is the promoters that may be differentially regulated during ethanol exposure and withdrawal. For example, calcium-dependent activation of a promoter that produces BDNF mRNA and targets to dendrites would be expected to have a different functional effect than an mRNA species that is translated in the soma. Knowledge of the promoter being regulated during these various states of intoxication and stress/anxiety may also provide clues to the mechanisms and neurotransmitter systems involved. A detailed time-course of the effects of ethanol on signal transduction pathways in a high-throughput assay combined with protein expression and

mRNA could be used to construct a temporal picture of changes in signaling that result in tolerance, compensatory regulation and subsequent neurodegeneration.

4. Is BDNF involved in FASD?

Neurotrophins regulate proliferation, migration and differentiation in the developing brain and therefore are likely to be involved in the toxicity of ethanol during brain development. Rodents exposed to ethanol in utero or during the early postnatal period (a period roughly equivalent to the third trimester of human development) display microcephaly, hyperactivity, reduced cerebellar Purkinje cells, ataxia and learning deficits, which are also hallmarks of the human disorder (reviewed by Berman and Hannigan, 2000; Burn *et al.*, 2003). The striking similarities between FASD models and BDNF haplodeficient mice suggest that the two phenotypes may be developmentally linked. There is considerable evidence that some of the teratogenic consequences of developmental ethanol exposure are mediated by inhibition of neurotrophin expression and function in a region-specific and time-dependent manner. This may contribute to apoptosis and impaired synaptogenesis, which are characteristic of FASD (reviewed by Olney 2004). The majority of the studies examining the effect of ethanol on BDNF and its associated signaling pathways during development have been performed in neonatal animals. This is likely because the regions where BDNF is expressed in the greatest amounts are late embryonic or postnatally developing structures. A variety of exposure paradigms have been employed and are summarized in Table 2.

The ontogeny of specific neuronal populations in the cerebellum, their migration, synaptogenesis and the factors modulating these processes have been well-characterized (reviewed by Sotelo *et al.*, 2004) making the developing cerebellum an ideal model for examining alcohol-induced teratogenesis. Cerebellar Purkinje cells are particularly vulnerable to ethanol during the first postnatal week but resistant to ethanol during the second postnatal week (West, 1993). The majority of studies examining BDNF levels, TrkB, or BDNF signaling have suggested that ethanol inhibits the neurotrophic activity of BDNF in the cerebellum (Table 2) and this may contribute to cerebellar Purkinje cell loss. Ge and colleagues (2004) performed a detailed dose-response, time-course analysis and found a rapid decrease in BDNF and TrkB mRNA in the cerebellum after exposure on PN4 but not PN9. In contrast, Heaton and colleagues (1999) found no differences in BDNF protein levels in cerebellum with numerous exposure paradigms that span this age but subsequently reported an increase immediately after exposure on PN4 that normalized by 2 hours (Heaton *et al.*, 2003a). Exposure on PN7 increased BDNF levels at 2 hours and decreased levels at 12 hours in this study.

Light and colleagues used immunohistochemistry to detect TrkB specifically on cerebellar Purkinje cells during the vulnerable first postnatal week. Ethanol decreased TrkB immunoreactivity at all ages examined. Furthermore, they also observed a decrease in mRNA for TrkB and TrkB-T1 (Light *et al.*, 2002). These data are particularly compelling since TrkB was detected and selectively decreased directly on the vulnerable cell population, suggesting that TrkB down-regulation and decreased secretion of BDNF by granule cells (Bhave *et al.*, 1999; Heaton *et al.*, 2004) are a prelude to apoptosis.

Many studies have examined changes in tissue homogenates but this represents total cerebellar protein or mRNA and Purkinje cells represent a small proportion of the total number of cerebellar cells (approximately 5%). In situ hybridization studies do not indicate that Purkinje cells contain more BDNF or TrkB mRNA than other cells and their large arbors are still developing and would therefore contribute less to the total protein or mRNA signal at earlier time-points (i.e. vulnerable vs. resistant periods). In addition, granule cells express both BDNF and TrkB mRNA and comprise the majority of cerebellar neurons thereby complicating the analysis of Purkinje cell proteins or mRNA in total cerebellar homogenates.

BDNF and TrkB expression have also been examined in cortex, striatum and hippocampus in developmental exposure models (Table 2). Several lines of evidence supported a role for decreased BDNF activity in the cortex in the development of FASD. Fetal exposure decreases cortical BDNF and increases TrkB/TrkB-T1 ratios as neonates (Climent *et al.*, 2002). Feng and colleagues (2005) reported similar findings, showing decreased BDNF expression in hippocampus and cortex with gestational exposure, but no change in TrkB. Moore and colleagues (2004) reported a decrease in hippocampal TrkB in males and an increase in cortical TrkB in females at PN1 with gestational exposure. Fattori and colleagues (2008) examined BDNF levels in cortex after neonatal exposure and, as with the majority of studies, also observed a decrease in BDNF mRNA. These data are in contrast with the results of Heaton and colleagues (2003c), who found bidirectional changes in BDNF cortex after exposure on PN7. This group also found no differences in corticostriatal BDNF levels with prenatal exposure but found an increase after neonatal exposure (Heaton *et al.*, 2000b). The discrepancy in these data is not likely due to timing of exposure since conflicting results were also obtained with gestational exposure, but may be due age of the animals at analysis. Heaton*et al.* (2000) measured levels at PN1, while Feng *et al.* (2005) examined BDNF levels at PN7 and Climent *et al.* (2002) measured levels at PN5, PN14, PN21 and PN35 after gestational exposure.

Similar discrepancies also exist for the effect in hippocampus, with Heaton and colleagues (2000) reporting a transient increase in hippocampal BDNF at PN10 with neonatal exposure, while Feng and colleagues (2005) report a decrease in BDNF with gestational exposure. This brings into question whether BDNF levels change as a result of ethanol exposure or change as a result of neuronal atrophy, impaired synaptogenesis and accelerated apoptosis. More experiments examining acute effects on signaling during exposure and compensatory regulation over time after the insult in specific cell types are required (i.e. Ge *et al.*, 2004; Light *et al.*, 2002). It is also unclear whether these changes result in a decrease in activity-dependent BDNF release or represent differences in dendritic complexity. Examination of BDNF signaling (discussed below) suggests that ethanol causes a decrease in the ability of TrkB to transduce signal and desensitize, which may lead to compensatory regulation of both BDNF and TrkB in vivo. The data from Feng *et al.* (2005) and Climent *et al.* (2002) suggest that the end result is a decrease in BDNF during post-exposure development that may be secondary to impaired synaptogenesis and neuronal apoptosis.

5. Effects of EtOH on BDNF signaling

The literature on BDNF and TrkB expression levels before, during, and after ethanol exposure is extensive; therefore it is surprising that relatively few studies have focused on the *direct* effect of ethanol on BDNF signaling. Animals exposed prenatally to ethanol have decreased TrkB phosphorylation at 1 week of age that is probably secondary to a decrease in BDNF (Climent *et al.*, 2002; Feng *et al.*, 2005; Fattori *et al.*, 2008). Chronic ethanol exposure increases ERK activity in cultured cortical neurons and pheochromocytoma cells (Kalluri and Ticku, 2003; Roivainen *et al.*, 1995) while acute ethanol administration decreases activation of ERK (Chandler and Sutton, 2005; Davis *et al.*, 1999; Han *et al.*, 2006; Kalluri and Ticku, 2002; Kalluri and Ticku 2003; Tsuji *et al.*, 2003; Fattori et al., 2008; Ohrtman et al., 2006, however, see Acquaah-Mensah et al., 2001).

Ethanol acutely inhibits TrkB signaling to ERK in cerebellar granule cells (Li *et al.*, 2004; Ohrtman *et al.*, 2006) and this is not due to a decrease in TrkB phosphorylation (Li *et al.*, 2004), suggesting an intracellular cite of action. Hippocampal slices prepared from neonatal rat pups stimulated with BDNF also show reduced nuclear translocation of phospho-ERK in CA1 hippocampal pyramidal cells (Davis *et al.*, 1999). A thorough pharmacological analysis TrkB-ethanol interactions in cerebellar granule cells revealed that inhibition of BDNF-stimulated ERK phosphorylation by ethanol is dependent on BDNF concentration and

independent of NMDA receptors (Ohrtman *et al.*, 2006). Low concentrations of ethanol (25 mM) inhibit ERK activation when BDNF is present at concentrations in the linear range of the high affinity concentration-response curve (less than or equal to 5 ng/mL; 185 pM). However, high concentrations of ethanol (100 mM) block the desensitization of the ERK response at high BDNF concentrations (>10 ng/mL). This may occur through inhibition of NMDA receptors by ethanol since APV blocks desensitization of ERK stimulated by TrkB (Davis and Ohrtman, unpublished observations). The mechanism for inhibition of BDNF-stimulated ERK activation by ethanol remains elusive but recent evidence suggests that it may involve the signaling intermediate Raf and Raf kinase inhibitor protein (Hellmann *et al.*, 2006). The decrease in ERK activity also correlates with an increase in protein kinase A activity and a decrease in calcium-sensitive protein kinase C activity (Davis *et al.*, 1999). Both of these pathways can cross-regulate ERK (reviewed by Liebmann 2001), therefore inhibition of TrkB signaling may also ultimately involve pathways previously known to be sensitive to ethanol.

Ethanol decreases Akt phosphorylation in the developing, but not adult, brain (Chandler and Sutton, 2005), suggesting that Akt may be involved in the selective vulnerability of the developing brain to ethanol. BDNF protects neurons from ethanol-induced cell death *in vitro* (Heaton *et al.*, 2000a; Bonthius *et al.*, 2003). NMDA also increases granule cell survival and protects from ethanol toxicity through up-regulation of BDNF (Bhave *et al.*, 1999; Marini *et al.*, 1998). The biochemical mechanisms and isozymes underlying this neuroprotection are less clear but likely involve PI 3-kinase. To address this question, Bhave and colleagues (1999) used pharmacological inhibitors of PI 3-kinase (100 nM wortmannin or 10 μm LY294002) to block the trophic effects of NMDA and its induction of BDNF. Inhibition of PI 3-kinase was toxic to granule cells in these experiments; however confirmatory western blot analysis further implicated PI 3-kinase in the neuroprotection. In a similar study, Heaton and colleagues (2000a) examined the mechanism of BDNF neuroprotection in cerebellar granule cell cultures and also suggested a role for PI 3-kinase. This study used wortmannin at 10–100 μm to reverse the neuroprotective effects of BDNF. The data for wortmannin alone were not presented but the authors stated that it was without effect. This is in contrast to Bhave and colleagues but may be due to the poor stability of wortmannin since the concentrations used are much higher than what is normally required to inhibit PI 3-kinase (D'Mello et al., 1997; Miller *et al.*, 1997; Bhave *et al.*, 1999). Isozyme-selective PI 3-kinase inhibitors have recently been developed (Redaelli *et al.*, 2006) that have reduced global toxicity and could be used to extend these studies. Given the role of PI 3-kinase in neuronal survival, BDNF is most likely mediating neuroprotection from ethanol toxicity through PI 3-kinase in cultured cerebellar granule cells. The situation *in vivo* is quite different, however, since the effect in the absence of neurotrophic factor supplementation may be a decrease in the ability of endogenous BDNF to increase survival during development secondary to a decrease in BDNF levels and the reduced ability of BDNF to transduce survival signals.

c-Jun N-terminal Kinase (JNK) is a MAP Kinase family protein that is activated under conditions of cellular stress (reviewed by Weston and Davis, 2007). This is isozyme-specific and JNKs can also participate in neuronal differentiation through phosphorylation of cytoskeletal proteins involved in neuronal migration (Reiner *et al*., 2004; Hirai *et al*., 2006) and axon formation (Oliva *et al.*, 2006). p46 JNK is also activated by BDNF (Davis and Hassoun, unpublished observations). Activation of the JNK pathway by ethanol has been suggested to be involved in apoptosis (Han *et al.*, 2006; Heaton *et al*., 2003). However, subsequent studies did not find a correlation between JNK activation and cell death (J.W. Olney, personal communication). Furthermore, BDNF stimulation of AP1 DNA binding activity is inhibited by ethanol in cerebellar granule cells (Li *et al.*, 2004), suggesting reduced genomic signaling through Fos/Jun. Activation of the NF-κB pathway has also been observed after EtOH exposure (Zou and Crews, 2006), and activation of this pathway correlates with cell death under conditions of cellular stress.

Other pathways such as those involving PLC and PKC that couple to TrkB are also regulated by G protein-coupled receptors, stress, integrins and calcium. Chronic ethanol has previously been shown to specifically decrease PLC activity and PLCβ1 expression *in vivo* and in neuroblastoma cells (Alling *et al*., 1993; Katsura *et al*., 1994; Pandey, 1996) and prenatal ethanol exposure decreases PLCβ1 activity in the hippocampus and cortex (Allan *et al.*, 1997). These changes in PLC may represent changes in G-protein coupled signaling since changes in PKCγ, which also couples to TrkB (Widmer *et al*., 1993), were not observed (Pandey *et al*., 1996). However, direct stimulation of PKCγ by epidermal growth factor is inhibited by ethanol (Thurston and Shukla, 1992). Many PKC isozymes are regulated by diacylglycerol that is generated by PLC activity and EtOH is known to modulate PKC activity but this depends on the isozyme of PKC examined and the duration of exposure (reviewed by Slater and Stubbs, 1999; Newton and Ron, 2007).

BDNF and multiple G protein-coupled and ionotropic receptors signal to CREB to modulate gene transcription and late phase synaptic plasticity. CREB is also a target of ethanol and multiple studies implicate CREB in ethanol-mediated changes in gene expression. A detailed discussion of region-specific regulation of CREB as it relates to behavioral phenotypes is presented in Section 6 but is introduced here in the context of cellular signaling. An *in silico* analysis of promoter elements in ethanol responsive genes has identified CREB as a potential mediator of ethanol effects on gene expression (Uddin and Singh 2007). Using mice expressing LacZ under a CRE, an acute exposure to ethanol increased CRE-mediated gene transcription (Asyyed *et al*., 2007) in NAc, PFC, Septum, BNST, Lateral Habenula, BLA, PVN, hippocampus, VTA and SN, in addition to hypothalamic nuclei. Pandey and colleagues (2004) identified CREB in the CeA and MeA as a potential mediator of ethanol consumption since reduced pCREB was associated with anxiety during withdrawal, and rats that prefer ethanol have lower levels of pCREB in this structure (Pandey *et al.*, 2005). These data are similar to the decrease in pCREB observed in the CeA and MeA after acute amphetamine exposure (McPherson *et al*., 2007) and may therefore be related to increased catecholamine levels during withdrawal. However, in contrast to Asyyed and colleagues (2007), Pandey and colleagues (2005) reported an increase in pCREB phosphorylation in CeA and MeA, but not BLA in ethanol preferring rats exposed to ethanol by either IP injection or self administration. Chandler and Sutton (2005) reported a decrease in CREB phosphorylation with acute ethanol exposure that was similar to the decreased observed in ERK activation in hippocampus and cortex. Again, this is in contrast to previous observations reporting a decrease in cortical pCREB and BDNF during withdrawal but not with ethanol exposure (Pandey *et al*., 1999). The different exposure paradigms (IP injection vs. long-term self administration and withdrawal), rodent strain or cell-type selectivity may explain these differences. Acute ethanol can activate adenylate cyclase, which can activate CREB through PKA (Asher et al., 2002; Constantinescu *et al.*, 2002). Chronic exposure leads to compensatory up-regulation of NMDA receptors, with withdrawal producing Ca^{2+}-dependent hyper-excitibility (Hoffman and Tabakoff, 1994). This may lead to increased CREB-mediated gene transcription through CaM kinases in certain regions. Therefore, CREB regulation, as with each of these pathways, may be mediated by multiple ethanol-sensitive pathways at different times during and after ethanol exposure. BDNF is just one factor mediating CREB phosphorylation, therefore any changes in pCREB must be carefully considered in the context of the circuit showing activation, the cell type and other ethanol-mediated changes in cell signaling induced by perturbation of ionotropic and G protein-coupled receptor signaling.

