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Neurotrophic Factor Signaling in Alcoholism

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Abstract

This article presents the proceedings of a symposium presented at the meeting of the International Society for the Biomedical Research on Alcoholism (ISBRA), held in Manheim, Germany, in September 2004. The organizers and chairpersons were Subhash C. Pandey and Toshikazu Saito. The presentations were (1) Ethanol and NMDA receptor coupling to ERK signaling, by L.J. Chandler; (2) Ethanol modulation of CREB: Role in neurogenesis, by Fulton Crews;(3) Serotonin dysfunction and alcohol preference in mice deficient in brain-derived neurotrophic factor (BDNF), by Julie G. Hensler; (4) BDNF gene and related signaling: role in anxiety and alcohol dependence and preference, by Subhash C. Pandey; (5) BDNF and CREB: role in ethanol induced neuronal damage, Wataru Ukai.

Keywords

CREB; BDNF; ERK; Alcohol; Neurogenesis; Anxiety; Brain

INTRODUCTION

Alcohol dependence is a complex disease and may involve adaptations in several neurotransmitters and neuromodulator systems in brain circuitry (Eckardt et al., 1998; Koob, 2003). Brain derived neurotrophic factor (BDNF) is a member of the nerve growth factor family of neurotrophic factors, which includes nerve growth factor, Neurotrophin-3, Neurotrophin-4/5 and BDNF (Thoenen, 1995; Poo, 2001; Lu, 2003). The neurotrophic factors play a critical role in the guidance and development of neuronal systems as well as being important in the survival, differentiation and function of neurons (Finkbeiner et al., 1997; Huang and Reichardt, 2001;Gorski et al., 2003). The action of BDNF is mediated by binding to tyrosine kinase B (Trk B) receptors and subsequently activation of the mitogen activated protein (MAP) kinase [also known as extracellular signal-regulated kinases 1/2 (ERK1/2)] signal transduction pathway (Chao, 2003; Pandey, 2004). Other kinases such as calcium calmodulin-dependent protein kinases and cAMP dependent protein kinase A also activate cyclic AMP responsive element binding (CREB) protein by phosphorylation at serine-133 (Silva et al.,1998; Soderling,

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1999; Pandey, 2004). BDNF is a cyclic AMP inducible gene and is regulated by the CREB gene transcription factor. Thus, CREB serves as both an up and downstream molecular target for the action of BDNF in the brain (Shieh et al., 1998; Chao, 2003).

The role of BDNF has been implicated in several neurological and psychiatric conditions such as synaptic plasticity associated with learning and memory, depression and stress (Poo, 2001; Lu, 2003; Nestler et al., 2002; Duman et al., 2000). Using various approaches during recent years, several investigators have pointed out that BDNF gene and its signaling (ERK1/2 and CREB) play a crucial role in alcohol dependence; preference and ethanol induced brain damage. This symposium provided an attractive forum to highlight the current developments in the role of BDNF and related signaling in the action of ethanol.

ETHANOL AND NMDA RECEPTOR COUPLING TO ERK SIGNALING

L.J. Chandler

NMDA receptors regulate neuronal development, and neuronal survival and death (Collingride and Lester, 1989). Accumulating evidence also indicates they are important sites of action of ethanol in both the adult and developing brain. Acutely, ethanol attenuates NMDA receptor activity, and this inhibitory effect may contribute to its intoxicating actions (Chandler et al., 1998). Alterations in NMDA receptor function by ethanol have been implicated in brain plasticity, including the plasticity of addiction (Chandler, 2003).

