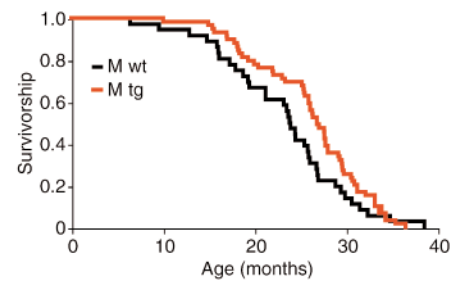
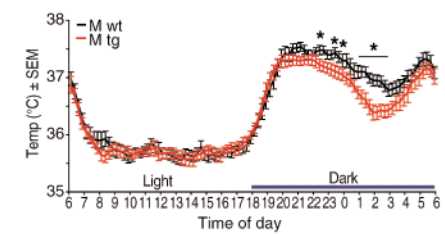
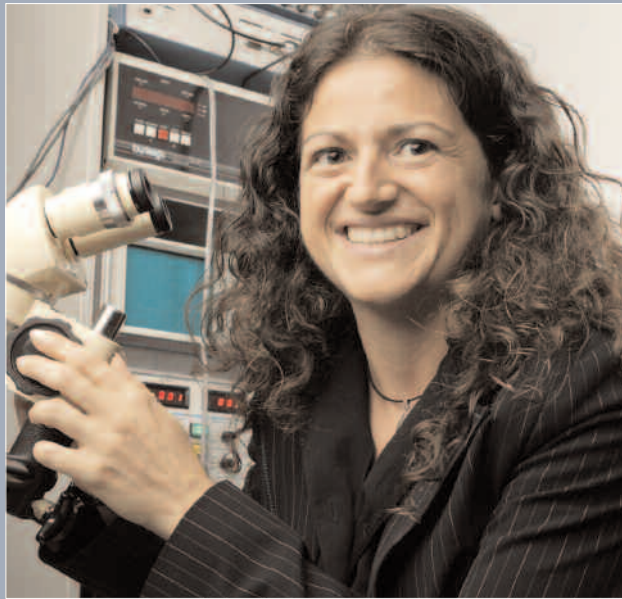


Molecular and Integrative Neurosciences

(Top) Transgenic mice (tg) engineered to overexpress the uncoupling protein-2 in the hypocretin neurons have a modest reduction of core body temperature in the dark/active part of the day. (Bottom) Fed ad libitum, these mice have a prolonged median lifespan compared to their wild-type littermates (wt). Work done by Bruno Conti, Ph.D., in the laboratory of Tamas Bartfai, Ph.D.





Marisa Roberto, Ph.D.
Assistant Professor
Molecular and Integrative
Neurosciences Department

**MOLECULAR AND
INTEGRATIVE
NEUROSCIENCES
DEPARTMENT**

STAFF

Tamas Bartfai, Ph.D.

Professor and Chairman

Serge Ahmed, Ph.D.

Adjunct Assistant Professor

Etienne Baulieu, Ph.D.

Adjunct Professor

Floyd E. Bloom, M.D.

Professor Emeritus

Jason Botten, Ph.D.

Assistant Professor

Benjamin Boutrel, Ph.D.

Professor Emeritus

Karen T. Britton, M.D., Ph.D.

Adjunct Associate Professor

Michael Buchmeier, Ph.D.

Professor

Iain L. Campbell, Ph.D.,

Adjunct Professor

Kathleen Cashman, Ph.D.

Professor Emeritus

Zhen Chai, Ph.D.

Adjunct Assistant Professor

Jerold Chun, M.D., Ph.D.

Adjunct Professor

Bruno Conti, Ph.D.

Assistant Professor

Jose Criado, Ph.D.

Adjunct Assistant Professor

Juan Carlos de la Torre, Ph.D.

Associate Professor

Cindy L. Ehlers, Ph.D.

Associate Professor

Howard S. Fox, M.D., Ph.D.

Associate Professor

Hermann H. Gram, Ph.D.

Adjunct Associate Professor

Donna L. Gruol, Ph.D.

Associate Professor

Steven J. Henriksen, Ph.D.

Adjunct Professor

Paul L. Herrling, Ph.D.

Adjunct Professor

Tomas Hokfelt, M.D., Ph.D.

Adjunct Professor

Danny Hoyer, Ph.D.

Adjunct Professor

Koki Inoue, Ph.D.

Adjunct Associate Professor

George F. Koob, Ph.D.

Professor

Harvey Karten, M.D.

Adjunct Professor

Henri Korn, M.D., Ph.D.

Adjunct Professor

Stefan Kunz, Ph.D.

Assistant Professor

Thomas Krucker, Ph.D.

Adjunct Assistant Professor

Cary Lai, Ph.D.

Associate Professor

Ulo Langel, Ph.D.

Adjunct Professor

Michel Le Moal, M.D., Ph.D.

Adjunct Professor

Jan O. Lundstrom, Ph.D.

Adjunct Professor

Athina Markou, Ph.D.

Associate Professor

Barbara J. Mason, Ph.D.

Professor

Dorian McGavern, Ph.D.

Assistant Professor

Madis Metsis, Ph.D.

Adjunct Associate Professor

Thomas Nelson, Ph.D.

Assistant Professor

Benjamin Neuman, Ph.D.

Assistant Professor

Michael B.A. Oldstone, M.D.

Professor

Shirley M. Otis, M.D.

Adjunct Professor

Loren Parsons, Ph.D.

Associate Professor

Tommy Phillips, Ph.D.

Adjunct Assistant Professor

John Polich, Ph.D.

Associate Professor

Luigi Pulvirenti, M.D.

Adjunct Associate Professor

Teresa Reyes, Ph.D.

Adjunct Assistant Professor

Catherine Rivier, Ph.D.

Adjunct Professor

Marisa Roberto, Ph.D.

Assistant Professor

Amanda Roberts, Ph.D.

Assistant Professor

Michael G. Rosenfeld, M.D.

Adjunct Professor

Pietro P. Sanna, M.D.

Associate Professor

Paul Schweitzer, Ph.D.

Associate Professor

George R. Siggins, Ph.D.

Professor

Craig Slawecki, Ph.D.

Assistant Professor

Antoine Tabarin, Ph.D.

Adjunct Associate Professor

Michael A. Taffe, Ph.D.

Assistant Professor

Lars Terenius, Ph.D.

Adjunct Professor

**Claes Wahlestedt, M.D.,
Ph.D.**

Adjunct Professor

Tammy Wall, Ph.D.

Adjunct Associate Professor

Friedbert Weiss, Ph.D.

Professor

**J. Lindsay Whitton, M.B.
Ch.B., Ph.D.**

Professor

Eric Zorilla, Ph.D.

Assistant Professor

STAFF SCIENTISTS

Walter Francesconi, Ph.D.

Bumsuk Hahm, Ph.D.

**Salvador Huitrón-Reséndiz,
Ph.D.**

Xiaoying Lu, Ph.D.

M. Cecilia Marcondes, Ph.D.

Remi Martin-Fardon, Ph.D.

Zhiguo Nie, Ph.D.

Robert Purdy, Ph.D.

Mitra Rebek, Ph.D.

Heather Richardson, Ph.D.

Svetlana Semenova, Ph.D.

SCIENCE ASSOCIATES

Elena Crawford

Caroline Lanigan, Ph.D.

Rong-Sheng Lee, Ph.D.

Hanna Lewicki

John Light

Sam Madamba

Antoinette Tishon

RESEARCH ASSOCIATES	Olivier George, Ph.D.	Sunmee Wee, Ph.D.	Noemi Sevilla, Ph.D. Universidad Autonoma de Madrid Madrid, Spain
Mehrdad Alirezaei, Ph.D.	Sandy Ghozland, Ph.D.	Jason Whitmire, Ph.D.	
Harinder Aujla, Ph.D.	Nicholas Gilpin, Ph.D.	Manisha Yadav, Ph.D.	
Michal Bajo, M.D., Ph.D.	Thomas Greenwell, Ph.D.	Ge Ying, Ph.D.	Christina Spiropoulou, Ph.D. Centers for Disease Control and Prevention Atlanta, Georgia
Hilda Bajova, D.V.M.	Hazuki Hagihara, Ph.D.	Yu Zhao, Ph.D.	
Marco A. Baptista, Ph.D.	Peter James, Ph.D.	Elina Zuniga, Ph.D.	Persephone Tough, M.D. Edward Jenner Institute for Vaccine Research Compton, England
Fulvia Berton, Ph.D.	Paul John Kenny, Ph.D.		
David Brooks, Ph.D.	Izabella Klein, Ph.D.	VISITING INVESTIGATORS	
Adriaan Bruijnzeel, Ph.D.	Henning Lauterbach, Ph.D.	Hedieh Badie, Ph.D. Genomics Institute of the Novartis Research Foundation San Diego, California	
Tricia Burdo, Ph.D.	Dusan Lekic, M.D., Ph.D.	Roberto Ciccocioppo, Ph.D. University of Camerino Camerino, Italy	
Renaud Jean Burrer, Ph.D.	Matthias Liechti, Ph.D.		
Veze Repunte Canonigo, Ph.D.	Li Ying Liou, Ph.D.	Urs Christen, Ph.D. La Jolla Institute for Allergy and Immunology La Jolla, California	
Althea Capul, Ph.D.	Fei Lu, Ph.D.		
Roberto Cervera, Ph.D.	Chitra Mandyam, Ph.D.	Jean E. Gairin, Ph.D. CNRS Toulouse, France	
Zhifeng Chen, Ph.D.	Monica Mendez-Diaz, Ph.D.		
Irene Yoon-Jin Choi, Ph.D.	Victor Mendoza-Fernandez, Ph.D.	Karine Guillem, Ph.D. University of Pennsylvania Philadelphia, Pennsylvania	
Christopher Cornell, Ph.D.	Covadonga Paneda, Ph.D.		
Cromwell Cornillez-Ty, Ph.D.	Neil Paterson, M.D.	Dirk Homann, M.D., Ph.D. University of Colorado Health Sciences Center Denver, Colorado	
Rebecca Crean, Ph.D.	Gurudutt Pendyala, Ph.D.		
Stephen J. Crocker, Ph.D.	Jilla Sabeti, Ph.D.	Shinchi Iwasaki, M.D., Ph.D. Osaka City University Medical School Osaka, Japan	
Chris Davis, Ph.D.	Valentina Sabino, Ph.D.		
Christopher Dayas, Ph.D.	Ana Sanchez, Ph.D.	Rolf Kiessling, Ph.D. Karolinska Institutet Stockholm, Sweden	
Andre Der-Avakian, Ph.D.	Manuel Sanchez-Alavez, M.D., Ph.D.		
Toby Escher, Ph.D.	Lisa Sharkey, Ph.D.	Denise Nanche, Ph.D. Universitat de Barcelona Barcelona, Spain	
Kurt Edelmann, Ph.D.	Nimish Sidhpura, Ph.D.		
Ralph Feuer, Ph.D.	Iustin Tabarean, Ph.D.		
Cindy Funk, Ph.D.	Matthew Trifilo, Ph.D.	Laura O'Dell, Ph.D. University of Texas El Paso, Texas	
Lucile Garidou, Ph.D.	Brendan Walker, Ph.D.		
Peter Gaskill, Ph.D.			



Tamas Bartfai, Ph.D.

Chairman's Overview

This has been a scientifically productive year for the researchers in the Molecular and Integrative Neurosciences Department. Our members have published 192 papers in top-tier journals and 2 acclaimed textbooks: *Neurobiology of Addiction* by George Koob and Michel Le Moal and *Drug Discovery* by Tamas Bartfai and Graham Lees.

We have made important breakthroughs in work on viruses such as severe acute respiratory syndrome (SARS), Lassa, and lymphocytic choriomeningitis virus and their interactions with different cell types in the brain. We have learned more about how viral infections are cleared and what strategies viruses use to remain dormant in neurons.

George Siggins and Marisa Roberto have identified the mechanism of action of the neuropeptide orphanin on neurons in the amygdala, and have drawn important conclusions about the contribution of this peptide to anxiety disorders and alcohol addiction. Bruno Conti's work on longevity and the work of Michael Buchmeier and Benjamin Neuman on SARS are discussed below.

Aging is considered by many scientists to be the result of the accumulation of free radicals-mediated cellular damage. Thus, a reduction in the formation of free radicals should slow aging and possibly prolong lifespan. Generation of free radicals is inherently asso-

ciated with the consumption of oxygen and nutrients required to produce ATP. This is what Lars Ernster called oxygen toxicity, and as long as we live on earth it can only be reduced by reducing our energy requirement.

One way to achieve this reduction is through calorie restriction (CR), a controlled dietary regimen that prolongs lifespan and delays the onset of certain diseases. CR is associated with a reduction in core body temperature (CBT), most likely an adaptive mechanism for coping with limited food resources. Considering that in homeotherms, most calorie intake is used to keep a constant CBT against a normally lower ambient temperature, Dr. Conti set out to test whether reduction of CBT per se could contribute to longevity in the absence of CR. To achieve this goal, he has relied on data showing that CBT is controlled centrally by temperature-sensitive neurons in the hypothalamic preoptic area, where the "core body thermostat" resides. By generating transgenic mice that produce heat locally in the vicinity of the preoptic area, he mimicked an increase of CBT. This triggered a thermoregulatory response that resulted in a modest but prolonged reduction of CBT. Fed ad libitum, these transgenic mice have similar calorie intake to that of their wild-type littermates but show increased metabolic efficiency and prolonged median lifespan, demonstrating that a reduction of CBT can contribute to longevity independent of CR. These mice will serve as a model for investigating thermo and metabolic regulation in mammals and the effects of CBT on aging.

The SARS virus has scared the world and has also given us an incredible dress rehearsal for how to rapidly identify pathogenic microbes before catastrophic pandemics take millions of lives. In a consortium that has utilized the best structural chemistry and nuclear magnetic resonance expertise at Scripps Research, Drs. Buchmeier and Neuman have shown that they can rapidly solve the structure of multiple surface proteins of new viruses and thereby identify targets for vaccine development in a very short timeframe. In collaboration with Ron Milligan and Mark Yeager, they have used electron cryomicroscopy to investigate the supramolecular architecture of the SARS virus. Working with Peter Kuhn, they have used x-ray crystallography to solve the structure of the SARS nsp10 protein, and have discovered a novel structural motif: a previously unknown fold containing 2 zinc-binding motifs. Finally, in collaboration with Kurt Wüthrich, the Nobel Prize-winning nuclear magnetic resonance researcher, they have solved the dynamic structure of a key SARS protein, the protein nsP7.

These investigators have been able to identify key functions in emerging viruses and have obtained a degree of detail on their structure that permits either the rapid synthesis of chemicals to combat viral replication or the selection of antibodies for passive immunization to treat the infected. Their research also permits identification of components for protective vaccines. Our hope is to be able to obtain the same type of information for the bird flu virus.

INVESTIGATORS' REPORTS

Studies of Severe Acute Respiratory Syndrome Virus and Other Coronaviruses

B.W. Neuman, R.J. Burrer, R. Aur, J.P.C. Ting, B.D. Adair, C. Yoshioka,* J. Quispe,* R.A. Milligan,* M. Yeager,* M.J. Buchmeier

* Department of Cell Biology, Scripps Research

Coronaviruses are an important family of human and veterinary pathogens that cause a wide range of diseases. The emergence of the coronavirus that causes severe acute respiratory syndrome (SARS) highlighted a need for structural information on coronavirus proteins and effective antiviral treatments.

ARCHITECTURE OF CORONAVIRUS PARTICLES

Coronaviruses derive their name from the protruding transmembrane spike glycoproteins, which are seated in the viral membrane via interactions with the 3-pass transmembrane matrix glycoprotein. A core containing nucleoprotein and the single-stranded RNA genome of approximately 30 kb is incorporated into virions at membranes of the endoplasmic reticulum–Golgi complex intermediate in a process mediated by interactions between the nucleoprotein and the matrix glycoprotein. We used electron cryomicroscopy and image analysis to examine the supramolecular structure of coronaviruses.

We found that coronavirus particles are enveloped, pleomorphic, and about 85 nm in diameter (Fig. 1A). The surface spikes consist of a globular head supported by a slender stalk. The predicted volume for the spike ectodomain is a close match for a homotrimer of spike molecules. A layer of density directly apposed to the inner bilayer leaflet and tightly packed intramembrane densities are ascribed to the matrix protein molecules. Punctate densities of about 5 nm are present at the underside of the viral membrane, distributed throughout the interior of the virion, and ascribed to complexes consisting of the nucleoprotein and RNA. The spike and nucleoprotein molecules closest to the viral membrane form overlapping 2-dimensional lattices, most likely due to protein-protein interactions during assembly. Using single-particle techniques, we reconstructed 3-dimensional models of spike and nucleoprotein densities (Figs. 1B and 1C) at a resolution of approximately 3 nm.

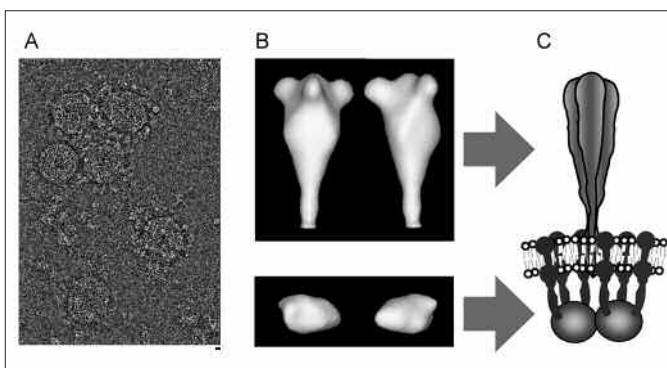


Fig. 1. Reconstruction of coronavirus spike and nucleoprotein molecules. A, Formalin-fixed feline coronavirus particles were imaged by using low-dose electron cryomicroscopy. Scale bar at lower right indicates 10 nm. B, Three-dimensional reconstructions of the spike (top) and nucleoprotein (bottom) densities were obtained by using single-particle image analysis. C, A schematic interpretation of the membrane proximal region shows spikes (top) in contact with membrane-embedded matrix protein, which in turn interacts with nucleoprotein-RNA complexes of the viral core (bottom).

ANTISENSE ANTIVIRAL AGENTS

Coronaviruses are the cause of important and emerging zoonotic infectious diseases in many parts of the world. To design a rational and consistent approach to combat these viruses, we used peptide-conjugated antisense morpholino oligomers (P-PMOs) designed to bind to duplex-specific sequences in the genomes of SARS virus and mouse hepatitis virus, a murine coronavirus. P-PMOs directed against regions in the 5' untranslated regions of the genomes reduced virus-associated cytopathologic changes and spread by decreasing viral amplification. Random-sequence control P-PMOs had low antiviral activity against both viruses. During prolonged treatment, the SARS virus developed contiguous point mutations at a P-PMO binding site, producing resistant but severely growth-attenuated virus.

In mice, treatment with P-PMOs reduced the levels of virus and cytopathologic changes in the liver and weight loss associated with infection. Treatment postponed death in animals infected with mouse hepatitis virus 3 and reduced mortality in animals infected with mouse hepatitis virus Alb139. These results suggest P-PMOs have powerful therapeutic and investigative potential in coronavirus infections.

Vaccination for Severe Acute Respiratory Syndrome – Associated Coronavirus

C.T. Cornillez-Ty, R.J. Burrer, B.W. Neuman, J.P.C. Ting, A. Sette,* J. Sidney,* M.J. Buchmeier

* La Jolla Institute for Allergy and Immunology, San Diego, California

In an effort to develop a multiepitope vaccine against severe acute respiratory syndrome–associated coronavirus (SARS-CoV), we are collaborating with investigators at the La Jolla Institute of Allergy and Immunology. We are attempting to identify epitopes within the 4 structural proteins (nucleoprotein, matrix, envelope, and spike) of the virus that can be presented on human MHC class I molecules. Although SARS-CoV contains at least 14 open reading frames, the 4 structural proteins have the highest level of expression in cells infected with the virus. Hence, in a natural infection, these 4 proteins are the ones most likely to be processed and presented on MHC class I molecules.

On the basis of a predictive algorithm, several potential epitopes within these 4 proteins have been identified. These predicted epitopes have been synthesized as 9-mer peptides and have been tested for in vitro binding affinities to MHC class I molecules of the A1, A2, A3, A24, B7, and B44 supertypes. This process has yielded a pool of peptides that must be further tested for their ability to elicit a CD8⁺ T-cell response in vivo.

To determine whether these peptides are immunogenic in vivo, we will use IFN- γ enzyme-linked immunospot assays (Fig. 1). Construction of recombinant vaccinia virus that expresses the 4 structural proteins of SARS-CoV has been completed, and immunization of HLA transgenic mice with the various peptides are under way.

