Bowles Center Member Wins 2005 Gordis Graduate Student Research Award

The Bowles Center for Alcohol Studies' faculty, post-doctoral fellows, and graduate students were all in attendance at the 2005 Research Society on Alcoholism's (RSA) Annual Scientific Meeting held in Santa Barbara, California. Each year, scientists from all over the world gather at the meeting to share their research, learn about advances in research and treatment and network with their colleagues. One highlight of this meeting was the presentation of one of the Society's awards to a Bowles Center researcher.

Jennie Hillmann

Jennie Hillmann, a neurobiology graduate student in the laboratory of Dr. Clyde Hodge, received the prestigious Enoch Gordis Award for her research and poster presentation, *Cessation of Chronic Alcohol Drinking: Effects on Depression-Like Behavior and Neurogenesis in Mice*. The award is presented to one postdoctoral fellow and graduate student to acknowledge their research accomplishments and professional integrity. Hillmann's research focuses on the connection between ethanol drinking and depression and the investigation of the effects of drinking on the creation of new brain cells, known as neurogenesis, as a possible mechanism underlying behavioral changes associated with drinking. "Dr. Hodge has provided me with the opportunity to perform this research as well as a tremendous amount of support, technical advice and mentoring," Hillmann said when speaking on her experience at the Center. She added, "Dr. Kim Nixon and Dr. Fulton Crews, who demonstrated that alcohol consumption can alter neurogenesis, contributed significantly to the birth and execution of this research project."

Overall, our Center contributed greatly to the success of this meeting by coordinating and participating in several symposia sessions. Three sessions were chaired and organized by our faculty: *Cytokines and Alcohol* (Dr. Fulton Crews, chair/coordinator; Dr. Michael Wheeler, participant), *Basis of the GABAmimetic Profile of Ethanol* (Drs. George Breese and Hugh Criswell, co-chairs/coordinators; Dr. A. Leslie Morrow, participant), and *Understanding How the Brain Perceives Alcohol: Neurobiological Basis of Ethanol Discrimination* (Dr. Clyde Hodge, co-chair; Dr. Joyce Besheer, participant). In addition, Center members presented 29 posters and served on several committees.

The Bowles Center for Alcohol Studies

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Center Line

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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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Research Sheds Light on Mechanisms Underlying Fetal Alcohol Spectrum Disorders

Among the most devastating consequences of the widespread use and abuse of alcohol are birth defects. All alcohol-induced birth defects are now referred to under the umbrella term "Fetal Alcohol Spectrum Disorders (FASD)". This includes full-blown Fetal Alcohol Syndrome (FAS; characterized by CNS abnormalities, distinctive facial features, and growth deficiency), and all other morphological, functional and behavioral abnormalities that result from prenatal alcohol exposure.

It is estimated that 40,000 babies per year are born in this country with FASD. This exceeds the combined number born with Down Syndrome and spina bifida. Indeed, maternal alcohol abuse is the leading known, yet preventable, cause of mental retardation in the US. Major factors accounting for the high prevalence of alcohol-induced birth defects include the common use of alcohol by reproductively active females, a high incidence of unplanned pregnancies, and the fact that alcohol can cause damage at any time from the third week of gestation (when pregnancy frequently has not yet been recognized) until term.

The US costs for the treatment and care of individuals with alcohol-induced birth defects are alarmingly high. For example, for FAS alone, NIAAA reported an annual cost estimate of more than $4 billion in 1998. Inclusion of costs for the entire spectrum of disorders greatly increases this number. Additionally, the toll on individuals and families is incalculable.

FASD research is essential in order to increase our understanding of the biological basis for these abnormalities as well as to aid in the development of strategies for their prevention. Dr. Shao-yu Chen, a Bowles Center researcher and Associate Professor of Cell and Developmental Biology, has worked for over ten years in collaboration with Dr. Kathy Sulik on the mechanisms underlying FASD. For this work, he has used both *in vitro* and *in vivo* model systems and has employed agents that can diminish the deleterious impact that alcohol has on embryonic development as a means of exploring alcohol's mechanisms of teratogenesis.

Fetal Toxicology Group: Zhong Lu, PhD, Debbie Dehart, Shao-yu Chen, PhD, Kathy Sulik, PhD, Marianne Meeker, PhD, Elizabeth Meyers, Scott Parnell, PhD

Noteworthy are the protective effects of antioxidants. Research in a number of fields, including cancer biology and aging, has shown the deleterious effects of free radicals and the benefit provided by agents including vitamins C and E and other free radical scavengers or antioxidants that can diminish free radical-mediated damage. Similarly, Dr. Chen's work has shown that antioxidants can prevent alcohol-induced cell death and subsequent structural abnormalities in embryos.