BDNF modulates synaptic plasticity in preparations made from mature brain and this has been extensively examined in the modulation of hippocampal LTP (Korte *et al.*, 1995; reviewed by Nagappan and Lu, 2005; Soule *et al.*, 2006). Ethanol inhibits NMDA receptors and NMDA mediated long-term plasticity (Blitzer *et al.*, 1990; Lovinger *et al.*, 1989; Lovinger *et al.*, 1990). BDNF potentiation of NMDA receptor function is inhibited by EtOH and this occurrs

at concentrations as low as 10 mM, which have relatively small direct effects on the current itself (Kolb *et al.*, 2005). These data suggest that BDNF-mediated regulation of NMDA receptor currents may be extrememly sensitive to ethanol.

Regional differences in activation vs. inhibition of BDNF-regulated signaling pathways may depend on the local concentration of BDNF, differential sensitivity of the neuronal poulation to ethanol, developmental state of the cell, duration of ethanol exposure and on compensatory regulation of the pathways (homeostasis and pharmacodynamic tolerance). Disinhibition of a circuit (i.e. inhibition of interneuron function) may also result in seemingly paradoxical activation of BDNF signaling pathways in specific neuronal populations by ethanol *in vivo*. In addition, the effect of ethanol on pro-survival pathways may depend on the differentiation state of the cells examined. Developing neurons are more sensitive to inhibition of Akt by ethanol (Chandler and Sutton, 2005), certain transformed cells show an increase in ERK activation in response to chronic ethanol exposure (Roivainen *et al.*, 1995; Ku *et al*., 2007), while terminally differentiated cells, for the most part, show inhibition of ERK after acute exposure (Davis *et al*., 1999; Chandler and Sutton, 2005; Li *et al*., 2004; Ohrtman *et al.*, 2006). Detailed temporal, global immunohistochemical mapping studies of activated protein kinases, neuronal activity and the determination of neuronal identities will be essential in resolving temporal and regional differences. In addition, selective BDNF deletion using the Lox-Cre system could be used to elucidate the role of BDNF in ethanol-induced changes in neuronal signaling in discrete neuronal populations.

6. BDNF in animal models of anxiety, depression and addiction

Addiction and memory share common biochemical substrates and produce long lasting changes in brain circuitry that lead to compulsive drug seeking (reviewed by Hyman *et al.*, 2006). The dopamine hypothesis of addiction-reward posits that increased dopamine release from VTA neurons onto neurons in the NAc underlies the rewarding properties of most drugs of abuse, including ethanol (reviewed by Koob, 2003). This system has been termed the mesocorticolimbic dopamine pathway and includes prefrontal cortex and the extended amygdala. According to this model, repeated exposure to a drug produces synaptic plasticity (LTP and LTD) in this circuit (and others) and changes the anxiety set point resulting ultimately in craving and addiction. The allostatic model for ethanol addiction posits that drug exposure resets the activity of neurons in these reward pathways. Reward and withdrawal-induced anxiety increases the desire to consume ethanol (Koob, 2003). Consumption leads to an increase in dopamine release from neurons located in the VTA onto neurons in the NAc, leading to “reward”. This is followed by cycles of withdrawal-induced anxiety, decreased CREB activation and decreased NPY production in the amygdala and continued drinking (Pandey, 2003). Rewarding properties are reduced with subsequent exposures and more drug must be consumed to feel “normal”. Theories of ethanol reward and addiction implicate the integration of these circuits in ethanol seeking and relapse.

This reward circuitry has recently been extended to include the lateral habenula, which inhibits DA neurons in VTA to block signaling the absence of reward (Matsumoto and Hikosaka, 2007). Ethanol administered by IP injection acutely increases CREB/CRE mediated gene transcription in the lateral habenula, suggesting that this region may also be involved in ethanol intoxication and acute adaptation/tolerance (Asyyed *et al.*, 2006) but how activity in this region changes with prolonged ethanol exposure and tolerance is unknown. In addition, the mesolimbic dopamine pathway regulates depressive-like behaviors in mice (Nestler and Carlezon, 2006). Projections from the VTA to the NAc are regulated by feedback from inhibitory projection neurons to the VTA, as well as excitatory projections to the NAc from the cortex (Nestler and Carlezon, 2006). These circuits are active in stress, anxiety, depression and addiction, therefore I will consider the effects of ethanol on BDNF pathways in the context

of what is known about these brain regions in substance abuse, depressive and anxiety-like behaviors.

Multiple lines of evidence suggest that BDNF is involved in VTA-NAc mediated addiction, craving and withdrawal. Initial observations indicated that BDNF infusion directly into the VTA led to a down-regulation of total ERK with no change in ERK activity. This occluded any further enhancement of the pathway by morphine or cocaine, thereby blocking the rewarding properties of the drugs (Berhow *et al.*, 1996). These data also suggest that the ERK pathway can be dysregulated by prolonged BDNF exposure. Amphetamine exposure increases BDNF in the BLA and within its projections to the CeA, striosomes, medial striatum and NAc (Meredith *et al.*, 2002). BDNF potentiates psychostimulant-induced increases in DA both acutely and during sensitization (Altar *et al*., 1994; Pu *et al.*, 2006; Horger *et al.*, 1999). Conditioned place preference in response to psychostimulants is also reduced in BDNF haplodeficient mice (Hall *et al*., 2003). Amphetamine increases TrkB expression (Meredith and Steiner, 2006) and BDNF participates in the rewarding properties of psychostimulants by potentiating pre and post synaptic activity (Horger *et al.*, 1999). BDNF also sensitizes midbrain DA neurons to potentiation during psychostimulant withdrawal (Pu *et al.*, 2006). A single infusion of BDNF into the VTA can potentiate cocaine craving for a month (Lu *et al.*, 2004). Taken together, these observations indicate that BDNF can modulate and potentiate reward pathways, but these become dysregulated with chronic exposure to pyschostimulants or infusion of BDNF itself.

In contrast, chronic stress and depression decrease BDNF levels in the hippocampus (Smith *et al.*, 1995). Antidepressants increase BDNF and TrkB levels in hippocampus and it has been posited that this is a mechanism by which antidepressants exert their actions (Nibuya *et al.*, 1995; reviewed by Duman and Monteggia 2006). These findings were extended with a forebrain-specific doxycycline inducible knock-out of BDNF. These mice show profound hippocampal dysfunction, attenuated antidepressant effects and hyperactivity if the gene is deleted late in embryonic development (Monteggia *et al.*, 2004). There were gender differences as well, with males showing more hyperactivity and females showing more depressive-like behaviors (Monteggia *et al.*, 2006). These data further implicate cortical and hippocampal sources of BDNF in antidepressant efficacy. In a related study (Zorner *et al.*, 2003), $TrkB^{CaMKII\text{-}CRE}$ mice were generated to screen for a role for BDNF in depressive-like behavior. This strategy relies on forebrain-specific expression of the Cre recombinase under the αCaMKII promoter, which is expressed after the major wave of cortical development. This strategy circumvents the developmental lethality of the deletion and allows for selective deletion of TrkB in the forebrain. These mice exhibited an amplified impulsive response to novelty and stereotyped hyperlocomotion, suggesting these mice may be a model for ADHD. It is interesting that both models point to a role for impaired forebrain BDNF signaling in the development of impulsivity and hyperactivity since these are characteristics of FASD with hyperactivity more prevalent in males. BDNF would be retained in the dopaminergic and absent in the corticostriatal terminals, possibly leading to an increase in dopaminergic transmission relative to glutamate from the cortex.

BDNF appears to be a key player in antidepressant efficacy, at least in hippocampus, but what about the systems that regulate anhedonia and depressive states? The VTA and projections from this area (i.e. ventral striatum and amygdala) are not only implicated in reward and anxiety but may also be involved in the eitiology of depression (Nestler and Carlezon, 2006). Surprisingly, direct delivery of BDNF into the VTA produced depressive-like behaviors in mice (Eisch *et al.*, 2003). Furthermore, this was reversed by expression of TrkB.T1 in NAc using stereotaxic viral injections. This observation was recently expanded upon to examine the role of BDNF in another depressive-like behavior, social defeat stress (Berton *et al.*, 2006). Mice exposed to a “bully” mouse (a male mouse of a larger strain) have increased mesolimbic

BDNF levels and target activity in NAc as measured by c-Fos induction. Therefore, increased TrkB activation is associated with decreased social interactions and heightened accumbal induction of Fos after exposure to the "bully" mouse. Selective deletion of BDNF in the VTA using viral expression of the Cre-recombinase in floxed-BDNF transgenic mice or antidepressant treatment with fluoxetine reversed these effects. This study was recently extended, further implicating BDNF in the VTA and it's projections in suceptiblity to social defeat stress (Krishnan *et al*., 2007).

In a complementary series of experiments, transgenic mice over-expressing BDNF under the αCaMKII promoter were generated to increase BDNF specifically in the forebrain (Govindarajan *et al*., 2006). These mice exhibited increased anxiety-like behavior and increased dendritic spines in BLA neurons but a decrease in depressive-like behavior in the Porsolt forced-swim test. Therefore, an increase in forebrain expression of BDNF increases anxiety but decreases depressive-like behaviors. These data indicate that precise activation of brain areas by BDNF is required for appropriate responses with induction of BDNF being correlated with antidepressant efficacy in the hippocampus but not in the VTA-NAc pathway. Furthermore, these studies suggest that depression, anxiety and locomotor activity are differentially regulated by BDNF, depending on the source.

Depression and anxiety often result from dysregulation of the serotonergic system (reviewed by Hariri and Holmes, 2006) and the role of BDNF in antidepressant efficacy has recently been reviewed in this journal (Kozisek *et al.*, 2007) It has been proposed that modulation of the 5-HT system might be a viable therapy for alcoholism in a sub-set of patients (Johnson 2004; Wrase *et al.*, 2006) and BDNF supports survival of serotonergic neurons (Altar, 1999; Madhav *et al.*, 2001). Lyons and colleagues (1999) found that $BDNF^{+/-}$ mice had abnormalities in the serotonergic system, with an age-related decline in 5HT and 5-HIAAA. They observed upregulation of $5\text{-}HT_{1B,}$ $5\text{-}HT_{2A}$ and $5\text{-}HT_{2C}$ in hypothalamus and cortex at 6–9 months of age. Hippocampal levels were slightly decreased, but only significantly for $5\text{-}HT_{1C}$. This is accompanied by a reduction in dexfenfluramine induction of c-Fos in the cortex, increased aggression, hyperphagia and degeneration of serotonergic neurons. It was subsequently reported that $5\text{-}HT_{1A}$ receptor-stimulated GTP-γS binding was modestly attenuated in the medial and dorsal raphe nuclei, anterior cingulate cortex, lateral septum, and significantly decreased in the hippocampus of BDNF haplodeficient female mice (Hensler *et al.*, 2003).

Unlike the studies discussed above, these $BDNF^{+/-}$ mice did not display hyperphagia as measured by an increased preference for saccharine or quinine, but showed a significant preference for ethanol in a 2-bottle choice paradigm. These studies were extended in the same mouse strain (McGough *et al.*, 2004) to suggest that induction of BDNF in the dorsal striatum and hippocampus negatively regulates ethanol consumption. Also using BDNF globally haplodeficient mice, this study replicated the observations of Hensler and colleagues with respect to increased ethanol consumption. In addition, $BDNF^{+/-}$ mice show increased conditioned place preference to ethanol (2 g/kg) and increased locomotor activity in response to ethanol in this study. Unlike previous studies with this strain (Duan *et al.*, 2003; Kernie *et al.*, 2000) and with forebrain-specific deletion (Monteggia *et al.*, 2004; Monteggia *et al.*, 2006; Rios *et al.*, 2001; Zorner *et al.*, 2003), these mice did not differ in basal locomotor activity but showed enhanced activity in response to ethanol injection. This is in contrast to cocaine sensitivity, where $BDNF^{+/-}$ mice show reduced locomotor effects and decreased conditioned place preference (Hall *et al.*, 2003). The increased consumption of ethanol in haplodeficient mice could be reversed by systemic delivery of TAT-RACK1. Ethanol increases RACK1 nuclear translocation (Ron *et al.*, 2000) and the TAT-RACK1 construct increases BDNF expression in striatal slices, in hippocampal cultures, and also after IP injection (McGough *et al.*, 2004). These are intriguing studies because they implicate the dorsal striatum, an area

involved in habit learning (reviewed by Gerdeman *et al.*, 2003; Yin and Knowlton, 2006), in ethanol self administration.

Array analysis has identified BDNF as an ethanol-responsive gene in PFC and NAc, but there are also discrepancies in these observations. Melendez and colleagues (2006) exposed mice by vapor inhalation for 2 “binges” of 64 hours separated by 2 weeks. PFC, NAc and hippocampus were examined but differences in BDNF were only observed in the PFC. This group observed a decrease in BDNF mRNA on the array, which was confirmed by RT-PCR. Protein levels were also examined and found to be reduced in the PFC immediately after the second exposure and 8 hours later. In a similar study, Kerns and colleagues identified NAc BDNF as an ethanol responsive gene in DBA/2J mice using mRNA from animals exposed to acute EtOH by IP injection. They compared low ethanol drinking DBA/2J mice to high ethanol drinking C57BL/6J mice using microarrays and found an increase in BDNF mRNA in the NAc in DBA/2J mice only. These data suggest that BDNF induction in the NAc may be associated with ethanol aversion or dysphoria, similar to the depressive-like effect of intra-VTA administration of BDNF (Eisch *et al.*, 2003) or induction by social defeat stress (Berton *et al.*, 2006). The source of the BDNF mRNA is also a mystery since it is not generally detected in the NAc or at significant levels in striatum under normal conditions (Altar *et al.*, 1997; Baquet *et al.*, 2004; Conner *et al.*, 1997; Zuccato *et al.*, 2001), but may indicate strain differences that mediate preference and aversion. Given that these studies used extremely sensitive techniques, this mRNA may represent increased expression in a small population of neurons or mRNA present in glia, stem cells, platelets or blood cell progenitors. Axonal mRNA from cortex seems unlikely since BDNF protein is transported to striatum from cortex and midbrain structures (Altar *et al.*, 1997; Conner *et al.*, 1997; Gauthier *et al.*, 2004; Strand *et al.*, 2007) but remains a possible explanation. Another intriguing possibility is that the source of BDNF mRNA is the dopaminergic terminals, which are the source of the BDNF that increases D3 receptor expression during cocaine sensitization (Guillin *et al*., 2001). Alternatively, BDNF induction may signal a significant pathological reaction to a toxic insult, as has been shown to occur with cocaine exposure (Liu *et al*., 2006) and excitotoxic lesions (Rite *et al*., 2005).

The role of the extended amygdala in fear, anxiety, hypothalamic-pituitary adrenal axis regulation, appetite and sensation has been studied extensively in rodent models of anxiety-like behaviors and fear conditioning. The amygdala is divided into at least 13 nuclei with multiple subdivisions in rodents based on location and afferent-efferent projections (reviewed by Sah *et al*., 2003). These nuclei are grouped anatomically into cortical, basolateral and centromedial but extensively subdivided, with signals generally flowing laterally to medially through the nuclei with numerous intranuclear connections and collaterals. The amygdala integrates information from multiple sensory systems, the cortex and the hippocampal formation. Monoaminergic brain stem innervation, particularly in the CeA and BNST, modulates the activity of the circuit. The CeA is considered the primary output, with projections to the hypothalamus, ventral striatum, PAG and BNST which mediate arousal. The basolateral complex also sends reciprocal projections to prefrontal cortex, BNST, VTA, mediotemporal lobe memory structures and the ventral striatum. The precise function of these connections is not well understood but the output of this structure regulates fear, startle, anxiety, pain perception and autonomic function through hypothalamic projections.