Pathogenesis of Coronavirus-Induced Demyelination

R.J. Burrer, C.T. Cornillez-Ty, L. Breakwell, M.J. Buchmeier

Mouse hepatitis virus (MHV) causes acute encephalomyelitis; in mice that survive, a demyelinating disease resembling the human disease multiple sclerosis develops. An adaptive immune response is required to survive the acute phase of MHV infection in the CNS, because the lack of CD8⁺, and

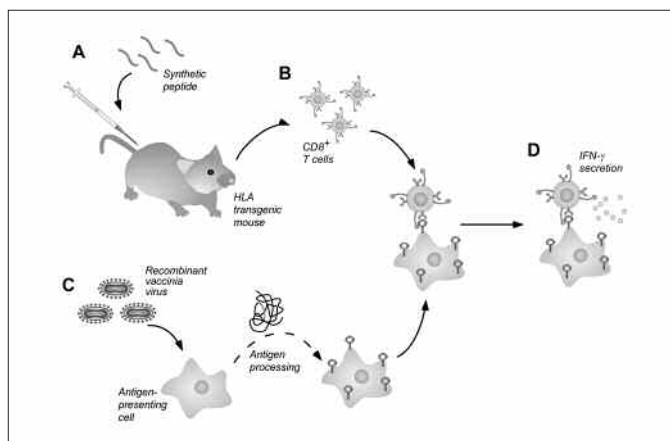


Fig. 1. IFN- γ enzyme-linked immunospot assay to determine the MHC I–restricted epitopes of SARS-CoV. A, HLA transgenic mice are immunized with a candidate 9-mer peptide. B, Spleens from immunized mice are harvested and CD8⁺ T cells are isolated. C, One day before isolation of CD8⁺ T cells, antigen-presenting cells are infected with a recombinant vaccinia virus that expresses 1 of the 4 SARS-CoV structural proteins. Antigen processing of the SARS-CoV protein will result in loading of certain epitopes onto MHC class I molecules. If the peptide used to immunize HLA transgenic mice corresponds to an epitope (light gray region) that can be processed and loaded onto an MHC class I molecule, exposure of isolated CD8⁺ T cells to the vaccinia-infected antigen-presenting cells will elicit a memory response. D, A memory response can be determined by assaying for secretion of IFN- γ .

to a lesser extent CD4⁺, T cells results in an impaired ability to survive sublethal doses of the virus. Innate immunity does play a protective role, and previous studies by us and by others have highlighted the importance of various chemokines in the immune response to MHV.

Toll-like receptors (TLRs) recognize specific molecular patterns that are usually associated with the presence of viruses or bacteria. Upon recognition of microbial pathogens, the receptors shape both the innate and the adaptive immune responses by initiating the release of inflammatory cytokine and chemokine mediators and upregulating costimulatory molecules on dendritic cells, respectively. Each TLR interacts with a specific combination of adapter proteins to trigger intracellular signaling pathways that result in the activation of transcription factors.

To determine if TLRs are required to survive MHV in the CNS, we monitored the outcome of a sublethal infection in wild-type (control) mice and in mice deficient in 2 of the adapter proteins, MyD88 and Trif/Ips2, because each of the TLRs signals through 1 or both of these proteins. Mortality was similar in wild-type mice and mice deficient in Trif/Ips2 and greatly increased

in mice deficient in MyD88. We are determining which TLRs and intracellular pathways are involved in the response to MHV infection.

HLA-Restricted Epitope Discovery From Pathogenic Arenaviruses

J.W. Botten, P.A. Barrowman, J.P.C. Ting, A. Sette,*
J.L. Whitton,** M.J. Buchmeier

* La Jolla Institute for Allergy and Immunology, San Diego, California

** Molecular and Integrative Neurosciences Department, Scripps Research

Arenaviruses are rodent-borne pathogens that cause significant morbidity and mortality in humans. Pathogenic arenaviruses include Lassa, lymphocytic choriomeningitis (LCMV), Junin, Machupo, Guanarito, Sabia, and Whitewater Arroyo viruses. Our understanding of the human immune response to arenavirus infection is limited. In the case of Lassa virus, infected individuals generate poor neutralizing antibody responses, indicating that cellular immunity most likely plays a primary role in viral clearance and protective immunity. Because of its central role, sensitive reagents are needed to measure the cell-mediated immunity that develops in response to naturally occurring infections with Lassa virus or to vaccines. The development of diagnostic assays requires identification of HLA-restricted class I and class II epitopes of the virus. These epitopes could be used not only to diagnose Lassa virus infection but also to determine the quality of immune responses, define correlates of protection and immunopathologic changes, and ultimately guide the selection of possible vaccines.

We have established a model system for identifying human CD8⁺ T-cell epitopes from pathogenic arenaviruses; we use Lassa virus as our test virus. In this system, bioinformatic predictions are used to identify possible epitopes, *in vitro* MHC-binding assays and *in vivo* immunogenicity studies in HLA transgenic mice are used to validate epitopes, and recombinant vaccinia virus-based challenge studies are used to evaluate whether epitopes protect against viral challenge.

Using this approach, we identified 4 HLA-A2 supertype-restricted epitopes encoded by the gene for the Lassa virus glycoprotein precursor. Two of these epitopes protected mice against challenge with a

recombinant vaccinia virus construct that expressed the glycoprotein precursor.

We are now applying this epitope identification approach to the remaining pathogenic arenaviruses. For LCMV, we have identified novel HLA-A2 supertype-restricted epitopes from the nucleoprotein, glycoprotein precursor, zinc-binding protein, and viral polymerase. Immunization of HLA-A2 transgenic mice with 2 of these epitopes led to significant reductions in viral titer after challenge with LCMV. Last, a subset of the identified Lassa virus and LCMV epitopes cross-react with the corresponding determinants in the remaining pathogenic arenaviruses.

The epitopes identified in these studies are potential diagnostic reagents and candidates for inclusion in genetically engineered or epitope-based vaccine constructs. Our approach is applicable to any pathogen with existing sequence data, does not require manipulation of the virulent pathogen or access to immune human donors, and should therefore be generally applicable to category A-C agents (pathogens that pose a risk to national security) and other emerging pathogens.

Structure and Function of the Arenavirus Signal Peptide

A.A. Saunders, B.W. Neuman, J.P.C. Ting, M.J. Buchmeier

The signal peptide of the surface glycoprotein of lymphocytic choriomeningitis virus (LCMV) has several unique characteristics. It is unusually long at 58 amino acids, it contains 2 hydrophobic domains, and its sequence is highly conserved among both Old and New World arenaviruses. To better understand the functions of the peptide, we created a panel of point and deletion mutants targeting many of the highly conserved elements within the peptide. In characterizing our mutant signal peptides, we discovered that in addition to translocation of the viral surface glycoprotein precursor into the lumen of the endoplasmic reticulum, the signal peptide is involved in glycoprotein expression, cleavage, and cell-surface localization; glycoprotein incorporation during assembly of viral particles; and glycoprotein-mediated fusion with a host cell.

PUBLICATIONS

Botten, J., Alexander, J., Pasquetto, V., Sidney, J., Barrowman, P., Ting, J., Peters, B., Southwood, S., Stewart, B., Rodriguez-Carreno, M.P., Mothe, B., Whitton, J.L., Sette, A., Buchmeier, M.J. Identification of protective Lassa virus epitopes that are restricted by HLA-A2. *J. Virol.* 80:8351, 2006.

Joseph, J.S., Saikatendu, K.S., Subramanian, V., Neuman, B.W., Brooun, A., Griffith, M., Moy, K., Yadav, M.K., Velasquez, J., Buchmeier, M.J., Stevens, R.C., Kuhn, P. Crystal structure of nonstructural protein 10 from the severe acute respiratory syndrome coronavirus reveals a novel fold with two zinc-binding motifs. *J. Virol.* 80:7894, 2006.

Neuman, B.W., Adair, B.D., Yoshioka, C., Quispe, J.D., Orca, G., Kuhn, P., Milligan, R.A., Yeager, M., Buchmeier, M.J. Supramolecular architecture of severe acute respiratory syndrome coronavirus revealed by electron cryomicroscopy. *J. Virol.* 80:7918, 2006.

Nussbaum, A.K., Rodriguez-Carreno, M.P., Benning, N., Botten, J., Whitton, J.L. Immunoproteasome-deficient mice mount largely normal CD8⁺ T cell responses to lymphocytic choriomeningitis virus infection and DNA vaccination. *J. Immunol.* 175:1153, 2005.

Peti, W., Johnson, M.A., Herrmann, T., Neuman, B.W., Buchmeier, M.J., Nelson, M., Joseph, J., Page, R., Stevens, R.C., Kuhn, P., Wüthrich, K. Structural genomics of the severe acute respiratory syndrome coronavirus: nuclear magnetic resonance structure of the protein nsP7. *J. Virol.* 79:12905, 2005.

Reignier, T., Oldenburg, J., Noble, B., Lamb, E., Romanowski, V., Buchmeier, M.J., Cannon, P.M. Receptor use by pathogenic arenaviruses. *Virology, in press.*

Saikatendu, K.S., Joseph, J.S., Subramanian, V., Clayton, T., Griffith, M., Moy, K., Velasquez, J., Neuman, B.W., Buchmeier, M.J., Stevens, R.C., Kuhn, P. Structural basis of severe acute respiratory syndrome coronavirus ADP-ribose-1''-phosphate dephosphorylation by a conserved domain of nsP3. *Structure* 13:1665, 2005.

Chronic Virus-Host Interaction in the CNS

H.S. Fox, M. Alirezaei, J. Boyd, C. Flynn, P. Gaskill, S. Huitrón-Reséndiz, C. Lanigan, C. Marcondes, R. Ojakian, G. Pendyala, D. Watry, M. Yadav, C. York-DeFalco, M. Zandonatti

The brain is a unique organ, not only functionally but also in terms of host response to events such as infection. We study processes in which this response leads to brain dysfunction; we have mostly focused on a degenerative and dementing condition that occurs after a known stimulus, infection with HIV.

The HIV pandemic continues worldwide. In the United States and certain other countries, antiviral treatment is available, leading to greatly prolonged survival. However, as HIV infection has turned into a chronic disease, the CNS disorders caused by the virus (neuroAIDS) continue to affect a significant proportion of those who are infected. Using infection of rhesus monkeys with simian immunodeficiency virus (SIV) as a model of neuroAIDS in humans, we are studying the virology, immunology, pathology, and neurobiology of the resulting CNS disease.

We have defined the different stages of SIV disease in the CNS in terms of interactions between the virus and the immune system, pathologic changes in CNS

function, and molecular mechanisms. Initial brain invasion by virus occurs early, by the second week after infection. At this time, a self-limited illness affects many physiologic systems, including the CNS. In addition, an innate immune response occurs in the brain, with an upregulation of genes induced by interferon and IL-6. The adaptive immune response is beginning. During the next 3 months, the brain CD8⁺ T-cell phenotype switches from a surveillance mode to an effector mode, SIV-specific CD8⁺ T cells can be detected in the brain, and level of virus in the brain decreases by 100-fold.

This chronic, relatively asymptomatic phase can last years. However, in both humans and rhesus monkeys, behavioral testing reveals cognitive alterations, and neurophysiologic analysis reveals abnormal CNS function. We found that 2 years after infection, the concentration of SIV in the brain remained low, but the virus was still present and the increased number of CD8⁺ T cells in the brain that occurred early after infection was preserved. Molecular analysis confirmed an active immune interaction in the brain. Intriguingly, the level of the chemokine RANTES/CCL5, which has numerous effects on neurons and on immune cells, was increased, and expression of the chemokine was localized to the brain-infiltrating cytotoxic T cells.

Thus, rather than being a quiescent state, the chronic phase is an important stage of the viral-host interaction. The original purpose of this interaction is to protect the brain from the virus, but in the long-term, the interaction can lead to brain damage.

Late in the disease course, the adaptive immune response wanes, and the amount of virus again increases in the brain. This increase is accompanied by a number of indications of CNS dysfunction, and an influx of macrophages into the infected brain occurs, leading to SIV encephalitis. At the molecular level, genes associated with innate immune responses are again upregulated, and macrophages as well as brain glia are activated.

Currently, we are focusing on the chronic stage of SIV infection, modeling the effects of patients who are infected with HIV but have not progressed to AIDS. We are investigating immune specificity in the brain vs the rest of the body and the mechanisms responsible for these differences. We are also examining the effects of highly active antiretroviral therapy on the interaction between the virus and the immune system in the brain. Understanding the mechanisms of dysfunction in the brain will define the pathogenesis of CNS HIV infec-

tion, as well as potentially other CNS disorders, and lead to preventive or therapeutic strategies.

PUBLICATIONS

Burdo, T.H., Marcondes, M.C., Lanigan, C.M., Penedo, M.C., Fox, H.S. Susceptibility of Chinese rhesus monkeys to SIV infection. *AIDS* 19:1704, 2005.

Everall, I., Salaria, S., Roberts, E., Corbeil, J., Sasik, R., Fox, H.S., Grant, I., Masliah, E., HNRC Group. Methamphetamine stimulates interferon inducible genes in HIV infected brain. *J. Neuroimmunol.* 170:158, 2005.

Roberts, E.S., Huitrón-Reséndiz, S., Taffe, M.A., Marcondes, M.C., Flynn, C.T., Lanigan, C.M., Hammond, J.A., Head, S.R., Henriksen, S.J., Fox, H.S. Host response and dysfunction in the CNS during chronic simian immunodeficiency virus infection. *J. Neurosci.* 26:4577, 2006.

Viral-Immunobiology Laboratory

M.B.A. Oldstone, J.C. de la Torre, S. Kunz, D.B. McGavern, B. Hahm, D. Brooks, A. Capul, R. Clemente, K. Edelmann, L. Garidou, H. Lauterbach, A. Lee, L. Liou, A. Sanchez, M. Trifilo, G. Ying, E. Zuniga, A. Tishon, H. Lewicki, E. Buset, A. Gundersen, P. Borrow,* E. Domingo,** J.E. Gairin,*** R. Kiessling,**** N. Sevilla,** Christina Spiropoulou*****

* Edward Jenner Institute for Vaccine Research, Compton, England

** Universidad Autonoma de Madrid, Madrid, Spain

*** CNRS, Toulouse, France

**** Karolinska Institutet, Stockholm, Sweden

***** Centers for Disease Control and Prevention, Atlanta, Georgia

The Viral-Immunobiology Laboratory encompasses the programs of 4 faculty members: Juan Carlos de la Torre, Stefan Kunz, Dorian B. McGavern, and Michael B.A. Oldstone. Each program is independent, but the interactions between the researchers and the use of different technologies provide an intellectual sum greater than any single part. Our studies of both viral and transmissible spongiform encephalopathies (e.g., prion diseases, scrapie) include basic analysis of the mechanisms by which viruses persist, escape immune recognition, and cause disease. Integral parts of the programs are understanding how viruses infect cells; defining the cellular receptors used by viruses; and mapping the trafficking of viruses into cells and the subsequent viral uncoating, replication, assembly, exit, and spread. Because the immune system has evolved to recognize, attack, and remove these foreign substances, we evaluate the immune response against viruses, probe how viruses subvert this response to provide a selective advantage for their survival, and study how the host can correct this subversion to allow termination of viral persistence.

Other interests include dissecting how viruses and immune cells traffic to the brain and interact there; how viruses are cleared from the brain; and how viruses alter the differentiation processes of cells they persistently infect, thereby disturbing homeostasis and causing disease. We also are investigating how viruses induce autoimmune disease or induce immunosuppression, and we are designing therapies to control viral infections. Because different viruses have different lifestyles, we focus on 3 RNA negative-stranded viruses: Borna disease virus, lymphocytic choriomeningitis virus, and measles virus. We also investigate the mechanism by which infectious agents cause transmissible spongiform encephalopathies.

Resurrection of Nonfunctional T Cells During Persistent Viral Infection

D. Brooks, D.B. McGavern, M.B.A. Oldstone

Persistent viral infections such as HIV disease and hepatitis C are major health problems. A fundamental obstacle in control of these infections is the functional inactivation of antiviral T cells. After a persistent infection is established, both CD4⁺ and CD8⁺ T cells rapidly lose their antiviral and immunostimulatory functions. Although this phenomenon has been recognized for years, the pertinent question is whether the immune response is programmed to fail or can be fixed to eliminate infection. Also unclear are the molecular events or factors involved.

We found that in contrast to T-cell expansion, which is hardwired during priming, T-cell functional responses are malleable and rely on continuous signals from the cells' antigenic environment. In accordance with this plasticity, function can be restored to nonresponsive CD4⁺ and CD8⁺ T cells during persistent infection by treatment with the antiviral drug ribavirin. Treatment that reduced the concentration of virus by as little as one log led to the removal of T-cell suppression factors. Removal of IL-10 initiated by viral infection resulted in restoration of T-cell function.

During persistent infection, CD8⁺ T cells with the highest affinity for viral antigens are physically deleted. Removal of these high-affinity cells results in the depletion of the effector population best equipped to fight

infection, thereby limiting the breadth, magnitude, and efficacy of the antiviral response. We found that deletion of high-affinity CD8⁺ T cells during persistent viral infection is a direct result of the inactivation of virus-specific CD4⁺ T cells. The deletion of high-affinity CD8⁺ T cells could be averted by therapeutically rescuing CD4⁺ T-cell activity in vivo, resulting in long-term cytolytic activity of CD8⁺ T cells against virus-infected cells and control of infection.

Dissecting the Molecular Role of CD4⁺ and CD8⁺ T Cells in Control of Acute Viral Infections

A. Tishon, H. Lewicki, K. Edelmann, M.B.A. Oldstone

Measles virus is one of the most infectious of human pathogens and today still infects more than 30 million persons each year, killing more than 500,000 persons annually. Death is primarily due to secondary microbial infections associated with immunosuppression or to CNS disease.

We used a transgenic mouse model to express receptors for measles virus in neurons in the CNS. Infecting the transgenic mice with measles virus in concert with depleting and reconstituting individual T-cell subsets and B cells alone or in combination revealed that neither CD8⁺ nor CD4⁺ nor B cells alone can control acute measles virus infection. Combinations of either (1) CD4⁺ cells and B cells or (2) CD4⁺ and CD8⁺ T cells were required, but CD8⁺ T cells with B cells were not effective. Both IFN- γ and neutralizing antibodies, but not perforin or TNF- α , were associated with clearance of the virus. Interestingly, lack of IFN- γ but not lack of TNF- α led to persistent measles virus infection.

Influenza virus remains a major concern for a returning infection with potential devastating results for the human population. To better understand how to control influenza virus infection of the lung, in collaboration with Y. Kawaoka, University of Wisconsin, Madison, we used reverse genetics to insert the known H-2^b-restricted immunodominant CD8⁺ T-cell epitope (GP 33–41) and the CD4⁺ T-cell epitope (GP 61–80) of lymphocytic choriomeningitis virus (LCMV) into the neuraminidase gene for WSN influenza virus. We then adoptively transferred fluorescently labeled LCMV-specific GP 33 CD8⁺ cells or GP 61 CD4⁺ T cells alone or in

combination and used fluorescence methods to identify and measure the trafficking of these specific T cells to the lung (Fig. 1). We also examined the effects of vari-

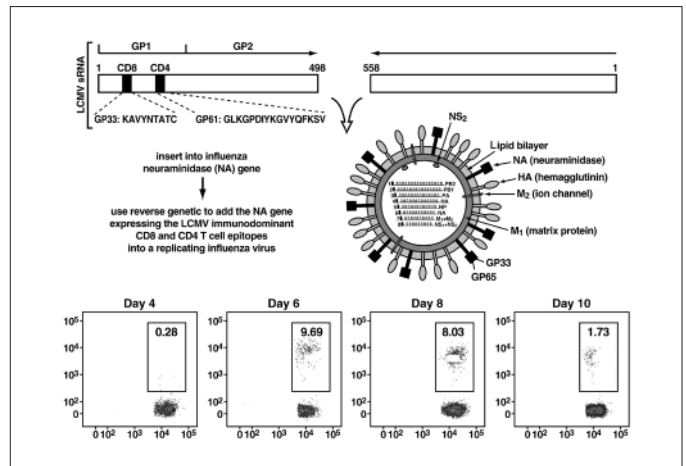


Fig. 1. Top, The genomic structure of LCMV and its immunodominant CD8⁺ (GP 33) and CD4⁺ (GP 65) epitopes. The GP 33 and GP 65 sequences are inserted into the neuraminidase gene of influenza WSN virus. Bottom, Infiltration into the lung of fluorescently labeled GP 33-specific T cells after intranasal inoculation with 1×10^5 plaque-forming units of WSN-LCMV influenza virus.

ous therapies on trafficking of these cells to the lung and control of the resultant immunopathologic injury.

Prion-Induced Amyloid CNS and Heart Disease With High Levels of Infectivity in Blood and a Transgenic Model for Chronic Wasting Disease

M.J. Trifilo, G. Ying, M.B.A. Oldstone

Transmissible spongiform encephalopathies, or prion diseases, are a group of infectious diseases due to abnormal folding of the normal cellular protein PrP. More than 98% of PrP exists as a membrane-bound, glycosylphosphatidylinositol-anchored protein. In collaboration with B. Chesebro, Rocky Mountain Laboratories, Hamilton, Montana, we produced transgenic mice in which the C-terminal 21 amino acids of PrP are not transcribed; in these mice more than 98% of PrP exists in an anchorless, non-membrane-bound form.

