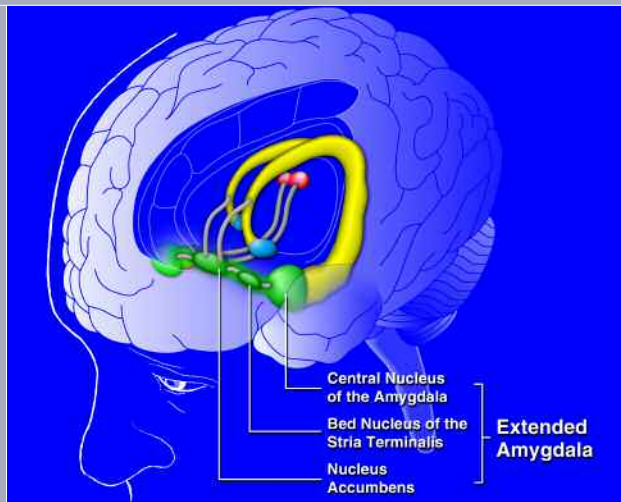


## Committee on the Neurobiology of Addictive Disorders

Functional observations suggest that the neuroanatomic regions for many of the motivational effects of opponent processes associated with drug dependence may involve a common neural circuitry that forms a separate entity, termed the "extended amygdala," within the basal forebrain. The extended amygdala is a macrostructure composed of several basal forebrain structures: the bed nucleus of



the stria terminalis, the central medial amygdala, and a transition zone in the posterior part of the medial nucleus accumbens (i.e., posterior shell). These structures have similarities in morphology, immunohistochemistry, and connectivity, and they receive afferent connections from limbic cortices, hippocampus, basolateral amygdala, midbrain, and lateral hypothalamus. Key elements of the extended amygdala include not only neurotransmitters associated with the positive reinforcing effects of drugs of abuse but also major components of the brain stress systems associated with the negative reinforcement of dependence. Reprinted from Pulvirenti, L., Koob, G.F. *Neurobiologia della dipendenza da cocaina* [in Italian]. *Scienze* 321:18, 1995.



*Eric Zorrilla, Ph.D., Associate Professor*

**COMMITTEE ON THE  
NEUROBIOLOGY OF  
ADDICTIVE DISORDERS**

**STAFF**

**George F. Koob, Ph.D.**  
Professor and Chairman

**Chitra D. Mandyam, Ph.D.**  
Assistant Professor

**Barbara J. Mason, Ph.D.**  
Professor  
Director, Laboratory of  
Clinical Neuropsychopharmacology

**Loren H. Parsons, Ph.D.**  
Associate Professor

**Marisa Roberto, Ph.D.**  
Associate Professor

**Paul Schweitzer, Ph.D.**  
Associate Professor

**Michael A. Taffe, Ph.D.**  
Associate Professor

**Eric P. Zorrilla, Ph.D.**  
Associate Professor

**SENIOR STAFF  
SCIENTIST**

**Robert H. Purdy, Ph.D.**

**STAFF SCIENTISTS**

**Rebecca Crean, Ph.D.**

**Heather N. Richardson,  
Ph.D.\***

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Adjunct Assistant Professor

**Etienne Baulieu, M.D.**  
Adjunct Professor

**Floyd E. Bloom, M.D.**  
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**Kim D. Janda, Ph.D.**  
Adjunct Professor

