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Spotlight on Young Investigators: Patrizia Porcu

Neurosteroids are endogenous neuromodulators that are produced in the brain, adrenal glands and gonads. These steroids have potent effects on neurotransmission mediated by gamma-aminobutyric acid type A (GABA-A) receptors inducing anti-anxiety, anticonvulsant, sedative and cognitive effects. The most potent and effective steroids of this type are known as allopregnanolone or THP and tetrahydrodeoxycorticosterone or THDOC.

Bowles CAS Investigator Patrizia Porcu, Ph.D., Assistant Professor of Psychiatry, studies the role of neurosteroids in alcohol sensitivity and alcohol's effects on levels of THP and THDOC in various species. To facilitate her studies, she recently developed a highly specific gas chromatography/mass spectrometry technique and validated the assay for accuracy and reproducibility. This assay has allowed her to extend her studies to humans, where she has demonstrated that administration of precursor steroids, including progesterone or pregnenolone, increases levels of GABAergic neurosteroids in serum.

Acute ethanol administration increases serum and brain levels of THP and THDOC in Sprague Dawley rats and this effect contributes to alcohol's actions and increases sensitivity to alcohol. Porcu has found that plasma and brain levels of the neurosteroid deoxycorticosterone are also correlated with ethanol sensitivity across 42 different mouse strains. She is exploring the genetic basis of this difference in deoxycorticosterone levels because low ethanol sensitivity is predictive of alcoholism risk in humans and heavy drinking in rodents. Indeed, she received pilot funding from a NIAAA-funded consortium to explore this idea and will present her genetic analysis this summer at the Research Society on Alcoholism meeting.

Patrizia Porcu, Ph.D.

Porcu has also studied species differences in the ability of alcohol to increase neurosteroids circulating in the blood. The results showed clear species differences in response to alcohol, among rats, mice and cynomolgus monkeys, with rats being the only species showing increased levels of THP and THDOC at comparable ethanol doses. Studies are underway to determine if these differences are related to the propensity to drink alcohol voluntarily.

Because neurosteroids contribute to ethanol sensitivity in both rats and humans, Porcu and colleagues in the Bowles CAS have proposed that they play a role in preventing excessive alcohol consumption in healthy subjects. Reducing excessive alcohol abuse reduces risk of alcoholism. Thus, neurosteroids may have therapeutic utility to increase ethanol sensitivity in those people with innate alcohol tolerance or restoring ethanol sensitivity in alcohol-dependent individuals. This remains an unanswered question, and Porcu's work will help lead us to the answer. ■

The Bowles Center for Alcohol Studies

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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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It's in the Molecules: Hodge Laboratory Elucidates Subcellular Mechanisms of Alcohol Dependence

I don't know why I took my first drink, and I don't know why I continue to drink. I do know that, from the first drink, alcohol felt right. It felt right for years, until my body and mind started to break down and my life as I knew it fell apart before my eyes. Now, even when alcohol does not feel right, when I know it is not right, I can't rid myself of it. I cannot stop drinking. It seems like alcohol has a grip on my very molecules.
~Jessie, an alcoholic

Describing her personal struggles with alcohol abuse, Jessie perhaps could not know how apt her characterization of alcoholism is from the perspective of addiction science. Alcoholism is not a failure of self-discipline or the sign of a weak will as it was regarded decades ago, before neuroscientific research began to elucidate its physiological underpinnings. Rather, alcoholism is a disease characterized by pathophysiological changes in the body and brain – a disease as real as asthma or cancer or hypertension – that leads to maladaptive behaviors. In recent years, pathophysiological correlates of alcoholism have been identified at the subcellular level, and molecular mechanisms that could mediate the inability to stop drinking are being discovered. The research bears out Jessie's assertion: alcoholism is in the molecules.

A fundamental aspect of human (and animal) nature is that we repeat those behaviors that accomplish a goal, or bring pleasure. The process by which certain actions become repetitive is called *positive reinforcement*. Simply defined, positive reinforcement is the ability of positive, or pleasurable, events to increase the likelihood of the behavior that produces the event. Much like food when we are hungry, shelter when we are cold, or companionship when we are lonely, alcohol is a powerful positive reinforcer. It is critically important from both a scientific and clinical perspective to understand how alcohol alters the brain to promote continued drinking.

Hodge Lab (left to right): Jaqueline Lee, Sara Faccidomo, PhD, Reggie Cannady, Marina Spanos, PhD, Clyde Hodge, PhD, Michael Salling, Rebecca Fanelli, Kristen Fisher, Joyce Besheer, PhD, Christina Galunas, and Sarah Holstein, PhD

Dr. Clyde W. Hodge, Professor in UNC's Departments of Psychiatry and Pharmacology, and member of the Bowles Center for Alcohol Studies, is at the forefront of research to elucidate the molecular underpinnings of how alcohol serves as such a powerful reinforcer. Using animal models, he seeks to understand the functional involvement of molecular signaling pathways in alcohol drinking and relapse. Hodge's work is based on the premise that, in alcoholism, as in other diseases, behavioral pathologies such as excessive drinking are mediated by molecular pathologies in brain systems that control natural behavioral processes, such as positive reinforcement. Hodge is particularly interested in the molecular pathologies that support behavioral pathology in the early stages of alcoholism—for example, increasing intake of alcohol over time.