When these transgenic mice were inoculated intracerebrally with the agent that causes murine scrapie, a

dramatic accumulation of abnormally folded prion protein, PrPres, occurred within the brain (Fig. 1A). PrPres

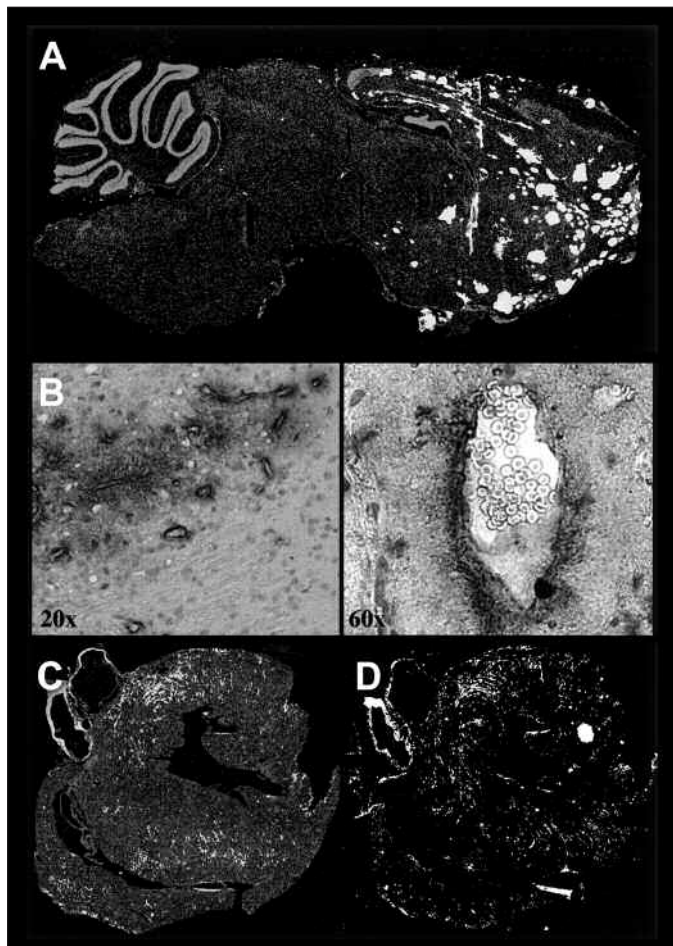


Fig. 1. Deposition of PrPres in the frontal cortex (A) and around endothelial cells (B) of the brain in transgenic mice with anchorless, non-membrane-bound PrP infected with the agent that causes murine scrapie. Both PrPres and infectious material enter the blood and are deposited in several extraneural tissues, including the heart (C and D). Deposits of PrPres (C) colocalize with amyloid deposits (D), preventing proper function of the heart.

was infectious and formed large amyloid plaques in the absence of overt clinical disease during observation times of up to 720 days. However, the infected mice had learning and memory deficits, including an inability to perform cued learning tests and a failure to induce long-term potentiation. In other studies, we showed that inhibition of learning and memory was associated with PrPres binding, upregulating, and signaling through the γ -aminobutyric acid receptor. These results indicate for the first time that PrP can function as a ligand for this receptor.

PrPres deposits in the brains of the infected transgenic mice also occurred within and around endothelial cells lining blood vessels in the brain (Fig. 1B). Examination of blood indicated that both infectivity

and PrPres could be readily detected. These findings were the first demonstration of PrPres in the blood and indicate that a way exists to determine the precise blood components involved and to detect or remove PrPres to safeguard blood supplies.

Additionally, multiple extraneural tissues, including the heart, had PrPres deposition (Fig. 1C). Studies indicated that similar to deposits in the brain, deposits of PrPres in the heart formed amyloid (Fig. 1D) that was infectious. In catheterization studies done in collaboration with K. Knowlton, University of California, San Diego, we found that the deposition of amyloidogenic PrPres within the hearts of infected transgenic mice caused significant alterations in both systolic (reduced compliance) and diastolic (stiffening of the heart) functions of the heart.

These results provided the first evidence that prion-mediated disease could occur outside the CNS. Last, in collaboration with Dr. Chesebro, we inserted the gene for normal deer PrP into mouse genes behind the PrP promoter and introduced this construct into mice in which the gene for mouse PrP had been inactivated. When such transgenic mice were inoculated intracerebrally or orally with the agent that causes deer scrapie, clinical and pathologic evidence of prion disease developed and normal cellular deer PrP was biochemically converted into deer PrPres. Thus, we now have an animal model that can be used to investigate the pathogenesis and mechanism of spread of chronic wasting disease in deer. Both of these characteristics are currently unknown, although chronic wasting disease is a major economic problem for those who hunt or breed deer and an unknown public health risk for humans.

PUBLICATIONS

Brooks, D.G., McGavern, D.B., Oldstone, M.B.A. Reprogramming of antiviral T cells prevents inactivation and restores T cell activity during persistent viral infection. *J. Clin. Invest.* 116:1675, 2006.

Homann, D., Dummer, W., Wolfe, T., Rodrigo, E., Theofilopoulos, A.N., Oldstone, M.B.A., von Herrath, M.G. Lack of intrinsic CTLA-4 expression has minimal effect on regulation of antiviral T-cell immunity. *J. Virol.* 80:270, 2006.

Kunz, S., Rojek, J.M., Kanagawa, M., Spiropoulou, C.F., Barresi, R., Campbell, K.P., Oldstone, M.B.A. Posttranslational modification of α -dystroglycan, the cellular receptor for arenaviruses, by the glycosyltransferase LARGE is critical for virus binding. *J. Virol.* 79:14282, 2005.

Oldstone, M.B.A. Molecular and cellular mechanisms, pathogenesis, and treatment of insulin-dependent diabetes obtained through study of a transgenic model of molecular mimicry. *Curr. Top. Microbiol. Immunol.* 296:65, 2005.

Oldstone, M.B.A. Molecular mimicry, microbial infection, and autoimmune disease: evolution of the concept. *Curr. Top. Microbiol. Immunol.* 296:1, 2005.

Oldstone, M.B.A. Viral persistence: parameters, mechanisms and future predictions. *Virology* 344:111, 2006.

Oldstone, M.B.A., Dales, S., Tishon, A., Lewicki, H., Martin, L. A role for dual viral hits in causation of subacute sclerosing panencephalitis. *J. Exp. Med.* 202:1185, 2005.

Rhode, A., Pauza, M.E., Barral, A.M., Rodrigo, E., Oldstone, M.B., von Herrath, M.G., Christen, U. Islet-specific expression of CXCL10 causes spontaneous islet infiltration and accelerates diabetes development. *J. Immunol.* 175:3516, 2005.

Tishon, A., Lewicki, H., Andaya, A., McGavern, D., Martin, L., Oldstone, M.B.A. CD4 T cell control primary measles virus infection of the CNS: regulation is dependent on combined activity with either CD8 T cells or with B cells: CD4, CD8 or B cells alone are ineffective. *Virology* 347:234, 2006.

Trifilo, M.J., Hahn, B., Zuniga, E.I., Edelmann, K.H., Oldstone, M.B.A. Dendritic cell inhibition: memoirs from immunosuppressive viruses. *J. Infect. Dis.*, *in press*.

Trifilo, M.J., Yajima, T., Gu, Y., Dalton, N., Peterson, K.L., Race, R.E., Meade-White, K., Portis, J.L., Masliah, E., Knowlton, K.U., Chesebro, B., Oldstone, M.B.A. Prion-induced amyloid heart disease with high blood infectivity in transgenic mice. *Science* 313:94, 2006.

Zuniga, E.I., Edelmann, K.H., Oldstone, M.B.A. Viruses and dendritic cells: a prominent mechanism for subverting the immune response. *In: Microbial Subversion of the Host Immune Response.* Lachmann, P., Oldstone, M.B.A. (Eds.). Horizon Scientific Press, London, 2006, p. 211.

Arenavirus Molecular and Cell Biology: Implications for Novel Antiviral Therapies

A.B. Sánchez, A. Capul, B. Cubitt, N. Nguyen, D. Rosario, J.C. de la Torre

Arenaviruses merit significant attention both as model systems for studies of acute and persistent viral infections and as important human pathogens, including Lassa virus and several other causative agents of severe hemorrhagic fever. Moreover, evidence indicates that the prototypic arenavirus lymphocytic choriomeningitis virus (LCMV) is a neglected human pathogen of clinical importance.

Arenaviruses are enveloped viruses with a bisegmented negative-stranded RNA genome. Each genomic RNA segment, L and S, uses an ambisense coding strategy to direct the synthesis of 2 polypeptides in opposite orientation, separated by an intergenic region. The S RNA encodes the viral glycoprotein and the nucleoprotein, whereas the L RNA encodes the viral RNA-dependent RNA polymerase and the small RING finger protein Z. We have developed a reverse genetics system for LCMV that provides a novel and powerful approach for elucidating the role of viral polypeptides and *cis*-acting sequences in the control of arenavirus RNA replication, gene expression, assembly, and budding and virus-cell protein interactions.

No licensed vaccines against arenavirus are available, and current therapies for arenavirus infections

are limited to the use of ribavirin, which is only partially effective and often causes severe secondary effects. The LCMV reverse genetics system is an excellent platform for investigating novel antiviral strategies to combat arenavirus infections. We identified sequence and structural constraints within the arenavirus genome promoter that revealed a new potential target for aminoglycoside-based drugs against arenaviruses.

Likewise, we showed that the arenavirus functional polymerase strictly requires an L-L interaction that is amenable to disruption by small molecules. We also identified the Z protein as the driving force of arenavirus budding. This process is mediated by proline-rich late (L) domain motifs similar to those known to control budding of several other viruses, including HIV and Ebola virus, via interaction with specific host-cell proteins. We are using genetic and proteomic approaches to identify cellular proteins that interact with Z and are required for arenavirus budding; these approaches may yield new antiviral strategies for targeting arenavirus budding.

LCMV is a Rosetta stone in viral immunology and pathogenesis. We can now generate recombinant LCMVs that have predetermined specific mutations within their genomes, or express additional foreign genes, and analyze their phenotypic expression *in vivo*, a novel and powerful approach for elucidating the molecular mechanisms that underlie arenavirus-host interactions and associated disease.

PUBLICATIONS

Flatz, L., Bergthaler, A., de la Torre, J.C., Pinschewer D.D. Recovery of an arenavirus entirely from RNA polymerase I/II-driven cDNA. *Proc. Natl. Acad. Sci. U. S. A.* 103:4663, 2006.

Kunz, S., de la Torre, J.C. Novel antiviral strategies to combat human arenavirus infections. *Curr. Mol. Med.* 5:735, 2005.

Merkler, D., Horvath, E., Bruck, W., Zinkernagel, R.M., de la Torre, J.C., Pinschewer, D.D. "Viral déjà vu" elicits organ-specific immune disease independent of reactivity to self. *J. Clin. Invest.* 116:1254, 2006.

Sánchez, A.B., de la Torre, J.C. Rescue of the prototypic arenavirus LCMV entirely from plasmid. *Virology* 350:370, 2006.

Sánchez, A.B., Perez, M., Cornu, T., de la Torre, J.C. RNA interference-mediated virus clearance from cells both acutely and chronically infected with the prototypic arenavirus lymphocytic choriomeningitis virus. *J. Virol.* 79:11071, 2005.

Sevilla, N., de la Torre, J.C. Arenavirus diversity and evolution: quasispecies *in vivo*. *Curr. Top. Microbiol. Immunol.* 299:315, 2006.

Virus-Cell Interactions in Persistently Infected Brains

R. Clemente, M. Perez, B. Cubitt, K. Hagiwara, D. Rosario, J.C. de la Torre

Persistent viral infections of the CNS can cause progressive neurologic disorders associated with diverse abnormalities. These findings led to the hypothesis that viruses can contribute to human mental disorders of unknown etiology. We use infection with Borna disease virus (BDV) as a model system to investigate virus-cell interactions that underlie nonlytic persistent viral infections of the CNS and associated disturbances.

BDV is an enveloped virus with a nonsegmented negative-stranded RNA genome, and is the prototypic member of the virus family Bornaviridae, within the order Mononegavirales. We established a reverse genetics system for BDV that enables us to investigate the mechanisms that control BDV RNA replication and gene expression and interactions between the virus and host cells within the CNS.

We found that the ectodomain of the BDV glycoprotein p56 is the sole entity responsible for recognition of the virus receptor and cell entry, and we developed reagents to identify cellular receptors of BDV responsible for the strong tropism of BDV for limbic system neurons. We also uncovered a receptor-independent cell-to-cell propagation of BDV that may play an important role in the biology of the virus.

Neonatal infection of rats with BDV causes distinct CNS neurodevelopmental and behavioral abnormalities that parallel those reported in certain neuropsychiatric disorders in humans. We identified changes in host gene expression associated with BDV persistence. We are using a variety of cell culture systems and animal models to examine the contribution of identified targets to BDV-induced CNS disturbances.

PUBLICATIONS

Perez, M., de la Torre, J.C. Identification of the Borna disease virus (BDV) proteins required for the formation of BDV-like particles. *J. Gen. Virol.* 86:1891, 2005.

Yanai, H., Kobayashi, T., Hayashi, Y., Watanabe, Y., Ohtaki, N., Zhang, G., de la Torre, J.C., Ikuta, K., Tomonaga, K. A methionine-rich domain mediates CRM1-dependent nuclear export activity of Borna disease virus phosphoprotein. *J. Virol.* 80:1121, 2006.

Persistent Viral Infection of the CNS

D.B. McGavern, P. Truong, H. Lauterbach, L. Garidou

We focus on developing and understanding strategies to purge tissues of persistent viral infections without killing or damaging the tissues. Prevention of persistent infections is traditionally attained via vaccination; however, vaccination is often ineffective once a virus establishes persistence. Another factor that must be considered in strategies to eradicate a chronic infection is the anatomic compartment in which the virus establishes persistence. The CNS, for example, resides behind a specialized blood-brain barrier, lacks standard lymphatic drainage, limits the expression of antigen-presenting machinery, and heavily regulates the activity of T cells. The CNS also has an intricate network of nonreplicative cells (neurons) that if targeted could result in dire, potentially irreparable consequences.

Because of the considerable challenges associated with eradicating persistent viral infections, we are using a remarkable therapeutic approach referred to as immunocytotherapy. In this approach, which has been used successfully in a clinical setting, persistently infected hosts are given competent, virus-specific memory T cells. Specifically, we exploit a well-established model in which mice are persistently infected from birth or in utero with lymphocytic choriomeningitis virus (LCMV). Mice infected in this manner become lifelong carriers of LCMV, and the virus establishes persistence in every tissue compartment, including CNS neurons. Quite remarkably, a single injection of LCMV-specific memory T cells into carrier mice achieves systemic eradication of the virus as well as clearance of virus from CNS neurons without evidence of injury.

We are developing novel visualization strategies to define the precise mechanisms by which adoptively transferred memory T cells purge neurons of a persistent viral infection. Recently, we used genetically tagged populations of memory T lymphocytes specific for LCMV glycoprotein in combination with 2- and 3-dimensional microscopy to show that LCMV-specific T cells arrive in the CNS soon after adoptive immunotherapy. The T cells then recruit antigen-presenting cells referred to as dendritic cells into the CNS parenchyma.

Importantly, dendritic cells are able to present antigen to the memory T cells and preferentially induce the

production of an antiviral cytokine (TNF- α) required for successful immunotherapy. Finally, we also showed through *in vivo* depletion studies that successful immunotherapy depends on dendritic cells, because in their absence immunotherapeutic clearance was impeded both in the CNS and in the periphery. On the basis of these results, we postulate that therapeutically enhanced recruitment of dendritic cells into the CNS may be an excellent strategy to promote (or accelerate) clearance of a persistent viral infection.

PUBLICATIONS

Brooks, D.G., McGavern, D.B., Oldstone, M.B.A. Reprogramming of antiviral T cells prevents inactivation and restores T cell activity during persistent viral infection. *J. Clin. Invest.* 116:1675, 2006.

Brooks, D.G., Teyton, L., Oldstone, M.B.A., McGavern, D.B. Intrinsic functional dysregulation of CD4 T cells occurs rapidly following persistent viral infection. *J. Virol.* 79:10514, 2005.

Lauterbach, H., Zuniga, E.I., Truong, P., Oldstone, M.B.A., McGavern, D.B. Adoptive immunotherapy induces CNS dendritic cell recruitment and antigen presentation during clearance of a persistent viral infection. *J. Exp. Med.* 203:1963, 2006.

McGavern, D.B. Immunotherapeutic relief from persistent infections and amyloid disorders. *Neurology* 66(Suppl. 1):S59, 2006.

McGavern, D.B. The role of bystander T cells in CNS pathology and pathogen clearance. *Crit. Rev. Immunol.* 25:289, 2005.

Interaction Between Lassa Virus and Its Receptor and Development of Novel Antiviral Drugs Against Lassa Fever

J.M. Rojek, A.T. Gundersen, D.L. Boger,* S. Kunz

* Department of Chemistry, Scripps Research

Lassa fever is the second most important viral hemorrhagic fever in humans; it accounts for more than 200,000 infections and several thousand deaths each year. We focus on the earliest steps of Lassa virus infection, the binding of the virus to its cellular receptor, and combine studies of fundamental mechanisms of virus–host cell interaction with the development of novel antiviral strategies against this devastating disease.

The cellular receptor for Lassa virus is α -dystroglycan, a cell-surface receptor for proteins of the extracellular matrix that provides a molecular link between the matrix and the cytoskeleton. Our recent studies indicate that binding of Lassa virus to α -dystroglycan involves a high-affinity interaction with specific sugar

residues of the receptor crucial for binding to the extracellular matrix. This finding suggests that the virus mimics the mechanism of receptor recognition of extracellular matrix proteins. As a consequence, Lassa virus efficiently competes with extracellular matrix proteins and perturbs the normal function of α -dystroglycan. Using a combination of biochemical and cell biological techniques, we are studying the impact of binding of the virus on α -dystroglycan–mediated signal transduction and cell-matrix adhesion.

A hallmark of fatal Lassa virus infection in humans is an overwhelming viral load and subsequent collapse of the host's immune system. Because rapid dissemination of the virus critically depends on viral attachment and entry into host cells, drugs that target these steps will be of great therapeutic value. A major goal of our current research is to develop novel antiviral drugs that can block these initial steps of infection. Using high-throughput screening assays for combinatorial small-molecule libraries obtained from the laboratory of D.L. Boger, Department of Chemistry, we have identified a number of compounds that specifically block attachment and entry of Lassa virus into human cells. The most promising compounds are being optimized and pharmacologically characterized, and their exact mechanism of action is being determined.

In contrast to the receptor for Lassa virus, the cellular receptors of the highly pathogenic South American human hemorrhagic fever viruses Junin, Machupo, and Guanarito are currently unknown. Because of the pivotal role of the virus-receptor interaction for infection and tissue tropism, identifying the cellular receptors used by these emerging viruses will substantially contribute to the understanding of their pathogenesis and provide promising new targets for antiviral strategies. After initial biochemical characterization of the receptors for these severe human pathogens, we will use a combination of genetic and biochemical techniques to identify these cellular receptor molecules.

PUBLICATIONS

Kunz, S., de la Torre, J.C. Novel antiviral strategies to combat human arenavirus infections. *Curr. Mol. Med.* 5:735, 2005.

Kunz, S., Rojek, J.M., Kanagawa, M., Spiropoulou, C.F., Barresi, R., Campbell, K.P., Oldstone, M.B.A. Posttranslational modification of α -dystroglycan, the cellular receptor for arenaviruses, by the glycosyltransferase LARGE is critical for virus binding. *J. Virol.* 79:14282, 2005.

Rojek, J.M., Spiropoulou, C.F., Kunz, S. Characterization of the cellular receptors for the South American hemorrhagic fever viruses Junin, Guanarito, and Machupo. *Virology* 349:476, 2006.

Viral Pathogenesis and Antiviral Immunity

J.L. Whitton, N. An, N. Benning, C. Cornell, S. Crocker, B. Eam, R. Feuer, R. Frausto, S. Harkins, I. Hunziker, F. Liu, A. Nussbaum, R. Pagarigan, M.P. Rodriguez-Carreno, J. Whitmire

ANTIVIRAL T-CELL FUNCTION

CD8⁺ T cells play a key role in combating most viral infections, either by killing virus-infected cells or by showering the cells with antiviral cytokines such as IFN- γ . During microbial infection, epitope-specific CD8⁺ T-cell responses usually exist as a hierarchy; responses to some epitopes are much stronger than responses to others. The stronger responses are termed dominant; the weaker, subdominant. The hierarchy is regulated by a poorly understood phenomenon called immunodominance.

We have found that immunodominance depends on expression of IFN- γ . Our current hypothesis is that the immunodominance hierarchy (i.e., the relative abundances of the various epitope-specific T-cell populations) is defined by the rate at which the various epitope-specific cells can initiate production of IFN- γ ; the fastest cells become the dominant population. Our most recent data indicate that expression of receptors for IFN- γ on CD8⁺ T cells is tightly regulated and that cells lacking these receptors are at a selective disadvantage. Therefore, evolution appears to have used IFN- γ to kill two birds with one stone; the cells that are best suited to combat viral infection (i.e., the cells that most rapidly elaborate IFN- γ) are the ones that are preferentially expanded in the host. These studies of T-cell regulation are being extended to include CD4⁺ T cells.

In most studies of T-cell function, including ours, synthetic peptides are used to stimulate T-cell responses *in vitro*. We developed a novel method to identify T cells, and other cell types, that are actively responding to contact with an authentic antigen *in vivo*. Using this approach, we showed that the *in vivo* response of CD8⁺ memory T cells to viral infection is explosive. This method not only will be useful for studies of immune responses to infection but also may facilitate a better understanding of autoimmune disease. In our analysis of antigen-specific activation *in vivo*, we also use *in situ* hybridization, and we have confirmed that the *in vivo* CD8⁺ T-cell IFN- γ response to antigen contact is very rapid and is regulated at the transcriptional level.

VIRAL PATHOGENESIS

Our ongoing studies of the molecular biology of coxsackievirus B3 have suggested a new explanation for how this class of virus evades the host cellular immune response: several of the viral proteins target the Golgi complex, inhibiting the function of the complex and leading to its eventual dissolution. Coxsackievirus B3 is an important human pathogen that causes a variety of clinical syndromes, including myocarditis and pancreatitis. Myocarditis is remarkably common (about 1 million cases per year in the United States), currently is not treatable, and can lead to dilated cardiomyopathy, which is the most common indicator for heart transplantation in young males. We previously showed the importance of CD4⁺ and CD8⁺ T cells in the control of virus-induced myocarditis and in the related immunopathologic changes.