**Michel Le Moal, M.D., Ph.D.**  
Adjunct Professor

**Pietro Sanna, M.D.**  
Adjunct Associate Professor

**Luis Stinus, M.D., Ph.D.**  
Adjunct Professor

**Luigi Pulvirenti, M.D.**  
Adjunct Associate Professor

**George R. Siggins, Ph.D.**  
Adjunct Professor

**Lars Terenius, Ph.D.**  
Adjunct Professor

**Friedbert Weiss, Ph.D.**  
Adjunct Professor

**SENIOR RESEARCH  
ASSOCIATES**

**Candice Contet, Ph.D.**

**Olivier George, Ph.D.**  
Laboratory Manager,  
Preclinical  
Neuropsychopharmacology

**Sunmee Wee, Ph.D.**

**RESEARCH ASSOCIATES**

**Camryn Allen, Ph.D.**

**Lily J. Alvarez, Ph.D.**

**Marc R. Azar, Ph.D.**

**Pietro Cottone, Ph.D.**

**Maureen Cruz, Ph.D.**

**Alessandra Dicitore, Ph.D.**

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**Laura Orió Ortiz, Ph.D.**

**Francisco J. Pavon Moron,  
Ph.D.**

**Valentina Sabino, Ph.D.**

**Antonia M. Serrano Criado,  
Ph.D.**

**SENIOR STATISTICAL  
CONSULTANT**

**John Light, Ph.D.**

**STATISTICIAN**

**Matthew Marler, Ph.D.\***

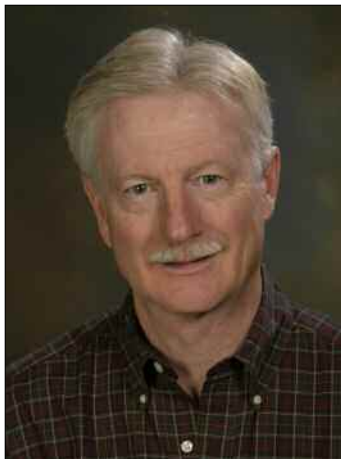
*\* Appointment completed*

## Chairman's Overview

The research programs of the Committee on the Neurobiology of Addictive Disorders are focused on the neurobiological mechanisms involved in motivated and emotional behavior and how these mechanisms are altered with the development of pathologic changes in the CNS, such as the alterations associated with addiction, stress, and eating disorders. Our overall goals are to integrate and translate basic research findings across multiple domains, with a common emphasis on understanding not only the function of the brain emotional systems but also how these systems malfunction in disease. The committee also forms the foundation for the Pearson Center for Alcoholism and Addiction Research, with the goal of developing novel medications for the treatment of addictive disorders.

Neuropharmacologic studies of addiction and stress in my laboratory are covered in the following report. Detailed results of activity in the clinical, neurochemical, neuropsychological, neuroendocrinologic, and neurophysiologic domains are covered in the reports of Drs. Mason, Parsons, Taffe, Zorrilla, Roberto, and Mandyam.

Highlights of the past year are as follows. Barbara Mason was awarded the newly endowed Pearson Family Chair and a developmental center grant from the National Institute on Drug Abuse to explore the neurobiological mechanisms involved in cannabis addiction in young adults. Loren Parsons discovered an exciting new role for endocannabinoids in alcohol and drug addiction. Eric Zorrilla developed novel animal models for binge eating and has begun to explore the neurobiological basis for such dysregulated eating. Marisa Roberto discovered important cellular interactions in the central nucleus of the amygdala linking drugs of abuse with the corti-



*George Koob, Ph.D.*

cotropin-releasing factor- $\gamma$ -aminobutyric acid system, pointing to a key role of alcohol in increasing both corticotropin-releasing factor and  $\gamma$ -aminobutyric acid during the development of dependence. Chitra Mandyam was recruited as a cell biologist to explore the effects of drugs of abuse on neurogenesis in adults, cell-cycle kinetics of proliferating progenitors, and the microenvironment that supports the neurogenic niche in brain structures known to contribute to the cognitive deficits associated with addiction.

## Investigators' Reports

### Neurobiology of Addiction and Stress

G.F. Koob, H.N. Richardson, R.H. Purdy,\* M. Le Moal,\*\* S.H. Ahmed, E. Riley,\*\*\* L. Stinus,\*\* L. Pulvirenti,\*\*\*\* A. Tabarin,\*\*\*\*\* S. Wee, N. Gilpin, C. Mandyam, C. Contet, O. George, L. Orio, K. Misra, D. Ji, S. Edwards, R. Lintz, E. Crawford, R. Schroeder, T. Kimber, M. Cole, M.A. Arends, M. Brennan, Y. Grant

\* VA Medical Center, San Diego, California

\*\* Université Victor Ségalen Bordeaux 2, Bordeaux, France

\*\*\* San Diego State University, San Diego, California

\*\*\*\* Claude Bernard Neuroscience Institute, Pozzilli, Italy

\*\*\*\*\* Université Victor Ségalen Bordeaux 2, Hôpital du Haut-Lévêque, Pessac, France

In the Laboratory of Psychopharmacology, we continue to focus on the neuropharmacologic mechanisms involved in emotional behavior, particularly those involved in stress and negative emotional states. A current major hypothesis is that dysregulation of the mechanisms controlling negative emotional states has a key role in the development of CNS pathologic conditions, such as addiction, stress, and other psychiatric disorders.

#### NICOTINE DEPENDENCE AND CORTICOTROPIN-RELEASING FACTOR

We continue to develop animal models for excessive drug intake and to chart neurocircuitry changes associated with such intake. Previously, we established that prolonged access to cocaine, methamphetamine, and heroin can produce progressive increases in drug intake that are paralleled by decreases in reward function and reflect the development of dependence. New studies suggest that similar escalation in drug intake occurs with prolonged access to nicotine.