Mouse embryos grown in culture, as well as cultured neural crest cells (NCCs), an alcohol-sensitive cell population, have been studied. Confocal imaging has made it possible to show that in cultured living NCCs, alcohol (ethanol) exposure results in the generation of free radicals. In addition, Dr. Chen has shown that these cells are sensitive to free radical damage resulting from iron overload and that this damage is exacerbated by co-administration of ethanol. Both ethanol and iron-induced NCC death can be prevented by the addition to the culture medium of the iron chelators desferoxamine and phenanthroline, as well as an antioxidant, N-acetylcysteine. Additionally, NCC death is diminished in culture with the addition of a naturally occurring free radical scavenger, superoxide dismutase. This agent also is capable of reducing the incidence of alcohol-induced abnormalities in cultured whole embryos.

With the availability of EUK-134, an agent that has both superoxide dismutase and catalase-like activity (i.e., the ability to scavenge both the superoxide anion and hydrogen peroxide), this work has been extended to an *in vivo* mouse model. In this model, EUK-134 inhibits ethanol-induced cell death and diminishes the incidence and

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Shao-yu Chen, PhD

Experience

Associate Professor, UNC Department of Cell and Developmental Biology and Bowles Center for Alcohol Studies

Education

PhD in biochemistry and plant physiology, Fujian Agricultural University, China, 1991; MS in biochemstry and plant physiology, Fujian Forestry College, China, 1985; BS in Agriculture, Fujian Forestry College, China, 1982

Recent Publications

Chen S-Y, Charness ME , Wilkemeyer MF, Sulik KK. Peptide-mediated protection from ethanol-induced neural tube defects. Dev Neurosci 2005, 27:13-19

Chen, S-Y, Dehart DB, Sulik KK. Protection from ethanol-induced limb malformations by the superoxide dismutase/catalase mimetic, EUK-134. FASEB J. 2004, 18: 1234-1236

Wilkemeyer MF, **Chen S-Y**, Menkari CE, Sulik KK, Charness ME. Ethanol antagonist peptides: structural specificity without stereospecificity. J Pharmacol Exp Ther. 2004, 309:1183-1189

Wilkemeyer MF, **Chen S-Y**, Carrie E. Menkari CE, Brenneman DE, Sulik KK, Charness ME. Differential effects of ethanol antagonism and neuroprotection in peptide fragment NAPVSIPQ prevention of ethanol-induced developmental toxicity. *Proceedings of the National Academy of Sciences of USA* 2003; 100: 8543-8548

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severity of resulting birth defects. Due to their ready induction by ethanol and the ability to easily study their pathogenesis, limb defects were chosen as the developmental end point for analyses. Although in humans the commonly referenced effects of prenatal ethanol exposure are on the CNS and the craniofacies, limb malformations also occur. In mice, acute ethanol exposure at a time in development that corresponds to the human fourth week after fertilization results in fewer digits than normal (see figure 1.A). At the early stage of development when the embryos are exposed to the ethanol, the limbs appear as bud-like outgrowths of tissue. At the distal aspect of the limb buds is a specialized cell population called the apical ectodermal ridge, the presence of which is essential for normal limb development. Alcohol selectively kills these cells. In Dr. Chen's recent study, concurrent maternal treatment with EUK-134 and ethanol substantially reduced the amount of cell death in the apical ectodermal ridge as compared to that observed in embryos exposed to ethanol alone. Additionally, the incidence of limb malformations noted in near term fetuses was reduced by approximately 50%. These results not only support the premise of a causal link between excessive ethanol-induced cell death and subsequent malformations, but also show that free radicals are major players with respect to ethanol's cellular toxicity. This work was published in *FASEB-J*, the journal of the Federation of American Societies for Experimental Biology. Says Dr. Chen: "The results of this investigation demonstrate the capacity of a potentially therapeutic antioxidant compound to significantly diminish major malformations caused by *in vivo* prenatal alcohol exposure. The results give hope that antioxidants could lessen the effects of prenatal alcohol exposure in the children of women who are unable or unwilling to curtail their alcohol abuse while pregnant". Currently, the laboratory is taking this work a step further - investigating the incidence and severity of birth defects in mice whose mothers self-administer ethanol and the potential of antioxidants added to the maternal diet to diminish alcohol's damage.

"The results give hope that antioxidants could lesson the effects of prenatal alcohol exposure in the children of women who are unable or unwilling to curtail their alcohol abuse when pregnant."

In addition to a free radical mechanism, alcohol appears to be damaging to a conceptus as a result of interference with the L1 cell adhesion molecule (L1). In collaboration with Dr. Michael Charness and co-workers at Harvard University, Dr. Chen has employed the whole embryo culture system to show that low concentrations of 1-octanol are able to antagonize ethanol-induced inhibition of L1 and diminish the apoptotic cell death and the adverse developmental effects of ethanol. These findings suggest that ethanol's disruption of L1-mediated cell adhesion contrib-