Hodge and his laboratory have focused on the mitogen-activated protein kinase (MAPK) system. Found in the cells of all plants and animals, the MAPK system is a group of molecular signaling pathways that mediate numerous cellular activities and functions such as growth, differentiation, and inflammation. MAPK signaling pathways can also regulate the activity of genes and thereby can affect long-term, or enduring, functions such as learning, memory, and addiction. Of several MAPK signaling pathways that have been discovered, one—the extracellular signal-regulated kinase

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Education and Training

Ph.D., Experimental Psychology and Behavioral Pharmacology, 1991; M.S., Experimental Psychology, 1989, Auburn University, Alabama; B.S., Psychology, University of Alabama, Birmingham, 1986.

Publications

Schroeder JP, Spanos M, Stevenson JR, Besheer J, Salling M, **Hodge CW**. Cue-induced reinstatement of alcohol-seeking behavior is associated with increased ERK1/2 phosphorylation in specific limbic brain regions: Blockade by the mGluR5 antagonist MPEP. Neuropharmacology 55(4):546-54; 2008.

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Besheer J, Grondin JJM, Cannady R, Sharko AC, Faccidomo S, **Hodge CW**. MGlu5 receptor activity in the nucleus accumbens is required for the maintenance of ethanol self-administration in a rat genetic model of high alcohol intake. Biological Psychiatry; 67(9): 812-22; 2010.

Website

www.med.unc.edu/alcohol/ hodge

(ERK)/MAPK pathway—appears to be particularly important in regulating gene activity to effect long-term changes in brain structure and function such as those involved in addiction. Because of its apparent role in general positive reinforcement, memory, and other adaptive processes, the ERK/MAPK pathway is a particular focus of the Hodge laboratory's work. Their recent research has been directed at testing the hypothesis that voluntary alcohol drinking, such as that involved in the early stages of alcoholism, produces adaptations in the ERK/MAPK pathway that functionally regulate behavioral pathologies associated with alcoholism.

In a recent series of experiments, Hodge and his colleagues have studied the effects of SL 327, a substance that inhibits the activity of the ERK/MAPK pathway, on self-administration of alcohol in rat and mouse models. The drug self-administration method is a means of studying the reinforcing, or rewarding, effects of drugs, including alcohol and other substances. In this method, animals are trained to make a response—to press a lever a certain number of times, for example—in order to obtain reinforcing substances such as alcohol or sugar. The frequency with which the animals press the lever to obtain the reinforcing substance reflects the degree to which the substance is "reinforcing." Generally speaking, the higher the frequency of responding for a substance, the more rewarding the substance is. Hodge and his colleagues first discovered that in models of relapse to ethanol self-administration activated a neuronal signaling pathway, e.g. the ERK/MAPK pathway. He then discovered that, in rats trained to press a lever for alcohol, administration of a low dose (30 mg/kg) of the ERK/MAPK pathway inhibitor SL 327 increased alcohol self-administration (Figure). At the low dose, SL 327 did not increase lever pressing for sucrose, a result demonstrating that the increased responding is at least to a degree specific to alcohol and does not apply to all rewarding substances. In addition, at the low dose, SL 327 increased alcohol self-administration without affecting the animal's locomotor activity, a finding that suggests that the increase in alcohol drinking is not secondary to an effect of SL 327 on locomotor activity. The results of this study suggest that activation of ERK/MAPK is a fundamental effect of alcohol in the brain that supports self-administration – that is, when ERK/MAPK activity was blocked, more alcohol was required to achieve the neural effect. Hodge's research is groundbreaking in being the first to show that this key pathway functionally regulates self-administration of alcohol.

The inhibition of alcohol self-administration by inhibition of the ERK/MAPK pathway appears to be specific to that pathway and does not apply to all MAPK pathways generally, as supported by the additional finding that inhibition of a MAPK pathway known as the c-Jun N-terminal kinase (JNK) pathway did not increase lever pressing for alcohol. Mice administered the JNK/MAPK pathway inhibitor AS 601245 did not increase lever pressing for alcohol as they did upon administration of low-dose SL 327. Instead, AS 601245 dose-dependently decreased lever pressing for alcohol (Figure).