The ERK cascade is a prototypical growth factor signaling pathway that regulates neuronal plasticity and survival (Sweatt, 2001). In addition, it is now clear that calcium-mobilizing receptors, including NMDA receptors, modulate neuronal function through ERK signaling events (Chandler, 2003). Interestingly, Dr. Chandler and his group have previously demonstrated that NMDA receptor coupling to ERK signaling is bidirectional. This bidirectionality is characterized by NMDA receptor coupling to opposing stimulatory and inhibitory pathways (Chandler et al., 2001). Based upon similar studies demonstrating that extrasynaptic NMDA receptors couple to a dominant inhibitory pathway that shuts-off CREB activation coupled to synaptic NMDA receptors, they hypothesized a similar spatial separation for NMDA receptor coupling to ERK signaling. As an initial test of this hypothesis, Dr. Chandler and his group utilized the MK-801 trapping technique to selectively block synaptic receptors while leaving extrasynaptic receptors unblocked. Following MK-801 block of synaptic receptors in primary cortical cultures, addition of NMDA resulted in a rapid reduction in basal (activity-dependent) phospho-ERK. These observations are consistent with their hypothesis that extrasynaptic NMDA receptors stimulate a dominant ERK shut-off pathway of synaptic activation of ERK signaling. However, it should be noted that these results do not indicate that the shut-off pathway is selectively coupled with extrasynaptic NMDA receptors as synaptic NMDA receptors could also couple to an inhibitory pathway. However, previous observations by this group and others that only ERK activation is associated with NMDA receptors regardless of how hard synaptic activity is driven, would argue against an inhibitory action of synaptic NMDA receptors (Chandler, 2003).

A number of recent studies have also implicated BDNF in the actions of ethanol in brain (Climent et al., 2002; McGough et al., 2004). Since a primary signaling pathway utilized by BDNF is the ERK signaling cascade, they next addressed the question of whether the NMDA receptor shut-off pathway, which exerts dominant control over the synaptic NMDA receptor activation pathway, exerts similar dominant inhibition of BDNF activation of ERK signaling. For these experiments, synaptic NMDA receptors were first selectively blocked with MK-801 that was followed by 1hr incubation with TTX to eliminate basal levels of phospho-ERK. Following this, BDNF was added with or without simultaneous addition of NMDA. In a separate set of experiments, addition of BDNF was delayed 10 min after the addition of NMDA.

Immunoblot analysis of phospho-ERK levels revealed that while BDNF produced a rapid increase in phospho-ERK levels, in neither instance did NMDA attenuate this increase.

The results of these experiments suggest that NMDA receptor-coupled ERK modulatory pathways are compartmentalized with spatially distinct populations of NMDA receptors. Furthermore, while NMDA receptor signaling plays an important role in transcriptional regulation of BDNF expression, NMDA and BDNF coupling to ERK activation functions independently (Chandler, 2003). Dr. Chandler and his group have also recently observed that prolonged ethanol exposure of cultured neurons leads to a selective increase in synaptic NMDA receptors without altering extrasynaptic receptors. This effect occurs through activity-dependent homeostatic processes that restore stability to the neuronal network in response to the inhibitory effects of ethanol. They propose a molecular model in which chronic ethanol-induced increases in synaptic NMDA receptors enhance BDNF expression (via enhanced NMDA receptor-ERK-CREB signaling) without directly altering BDNF activation of ERKs (Chandler, 2003).

ETHANOL MODULATION OF CREB: ROLE IN NEUROGENESIS

Fulton Crews and Kim Nixon

Genes induced by activated CREB play a critical role in many process such as neuronal plasticity, long term memory (Lonze and Ginty, 2002; Silva et al., 1998), drug addiction (Nestler, 2002) and the promotion of neuronal cell survival through regulating the transcription of pro-survival factors (Dawson and Ginty, 2002; Lonze and Ginty, 2002; Riccio et al., 1999). CREB also promotes neurogenesis by contributing to proliferation, differentiation and survival of newborn neuronal cells. New born immature neurons are CREB positive in mouse and rat dentate gyrus (Nakagawa et al., 2002a; 2002b). Adult neurogenesis (the formation of new neurons) has recently been discovered as a form of neural plasticity that contributes to memory, learning and mood (Crews et al., 2003; Eisch, 2002). Thus, phosphorylated CREB (p-CREB) and the associated transcriptional activation by p-CREB underlie brain neural plasticity, growth and vitality through activation of neuronal growth and survival processes and increased neurogenesis.