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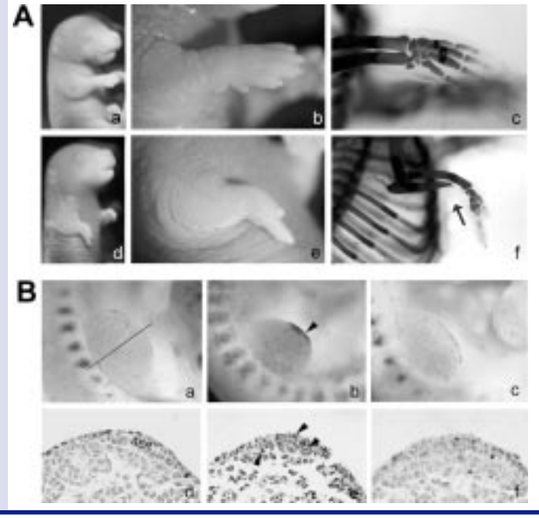

Figure 1. A. Illustrated are the right forelimbs of control (*a - c*) and ethanol-exposed (*d – f*) near term C57BL/6J mouse fetuses. Pictured in (*c*) and (*f*) are skeletal preparations showing loss of the distal part of the ulna (arrow), as well as of bones of the wrist and paw in the ethanol-exposed animal. **B**. Images of whole (*a – c*) and histologically sectioned (*d – f*) limb buds of normal (*a, d*), ethanol-exposed (*b, e*) and ethanol/EUK-134 co-treated (*c, f*) embryos are shown. The plane of section for the images in (*d – f*) is indicated by the line in (*a*). Excessive cell death in the apical ectodermal ridge of the ethanol-exposed limb buds (arrowheads in *b, e*) is evident, as is diminished cell death resulting from EUK-134 co-treatment (*c, f*). (From: *FASEB-J 18:1234-1236, 2004)*

The Director's Column

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

Oxidative stress is a common mechanism of alcoholic pathology. Anti-oxidants have been found to reduce damage in Fetal Alcohol Syndrome (FAS) models as well as models of alcoholic liver disease, pancreatic disease, and some carcinogenic and neurotoxicity studies. Oxidative stress could be caused by induction of cytochrome P450 2E1, cyclo-oxygenase 2, NADPH oxidase, nitric oxide synthetase and other enzymes that generate oxidative free radicals as well as uncoupling of mitochondrial oxidative phosphorylation. These processes are common to multiple tissues. The source of oxidative free radicals in the fetus is unknown, although all of the mechanisms listed above can contribute to alcoholic liver disease.

Cellular oxidative balance is genetically influenced by induction of oxidative free radical producing enzymes as well as the anti-oxidant factors that protect against oxidative free radicals. The environment also impacts oxidative free radical balance including nutrient anti-oxidants and pro-oxidants and other factors that induce oxidative stress such as the components of tobacco smoke. Drs. Chen and Sulik's studies on oxidative stress and alcoholic cellular pathology contribute to our general knowledge of how birth defects occur and how they could be reduced, as well as the mechanisms of similar pathology in liver, heart and other organs.

The basic biological processes that drive alcoholic pathology provide insight into new therapies; however, with alcoholism we are confronted with the challenge of treating both tissue pathology and the alcoholism that causes the excessive drinking. Much effort needs to be focused on treating the alcoholism, especially with regard to FAS. Unfortunately, the best predictor of a woman having an FAS baby is the history of a prior FAS baby. Thus, the tragedy of FAS requires treatment of the mother, and we are not successfully treating a woman's alcoholism if they have even one FAS child. Treatment of women is being studied more fully. Through efforts on all fronts we hope that these studies of fetal development and toxicity lead to healthier babies who grow up to learn how to be healthier parents. Ultimately, the cure for FAS is healthy moms who do not drink.

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utes to its teratogenic actions through the induction of cell death.

Other agents that can reduce alcohol's insult are also being studied in collaboration with the Harvard University group. Of particular interest are the peptides NAP and SAL, which are small fragments of the glial-derived activity dependent neuroprotective protein and activity dependent neurotrophic factor, respectively. Both NAP and SAL are protective at femtomolar concentrations against the neural toxicity of a wide range of compounds and cellular insults. As published by Dr. Chen and his collaborators in the *Proceedings of the National Academy of Sciences*, NAP protects against alcohol-induced embryo toxicity and growth retardation in mice. NAP's antagonism of ethanol inhibition of L1, rather than its broad neuroprotective action, appears to be central to its anti-teratogenic effect. These findings highlight the potential importance of ethanol's effect on L1-mediated cell adhesion in the pathophysiology of FASD.

Extending these investigations, Dr. Chen has recently begun to use a proteomics approach to identify and classify protein networks and pathways that mediate critical events in ethanol sensitive versus non-sensitive regions of the developing mouse brain. The research is designed to reveal proteins that may predispose cells to, or protect them from, ethanol-induced cell death. To date, a number of proteins have been identified whose expression is altered within hours of ethanol exposure. Changes in protein expression have been observed in both non-sensitive and sensitive cell populations. "Identification of those proteins that are differentially expressed in control and ethanol-exposed mouse brains promises to increase our knowledge of the signaling cascades that mediate ethanol-induced cell death and provide insight into the selective vulnerability of certain brain regions affected in human alcohol-related neurodevelopmental disorders," says Dr. Chen. "However, sorting out the key players in the cellular cascades that lead to FASD remains a challenge."