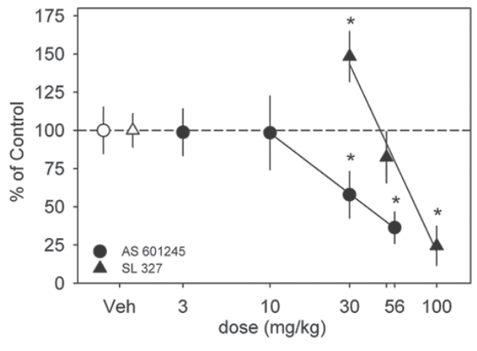

Figure: Modulation of ethanol self-administration by the MAPK inhibitors AS 601245 (filled circles), and SL 327 (filled triangles). AS 601245 inhibits the JNK/MAPK pathway; SL 327 inhibits the ERK/MAPK pathway. Data are pictured as a percentage difference from control animals (represented by the open symbols) in the dose of alcohol consumed in the lever-press alcohol self-administration paradigm. Control animals received an inactive saline vehicle rather than active AS 601245 or SL 327. The asterisks denote statistically significant differences from saline vehicle. From Faccidomo et al. Psychopharmacology 2009;204:135-147.

The Director's Column

Fulton T. Crews, Ph.D.
Director,
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The biological factors that underlie alcohol-induced actions in brain are multifaceted and complex. Dr. Porcu's studies on neuroactive steroids that are elevated by alcohol are at the cutting edge of factors that regulate the acute response to alcohol, which in part determines how much you drink. Although alterations in steroids have long been associated with alcohol abuse and other mental diseases, the role of neuroactive steroids has only recently been elucidated and new types of neuroactive steroids are continually being found. Porcu's discoveries are notable in that she has both developed methods to measure neuroactive steroids and used these methods to establish principle compounds and responses to alcohol. Neurosteroids appear to regulate alcohol sedative responses. Studies have found that young individuals with a low sedative response to alcohol have a greater risk of becoming alcohol dependent in their lifetime. The increased risk is likely due to increased drinking, since sedated individuals will reduce their drinking. Understanding how neurosteroids impact responses to ethanol will contribute to understanding what regulates the quantity of alcohol people drink. Alcohol abuse, stress and steroid hormones converge on a variety of mental dysfunctions within unknown etiology. These studies will contribute to understanding the biological mechanisms that control acute alcohol intake and sensitivity to alcohol sedation.

The Hodge laboratory models drinking behavior and relapse drinking, a model of increased drinking following drinking experience followed by abstinence with a return to access to alcohol that results in a pronounced increase in drinking quantity and effort (lever pressing) to obtain alcohol. His discovery of MAP kinase activation during drinking following abstinence is a new target in his efforts to find medications useful for alcohol dependence. His earlier discoveries of mGLuR5 regulation of drinking behavior have exploded within the addiction community with large numbers of investigators currently studying this potential therapeutic target. The Hodge laboratory approach of identifying targets and then following with pharmacological interventions continues to define new and novel ways to reverse compulsive, excessive alcohol abuse. In time, these studies will improve efforts to treat and reverse excessive drinking. ■

CONGRATULATIONS
to
Dr. Clyde Hodge,
UNC Faculty Mentoring Awardee

Sponsored by the Carolina Women's Leadership Council, this award honors faculty members who go the extra mile to guide, mentor and lead students and junior faculty as they embark on an innovative career in research.

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In contrast to the effect of low-dose SL 327, which increased alcohol self-administration, high doses of SL 327 decreased alcohol self-administration as well as sucrose self-administration. Similarly, high doses of the JNK/MAPK pathway inhibitor AS 601245 decreased both alcohol self-administration and sucrose self-administration. The reduced lever pressing for alcohol and sucrose at higher doses of the MAPK pathway inhibitors is likely to have been secondary to an effect on locomotor activity: the higher doses of the MAPK pathway inhibitors reduced locomotor activity, as well as alcohol self-administration, in the mice. These findings suggest sedation contributes to reduced responding to ethanol and sucrose self-administration.

In other work, the Hodge lab is studying how ERK/MAPK activity changes in the brain under different conditions of alcohol exposure including voluntary drinking, abstinence, and re-exposure to alcohol after a period of abstinence. Their research suggests that chronic voluntary drinking of alcohol decreases ERK activity in the amygdala and the nucleus accumbens—two brain areas known to be important in addiction and reward. In contrast, ERK activity is elevated in the amygdala after 2 days of abstinence. These experiments should shed light on molecular processes involved in relapse as well as those involved during early alcoholism in the escalation of drinking that contributes to the alcoholic's dependence on alcohol.

"In the early stages of alcoholism, individuals engage in voluntary alcohol drinking and abstinence in the absence of physical dependence on alcohol," says Hodge. "This same cycle of drinking followed by abstinence is also observed in the alcohol-dependent individual. Evidence from our laboratory and other researchers indicates that abstinence increases alcohol self-administration even in non-dependent animals. Thus, the neural adaptations that occur in non-dependent drinkers—changes in the activity of the ERK/MAPK pathway, for instance— may initiate the progressive increased drinking associated with alcoholism. By characterizing the role of the ERK/MAPK pathway in the behavioral pathologies associated with excessive alcohol drinking, we are paving the way to understanding the underlying causes of alcoholism and to identify treatments that target the source of the problem. Perhaps one day this research will lead the way to therapeutic interventions that will help reduce the grip that alcohol has on people's lives." ■