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SCHOOL OF MEDICINE

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Crews Leads Adolescent Alcohol Research Initiative

Bowles Center for Alcohol Studies Director Fulton Crews, Ph.D., will lead a multi-institutional, multidisciplinary research initiative entitled, "Neurobiology of Adolescent Drinking in Adulthood" (NADIA), with funding from the National Institute of Alcohol Abuse and Alcoholism, a component of the National Institutes of Health. The five-year collaborative initiative includes faculty from eight universities and research centers across the US focused on understanding the impact of underage drinking on the brain. The ultimate goal of the NADIA Project is to determine if underage drinking causes changes in brain function that persist in adulthood.

Initial studies will focus on the maturation of the brain during the adolescent years. Studies of earlier brain development are based on clear morphological changes that permit definition of specific stages during childhood. The adolescent brain is physically similar to that of adults, making it more difficult to chart later brain development. Additionally, puberty occurs during adolescence and the associated hormonal changes can influence behavior. Recent studies indicate that human brain continues to mature into the third decade of life, so post-pubertal influences are likely important as well.

NADIA investigations plan to relate maturation of motivation, mood, impulsiveness and decision making to changes in brain anatomy, chemistry, neurocircuitry and physiology and therefore provide a more precise definition of adolescent brain development and the mechanisms involved. This may help to explain why adolescents are relatively resistant to the sleep inducing effects of alcohol and particularly sensitive to its cognitive effects.

Delayed development of the frontal cortex, the part of the brain that helps to control emotions and impulses, is thought to contribute to adolescent risk taking, thrill seeking, binge-drinking and poor behavioral control. Brain development is sensitive to many factors, including genetics, environment, stress and drinking history that can complicate interpretation of the consequences of adolescent drinking in humans. The NADIA Project uses rat models of adolescent alcohol exposure that can control all of these variables. Investigators will use these models to mimic episodic human underage drinking and determine the long-term molecular, cellular, physiological, gene expression, neuroanatomical and behavioral consequences. Identification of alcohol-related changes in adult brain and behavior will provide evidence for an "adolescent alcohol syndrome."

The NADIA Project investigators provide a broad range of expertise in neuroanatomy, pharmacology, neurochemistry and psychology. In addition to Crews, the team includes Judson Chandler, Ph.D., (Medical University of South Carolina), Cindy Ehlers, Ph.D., (Scripps Research Institute), Athina Markou, Ph.D., (UC, San Diego), Subhash Pandey, Ph.D., (University of Illinois, Chicago), Catherine Rivier, Ph.D., (Salk Institute for Biological Studies), Linda Spear, Ph.D., (Binghamton University), Martin Styner, Ph.D., (UNC), and Scott Swartzwelder, Ph.D., (Duke University). ■

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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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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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Kash Elucidates the Neurocircuitry of the Brain's Stress and Anti-Stress Systems During Alcohol Drinking and Dependence

Stress is one of the most frequently cited reasons for excessive drinking in survey research and in experimental studies designed to elucidate the motivation for drinking alcohol. Stress caused by work, relationships, finances, and acute stressors such as traumatic events are common self-reported reasons for excessive alcohol consumption. Consistent with these self-reports, some studies show that alcohol consumption can increase with stress in both humans and animals and that often the more severe and chronic the stressor, the greater the alcohol consumption. Other research implicates stress as a motivation for continued drinking. Alcoholism is a chronic relapsing disorder that progressively alters stress responses to increase anxiety and negative feelings that promote drinking or relapse to drinking in individuals with alcohol dependence. Stress, accordingly, figures prominently in addiction scientists' models of alcohol abuse and alcoholism and in psychological and behavioral approaches to treatment of alcoholism. The widely acknowledged role of stress in alcohol abuse and alcoholism notwithstanding, most of the specific mechanisms and determinants of stress-associated excessive drinking remain unknown, and the study of the biological contribution of stress to alcohol consumption and dependence is in its infancy. For example, whether or not stress evokes drinking in a given individual appears to depend on numerous factors: genetics, expectations, sense of control over the stressor, past behavior, habitual coping responses, the availability of social support, and the accessibility of alcohol to name a few. Also, the specific cellular and molecular means by which stress influences alcohol consumption and dependence remain largely unidentified. Dr. Thomas Kash, Assistant Professor in UNC's Department of Pharmacology and the Bowles Center for Alcohol Studies, is working to fill some of these knowledge gaps. His research is directed at elucidating the cellular and molecular workings of brain structures and modulators that mediate behavioral responses to stress. Kash refers to the brain structures that promote drinking and anxiety as "stress systems" and those that protect against drinking and anxiety as the "anti-stress systems." The goal of his research group is to understand how these systems shape alcohol and addiction related behaviors on a cellular level, with an eye towards developing new methods of treating these age-old problems.