We are extending our studies of coxsackievirus B-specific immune responses to ask why this virus does not induce strong CD8⁺ T-cell responses, despite reaching very high concentrations in various tissues. We are also investigating prophylactic measures by evaluating RNA immunization in the coxsackievirus B3 model by using variant viral genomes with directed mutations that are intended to retain immunogenicity while reducing virulence. Recently, we found that blockade of the protein tissue inhibitor of metalloproteinase 1 can ameliorate myocarditis and its consequences; this observation may be of substantial clinical usefulness. Finally, our studies of coxsackievirus B3 infection of the CNS in neonates indicated that the virus may preferentially infect stem cells and be carried into the brain parenchyma by these cells as the cells migrate toward their final destinations.

AUTOIMMUNITY

Together with colleagues at the University of Utah and the La Jolla Institute of Allergy and Immunology, we are studying the molecular basis of autoimmunity induced by viral infection. Some autoimmune diseases (e.g., multiple sclerosis) appear to be triggered and/or exacerbated by a wide variety of viral infections. Two general mechanisms, molecular mimicry and bystander activation, have been proposed to explain this phenomenon. We have suggested an alternative explanation that is based on changes in antigen presentation that occur during almost all viral infections.

DNA IMMUNIZATION

With our colleague M.J. Buchmeier, Molecular and Integrative Neurosciences Department, we are evaluating

DNA vaccines against the highly pathogenic arenavirus Lassa virus. We have developed a DNA vaccine that encodes Lassa virus proteins, shown that this vaccine induces Lassa virus-specific immune responses in mice, and shown that these mice are protected against challenge with a related, but less pathogenic, arenavirus.

PUBLICATIONS

Fujinami, R.S., von Herrath, M.G., Christen, U., Whitton, J.L. Molecular mimicry, bystander activation, or viral persistence: infections and autoimmune disease. *Clin. Microbiol. Rev.* 19:80, 2006.

Liu, F., Feuer, R., Hassett, D.E., Whitton, J.L. Peptide vaccination of mice immune to LCMV or vaccinia virus causes serious CD8 T cell-mediated, TNF-dependent immunopathology. *J. Clin. Invest.* 116:465, 2006.

Nussbaum, A.K., Rodriguez-Carreno, M.P., Benning, N., Botten, J., Whitton, J.L. Immunoproteasome-deficient mice mount largely normal CD8⁺ T cell responses to lymphocytic choriomeningitis virus infection and DNA vaccination. *J. Immunol.* 175:1153, 2005.

Whitmire, J.K., Benning, N., Whitton, J.L. Cutting edge: early IFN- γ signaling directly enhances primary antiviral CD4⁺ T cell responses. *J. Immunol.* 175:5624, 2005.

Whitmire, J.K., Benning, N., Whitton, J.L. Precursor frequency, nonlinear proliferation, and functional maturation of virus-specific CD4⁺ T cells. *J. Immunol.* 176:3028, 2006.

Whitton, J.L. Adaptive immune responses. *In: Molecular Pathogenesis of Virus Infections.* Digard, P., Nash, A.A., Randall, R.E. (Eds.). Cambridge University Press, New York, 2005, p. 1. Society for General Microbiology Symposia. Scourfield, M. (Series Ed.).

Whitton, J.L., Cornell, C.T., Feuer, R. Host and virus determinants of picornavirus pathogenesis and tropism. *Nat. Rev. Microbiol.* 3:765, 2005.

Laboratory of Translational Neurophysiology and the San Diego Substance Abuse and Minorities Project

C.L. Ehlers, C. Agneta, L. Corey, G. Finerman, D.A. Gilder, J.W. Havstad, P. Lau, S.L. Lopez, E. Phillips, J. Roth, D. Wills

Rates of alcoholism and drug dependence within a population are thought to reflect an almost equal combination of sociocultural (environmental) and biological (genetically determined) factors. Our goal is to identify genes that may encode the neurophysiologic processes that underlie drug dependence in selected populations at high risk for drug dependence, including Native American Indians, Mexican Americans, and islanders of Trinidad and Tobago.

The prevalence of drug and alcohol dependence among ethnic groups varies widely. These differences provide an opportunity to investigate how genetic variation may influence substance abuse. One difference

between ethnic groups is a natural variation in the genes that encode the structure of the enzymes that metabolize alcohol. We were the first to identify a role for genetic variations in 2 genes, the gene for alcohol dehydrogenase (*ADH1B*3*) and the gene for cytosolic aldehyde dehydrogenase (*ALDH1A*1*), in African Americans, Southwest California Indians, and islanders on Trinidad and Tobago. We also showed that the response to alcohol in Asians is highly dependent on variants of the gene for the mitochondrial enzyme aldehyde dehydrogenase (*ALDH2*2*). In Mexican Americans, the presence of another form of alcohol dehydrogenase (*ADH1B*2*) can provide some protection from heavy drinking and alcohol dependence. Our recent studies in Trinidad and Tobago have indicated that individuals with the *ADH1C2*2* genotype are more at risk for alcoholism and alcoholic liver disease than are individuals without that genotype.

Although variations in alcohol-metabolizing enzymes clearly confer protection from or risk for alcoholism, other genes also add to the genetic variance in risk for the disorder. To further evaluate other genetic factors associated with substance dependence, we conducted a genome scan in Southwest California Indian families for alcoholism and behaviors related to substance abuse. We found that chromosomes 4 and 12 appear to have genes linked to the severity of an individual's drinking; chromosomes 6, 15, 16 have genes linked to a severe form of alcoholism with symptoms of withdrawal; and a locus on chromosome 5 is linked to "craving" for alcohol.

Additional genome scans were conducted for use of tobacco, dependence on marijuana, and dependence on stimulants. We discovered that a unique locus on chromosome 14 is linked to marijuana dependence; a locus on chromosome 1, to stimulant dependence; and a locus on chromosome 4 and 8, to use of tobacco. In addition, several sites in the genome are linked not only to use of multiple drugs of abuse but also to body mass.

One theoretical assumption concerning Native Americans is that the long history of dependence on foraging and subsistence agriculture may have led to selective enrichment of traits that improve genetic fitness, so-called thrifty or fat-sparing genes. In addition, such genes might influence fat accumulation during food availability, thus improving survival during times of shortage. The same selective pressure may have enriched for genetic variants that increase the risk for consumption of energy-rich beverages such as alcohol and perhaps for consumption of other drugs of abuse. Taken together, our data lend support for the hypothesis that some genes

confer risk and/or protection for the abuse of specific drugs and that other genes influence common behaviors associated with addiction such as the consumptive drive.

PUBLICATIONS

Cook, T.A.R., Luczak, S.E., Shea, S.H., Ehlers, C.L., Carr, L.G., Wall, T.L. Associations of *ALDH2* and *ADH1B* genotypes with response to alcohol in Asian Americans. *J. Stud. Alcohol* 66:196, 2005.

Ehlers, C.L., Slutske, W., Gilder, D.A., Lau, P. Age of first marijuana use and the development of abuse and dependence in Southwest California Indians. *Pharmacol. Biochem. Behav.*, *in press*.

Ehlers, C.L., Slutske, W., Gilder, D.A., Lau, P., Wilhelmsen, K.C. Age at first intoxication and the development of alcohol dependence in Southwest California Indians. *Alcohol. Clin. Exp. Res.*, *in press*.

Ehlers, C.L., Wall, T.L., Dixon, M., Corey, L., Lau, P., Gilder, D.A., Wilhelmsen, K.C. Heritability of illicit drug use and transition to dependence in Southwest California Indians. *Psychiatr. Genet.*, *in press*.

Ehlers, C.L., Wilhelmsen, K.C. Genomic screen for loci associated with tobacco usage in Mission Indians. *BMC Med. Genet.* 7:9, 2006.

Ehlers, C.L., Wilhelmsen, K.C. Genomic screen for substance dependence and body mass index in Southwest California Indians. *Genes Brain Behav.*, *in press*.

Gilder, D.A., Lau, P., Dixon, M., Corey, L., Phillips, E., Ehlers, C.L. Comorbidity of select anxiety, affective, and psychotic disorders with cannabis dependence in Southwest California Indians. *J. Addict. Dis.*, *in press*.

Irwin, M.R., Valladares, E., Motivala, S., Thayer, J.F., Ehlers, C.L. Association between nocturnal vagal tone and sleep depth, sleep quality, and fatigue in alcohol dependence. *Psychosom. Med.* 68:159, 2006.

Venner, K.L., Wall, T.L., Lau, P., Ehlers, C.L. Testing of an orthogonal measure of cultural identification with adult Mission Indians. *Cultur. Divers. Ethnic Minor. Psychol.*, *in press*.

Wilhelmsen, K.C., Ehlers, C. Heritability of substance dependence in a Native American population. *Psychiatr. Genet.* 15:101, 2005.

Cellular and Molecular Mechanisms of Neuronal Signaling in the CNS

D.L. Gruol, T.E. Nelson, J. Cho,* J. Sabeti, H. Bajova, S. Chow, E. Vereyken,** P.N.E. de Graan**

* Dongguk University, Gyeong Buk, Korea

** University Medical Center Utrecht, Utrecht, the Netherlands

DEVELOPMENTAL REGULATION OF ION CHANNEL FUNCTION IN CNS NEURONS

CNS neurons express a variety of ion channels with specific roles in the generation and regulation of neuronal properties and functions. Of particular importance are the voltage-gated calcium channels (VGCCs). Although several types of these channels are expressed by neurons, calcium signaling through L-type VGCCs is particularly important in many fundamental neuronal processes, including neuronal development, neuronal excitability, and calcium homeostasis.

Extensive studies of VGCCs in cerebellar Purkinje neurons from adult animals, a neuronal type that plays a critical role in fine motor control, have indicated that the channels are essential for dendritic excitability. The primary VGCCs involved in this function are the P/Q-type VGCCs, which are expressed in abundance in Purkinje neurons. Our recent immunohistochemical studies revealed that in addition to P/Q-type VGCCs, Purkinje neurons express L-type VGCCs, both in the mature state and at early stages of development. In the mature neurons, the L-type channels were located primarily in the somatic region, whereas the P/Q-type channels were prominent in both the somatic and the dendritic regions.

To examine the role of L-type VGCCs in Purkinje neurons, we used combined recordings of intracellular calcium and electrical activity in cultured Purkinje neurons at different developmental stages. The results showed that L-type VGCCs contribute to somatic excitability and calcium signaling both early in development, when each Purkinje neuron consists of just a soma and fine perisomatic processes, and at mature stages, when dendrites are present. Interestingly, L-type VGCCs played a prominent role in the somatic excitability in immature Purkinje neurons but only a modest role in mature Purkinje neurons.

Calcium signaling involving L-type VGCCs is an important pathway to gene expression, especially during development. Consistent with such a role for L-type channels in immature Purkinje neurons, our recent studies showed that the calcium signals through L-type VGCCs were communicated to the nucleus and were associated with activation of the transcription factors CREB and c-Fos. These results are consistent with a role for activity-dependent gene expression involving L-type VGCCs in early developing Purkinje neurons.

REGULATION OF MEMORY MECHANISMS BY NEUROACTIVE STEROIDS

The hippocampus is an integral component of the limbic circuitry and plays a central role in memory formation. Through effects on memory-related mechanisms in the hippocampus, exposure to stress can result in strong intrusive memories that interfere with normal memory processing. A diverse class of biological signaling molecules is thought to play a role in stress-related changes in hippocampal memory processes, including the glucocorticoids (i.e., cortisol in humans and corticosterone in rodents), which are considered the major stress hormones; pregnenolone sulfate; and progesterone and its reduced metabolites. These stress

steroids are designated neuroactive steroids because of their ability to modify neuronal activity via both rapid (i.e., membrane receptor- or second messenger-mediated) and delayed (i.e., genomic) actions.

The involvement of glucocorticoids in the brain stress response has been intensely studied, especially in hippocampus; considerably less is known about the role of other stress-induced neuroactive steroids. To address this question, we investigated the actions of pregnenolone sulfate on hippocampal long-term potentiation (LTP), considered the cellular basis for memory and learning, by combining pharmacologic studies with electrophysiology. We found that pregnenolone sulfate selectively facilitated the induction of a slow-developing LTP that was contingent on high-frequency (100 Hz) stimulation of afferent neurons. This LTP was independent of activation of receptors for *N*-methyl-D-aspartate but dependent on L-type VGCCs. Additional studies showed that pregnenolone sulfate produced concentration-dependent inhibition of LTP dependent on receptors for *N*-methyl-D-aspartate. These findings indicate that pregnenolone sulfate produces opposing actions on 2 pharmacologically distinct forms of hippocampal LTP, effects that could be important in the formation of stress-related memories.

PUBLICATIONS

Gruol, D.L., Nelson, T.E. Purkinje neuron physiology is altered by the inflammatory factor interleukin-6. *Cerebellum* 4:198, 2005.

Gruol, D.L., Netzeband, J.G., Quina, L.A., Blakely-Gonzalez, P.K. Contribution of L-type channels to Ca²⁺ regulation of neuronal properties in early developing Purkinje neurons. *Cerebellum* 4:128, 2005.

Gruol, D.L., Netzeband, J.G., Schneeloch, J., Gullette, C.E. L-type Ca²⁺ channels contribute to current-evoked spike firing and associated Ca²⁺ signals in cerebellar Purkinje neurons. *Cerebellum* 5:146, 2006.

Gruol, D.L., Quina, L.A., Netzeband, J.G., Nguyen, D., Gullette, C.E. Developmental changes in Ca²⁺-regulated functions of early postnatal Purkinje neurons. *J. Neurosci. Res.* 83:1381, 2006.

Nelson, T.E., Ur, C.L., Gruol, D.L. Chronic intermittent ethanol exposure enhances NMDA-receptor-mediated synaptic responses and NMDA receptor expression in hippocampal CA1 region. *Brain Res.* 1048:69, 2005.

van Gassen, K.L.I., Netzeband, J.G., de Graan, P.N.E., Gruol, D.L. The chemokine CCL2 modulates Ca²⁺ dynamics and electrophysiological properties of cultured cerebellar Purkinje neurons. *Eur. J. Neurosci.* 21:2949, 2005.

Neurobiology of Addiction and Stress

G.F. Koob, M. Le Moal,* S. Ahmed, E. Riley,** L. Stinus,* L. Pulvirenti,*** R. Purdy,**** H. Richardson, K. Inoue,***** A. Tabarin,† C. Funk, S. Ghozland, T. Greenwell, B. Walker, S. Wee, N. Gilpin, C. Mandyam, O. George, R. Lintz, E. Crawford, R. Schroeder, T. Kimber, M. Cole, M. Arends, M. Brennan, R. Smith, Y. Grant

* Université Victor Ségalen Bordeaux 2, Bordeaux, France

** San Diego State University, San Diego, California

*** Claude Bernard Neuroscience Institute, Pozzilli, Italy

**** Veterans Affairs Medical Center, San Diego, California

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

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

ADDICTION

In studies on the neurobiology of addiction, we continue to focus on the neuropharmacologic mechanisms involved in motivated and emotional behavior and how these mechanisms are altered in addiction, stress, and genetic variability.

We are exploring the role of neurochemical systems in the extended amygdala in the neuroadaptations associated with the transition from drug taking to drug dependence that is an integral part of the development of addiction. We are also developing animal models for excessive drug intake and charting the changes in neurocircuitry associated with excessive drug intake. Previous results established that prolonged access to cocaine can produce progressive increases in drug intake that are paralleled by decreases in reward function. This escalation is paralleled by increased activity in the brain stress system mediated by corticotropin-releasing factor (CRF). New studies suggest that cocaine also activates the neuropeptide hypocretin to produce cocaine-seeking behavior via an arousal action originating in the hypothalamus. Consistent with this observation, animals that escalate cocaine intake have a remodeling of lateral hypothalamic circuitry as measured by gene array studies. These results suggest that the reward dysregulation associated with extended access to drugs of abuse that leads to addiction may depend not only on neuroadaptive changes in basal forebrain systems but also on activation of hypothalamic systems.

Studies in animal models of nicotine dependence revealed similar neuropharmacologic adaptations to chronic administration of nicotine. We found that chronic

exposure to nicotine increased nicotine self-administration in rats and that this increase could be exaggerated by intermittent periods of abstinence from nicotine. A CRF₁ antagonist effectively blocked the development of anxiety-like responses to precipitated nicotine withdrawal. These findings suggest that excessive exposure to nicotine can produce some of the same neuroadaptive changes in the brain that occur with excessive access to other drugs of abuse.

We continue to develop animal models for excessive drinking of alcohol that will be useful in identifying compounds with potential as medications for treatment of drug addiction. The excessive drinking associated with alcohol dependence can be exacerbated by intermittent repeated withdrawal from chronic alcohol exposure. Research with highly selective CRF₁ small molecular antagonists has shown that CRF₁ receptors may be effective in selectively blocking excessive drinking associated with dependence but have no effect in nondependent animals. Because exposure to stressors is a major stimulus for relapse in humans with alcoholism, these data suggest a potential novel role for the CRF system, via CRF₁ receptors, in vulnerability to relapse.

We are refining our conceptual framework that the neurochemical changes in brain stress neurotransmitter systems lead to an allostatic change in motivated behavior. Consistent with a role for self-medication for treatment of emotional states in humans with drug addiction, in the allostatic view, individuals, through genetic vulnerability or environmental events, may use drugs in an attempt to return to a nonstressed state (i.e., to return to motivational homeostasis). Because of the time lag between cause and effect in the neuroadaptive capabilities of the brain motivational systems, however, such individuals do not return to a nonstressed state, and the continued use of drugs further exacerbates the situation. Further refinement of this hypothesis was explored both in the context of a model of negative reinforcement for drug seeking and in the context of the conceptual framework of a brain antireward system.

NEUROPEPTIDES AND STRESS

We are examining the functional significance of members of the CRF brain stress neurotransmitter system. *In vivo* tests with rats and defensive burying showed efficacy for highly selective small-molecule CRF₁ antagonists, confirming a role for the CRF₁ receptor in anxiety-like responses. The CRF₂ receptor-selective agonist murine urocortin 3 had similar anxiolytic-like effects in the defensive burying test. Thus, both blockade of CRF₁

receptor systems and activation of CRF₂ receptor systems produce anti-anxiety-like effects. These results emphasize the selectivity of the actions of the brain CRF and urocortin receptor systems and provide a basis for future studies of the pathophysiology of stress disorders.

PUBLICATIONS

Ahmed, S.H., Koob, G.F. Transition to drug addiction: a negative reinforcement model based on an allostatic decrease in reward function. *Psychopharmacology (Berl.)* 180:473, 2005.

Ahmed, S.H., Lujens, R., van der Stap, L.D., Lekic, D., Romano-Spica, V., Morales, M., Koob, G.F., Repunte-Canonigo, V., Sanna, P.P. Gene expression evidence for remodeling of lateral hypothalamic circuitry in cocaine addiction. *Proc. Natl. Acad. Sci. U. S. A.* 102:11533, 2005.

Boutrel, B., Kenny, P.J., Markou, A., Koob, G.F. Hypocretin and brain reward function. *In: Hypocretins: Integrators of Physiological Functions.* de Lecea, L., Sutcliffe, J.G. (Eds.). Springer, New York, 2005, p. 315.

Boutrel, B., Kenny, P.J., Specio, S.E., Martin-Fardon, R., Markou, A., Koob, G.F., de Lecea, L. Role for hypocretin in mediating stress-induced reinstatement of cocaine-seeking behavior. *Proc. Natl. Acad. Sci. U. S. A.* 102:19168, 2005.

De Witte, P., Littleton, J., Parot, P., Koob, G. Neuroprotective and abstinence-promoting effects of acamprosate: elucidating the mechanism of action. *CNS Drugs* 19:517, 2005.

Frantz, K.J., Koob, G.F. The neurobiology of addiction. *In: Addiction Counseling Review: Preparing for Comprehensive, Certification, and Licensing Examinations.* Coombs, R.H. (Ed.). Lawrence Erlbaum, Mahwah, NJ, 2005, p. 33.

Guillemin, K., Vouillac, C., Azar, M.R., Parsons, L.H., Koob, G.F., Cadore, M., Stinus, L. Monoamine oxidase inhibition dramatically increases the motivation to self-administer nicotine in rats. *J. Neurosci.* 25:8593, 2005.

Heinrichs, S.C., Koob, G.F. Application of experimental stressors in laboratory rodents. *In: Current Protocols in Neuroscience*, 2nd ed. Crawley, J.N., et al. (Eds.). Wiley & Sons, New York, 2005, p. 8.4.1.

Izzo, E., Sanna, P.P., Koob, G.F. Impairment of dopaminergic system function after chronic treatment with corticotropin-releasing factor. *Pharmacol. Biochem. Behav.* 81:701, 2005.

Johnson, B.A., Koob, G.F., Schuckit, M.A., Mason, B.J., Ait-Daoud, N. Understanding and treating alcohol dependence. *Alcohol. Clin. Exp. Res.* 30:567, 2005.

Kenny, P.J., Boutrel, B., Gasparini, F., Koob, G.F., Markou, A. Metabotropic glutamate 5 receptor blockade may attenuate cocaine self-administration by decreasing brain reward function in rats. *Psychopharmacology (Berl.)* 179:247, 2005.

Koob, G.F. The neurocircuitry of addiction: implications for treatment. *Clin. Neurosci. Res.* 5:89, 2005.

Koob, G.F., Le Moal, M. Plasticity of reward neurocircuitry and the "dark side" of drug addiction. *Nat. Neurosci.* 8:1442, 2005.