In rats with 23-hour access to nicotine, motivational and physical dependence developed, as indicated by physical signs, anxiety, and measures of meal patterns and diurnal activity. A strong increase in nicotine intake occurred with 23-hour access after deprivation. This nicotine deprivation effect was long-lasting and increased with repeated deprivations. In addition, withdrawal from chronic nicotine produced increased anxiety-like responses that were blocked by administration of a selective antagonist of the corticotropin-releasing factor 1 (CRF<sub>1</sub>) receptor. During nicotine withdrawal, increases in extracellular

CRF were detected in the amygdala by using in vivo microdialysis. More compelling, the deprivation-induced increases in nicotine self-administration were blocked by administration of a CRF<sub>1</sub> receptor antagonist, and the antagonist had no effect in nondependent animals self-administering nicotine for 1 hour per day.

#### ALCOHOL DEPENDENCE AND CRF

The animal models of excessive drug intake were extended to alcohol; animals were trained to self-administer alcohol, made dependent on alcohol, and then allowed access to it during acute withdrawal. Withdrawal-induced drinking in these animals was 3–4 times greater than drinking in nondependent animals. Dependence-induced drinking was selectively blocked by systemic administration of selective CRF<sub>1</sub> receptor antagonists, whereas the antagonists had no effect in nondependent animals.

The neurobiological site affected by CRF antagonism appears to be the central nucleus of the amygdala. Previously, we found that administration of a combined CRF<sub>1</sub>/CRF<sub>2</sub> peptide antagonist into the central nucleus of the amygdala dose dependently reversed excessive drinking associated with alcohol dependence. Similar administration of a CRF<sub>2</sub> peptide agonist also dose dependently reversed excessive drinking associated with alcohol dependence. These results suggest a powerful contribution of increased CRF<sub>1</sub> receptor activity and decreased CRF<sub>2</sub> receptor activity in the central nucleus of the amygdala in the motivation for excessive drinking in alcohol dependence. Mice lacking the gene for the CRF<sub>1</sub> receptor also had no withdrawal-induced increases in alcohol self-administration. This finding supports the hypothesis that CRF receptors have a key role in driving the compulsiveness associated with excessive drug intake that is generated from dependence-induced activation of brain stress systems.

#### NEUROBIOLOGICAL ELEMENTS OF COMPULSIVITY IN ADDICTION

These results suggest that a common component of extended access to drugs is the development of compulsive use concomitant with the development of dependence. This compulsive use has face validity for the human condition and heuristic value for exploring the neurobiological mechanisms associated with the development of dependence. A common element in compulsive drug intake associated with all drugs of abuse is activation of brain stress systems such as the CRF system. Preliminary evidence also suggests that the basal forebrain has antistress modulatory systems that may be compromised during the development of dependence

and that contribute to the increased drug seeking. Viral vector-induced overexpression of neuropeptide Y in the central nucleus of the amygdala produced a decrease in alcohol consumption in dependent rats, suggesting that the neuropeptide Y system may act as a neurochemical buffer to the CRF system.

#### MEDICATIONS FOR TREATMENT OF ADDICTION

An additional thrust of the Laboratory of Psychopharmacology and the Pearson Center for Alcoholism and Addiction Research has been to develop novel medications for the treatment of addiction. The combination of information from animal models and data from laboratory studies in humans provides a conceptual framework for developing new medications for the clinic and for validating the animal models. Animal models that have indicated targets hypothesized to be selective for the treatment of addiction include those for extended-access intravenous self-administration, dependence-induced drinking, and binge drinking. The efficacy of drugs such as naltrexone and acamprosate (which are approved by the Food and Drug Administration for the treatment of alcoholism) provide validation for such animal models.

Using the alcohol model of withdrawal-induced drinking, we found that baclofen, a  $\gamma$ -aminobutyric acid-B receptor antagonist, can block alcohol self-administration with an increased effectiveness in dependent animals compared with nondependent animals. Although baclofen has marked sedative effects, other agonists of this receptor may be a target for clinical development.

Additional studies in the development of medications include a collaboration with K.D. Janda, Department of Chemistry, in which novel immunologic and chemical-immunologic approaches are used in the pharmacologic characterization of novel approaches to drug elimination. We found that methamphetamine glycation products induced a self-vaccination that chemically links chronic drug abuse and cardiovascular disease. Chemical-immunologic approaches may be useful for future novel treatments of addiction. In a collaboration with E. Roberts, Department of Chemistry, we are developing novel peptide antagonists. Antagonists to vasopressin  $V_{1a}$  and  $V_{1b}$  receptors are currently being explored in animal models of dependence.