Ethanol (alcohol) is known to alter a variety of signaling pathways, particularly receptor operated ion channels such as the NMDA-glutamate receptor and G protein receptors coupled to adenylate cyclase (Chandler et al., 1998; Crews et al., 1996). These signaling pathways produce complex effects on CREB phosphorylation depending upon the brain region, kinases present and other signal modulators within each brain region. Chronic binge ethanol models of dependence also cause neurodegeneration depending upon the region, including forebrain olfactory areas, insular, perirhinal, piriform and entorhinal association cortical areas; and hippocampal dentate gyrus (Crews et al., 2000; Crews et al., 2004; Obernier et al., 2002a). The hippocampus is an important brain region for learning, memory and mood that is altered by ethanol. Alcoholism results in cognitive deficits in spatial learning and memory, short term and declarative memory, and impulsivity, that are consistent with hippocampal dysfunction (Brandt et al., 1983; Parsons, 1993; Sullivan et al., 2000). Hippocampal neuropathology in human alcoholics (Agartz et al., 1999; Bengochea and Gonzalo, 1990; Laakso et al., 2000; Sullivan et al., 1995) is consistent with hippocampal cell loss and dentate gyrus neurodegeneration found in animal models of chronic alcohol consumption (Collins et al., 1996; Obernier et al., 2002b; Walker et al., 1980). The dentate gyrus region of the hippocampus contains resident neural progenitor cells that proliferate and differentiate into new neurons throughout life. The inhibition of neurogenesis by chronic alcohol may contribute to neurodegeneration via lack of cell generation (Nixon and Crews, 2002). Because p-CREB contributes to both pro-survival signals in the promotion of cell survival and neurogenesis, it may play a key role in neuronal plasticity. Alcohol alters neuronal plasticity via impacting cell

survival and cell birth. Thus, alterations in p-CREB associated gene transcription, as a mediator of these plasticity mechanisms affected in chronic ethanol exposure, could contribute to brain changes associated with chronic alcoholism.

Indeed, p-CREB immunoreactivity in hippocampal dentate gyrus is reduced during ethanol dependence (Bison and Crews, 2003). In addition, hippocampal neurogenesis is markedly decreased during binge ethanol exposure (Nixon and Crews, 2002). Ethanol self-administration decreases neural progenitor cell proliferation in a variety of brain regions (Crews et al., 2004), likely due to increased progenitor cell death and reduced differentiation of progenitors (He et al., 2005). Ethanol induced decreases in hippocampal p-CREB likely contribute to ethanol inhibition of hippocampal neurogenesis and increased neurodegeneration.

Although hippocampal p-CREB appears to be reduced during chronic ethanol administration, 72 hrs of alcohol withdrawal result in increased p-CREB immunohistochemistry in the hippocampal dentate gyrus (Bison and Crews, 2003). Withdrawal from ethanol also is associated with an increase in progenitor cell proliferation and neurogenesis in hippocampus (Nixon and Crews, 2004). Improvements in learning and memory are associated with increased neurogenesis (van Praag et al., 1999) and increased p-CREB (Nagakura et al., 2002; Zhang et al., 2000). Abstinence from alcohol in humans is associated with a reversal of hippocampal neurodegeneration and a partial return of cognitive abilities (Carlen et al., 1978; Harper, 1998). p-CREB is associated with the recovery of hippocampal volume during recovery from depression (Duman et al., 2001) and could contribute to improvements in mood associated with recovery from alcoholism. A withdrawal-abstinence induced increase in pCREB in hippocampus would be expected to contribute to the increased neurogenesis through increased proliferation, reduced cell death and enhanced differentiation (Fig. 1).

In summary, p-CREB appears to contribute to brain vitality by inducing pro-survival genes that prevent neuronal death and by stimulating neural progenitor cells to form new neurons. Chronic ethanol administration causes neurodegeneration, inhibits neurogenesis and reduces p-CREB in hippocampal dentate gyrus, whereas abstinence results in increases in neurogenesis, loss of degeneration and increased hippocampal p-CREB. It is possible that pCREB represents a key component of the long-term effects of chronic alcohol on brain as well as the changes associated with recovery from alcohol dependence.

SEROTONIN DYSFUNCTION AND ALCOHOL PREFERENCE IN MICE DEFICIENT IN BRAIN-DERIVED NEUROTROPHIC FACTOR (BDNF)

Julie G. Hensler

Disturbances of serotonergic neurotransmission in the brain have been implicated in the pathogenesis and maintenance of alcoholism, as well as behavioral patterns relevant to alcohol abuse. Both preclinical and clinical studies suggest an inverse relationship between central serotonergic activity and alcoholism, impulse control disorders, aggression, negative mood and low response to alcohol (LeMarquand et al., 1994a; Heinz et al., 2001). Decreased function of central serotonergic systems is associated with increased alcohol preference and consumption in rats. Depletion of serotonin in brain by inhibiting serotonin synthesis or by using neurotoxins increases alcohol intake, impulsive behavior and aggression in laboratory animals (LeMarquand et al., 1994b; Nelson and Chiavegatto, 2001). Pharmacological interventions that increase serotonergic neurotransmission decrease alcohol consumption and behavioral patterns relevant to alcoholism (LeMarquand et al., 1994a, b; Heinz et al., 2001; Nelson and Chiavegatto, 2001).