One of many self-medication hypotheses for the development of alcoholism exploits the anxiolytic properties of ethanol in the amygdala. Ethanol increases GABAergic transmission through enhanced release of GABA in the CeA and BLA (Roberto *et al.*, 2003a; Zhu and Lovinger, 2006) and can potentiate $GABA_A$ receptor function, although this is controversial (reviewed by Lovinger and Homanics, 2007). In addition, a recent study indicates that there are 2 ethanol-sensitive populations in the BLA (Silberman *et al.*, 2007). The first population

is represented by local interneurons and responds to ethanol with enhanced release through a $GABA_B$-dependent mechanism. The second population, paracapsular neurons, responds to ethanol through norepinepherine receptors. These data suggest that synaptic transmission in BLA target areas would be decreased by ethanol.

Chronic ethanol also increases the sensitivity of CeA neurons to inhibition of NMDA receptors by ethanol (Roberto *et al.*, 2004). However, the mechanism for this effect is still being debated, with Roberto and colleagues suggesting that this is due to changes in receptor expression. Others have shown no differences in receptor levels (Läck *et al.*, 2005), instead implicating post-translational regulation of NMDA receptors in the CeA in neuroadaptation to chronic ethanol and possibly withdrawal-induced anxiety. The BLA has been extensively examined for roles in fear conditioning, addiction and anxiety (reviewed by Davis, 2006; Shekhar *et al.*, 2005). BDNF in the BLA plays a role in Pavlovian fear conditioning (Rattiner *et al.*, 2005) and consolidation of fear extinction (Chhatwal *et al.*, 2006). Other studies have implicated the BNST in anxiety-like behaviors and afferent modulation of the acoustic startle reflex, with the CeA modulating fear conditioning (reviewed by Davis and Shi, 1999; Davis 2006).

Surprisingly, administration of a BDNFantisense oligodeoxynucleotides into the CeA or MeA, but not the BLA, increased anxiety and increased ethanol consumption in rats (Pandey *et al.*, 2005). This was also reflected in reduced activation of ERK and CREB with oligodeoxynucleotide infusion. The increased ethanol consumption is recapitulated in CREB deficient mice (Pandey *et al.*, 2004b), suggesting that reduced CREB activity and decreased BDNF and NPY levels in the CeA and MeA contribute to ethanol drinking. This is in contrast to observations in the hippocampus and cortex, reporting a decrease in pCREB and NPY with ethanol exposure and an increase in both pCREB and NPY in the dentate gyrus/hilus during withdrawal (Bison and Crews, 2003). Opiate withdrawal also induces anxiety, but this involves A1/A2 noradrenergic projections and *increased* CREB phosphorylation in the CeA and BNST (reviewed by Aston-Jones and Harris, 2004). The identity of the cells expressing BDNF is also of interest. The striatum and CeA, but not the MeA, share a common embryonic origin in the lateral ganglionic eminence (García-López *et al.*, 2008). The majority of the cells in the CeA are of the medium spiny type and presumably GABAergic (McDonald, 1982). Therefore, increased activity in these neurons would presumably lead to decreased activity in target structures such as the brain stem, PVN and VTA but this has not been established.

As discussed above, these data are in contrast to anatomical studies showing no BDNF mRNA in CeA, but heavy staining of fibers and terminals in this area (Conner *et al.*, 1997; Krause *et al.*, 2008). Ethanol induces expression of BDNF mRNA in structures not identified in classical expression studies in normal animals, such as the CeA (Pandey *et al.*, 2005) and dorsolateral striatum (McGough *et al.*, 2004). McGough and colleagues used PCR amplification for detection but did not examine protein levels in these structures. Pandey and colleagues (2005) used in situ RT-PCR and immunogold to detect both mRNA and protein. The speckled distribution of the immunogold particles suggests that BDNF might be in terminals but this study did not specifically address this possibility and in situ PCR for BDNF clearly showed somatic localization but no immunoreactive fibers from the BLA projections, as have previously been described (for images of these projections, see Conner *et al.*, 1997). In addition, ethanol-induced increases in glutamatergic function in the BLA do not affect BDNF expression in the BLA (B.A. McCool, personal communication), suggesting that the BLA is not the source of this change in BDNF. Recent high resolution immunodetection of BDNF protein in the CeA identified immunoreactivity in pericellular baskets surrounding the somata of CeA neurons (Krause *et al*., 2008). This study used a sheep anti-BDNF antibody that was extensively characterized by Western blot and antigen preabsorption. It is possible that the antibodies employed in the two studies recognize different epitopes present on the pro and mature forms

of BDNF or have cross reactivity with other neurotrophins (as has been shown for the Santa Cruz anti-BDNF antibody SC 564, www.scbt.com). Pathological conditions such as excitotoxic lesions (Rite *et al*., 2005) and cocaine (Liu *et al*., 2006) can induce striatal BDNF mRNA, suggesting that BDNF induction in these areas may have significant biological consequences, but it is uncertain what this means in relation to addiction or alcoholism. It is also worth noting that NT-3 binding and TrkC expression are high in the striatum (Altar *et al.*, 1994), yet there are few functional studies with this neurotrophin. Immuno-identification of specific ethanol-sensitive populations within the extended amygdala and striatum combined with anterograde tracing could resolve these neuroanatomical interactions and identify the active circuits before, during and after ethanol consumption. The use of colchicine (Altar *et al.*, 1997) to allow BDNF to accumulate in the cell body of origin might also aid in mapping the circuit.

These disparate data between depression/anxiety and ethanol consumption might be explained by the regional and cellular differences in expression (global vs. forebrain principle neurons vs. amygdalar nuclei), disinhibition, or by regional differences in sensitivity of TrkB to activation and desensitization. Most ethanol studies to date have focused on the BLA, CeA and MeA. As discussed above, ethanol enhances GABAergic transmission in the BLA through both paracapsular interneurons and local interneurons (Zhu and Lovinger, 2006; Silberman *et al.*, 2007). Neurons in the BLA are also hyper-excitible during withdrawal (Floyd *et al.*, 2003; Läck *et al*., 2007) and CRF is increased with chronic ethanol treatment in the CeA (Läck *et al*., 2005). The BLA projects to the CeA and to the paracapsular intercalated cell masses, which are GABAergic and located at the medial border of the BLA (Royer *et al.*, 1999). The intercalated cell masses and paracapsular neurons express high levels of D1 receptors and are also regulated by inputs from the prefrontal cortex and by catacholamines (Fuxe *et al*., 2003; Marowski *et al*., 2005). Ultimately, this provides feed forward inhibition of neurons in the CeA that is modulated by cortical inputs and catacholamines. Therefore, the effects of ethanol exposure on BLA projections to the CeA would be expected to be inhibitory acutely, while chronic exposure produces hyperexcitibility. However, acute ethanol can increase BDNF and pCREB in the CeA, suggesting excitation in this nucleus. Inhibition of the feed forward GABAergic neurons in the intercalated cell masses might be a homeostatic pathway that regulates CeA function and would be modulated by inputs from PFC, similar to the pathway described by Nestler and Carlezon (2006) that regulates excitability of the mesolimbic dopamine pathway in depression. Recent observations suggest that withdrawal causes a decrease in GABA release probability from the lateral intercalated cells with no effect on the local, feed-back type neurons (B.A. McCool, personal communication), suggesting a decrease in feed forward inhibition to the BLA during withdrawal that contributes to hyperexcitibility in the BLA. Interestingly, neurons in the CeA show a decrease in pCREB during ethanol withdrawal (Pandey *et al*., 1999) and acute amphetamine exposure also decreases pCREB in this region (McPherson *et al.*, 2007). Therefore, these data might be explained by the relative activity of ethanol at each cell type within this local circuit or may reflect withdrawal-induced catecholamine release. These studies also suggest dissociation between BDNF and pCREB that can be modulated by catacholamines in the extended amygdala.

An intriguing target area of the amygdalar projections from the BLA and, to a lesser degree the CeA, neurons is the BNST, which is hyper-excitable during opiate withdrawal through norephinepherine (Aston-Jones and Harris, 2004). Output of this nucleus is also regulated presynaptically by NPY and postsynaptically by CRF/Urocortin (Kash and Winder, 2006). Given the partial efficacy of opiate and CRF antagonists in reducing ethanol consumption and the efficacy of benzodiazepines in treating withdrawal-induced anxiety and seizures, this circuit may be mediating withdrawal. Projections from the CeA and BNST also regulate stress hormone production, which could be correlated with synaptic activity as an in vivo surrogate for activity in this circuit. A complete understanding of the role of BDNF in the amygdala and

stress circuitry will require integration of the electrophysiological data with fine mapping of the time course of transcription factor activation and gene transcription both during exposure and after withdrawal. Complex, multi-electrode electrophysiological recordings have been used to elucidate the circuitry of the amygdala (Woodruff *et al.*, 2006) and the effect of ethanol on this structure is a question well suited for this technique. Combined immunodetection would not be possible in real time but the use of a GFP reporter construct (e.g. synaptically driven translation or immediate early gene promoters driving somatic GFP expression) could be used to monitor activity in combination with electrophysiological studies, which may aid in mapping this circuit.

7. BDNF-TrkB polymorphisms in humans: depression, anxiety and addiction

There have been reports of isolated cases of deletions or inactivating mutations in the human TrkB-BDNF system that lead to severe hyperphagia, obesity, hyperactivity and impaired cognitive function (Yeo *et al.*, 2004; Gray *et al.*, 2006). BDNF polymorphisms have been linked to eating disorders in humans (Koizumi *et al*., 2004; Monteleone *et al*., 2006; Mercader *et al.*, 2007) and the human phenotype of FASD is remarkably similar to that described for BDNF haplodeficient mice. Therefore, BDNF is involved in neuronal systems that regulate multiple aspects of alcoholism and alcohol use disorders.

Genetic variation at the TrkB gene is associated with alcohol dependence and antisocial personality disorder in a Finnish population (Xu *et al.*, 2007). In particular, a haplotype was detected at a higher frequency in non-alcohol dependent subjects compared with alcohol dependent individuals, suggesting that some genotypes may be “protective.” Whether variation at the TrkB gene alters receptor expression or function is unknown. The BDNF coding sequence is also polymorphic in human populations and several studies have linked these polymorphisms with substance abuse and psychiatric disorders. A dinucleotide repeat in the BDNF gene was recently shown to be associated with drug abuse vulnerability (Uhl *et al.*, 2001). This repeat is located in an intron 5′ to the first coding exon. This study did not specifically examine alcoholics and excluded subjects who did not abuse at least 1 illegal substance, however.

A polymorphism in the coding sequence in the pro region of the protein was also recently identified. This single nucleotide polymorphism (G196A) results in either a valine or a methionine residue at codon 66. BDNF Met66Met shows impaired stimulus-induced secretion and decreased localization in secretory granules (Egan *et al.*, 2003; Chen *et al.*, 2004). Humans with the Met66Met genotype have impaired hippocampal function, with poorer episodic memory and less activation in the hippocampus as measured by functional magnetic resonance imaging (Egan *et al.*, 2003; Hariri *et al.*, 2003). The Met66 allele is also linked to reduced hippocampal volume and depression (Bueller *et al.*, 2006; Pezawas *et al.*, 2004; see Dumas and Monteggia 2006 for review).

Met66Met was recently shown to be associated with alcoholism in violent alcoholics (Matsushita *et al.*, 2004), but this was not replicated in a small sample of Chinese alcoholics with violent tendencies (Tsai *et al.*, 2005). In a follow-up study, the Met66Met genotype was associated with harm-avoidant personality types and depression/anxiety but this may be modulated by the presence of a polymorphism in the promoter with lower DNA binding and reporter activity (Jiang *et al.*, 2005). Val66 was shown to be positively associated with methamphetamine and heroin abuse (Cheng *et al.*, 2005). Interestingly, this allele has also been associated with bipolar disorder (Sklar *et al.*, 2002; Lohoff *et al.*, 2005) and ADHD (Kent *et al.*, 2005). Like humans, Met66Met mice exhibit increased anxiety (Chen *et al.*, 2006).

BDNF is present in plasma and has been used as a surrogate marker for central function (Karege *et al.*, 2002). Joe and colleagues (2007) measured BDNF levels in alcoholic Korean patients and found a decrease in plasma BDNF. This difference was further magnified when examined

in terms of family history, with family history positive individuals having consistently lower levels and less variability between subjects. There was significantly more variability in the BDNF levels measured in patients with no family history of alcoholism. Changes in BDNF did not correlate with anxiety or depression in this patient population, which is in contrast to animal studies. Unfortunately this study did not report the genotype of these patients with respect to the Met/Val BDNF polymorphism.

Patients with the Val66Val genotype would be expected to have globally higher BDNF levels. Higher BDNF levels may protect against depression in the hippocampus and through increased survival of serotonergic neurons, but higher levels of BDNF are associated with addiction and stress in the mesolimbic dopamine system in rodent models. In addition, BDNF haplodeficient mice show decreased psychostimulant-induced conditioned place preference, suggesting that patients with lower BDNF secretion might me more resistant to stimulant abuse. BDNF is trophic for GABAergic neurons (Carrasco et al., 2007; Baquet *et al.*, 2004; Strand *et al.*, 2007); therefore, the Met66Met allele might lead to reduced interneuron function, thereby contributing to the anxious phenotype. The Val66Met polymorphism may predispose to addiction in a substance-selective manner but this seems unlikely since the Val66 allele is over-represented in bipolar patients and they show little selectivity in the drugs they chose to abuse (Levin and Hennessy, 2004). Alcoholism and substance abuse has historically been prevalent in writers and artists with comorbid psychiatric disorders who are unlikely to have impaired hippocampal function. A more likely scenario invokes the archaic self-medication theories. Several genes predisposing to alcoholism have been described and progress is slowly being made towards integrating these polymorphisms with behavioral phenotypes. Knowledge of the BDNF genotype and underlying psychopathology may ultimately help determine the motivation to seek alcohol and may eventually be used to tailor therapeutics.

8. BDNF as a therapeutic target for alcoholism?

Specific strategies targeting BDNF therapeutically have a long history of partial successes and failures (reviewed by Pezet and Malcangio, 2004). Therapeutic BDNF was first explored by pharmaceutical companies for the treatment of neurodegenerative disorders. BDNF is trophic for dopaminergic neurons and early preclinical studies suggested that BDNF infusion or transduction of the gene into the striatum could halt degeneration of dopaminergic neurons in the substantia nigra. These types of studies have been tedious due to problems administering a protein, whether systemically, virally, microencapsulated or through a cannula. Since BDNF has a narrow window of activity and TrkB desensitizes (Carter *et al.*, 1995; Segal *et al.*, 1992; Ohrtman *et al.*, 2006), regulated and well controlled levels in selected brain regions are also essential for this type of therapy.