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## Laboratory of Clinical Psychopharmacology

B.J. Mason, K. Coveney, N. Crane, R. Crean, A. Feng, R. Forsyth, H. Hargett, M. Jacobs, J. Light,\* S. Quello, J. Diamant,\*\* F. Shadan,\*\* M. Kyle,\*\* M. Adusumalli,\*\* S. Rao,\*\* A. Begovic,\*\* J. Gleason,\*\* D. Drobos\*\*\*

\* Oregon Research Institute, Eugene, Oregon

\*\* Scripps Green Hospital, La Jolla, California

\*\*\* University of South Florida, Tampa, Florida

The focus of research in the Laboratory of Clinical Psychopharmacology, the clinical component of the Pearson Center for Alcoholism and Addiction Research, is early phase 2 evaluation of medications for treatment of substance dependence. Our primary aim is to reduce the risk of relapse and the signs and symptoms of protracted abstinence, such as disturbances of mood and sleep, associated with an increased risk for relapse. Projects range from proof-of-concept early-phase laboratory studies in humans to longer term, double-blind, placebo-controlled studies of clinical efficacy.

A critical aspect of our conceptual framework is dynamic feedback from the scientists involved in pre-clinical and clinical studies, which are designed to streamline information and provide converging evidence for ultimate clinical use of medications. We also anticipate that the results of the clinical laboratory studies will in turn be useful in the preclinical animal studies to further refine basic research involving animal models and the neuropharmacologic approach. Using this approach, we have identified areas of research that are being translated into long-term studies of the clinical efficacy of various medications for treatment of alcohol, nicotine, and cannabis dependence.

### DEVELOPMENT OF A HUMAN LABORATORY MODEL

We developed a human experimental model of various components of the alcoholism cycle. We use cue

reactivity techniques in the laboratory and assessment of substance use, mood, and sleep under natural conditions. The parameters of the model are used to evaluate potential treatments for various components of the alcoholism cycle. A Merit award and a 5-year renewal of this project by the National Institute on Alcohol Abuse and Alcoholism enable us to use the model to rapidly screen medications that may prevent relapse to drinking.

### CLINICAL RESEARCH

We obtained positive results in our proof-of-concept human laboratory study of a nonaddicting GABAergic modulator in non-treatment-seeking individuals with alcohol dependence. For example, compared with a placebo, the modulator significantly reduced measures of craving in response to alcohol cues. We are conducting a 12-week, double-blind, placebo-controlled, dose-ranging study to evaluate this drug as a treatment for drinking relapse and for the signs and symptoms of protracted abstinence, such as craving, anxiety, and insomnia, that may occur after acute alcohol withdrawal and that can precipitate a relapse to drinking. The study sample will consist of 150 recently abstinent outpatient volunteers who are alcohol dependent. This project is funded by the National Institute on Alcohol Abuse and Alcoholism.

We also received funding from the National Institute on Drug Abuse to conduct a 12-week, double-blind, placebo-controlled pilot study of a GABAergic modulating drug as a potential treatment for cannabis dependence. We found that this drug improved symptoms of cannabis withdrawal and decreased risk and severity of relapse to cannabis use. A confirmatory, large-scale trial is planned.

We recently received funding from the National Institute on Drug Abuse to develop a translational research center for neurobiological studies on the development of cannabis dependence. As designed, the center draws together a critical mass of investigators to explore the neurobiological basis for the development of cannabis dependence, identify the mechanisms for vulnerability to cannabis dependence, and characterize the potential cognitive pathologic changes associated with cannabis abuse and dependence.

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## Neurochemistry of Addiction

L.H. Parsons, L. Alvarez-Jaimes, A. Serrano-Criado,  
J. Pavon-Moron, I. Polis, D. Stouffer

**E**ndocannabinoid signaling is involved in functions such as memory, emotional state, motivation, and reward processing, and converging evidence implicates the endocannabinoid system in the etiology of drug addiction. We focus primarily on the involvement of endogenous cannabinoid signaling in the modulation of drug reward and the development of drug dependence. Our current emphasis is on alcohol-related behaviors and neurochemistry.

We found that voluntary ethanol self-administration is reduced by blockade of cannabinoid-1 (CB<sub>1</sub>) receptors in the nucleus accumbens and posterior ventral tegmental area. In contrast, ethanol intake is not altered by administration of CB<sub>1</sub> receptor antagonists into the prefrontal cortex and basolateral amygdala despite the known influence of these regions on ethanol intake and the relatively high expression of CB<sub>1</sub> receptors in these areas. Using *in vivo* microdialysis, we found that ethanol increases levels of the endocannabinoid 2-arachidonoylglycerol (2-AG) in the nucleus accumbens and ventral tegmental area, but not in the prefrontal cortex. The correlation between the regional selectivity of ethanol-induced increases in 2-AG with the regionally selective effects of CB<sub>1</sub> receptor blockade on ethanol intake supports a hypothesized facilitory influence of 2-AG on alcohol intake.