O'Brien, C.P., Anthony, J.C., Carroll, K., Childress, A.R., Dackis, C., Diamond, G., Hornik, R., Johnston, L.D., Jones, R., Koob, G.F., Kosten, T., Lerman, C., McLellan, A.T., Moss, H., Pettinati, H., Spoth, R. Defining substance use disorders. *In: Treating and Preventing Adolescent Mental Health Disorders: What We Know and What We Don't Know—A Research Agenda for Improving the Mental Health of Our Youth.* Evans, D.L., et al. (Eds.). Oxford University Press, New York, 2005, p. 335.

O'Dell, L.E., Purdy, R.H., Covey, D.F., Richardson, H.N., Roberto, M., Koob, G.F. Epipregnanolone and a novel synthetic neuroactive steroid reduce alcohol self-administration in rats. *Pharmacol. Biochem. Behav.* 81:543, 2005.

Qi, L., Yamamoto, N., Meijler, M.M., Altobelli, L.J. III, Koob, G.F., Wirsching, P., Janda, K.D. Δ^9 -Tetrahydrocannabinol immunochemical studies: haptens, monoclonal antibodies, and a convenient synthesis of radiolabeled Δ^9 -tetrahydrocannabinol. *J. Med. Chem.* 48:7389, 2005.

Sanchis-Segura, C., Grisel, J.E., Olive, M.F., Ghazizadeh, S., Koob, G.F., Roberts, A.J., Cowen, M.S. Role of the endogenous opioid system on the neuropsychopharmacological effects of ethanol: new insights about an old question. *Alcohol. Clin. Exp. Res.* 29:1522, 2005.

Tabarin, A., Chaves, Y.D., Carmona, M.D., Catargi, B., Zorrilla, E.P., Roberts, A.J., Coscina, D.V., Rousset, S., Redonnet, A., Parker, G.C., Inoue, K., Ricquier, D., Penicaud, L., Kieffer, B.L., Koob, G.F. Resistance to diet-induced obesity in μ -opioid receptor-deficient mice: evidence for a "thrifty gene." *Diabetes* 54:3510, 2005.

Zorrilla, E.P., Inoue, K., Fekete, E.M., Tabarin, A., Valdez, G.R., Koob, G.F. Measuring meals: structure of prandial food and water intake of rats. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 288:R1450, 2005.

Zorrilla, E.P., Koob, G.F. The roles of urocortins 1, 2 and 3 in the brain. *In: Handbook of Stress and the Brain, Part 1: The Neurobiology of Stress.* Steckler, T., Kalin, N.H., Reul, J.M.H.M. (Eds.). Elsevier Science, New York, 2005, p. 179. *Techniques in the Behavioral and Neural Sciences*; Vol. 15.

Role of the Neuregulins in the Nervous System

C. Lai, J.L. Weber, J. Tan, A. Dowell, R. Salazar, D. Hom

The focus of our research is understanding the signaling mechanisms that underlie the establishment and maintenance of mature neuronal and glial cell phenotypes. We are studying the roles played by a subfamily of receptor protein-tyrosine kinases, the ErbBs (EGFR, ErbB2, ErbB3, and ErbB4), and their ligands, the neuregulins (NRG-1–NRG-4). NRG-1 was first recognized as the Schwann cell mitogen glial growth factor. NRG-1 was also termed ARIA (for acetylcholine receptor inducing activity), which was thought to regulate expression of acetylcholine receptors at developing neuromuscular junctions. These distinct functions are now thought to be served by discrete types of NRG-1 (I, II, and III) that arise by alternative splicing. A primary goal of our research is to understand the specific roles of each of these types of NRG-1 in the nervous system.

NRG-1 supports survival of Schwann cells and regulates the number of premyelinating Schwann cells. The results of genetic studies suggested that the type III isoform serves in this capacity, and we helped determine that this isoform also plays a key role in regulating the thickness of the myelin sheath. The emerging picture is that different NRG-1 isoforms serve as signaling molecules from neuron to glial cell and from neuron to muscle to carry out distinct biological activities. We are also pursuing the roles of these NRG-1 isoforms in the brain, which became an area of considerable interest after NRG-1 was identified as a susceptibility gene for schizophrenia.

We have 4 areas of primary interest. The first is the roles of the 3 types of NRG-1 in the developing and mature nervous system. We developed transgenic mice that permit the tetracycline-regulated expression of specific NRG-1 isoforms. With these mice, we can assess the distinct biological functions served by each isoform.

The second area is neurogenesis and migration. We found that the neuregulin receptor ErbB4 is expressed by multiple tangentially migrating populations of neuronal cells in the developing and mature nervous systems. ErbB4 is expressed at high levels in the mature subventricular zone and rostral migratory stream, one of the few regions in the brain in rats where neurogenesis occurs in adults. We are searching for the endogenous ligands and are testing the effects of the NRGs on cells derived from the subventricular zone. Our data suggest that ErbB4 influences both the proliferation of neural progenitor cells and the migration of neuroblasts in the rostral migratory stream.

The third area of interest is the effects of the loss of ErbB4 function in the mature brain. We are analyzing the phenotype of mice that lack the gene for ErbB4 in the nervous system. These animals have a reduction in anxiety-like behavior, and we are testing the hypothesis that the loss of ErbB4 in the amygdala underlies this defect.

Last, we are developing transgenic tools that allow regulated gene expression in specific subsets of neurons. We developed lines of mice that permit regulated gene expression in cholinergic neurons, and we are evaluating similar lines that permit regulated expression in either dopaminergic neurons or the medium spiny neurons of the striatum. These animal models may be useful for investigating addiction and neurodegenerative disorders such as Alzheimer's, Parkinson's and Huntington's diseases.

PUBLICATIONS

Ghashghaei, H.T., Weber, J.L., Pevny, L., Schmid, R., Schwab, M.H., Lloyd, K.C., Eisenstat, D.D., Lai, C., Anton, E.S. The role of neuregulin-ErbB4 interactions on the proliferation and organization of cells in the subventricular zone. *Proc. Natl. Acad. Sci. U. S. A.* 103:1930, 2006.

Ponomareva, O., Ma, H., Dakour, R., Raabe, T.D., Lai, C., Rimer, M. Stimulation of acetylcholine receptor transcription by neuregulin-2 requires an N-box response element and is regulated by alternative splicing. *Neuroscience* 134:495, 2005.

Neurobiology of Reward, Motivation, and Emotion in Psychiatric Disorders

A. Markou, K. Fish, S.G. Semenova, N.E. Paterson, P.J. Kenny, M. Liechti, A. Bruijnzeel, A. Barr, B. Boutrel, B. Henry, N. Amitai, S. Jonkman, J. Benedict, G. Finnerman, B. Silbaugh, J. Cryan,* W. Froestl,* D. Slattery,* A. Bespalov,** T. Svensson***

* Novartis Pharma AG, Basel, Switzerland

** Pavlov Medical University, St. Petersburg, Russia

*** Karolinska Institutet, Stockholm, Sweden

The focus of our research is the neurobiology of reward, motivation, and emotion in 3 psychiatric disorders: drug abuse, depression, and schizophrenia.

Two factors that contribute to habitual tobacco smoking are the euphorogenic and other reinforcing (e.g., cognitive enhancement, anxiolytic) effects of nicotine and the depression-like symptoms that occur when a person quits smoking. The depressive symptoms motivate the reinitiation of tobacco use to alleviate this negative affective state. Because nicotine is the main ingredient in tobacco that leads to addiction, we are studying the neurobiology of nicotine reinforcement and dependence. During the past few years, we have focused on glutamatergic and γ -aminobutyric acid modulation of nicotine reinforcement and dependence.

Nicotine activates nicotinic acetylcholine receptors on glutamate-releasing neurons, terminals, leading to increased glutamate release. This finding suggests that drugs that act on glutamate receptors may alter behavioral effects of nicotine related to dependence. In rats, blockade of postsynaptic metabotropic glutamate 5 (mGlu5) receptors with 2-methyl-6-(phenylethynyl)pyridine hydrochloride, a selective mGlu5 receptor antagonist, and blockade of postsynaptic ionotropic *N*-methyl-D-aspartate (NMDA) receptors with LY235959, an NMDA receptor antagonist, decreased nicotine self-administration at doses that had no effect on responding for food. Treatment with 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo(F)quinoxaline, an antagonist of α -amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid/kainate receptors, did not alter nicotine self-administration. Further, blockade of mGlu5 receptors decreased incentive-motivation for nicotine and nicotine-seeking behavior in rats. In addition, NMDA receptor antagonism reversed the reward-facilitating effects of nicotine, thus blocking

a property of nicotine that contributes to its potential for abuse. In addition, in a study of the reward-facilitating effects of nicotine in rats self-administering nicotine, chronic nicotine self-administration led to plastic changes in NMDA receptor activity reflected by increased sensitivity to NMDA receptor antagonism.

We also examined the role of mGlu2/3 receptors, which are inhibitory autoreceptors on glutamate-releasing neurons, in nicotine-related behaviors. Activation of these receptors with LY379268, an mGlu2/3 receptor agonist, decreased nicotine self-administration at doses that had no effect on responding for food; this effect persisted with chronic LY379268 administration for 5 days before tolerance developed. Further, LY379268 blocked cue-induced reinstatement of nicotine-seeking behavior. Interestingly, rats chronically treated with nicotine had increased activity of mGlu2/3 receptors; treatment with the mGlu2/3 receptor agonist LY314582 precipitated withdrawal-like reward deficits. Consistent with this finding, the mGlu2/3 receptor antagonist LY341495 reversed the reward deficits that occurred during the early phase of spontaneous nicotine withdrawal.

These data indicate that actions of nicotine on glutamate transmission are critically involved in mediating several behavioral effects of nicotine related to its potential for abuse and its dependence-inducing properties. Blockade of the stimulatory effects of nicotine on glutamate transmission, through antagonism of excitatory postsynaptic glutamate receptors or activation of inhibitory presynaptic glutamate autoreceptors, attenuates the reinforcing effects of nicotine and nicotine-seeking behaviors.

Further, chronic exposure to nicotine results in adaptations in presynaptic inhibitory and postsynaptic excitatory glutamate receptors, most likely to counteract the acute effects of nicotine on glutamate transmission. Such adaptations in the activity of glutamate receptors most likely lead to the behavioral effects associated with dependence on nicotine, such as reward facilitation, aversive withdrawal signs shortly after cessation of nicotine administration, and increased vulnerability to relapse during extended abstinence.

These findings suggest several new targets for the development of antismoking medications. In addition, on the basis of the phenomenologic and neurobiological similarities between drug withdrawal and non-drug-induced depressions, we hypothesize that mGlu2/3 receptor antagonists may also have therapeutic antidepressant properties for non-drug-induced depression.

In other ongoing work, we are developing and using rat and murine models of the cognitive deficits of schizophrenia in humans, and we are extending our investigations on nicotine dependence to mice, the mostly commonly used species in genetic studies.

PUBLICATIONS

Barr, A.M., Powell, S.B., Markou, A., Geyer, M.A. Iloperidone reduces sensorimotor gating deficits in pharmacological models, but not a developmental model, of disrupted prepulse inhibition in rats. *Neuropharmacology*, *in press*.

Bespalov, A.Y., Dravolina, O.A., Sukhanov, I., Zakharova, E., Blokhina, E., Zvartau, E., Danysz, W., van Heeke, G., Markou, A. Metabotropic glutamate receptor (mGluR5) antagonist MPEP attenuated cue- and schedule-induced reinstatement of nicotine self-administration behavior in rats. *Neuropharmacology* 49(Suppl. 1):167, 2005.

Boutrel, B., Kenny, P.J., Specio, S.E., Martin-Fardon, R., Markou, A., Koob, G.F., de Lecea, L. Role for hypocretin in mediating stress-induced reinstatement of cocaine-seeking behavior. *Proc. Natl. Acad. Sci. U. S. A.* 102:19168, 2005.

Brujinzeel, A.W., Markou, A. Decreased sensitivity to the effects of dopamine D1-like, but not D2-like, receptor antagonism in the posterior hypothalamic region/anterior ventral tegmental area on brain reward function during chronic exposure to nicotine in rats. *Brain Res.* 1058:91, 2005.

Jonkman, S., Markou, A. Blockade of nicotinic acetylcholine or dopamine D1-like receptors in the central nucleus of the amygdala or the bed nucleus of the stria terminalis does not precipitate nicotine withdrawal in nicotine-dependent rats. *Neurosci. Lett.* 400:140, 2006.

Kenny, P.J., Chen, S.C., Kitamura, O., Markou, A., Koob, G.F. Conditioned withdrawal drives heroin consumption and decreases reward sensitivity. *J. Neurosci.* 26:5894, 2006.

Kenny, P.J., Markou, A. Conditioned nicotine withdrawal profoundly decreases the activity of brain reward systems. *J. Neurosci.* 25:6208, 2005.

Kenny, P.J., Markou, A. Nicotine self-administration acutely activates brain reward systems and induces a long-lasting increase in reward sensitivity. *Neuropsychopharmacology* 31:1203, 2006.

Lindblom, N., de Villiers, S.H., Semenova, S., Kalayanov, G., Gordon, S., Schilström, B., Johansson, A., Markou, A., Svensson, T.H. Active immunisation against nicotine blocks the reward facilitating effects of nicotine and partially prevents nicotine withdrawal in the rat as measured by dopamine output in the nucleus accumbens, brain reward thresholds and somatic signs. *Naunyn Schmiedeberg's Arch. Pharmacol.* 372:182, 2005.

Markou, A. Metabotropic glutamate receptor antagonists: novel therapeutics for nicotine dependence and depression? *Biol. Psychiatry*, *in press*.

Matta, S.G., Balfour, D.J., Benowitz, N.L., Boyd, R.T., Buccafusco, J.J., Caggiula, A.R., Craig, C.R., Collins, A.C., Corrigan, W.A., Damaj, M.A., Donny, E.C., Gardner, P.S., Grady, S.R., Heberlein, U., Leonard, S.S., Levin, E.D., Lukas, R.J., Markou, A., Marks, M.J., McCallum, S.E., Parameswaran, N., Perkins, K.A., Picciotto, M.R., Quik, M., Rose, J.E., Rothenflut, A., Schafer, W.R., Stolerman, I.P., Tyndale, R.F., Wehner, J.M., Zirger, J.M. Guidelines on nicotine dose selection for in vivo research. *Psychopharmacology (Berl.)*, *in press*.

O'Dell, L.E., Brujinzeel, A.W., Smith, R.T., Parsons, L.H., Merves, M.L., Goldberger, B.A., Richardson, H.N., Koob, G.F., Markou, A. Diminished nicotine withdrawal in adolescent rats: implications for vulnerability to addiction. *Psychopharmacology (Berl.)* 186:629, 2006.

Paterson, N.E., Brujinzeel, A.W., Kenny, P.J., Wright, C.D., Froestl, W., Markou, A. Prolonged nicotine exposure does not alter GABA_B receptor-mediated regulation of brain reward function. *Neuropharmacology* 49:953, 2005.

Paterson, N.E., Markou, A. Design of animal models and treatments for addiction and depression comorbidity. *Neurotox. Res.*, *in press*.

Paterson, N.E., Markou, A. The metabotropic glutamate 5 antagonist MPEP decreased break points for nicotine, cocaine and food in rats. *Psychopharmacology (Berl.)* 179:255, 2005.

Slattery, D.A., Markou, A., Froestl, W., Cryan, J.F. The GABA_B receptor-positive modulator GS39783 and the GABA_B receptor agonist baclofen attenuate the reward-facilitating effects of cocaine: intracranial self-stimulation studies in the rat. *Neuropsychopharmacology* 30:2065, 2005.

Medication Development in Substance Dependence

B.J. Mason, K. Buffkins, K. Coveney, R. Crean, T. Escher, J. Light, S. Nash, S. Payton, S. Quello, J. Reiter, J. Diamant,* F. Shadan,* M. Kyle,* S. Rao,* M. Adusumalli,* J. Gleason,* D. Drobos**

* Scripps Green Hospital, La Jolla, California

** University of South Florida, Tampa, Florida

The focus of our research is the clinical 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 long-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 hope 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.

CLINICAL RESEARCH

Alcohol and nicotine dependence are major public health problems that tend to occur together. Existing treatments address each disorder independently and are of limited efficacy. The opioid antagonist naltrexone (ReVia) is approved by the Food and Drug Administration for the treatment of alcohol dependence. Some laboratory and clinical studies suggest that administration of opioid antagonists also reduces signs and symptoms of nicotine dependence, but the findings vary.

We recently determined the efficacy of naltrexone for treatment of outpatients with concurrent nicotine and

alcohol dependence in a double-blind, placebo-controlled 12-week trial of either a fixed daily dose of naltrexone or a daily placebo. In order to control for over-the-counter availability of nicotine replacement products and to examine potential drug-drug interactions, patients who set a smoking quit date during the first 6 weeks of study were also randomized to receive a nicotine replacement patch (Nicotrol) or a placebo patch.

In an interim analysis of the first 53 subjects, compared with the other treatments, treatment with naltrexone was associated with a higher rate of premature termination of therapy and no beneficial effects on drinking or smoking outcomes. Patients who used the nicotine replacement patch stayed in treatment longer and had more nonsmoking days, fewer heavy smoking days, longer time to smoking relapse, less irritability, and more nondrinking days than did patients who did not use the patch. These results suggest that nicotine replacement (agonist) therapy may result in longer compliance with treatment and better smoking and drinking outcomes than does naltrexone (antagonist) treatment in patients with concurrent alcohol and tobacco dependence.

We are currently conducting a 12-week, double-blind, placebo-controlled dose-ranging study to evaluate gabapentin, an anticonvulsant with favorable side effects, as a treatment for signs and symptoms such as anxiety and insomnia that may occur after 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 have also received funding from the National Institute on Drug Abuse to conduct a 12-week, double-blind, placebo-controlled study of gabapentin as a potential treatment for cannabis dependence. We hypothesize that gabapentin will improve signs and symptoms of cannabis withdrawal and, as a result, facilitate setting a quit date, promote longer-term abstinence, and decrease risk and severity of relapse to cannabis use.

DEVELOPMENT OF A HUMAN LABORATORY MODEL

We developed a human experimental model of various components of the alcoholism cycle. We use cue reactivity and mood induction techniques in the laboratory and assessment of drinking, mood, and sleep under natural conditions. The parameters of the model are used to evaluate potential treatments for various components of the alcoholism cycle.

In an important validation study of the model, we found that acamprosate, a medication approved by the

Food and Drug Administration to reduce relapse in early abstinence from alcohol, is highly involved in mediating responsivity to affective as well as alcohol cues in recently abstinent patients with alcoholism. A 5-year renewal of this project by the National Institute on Alcohol Abuse and Alcoholism enables us to use the model to rapidly screen medications that may prevent relapse to drinking.

PUBLICATIONS

Anton, R.F., O'Malley, S.S., Ciraulo, D.A., Cisler, R.A., Couper, D., Donovan, D.M., Gastfriend, D.R., Hosking, J.D., Johnson, B.A., LoCastro, J.S., Longabaugh, R., Mason, B.J., Mattson, M.E., Miller, W.R., Pettinati, H.M., Randall, C.L., Swift, R., Weiss, R.D., Williams, L.D., Zweben, A., the COMBINE Study Research Group. Combined pharmacotherapies and behavioral interventions for alcohol dependence: the COMBINE study: a randomized controlled trial. *JAMA* 295:2003, 2006.

Johnson, B.A., Koob, G.F., Schuckit, M.A., Mason, B.J., Ait-Daoud, N. Understanding and treating alcohol dependence. *Alcohol. Clin. Exp. Res.* 30:567, 2006.

Kranzler, H.R., Mueller, T., Cornelius, J., Pettinati, H.M., Moak, D., Martin, P.R., Anthenelli, R., Brower, K.J., O'Malley, S., Mason, B.J., Hasin, D., Keller, M. Sertraline treatment of co-occurring alcohol dependence and major depression. *J. Clin. Psychopharmacol.* 26:13, 2006.

Mason, B.J. Acamprosate in the treatment of alcohol dependence. *Expert Opin. Pharmacother.* 6:2103, 2005.

Mason, B.J., Goodman, A.M., Chabac, S., Leher, P. Effect of oral acamprosate on abstinence in patients with alcohol dependence in a double-blind, placebo-controlled trial: the role of patient motivation. *J. Psychiatr. Res.* 40:383, 2006.

Chemokine Effects on Neuronal Physiology

T.E. Nelson, D.L. Gruol, H. Bajova, J. Cho*

* Dongguk University, Gyeong Buk, South Korea

Chemokines are members of the cytokine family of immunoregulators whose primary role is the activation and trafficking of leukocytes to sites of infection or injury. Expression of chemokines in the CNS is upregulated in a number of neurologic diseases and disorders, including HIV-associated dementia, multiple sclerosis, Alzheimer's disease, brain tumors, CNS trauma, and stroke. In the CNS, chemokines are expressed predominantly by glial cells (astrocytes and microglia), whereas chemokine receptors are expressed by both neurons and glial cells, indicating that the latter 2 cell types are potential targets of chemokine actions in the CNS. In addition to their role in neuroinflammation, chemokines are also involved in regulating normal neural processes, including neuronal migration, modulation of synaptic activity and plasticity, and neuronal survival.

Currently, we are using primary organotypic cultures of rat hippocampus to investigate the effects of

acute and chronic chemokine exposure on neuronal function. We have focused on the chemokine CXCL10 (previously known as IFN- γ -inducible protein-10 or IP-10). Elevated levels of CXCL10 are highly prevalent in the cerebrospinal fluid of patients with HIV type 1 infection and correlate strongly with the severity of the neurologic disorders associated with the infection.

