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Pandey Receives 2010 Bowles Lectureship Award

Subhash C. Pandey, Ph.D., received the 2010 Bowles Lectureship Award on April 26 for his contributions to our understanding of the causes, prevention and/or treatment of alcoholism and alcohol abuse. Pandey was selected for his outstanding discoveries of epigenetic effects of alcohol exposure that promote anxiety and lead to greater alcohol self-administration in animal models. His groundbreaking work identifies new targets for alcoholism therapy and demonstrates how alcohol exposure can have long-lasting effects on gene function through alterations in DNA methylation.

Left to Right: Kathleen Sulik, PhD, Clyde Hodge, PhD, Subhash Pandey, PhD, Fulton Crews, PhD, and A. Leslie Morrow, PhD.

CAS Director Fulton Crews presented Pandey with the prestigious Award that carries a $5,000 stipend. Pandey graciously accepted the honor and presented his lecture entitled, "Alcoholism and Anxiety: A Perspective from Molecular and Epigenetic Studies," to colleagues, faculty and students at UNC. Pandey is the Director of Neuroscience Alcoholism Research and Professor of Biochemistry in Psychiatry, Anatomy and Cell Biology at the University of Illinois at Chicago. He has become a leader in the field of alcoholism research. Pandey hopes to one day provide a basis for designing drugs to treat alcohol abuse and anxiety disorders.

"Epigenetic effects of alcohol are profound, showing how environment and genetics interact to increase alcoholism risk," said Bowles Associate Director A. Leslie Morrow. "Pandey's work has challenged all of us to consider the role of epigenetic mechanisms in the myriad of pathways influenced by ethanol abuse. His work will likely lead to an explosion of new evidence in this area."

Introduced in 1997, the annual Bowles Lectureship Award honors distinguished researchers that have made significant contributions in the alcohol research field. Please visit our web site to view a list of previous awardees: http://www.med.unc.edu/alcohol/bowles. ■

The Bowles Center for Alcohol Studies **Tel.** 919-966-5678 **Fax.** 919-966-5679 **Web** www.med.unc.edu/alcohol

To become involved in our mission, call Elizabeth Thomas at 919-966-4977 or email ethomas@med.unc.edu.
For treatment information call UNC Health Care's Alcohol and Substance Abuse Program at 919-966-6039 or 888-457-7457.

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A. Leslie Morrow, Ph.D., Editor-in-Chief; Elizabeth Thomas, Managing Editor; Jane Saiers, Ph.D., Science Writer
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Center Line

Bowles Center for Alcohol Studies • School of Medicine, University of North Carolina at Chapel Hill
Our mission is to conduct, coordinate, and promote basic and clinical research on the causes, prevention, and treatment of alcoholism and alcoholic disease.
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Crews Lab Shows that Neuroimmune Activation Contributes to the Neurobiology of Addiction

The nervous and immune systems are key sensors of the environment. Neuro-immune communication improves survival through multiple complex mechanisms that are poorly understood. Neural-immune communication involves the central and peripheral nervous systems, the endocrine system and innate immune signaling first discovered in white blood cells, particularly monocyte-like cells. Infections that activate immune responses also change hormone release and brain activity-modifying behavior. For example, multiple forms of illness that activate innate immune signaling cause "sickness behavior," a syndrome that includes lethargy, depression, anxiety, loss of appetite, loss of energy and motivation, hyperalgesia, and difficulty concentrating.

Crews Research Team: (Left to Right) Leon Coleman, PhD, Qian Jiang, Tonya Hurst, Liya Qin, Ph.D., Fulton Crews, PhD, Shriya Soora Jian Zou, PhD., and Wen Liu, PhD.

Dr. Fulton Crews, John Andrews Distinguished Professor, Professor of Pharmacology and Psychiatry, and Director of UNC's Bowles Center for Alcohol Studies, has advanced the understanding of the role of innate immune signaling in the brain including the discovery that ethanol induction of innate immune signals contributes to the neurobiology of addiction. In a series of studies, Crews first found an important role for chemokines, cytokines, proteases and oxidase enzymes that contribute to alcohol-induced neurodegeneration. Although neurodegeneration is most often associated with Alzheimer's Disease, dementia and neurodegeneration also occur with chronic alcoholism. Crews found neuro-immune activation by infections and/or alcohol create signaling cascades that continue through multiple loops of self-sustaining activation (Fig. 1). Crews and his laboratory discovered that these positive loops of activation of proinflammatory signals contribute to a slow, progressive degeneration that changes the brain and behavior.