However, not all data clearly support this hypothesis. For example, although infusions of the CB<sub>1</sub> agonist WIN 55,212-2 into the nucleus accumbens dose dependently increased ethanol consumption, infusions of exogenous 2-AG or the putative 2-AG clearance inhibitor URB602 reduced ethanol intake. Saccharin self-administration was unaltered by administration of 2-AG into the nucleus accumbens, suggesting some specificity of these manipulations to alcohol. Furthermore, microdialysis data gathered in mice deficient in fatty acid amide hydrolase suggest an inverse relationship between levels of 2-AG in the nucleus accumbens and alcohol consumption. Thus, although our data consistently show that ethanol alters endocannabinoid levels in the CNS, the resulting influence of elevated levels of 2-AG signaling on ethanol intake and the receptor system through which this influence occurs remain to be determined.

Endocannabinoids are present in stress-responsive neural circuits, and a growing body of evidence indicates that stress-induced increases in endocannabinoid tone provide negative feedback to attenuate stress responses and reestablish homeostasis. Disrupted endocannabinoid signaling is associated with an inability to adapt to stress and has been implicated in affective disorders, such as anxiety and depression. Maladaptive stress responses, anxiety, and depression are characteristics of acute and prolonged withdrawal in alcoholics, and these negative affective states can be relieved by resumed alcohol intake. This self-medication behavior (negative reinforcement) is theorized to contribute to relapse drinking and excessive alcohol intake.

We have found that ethanol dependence is associated with dysregulated endocannabinoid function in the central nucleus of the amygdala, a brain region critically involved in mediating stress responses. Baseline dialysate levels of 2-AG were significantly reduced in the central nucleus of the amygdala after a 4- to 8-week period of intermittent exposure to ethanol vapor. A further decrease in both 2-AG and anandamide levels occurred during a 12-hour withdrawal period, and deficits developed in CB<sub>1</sub> receptor signaling in the central nucleus of the amygdala during this time. Although renewed ethanol consumption can ameliorate withdrawal-associated deficits in 2-AG in the central nucleus of the amygdala, ethanol-dependent animals were tolerant to this effect.

Because withdrawal-associated excessive ethanol self-administration is reduced by antagonism of receptors for corticotropin-releasing factor, a stress-related peptide, in the central nucleus of the amygdala, we theorized that restoration of cannabinoid signaling in this area of the brain also may reduce withdrawal-associated excessive ethanol intake by reestablishing feedback inhibition of this stress response. Consistent with this theory, infusion of the CB<sub>1</sub> agonist WIN 55,212-2 into the central nucleus of the amygdala dose dependently reduced ethanol self-administration by ethanol-dependent animals, whereas the same doses of the agonist did not alter ethanol intake by nondependent control animals. This finding suggests that chronic ethanol intake and withdrawal produce deficits in amygdalar endocannabinoid signaling that contribute to excessive alcohol consumption by ethanol-dependent rats.

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## Primate Neurobehavioral Laboratory

M.A. Taffe, S.A. Davis

The overuse and abuse of alcohol among adolescents remains a significant and continuing problem. According to the *Monitoring the Future* survey, in 2006, 17% of 8th graders, 34% of 10th graders, and 45% of 12th graders reported consuming alcohol within the 30 days preceding the survey. The majority of 12th graders who use alcohol reported consuming 5 or more drinks on at least one occasion in the preceding 2 weeks. Individuals who begin drinking before they are 15 years old are 4 times more likely to become alcohol dependent than are those who delay drinking until they are 21 years old, suggesting a developmental window for vulnerability to alcohol. Studies indicate that chronic heavy drinking is associated with adverse effects on the CNS and brain functioning, and individuals at familial risk for later alcohol abuse may have premorbid cognitive deficits. The direction of causality linking alcohol exposure with poor cognition, therefore, is difficult to establish in humans, and good animal models are required to determine the mechanisms by which long-term alcohol drinking may interfere with cognitive development.

We created a model in which adolescent and young adult monkeys voluntarily consume binge quantities of alcohol, generating blood alcohol levels in excess of the legal limit, obvious intoxication, and impaired motor performance. We found that individual animals titrate their alcohol intake under a variety of conditions, and it appears that each animal seeks a stable level of intoxication. Drinking patterns are maintained for more than 1 year of chronic access to alcohol twice per day, 5 days per week. We also found that individual differences in impulsivity observed before any access to alcohol are predictive of an individual animal's propensity to consume alcohol when given access. Furthermore, animals with a low level of serotonergic function, indicated by

analysis of cerebrospinal fluid, have a propensity to drink more alcohol than do animals with a higher level. These studies are helping us determine the neurobiological and behavioral variables that have been associated with liability for alcoholism in human adolescents and that are predictive of high alcohol consumption in nonhuman primates.