Ethanol inhibits BDNF signaling and BDNF is neuroprotective in several in vitro models of developmental ethanol exposure. BDNF is a stimulus for migration for multiple neuronal populations. Dysregulation of BDNF, TrkB, or the associated signaling pathways during critical migratory periods would presumably be irreversible. Therefore, the ultimate intervention would be aimed at the mother. Despite intervention and close monitoring, a significant number of newborns are still diagnosed with FASDs. Fortunately the developing brain is remarkably plastic and postnatal intervention with environmental enrichment (Hannigan *et al.*, 2006), appropriate nutritional supplementation (Thomas *et al.*, 2004) and motor training (Klintsova *et al.*, 2000) can reverse some of the deficits caused by gestational ethanol exposure. In addition, the characterization of factors governing stem cell production during intoxication and repair may lead to regererative therapies (Nixon 2006). These interventions ultimately involve BDNF and may be augmented by BDNF peptidomimentics. O'Leary and Hughes (2003) recently developed a peptidomimetic partial agonist of TrkB that may prove useful therapeutically. A partial agonist would presumably not show the same

desensitization kinetics as the full agonist, which could expand the therapeutic window. Interestingly, exercise is sufficient to induce BDNF (Cotman and Berchtold, 2002) and may also augment other behavioral and nutritional interventions.

A feasible approach to be investigated in alcoholism is pharmacological modulation of BDNF levels. As discussed earlier, antidepressants increase BDNF levels in the hippocampus, however, it was recently shown that high doses of venlafaxine actually decrease hippocampal BDNF (Xu *et al.*, 2003). This decrease is a property shared with the structurally unrelated antidepressant bupropion, which has been shown to be effective in the treatment of cocaine abusers with and without comorbid ADHD (Levin *et al.*, 2002; Margolin *et al.*, 1991). Polymorphisms in the BDNF gene have also been linked to antidepressant efficacy. Patients with the Met66Met genotype have a better response to fluvoxamine, milnacipran and citalopram (Choi *et al.*, 2006; Yoshida *et al.*, 2006). However, therapeutics that globally target TrkB may not be universally efficacious in depression or anxiety where individual variation and regional differences exist in the effect of BDNF.

Pharmacological agents useful in a particular subset of individual patients might be deduced a priori based on serum BDNF levels and genotype. Alcoholic patients with underlying anxiety, a Met66Met genotype and low serum BDNF might be more likely to benefit from SSRI antidepressant therapy for alcoholism. A polysubstance abusing patient (addictopathic) or sociopathic with a Val66Val genotype might benefit from bupropion. Alcoholism, depression and anxiety are polygenic disorders, therefore there are likely to be substantial interactions between BDNF the many systems it regulates, such as catecholamines, CRF and NPY that influence both mood and alcohol consumption (reviewed by Oroszi and Goldman, 2004; Thorsell *et al.*, 2006).

Acknowledgements

This work was supported by the NIAAA Division of Intramural Clinical and Biomedical Research. I would like to thank Drs. Robert Lipsky, Andrew Holmes and Brian McCool for useful comments, conversations and suggestions during the preparation of this manuscript. I would also like to thank Ms. Amani Hassoun for clerical assistance.

Abbreviations

ADHD
Attention Deficit Hyperactivity Disorder

BDNF
Brain-Derived Neurotrophic Factor

BLA
Basolateral Amygdala Complex

BNST
Bed Nucleus of the Stria Terminalis

CaMK
Calmodulin-dependent Kinase

CeA
Central Amygdala

CNS
Central Nervous System

CREB

Cyclic 3′5′ adenosine monophosphate Response Element Binding protein

DA
Dopamine

ERK
Extracellular-signal-Regulated Kinase

FAS
Fetal Alcohol Syndrome

FASD
Fetal Alcohol Spectrum Disorder

LA
Lateral Amygdala

MeA
Medial Amygdala

NAc
Nucleus Accumbens

NGF
Nerve Growth Factor

NPY
Neuropeptide Y

PI 3-kinase
Phosphatidyl inositol 3-OH kinase

PN
Postnatal

SN
Substantia Nigra

Trk
Tropomyosin-related kinase

VTA
Ventral Tegmental Area

Literature Cited

Acquaah-Mensah GK, Leslie SW, Kehrer JP. Acute exposure of cerebellar granule neurons to ethanol suppresses stress-activated protein kinase-1 and concomitantly induces AP-1. Toxicol Appl Pharmacol 2001;175:10–8. [PubMed: 11509022]

Agassandian K, Gedney M, Cassell MD. Neurotrophic factors in the central nucleus of amygdala may be organized to provide substrates for associative learning. Brain Res 2006;1076:78–86. [PubMed: 16473337]

Aid T, Kazantseva A, Piirsoo M, Palm K, Timmusk T. Mouse and rat BDNF gene structure and expression revisited. J Neurosci Res 2007;85:525–35. [PubMed: 17149751]

Akbarian S, Rios M, Liu RJ, Gold SJ, Fong HF, Zeiler S, et al. Brain-derived neurotrophic factor is essential for opiate-induced plasticity of noradrenergic neurons. J Neurosci 2002;22:4153–4162. [PubMed: 12019333]

Allan AM, Weeber EJ, Savage DD, Caldwell KK. Effects of prenatal ethanol exposure on phospholipase C-beta 1 and phospholipase A2 in hippocampus and medial frontal cortex of adult rat offspring. Alcohol Clin Exp Res 1997;21:1534–41. [PubMed: 9394128]

Allen GC, West JR, Chen WJ, Earnest DJ. Developmental alcohol exposure disrupts circadian regulation of BDNF in the rat suprachiasmatic nucleus. Neurotoxicol Teratol 2004;26:353–358. [PubMed: 15113597]

Alling C, Bergman O, Larsson C, Simonsson P. Evaluation of ethanol effects on PLC signal transduction pathways using cell lines of neuronal origin. Alcohol Alcohol Supp 1993;2:295–9.

Altar CA. Neurotrophins and depression. Trends Pharmacol Sci 1999;20:59–61. [PubMed: 10101965]

Altar CA, Boylan CB, Jackson C, Hershenson S, Miller J, Wiegand SJ, et al. Brain-derived neurotrophic factor augments rotational behavior and nigrostriatal dopamine turnover in vivo. Proc Natl Acad Sci U S A 1992;89:11347–11351. [PubMed: 1454818]

Altar CA, Siuciak JA, Wright P, Ip NY, Lindsay RM, Wiegand SJ. In situ hybridization of trkB and trkC receptor mRNA in rat forebrain and association with high-affinity binding of [125I]BDNF, [125I] NT-4/5 and [125I]NT-3. Eur J Neurosci 1994;6:1389–1405. [PubMed: 8000564]

Altar CA, Cai N, Bliven T, Juhasz M, Conner JM, Acheson AL, et al. Anterograde transport of brain-derived neurotrophic factor and its role in the brain. Nature 1997;389:856–60. [PubMed: 9349818]

Asher O, Cunningham TD, Yao L, Gordon AS, Diamond I. Ethanol stimulates cAMP-responsive element (CRE)-mediated transcription via CRE-binding protein and cAMP-dependent protein kinase. J Pharmacol Exp Ther 2002;301:66–70. [PubMed: 11907158]

Aston-Jones G, Harris GC. Brain substrates for increased drug seeking during protracted withdrawal. Neuropharmacology 2004;47:167–179. [PubMed: 15464135]

Asyyed A, Storm D, Diamond I. Ethanol activates cAMP response element-mediated gene expression in select regions of the mouse brain. Brain Res 2007;23:63–71.

Baek JK, Heaton MB, Walker DW. Up-regulation of high-affinity neurotrophin receptor, trk B-like protein on western blots of rat cortex after chronic ethanol treatment. Brain Res Mol Brain Res 1996;40:161–164. [PubMed: 8840027]

Baquet ZC, Gorski JA, Jones KR. Early striatal dendrite deficits followed by neuron loss with advanced age in the absence of anterograde cortical brain-derived neurotrophic factor. J Neurosci 2004;24:4250–4258. [PubMed: 15115821]

Baquet ZC, Bickford PC, Jones KR. Brain-derived neurotrophic factor is required for the establishment of the proper number of dopaminergic neurons in the substantia nigra pars compacta. J Neurosci 2005;25:6251–6259. [PubMed: 15987955]

Barbacid M. Neurotrophic factors and their receptors. Curr Opin Cell Biol 1995;7:148–155. [PubMed: 7612265]

Barde YA, Edgar D, Thoenen H. Purification of a new neurotrophic factor from mammalian brain. EMBO J 1982;1:549–553. [PubMed: 7188352]

Barde YA. Neurotrophins: a family of proteins supporting the survival of neurons. Prog Clin Biol Res 1994;390:45–56. [PubMed: 7724649]

Berhow MT, Hiroi N, Nestler EJ. Regulation of ERK (extracellular signal regulated kinase), part of the neurotrophin signal transduction cascade, in the rat mesolimbic dopamine system by chronic exposure to morphine or cocaine. J Neurosci 1996;16:4707–4715. [PubMed: 8764658]

Berman RF, Hannigan JH. Effects of prenatal alcohol exposure on the hippocampus: spatial behavior, electrophysiology, and neuroanatomy. Hippocampus 2000;10:94–110. [PubMed: 10706221]

Berton O, McClung CA, Dileone RJ, Krishnan V, Renthal W, Russo SJ, et al. Essential role of BDNF in the mesolimbic dopamine pathway in social defeat stress. Science 2006;311:864–868. [PubMed: 16469931]

Bhave SV, Snell LD, Tabakoff B, Hoffman PL. Ethanol sensitivity of NMDA receptor function in developing cerebellar granule neurons. Eur J Pharmacol 1999;369:247–259. [PubMed: 10206186]

Bison S, Crews F. Alcohol withdrawal increases NPY immunoreactivity in rat brain. Alcohol Clin Exp Res 2003;27:1173–1183. [PubMed: 12878925]

Blitzer RD, Gil O, Landau EM. Long-term potentiation in rat hippocampus is inhibited by low concentrations of ethanol. Brain Res 1990;537:203–2088. [PubMed: 2150775]

Bolanos CA, Nestler EJ. Neurotrophic mechanisms in drug addiction. Neuromolecular Med 2004;5:69–83. [PubMed: 15001814]

Bonthius DJ, Karacay B, Dai D, Pantazis NJ. FGF-2, NGF and IGF-1, but not BDNF, utilize a nitric oxide pathway to signal neurotrophic and neuroprotective effects against alcohol toxicity in cerebellar granule cell cultures. Brain Res Dev Brain Res 2003;140:15–28.

Bowers BJ, Radcliffe RA, Smith AM, Miyamoto-Ditmon J, Wehner JM. Microarray analysis identifies cerebellar genes sensitive to chronic ethanol treatment in PKCγ mice. Alcohol 2006;40:19–33. [PubMed: 17157717]

Bruns MB, Miller MW. Neurotrophin ligand-receptor systems in somatosensory cortex of adult rat are affected by repeated episodes of ethanol. Exp Neurol 2007;204:680–92. [PubMed: 17320080]

Bueller JA, Aftab M, Sen S, Gomez-Hassan D, Burmeister M, Zubieta JK. BDNF Val66Met allele is associated with reduced hippocampal volume in healthy subjects. Biol Psychiatry 2006;59:812–5. [PubMed: 16442082]

Burd L, Klug MG, Martsolf JT, Kerbeshian J. Fetal alcohol syndrome: neuropsychiatric phenomics. Neurotoxicol Teratol 2003;25:697–705. [PubMed: 14624969]

Cardoso JM, Barbosa A, Ismail F, Pombo S. NETER alcoholic typology (NAT). Alcohol Alcohol 2006;41:133–139. [PubMed: 16314426]

Carrasco MA, Castro P, Sepulveda FJ, Tapia JC, Gatica K, Davis MI, et al. Regulation of glycinergic and GABAergic synaptogenesis by brain-derived neurotrophic factor in developing spinal neurons. Neuroscience 2007;145:484–94. [PubMed: 17306467]

Carter BD, Zirrgiebel U, Barde YA. Differential regulation of p21ras activation in neurons by nerve growth factor and brain-derived neurotrophic factor. J Biol Chem 1995;270:21751–2177. [PubMed: 7665594]

Cavanaugh JE, Ham J, Hetman M, Poser S, Yan C, Xia Z. Differential regulation of mitogen-activated protein kinases ERK1/2 and ERK5 by neurotrophins, neuronal activity, and cAMP in neurons. J Neurosci 2001;21:434–443. [PubMed: 11160424]

Chandler LJ, Sutton G. Acute ethanol inhibits extracellular signal-regulated kinase, protein kinase B, and adenosine 3′:5′-cyclic monophosphate response element binding protein activity in an age- and brain region-specific manner. Alcohol Clin Exp Res 2005;29:672–682. [PubMed: 15834234]

Chao MV, Hempstead BL. p75 and Trk: a two-receptor system. Trends Neurosci 1995;18:321–326. [PubMed: 7571013]

Chen WG, Chang Q, Lin Y, Meissner A, West AE, Griffith EC, et al. Derepression of BDNF transcription involves calcium-dependent phosphorylation of MeCP2. Science 2003;302:885–9. [PubMed: 14593183]

Chen WG, West AE, Tao X, Corfas G, Szentirmay MN, Sawadogo M, et al. Upstream stimulatory factors are mediators of Ca2+-responsive transcription in neurons. J Neurosci 2003;23:2572–81. [PubMed: 12684442]

Chen ZY, Patel PD, Sant G, Meng CX, Teng KK, Hempstead BL, et al. Variant brain-derived neurotrophic factor (BDNF) (Met66) alters the intracellular trafficking and activity-dependent secretion of wild-type BDNF in neurosecretory cells and cortical neurons. J Neurosci 2004;24:4401–4411. [PubMed: 15128854]

Chen ZY, Jing D, Bath KG, Ieraci A, Khan T, Siao CJ, et al. Genetic variant BDNF (Val66Met) polymorphism alters anxiety-related behavior. Science 2006;314:140–143. [PubMed: 17023662]

Cheng CY, Hong CJ, Yu YW, Chen TJ, Wu HC, Tsai SJ. Brain-derived neurotrophic factor (Val66Met) genetic polymorphism is associated with substance abuse in males. Brain Res Mol Brain Res 2005;140:86–90. [PubMed: 16109452]

Chhatwal JP, Stanek-Rattiner L, Davis M, Ressler KJ. Amygdala BDNF signaling is required for consolidation but not encoding of extinction. Nat Neurosci 2006;9:870–872. [PubMed: 16783370]

Choi MJ, Kang RH, Lim SW, Oh KS, Lee MS. Brain-derived neurotrophic factor gene polymorphism (Val66Met) and citalopram response in major depressive disorder. Brain Res 2006;1118:176–82. [PubMed: 16979146]

Clary DO, Reichardt LF. An alternatively spliced form of the nerve growth factor receptor TrkA confers an enhanced response to neurotrophin 3. Proc Natl Acad Sci U S A 1994;91:11133–11137. [PubMed: 7972023]

Climent E, Pascual M, Renau-Piqueras J, Guerri C. Ethanol exposure enhances cell death in the developing cerebral cortex: role of brain-derived neurotrophic factor and its signaling pathways. J Neurosci Res 2002;68:213–225. [PubMed: 11948666]

Cloninger CR, Bohman M, Sigvardsson S. Inheritance of alcohol abuse. Cross-fostering analysis of adopted men. Arch Gen Psychiatry 1981;38:861–868. [PubMed: 7259422]

Conner JM, Lauterborn JC, Yan Q, Gall CM, Varon S. Distribution of brain-derived neurotrophic factor (BDNF) protein and mRNA in the normal adult rat CNS: evidence for anterograde axonal transport. J Neurosci 1997;17:2295–2313. [PubMed: 9065491]

Conover JC, Erickson JT, Katz DM, Bianchi LM, Poueymirou WT, McClain J, et al. Neuronal deficits, not involving motor neurons, in mice lacking BDNF and/or NT4. Nature 1995;375:235–238. [PubMed: 7746324]