In the brain, cells known as microglia sense their surroundings, producing innate immune-signaling molecules. In an animal model of binge alcohol drinking, Crews' group found that alcohol activated microglia and increased brain levels of the proinflammatory chemokines-cytokines, TNFa, IL-1b, and MCP-1(CCL2), as well as oxidases and proteases involved in innate immune activation. The alcohol-induced

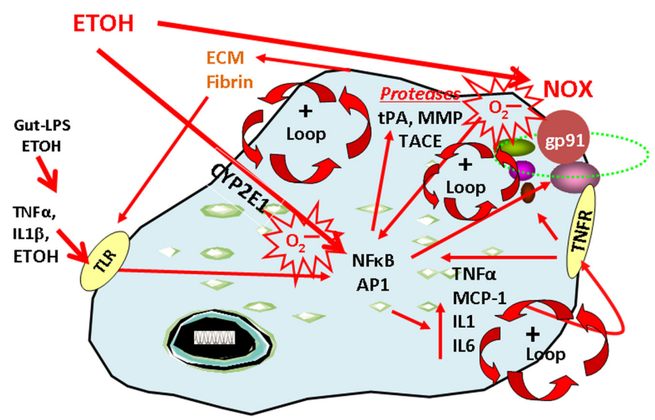

Figure 1: Mechanisms of Alcohol Induction of Brain Innate Immune Genes. Alcohol (ethanol, ETOH) directly activates NFkB transcription, likely through increased levels of reactive oxygen species (O_2^-) from NOX (NADPH oxidase), an enzyme that produces superoxide or through cytochrome P4502E1 (CYP2E1), an enzyme that metabolizes ethanol. Activation of NFkB transcription leads to increased synthesis of cytokine-chemokines, e.g. TNFa, MCP1, IL1 and IL6. Cytokine-chemokines act on cellular receptors to further increase NFkB transcription of innate immune genes in additional brain cells. The NFkB to chemokine to NFkB loop crosses various cell types in brain leading to persistent activation. Activation of NFkB also increases NOX transcription, particularly gp91, creating a NOX-oxidation loop. A third loop involves NFkB to protease-TLR receptor induction to NFkB. Together these loops cause persistent NFkB transcription that alters the neurobiology of the brain. Repeated exposure promotes loops that activate limbic anxiety and disrupt the frontal cortex leading to degeneration.

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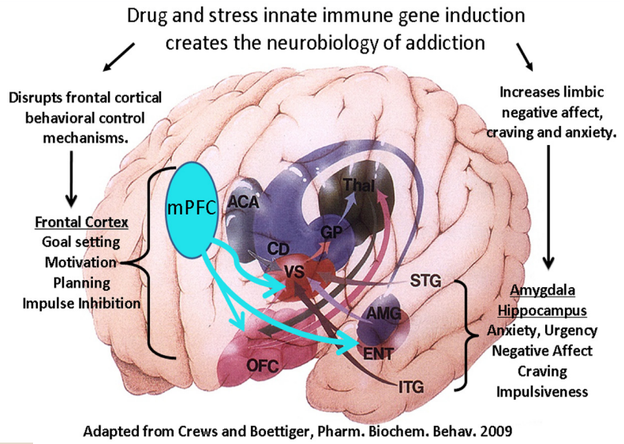

Adapted from Crews and Boettiger, Pharm. Biochem. Behav. 2009

Figure 2: The Neurobiology of Addiction and Innate Immune Gene Induction. Addiction involves compulsive behaviors, due in part, to loss of frontal cortex behavioral control and attention. Frontal cortical disruption and degeneration due to innate immune gene induction alters neurotransmission and increases impulsivity and compulsivity. Innate Immune gene induction in the amygdala, hippocampus and other limbic brain regions alters peptides and other transmitter signaling. Innate immune gene induction increases anxiety, negative affect, craving and wanting - promoting impulsiveness. Innate immune genes induced by drugs and/or stress reduce hippocampal neurogenesis contributing to these symptoms. Thus, innate immune gene induction is hypothesized to create the neurobiology that leads to addictive behavior.