Kash Research Team: (Left to Right): José Peña, Ana Jijón, Chia Li, Kristen Pleil, Ph.D, Thomas Kash, Ph.D.

A relative newcomer to North Carolina, Kash was recruited to the Bowles Center and UNC, arriving in April 2009 from Tennessee's Vanderbilt University, where he completed his postdoctoral research. Since joining the faculty, he has received several research grants from sources including the National Institutes of Alcohol Abuse and Alcoholism, the Department of Defense, ABMRF/The Foundation for Alcohol Research, and the Integrative Neuroscience Initiative on Alcoholism (INIA). Each grant is devoted to elucidating specific aspects of the brain processes regulating stress and anti-stress systems related to alcoholism, drug addiction, and other neuropsychiatric conditions.

Some of Kash's research focuses on a brain area known as the bed nucleus of the stria terminalis (BNST). The BNST is a key component of the brain's circuitry for mediating stress responses, fear, and anxiety. This region is thought to act as a critical 'way-station' in the brain, receiving information about both the external environment and internal 'mental state' of the animal and then activating distinct responses, such as anxiety, arousal, and stress hormone secretion (Figure). One of the most interesting aspects of this brain circuitry is that the BNST both receives projections from and sends projections to brain regions important for regulation of emotional behavior. This interaction is normally controlled with feedback inhibition, but it has been suggested in certain pathological states, such as anxiety or depression, these interactions may lose their inhibition and become over-active. Human brain imaging studies support this possibility, as activity in the BNST is correlated with anxiety during a behavioral challenge.

In one series of experiments, Kash is characterizing changes of the neurotransmitter serotonin in the BNST and the association of changes in BNST serotonin functioning

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with behavioral manifestations of alcohol withdrawal. Serotonin is known to be involved in psychiatric conditions such as anxiety and depression, and some evidence suggests that serotonin signaling is involved in the high anxiety that can occur during alcohol withdrawal. In preliminary studies, Kash has shown that alcohol exposure alters both serotonin levels and expression of a specific serotonin receptor in the BNST that can increase anxiety. Kash is now testing the hypothesis that serotonergic signaling in the BNST is altered after alcohol exposure with resultant changes in brain function and anxiety-like behavior.

In another series of experiments, Kash is assessing the role of the neurotransmitter dopamine and its receptors in the BNST in acute responses to alcohol. In animals, dopamine appears to be elevated in the BNST after administration of alcohol, and disruption of dopamine signaling in the BNST impairs alcohol-seeking behavior. Based on preliminary data, Kash hypothesizes that the source of the dopamine that underlies alcohol responses in the BNST is a little-studied group of dopamine neurons in a brain region known as the ventral lateral periaqueductal gray (PAG). The PAG has been implicated in a number of stress-regulated behaviors including anxiety, panic responses, and ethanol withdrawal. The cells in the PAG project to the BNST and release dopamine when they are activated. Kash is testing the possibility that that chronic alcohol exposure activates dopaminergic neurons in the PAG with resultant dopamine release in the BNST, and that this activity contributes to aberrant behavior associated with alcohol dependence and withdrawal. If his hypotheses are confirmed, this relatively unstudied population of dopamine-releasing neurons in the PAG could represent a novel target for treatment of alcoholism.

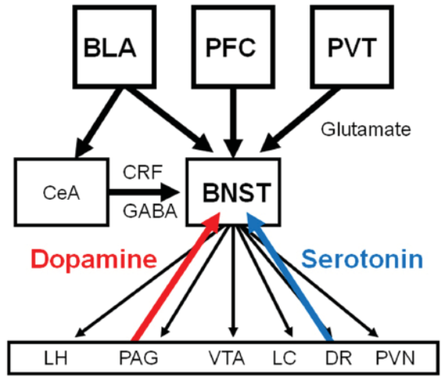

Figure. Diagram outlining the connectivity of the BNST. The BNST receives projections from the basolateral amygdala (BLA), prefrontal cortex (PFC), and thalamus (PVT) as well as the central nucleus of the amygdala (CeA). The BNST projects back to the CeA as well as to numerous brainstem regions important in stress and anxiety responses (LH=lateral hypothalamus; PAG=periaqueductal gray; DR=dorsal raphe; VTA=ventral tegmental area; PVN=paraventricular nucleus of the hypothalamus). New evidence from Kash's work shows a dopamine projection from the PAG to the BNST and a serotonin projection from the raphe to the BNST, as discussed in this article.