We have also been examining the effects of chronic alcohol drinking on the gradual acquisition of complex cognitive skills in a model of adolescent schooling. Humans with alcoholism are cognitively impaired, but preexisting differences in cognitive function and preference for alcohol make it difficult to assign lasting cognitive impairment to alcohol exposure. Our major goal was to rule out the contributing effect of alcohol preference and prealcohol cognitive ability by balancing the experimental groups according to these factors.

Our data indicate that repeated alcohol drinking interferes with the ability of animals to learn a visuospatial associative memory task. Furthermore, the deficits in the incremental learning of this task may be related to a specific effect on short-term memory. In a memory-retention task, the alcohol-drinking animals were impaired at retaining information for as short a duration as 5–10 seconds. The pattern of deficits observed suggest an effect of chronic alcohol on frontal cortical mechanisms involved in spatial memory.

We also used this model, in collaboration with H.S. Fox, Molecular and Integrative Neurosciences Department, to show that alcohol use alters the pathogenicity of simian immunodeficiency virus infection in the rhesus model of neuroAIDS. In these studies, chronic alcohol exposure interfered with the initial immune response to infection in the gut, liver, and brain, illustrating potential mechanisms by which humans with alcoholism may be at increased risk of infection after exposure to HIV.

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## Neurobiology of Feeding, Motivation, and Stress

E.P. Zorrilla, L. Steardo,\* A. Tabarin,\*\* S. Iwasaki,\*\*\*  
É. Fekete, Y. Zhao, V. Sabino, P. Cottone, M. Brennan,  
J. Helfers

\* University of Palermo, Palermo, Italy

\*\* Université Victor Ségalen Bordeaux 2, Hopital du Haut-Lévêque, Pessac,  
France

\*\*\* Osaka City University Medical School, Osaka, Japan

**W**e continue to study motivated behavior, with an emphasis on brain reward and stress neurocircuits that control food intake and appetitive behavior. In an ongoing collaboration with B. Conti and M. Sanchez-Alavez, Molecular and Integrative Neurosciences Department, we identified a regulatory role for the cytokine IL-18 in energy homeostasis. Male and female mice that lacked IL-18 overate and became obese in adulthood. Conversely, central or peripheral administration of IL-18 suppressed food intake in fasted wild-type mice without producing malaise. We also showed a metabolic function for IL-18. Female mice that lacked this cytokine had reduced energy expenditure, whereas administration of IL-18 reduced feed efficiency in fasted mice.

We also studied the hedonic control of food intake by using diet-cycling models. Intermittent access to palatable (i.e., highly preferred) food decreased the reinforcing efficacy and intake of a less preferred, but otherwise acceptable, food. Rats withdrawn from access to palatable food also had increased anxiety-like behavior and activation of stress-regulatory corticotropin-releasing factor (CRF) systems in the central nucleus of the amygdala, changes not detected when palatable food was available. Restoration of access to palatable food led to overeating. Administration of a CRF<sub>1</sub> receptor antagonist blocked the increase in anxiety-like behavior and normalized the reinforcing efficacy of less preferred food while also blunting the observed changes in food intake. Despite eating no more overall than rats that were fed chow, diet-cycled rats ultimately became heavier and fatter, with elevated levels of adipokines associated with metabolic syndrome. Thus, intermittent access to palatable food had several effects that resembled those of addictive substances and which were reduced by CRF<sub>1</sub> receptor antagonists.

We also combined pharmacologic and transgenic approaches to study the functional importance of urocortin-CRF<sub>2</sub> receptor systems in the control of food

intake. Local injection of urocortin 3 into the hypothalamus suppressed food intake via a CRF<sub>2</sub> mechanism more potently than did administration into the hind-brain. Urocortin 3 prolonged satiety after a meal and slowed the rate and regularity of feeding within meals. Intracranial administration of urocortin 3 did not elicit anxiety-like or malaise-like behavior, whereas selective CRF<sub>1</sub> receptor agonists did. Mutant mice lacking CRF<sub>2</sub> had increased nocturnal food intake and abbreviated stress-induced anorexia, supporting a physiologic role for the CRF<sub>2</sub> receptor in the control of food intake. Most recently, we showed that urocortin 2 is less potent, but has normal efficacy, in rats genetically vulnerable to diet-induced obesity compared with rats resistant to obesity. The ability of urocortin 2 to suppress high-fat food intake in obesity models is being evaluated.