BDNF promotes serotonergic neurotransmission by increasing serotonin synthesis and release, and promotes the structural plasticity of serotonergic neurons in the adult brain. Thus, BDNF

has an important role in the adult brain in maintaining the functional architecture of serotonergic neurons (Mamounas et al., 2000). There is substantial evidence for reciprocal interactions between BDNF and serotonin (Duman et al., 2002; MacQueen et al., 2003). Because, BDNF promotes serotonergic neurotransmission and the functional architecture of serotonergic neurons, it is tempting to speculate that deficiency in BDNF may cause abnormalities in serotonin systems.

Heterozygous animals with one functional BDNF gene have forebrain BDNF mRNA and protein levels that are 50% of that of wild-type mice and exhibit nonlethal abnormalities in central serotonergic systems. In BDNF heterozygons (+/−) mice, the induction of c-Fos by the specific serotonin releaser dexfenfluramine (an indication of neuronal activation in response to serotonin) is blunted in many forebrain areas, including lateral frontal cortex, bed nucleus of the stria terminalis, central nucleus of the amygdala and the shell region of the nucleus accumbens (Lyons et al., 1999). These brain areas are key components of the forebrain macrostructure “the extended amygdala,” the brain reward system implicated in the development of alcoholism (Koob and LeMoal, 2001; Pandey, 2004). Consistent with these deficits in serotonergic neurotransmission, BDNF heterozygons (+/−) mice exhibit enhanced inter-male aggressiveness, which is reversed by chronic administration of the SSRI fluoxetine. BDNF heterozygons (+/−) mice also develop premature age-associated decrements in forebrain serotonin levels and fiber density (Lyons et al., 1999).

Dr. Hensler and her group have examined the alcohol drinking behavior in female BDNF heterozygons (+/−) mice. BDNF heterozygons (+/−) mice display increased ethanol intake and preference in the two-bottle choice procedure. There is no difference in the preference ratio for nonalcoholic tastants (i.e., quinine or saccharin) between genotypes. Thus, the increased ethanol consumption of BDNF heterozygons (+/−) *versus* wild-type mice appears not to reflect a general difference in taste preference between genotypes, but may be related to the pharmacological effects of ethanol, or perhaps an attenuation of the physiological or subjective effects of alcohol in BDNF heterozygons (+/−) mice (Hensler et al., 2003).

The serotonin-1A (5-HT_{1A}) receptor has been implicated in aggressive behavior and impulse control disorders, as well as alcohol abuse. The 5-HT_{1A} receptor, located on serotonergic cell bodies in the brainstem, functions as the somatodendritic autoreceptor and therefore plays a key role in regulating serotonergic neurotransmission. The 5-HT_{1A} receptor is also located postsynaptically to serotonergic neurons and is present in high density in cortical and limbic structures. Dr. Hensler and her group have examined the function of 5-HT_{1A} receptors at the level of 5-HT_{1A} receptor-G protein interaction in the brains of alcohol-naive BDNF heterozygons (+/−) mice. The capacity of 5-HT_{1A} receptors in cortical and limbic structures to activate G proteins is decreased in BDNF heterozygons (+/−) mice, indicating that postsynaptic 5-HT_{1A} receptor function is diminished in many forebrain areas. Somatodendritic 5-HT_{1A} receptor function at the level of receptor-G protein interaction is also reduced in BNDF (±) mice. However, the total number of 5-HT_{1A} receptor is not different between genotypes in any area of brain examined (Hensler et al., 2003). Thus, attenuated 5–HT_{1A} receptor function may by a factor in alcohol abuse, as well as aggressive behavior in BDNF deficient mice.