Constantinescu A, Gordon AS, Diamond I. cAMP-dependent protein kinase types I and II differentially regulate cAMP response element-mediated gene expression: implications for neuronal responses to ethanol. J Biol Chem 2002;277:18810–6. [PubMed: 11886856]

Cotman CW, Berchtold NC. Exercise: a behavioral intervention to enhance brain health and plasticity. Trends Neurosci 2002;25:295–301. [PubMed: 12086747]

Davis M, Shi C. The extended amygdala: are the central nucleus of the amygdala and the bed nucleus of the stria terminalis differentially involved in fear versus anxiety? Ann N Y Acad Sci 1999;877:281–291. [PubMed: 10415655]

Davis M. Neural systems involved in fear and anxiety measured with fear-potentiated startle. Am Psychol 2006;61:741–756. [PubMed: 17115806]

Davis MI, Szarowski D, Turner JN, Morrisett RA, Shain W. In vivo activation and in situ BDNF-stimulated nuclear translocation of mitogen-activated/extracellular signal-regulated protein kinase is inhibited by ethanol in the developing rat hippocampus. Neurosci Lett 1999;272:95–98. [PubMed: 10507550]

Dechant G, Barde YA. The neurotrophin receptor p75(NTR): novel functions and implications for diseases of the nervous system. Nat Neurosci 2002;5:1131–1136. [PubMed: 12404007]

Diamond I, Gordon AS. Cellular and molecular neuroscience of alcoholism. Physiol Rev 1997;77:1–20. [PubMed: 9016298]

D'Mello SR, Borodezt K, Soltoff SP. Insulin-like growth factor and potassium depolarization maintain neuronal survival by distinct pathways: possible involvement of PI 3-kinase in IGF-1 signaling. J Neurosci 1997;17:1548–1560. [PubMed: 9030615]

Duan W, Guo Z, Jiang H, Ware M, Mattson MP. Reversal of behavioral and metabolic abnormalities, and insulin resistance syndrome, by dietary restriction in mice deficient in brain-derived neurotrophic factor. Endocrinology 2003;144:2446–2453. [PubMed: 12746306]

Duman RS, Monteggia LM. A neurotrophic model for stress-related mood disorders. Biol Psychiatry 2006;59:1116–1127. [PubMed: 16631126]

Egan MF, Kojima M, Callicott JH, Goldberg TE, Kolachana BS, Bertolino A, et al. The BDNF val66met polymorphism affects activity-dependent secretion of BDNF and human memory and hippocampal function. Cell 2003;112:257–269. [PubMed: 12553913]

Eide FF, Vining ER, Eide BL, Zang K, Wang XY, Reichardt LF. Naturally occurring truncated trkB receptors have dominant inhibitory effects on brain-derived neurotrophic factor signaling. J Neurosci 1996;16:3123–3219. [PubMed: 8627351]

Eisch AJ, Bolanos CA, de Wit J, Simonak RD, Pudiak CM, Barrot M, et al. Brain-derived neurotrophic factor in the ventral midbrain-nucleus accumbens pathway: a role in depression. Biol Psychiatry 2003;54:994–1005. [PubMed: 14625141]

Ernfors P, Lee KF, Jaenisch R. Mice lacking brain-derived neurotrophic factor develop with sensory deficits. Nature 1994;368:147–150. [PubMed: 8139657]

Esteban PF, Yoon HY, Becker J, Dorsey SG, Caprari P, Palko ME, et al. A kinase-deficient TrkC receptor isoform activates Arf6-Rac1 signaling through the scaffold protein tamalin. J Cell Biol 2006;173:291–299. [PubMed: 16636148]

Fattori V, Abe SI, Kobayashi K, Costa LG, Tsuji R. Effects of postnatal ethanol exposure on neurotrophic factors and signal transduction pathways in rat brain. J Appl Toxicol 2008;28:370–376. [PubMed: 17685400]

Feng MJ, Yan SE, Yan QS. Effects of prenatal alcohol exposure on brain-derived neurotrophic factor and its receptor tyrosine kinase B in offspring. Brain Res 2005;1042:125–132. [PubMed: 15854584]

Floyd DW, Jung KY, McCool BA. Chronic ethanol ingestion facilitates N-methyl-D-aspartate receptor function and expression in rat lateral/basolateral amygdala neurons. J Pharmacol Exp Ther 2003;307:1020–9. [PubMed: 14534353]

Frank L, Ventimiglia R, Anderson K, Lindsay RM, Rudge JS. BDNF down-regulates neurotrophin responsiveness, TrkB protein and TrkB mRNA levels in cultured rat hippocampal neurons. Eur J Neurosci 1996;8:1220–1230. [PubMed: 8752592]

Frank L, Wiegand SJ, Siuciak JA, Lindsay RM, Rudge JS. Effects of BDNF infusion on the regulation of TrkB protein and message in adult rat brain. Exp Neurol 1997;145:62–70. [PubMed: 9184109]

Fuxe K, Jacobsen KX, Höistad M, Tinner B, Jansson A, Staines WA, et al. The dopamine D1 receptor-rich main and paracapsular intercalated nerve cell groups of the rat amygdala: relationship to the dopamine innervation. Neuroscience 2003;119:733–46. [PubMed: 12809694]

García-López M, Abellán A, Legaz I, Rubenstein JL, Puelles L, Medina L. Histogenetic compartments of the mouse centromedial and extended amygdala based on gene expression patterns during development. J Comp Neurol 2008;506:46–74. [PubMed: 17990271]

Gauthier LR, Charrin BC, Borrell-Pages M, Dompierre JP, Rangone H, Cordelieres FP, et al. Huntingtin controls neurotrophic support and survival of neurons by enhancing BDNF vesicular transport along microtubules. Cell 2004;118:127–138. [PubMed: 15242649]

Ge Y, Belcher SM, Light KE. Alterations of cerebellar mRNA specific for BDNF, p75NTR, and TrkB receptor isoforms occur within hours of ethanol administration to 4-day-old rat pups. Brain Res Dev Brain Res 2004;151:99–109.

Gerdeman GL, Partridge JG, Lupica CR, Lovinger DM. It could be habit forming: drugs of abuse and striatal synaptic plasticity. Trends Neurosci 2003;26:184–192. [PubMed: 12689769]

Ginty DD, Segal RA. Retrograde neurotrophin signaling: Trk-ing along the axon. Curr Opin Neurobiol 2002;12:268–274. [PubMed: 12049932]

Glorioso C, Sabatini M, Unger T, Hashimoto T, Monteggia LM, Lewis DA, et al. Specificity and timing of neocortical transcriptome changes in response to BDNF gene ablation during embryogenesis or adulthood. Mol Psychiatry 2006;11:633–648. [PubMed: 16702976]

Goldstein BI, Diamantouros A, Schaffer A, Naranjo CA. Pharmacotherapy of alcoholism in patients with co-morbid psychiatric disorders. Drugs 2006;66:1229–1237. [PubMed: 16827599]

Gorski JA, Balogh SA, Wehner JM, Jones KR. Learning deficits in forebrain-restricted brain-derived neurotrophic factor mutant mice. Neuroscience 2003;121:341–354. [PubMed: 14521993]

Govindarajan A, Rao BS, Nair D, Trinh M, Mawjee N, Tonegawa S, et al. Transgenic brain-derived neurotrophic factor expression causes both anxiogenic and antidepressant effects. Proc Natl Acad Sci U S A 2006;103:13208–13213. [PubMed: 16924103]

Gray J, Yeo GS, Cox JJ, Morton J, Adlam AL, Keogh JM, et al. Hyperphagia, severe obesity, impaired cognitive function, and hyperactivity associated with functional loss of one copy of the brain-derived neurotrophic factor (BDNF) gene. Diabetes 2006;55:3366–3371. [PubMed: 17130481]

Guillin O, Diaz J, Carroll P, Griffon N, Schwartz JC, Sokoloff P. BDNF controls dopamine D3 receptor expression and triggers behavioral sensitization. Nature 2001;411:86–89. [PubMed: 11333982]

Hall FS, Drgonova J, Goeb M, Uhl GR. Reduced behavioral effects of cocaine in heterozygous brain-derived neurotrophic factor (BDNF) knockout mice. Neuropsychopharmacology 2003;28:1485–1490. [PubMed: 12784114]

Han JY, Jeong JY, Lee YK, Roh GS, Kim HJ, Kang SS, et al. Suppression of survival kinases and activation of JNK mediate ethanol-induced cell death in the developing rat brain. Neurosci Lett 2006;398:113–117. [PubMed: 16414187]

Hannigan JH, O'leary-Moore SK, Berman RF. Postnatal environmental or experiential amelioration of neurobehavioral effects of perinatal alcohol exposure in rats. Neurosci Biobehav Rev 2006;31:202–211. [PubMed: 16911827]

Hariri AR, Goldberg TE, Mattay VS, Kolachana BS, Callicott JH, Egan MF, et al. Brain-derived neurotrophic factor val66met polymorphism affects human memory-related hippocampal activity and predicts memory performance. J Neurosci 2003;23:6690–6694. [PubMed: 12890761]

Hariri AR, Holmes A. Genetics of emotional regulation: the role of the serotonin transporter in neural function. Trends Cogn Sci 2006;10:182–191. [PubMed: 16530463]

Harper C, Matsumoto I. Ethanol and brain damage. Curr Opin Pharmacol 2005;5:73–78. [PubMed: 15661629]

Hartmann M, Brigadski T, Erdmann KS, Holtmann B, Sendtner M, Narz F, et al. Truncated TrkB receptor-induced outgrowth of dendritic filopodia involves the p75 neurotrophin receptor. J Cell Sci 2004;117:5803–5814. [PubMed: 15507485]

Heaton MB, Mitchell JJ, Paiva M. Ethanol-induced alterations in neurotrophin expression in developing cerebellum: relationship to periods of temporal susceptibility. Alcohol Clin Exp Res 1999;23:1637–1642. [PubMed: 10549996]

Heaton MB, Kim DS, Paiva M. Neurotrophic factor protection against ethanol toxicity in rat cerebellar granule cell cultures requires phosphatidylinositol 3-kinase activation. Neurosci Lett 2000;291:121–125. [PubMed: 10978589]

Heaton MB, Mitchell JJ, Paiva M, Walker DW. Ethanol-induced alterations in the expression of neurotrophic factors in the developing rat central nervous system. Brain Res Dev Brain Res 2000;121:97–107.

Heaton MB, Moore DB, Paiva M, Madorsky I, Mayer J, Shaw G. The role of neurotrophic factors, apoptosis-related proteins, and endogenous antioxidants in the differential temporal vulnerability of neonatal cerebellum to ethanol. Alcohol Clin Exp Res 2003;27:657–669. [PubMed: 12711928]

Heaton MB, Paiva M, Madorsky I, Mayer J, Moore DB. Effects of ethanol on neurotrophic factors, apoptosis-related proteins, endogenous antioxidants, and reactive oxygen species in neonatal striatum: relationship to periods of vulnerability. Brain Res Dev Brain Res 2003;140:237–252.

Heaton MB, Paiva M, Madorsky I, Shaw G. Ethanol effects on neonatal rat cortex: comparative analyses of neurotrophic factors, apoptosis-related proteins, and oxidative processes during vulnerable and resistant periods. Brain Res Dev Brain Res 2003;145:249–262.

Heaton MB, Madorsky I, Paiva M, Siler-Marsiglio KI. Ethanol-induced reduction of neurotrophin secretion in neonatal rat cerebellar granule cells is mitigated by vitamin E. Neurosci Lett 2004;370:51–54. [PubMed: 15489016]

Hellmann JD, Wernicke C, Rommelspacher H. Chronic ethanol exposure impairs neuronal differentiation of SH-SY5Y cells involving ERK-1/2 responsiveness to BDNF and raf kinase inhibitor protein. Society for Neurosci Symposium. 2006

Hensler JG, Ladenheim EE, Lyons WE. Ethanol consumption and serotonin-1A (5-HT1A) receptor function in heterozygous BDNF (+/−) mice. J Neurochem 2003;85:1139–1147. [PubMed: 12753073]

Hirai S, de Cui F, Miyata T, Ogawa M, Kiyonari H, Suda Y, et al. The c-Jun N-terminal kinase activator dual leucine zipper kinase regulates axon growth and neuronal migration in the developing cerebral cortex. J Neurosci 2006;26:11992–2002. [PubMed: 17108173]

Hoffman PL, Tabakoff B. The role of the NMDA receptor in ethanol withdrawal. EXS 1994;71:61–70. [PubMed: 8032173]

Holtzman DM, Li Y, Parada LF, Kinsman S, Chen CK, Valletta JS, Zhou J, et al. p140trk mRNA marks NGF-responsive forebrain neurons: evidence that trk gene expression is induced by NGF. Neuron 1992;9:465–478. [PubMed: 1524827]

Horger BA, Iyasere CA, Berhow MT, Messer CJ, Nestler EJ, Taylor JR. Enhancement of locomotor activity and conditioned reward to cocaine by brain-derived neurotrophic factor. J Neurosci 1999;19:4110–4122. [PubMed: 10234039]

Hyman SE, Malenka RC, Nestler EJ. Neural mechanisms of addiction: the role of reward-related learning and memory. Annu Rev Neurosci 2006;29:565–598. [PubMed: 16776597]

Impey S, Goodman RH. CREB signaling--timing is everything. Sci STKE 2001;82:PE1. [PubMed: 11752651]

Ip NY, Li Y, Yancopoulos GD, Lindsay RM. Cultured hippocampal neurons show responses to BDNF, NT-3, and NT-4, but not NGF. J Neurosci 1993;13:3394–3405. [PubMed: 7688038]

Ip NY, Stitt TN, Tapley P, Klein R, Glass DJ, Fandl J, et al. Similarities and differences in the way neurotrophins interact with the Trk receptors in neuronal and nonneuronal cells. Neuron 1993;10:137–149. [PubMed: 7679912]

Jeanneteau F, Chao MV. Promoting neurotrophic effects by GPCR ligands. Novartis Found Symp 2006;276:181–189. [PubMed: 16805430]

Jiang X, Xu K, Hoberman J, Tian F, Marko AJ, Waheed JF, et al. BDNF variation and mood disorders: a novel functional promoter polymorphism and Val66Met are associated with anxiety but have opposing effects. Neuropsychopharmacology 2005;30:1353–1361. [PubMed: 15770238]

Joe KH, Kim YK, Kim TS, Roh SW, Choi SW, et al. Decreased plasma brain derived neurotrophic factor levels in patients with alcohol dependence. Alcohol Clin Exp Res 2007;31:1–6. [PubMed: 17207095]

Johnson BA. Role of the serotonergic system in the neurobiology of alcoholism: implications for treatment. CNS Drugs 2004;18:1105–1118. [PubMed: 15581381]

Johnson BA, Rosenthal N, Capece JA, Wiegand F, Mao L, Beyers K, McKay A, et al. Topiramate for treating alcohol dependence: a randomized controlled trial. JAMA 2007;298:1641–1651. [PubMed: 17925516]

Johnson BA. Update on neuropharmacological treatments for alcoholism: Scientific basis and clinical findings. Biochem Pharmaco 2008;75:34–56.