Previously, in a collaborative study with K.D. Janda, Department of Chemistry, we showed that active immunization against the N-terminal residues of ghrelin, an acylated 28-residue stomach hormone that stimulates appetite and reduces lipid oxidation, slowed fat gain in rats proportional to the degree to which the rats generated antibodies that bind ghrelin. This research on active immunization against ghrelin continues, with a focus on the development of haptens, conjugated to feasible carrier proteins and designed not to elicit undesired cytotoxic T-cell responses, that can be commercialized. This work also involves further study of mechanism, efficacy, and safety. In addition, we recently found that acute administration of a catalytic antibody that facilitates degradation of ghrelin to its inactive desacyl form increases energy expenditure in fasting mice and blunts refeeding after fasting. This finding suggests the practicality of passive administration approaches, which we are investigating by using several high-affinity, N terminally directed monoclonal antibodies.

Finally, we also found, partly in collaboration with G.F. Koob, Committee on the Neurobiology of Addictive Disorders, and A. Roberts, Molecular and Integrative Neurosciences Department, that previously known and novel small-molecule selective CRF<sub>1</sub> receptor antagonists reduce anxiety-like behavior and blunt the excess drug self-administration that occurs in rodent models of alcohol, nicotine, and cocaine dependence.

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## Neurophysiology of Peptides and Drugs of Abuse

M. Roberto, M. Bajo,\* M.T. Cruz, N. Gilpin, P.J. Kenny,\*\* G.F. Koob, R. Messing,\*\*\* L.H. Parsons, P. Schweitzer, G.R. Siggins,\* B. Tabakoff,\*\*\*\* E.P. Zorrilla

\* Molecular and Integrative Neurosciences Department, Scripps Research

\*\* Department of Molecular Therapeutics, Scripps Research

\*\*\* University of California, San Francisco, California

\*\*\*\* University of Colorado, Denver, Colorado

The central nucleus of the amygdala is a brain region crucial in mediating the behavioral effects of acute and chronic drug consumption. Our overall aims are to uncover the physiologic mechanisms that underlie the acute action of ethanol on synaptic transmission in the central nucleus of the amygdala; the involvement of neuropeptides such as corticotropin-releasing factor (CRF), neuropeptide Y, and those of the endocannabinoid and opioid systems; and the neuroadaptations associated with ethanol dependence.

CRF is an important mediator of anxiety and ethanol intake. The CRF and  $\gamma$ -aminobutyric acid (GABA) systems are intimately related and balanced at the synaptic level. Our in vitro and in vivo studies have shown that ethanol acts in the central nucleus of the amygdala via the CRF<sub>1</sub> receptor to enhance GABA release and inhibit amygdala discharge. However, nociceptin, neuropeptide Y, and cannabinoids decrease GABA release and oppose ethanol effects. We are studying the interactions between these transmitter systems.

Mutant mice lacking the  $\epsilon$  isoform of protein kinase C (PKC $\epsilon$ ) have reduced anxiety-like behavior and alcohol consumption. In collaboration with R. Messing, University of California, San Francisco, we used genetic and pharmacologic approaches to investigate whether the PKC $\epsilon$  signaling pathway lies downstream of CRF<sub>1</sub> receptors in the central nucleus of the amygdala and to determine the role of PKC $\epsilon$  in CRF- and ethanol-mediated actions on GABAergic transmission. Our findings indicate that PKC $\epsilon$  is a key regulator of basal and CRF- and ethanol-stimulated GABA release from neurons in the central nucleus.

We also characterized neuroadaptations of the GABAergic system associated with alcohol dependence. In collaboration with G.F. Koob, Committee on the Neurobiology of Addictive Disorders, we found that gabapentin, a structural analog of GABA, reverses behavioral effects of ethanol dependence. In turn, dependence reverses the effects of gabapentin on neurons in the central nucleus of the amygdala. These behavioral and cellular findings with gabapentin suggest a potential medication for the treatment of alcoholism.

We previously reported that acute and chronic ethanol inhibits responses mediated by receptors for *N*-methyl-D-aspartate (NMDA) in neurons in the central nucleus of the amygdala. In collaboration with P.J. Kenny, Department of Molecular Therapeutics, we found that nicotine increases NMDA receptor-mediated transmission by facilitating glutamate release. These physiologic data correlate with molecular studies indicating that chronic nicotine increased expression of NMDA receptor subunits in the central nucleus of the amygdala. Taken together, these data suggest that NMDA receptors in the central nucleus gate, in part, the effects of nicotine on brain reward systems and may thereby regulate motivation for drug consumption. Dissecting these drug interactions and cellular mechanisms in the central nucleus of the amygdala could uncover a key neuroadaptive site and may provide insights into the transition to addiction and into possible treatments.