Using fluorescence-based calcium imaging and intracellular electrophysiologic recording, we found that acute exposure to CXCL10 enhanced ongoing electrical activity of hippocampal neurons maintained in culture and that this enhancement coincided with increased activity-dependent elevations of intracellular calcium in these cells. In addition, using immunoblotting and immunohistochemical methods, we investigated (1) effects of acute CXCL10 exposure on signal transduction mediated by extracellular-signal-regulated kinase 1/2 (ERK1/2) in hippocampal neurons and (2) alterations in expression of neurotransmitter receptors induced in hippocampal cultures by chronic exposure to CXCL10. We found that acute exposure (5 minutes) to CXCL10 activated ERK1/2 in hippocampal neurons; after longer exposure (15–30 minutes), ERK1/2 activity was decreased relative to control levels. In addition, 30–40 minutes of exposure inhibited ERK1/2 activation mediated by the *N*-methyl-D-aspartate (NMDA) subtype of glutamate receptors. After chronic CXCL10 exposure (7 days), protein levels of the NR1, NR2A, and NR2B subunits of the NMDA receptor were increased.

Functional studies indicated that calcium signaling mediated by NMDA receptors in hippocampal neurons was enhanced after chronic CXCL10 exposure, corresponding to the increased expression of the receptor subunits. In contrast, expression of the R1 subunit of the γ -aminobutyric acid B receptor was reduced by chronic CXCL10 exposure. Chronic exposure also was associated with a decrease in the effect of CGP55845A, an antagonist of the γ -aminobutyric acid B receptor, on spontaneous calcium oscillations in the neuronal network of hippocampal neurons. Our results indicate that chemokines modulate CNS function under normal physiologic conditions as well as during periods of immune challenge, neurotrauma, or neurologic disease.

PUBLICATIONS

Gruol, D.L., Nelson, T.E. Purkinje neuron physiology is altered by the inflammatory factor interleukin-6. *Cerebellum* 4:198, 2005.

Nelson, T.E., Ur, C.L., Gruol, D.L. Chronic intermittent ethanol exposure enhances NMDA-receptor-mediated synaptic responses and NMDA receptor expression in hippocampal CA1 region. *Brain Res.* 1048:69, 2005.

Neurochemistry of Addiction

L.H. Parsons, L. Alvarez, I. Polis, D. Stouffer

Endogenous cannabinoids such as anandamide and 2-arachidonoylglycerol (2-AG) modulate several functions, including memory, emotional behavior, and pain perception. Endocannabinoids are also involved in motivation and reward processes, and converging evidence from studies in humans and animals implicates the endogenous cannabinoid system in the etiology of drug addiction.

We have developed an *in vivo* microdialysis method for monitoring extracellular endocannabinoid levels in the brains of rats during ongoing behavioral tasks. Using this technique, we found that voluntary self-administration of ethanol, heroin, and cocaine in rats induces distinct alterations in extracellular endocannabinoid levels in the nucleus accumbens, a brain region critically involved in drug reward.

Oral self-administration of ethanol produced a strong increase in extracellular 2-AG levels with no concomitant alteration in extracellular levels of anandamide. Conversely, heroin self-administration significantly increased anandamide levels while inducing a slight but significant decrease in 2-AG levels. The relative changes in endocannabinoid levels in the nucleus accumbens induced by ethanol and heroin were significantly correlated with the amount of drug consumed by each animal, indicating a dose-dependent pharmacologic process. In contrast, neither the anandamide level nor the 2-AG level in the nucleus accumbens was altered by cocaine self-administration.

These observations are consistent with our finding that self-administration of ethanol and of heroin is reduced by blockade of cannabinoid-1 (CB₁) receptors, whereas cocaine self-administration is not. Collectively these findings suggest that the motivational effects of ethanol and heroin are mediated in part by drug-induced formation of endocannabinoids.

In support of this hypothesis, we found that pharmacologic manipulations of endocannabinoid signaling altered ethanol self-administration behavior only when sufficient blood alcohol levels were achieved. Blood alcohol levels after self-administration were nearly 3-fold higher when rats were presented with a 10% ethanol solution than when they were presented with a 2% ethanol solution. Lever pressing for a 10% ethanol

solution was decreased by treatment with the CB₁ receptor antagonist rimonabant and increased by treatment with the endocannabinoid clearance inhibitor AM404; treatment with these compounds did not alter the self-administration of 2% ethanol. In contrast, treatment with the synthetic CB₁ receptor agonist WIN 55,212-2 significantly increased lever pressing for both 10% and 2% concentrations of ethanol. These data are consistent with a dose-dependent effect of ethanol on the formation of endocannabinoids and indicate that ethanol-induced increases in endocannabinoid formation participate in the regulation of alcohol consumption.

We also found evidence for a sensitization of ethanol-induced increases in brain levels of 2-AG after chronic exposure to ethanol. In ethanol-naïve rats, acute intraperitoneal administration of ethanol increased levels of 2-AG in the nucleus accumbens to approximately 150% of baseline; this effect dissipated within 60 minutes of ethanol administration. However, this same dose increased levels of 2-AG in the nucleus accumbens to approximately 280% of baseline for a period of more than 2 hours in rats previously exposed to ethanol for 14 days via a liquid-diet procedure. The potentiation of ethanol-induced formation of endocannabinoids after chronic ethanol exposure suggests a potential involvement of endocannabinoids in the development of ethanol dependence.

Finally, we have been testing the influence of endocannabinoid signaling on the vulnerability to drug relapse. We found that intraperitoneal administration of rimonabant significantly decreased the reinstatement of drug-seeking behavior induced by presentation of an environmental cue that signals the availability of heroin. Several brain regions were implicated in the mediation of cue-induced drug-seeking behavior, including the core subregion of the nucleus accumbens, the basolateral nucleus of the amygdala, and the medial prefrontal cortex. Localized administration of rimonabant into the core subregion of the nucleus accumbens dose-dependently attenuated cue-induced drug seeking, whereas administration of this CB₁ antagonist into the basolateral amygdala did not.

Collectively our observations indicate an involvement of drug-induced formation of endocannabinoids in the regulation of ethanol and heroin intake and suggest a possible role for endocannabinoids in the development of drug dependence and the vulnerability toward drug relapse.

PUBLICATIONS

Breese, G.R., Criswell, H.E., Carta, M., Dodson, P.D., Hanchar, H.J., Khisti, R.T., Mamelli, M., Ming, Z., Morrow, A.L., Olsen, R.W., Otis, T.S., Parsons, L.H., Penland, S.N., Roberto, M., Siggins, G.R., Valenzuela, C.F., Wallner, M. Basis of the GABA_{mimetic} profile of ethanol. *Alcohol. Clin. Exp. Res.* 30:731, 2006.

Caillé, S., Parsons, L.H. Cannabinoid modulation of opiate reinforcement through the ventral striatopallidal pathway. *Neuropsychopharmacology* 31:804, 2006.

Frantz, K.J., O'Dell, L.E., Parsons, L.H. Behavioral and neurochemical responses to cocaine in periadolescent and adult rats. *Neuropsychopharmacology*, *in press*.

O'Dell, L.E., Manzardo A., Polis, I., Parsons, L.H. Biphasic alterations in 5-HT_{1B} receptor function during abstinence from extended cocaine self-administration. *J. Neurochem.*, *in press*.

Purdy, R.H., Fitzgerald, R.L., Alomary, A.A., Parsons, L.H. The analysis of neuroactive steroids by mass spectrometry. *In: Handbook of Neurochemistry and Molecular Neurobiology*, 3rd ed. Baker, G., et al. (Eds.). Springer, New York, *in press*. *Practical Neurochemistry Methods*.

Selvaige, D.J., Parsons, L., Rivier, C. Role played by brainstem neurons in regulating testosterone secretion via a direct neuronal pathway between the hypothalamus and the testes. *Endocrinology* 147:3070, 2006.

Brain-Computer Interface: New Directions

B.Z. Allison, J. Polich

Millions of persons have neuromuscular disorders due to central or peripheral nervous system injury, amyotrophic lateral sclerosis, stroke, cerebral palsy, muscular dystrophy, multiple sclerosis, Guillian-Barré syndrome, and other causes. Amputees also need alternative interfaces to control prosthetic devices. Many patients with disabilities cannot use keyboards, computer mice, or other conventional interfaces and rely on alternative means of communication that require voluntary control of movement, such as speech, gaze shifting, muscle activity, and breathing. Some patients cannot use any interface that requires motor control.

A brain-computer interface (BCI) is a communication system in which messages or commands that a person sends to the external world do not pass through the normal output pathways of peripheral nerves and muscles. The system infers the user's intent via direct measures of brain activity.

BCI COMPONENTS

Figure 1 schematically illustrates the 4 BCI components. The first component is signal acquisition. Nearly all BCI systems rely on measures of electroencephalographic activity collected via electrodes placed on the scalp. These noninvasive systems do not require expensive or bulky equipment, highly trained personnel, surgery, or long preparation times.

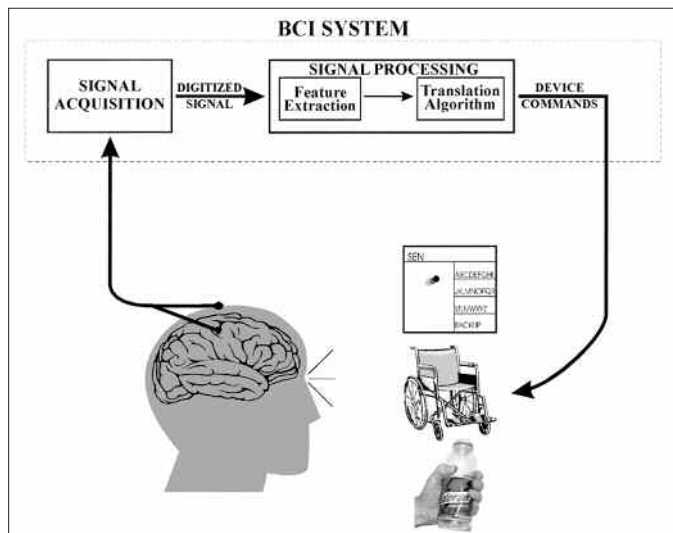


Fig. 1. Schematic illustration of the 4 major BCI components: signal acquisition, signal processing, output device, and operating protocol. Reprinted from Wolpaw, J.R., Birbaumer, N., McFarland, D.J., Pfurtscheller, G., Vaughan, T.M. Brain-computer interfaces for communication and control. *Clin. Neurophysiol.* 113:767. Copyright 2002, with permission from International Federation of Clinical Neurophysiology.

The second component is signal processing, which includes feature extraction and a translation algorithm. Electroencephalographic features of interest are isolated and then translated into instructions for the output device. Some signal-processing mechanisms are more adaptive than others are. An ideal BCI would respond to initial identification of a user's brain wave features, adjust to phasic changes, and update as the user adapts.

The third component, the output device, implements the messages or commands conveyed by the translation algorithm. The most common BCI output device is a computer monitor, although other output devices have been developed to control appliances, robotic arms, mobile robots, and functional electrical stimulators.

The fourth component is the operating protocol, which reflects how the user and the BCI interact. The output device is hardware, whereas the operating protocol is software that includes how the user can affect the hardware. Monitor-based BCIs have been developed in which users control a switch, move a cursor in one or more dimensions, directly select one of two or more choices, select items from a scrolling or iterative menu, browse the Internet, or navigate a virtual environment. Additional issues include the timing of trials and sessions, feedback, word and sentence completion, and error-correction mechanisms based on response verification, a backspace option, or event-related brain potentials such as the P300 component.

SCRIPPS BCI RESEARCH GROUP

A major limitation of current BCIs is poor information throughput compared with that of other assistive interfaces. This difficulty hampers direct communication between patients and other persons, monitors, or devices. Researchers in the Cognitive Electrophysiology Laboratory in the Molecular and Integrative Neurosciences Department have begun a series of studies to improve traditional BCIs that enable spelling and other communication by combining the BCIs with steady-state stimulation to produce highly readable brain wave signals. Initial investigations with these "hybrid BCI" approaches have shown promise for improving performance. These methods are more reliable than conventional systems and are easier to use with different technologies. If successful, this approach will greatly accelerate development of system applications to critical populations of patients.

PUBLICATIONS

Cahn, B.R., Polich, J. Meditation states and traits: EEG, ERP, and neuroimaging studies. *Psychol. Bull.* 132:180, 2006.

Combs, L.A., Polich, J. P3a from white noise stimuli. *Clin. Neurophysiol.* 117:1106, 2006.

Conroy, M.A., Polich, J. Affective valence and P300 when stimulus arousal level is controlled. *Cogn. Emot., in press.*

Hagen, G.F., Gatherwright, J.R., Lopez, B.A., Polich, J. P3a from visual stimuli: task difficulty effects. *Int. J. Psychophysiol.* 59:8, 2006.

Polich, J., Corey-Bloom, J. Alzheimer's disease and P300: review and evaluation of task and modality. *Cur. Alzheimer Res.* 2:515, 2005.

Polich, J., Criado, J.R. Neuropsychology and neuropharmacology of P3a and P3b. *Int. J. Psychophysiol.* 60:172, 2006.

Cellular Physiology of Neuropeptides and Drugs of Abuse in the Brain

M. Roberto, P. Schweitzer, G.R. Siggins, L.H. Parsons

We study the effects of ethanol on neuronal communication by focusing on the nucleus of the central amygdala. Our aim is to uncover (1) the physiologic mechanisms that underlie the acute action of ethanol and the involvement of neuropeptides such as corticotropin-releasing factor (CRF) and nociceptin and (2) the neuroadaptations associated with ethanol dependence.

Using an electrophysiologic approach, we found that acute exposure to ethanol increases inhibitory trans-

mission in neurons of the central amygdala in both naive and ethanol-dependent rats, in part by enhancing the vesicular release of γ -aminobutyric acid (GABA) in the central amygdala. We confirmed these in vitro findings with in vivo microdialysis studies in which we found that both acute and chronic ethanol exposure increased GABA release in the central amygdala. In addition, we found a critical mimicry between ethanol and CRF effects on GABAergic transmission that showed marked adaptations during the development of ethanol dependence. Nociceptin, however, decreased GABA release and opposed both ethanol and CRF effects. Chronic ethanol exposure enhances the sensitivity of GABAergic systems in central amygdala to these 2 neuropeptides. On the basis of this evidence, we hypothesize that nociceptin functions as a "brake" to limit the enhancement of inhibitory transmission in the neural circuitry involved in the reinforcing actions of ethanol.

We also recently discovered that acute exposure to ethanol inhibits responses in neurons in the central amygdala mediated by receptors for *N*-methyl-D-aspartate (NMDA), principally at postsynaptic sites. Prolonged exposure to ethanol postsynaptically increased the sensitivity of NMDA receptors and increased glutamate release. Importantly, these changes were reversed with ethanol withdrawal. These physiologic data were correlated with molecular studies showing that chronic exposure to ethanol increased the levels of messenger RNA and protein of selective NMDA receptor subunits in neurons in the central amygdala, indicating that ethanol elicits reversible neuroadaptations in synaptic function, gene expression, and protein composition of NMDA receptors in these neurons.

Understanding the cellular adaptations induced by chronic exposure to alcohol will provide insight into how the transition to alcoholism occurs, and possibly treatments for alcoholism. Currently, we are examining how these changes contribute to the development of ethanol dependence and protracted abstinence.

PUBLICATIONS

Bajo, M., Crawford, E.F., Roberto, M., Madamba, S.G., Siggins, G.R. Chronic morphine treatment alters expression of *N*-methyl-D-aspartate receptor subunits in the extended central amygdala. *J. Neurosci. Res.* 83:532, 2006.

Breese, G.R., Criswell, H.E., Carta, M., Dodson, P.D., Hanchar, H.J., Khisti, R.T., Mamedi, M., Ming, Z., Morrow, A.L., Olsen, R.W., Otis, T.S., Parsons, L.H., Penland, S.N., Roberto, M., Siggins G.R., Valenzuela, C.F., Wallner, M. Basis of the GABA-mimetic profile of ethanol. *Alcohol. Clin. Exp. Res.* 30:731, 2006.

O'Dell, L.E., Purdy, R.H., Covey, D.F., Richardson, H.N., Roberto, M., Koob, G.F. Epipregnanolone and a novel synthetic neuroactive steroid reduce alcohol self-administration in rats. *Pharmacol. Biochem. Behav.* 81:543, 2005.

Roberto, M., Bajo, M., Crawford, E., Madamba, S.G., Siggins, G.R. Chronic ethanol exposure and protracted abstinence alter NMDA receptors in central amygdala. *Neuropsychopharmacology* 31:988, 2006.

Roberto, M., Siggins, G.R. Nociceptin/orphanin FQ presynaptically decreases GABAergic transmission and blocks the ethanol-induced increase of GABA release in central amygdala. *Proc. Natl. Acad. Sci. U. S. A.* 103:9715, 2006.

Roberto, M., Treistman, S.N., Pietrzykowski, A.Z., Weiner, J., Galindo, R., Mamedi, M., Valenzuela, F., Zhu, P.J., Lovinger, D., Zhang, T.A., Hendricson, A.H., Morrisett, R., Siggins, G.R. Actions of acute and chronic ethanol on presynaptic terminals. *Alcohol. Clin. Exp. Res.* 30:222, 2006.

Siggins, G.R., Roberto, M., Nie, Z. The tipsy terminal: presynaptic effects of ethanol. *Pharmacol. Ther.* 107:80, 2005.

Slanina, K.A., Roberto, M., Schweitzer, P. Endocannabinoids restrict hippocampal long-term potentiation via CB1. *Neuropharmacology* 49:660, 2005.

Woodward, J.J., Ron, D., Winder, D., Roberto, M. From blue states to up states: a regional view of NMDA-ethanol interactions. *Alcohol. Clin. Exp. Res.* 30:359, 2006.

Alcohol and Drug Self-Administration and the Mouse Behavioral Assessment Core Facility

A.J. Roberts, C.L. Levy, L. Underwood, C. Pañeda

Our overall goal is to investigate the neural bases of behavior by using mouse models. In particular, we are interested in motivated behaviors such as drug and alcohol self-administration and exploratory drive.

We are involved in a multisite integrated neuroscience initiative on alcoholism sponsored by the National Institute on Alcohol Abuse and Alcoholism in which the overall goal is to examine the neural basis of excessive alcohol drinking. We developed a model of excessive alcohol drinking after a period of abstinence in alcohol-dependent mice that we are using in both genetic and neuropharmacologic experiments. For example, recently we found that mice lacking the corticotropin-releasing factor (CRF) receptor 1 do not have increased ethanol self-administration after alcohol dependence. In parallel, we showed that intra-amygdalar injections of a CRF receptor antagonist block increases in ethanol self-administration induced by alcohol dependence in normal mice. These complementary studies support a role for the brain CRF system in alcohol addiction.

Another focus of our group is studies of self-administration of intravenous cocaine, morphine, and methamphetamine in mice. We developed a model of relapse

to drug-seeking behavior in which mice are trained to press a lever for cocaine and then this behavior is extinguished by removing the cocaine. Then the mice are subjected to various manipulations, such as exposure to stressors or administration of neuroactive compounds, and how the animals respond to the lever previously associated with cocaine is recorded. We found that treatment with the newly discovered neuroactive peptide neuropeptide S results in reinstatement of lever pressing in this model, suggesting a novel relapse mechanism. Understanding the underlying neural mechanisms of relapse can enhance the ability to treat and prevent addictive disorders.

In the past year, we started a mouse behavioral assessment core facility at Scripps Research. The purpose of the facility is to provide high-quality mouse behavioral assessments to neuroscientists located near Scripps Research. A primary focus is to provide tests that allow investigators to make transitions from laboratory findings to clinical applications by enabling the modeling of human diseases and the development of medications and other treatment strategies. For example, test batteries have been developed for several neuropsychiatric disorders, including anxiety disorders, depressive disorders, disorders of learning and memory, disorders of motor functioning, drug and alcohol abuse and dependence, eating disorders, and other compulsive and impulsive disorders.

Our services include specific behavioral tests, general test batteries, surgical procedures, drug administration protocols, and training in all of these areas. Included in these services is advice on experimental design, assistance in submitting protocols for studies in animals, data analysis, interpretation of results, and assistance in writing descriptions of these tests, results, and interpretations in grant proposals and manuscripts. In the past few months, we have done work for investigators in the Departments of Molecular Biology, Molecular and Experimental Medicine, Cell Biology, and Chemistry and in the Molecular and Integrative Neurosciences Department.

PUBLICATIONS

Carrera, M.R., Trigo, J.M., Wirsching, P., Roberts, A.J., Janda, K.D. Evaluation of the anticocaine monoclonal antibody GNC92H2 as an immunotherapy for cocaine overdose. *Pharmacol. Biochem. Behav.* 81:709, 2005.

Chen, A., Zorrilla, E., Smith, S., Rousso, D., Levy, C., Donaldson, C., Roberts, A., Lee, K-F., Vale, W. Urocortin 2-deficient mice exhibit gender-specific alterations in circadian hypothalamus-pituitary-adrenal axis and depressive-like behavior. *J. Neurosci.* 26:5500, 2006.

Ghozland, S., Chu, K., Kieffer, B.L., Roberts, A.J. Lack of stimulant and anxiolytic-like effects of ethanol and accelerated development of ethanol dependence in μ -opioid receptor knockout mice. *Neuropharmacology* 49:493, 2005.