Kash also studies the brain's anti-stress systems. "It is relatively easy to make things go bad and to make brain systems malfunction," says Kash. "We are interested in studying what we can do to make things 'better' for the brain and the individual. We want to identify brain systems and brain modulators that lend protection and resiliency against stress. These brain systems and modulators could prove to be targets for interventions that can optimize protection against the negative impact of stressors." Kash is currently using behavioral and cellular techniques to assess the role of neuropeptide Y, a protein hypothesized to be part of the brain's anti-stress systems, in neural signaling in brain areas involved in stress. As part of this research, he seeks to determine whether stress-associated changes in neuropeptide Y regulation are associated with changes in drinking in animal models.

Kash is also attempting to shed light on the genetic characteristics that could underlie resilience to stress. Kash and his colleagues have recently demonstrated that different genetic strains of mice differ in the way that they respond behaviorally to chronic stress. One strain, known as C57BL/6J, exhibits an active coping response to stress—that is, it shows reduced anxiety-like behavior following stress. The other strain, known as DBA/2J, exhibits a passive-coping response by showing increased anxiety-like behavior. In both humans and animals, active coping is generally considered the more effective strategy because it can terminate the stress while serving to maintain engagement with life; the passive coping response is associated with the development of despondence and feelings of helplessness (or behaviors reflecting perceived helplessness, in the case of animals). Kash identified genetic correlates of the differences in coping strategies between the C57BL/6J strain and the DBA/2J strain by using a molecular biological technique known as microarray analysis, in which the genetic contents of a cell are analyzed using high-throughput screening methods to identify genes that are active. Results of his preliminary studies demonstrate that these mouse strains that differ in their coping responses also differ in the expression of genes that regulate the activity of glutamate—the brain's major excitatory neurotransmitter—in the amygdala. The findings are consistent with the possible involvement of glutamatergic activity in the amygdala in coping responses. Kash's results supplement a body of previous research that implicates glutamatergic function in the amygdala in the pathophysiology of several neuropsychiatric disorders, including anxiety disorders and post-traumatic stress disorder (PTSD), which involve maladaptive coping with stress. Kash and his colleagues are following up these findings by exploring the consequences of the different patterns of gene expression in the two mouse strains with respect to function of the amygdala. They are also testing whether manipulations that affect glutamate function can promote active coping responses. Results of these experiments could provide important insights into the genetics of susceptibility and resiliency to stress and could point the way to biological targets for treatments for people suffering from disorders such as PTSD and anxiety disorders. ■

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Publications

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Website
http://www.med.unc.edu/alcohol/kash

The Director's Column: Hope for the Best, Prepare for the Worst

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

Declining financial support over the past several years and a discouraging outlook for the future requires the Bowles Center for Alcohol Studies to prepare and adjust to the ongoing loss of support. This requires reductions in staff and activities. One such loss is this publication. This will be the last Center Line.

The Center Line has been supported primarily by donations. Our donor base has been hurt by the financial crisis. Additional financial pressures come from the State of North Carolina and the National Institutes of Health (NIH). The State has reduced our budget every year for the past five years, and this year we are likely to receive the largest budget cuts in our history. The UNC School of Medicine is under considerable financial pressure and has appointed a committee to make recommendations on the difficult decision of which programs to cut. Further, the NIH has decided to dissolve the National Institute on Alcohol Abuse and Alcoholism (NIAAA). NIAAA grants are the major source of funding for our Center. Federal budget issues will likely reduce the entire NIH budget as well. These changes require us to make difficult decisions to prepare for additional loss of support.

When I became Director of the Bowles Center in 1995, I re-started the Center Line, publishing four issues per year, with Leslie Morrow serving as editor for the past ten years. In addition, we have held the Carolina's Conference on Addiction and Recovery in Chapel Hill, partnering with the Addiction Recovery Institute, for almost a decade. These outreach education activities will also unfortunately come to an end. The mission of the Bowles Center for Alcohol Studies is to conduct, coordinate and promote basic and clinical research on the causes, prevention and treatment of alcoholism and alcoholic disease. The Center will maintain our efforts, but the continuing loss of support and likely loss of future NIH funding require hard choices. We will reduce staff and non-essential activities but plan to continue updating our website with new articles on discoveries. I hope our readers will continue to follow our progress through this site (http://www.med.unc.edu/alcohol/).

The Bowles Center for Alcohol Studies is excited about Tom Kash, our new faculty member, and the NADIA initiative to understand the neurobiology of underage drinking. These exciting events are likely to improve our understanding of the neurobiology of addiction. Understanding this neurobiology will allow efforts to prevent and block the progression to addiction and to help those with chronic relapsing alcoholism stay abstinent. Our faculty are broadening their research to include Post Traumatic Stress Disorder and Depression, two mental diseases with significant co-morbidity and overlapping neurobiological mechanisms with alcoholism. This broadens our research base for funding while staying on mission. Although our financial future is uncertain, our research continues to make discoveries that will brighten the future for all. We are confident that we can adjust to the coming challenges and in time reduce addiction in our communities. ■

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