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## Drug Abuse and Plasticity in the Brain in Adults

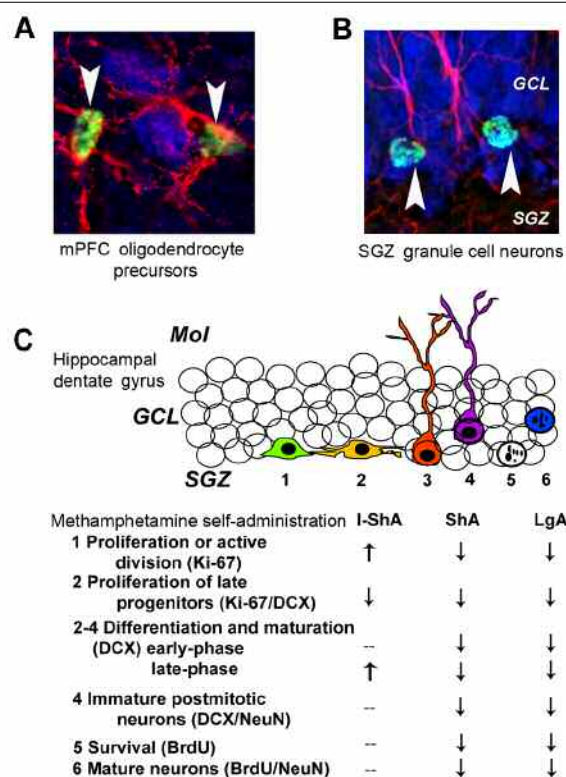
C. Mandyam, S. Wee, E. Crawford, A. Eisch,\* H. Richardson, G.F. Koob

\* University of Texas Southwestern Medical Center, Dallas, Texas

The ability of the brain in mammals, including humans, to endlessly generate new precursor cells (stem and/or progenitor cells) throughout adulthood that maintain the structure and function of the brain changed the dogma that new glial cells and/or neurons arose solely during development. Emerging research has indicated that "adult neurogenesis," the persistence of the entire process of neuronal development, occurs in several regions, including the medial prefrontal cortex and the hippocampal subgranular zone. Progenitors generated in the medial prefrontal cortex are mostly gliogenic (oligodendrocytes; Fig. 1A), whereas those generated in the subgranular zone are mostly neurogenic (Fig. 1B).

Importantly, the medial prefrontal cortex and the hippocampus are involved in drug abuse. For example, the medial prefrontal cortex has been implicated in several neurobiological phenomena associated with compulsive drug seeking, and the hippocampus has been implicated in forming context-specific memories associated with reinstatement of drug seeking. Such roles associated with compulsive drug seeking and context-specific memories may involve gliogenic and neurogenic mechanisms. Our goal is to determine if and how newly generated cells in the medial prefrontal cortex and subgranular zone are affected by drugs of abuse.

Compulsive intake or escalation of intravenous drug self-administration occurs in rodents with extended access to many drugs of abuse. Therefore, intravenous self-administration has marked clinical relevance and may



**Fig. 1.** In adults, proliferating cells in the medial prefrontal cortex (mPFC) of the brain become oligodendrocytes (A), whereas proliferating cells in the hippocampal subgranular zone (SGZ) become neurons (B). C, In adult rodents, intermittent brief access to methamphetamine increased active division in the hippocampus but did not enhance differentiation and adult neurogenesis, most likely because of a decreased neurogenic environment. Brief and long-term access to the drug decreased proliferation, differentiation, maturation, and neurogenesis in the hippocampus, most likely because of a decreased proliferative and neurogenic capacity. GCL, granule cell layer; Mol, molecular layer; --, no change; ↑, increase; ↓, decrease.

be a useful approach to understanding the neurobiological mechanisms responsible for the transition from drug use to escalation in intake. Importantly, the voluntary behavioral model for compulsive drug intake may be a particularly suitable one for testing the hypothesis that alteration in brain plasticity, such as gliogenesis and neurogenesis, in adults by a drug is partly responsible for the addictive behavior.

We have shown that self-administration of methamphetamine (a highly abused drug in the Southern California region, with street names such as speed, meth, chalk, ice, crystal, and glass) in adult rodents decreases gliogenesis in the medial prefrontal cortex and neurogenesis in the subgranular zone (Fig. 1C).

Ongoing research will determine the underlying cellular and molecular mechanisms that contribute to methamphetamine-induced decreases in cortical and

hippocampal plasticity. Comprehension of how psychostimulants such as methamphetamine inhibit new precursors or progenitors in the brain in adults will likely shed light on the basic mechanisms that regulate neural stem cells in adults. Our findings will improve our understanding of the complex mechanisms by which psychostimulants affect brain function and may suggest better therapies for methamphetamine addiction.

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