Sanchis-Segura, C., Grisel, J.E., Olive, M.F., Ghozland, S., Koob, G.F., Roberts, A.J., Cowen, M.S. Role of the endogenous opioid system on the neuropsychopharmacological effects of ethanol: new insights about an old question. *Alcohol. Clin. Exp. Res.* 29:1522, 2005.

Tabarin, A., Chaves, Y.D., Carmona, M.C., Catargi, B., Zorrilla, E.P., Roberts, A.J., Coscina, D.V., Rousset, S., Redonnet, A., Parker, G.C., Inoue, K., Ricquier, D., Penicaud, L., Kieffer, B.L., Koob, G.F. Resistance to diet-induced obesity in μ -opioid receptor-deficient mice: evidence for a "thrifty gene." *Diabetes* 54:3510, 2005.

Tallent, M.K., Fabre V., Qiu, C., Calbet, M., Lamp, T., Baratta, M.V., Suzuki, C., Levy, C.L., Siggins, G.R., Henriksen, S.J., Criado, J.R., Roberts, A.J., de Lecea, L. Cortistatin overexpression in transgenic mice produces deficits in synaptic plasticity and learning. *Mol. Cell. Neurosci.* 30:465, 2005.

Molecular Mechanisms of Adaptive and Maladaptive Neuronal Plasticity

P.P. Sanna, F. Berton, V. Canonigo, D. Lekic, K. Hagihara, M. Mendez-Diaz, V. Mendoza-Fernandez, D. Thurbon, L. van der Stap, W. Francesconi

As part of our effort to apply microarray-based strategies to studies of the neurobiology of drug abuse, we profiled gene expression in reward-related regions of the brains in rats that had escalated cocaine intake after extended access to cocaine. We compared 4 methods of analysis used to generate gene expression values: 2 that use perfect-match-minus-mismatch models and 2 that use perfect-match-only models. Results were validated by using reverse transcriptase-polymerase chain reaction of RNA from individual animals from an independent replication of the experiment.

We found that a small number of genes are selectively associated with escalated cocaine intake. Unexpectedly, of the brain regions examined (prefrontal cortex, nucleus accumbens, septum, lateral part of the hypothalamus, amygdala, and ventral tegmental area), the lateral part of the hypothalamus was the most transcriptionally responsive in escalation of cocaine intake. Most of the genes associated with escalated cocaine intake are also expressed during synaptogenesis and synaptic plasticity and include genes that code for several presynaptic and postsynaptic proteins involved in neurotransmission.

These results suggest that the intrinsic circuitry of the lateral part of the hypothalamus undergoes a structural reorganization during escalation of cocaine use. This remodeling could contribute to the chronic deficit in reward function hypothesized to drive the transition

to drug addiction. The results also support the value of using multiple analysis strategies to detect the strongest changes in gene expression and to compensate for the biases that affect each strategy.

In other studies, we discovered a novel form of neuronal plasticity in the juxtacapsular bed nucleus of the stria terminalis (BNST) characterized by a decrease in the firing threshold and an increase in the temporal fidelity of firing. The plasticity was impaired during protracted withdrawal from self-administration of various drugs of abuse, including alcohol, cocaine, and heroin. This impairment was more pronounced in rats with a history of drug dependence. The BNST has been implicated in stress responses and in the motivational dysregulation associated with drug dependence.

Interestingly, the long-term potentiation of the intrinsic excitability in the juxtacapsular BNST that was impaired in animals with a history of dependence on alcohol, cocaine, or heroin was a potentiated population spike due to enhanced intrinsic excitability and temporal fidelity of firing. Intracellular recordings revealed that high-frequency stimulation of the stria terminalis induced a long-lasting shift toward hyperpolarization of the threshold for the generation of action potentials in neurons in the juxtacapsular BNST in normal rats. This shift was accompanied by a protracted increase in firing probability that was not observed in rats with histories of dependence on alcohol, cocaine, or heroin, consistent with the impaired long-term potentiation of field potentials in these rats.

The activity-dependent decrease in the firing threshold of juxtacapsular BNST neurons was mediated by changes in the D-type potassium current. Using DNA microarrays and reverse transcriptase–polymerase chain reaction, we found that the expression of the Kv1.2 channel, the main contributor to the D-type potassium current, was significantly increased in the juxtacapsular BNST in rats dependent on alcohol, cocaine, or heroin, suggesting that increased density of Kv1.2 subsequent to its increased gene expression may contribute to the refractoriness to long-term potentiation of population spike in animals with histories of drug dependence.

Reduced capacity for plasticity of intrinsic excitability and temporal fidelity of firing of juxtacapsular BNST neurons would result in inadequate feedback inhibition of the central nucleus of the amygdala. Unrestrained activation of the central nucleus of the amygdala is expected to result in increased emotional arousal through the various projection areas of the structure, including

the paraventricular nucleus of the hypothalamus and the lateral part of the hypothalamus that are thought to be key in the expression of “emotional memories” by providing an interface between the central stress and autonomic systems. Thus, the impaired plasticity of the juxtacapsular BNST could contribute to the emotional dysregulation associated with abstinence in humans with drug dependence.

PUBLICATIONS

Ahmed, S.H., Lutjens, R., van der Stap, L.D., Lekic, D., Romano-Spica, V., Morales, M., Koob, G.F., Repunte-Canonigo, V., Sanna, P.P. Gene expression evidence for remodeling of lateral hypothalamic circuitry in cocaine addiction. *Proc. Natl. Acad. Sci. U. S. A.* 102:11533, 2005.

Izzo, E., Sanna, P.P., Koob, G.F. Impairment of dopaminergic system function after chronic treatment with corticotropin-releasing factor. *Pharmacol. Biochem. Behav.* 81:701, 2005.

Karpova, A., Sanna, P.P., Behnisch, T. Involvement of multiple phosphatidylinositol 3-kinase-dependent pathways in the persistence of late-phase long term potentiation expression. *Neuroscience* 137:833, 2006.

Lu, X., Mazarati, A., Sanna, P., Shinmei, S., Bartfai, T. Distribution and differential regulation of galanin receptor subtypes in rat brain: effects of seizure activity. *Neuropeptides* 39:147, 2005.

Sanna, P.P., King, A.R., van der Stap, L.D., Repunte-Canonigo, V. Gene profiling of laser-microdissected brain regions and sub-regions. *Brain Res. Brain Res. Protoc.* 15:66, 2005.

Cellular Physiology of Brain Cannabinoids and Peptides

P. Schweitzer, M. Roberto, G.R. Siggins, B. Lamboloz,* D. Piomelli**

* Ecole Supérieure de Physique et de Chimie Industrielles, Paris, France

** University of California, Irvine, California

NEUROBIOLOGY OF CANNABINOID SUBSTANCES

Cannabinoid substances contained in marijuana have powerful psychoactive properties and alter cognitive processes via activation of cannabinoid-1 (CB₁) receptors, but in order to function properly, the brain produces its own cannabinoid ligands. Our objectives are to uncover the cellular mechanisms that underlie the central effects of cannabinoid ligands and to determine the role played by endogenously formed cannabinoids.

Using a physiologic approach, we are investigating the modulation of synaptic transmission and plasticity. In this approach, we record from neurons in brain tissue from the hippocampus and neocortex, 2 structures that have high levels of CB₁ receptors and are involved in learning and memory processes. In collaboration with D. Piomelli, University of California, Irvine, we are using

various pharmacologic tools to study the routes of degradation of endogenous cannabinoids. In our collaboration with B. Lambolez, Ecole Supérieure de Physique et de Chimie Industrielles, we are using single-cell reverse transcriptase–polymerase chain reaction after whole-cell recording to characterize the neuronal populations that express transcripts for CB₁ receptors.

The role played by endogenous cannabinoids in hippocampal and neocortical excitatory synaptic transmission and plasticity remains controversial. We found that endogenous cannabinoids acting at CB₁ receptors in the hippocampus selectively decrease excitatory transmission and restrict synaptic plasticity. Consistent with such a role for endogenous cannabinoids, our research in collaboration with Dr. Lambolez indicates that more than half of pyramidal neurons express CB₁ receptors in the neocortex. The expression of the receptors also did not appear to be restricted to large cholecystokinin interneurons as previously reported, because a majority of neurons containing somatostatin and vasoactive intestinal peptide also expressed CB₁ receptors.

Using a physiologic approach, we confirmed that functional CB₁ receptors modulate neocortical networks. We also discovered that cyclooxygenase-2 has a predominant role in controlling the tonic level of endogenous cannabinoids that modulate synaptic activity and plasticity. Thus, the endogenous cannabinoid system interacts with the cyclooxygenase-2 pathway, and inhibitors of this enzyme may raise the levels of endogenous cannabinoids.

NEUROPEPTIDES AND ALCOHOL

Neuropeptides are found throughout the brain and strongly influence neuronal activity. Peptides such as corticotropin-releasing factor (CRF) and nociceptin are involved in the CNS effects of ethanol. In other studies, we are examining the actions of ethanol in the central amygdala, a brain region prominently involved in alcohol dependence and reinforcement. Our results indicate that CRF₁ receptors mediate the ethanol enhancement of inhibitory transmission, providing a cellular mechanism for the involvement of CRF in the effects of ethanol and supporting a role for the peptide in the motivational effects of ethanol. CRF₁ receptors could be an important therapeutic target for the treatment of stress-induced alcohol drinking.

PUBLICATIONS

Slanina, K.A., Roberto, M., Schweitzer, P. Endocannabinoids restrict hippocampal long-term potentiation via CB1. *Neuropharmacology* 49:660, 2005.

Slanina, K.A., Schweitzer, P. Inhibition of cyclooxygenase-2 elicits a CB1-mediated decrease of excitatory transmission in rat CA1 hippocampus. *Neuropharmacology* 49:653, 2005.

Neurophysiology of Alcohol Exposure During Adolescence and Adulthood: Role of Neuropeptides

C.J. Slawecki, J. Roth, D. Wills, C.L. Ehlers

CONSEQUENCES OF EXPOSURE TO DRUGS DURING ADOLESCENCE

Neurobiological, genetic, and environmental factors influence alcoholism. Exposure to a drug of abuse during adolescence greatly increases the likelihood that a person will become dependent on that drug. We have developed animal models to assess the environmental and genetic consequences of exposure to alcohol and nicotine during adolescence and adulthood. In these models, rats are exposed to alcohol or nicotine and then brain function and behavior are examined. Overall, these studies have shown the unique effects of exposure to alcohol or nicotine during adolescence.

We examined the effects of exposure to alcohol during adolescence during acute withdrawal and protracted abstinence. We found that when exposure abruptly ended, several signs of withdrawal were more pronounced in adolescents than in adults. Specifically, brain hyperarousal and behavioral hypoactivity developed more rapidly in adolescents. After more protracted periods of abstinence from alcohol, function in multiple brain regions known to mediate cognition was altered. Comparable levels of exposure to alcohol affected behavioral indices of cognitive function more profoundly in adults.

Distinct changes in behavior and brain function also occur in adolescents after exposure to nicotine. After nicotine exposure ends, pronounced increases in anxiety occur in several animal models. Alterations in selected brain peptides could account for the behavioral changes in rats exposed to nicotine during adolescence. Brain peptides, such as corticotropin-releasing factor and neuropeptide Y, are altered in critical brain sites known to mediate anxiety.

NEUROBIOLOGICAL MEDIATORS OF ALCOHOL CONSUMPTION

We also investigate how environmental and genetic factors mediate alcohol drinking in rats. Using newly developed models, we found that exposure to alcohol for 7–8 weeks during adulthood produced a persistent

20% increase in alcohol drinking. Neurochemical and behavioral studies revealed that changes in neuropeptide Y could be partially responsible for the neuroadaptive changes that increase alcohol drinking after prolonged alcohol exposure. More recently, we used this model to assess the sensitivity of adolescent rats to this effect of alcohol exposure. We found that 2 weeks of alcohol exposure was sufficient to increase ethanol intake in adolescent rats. This same 2-week period of exposure was not sufficient to affect alcohol drinking in adult rats. These data further indicate that age of exposure is a critical factor in alcohol-drinking behavior. This finding is consistent with the increased risk of alcohol dependence associated with early onset of alcohol drinking.

PUBLICATIONS

Barron, S., White, A., Swartzwelder, H.S., Bell, R.L., Ross, Z.A., Slawecki, C.J., Ehlers, C.L., Levin, E.D., Rezvani, A.H., Spear, L.P. Adolescent vulnerabilities to chronic alcohol or nicotine exposure: findings from rodent models. *Alcohol. Clin. Exp. Res.* 29:1720, 2005.

Slawecki, C.J. Comparison of anxiety-like behavior in adolescent and adult Sprague-Dawley rats. *Behav. Neurosci.* 119:1477, 2005.

Slawecki, C.J., Ehlers, C.L. Enhanced prepulse inhibition following adolescent ethanol exposure in Sprague-Dawley rats. *Alcohol. Clin. Exp. Res.* 29:1829, 2005.

Slawecki, C.J., Roth, J. Assessment of sustained attention in ad libitum fed Wistar rats: effects of MK-801. *Physiol. Behav.* 85:346, 2005.

Slawecki, C.J., Roth, J., Gilder, A. Neurobehavioral profiles during the acute phase of ethanol withdrawal in adolescent and adult Sprague-Dawley rats. *Behav. Brain Res.* 170:41, 2006.

Slawecki C.J., Thorsell, A., Ehlers, C.L. Antagonism of neuropeptide Y Y1 receptors does not inhibit ethanol's effects on cortical EEG and ERPs in Wistar rats. *J. Stud. Alcohol* 66:559, 2005.

Thorsell, A., Slawecki, C.J., El Khoury, A., Mathé, A.A., Ehlers, C.L. The effects of social isolation on neuropeptide Y levels, exploratory and anxiety-related behaviors in rats. *Pharmacol. Biochem. Behav.* 83:28, 2006.

Thorsell, A., Slawecki, C.J., Khoury, A., Mathé, A.A., Ehlers, C.L. Effect of social isolation on ethanol consumption and substance P/neurokinin expression in Wistar rats. *Alcohol* 36:91, 2006.

Neuronal Communication, Neuropeptides, and Drugs of Abuse

G.R. Siggins, P. Schweitzer, M. Roberto, T. Bartfai, S. Madamba, Z. Nie, M. Bajo, L. Sharkey, R. Vlkolinsky, L.H. Parsons, A.J. Roberts, L. de Lecea,* S. Moore**

* Department of Molecular Biology, Scripps Research

** Duke University, Raleigh-Durham, North Carolina

We study the effects of neuropeptides and abused drugs on electrophysiologic and molecular mechanisms of neuronal and synaptic

function. We use extracellular, intracellular, and patch recording of brain neurons in vitro. We administer transmitters, peptides, drugs, cytokines, and neurotoxins by micropipettes and by superfusion, and we activate synaptic transmission via stimulating electrodes. We also use molecular methods to assess drug-induced alterations of transmitter receptors.

We investigate synaptic mechanisms and drug effects in the hippocampus, nucleus accumbens, and central amygdala, brain regions involved in memory, learning, stress, and drug abuse. In previous studies, we discovered inhibitory roles in the hippocampus for the neuropeptides somatostatin, cortistatin, and the opioid-like peptide nociceptin. All 3 peptides often depressed hippocampal epileptiform events. Patch-clamp studies of neurons in the central amygdala indicated that nociceptin decreases presynaptic vesicular release of the inhibitory transmitter γ -aminobutyric acid (GABA) and reverses the effect of ethanol in enhancing GABA release. A δ opioid receptor agonist also appears to reduce transmitter release in neurons in the central amygdala in rats, with little effect on the properties of postsynaptic membranes.

Our previous findings suggested that glutamatergic synapses, particularly receptors for *N*-methyl-D-aspartate (NMDA), play a role in opiate and ethanol dependence. In patch-clamp recording and pharmacologic studies, chronic morphine treatment altered several pharmacologic and biophysical properties of NMDA receptor-mediated excitatory postsynaptic potentials (EPSPs) in slices and freshly isolated neurons from the nucleus accumbens, suggesting changes in the function or composition of the subunits of NMDA receptors. Using quantitative real-time polymerase chain reaction and Western blots of NMDA receptor subunits in tissue from the nucleus accumbens, we found that RNA for the 3 major subunits (NR1, NR2A, and NR2B) did not change in morphine-dependent rats, but the protein levels of NR1 and NR2B increased significantly, suggesting a posttranscriptional effect of chronic morphine treatment. However, in the central amygdala, chronic treatment with morphine significantly increased RNA levels for the NR1 subunit but had no effect on the protein levels of any of the 3 subunits. These data suggest that morphine dependence causes regional- and subunit-specific changes in NMDA receptors.

In previous studies, in slices of the central amygdala from rats never exposed to ethanol and rats chronically treated with ethanol, acute exposure to ethanol

increased the amplitude of GABAergic inhibitory postsynaptic potentials (IPSPs) and decreased glutamatergic EPSPs, indicating a reciprocal alteration of GABAergic and glutamatergic systems with no tolerance. Quantal synaptic analysis and microdialysis studies indicated that the action of ethanol on IPSPs is predominantly presynaptic, enhancing vesicular GABA release.

Corticotropin-releasing factor (CRF), a neuropeptide most likely involved in stress-induced alcohol drinking, also presynaptically augmented IPSPs in the central amygdala in both mice and rats. CRF₁ receptor antagonists and a mutation that deleted the gene for the CRF₁ receptor abolished the effects of both CRF and ethanol, whereas CRF₂ antagonists and agonists had no effect, indicating that the effects of ethanol are mediated by activation of endogenous CRF₁ receptors. Notably, the CRF augmentation of IPSPs increased after chronic ethanol treatment, suggesting that this neuropeptide-ethanol interaction represents a novel synaptic neuroadaptation underlying ethanol dependence. In addition, in recent studies with S. Moore, Duke University, we found that ethanol increases vesicular GABA release in neurons in the central amygdala in mice with null mutations in either δ or μ opioid receptors significantly more than in neurons from control mice. Furthermore, quantal analysis indicated that ethanol augments presynaptic GABA release more after δ opioid receptors are pharmacologically blocked and that activation of δ opioid receptors diminishes IPSPs, suggesting that endogenous opioid peptides, such as enkephalin, act opposite to the actions of CRF, presynaptically dampening the effects of ethanol on the GABAergic system.

As we reported previously, acute ethanol also reduced glutamatergic transmission in the central amygdala mediated by both non-NMDA and NMDA receptors, in part postsynaptically. However, the depressant effect of ethanol on NMDA-EPSPs was enhanced, and glutamate release was increased, after chronic ethanol treatment and withdrawal, suggesting both presynaptic and postsynaptic mechanisms of sensitization to ethanol. The postsynaptic effect of chronic treatment with ethanol may involve recomposition of NMDA receptors in the central amygdala to a preponderance of NR2B subunits; this possibility is supported by the finding that the sensitivity of NMDA receptors to an NR2B-selective antagonist increases and that NR2B mRNA and protein levels increase after chronic exposure to ethanol. This change in the NMDA receptor subunit associated with long-term ethanol treatment may indicate another cellular neuroadaptation that underlies ethanol dependence.

Finally, we are studying the effects of the neuropeptide galanin on neurons of the dorsal raphe nucleus, known to contain both galanin and its receptors. Our findings to date indicate that galanin decreases the size of evoked IPSPs in dorsal raphe, probably via a presynaptic decrease in GABA release.

PUBLICATIONS

Bajo, M., Crawford, E.F., Roberto, M., Madamba, S.G., Siggins, G.R. Chronic morphine treatment alters expression of *N*-methyl-D-aspartate receptor subunits in the extended amygdala. *J. Neurosci. Res.* 83:532, 2006.

Breese, G.R., Criswell, H.E., Carta, M., Dodson, P.D., Hanchar, H.J., Khisti, R.T., Mameli, M., Ming, Z., Morrow, A.L., Olsen, R.W., Otis, T.S., Parsons, L.H., Penland, S.N., Roberto, M., Siggins, G.R., Valenzuela, C.F., Wallner, M. Basis of the GABA_A profile of ethanol. *Alcohol. Clin. Exp. Res.* 30:731, 2006.

Roberto, M., Bajo, M., Crawford, E., Madamba, S.G., Siggins, G.R. Chronic ethanol exposure and protracted abstinence alter NMDA receptors in central amygdala. *Neuropsychopharmacology* 31:988, 2006.

Roberto, M., Siggins, G.R. Nociceptin/orphanin FQ presynaptically decreases GABAergic transmission and blocks the ethanol-induced increase of GABA release in central amygdala. *Proc. Natl. Acad. Sci. U. S. A.* 103:9715, 2006.

Roberto, M., Treisman, S.N., Pietrzykowski, A.Z., Weiner, J., Galindo, R., Mameli, M., Valenzuela, F., Zhu, P.J., Lovinger, D., Zhang, T.A., Hendricson, A.H., Morrisett, R., Siggins, G.R. Actions of acute and chronic ethanol on presynaptic terminals. *Alcohol. Clin. Exp. Res.* 30:222, 2006.

Tallent, M.K., Fabre, V., Qiu, C., Calbet, M., Lamp, T., Baratta, M.V., Suzuki, C., Levy, C.L., Siggins, G.R., Henriksen, S.J., Criado, J.R., Roberts, A., de Lecea, L. Cortistatin overexpression in transgenic mice produces deficits in synaptic plasticity and learning. *Mol. Cell. Neurosci.* 30:465, 2005.