A decrease in 5-HT_{1A} autoreceptor function in the raphe nuclei would be expected to result in a decrease in alcohol intake, given the well-established inverse relationship between serotonergic neurotransmission and alcohol consumption discussed above. This is because diminished 5–HT_{1A} autoreceptor function would be expected to be associated with an increase in serotonergic neurotransmission. However, the capacity of 5-HT_{1A} receptors in cortical and limbic structures to activate G proteins is decreased in BDNF heterozygons (+/−) mice, indicating that postsynaptic 5-HT_{1A} receptor function is diminished in forebrain areas. Interestingly, these mice also show a blunted c-Fos induction by the specific serotonin releaser

dexfenfluramine, a measure of neuronal activation in response to serotonin, in several forebrain regions. Thus, serotonin neurotransmission appears to be attenuated in these animals.

In conclusion, a partial disruption in BDNF expression results in functional deficits in serotonergic neurotransmission, and in serotonin-related behavioral abnormalities, i.e., heightened aggression and increased alcohol consumption and preference.

BDNF GENE AND RELATED SIGNALING: ROLE IN ANXIETY AND ALCOHOL DEPENDENCE AND PREFERENCE

Subhash C. Pandey

The neurotrophin, BDNF, is a CREB target gene (Shieh et al., 1998; Pandey et al., 2004), which plays a crucial role in synaptic plasticity in the brain (Schinder and Poo, 2000; Poo, 2001). Dr. Pandey and his group reported earlier that CREB phosphorylation is decreased in the central and medial but not in basolateral amygdala during ethanol withdrawal in rats treated chronically with ethanol. The central nucleus of amygdala is an important brain region, which plays a role in anxiety and motivational aspects of alcohol drinking (McBride, 2002; Koob 2003). They reported that reduction in CREB phosphorylation in the central amygdala is involved in ethanol withdrawal related anxiety in rats (Pandey et al., 2003). Interestingly, they also found that CREB is causally involved in anxiolytic properties of ethanol and a deficiency in CREB promotes higher alcohol drinking behaviors (Pandey et al., 2004). Recently it has been found that the BDNF may be linked with vulnerability to alcohol abuse (Uhl et al., 2001; McGough et al., 2004). Furthermore, ethanol exposure caused changes in BDNF levels in rat forebrain areas (Pandey et al., 1999; Miller, 2004). The role of BDNF in anxiety is less clear. For example, it has been shown that BDNF heterozygous mice do not show anxiety-like behaviors (Montkowski and Holsboer, 1997). On the other hand, BDNF conditional knock out mice are more prone to anxiety-like behaviors (Rios et al., 2001).

Dr. Pandey hypothesized that decreased BDNF signaling in amygdaloid structures may be involved in anxiety and alcohol drinking behaviors (Pandey, 2003; 2004). They examined the role of amygdaloid BDNF signaling in alcohol dependence and preference and also in anxiety-like behaviors in rats. They found that chronic ethanol treatment had no effect on protein and mRNA levels of BDNF as well as phosphorylation of extracellular-regulated protein kinases (p-ERK1/2) in the various structures of amygdala. However, ethanol withdrawal (24 hrs after 15 days of ethanol exposure) produced significant reductions in the protein and mRNA levels of BDNF as well as phosphorylation of ERK1/2 in the central, and medial but not in basolateral amygdala. These results suggest the possibility that the decreased expression and function of BDNF in the central and medial amygdala may be associated with the process of ethanol dependence such as development of anxiety-like behaviors during withdrawal, and this may be promoting the continued consumption of ethanol (Pandey, 2004). Several clinical and preclinical studies suggest an association between anxiety and alcohol drinking behaviors (Koob, 2003;Pandey, 2003; Cappell and Herman, 1972). Dr. Pandey and his group altered the expression of BDNF in various amygdaloid structures using an antisense strategy and then measured anxiety and alcohol drinking behaviors in normal rats. It was observed that BDNF antisense oligodeoxynucleotides (ODNs) infusions into the central or medial but not into basolateral amygdala provoked anxiety-like behaviors and also increased the alcohol preference in rats, which was prevented by BDNF coinfusion. The mRNA and protein levels of BDNF as well as protein levels of p-ERK1/2 and p-CREB were decreased by BDNF antisense but not by sense ODNs infusions, which were restored to normal following BDNF coinfusions. In support of this finding, they have also found that CREB-haplodeficient mice have lower expression of BDNF in the brain and these mice are behaviorally anxious and have higher preference to ethanol (Pandey et al., 2004). Taken together, these results indicate that

decreased BDNF function in the central and medial amygdala may be involved in alcohol preference and dependence and also in anxiety-like behaviors.