Jones KR, Farinas I, Backus C, Reichardt LF. Targeted disruption of the BDNF gene perturbs brain and sensory neuron development but not motor neuron development. Cell 1994;76:989–999. [PubMed: 8137432]

Juric DM, Miklic S, Carman-Krzan M. Monoaminergic neuronal activity up-regulates BDNF synthesis in cultured neonatal rat astrocytes. Brain Res 2006;1108:54–62. [PubMed: 16828062]

Kalluri HS, Ticku MK. Ethanol-mediated inhibition of mitogen-activated protein kinase phosphorylation in mouse brain. Eur J Pharmacol 2002;439:53–58. [PubMed: 11937092]

Kalluri HS, Ticku MK. Regulation of ERK phosphorylation by ethanol in fetal cortical neurons. Neurochem Res 2003;28:765–769. [PubMed: 12716028]

Kao S, Jaiswal RK, Kolch W, Landreth GE. Identification of the mechanisms regulating the differential activation of the mapk cascade by epidermal growth factor and nerve growth factor in PC12 cells. J Biol Chem 2001;276:18169–18177. [PubMed: 11278445]

Karege F, Perret G, Bondolfi G, Schwald M, Bertschy G, Aubry JM. Decreased serum brain-derived neurotrophic factor levels in major depressed patients. Psychiatry Res 2002;109:143–148. [PubMed: 11927139]

Karege F, Schwald M, Cisse M. Postnatal developmental profile of brain-derived neurotrophic factor in rat brain and platelets. Neurosci Lett 2002;328:261–4. [PubMed: 12147321]

Kash TL, Winder DG. Neuropeptide Y and corticotropin-releasing factor bi-directionally modulate inhibitory synaptic transmission in the bed nucleus of the stria terminalis. Neuropharmacology 2006;51:1013–22. [PubMed: 16904135]

Katsura M, Ohkuma S, Chen DZ, Kuriyama K. Ethanol-induced alteration in activities of cerebral phosphatidylinositol 4,5-biphosphate-specific and cytosolic phospholipase C in the brain: analysis using NG 108–15 cells and brains from ethanol-inhaled mice. Neurochem Int 1994;24:541–7. [PubMed: 7981635]

Kent L, Green E, Hawi Z, Kirley A, Dudbridge F, Lowe N, et al. Association of the paternally transmitted copy of common Valine allele of the Val66Met polymorphism of the brain-derived neurotrophic factor (BDNF) gene with susceptibility to ADHD. Mol Psychiatry 2005;10:939–943. [PubMed: 15940292]

Kermani P, Hempstead B. Brain-derived neurotrophic factor: a newly described mediator of angiogenesis. Trends Cardiovasc Med 2007;17:140–3. [PubMed: 17482097]

Kernie SG, Liebl DJ, Parada LF. BDNF regulates eating behavior and locomotor activity in mice. EMBO J 2000;19:1290–1300. [PubMed: 10716929]

Kerns RT, Ravindranathan A, Hassan S, Cage MP, York T, Sikela JM, et al. Ethanol-responsive brain region expression networks: implications for behavioral responses to acute ethanol in DBA/2J versus C57BL/6J mice. J Neurosci 2005;25:2255–2266. [PubMed: 15745951]

Klein R. Role of neurotrophins in mouse neuronal development. FASEB J 1994;8:738–744. [PubMed: 8050673]

Klintsova AY, Goodlett CR, Greenough WT. Therapeutic motor training ameliorates cerebellar effects of postnatal binge alcohol. Neurotoxicol Teratol 2000;22:125–132. [PubMed: 10642121]

Koizumi H, Hashimoto K, Itoh K, Nakazato M, Shimizu E, Ohgake S, et al. Association between the brain-derived neurotrophic factor 196G/A polymorphism and eating disorders. Am J Med Genet B Neuropsychiatr Genet 2004;127:125–7. [PubMed: 15108194]

Kolb JE, Trettel J, Levine ES. BDNF enhancement of postsynaptic NMDA receptors is blocked by ethanol. Synapse 2005;55:52–57. [PubMed: 15515007]

Koob GF. Alcoholism: allostasis and beyond. Alcohol Clin Exp Res 2003;27:232–243. [PubMed: 12605072]

Korte M, Carroll P, Wolf E, Brem G, Thoenen H, Bonhoeffer T. Hippocampal long-term potentiation is impaired in mice lacking brain-derived neurotrophic factor. Proc Natl Acad Sci U S A 1995;92:8856–60. [PubMed: 7568031]

Kozisek ME, Middlemas D, Bylund DB. Brain-derived neurotrophic factor and its receptor tropomyosin-related kinase B in the mechanism of action of antidepressant therapies. Pharmacol Ther. 2007In press

Krause S, Schindowski K, Zechel S, von Bohlen Und Halbach O. Expression of trkB and trkC receptors and their ligands brain-derived neurotrophic factor and neurotrophin-3 in the murine amygdala. J Neurosci Res 2008;86:411–21. [PubMed: 17828769]

Krimm RF. Factors that regulate embryonic gustatory development. BMC Neurosci 2007;8(Suppl 3):S4. [PubMed: 17903280]

Krishnan V, Han MH, Graham DL, Berton O, Renthal W, Russo SJ, et al. Molecular adaptations underlying susceptibility and resistance to social defeat in brain reward regions. Cell 2007;131:391–404. [PubMed: 17956738]

Kruse N, Cetin S, Chan A, Gold R, Luhder F. Differential expression of BDNF mRNA splice variants in mouse brain and immune cells. J Neuroimmuno 2007;182:13–21.

Ku BM, Lee YK, Jeong JY, Mun J, Han JY, Roh GS, et al. Ethanol-induced oxidative stress is mediated by p38 MAPK pathway in mouse hippocampal cells. Neurosci Lett 2007;419:64–7. [PubMed: 17420100]

Kuruvilla R, Ye H, Ginty DD. Spatially and functionally distinct roles of the PI3-K effector pathway during NGF signaling in sympathetic neurons. Neuron 2000;27:499–512. [PubMed: 11055433]

Läck AK, Floyd DW, McCool BA. Chronic ethanol ingestion modulates proanxiety factors expressed in rat central amygdala. Alcohol 2005;36:83–90. [PubMed: 16396741]

Läck AK, Diaz MR, Chappell A, DuBois DW, McCool BA. Chronic ethanol and withdrawal differentially modulate pre- and postsynaptic function at glutamatergic synapses in rat basolateral amygdala. J Neurophysiol 2007 Dec;98(6):3185–96. [PubMed: 17898152]Epub 2007 Sep 26

Lebrun B, Bariohay B, Moyse E, Jean A. Brain-derived neurotrophic factor (BDNF) and food intake regulation: a minireview. Auton Neurosci 2006;30:126–127.

Lee FS, Rajagopal R, Chao MV. Distinctive features of Trk neurotrophin receptor transactivation by G protein-coupled receptors. Cytokine Growth Factor Rev 2002;13:11–17. [PubMed: 11750876]

Levi-Montalcini R, Cohen S. Effects of the extract of the mouse submaxillary salivary glands on the sympathetic system of mammals. Ann N Y Acad Sci 1960;85:324–341. [PubMed: 14416187]

Levi-Montalcini R. The nerve growth factor 35 years later. Science 1987;237:1154–1162. [PubMed: 3306916]

Levin FR, Evans SM, McDowell DM, Brooks DJ, Nunes E. Bupropion treatment for cocaine abuse and adult attention-deficit/hyperactivity disorder. J Addict Dis 2002;21:1–16. [PubMed: 11916368]

Levin FR, Hennessy G. Bipolar disorder and substance abuse. Biol Psychiatry 2004;56:738–748. [PubMed: 15556118]

Li Z, Ding M, Thiele CJ, Luo J. Ethanol inhibits brain-derived neurotrophic factor-mediated intracellular signaling and activator protein-1 activation in cerebellar granule neurons. Neuroscience 2004;126:149–162. [PubMed: 15145081]

Liebl DJ, Mbiene JP, Parada LF. NT4/5 mutant mice have deficiency in gustatory papillae and taste bud formation. Dev Biol 1999;213:378–389. [PubMed: 10479455]

Liebmann C. Regulation of MAP kinase activity by peptide receptor signaling pathway: paradigms of multiplicity. Cell Signal 2001;13:777–785. [PubMed: 11583913]

Light KE, Ge Y, Belcher SM. Early postnatal ethanol exposure selectively decreases BDNF and truncated TrkB-T2 receptor mRNA expression in the rat cerebellum. Brain Res Mol Brain Res 2001;93:46–55. [PubMed: 11532337]

Light KE, Brown DP, Newton BW, Belcher SM, Kane CJ. Ethanol-induced alterations of neurotrophin receptor expression on Purkinje cells in the neonatal rat cerebellum. Brain Res 2002;924:71–81. [PubMed: 11743997]

Liu QR, Lu L, Zhu XG, Gong JP, Shaham Y, Uhl GR. Rodent BDNF genes, novel promoters, novel splice variants, and regulation by cocaine. Brain Res 2006 Jan 5;1067(1):1–12. [PubMed: 16376315]

Lohoff FW, Sander T, Ferraro TN, Dahl JP, Gallinat J, Berrettini WH. Confirmation of association between the Val66Met polymorphism in the brain-derived neurotrophic factor (BDNF) gene and bipolar I disorder. Am J Med Genet B Neuropsychiatr Genet 2005;139:51–53. [PubMed: 16152572]

Lovinger DM, Homanics GE. Tonic for what ails us? high-affinity GABAA receptors and alcohol. Alcohol 2007;41:139–43. [PubMed: 17521844]

Lovinger DM, White G, Weight FF. Ethanol inhibits NMDA-activated ion current in hippocampal neurons. Science 1989;243:1721–1724. [PubMed: 2467382]

Lovinger DM, White G, Weight FF. NMDA receptor-mediated synaptic excitation selectively inhibited by ethanol in hippocampal slice from adult rat. J Neurosci 1990;10:1372–1379. [PubMed: 2158533]

Lu B. Pro-region of neurotrophins: role in synaptic modulation. Neuron 2003;39:735–738. [PubMed: 12948441]

Lu L, Dempsey J, Liu SY, Bossert JM, Shaham Y. A single infusion of brain-derived neurotrophic factor into the ventral tegmental area induces long-lasting potentiation of cocaine seeking after withdrawal. J Neurosci 2004;24:1604–1611. [PubMed: 14973246]

Lyons WE, Mamounas LA, Ricaurte GA, Coppola V, Reid SW, Bora SH, et al. Brain-derived neurotrophic factor-deficient mice develop aggressiveness and hyperphagia in conjunction with brain serotonergic abnormalities. Proc Natl Acad Sci U S A 1999;96:15239–15244. [PubMed: 10611369]

MacInnis BL, Campenot RB. Retrograde support of neuronal survival without retrograde transport of nerve growth factor. Science 2002;295:1536–1539. [PubMed: 11799202]

MacLennan AJ, Lee N, Walker DW. Chronic ethanol administration decreases brain-derived neurotrophic factor gene expression in the rat hippocampus. Neurosci Lett 1995;197:105–108. [PubMed: 8552271]

Madhav TR, Pei Q, Zetterstrom TS. Serotonergic cells of the rat raphe nuclei express mRNA of tyrosine kinase B (trkB), the high-affinity receptor for brain derived neurotrophic factor (BDNF). Brain Res Mol Brain Res 2001;93:56–63. [PubMed: 11532338]

Maier SE, Cramer JA, West JR, Sohrabji F. Alcohol exposure during the first two trimesters equivalent alters granule cell number and neurotrophin expression in the developing rat olfactory bulb. J Neurobiol 1999;41:414–423. [PubMed: 10526319]

Mann K. Pharmacotherapy of alcohol dependence: a review of the clinical data. CNS Drugs 2004;18:485–504. [PubMed: 15182219]

Margolin A, Kosten T, Petrakis I, Avants SK, Kosten T. Bupropion reduces cocaine abuse in methadone-maintained patients. Arch Gen Psychiatry 1991;48:87. [PubMed: 1898631]

Marini AM, Rabin SJ, Lipsky RH, Mocchetti I. Activity-dependent release of brain-derived neurotrophic factor underlies the neuroprotective effect of N-methyl-D-aspartate. J Biol Chem 1998;273:29394–29399. [PubMed: 9792641]

Marini AM, Jiang X, Wu X, Tian F, Zhu D, Okagaki P, Lipsky RH. Role of brain-derived neurotrophic factor and NF-κB in neuronal plasticity and survival: From genes to phenotype. Restor Neurol Neurosci 2004;22(2):121–30. [PubMed: 15272146]

Matsumoto M, Hikosaka O. Lateral habenula as a source of negative reward signals in dopamine neurons. Nature 2007 Jun 28;447(7148):1111–5. [PubMed: 17522629]

Matsushita S, Kimura M, Miyakawa T, Yoshino A, Murayama M, Masaki T, et al. Association study of brain-derived neurotrophic factor gene polymorphism and alcoholism. Alcohol Clin Exp Res 2004;28:1609–1612. [PubMed: 15547445]

McDonald AJ. Cytoarchitecture of the central amygdaloid nucleus of the rat. J Comp Neurol 1982;208:401–418. [PubMed: 7119168]

McElroy SL, Shapira NA, Arnold LM, Keck PE, Rosenthal NR, Wu SC, et al. Topiramate in the long-term treatment of binge-eating disorder associated with obesity. J Clin Psychiatry 2004;65:1463–1469. [PubMed: 15554757]

McGinnis JM, Foege WH. Mortality and morbidity attributable to use of addictive substances in the United States. Proc Assoc Am Physicians 1999;111:109–118. [PubMed: 10220805]

McGough NN, He DY, Logrip ML, Jeanblanc J, Phamluong K, Luong K, et al. RACK1 and brain-derived neurotrophic factor: a homeostatic pathway that regulates alcohol addiction. J Neurosci 2004;24:10542–10552. [PubMed: 15548669]

McPherson CS, Featherby T, Krstew E, Andrew JL. Quantification of phosphorylated cAMP-response element-binding protein expression throughout the brain of amphetamine-sensitized rats: activation of hypothalamic orexin A-containing neurons. J Pharmacol Exp Ther 2007;323:805–12. [PubMed: 17878407]

Mercader JM, Ribasés M, Gratacòs M, González JR, Bayés M, de Cid R, et al. Altered brain-derived neurotrophic factor blood levels and gene variability are associated with anorexia and bulimia. Genes Brain Behav 2007;6:706–16. [PubMed: 17376155]

Melendez RI, Kalivas PW, McGinty JF, Becker HC. Differential gene expression alterations induced by chronic ethanol exposure and withdrawal in C57BL/6J mice. Society for Neurosci Abst. 2006

Meredith GE, Callen S, Scheuer DA. Brain-derived neurotrophic factor expression is increased in the rat amygdala, piriform cortex and hypothalamus following repeated amphetamine administration. Brain Res 2002;949:218–227. [PubMed: 12213320]

Meredith GE, Steiner H. Amphetamine increases tyrosine kinase-B receptor expression in the dorsal striatum. Neuroreport 2006;17:75–78. [PubMed: 16361954]

Middlemas DS, Lindberg RA, Hunter T. trkB, a neural receptor protein-tyrosine kinase: evidence for a full-length and two truncated receptors. Mol Cell Biol 1991;11:143–53. [PubMed: 1846020]

Miller MW, Mooney SM. Chronic exposure to ethanol alters neurotrophin content in the basal forebrain-cortex system in the mature rat: effects on autocrine-paracrine mechanisms. J Neurobiol 2004;60:490–498. [PubMed: 15307153]

Miller MW. Repeated episodic exposure to ethanol affects neurotrophin content in the forebrain of the mature rat. Exp Neurol 2004;189:173–81. [PubMed: 15296847]

Miller R, King MA, Heaton MB, Walker DW. The effects of chronic ethanol consumption on neurotrophins and their receptors in the rat hippocampus and basal forebrain. Brain Res 2002;950:137–147. [PubMed: 12231238]

Miller TM, Tansey MG, Johnson EM, Creedon DJ. Inhibition of phosphatidylinositol 3-kinase activity blocks depolarization- and insulin-like growth factor I-mediated survival of cerebellar granule cells. J Biol Chem 1997;272:9847–9853. [PubMed: 9092520]

Mitra G, Martin-Zanca D, Barbacid M. Identification and biochemical characterization of p70TRK, product of the human TRK oncogene. Proc Natl Acad Sci U S A 1987;84:6707–6711. [PubMed: 3477801]

Monteggia LM, Barrot M, Powell CM, Berton O, Galanis V, Gemelli T, et al. Essential role of brain-derived neurotrophic factor in adult hippocampal function. Proc Natl Acad Sci U S A 2004;101:10827–10832. [PubMed: 15249684]

Monteggia LM, Luikart B, Barrot M, Theobold D, Malkovska I, Nef S, et al. Brain-Derived Neurotrophic Factor Conditional Knockouts Show Gender Differences in Depression-Related. Behaviors Biol Psychiatry 2006;61:187–197.