Fulton T. Crews, Ph.D.
Director, Bowles Center for Alcohol Studies

Recent Publications

Induction of innate immune gene expression cascades in brain slice cultures by ethanol: key role of NF-kB and proinflammatory cytokines. Zou J, Crews FT. Alcohol Clin Exp Res. 2010;34(5):777-89.

Long-term suppression of forebrain neurogenesis and loss of neuronal progenitor cells following prolonged alcohol dependence in rats. Hansson AC, Nixon K, Rimondini R, Damadzic R, Sommer WH, Eskay R, Crews FT, Heilig M. Int J Neuropsychopharmacol. 2010;13:583-93.

Impulsivity, frontal lobes and risk for addiction. Crews FT, Boettiger CA. Pharmacol Biochem Behav. 2009;93(3):237-47.

Abstinence following alcohol drinking produces depression-like behavior and reduced hippocampal neurogenesis in mice. Stevenson JR, Schroeder JP, Nixon K, Besheer J, Crews FT, Hodge CW. Neuropsychopharmacology. 2009;34(5):1209-22.

Mechanisms of neurodegeneration and regeneration in alcoholism. Crews FT, Nixon K. Alcohol Alcohol. 2009;44(2):115-27.

Distinct cell proliferation events during abstinence after alcohol dependence: microglia proliferation precedes neurogenesis. Nixon K, Kim DH, Potts EN, He J, Crews FT. Neurobiol Dis. 2008;31(2):218-29.

Increased systemic and brain cytokine production and neuroinflammation by endotoxin following ethanol treatment. Qin L, He J, Hanes RN, Pluzarev O, Hong JS, Crews FT. J Neuroinflammation. 2008;5:10-27.

Increased MCP-1 and microglia in various regions of the human alcoholic brain. He J, Crews FT. Exp Neurol. 2008;210(2):349-58.

CREB and NF-kB transcription factors regulate sensitivity to excitotoxic and oxidative stress induced neuronal cell death. Zou J, Crews F. Cell Mol Neurobiol. 2006;26(4-6):385-405.

BHT blocks NF-kB activation and ethanol-induced brain damage. Crews F, Nixon K, Kim D, Joseph J, Shukitt-Hale B, Qin L, Zou J. Alcohol Clin Exp Res. 2006;30(11):1938-49.

innate immune response was associated with cortical brain regions that show binge drinking-induced brain damage in rats. Crews hypothesized that the innate immune gene induction was not due to the brain damage, but rather, it was the cause of the binge drinking-induced brain damage. Crews' group first extended previous studies suggesting that alcohol activated a key transcription factor, NF-kB, in brain. Crews linked binge drinking levels of alcohol in brain with activation of brain NF-kB and increased expression of chemokines-cytokines, oxidases and proteases. Indeed, Crews demonstrated that alcohol exposure increased NF-kB DNA binding in rat brain, activated microglia, and caused brain damage. Blockade of NF-kB transcription protected against brain damage. The results suggest a crucial role of NF-kB in alcohol-induced brain damage and support the hypothesis that innate immune gene induction contributes to alcohol-induced frontal cortical damage that could underlie the loss of behavioral control associated with alcohol addiction (Fig. 2).

Crews' lab has also investigated the effects of binge levels of alcohol intoxication on stem cells in the hippocampus, a key part of the brain that encodes mood and memory. They found that ethanol inhibition of neurogenesis, the formation of new neurons in brain, was related to ethanol induction of innate immune genes. Loss of hippocampal neurogenesis is associated with bad feelings and depression. In collaboration with Bowles Center Professor Dr. Clyde Hodge, chronic alcohol drinking was found to inhibit neurogenesis and induce depression-like behavior, and both effects could be reversed by antidepressants. Other studies showed that stress-induced loss of neurogenesis is related to innate immune genes, and anti-depressants reverse stress-induced depression-like behavior and stress inhibition of neurogenesis. These and other studies are consistent with Crews' hypothesis that the anxiety and depression-like feelings of alcohol withdrawal overlap with "sickness behaviors" and the psychopathology of many mental diseases and other addictions (Fig 2).