BDNF AND CREB: ROLE IN ETHANOL INDUCED NEURONAL DAMAGE

Wataru Ukai, and Toshikazu, Saito

Dr. Ukai and his group investigated the mechanisms of the disruption of neural network by the treatment of ethanol on the process of neural network formation. In their previous study, they demonstrated the ethanol-induced cortical neuronal survival change in the MTT metabolism assay and LDH assay, and the contribution of transcriptional factor CREB reduction to their function change (Sakai et al., 2004). Here, they investigated the effect of BDNF on ethanol-induced neuronal damage. Cortical neurons were treated with 200 mM ethanol for 48 hr in the presence or absence BDNF. Although exposure to ethanol significantly reduced neuronal survival, the reduction was recovered significantly by the treatment of BDNF dose-dependently. From these results they concluded that BDNF might play a protective role in ethanol-induced reduction in neuronal survival. They next investigated the precise BDNF production change by ethanol in the primary cortical neurons, and compared it to the alteration of neuronal survival by ethanol. More than 200 mM ethanol can significantly affect neuronal survival. However, a lower concentration of ethanol significantly decreased the amount of BDNF secreted from the cortical neurons. These results suggest that the low concentration of ethanol effects neuronal BDNF production, and induces a change in neuronal function that may include alterations in synaptic formation, before the induction of neuronal survival disruption.

Many investigators have indicated the function of BDNF in the regulation of neuronal survival (Huang and Reichardt, 2001; Schinder and Poo, 2000). However, BDNF is known to regulate the structure and function of synapses during development, and also regulate refinement of neuronal connectivity in the adult brain (Gorski et al., 2003). So, they next analyzed the neuronal developmental change by ethanol, focusing on the neuronal process formation in the primary cortical neurons. They aimed to investigate whether low concentration of ethanol effects neuronal process formation at a concentration that does not significantly change neuronal cell survival. For the evaluation of process formation, they used immature primary cortical neurons on 1DIV, and observed their morphological change for 48 hr. For the evaluation of neuronal survival change, they used mature cortical neurons on 10 DIV, and detected their morphology and viability change. The exposure to 100 mM ethanol for 48 hr did not influence mature neuronal survival, and the same result obtained in the additional neuronal viability assay of MTT. On the other hand, the suppression of neuronal process formation was observed in the immature 3DIV neurons by exposure to 100 mM ethanol for 48 hr. Then, they investigated cellular signaling changes in regards to this neuronal function change. In the process forming neurons, the BDNF-induced increase in phosphorylated Akt was not changed by the co-treatment with MEK inhibitor (U0126) or 100 mM ethanol, in the western blot analysis. On the other hand, the BDNF-increase in induced phosphorylated ERK and phosphorylated CREB were reduced by the co-treatment with U0126 or ethanol. These results indicate that the ERK/CREB signaling pathway plays an important role for the neuronal function of process formation, and ethanol inhibits process formation through reduction of ERK/CREB signaling in the process forming neurons. The other neurotrophic factor-signaling molecule, Akt seemed to be less important in the function of process formation.

Alteration of the cAMP-signaling pathway, especially a BDNF production change by ethanol, seems to induce neuronal cell damage. Ethanol inhibited neuronal process formation in the lower concentration that has little effect on neuronal cell survival. This may be also related to the reduction in BDNF production by ethanol exposure. The above results suggest that changes

in ethanol-induced BDNF production may cause impairment of brain networks by both reduction of survival and inhibition of neuronal process formation.

SUMMARY

The data presented in this symposium suggest the complex role of BDNF signaling in behavioral and pathologic consequences of ethanol exposure. Data presented by Dr. Chandler suggest that alcohol may enhance the BDNF expression via NMDA receptor linked activation of ERK and CREB signaling without directly modulating the BDNF activation in cortical neurons. Further data presented by Dr. Ukai provided evidence that BDNF is important in ethanol-induced reduction in survival of cortical neurons. Dr. Crews presented data, which suggests that CREB is crucial in the regulation of ethanol-induced neurogenesis in the hippocampus. Dr. Hensler's data provided evidence that BDNF regulates the function of 5-HT1A receptors and decreased function of these receptors in BDNF deficient mice may be involved in higher alcohol preference. The data presented by Dr. Pandey suggest that decreased function of BDNF in the amygdala may be important in the anxiety-like and alcohol drinking behaviors of rats. Taken together these data provided evidence to suggests that BDNF and related signaling play an important role in pathophysiology of alcoholism.