Monteleone P, Zanardini R, Tortorella A, Gennarelli M, Castaldo E, Canestrelli B, et al. The 196G/A (val66met) polymorphism of the BDNF gene is significantly associated with binge eating behavior in women with bulimia nervosa or binge eating disorder. Neurosci Lett 2006;406:133–7. [PubMed: 16901635]

Moore DB, Madorsky I, Paiva M, Barrow Heaton M. Ethanol exposure alters neurotrophin receptor expression in the rat central nervous system: Effects of prenatal exposure. J Neurobiol 2004;60:101–13. [PubMed: 15188276]

Moore DB, Madorsky I, Paiva M, Barrow Heaton M. Ethanol exposure alters neurotrophin receptor expression in the rat central nervous system: Effects of neonatal exposure. J Neurobiol 2004;60:114–26. [PubMed: 15188277]

Mukherjee RA, Hollins S, Turk J. Fetal alcohol spectrum disorder: an overview. J R Soc Med 2006;99:298–302. [PubMed: 16738372]

Nagappan G, Lu B. Activity-dependent modulation of the BDNF receptor TrkB: mechanisms and implications. Trends Neurosci 2005;28:464–471. [PubMed: 16040136]

Nestler EJ, Carlezon WA Jr. The mesolimbic dopamine reward circuit in depression. Biol Psychiatry 2006;59:1151–9. [PubMed: 16566899]

Newton PM, Ron D. Protein kinase C and alcohol addiction. Pharmacol Res 2007;55:570–7. [PubMed: 17566760]

Nibuya M, Morinobu S, Duman RS. Regulation of BDNF and trkB mRNA in rat brain by chronic electroconvulsive seizure and antidepressant drug treatments. J Neurosci 1995;15:7539–7547. [PubMed: 7472505]

Nixon K. Alcohol and adult neurogenesis: roles in neurodegeneration and recovery in chronic alcoholism. Hippocampus 2006;16:287–95. [PubMed: 16421863]

Numan S, Seroogy KB. Expression of trkB and trkC mRNAs by adult midbrain dopamine neurons: a double-label in situ hybridization study. J Comp Neurol 1999;403:295–308. [PubMed: 9886032]

Ohrtman JD, Stancik EK, Lovinger DM, Davis MI. Ethanol inhibits brain-derived neurotrophic factor stimulation of extracellular signal-regulated/mitogen-activated protein kinase in cerebellar granule cells. Alcohol 2006;39:29–37. [PubMed: 16938627]

Okamoto H, Miki T, Lee KY, Yokoyama T, Kuma H, Gu H, et al. Effects of chronic ethanol administration on the expression levels of neurotrophic factors in the rat hippocampus. Okajimas Folia Anat Jpn 2006;83:1–6. [PubMed: 16862745]

O'Leary PD, Hughes RA. Design of potent peptide mimetics of brain-derived neurotrophic factor. J Biol Chem 2003;278:25738–25744. [PubMed: 12730196]

Oliva AA Jr, Atkins CM, Copenagle L, Banker GA. Activated c-Jun N-terminal kinase is required for axon formation. J Neurosci 2006;26:9462–70. [PubMed: 16971530]

Olney JW. Fetal alcohol syndrome at the cellular level. Addict Biol 2004;9:137–149. [PubMed: 15223539]

Oroszi G, Goldman D. Alcoholism: genes and mechanisms. Pharmacogenomics 2004;5:1037–1048. [PubMed: 15584875]

Pandey SC. Acute and chronic ethanol consumption effects on the immunolabeling of Gq/11 alpha subunit protein and phospholipase C isozymes in the rat brain. J Neurochem 1996;67:2355–61. [PubMed: 8931467]

Pandey SC, Zhang D, Mittal N, Nayyar D. Potential role of the gene transcription factor cyclic AMP-responsive element binding protein in ethanol withdrawal-related anxiety. J Pharmacol Exp Ther 1999;288:866–878. [PubMed: 9918601]

Pandey SC. Anxiety and alcohol abuse disorders: a common role for CREB and its target, the neuropeptide Y gene. Trends Pharmacol Sci 2003;24:456–460. [PubMed: 12967770]

Pandey SC. The gene transcription factor cyclic AMP-responsive element binding protein: role in positive and negative affective states of alcohol addiction. Pharmacol Ther 2004;104:47–58. [PubMed: 15500908]

Pandey SC, Roy A, Zhang H, Xu T. Partial deletion of the cAMP response element-binding protein gene promotes alcohol-drinking behaviors. J Neurosci 2004;24:5022–5030. [PubMed: 15163695]

Pandey SC, Zhang H, Roy A, Misra K. Central and medial amygdaloid brain-derived neurotrophic factor signaling plays a critical role in alcohol-drinking and anxiety-like behaviors. J Neurosci 2005;26:8320–8331. [PubMed: 16899727]

Pandey SC, Zhang H, Roy A, Xu T. Deficits in amygdaloid cAMP-responsive element-binding protein signaling play a role in genetic predisposition to anxiety and alcoholism. J Clin Invest 2005;115:2762–73. [PubMed: 16200210]

Pang PT, Lu B. Regulation of late-phase LTP and long-term memory in normal and aging hippocampus: role of secreted proteins tPA and BDNF. Ageing Res Rev 2004;3:407–430. [PubMed: 15541709]

Pezawas L, Verchinski BA, Mattay VS, Callicott JH, Kolachana BS, Straub RE, et al. The brain-derived neurotrophic factor val66met polymorphism and variation in human cortical morphology. J Neurosci 2004;24:10099–10102. [PubMed: 15537879]

Pezet S, Malcangio M. Brain-derived neurotrophic factor as a drug target for CNS disorders. Expert Opin Ther Target 2004;8:391–399.

Pruunsild P, Kazantseva A, Aid T, Palm K, Timmusk T. Dissecting the human BDNF locus: bidirectional transcription, complex splicing, and multiple promoters. Genomics 2007;90:397–406. [PubMed: 17629449]

Pu L, Liu QS, Poo MM. BDNF-dependent synaptic sensitization in midbrain dopamine neurons after cocaine withdrawal. Nat Neurosci 2006;9:605–607. [PubMed: 16633344]

Rattiner LM, Davis M, Ressler KJ. Brain-derived neurotrophic factor in amygdala-dependent learning. Neuroscientist 2005;11:323–333. [PubMed: 16061519]

Redaelli C, Granucci F, De Gioia L, Cipolla L. Synthesis and biological activity of Akt/PI3K inhibitors. Mini Rev Med Chem 2006;6:1127–36. [PubMed: 17073713]

Reichardt LF. Neurotrophin-regulated signaling pathways. Philos Trans R Soc Lond B Biol Sci 2006;361:1545–1564. [PubMed: 16939974]

Reiner O, Gdalyahu A, Ghosh I, Levy T, Sapoznik S, Nir R, et al. DCX's phosphorylation by not just another kinase (JNK). Cell Cycle 2004;3:747–51. [PubMed: 15118415]

Rios M, Fan G, Fekete C, Kelly J, Bates B, Kuehn R, et al. Conditional deletion of brain-derived neurotrophic factor in the postnatal brain leads to obesity and hyperactivity. Mol Endocrinol 2001;15:1748–1757. [PubMed: 11579207]

Rite I, Machado A, Cano J, Venero JL. Divergent regulatory mechanisms governing BDNF mRNA expression in cerebral cortex and substantia nigra in response to striatal target ablation. Exp Neurol 2005;192:142–55. [PubMed: 15698628]

Roberto M, Madamba SG, Moore SD, Tallent MK, Siggins GR. Ethanol increases GABAergic transmission at both pre- and postsynaptic sites in rat central amygdala neurons. Proc Natl Acad Sci U S A 2003;100:2053–2058. [PubMed: 12566570]

Roberto M, Nelson TE, Ur CL, Brunelli M, Sanna PP, Gruol DL. The transient depression of hippocampal CA1 LTP induced by chronic intermittent ethanol exposure is associated with an inhibition of the MAP kinase pathway. Eur J Neurosci 2003;17:1646–1654. [PubMed: 12752382]

Roberto M, Schweitzer P, Madamba SG, Stouffer DG, Parsons LH, Siggins GR. Acute and chronic ethanol alter glutamatergic transmission in rat central amygdala: an in vitro and in vivo analysis. J Neurosci 2004;24:1594–1603. [PubMed: 14973247]

Roivainen R, Hundle B, Messing RO. Ethanol enhances growth factor activation of mitogen-activated protein kinases by a protein kinase C-dependent mechanism. Proc Natl Acad Sci U S A 1995;92:1891–1895. [PubMed: 7534406]

Ron D, Vagts AJ, Dohrman DP, Yaka R, Jiang Z, Yao L, et al. Uncoupling of betaIIPKC from its targeting protein RACK1 in response to ethanol in cultured cells and mouse brain. FASEB J 2000;14:2303–2314. [PubMed: 11053252]

Rose CR, Blum R, Pichler B, Lepier A, Kafitz KW, Konnerth A. Truncated TrkB-T1 mediates neurotrophin-evoked calcium signaling in glial cells. Nature 2003;426:74–78. [PubMed: 14603320]

Royer S, Martina M, Paré D. An inhibitory interface gates impulse traffic between the input and output stations of the amygdala. J Neurosci 1999;19:10575–83. [PubMed: 10575053]

Sah P, Faber ES, Lopez De Armentia M, Power J. The amygdaloid complex: anatomy and physiology. Physiol Rev 2003;83:803–834. [PubMed: 12843409]

Sakai R, Ukai W, Sohma H, Hashimoto E, Yamamoto M, Ikeda H, et al. Attenuation of brain derived neurotrophic factor (BDNF) by ethanol and cytoprotective effect of exogenous BDNF against ethanol damage in neuronal cells. J Neural Transm 2005;112:1005–1013. [PubMed: 15583957]

Seabold GK, Luo J, Miller MW. Effect of ethanol on neurotrophin-mediated cell survival and receptor expression in cultures of cortical neurons. Brain Res Dev Brain Res 1998;108:139–145.

Segal RA, Takahashi H, McKay RD. Changes in neurotrophin responsiveness during the development of cerebellar granule neurons. Neuron 1992;9:1041–1152. [PubMed: 1463606]

Shapira NA, Goldsmith TD, McElroy SL. Treatment of binge-eating disorder with topiramate: a clinical case series. J Clin Psychiatry 2000;61:368–72. [PubMed: 10847312]

Shekhar A, Truitt W, Rainnie D, Sajdyk T. Role of stress, corticotrophin releasing factor (CRF) and amygdala plasticity in chronic anxiety. Stress 2005;8:209–219. [PubMed: 16423710]

Shelton DL, Sutherland J, Gripp J, Camerato T, Armanini MP, Phillips HS, et al. Human trks: molecular cloning, tissue distribution, and expression of extracellular domain immunoadhesins. J Neurosci 1995;15:477–491. [PubMed: 7823156]

Shieh PB, Hu SC, Bobb K, Timmusk T, Ghosh A. Identification of a signaling pathway involved in calcium regulation of BDNF expression. Neuron 1998;20:727–40. [PubMed: 9581764]

Silberman Y, Shi L, Brunso-Bechtold JK, Weiner JL. Distinct Mechanisms of Ethanol Potentiation of Local and Paracapsular GABAergic Synapses in the Rat Basolateral Amygdala. J Pharmacol Exp Ther. 2007In press

Sklar P, Gabriel SB, McInnis MG, Bennett P, Lim YM, Tsan G, et al. Family-based association study of 76 candidate genes in bipolar disorder: BDNF is a potential risk locus. Brain-derived neutrophic factor. Mol Psychiatry 2002;7:579–593. [PubMed: 12140781]

Smith MA, Makino S, Kvetnansky R, Post RM. Effects of stress on neurotrophic factor expression in the rat brain. Ann N Y Acad Sci 1995;771:234–239. [PubMed: 8597402]

Soule J, Messaoudi E, Bramham CR. Brain-derived neurotrophic factor and control of synaptic consolidation in the adult brain. Biochem Soc Trans 2006;34:600–604. [PubMed: 16856871]

Sotelo C. Cellular and genetic regulation of the development of the cerebellar system. Prog Neurobiol 2004;72:295–339. [PubMed: 15157725]

Strand AD, Baquet ZC, Aragaki AK, Holmans P, Yang L, Cleren C, et al. Expression profiling of Huntington's Disease models suggests that brain-derived neurotrophic factor depletion plays a major role in striatal degeneration. J Neurosci 2007;27:11758–11768. [PubMed: 17959817]

Stubbs CD, Slater SJ. Ethanol and protein kinase C. Alcohol Clin Exp Res 1999;23:1552–60. [PubMed: 10512323]

Szabò G, Hoffman PL. Brain-derived neurotrophic factor, neurotrophin-3 and neurotrophin-4/5 maintain functional tolerance to ethanol. Eur J Pharmacol 1995;287:35–41. [PubMed: 8666023]

Tao X, Finkbeiner S, Arnold DB, Shaywitz AJ, Greenberg ME. Ca2+ influx regulates BDNF transcription by a CREB family transcription factor-dependent mechanism. Neuron 1998;20:709–26. [PubMed: 9581763]

Tapia-Arancibia L, Rage F, Givalois L, Dingeon P, Arancibia S, Beauge F. Effects of alcohol on brain-derived neurotrophic factor mRNA expression in discrete regions of the rat hippocampus and hypothalamus. J Neurosci Res 2001;63:200–208. [PubMed: 11169630]

Thomas JD, Garrison M, O'Neill TM. Perinatal choline supplementation attenuates behavioral alterations associated with neonatal alcohol exposure in rats. Neurotoxicol Teratol 2004;26:35–45. [PubMed: 15001212]

Thorsell A, Karlsson RM, Heilig M. NPY in alcoholism and psychiatric disorders. EXS 2006;95:183–192. [PubMed: 16383007]

Thurston AW Jr, Shukla SD. Ethanol modulates epidermal growth factor-stimulated tyrosine kinase and phosphorylation of PLC-gamma 1. Biochem Biophys Res Commun 1992;185:1062–8. [PubMed: 1320873]

Timmusk T, Palm K, Metsis M, Reintam T, Paalme V, Saarma M, et al. Multiple promoters direct tissue-specific expression of the rat BDNF gene. Neuron 1993;10:475–89. [PubMed: 8461137]

Tongiorgi E, Domenici L, Simonato M. What is the biological significance of BDNF mRNA targeting in the dendrites? Clues from epilepsy and cortical development. Mol Neurobiol 2006;33:17–32. [PubMed: 16388108]

Tsai SJ. Possible involvement of the BDNF-dependent pathway in treatment-emergent suicidality or decreased response to antidepressants. Med Hypotheses 2005;65:942–946. [PubMed: 16002234]

Uddin RK, Singh SM. Ethanol-responsive genes: identification of transcription factors and their role in metabolomics. Pharmacogenomics J 2007;7:38–47. [PubMed: 16652119]