Binge drinking-induced frontal cortical damage is common in alcoholism and associated with cognitive deficits and compulsive behaviors that are characteristic of addiction. Crews' group found that binge drinking induced long lasting

The Director's Column

A. Leslie Morrow, *Ph.D. Associate Director, Bowles Center for Alcohol Studies*

Each year the Bowles Center for Alcohol Studies honors a scientist whose contributions to alcohol research have enduring value to the field. Last May, we honored Subhash Pandey for his key contributions to understanding the acute and epigenetic mechanisms that underlie alcohol drinking and its relationship to anxiety. The Bowles Lectureship Award benefits our faculty and students by the exposure to other leaders in our field. We think the Bowles Award also benefits the field by calling attention to the importance of alcohol research for all humanity. This disease must be stopped in our lifetime, so we work to promote the cause every day and in every way we know.

Crews' work on the mechanisms of alcohol pathology has pointed to pro-inflammatory neuroimmune responses as a major contributor to the detrimental effects of binge alcohol exposure that leads to alcohol addiction. His lab has led the field in this area demonstrating the role of inflammatory cytokines in both animal models and post-mortem human brain. The activation of NF-Band the subsequent inflammatory signals promote not only neuronal loss but also behavioral maladaptations secondary to heavy alcohol exposure. More importantly, Crews' work shows that effects of alcohol on the pro-inflammatory pathways in brain are long–lasting. He is uncovering new mechanisms that will lead to better therapeutics for alcohol-related disease.

This work has important public health implications. The dangers of heavy drinking may not be well recognized by society, particularly those who think they can recover from a big party weekend. Crews' work establishes the long term consequences of heavy drinking – consequences that ultimately affect our mental health as well as performance in all life skills. The science of alcohol exposure is making it abundantly clear that binge drinking is dangerous to our health, with long term consequences that matter to both individuals and society. This knowledge is important for prevention efforts. Tell your friends and family. ■

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reversal learning deficits in both rats and mice. Reversal learning deficits are a form of cognitive dysfunction that is common in addiction when patients perseverate upon an incorrect choice and can't seem to relearn the correct choice in a task. This work prompted studies of human alcoholic brain. Crews undertook a postmortem study of the brains of alcoholics and found that human alcoholic brain showed increased innate immune molecules similar to those found in rat and mouse brain following binge alcohol treatment. Post-mortem human alcoholic brain showed increased innate immune gene expression, specifically increased the proinflammatory chemokine MCP-1 and increased microglial markers. In mice, binge alcohol treatment leads to a persistent increase in MCP-1, consistent with activation of the cytokine and chemokine loops that sustain innate immune activation (Fig.1). These studies suggest that innate immune genes contribute to frontal cortical neurodegeneration and the cognitive deficits associated with the poor decision-making common in alcoholism. Further, more recent studies in other laboratories have shown that innate immune genes regulate alcohol drinking behavior and may contribute to the genetics of alcoholism. Together, these studies suggest that many of the behavioral manifestations of abstinence that promote alcoholic relapse are also related to increases in innate immune gene expression in brain.

"We are interested not only in the neural correlates of excessive alcohol consumption but also the behavioral correlates as they relate to innate immune gene induction," says Crews. "What does alcohol-induced brain damage mean for the functioning of the alcoholic? Our research, considered in the context of others' findings, suggests that excessive alcohol consumption creates a neuroinflammatory response that mimics the neurobiological changes associated with addiction. One aspect involves a hyperglutamatergic-diffuse excitation state that contributes to confused decisions. Due to this decreased ability to focus and discriminate, the alcoholic defaults to automatic behaviors, to habits, and to anxious "wanting" that is often maladaptive. Defaulting to automatic behavior is a big part of addiction.

"We hope to use our knowledge of the molecular mechanisms of innate immune activation loops to work toward interventions and treatments that improve mental health and brain function for alcoholics and others suffering from brain diseases related to innate immune activation in brain." ■

Congratulations!

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Dr. A. Leslie Morrow

on Receiving the

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Distinguished Professorship