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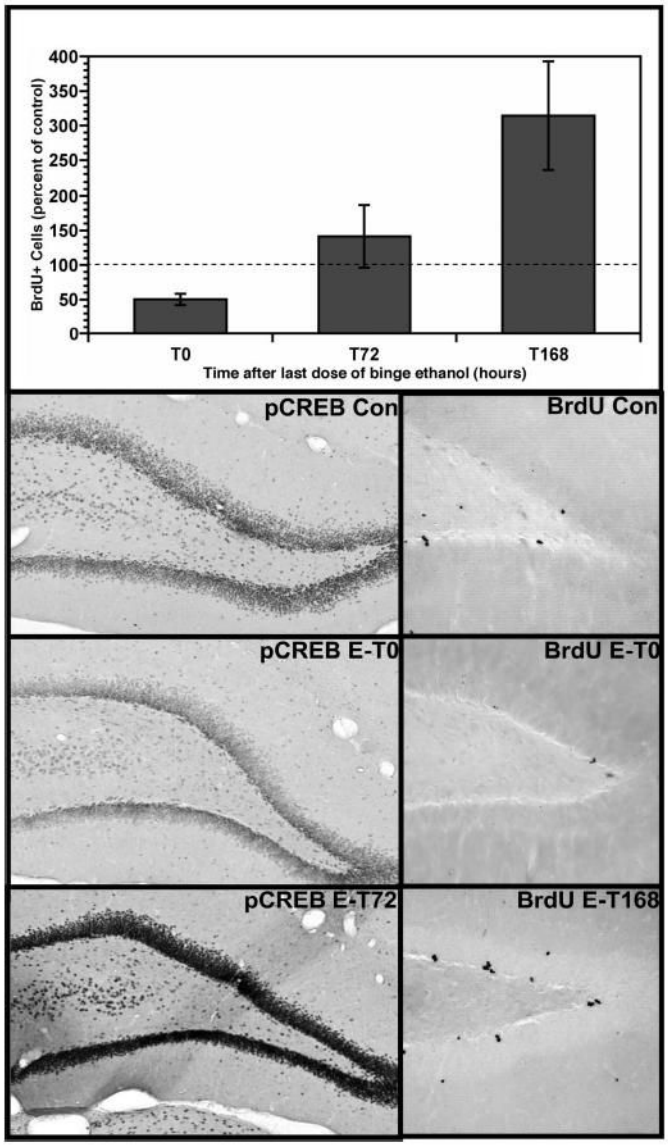

Fig. 1.

Neurogenesis and pCREB Immunohistochemistry in dentate gyrus of binge alcohol treated rats. Rats were treated with a 4-day binge treatment that induces alcohol dependence, causes dentate gyrus degeneration and reduced neurogenesis. The upper bar graph shows neurogenesis at three time points relative to pair fed controls. Note that after four days of binge ethanol treatment (T0), neurogenesis is decreased whereas during abstinence for 72 hrs (T72) or 168hrs (T168) neurogenesis is progressively increased. Left panels: pCREB immunohistochemical staining in control rats (upper left panel, pCREB Con) is greater than that found in rats after four days of binge intoxication (middle left panel, pCREB E-TO). In rats abstinent from alcohol for 72 hrs staining for pCREB is greatly increased (lower left, pCREB E-T72; Bison and Crews, 2003). Right panels: BrdU immunohistochemistry is used to assess proliferation of neuroprogenetor cells. Adult controls have a modest number of cells stained (upper right, BrdU Con), whereas after four days of binge treatment there are fewer cells (middle right, BrdU E-T0). However, during abstinence for 196 hrs there is a tremendous increase in BrdU+IR cells in the dentate gyrus (lower right, BrdU E-T168). Chronic treatment with rolipram to increase pCREB can increase neurogenesis (Nakagawa et al., 2002). It is possible that there is a delay

between the increase in pCREB and the time required to induce prosurvival and trophic factors that contribute to the increased neurogenesis observed during ethanol abstinence (Nixon and Crews, 2002; 2004).