Tsuji R, Guizzetti M, Costa LG. In vivo ethanol decreases phosphorylated MAPK and p70S6 kinase in the developing rat brain. Neuroreport 2003;14:1395–9. [PubMed: 12876481]

Turjanski AG, Vaqué JP, Gutkind JS. MAP kinases and the control of nuclear events. Oncogene 2007;14:3240–53. [PubMed: 17496919]

Uhl GR, Liu QR, Walther D, Hess J, Naiman D. Polysubstance abuse-vulnerability genes: genome scans for association, using 1,004 subjects and 1,494 single-nucleotide polymorphisms. Am J Hum Genet 2001;69:1290–1300. [PubMed: 11704927]

Vaudry D, Stork PJ, Lazarovici P, Eiden LE. Signaling pathways for PC12 cell differentiation: making the right connections. Science 2002;296:1648–1649. [PubMed: 12040181]

Walker DW, Barnes DE, Zornetzer SF, Hunter BE, Kubanis P. Neuronal loss in hippocampus induced by prolonged ethanol consumption in rats. Science 1980;209:711–713. [PubMed: 7394532]

Wang H, Ward N, Boswell M, Katz DM. Secretion of brain-derived neurotrophic factor from brain microvascular endothelial cells. Eur J Neurosci 2006;23:1665–70. [PubMed: 16553631]

West JR. Acute and long-term changes in the cerebellum following developmental exposure to ethanol. Alcohol Alcohol Suppl 1993;2:199–202. [PubMed: 7748300]

Weston CR, Davis RJ. The JNK signal transduction pathway. Curr Opin Cell Biol 2007;19:142–9. [PubMed: 17303404]

Widmer HR, Kaplan DR, Rabin SJ, Beck KD, Hefti F, Knüsel B. Rapid phosphorylation of phospholipase C gamma 1 by brain-derived neurotrophic factor and neurotrophin-3 in cultures of embryonic rat cortical neurons. J Neurochem 1993 Jun;60(6):2111–23. [PubMed: 8492120]

Woo NH, Teng HK, Siao CJ, Chiaruttini C, Pang PT, Milner TA, et al. Activation of p75NTR by proBDNF facilitates hippocampal long-term depression. Nat Neurosci 2005;8:1069–1077. [PubMed: 16025106]

Woodruff AR, Monyer H, Sah P. GABAergic excitation in the basolateral amygdala. J Neurosci 2006;26:11881–7. [PubMed: 17108161]

Wrase J, Reimold M, Puls I, Kienast T, Heinz A. Serotonergic dysfunction: brain imaging and behavioral correlates. Cogn Affect Behav Neurosci 2006;6:53–61. [PubMed: 16869229]

Xu H, Steven Richardson J, Li XM. Dose-related effects of chronic antidepressants on neuroprotective proteins BDNF, Bcl-2 and Cu/Zn-SOD in rat hippocampus. Neuropsychopharmacology 2003;28:53–62. [PubMed: 12496940]

Xu K, Anderson TR, Neyer KM, Lamparella N, Jenkins G, Zhou Z, et al. Nucleotide sequence variation within the human tyrosine kinase B neurotrophin receptor gene: association with antisocial alcohol dependence. Pharmacogenomics J. 2007

Yan QS, Feng MJ, Yan SE. Different expression of brain-derived neurotrophic factor in the nucleus accumbens of alcohol-preferring (P) and -nonpreferring (NP) rats. Brain Res 2005;1035:215–218. [PubMed: 15722062]

Yeo GS, Connie Hung CC, Rochford J, Keogh J, Gray J, Sivaramakrishnan S, et al. A de novo mutation affecting human TrkB associated with severe obesity and developmental delay. Nat Neurosci 2004;7:1187–1189. [PubMed: 15494731]

Yin HH, Knowlton BJ. The role of the basal ganglia in habit formation. Nat Rev Neurosci 2006;7:464–476. [PubMed: 16715055]

Yin Y, Edelman GM, Vanderklish PW. The brain-derived neurotrophic factor enhances synthesis of Arc in synaptoneurosomes. Proc Natl Acad Sci U S A 2002;99:2368–2373. [PubMed: 11842217]

York RD, Yao H, Dillon T, Ellig CL, Eckert SP, McCleskey EW, et al. Rap1 mediates sustained MAP kinase activation induced by nerve growth factor. Nature 1998;392:622–626. [PubMed: 9560161]

Yoshida K, Higuchi H, Kamata M, Takahashi H, Inoue K, Suzuki T, et al. The G196A polymorphism of the brain-derived neurotrophic factor gene and the antidepressant effect of milnacipran and fluvoxamine. J Psychopharmacol 2007;21:650–656. [PubMed: 17092970]

Zafra F, Lindholm D, Castrén E, Hartikka J, Thoenen H. Regulation of brain-derived neurotrophic factor and nerve growth factor mRNA in primary cultures of hippocampal neurons and astrocytes. J Neurosci 1992;12:4793–9. [PubMed: 1281495]

Zhang L, Dhillon HS, Barron S, Hicks RR, Prasad RM, Seroogy KB. Effects of chronic ethanol administration on expression of BDNF and trkB mRNAs in rat hippocampus after experimental brain injury. Brain Res Mol Brain Res 2000;79:174–179. [PubMed: 10925157]

Zhu PJ, Lovinger DM. Ethanol potentiates GABAergic synaptic transmission in a postsynaptic neuron/synaptic bouton preparation from basolateral amygdala. J Neurophysiol 2006;96:433–441. [PubMed: 16624993]

Zorner B, Wolfer DP, Brandis D, Kretz O, Zacher C, Madani R, et al. Forebrain-specific trkB-receptor knockout mice: behaviorally more hyperactive than “depressive”. Biol Psychiatry 2003;54:972–82. [PubMed: 14625139]

Zou J, Crews F. CREB and NF-kappaB Transcription Factors Regulate Sensitivity to Excitotoxic and Oxidative Stress Induced Neuronal Cell Death. Cell Mol Neurobiol 2006;26:383–403.

Zuccato C, Ciammola A, Rigamonti D, Leavitt BR, Goffredo D, Conti L, et al. Loss of huntingtin-mediated BDNF gene transcription in Huntington’s disease. Science 2001;293:493–498. [PubMed: 11408619]

Table 1

Summary of studies examining the effect of ethanol on TrkB or BDNF levels in mature animals and in vitro. Blood ethanol concentrations are noted if reported.

Model	Exposure	Protein/mRNA	Change	Reference
Human Patients	Self administration	Plasma BDNF Protein	↓ in alcoholic patients ↓↓with + family history	Joe *et al.*, 2007
C57BL/6J DBA/J2 Mice	IP injection 2g/kg	BDNF mRNA Array RT-PCR	↑NAc DBA/J2 only ↔ PFCx	Kerns *et al.*, 2005
C57BL/6J Mice	Vapor inhalation 2 × 64 hrs, 1 week apart 150–200 mg/dL	BDNF mRNA Array RT-PCR Western blot	↓PFC mRNA 0 HPE ↓protein 0 and 8 HPE in PFC ↔HP, vStr	Melendez *et al.*, 2006
P and NP rats	No exposure	BDNF ELISA normalized to protein	↓ in NAc of P rats	Yan *et al.*, 2005
PKCγ ko mice	Liquid diet 11 days 213 mg/dL	BDNF array BDNF RT-PCR	↓ in KO by array ↔ by PCR cerebellum	Bowers *et al.*, 2006
Rat	Liquid diet 19 weeks	BDNF mRNA RT-PCR	↔ hippocampus	Okamoto *et al.*, 2006
Rat	Liquid diet 28 weeks CET 24 hr WD 150–175 mg/dL	TrkB mRNA Northern blot	↑ Cortex	Baek *et al.*, 1996
Rat	Liquid diet 15 days 101 mg/dL	BDNF Western blot	↔ on diet ↓ at 24 hr WD Cortex	Pandey *et al.*, 1999
Rat	Liquid diet 28 weeks CET 24 hr WD 150–175 mg/dL	BDNF mRNA Northern blot	↓ in HP	MacLennan *et al.*, 1995
Rat	Liquid diet 3 days/week 6–24 weeks	IHC stereology	↑ number of $BDNF^+$ cells ↑ BDNF/cell Somatosensory cortex (layer V)	Bruns and Miller 2007
Rat	Liquid diet 8–24 weeks	BDNF ELISA Normalized to protein	↑ parietal Cx 24 weeks ↑ basal FB 8 and 24 weeks ↑ septum 24 weeks ↓ HP at 8 weeks only	Miller M. and Mooney 2004
Rat	Liquid diet Chronic Episodic exposure 6–24 weeks 137 mg/dL	BDNF ELISA Normalized to protein	↑ parietal Cx ↔ Enterhinal Cx ↑ septum ↑ HP early	Miller M. 2004
Rat	Liquid diet 28 weeks 150–175 mg/dL	BDNF mRNA TrkB mRNA RT-PCR	↔HP ↔ basal FB	Miller R. *et al.*, 2002
Rat	Liquid diet 6 weeks 142 mg/dL	TrkB mRNA BDNF mRNA	↔	Zhang *et al.*, 2000
Rat	Vapor inhalation 4 weeks 94–103 mg/dL	BDNF mRNA RT-PCR ISHH TrkB mRNA RPA	↓ during exposure HP (CA1 and DG) and SON ↑ CA3, DG, SON 12 hr WD ↑ SON, HP TrkB mRNA	Tapia-Arancibia *et al.*, 2001
C57BL/6 mice	2 bottle choice	BDNF mRNA RT-PCR	↑ DStr ↔ PFC, HP	McGough *et al.*, 2004
C57BL/6 mice	IP injection 2g/kg	BDNF mRNA RT-PCR	↑ DStr, HP Not detected NAc	
Hippocampal neurons	10–100 mM	BDNF mRNA RT-PCR	↑ 0.5–2 hr ↓ 24–48 hr	
Striatal slices	100 mM acute	BDNF mRNA RT-PCR	↑	
SH-SY5Y Cells	100 mM	BDNF ELISA	↓ secretion in differentiated cells	Sakai *et al.*, 2005
Cortical neurons	200 mg/dL 48 hr	TrkB Western blot	↔	Seabold *et al.*, 1998
Hippocampal explant cultures	100 mM 4 hr	BDNF mRNA RT-PCR	↓ EtOH combined with TNF, glutamate and H_20_2	Zou and Crews 2006
Cerebellar granule cells	400–1,600 mg/mL	BDNF ELISA	↓ secretion	Heaton *et al.*, 2004
Cerebellar granule cells	100 mM	BDNF ELISA	↓ secretion	Bhave *et al.*, 1999

Abbreviations: DG, dentate gyrus; Cx, cortex; HP, hippocampus; ISHH, in situ hybridization histochemistry; NAc, nucleus accumbens; PFC, prefrontal cortex; RT-PCR, quantitative reverse transcription polymerase chain reaction; SON, supraoptic nucleus; Str, striatum, TNF, tumor necrosis factor; vStr, ventral striatum; WD, withdrawal.

Table 2

Summary of experiments examining the effect of ethanol on BDNF or TrkB levels during development. Blood ethanol concentrations are noted if reported.

Model/region	Age and exposure	Protein/mRNA	Change	Reference
Neonatal rat SCN HP	artificial rearing 4.5g/kg PN4–9 BAC 333 mg/dL	BDNF ELISA @5–6 mos Normalized to protein	↓ Suprachiasmic nucleus ↓ HP	Allen *et al.*, 2004
Prenatal rat Olfactory bulb	Intubation of dam 6g/kg/day 380 mg/dL	BDNF mRNA Southern blot	↓ at birth and PN10 Olfactory bulb	Maier *et al.*, 1999
Neonatal rat	Gavage 5g/kg PN 5–8	BDNF mRNA	↓ Cortex	Fattori *et al.*, 2008
Neonatal rat Cerebellum 0–24 hr	PN4, PN7 Vapor 2 hr 45 min Peak BAC ~290 mg/dL	BDNF ELISA Normalized to wet weight	↑ PN4 0 HPE ↔ 2–12 HPE ↑ PN7 2 HPE ↓ PN7 12 HPE	Heaton *et al.*, 2003a
Neonatal rat Cerebellum	Vapor inhalation 2.5 hr PN4–5, PN7–8 PN4–10 Peak BAC ~300 mg/dL	BDNF ELISA Normalized to wet weight	↔ cerebellum with any exposure paradigm 1.5 HPE	Heaton *et al.*, 1999
Neonatal rat Cortex 0–24 hrs	PN7, PN21 Vapor 2 hr 45 min Peak BAC ~290 mg/dL	BDNF ELISA Normalized to wet weight	↑ 0 and 12 HPE ↓ at 2 HPE ↔ PN21	Heaton *et al.*, 2003c
Neonatal rat Striatum 0–24 hr	PN3 PN14 Vapor 2 hr 45 min ~290 mg/dL	BDNF ELISA Normalized to wet weight	↑ only 24 hr WD PN3 ↔ PN14	Heaton *et al.*, 2003b
Prenatal/neonatal multiple regions	Vapor inhalation dam or PN4–10 2.5 hrs/day 161 mg/dL (dam) ~300 mg/dL (neonatal)	BDNF Normalized to wet weight	↔ HP, Septum, Cx/Str or CB at PN1 (prenatal) ↑ Increase Cx/Str,HP PN10 (neonatal) ↔ at PN21	Heaton *et al.*, 2000
Prenatal Multiple regions	Liquid diet 161 mg/dL	TrkB protein Normalized to cyclophilin	TrkB p95 ↓Septum (female, PN 1, PN 10) ↑Cx (male & female) ↓ HP (male PN1) TrkB p145 ↑Cx, CB (female PN1) ↓Septum (female, PN1)	Moore *et al.*, 2004a
Neonatal Multiple regions	Vapor Inhalation PN4–10 266 mg/dL	TrkB protein Normalized to cyclophilin	TrkB p95 ↓Cx (male & female) TrkB p145 ↓HP (female PN10) ↑Septum (male & female) No effect at PN21 ↔CB	Moore *et al.*, 2004b
Prenatal Multiple regions	Intubation of dam 1 and 3 g/kg/day Examined at PN7	BDNF ELISA, normalized to protein BDNF mRNA TrkB pTrkB	↓ HP, Cx, NS decrease in Str (protein) ↓mRNA ↔cerebellum ↔TrkB ↓ TrkB phosphorylation	Feng *et al.*, 2005
Neonatal rat cortex	Intubation of dam during gestation and lactation ~107 mg/dL	BDNF ELISA normalized to protein TrkB mRNA RPA	↓ in cortex PN5–21 ↑ full length and truncated TrkB	Climent *et al.*, 2002
Neonatal rat	PN2–7, in bins intubation 300 mg/dL	TrkB TrkBT1 IHC	↓ on Purkinje cells at all ages	Light *et al.*, 2002
Neonatal rat cerebellum	PN2–3 Analyzed PN4 intubation 300 mg/dL	TrkB ECD and TK TrkB-T2 mRNA BDNF RT-PCR	↓ TrkB.T2 and Total ECD ↔ TrkB-TK or TrkB.T1 ↓ BDNF	Light *et al.*, 2001
Neonatal rat Cerebellum	Time course PN4, PN9 1.5–6 g/kg intubation 135–547 mg/dL	BDNF mRNA TrkB mRNA	dose-dependent ↓ 1–8 HPE PN4 ↔ PN9	Ge *et al.*, 2004

Abbreviations: CB, cerebellum; Cx, cortex; ECD, extracellular domain; HP, hippocampus; HPE, hours post exposure; IHC, immunohistochemistry; PN, postnatal age; RPA, ribonuclease protection assay; SCN, suprachiasmic nucleus; Str, striatum; WD, withdrawal.