Primate Neurobehavioral Laboratory

M.A. Taffe, S.N. Katner, R.D. Crean, S.A. Davis, C.C. Lay, S.N. Von Huben

We continue to focus on the behavioral and physiologic effects of exposure to drugs of abuse. Currently, we are examining the effects of 3,4-methylenedioxymethamphetamine (MDMA), known as "Ecstasy," and alcohol. Persons who use MDMA recreationally report cognitive, mood, and sleep disturbances even after prolonged abstinence from the drug. Many laboratory studies have indicated that MDMA can produce a selective reduction of serotonergic function in the brain in most species, including in nonhuman primates. However, whether such changes in the brain produce the cognitive or mood disruptions observed in human users is unclear. We are determining how MDMA-induced brain changes may impair cognition, mood, circadian patterns of temperature and activity, and brain electrophysiologic characteristics.

This past year, we focused on the impact of the disruption in body temperature produced by MDMA.

An elevation in temperature is a critical factor in MDMA-induced neurotoxic effects in rodents, but the thermoregulatory response differs in larger bodied primates. We found that the temperature of rhesus monkeys was consistently elevated by MDMA even under conditions in which body temperature in rats was decreased. In monkeys, but not in rodents, MDMA produced temperature elevations independently of significant increases in locomotor activity.

Thus, our data suggest that the thermoregulatory responses to MDMA in primates, including humans, may be even more important than would be predicted on the basis of studies in rodents. Our ongoing research will determine if such sensitivity is also associated with increased risk for the neurotoxic effects of MDMA.

In other studies, we are addressing medical emergencies (and fatalities) in humans who use MDMA. Patients for whom MDMA use is confirmed often have malignant hyperthermia and seizures. Using our models, we found that seizure can precede and cause hyperthermia after MDMA exposure in monkeys. Furthermore, repeated intermittent exposure to MDMA can lower the threshold for seizure. These results provide new insight into the etiology of medical emergencies observed in recreational users of MDMA.

We are also investigating the cognitive domains that are disrupted by chronic alcohol drinking in periadolescent monkeys. We found that monkeys who drank alcohol 5 days per week were impaired in learning the spatial location of objects. These effects became more pronounced when the monkeys were required to remember the location over several seconds, indicating a specific impairment of spatial short-term working memory.

PUBLICATIONS

Taffe, M.A., Lay, C.C., Von Huben, S.N., Davis, S.A., Crean, R.D., Katner, S.N. Hyperthermia induced by 3,4-methylenedioxymethamphetamine in unrestrained rhesus monkeys. *Drug Alcohol Depend.* 82:276, 2006.

Von Huben, S.N., Davis, S.A., Lay, C.C., Katner, S.N., Crean, R.R., Taffe, M.A. Differential contributions of dopaminergic D₁-like and D₂-like receptors to cognitive function in rhesus monkeys. *Psychopharmacology (Berl.)*, *in press*.

Von Huben, S.N., Lay, C.C., Crean, R.D., Davis, S.A., Katner, S.N., Taffe M.A. Impact of ambient temperature on hyperthermia induced by (±)3,4-methylenedioxymethamphetamine in rhesus macaques. *Neuropsychopharmacology*, *in press*.

Neurobiology of Addiction

F. Weiss, R. Ciccocioppo,* M. Heilig,** R. Martin-Fardon, E.P. Zorrilla, C.V. Dayas, H. Aujila, N. Sidhpura, Y. Zhao, M.A. Baptista, T.M. Kerr, N.D. Stuempfig, J.R. Lewis

* University of Camerino, Camerino, Italy

** National Institute on Alcohol Abuse and Alcoholism, Bethesda, Maryland

We study neuroadaptive changes in signaling mechanisms and neurocircuitries responsible for the development of chronic vulnerability to relapse, a defining feature of substance dependence and a central issue for the successful treatment of drug addiction. We recently discovered that group II metabotropic glutamate receptors (mGluRs) and the nociceptin/orphanin FQ (N/OFQ) opioid peptide system are novel regulatory mechanisms for hyperresponsiveness to stress and drug craving, conditions implicated clinically as major risk factors for relapse to drug use.

ROLE OF GROUP II mGluRs IN BEHAVIORAL CHANGES

This past year we began investigating the role of group II mGluRs in behavioral changes such as increased anxiety and sensitivity to stress that emerge in animals after chronic self-administration of cocaine. In contrast to the steady-state, nonescalating drug self-administration that occurs in rats maintained on limited access to cocaine, in rats given extended daily access to cocaine, cocaine intake escalates. This escalation model provides an opportunity to study neurobiological consequences of cocaine addiction in rats under conditions that mimic high-dose "bingelike" cocaine abuse in humans.

We found that a history of escalated cocaine intake was associated with profound and long-lasting increases in susceptibility to anxiogenic and stressful stimuli during withdrawal that was still unabated after 3 months of abstinence. The magnitude and persistence of these pathologic changes most likely contribute to the chronic relapsing associated with cocaine addiction.

Increased glutamate neurotransmission plays a major role in hyperresponsiveness to stress or anxiogenic stimuli. Recent advances in understanding the functional role of mGluRs have implicated group II mGluRs (mGluR_{2/3}) in modulating responses to anxiety and stress. Group II mGluRs act as autoregulatory receptors and dampen neural excitability by reducing the presynaptic release of glutamate. We therefore hypothesized that activation of group II mGluRs attenuates heightened anxiety-like behavior in rats with cocaine escalation.

As predicted, a selective mGluR_{2/3} agonist (LY379268) effectively reversed exacerbated anxiety-

like responses during protracted cocaine withdrawal. Moreover, this agent had greater anxiolytic potency in rats with cocaine escalation than in rats never exposed to cocaine, suggesting altered mGluR_{2/3} function in rats subjected to the cocaine escalation regimen. Thus, activation of group II mGluRs not only attenuates drug seeking induced by environmental challenges such as drug cues or exposure to stress but also ameliorates abnormal responses to stress and anxiety. Group II mGluRs therefore most likely will be a highly promising pharmacologic treatment target for preventing relapse.

N/OFQ AND ITS RECEPTOR AS TARGETS FOR PREVENTING RELAPSE TO ALCOHOL ABUSE

Our previous findings implicated the opioid-like peptide N/OFQ and its receptor, NOP, as promising targets for preventing relapse to alcohol abuse. We have extended our studies to the role of the N/OFQ system in the rewarding and dependence-inducing actions of alcohol. In contrast to our finding that central administration of N/OFQ suppresses alcohol seeking in animal models of relapse both in genetically heterogenous Wistar rats and genetically selected Marchigian sP rats, N/OFQ attenuated actual ethanol intake in Marchigian sP rats only. Genetically determined dysregulation of the N/OFQ system in Marchigian sP rats therefore may contribute to both increased spontaneous ethanol intake and sensitivity to modification of ethanol consumption by N/OFQ in this line of rats. Consistent with this possibility, we found that Marchigian sP rats had overexpression of the genes for N/OFQ and NOP within regions of the central amygdala and bed nucleus of the stria terminalis.

These abnormalities in N/OFQ and NOP gene expression in brain regions linked to the regulation of behavioral responses to stress may contribute to increased spontaneous ethanol intake in animal genetic models of alcoholism. Tests of this hypothesis are in progress.

We next examined whether chronic ethanol intoxication can produce neuroadaptive changes in N/OFQ-NOP function in Wistar rats and thereby contribute to the high ethanol intake that occurs in these animals after intoxication and withdrawal. Central administration of N/OFQ reduced ethanol self-administration in rats previously dependent on alcohol but not in nondependent control rats. The differential pharmacologic effect of N/OFQ on ethanol intake as a function of ethanol history tentatively suggests that ethanol intoxication dysregulates the N/OFQ system. Related to this hypothesis, we identified a role of the N/OFQ system in ethanol withdrawal in Wistar rats. In rats tested

2–24 hours after removal from a chronic ethanol liquid diet, treatment with N/OFQ significantly lowered 3 of 4 major behavioral indications of ethanol withdrawal. The mechanism of action for this effect is unclear. However, on the basis of our earlier findings that N/OFQ acts as a functional antagonist of the stress peptide corticotropin-releasing factor, most likely reduction of transmission of this factor is a key element in this pharmacologic action. Overall, these findings implicate N/OFQ and its receptor as an important regulatory system as well as a promising treatment target for alcohol abuse and addiction.

PUBLICATIONS

Dayas, C.V., Liu, X., Simms, J.A., Weiss, F. Distinct patterns of neural activation associated with ethanol seeking: effects of naltrexone. *Biol. Psychiatry*, *in press*.

Economidou, D., Fedeli, A., Martin-Fardon, F., Weiss, F., Massi, M., Cioccioppo, R. Effect of novel FQ-NOP receptor ligands on ethanol drinking in alcohol-preferring msP rats. *Peptides*, *in press*.

Martin-Fardon, R., Lorentz, C.U., Stuempfig, N.D., Weiss, F. Priming with BTCP, a dopamine uptake blocker, reinstates cocaine-seeking and enhances cocaine cue-induced reinstatement. *Pharmacol. Biochem. Behav.* 82:46, 2005.

Zhao, Y., Dayas, C.V., Aujla, H., Baptista, M.A.S., Martin-Fardon, R., Weiss, F. Activation of group II metabotropic glutamate receptors attenuates both stress and cue-induced ethanol-seeking and modulates c-fos expression in the hippocampus and amygdala. *J. Neurosci.* 26:9967, 2006.

Neurobiology of Feeding and Stress

E.P. Zorrilla, L. Steardo,* K. Inoue,** A. Tabarin,***
K. Rice,**** S. Iwasaki,** A. Chen,***** E. Fekete, Y. Zhao,
V. Sabino, P. Cottone, M. Brennan, M. Mattock, Y. Grant

* University of Palermo, Palermo, Italy

** Osaka City University Medical School, Osaka, Japan

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

**** National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda, Maryland

***** Weizmann Institute of Science, Rehovot, Israel

We study motivated behavior, with emphasis on brain reward and stress neurocircuits that control food intake. Understanding food intake requires understanding how the brain organizes units of ingestive behavior, or the “feeding microstructure.” This past year we pioneered a new way to study feeding microstructure in rats; the method is based on the recognition that eating and drinking are behaviorally integrated. We developed further tools to study dynamic

changes in the rate and regularity of eating within a meal. We generalized the methods so we could study control of food intake in mutant transgenic mice. Studies with these models will help define the biological bases for specific dysfunctional feeding patterns of obesity and eating disorders and target therapies for these abnormalities appropriately.

For example, we showed that type 2 urocortins produced a leptinlike facilitation of satiety, acting through hypothalamic receptors for corticotropin-releasing factor 2. Studies with female mice deficient in urocortin 2 further revealed a regulatory role for this urocortin in the expression of vasopressin in hypothalamic magnocellular neurons associated with a phenotype of altered circadian regulation of stress hormones by the hypothalamic-pituitary-adrenal axis, homeostasis of body fluids, and resistance to depressive-like behavior.

We also study ghrelin, a 28-residue stomach hormone hypothesized to signal "energy insufficiency" to the brain. Ghrelin may hinder consolidation of weight loss through its anabolic properties. In collaboration with K.D. Janda, Department of Chemistry, we found that n-octanoylation and the N-terminal third residue in the amino acid sequence of ghrelin are critical for the ability of the hormone to induce feeding. Accordingly, active immunization with haptens that included the N-terminal residues of ghrelin slowed the accrual of body weight and fat in rats in proportion to the acquired capacity of the rats' plasma to bind ghrelin. We now are using passive immunization with transfer of antibodies (whole immunoglobulin G or single-chain variable fragments) to the acylated ghrelin N terminus (residues 1–10).

Finally, we developed models of the hedonic (rather than homeostatic) control of food intake. Rats with intermittent, limited access to highly preferred foods will eat large quantities of these foods when the foods are available, even when fed to satiation before given access to the preferred foods. Conversely, with increasing experience with palatable foods, rats will reject less palatable rat chow for 5 days or longer, despite resulting weight loss. Thus, because of hedonic factors, rats in this model violate regulators of short-term homeostasis in both positive ("binge") and negative ("finickiness") directions. Despite taking in less energy (calories) overall than chow-maintained rats do, the rats in the model ultimately become heavier and fatter, have elevated levels of adipokines associated with metabolic complications of obesity, and appear anxious when their pre-

ferred food is not available. Treatment with opioid receptor antagonists reduced the finickiness and binge-like eating of rats given intermittent, limited access to highly preferred foods.

PUBLICATIONS

Chen, A., Zorrilla, E., Smith, S., Rousso, D., Levy, C., Vaughn, J., Donaldson, C., Roberts, A., Lee, K.-F., Vale, W. Urocortin 2-deficient mice exhibit gender-specific alterations in circadian hypothalamic-pituitary-adrenal axis and depressive-like behavior. *J. Neurosci.* 26:5500, 2006.

Chen, S.A., O'Dell, L.E., Hoefer, M.E., Greenwell, T.N., Zorrilla, E.P., Koob, G.F. Unlimited access to heroin self-administration: independent motivational markers of opiate dependence. *Neuropsychopharmacology*, *in press*.

Funk, C.K., Zorrilla, E.P., Lee, M.-J., Rice, K.C., Koob, G.F. CRF₁ antagonists selectively reduce ethanol self-administration in ethanol-dependent rats. *Biol. Psychiatry*, *in press*.

Richardson, H.N., Zorrilla, E.P., Mandyam, C.D., Rivier, C.L. Exposure to repetitive versus varied stress during prenatal development generates two distinct anxiogenic and neuroendocrine profiles in adulthood. *Endocrinology* 147:2506, 2006.

Tabarin, A., Chaves, Y.D., Carmona, M.C., Catargi, B., Zorrilla, E.P., Roberts, A.J., Coscina, D.V., Rousset, S., Redonnet, A., Parker, G.C., Inoue, K., Ricquier, D., Penicaud, L., Kieffer, B.L., Koob, G.F. Resistance to diet-induced obesity in μ -opioid receptor-deficient mice: evidence for a "thrifty gene." *Diabetes* 54:3510, 2005.

Valdez, G.R., Zorrilla, E.P., Koob, G.F. Homeostasis within the corticotropin-releasing factor system via CRF₂ receptor activation: a novel approach for the treatment of anxiety and depression. *Drug Dev. Res.*, *in press*.

Zorrilla, E.P., Koob, G.F. The roles of urocortins 1, 2 and 3 in the brain. *In: Handbook of Stress and the Brain, Part 1: The Neurobiology of Stress.* Steckler, T., Kalin, N.H., Reul, J.M.H.M. (Eds.). Elsevier Science, New York, 2005, p. 179. *Techniques in the Behavioral and Neural Sciences*; Vol. 15.

Galanin, a Neuropeptide Involved in Depression

T. Bartfai, X. Lu, H. Badie-Mahdavi, A. Barr, J. Kinney, L. Sharkey,* G.R. Siggins*

* Molecular and Integrative Neurosciences Department, Scripps Research

Galanin is a neuropeptide involved in regulation of cognition, mood, seizure, and pain threshold. After treating rats with selective serotonin reuptake inhibitors such as fluoxetine, electroconvulsive shock, and sleep deprivation, measures used to treat depression, we used transcriptional profiling to examine different areas of the brain. We found that galanin mRNA was upregulated in cells of the dorsal raphe nucleus and the locus coeruleus that are the major monoaminergic nuclei. We also found that levels of the type 2 galanin receptor were elevated in the dorsal raphe nucleus, suggesting that galanin acting at this depolarizing galanin receptor subtype may promote release of serotonin and contribute to antidepressant actions.

Electrophysiologic studies on serotonergic cells in the dorsal raphe nucleus revealed that galanin has strong synaptic effects. In rodent models of the efficacy of antidepressant agents, galnol, a galanin receptor agonist, produced antidepressant-like effects similar to those of the antidepressants fluoxetine and imipramine. Many antidepressants also promote neurogenesis, and we found that the type 2 galanin receptor mediates effects that promote neuroprotection or neurogenesis in the hippocampus.

On the basis of these data, we have made several important steps toward defining the galanin receptor 2 as a putative drug target for a new class of antidepressant drugs. We have also made progress toward a chemical proof of principle of galanin receptor 2 ligands as antidepressant agents.

Search for Fast-Acting Antidepressants

B. Conti, J.D. Hoyer, G. Bilbe, T. Bartfai

Current antidepressants such as the selective serotonin reuptake inhibitors fluoxetine (Prozac) and paroxetine (Paxil) must be taken for 14–21 days before clinically significant improvement occurs. This delay is a large problem, particularly in the treatment of patients with depression who are at high risk for suicide. In collaboration with scientists at Novartis Pharma, Basel, Switzerland, we are trying to develop a fast-acting antidepressant.

We are using 2 techniques that produce rapid, albeit short lived, antidepressant effects in rats: sleep deprivation and electroconvulsive therapy. We are comparing the transcriptional changes that occur in different brain areas in response to these treatments with the changes produced by 14 days of treatment with fluoxetine. We have identified several putative new drug targets, including sulfotransferase 1, serum- and glucocorticoid-inducible kinases, and G protein-coupled receptor 88, that if validated in behavioral experiments such as learned helplessness may form the basis of efforts to develop a fast-acting antidepressant.

Transcription of G protein-coupled receptor 88 is affected by lithium, the most common therapy for manic depression/bipolar disorder, and most surprisingly, the levels of this receptor are also altered in lactating females, who are considered to be in a “stress resis-

tant blisslike state.” We are doing genetic and chemical experiments to examine the mechanisms in which ligands for the receptor are involved.

Mechanisms of Thermoregulation: Thermosensitivity in the Brain

I. Tabarean, B. Conti, M. Sánchez-Alavez, C. Davis, H. Korn, T. Bartfai

The thermosensitivity of some peripheral sensory neurons with nerve endings in the skin underlies the ability to sense cold and heat as painful stimuli. Using cell cultures of primary neurons and slice preparations, we are examining neurons in the anterior part of the hypothalamus that sense temperature and regulate core body temperature.

We showed that individual neurons without the presence of a neuronal network can sense cold and warm temperatures and can change firing rate in response to these temperature changes. These neurons express receptors for several agents that cause fever, such as prostaglandin E₂, IL-1, and calcitonin gene-regulated peptide, which are involved in mediating fever in response to inflammation and infection and in hot flashes.

We also identified substances that reduce temperature sensitivity, such as adenosine and histamine, and we are defining the receptor subtypes through which these effects are exerted. A molecular and cellular understanding of the central temperature set point will be helpful in the treatment of feeding and sleep disorders because these phenomena are closely coordinated with and depend mutually on changes in the temperature set point.

PUBLICATIONS

Conti, B., Davis, C.N., Behrens, M.M., Rebek, J., Bartfai, T. Toll-like receptors as pharmacological targets. *In: Toll-Like Receptors in Inflammation*. O'Neill, L.A.J., Brint, E. (Eds.) Birkhäuser, Boston, 2005, p. 223. *Progress in Inflammation Research*. Parnham, M.J. (Series Ed.).

Davis, C.N., Mann, E., Behrens, M.M., Gaidarova, S., Rebek, M., Rebek, J., Jr., Bartfai, T. MyD88-dependent and -independent signaling by IL-1 in neurons probed by bifunctional Toll/IL-1 receptor domain/BB-loop mimetics. *Proc. Natl. Acad. Sci. U. S. A.* 103:2953, 2006.

Davis, C.N., Tabarean, I.V., Gaidarova, S., Behrens, M.M., Bartfai, T. IL-1 β induces a MyD88-dependent and ceramide-mediated activation of Src in anterior hypothalamic neurons. *J. Neurochem.* 98:1379, 2006.

Florén, A., Sollenberg, U., Lundström, L., Zorko, M., Stojan, J., Budihna, M., Wheatley, M., Martin, N.P., Kilk, K., Mazarati, A., Bartfai, T., Lindgren, M., Langel, Ü. Multiple interaction sites of galanin trigger its biological effects. *Neuropeptides* 39:547, 2005.

Kinney, J.W., Davis, C.N., Tabarean, I., Conti, B., Bartfai, T., Behrens, M.M. A specific role for NR2A-containing NMDA receptors in the maintenance of parvalbumin and GAD67 immunoreactivity in cultured interneurons. *J. Neurosci.* 26:1604, 2006.

Kovacs, Z., Kekesi, K.A., Szilagyi, N., Abraham, I., Szekacs, D., Kiraly, N., Papp, E., Csaszar, I., Szego, E., Barabas, K., Peterfy, H., Erdei, A., Bartfai, T., Juhasz, G. Facilitation of spike-wave discharge activity by lipopolysaccharides in Wistar Albino Glaxo/Rijswijk rats. *Neuroscience* 140:731, 2006.

Lundström, L., Elmquist, A., Bartfai, T., Langel, Ü. Galanin and its receptors in neurological disorders. *Neuromolecular Med.* 7:157, 2005.

Ostenson, C.G., Gaisano, H., Sheu, L., Tibell, A., Bartfai, T. Impaired gene and protein expression of exocytotic soluble *N*-ethylmaleimide attachment protein receptor complex proteins in pancreatic islets of type 2 diabetic patients. *Diabetes* 55:435, 2006.

Sánchez-Alavez, M., Tabarean, I.V., Behrens, M.M., Bartfai, T. Ceramide mediates the rapid phase of febrile response to IL-1 β . *Proc. Natl. Acad. Sci. U. S. A.* 103:2904, 2006.

Shi, T.J., Hua, X.Y., Lu, X., Malkmus, S., Kinney, J., Holmberg, K., Wirz, S., Ceccatelli, S., Yaksh, T., Bartfai, T., Hokfelt, T. Sensory neuronal phenotype in galanin receptor 2 knockout mice: focus on dorsal root ganglion neurone development and pain behaviour. *Eur. J. Neurosci.* 23:627, 2006.

Tabarean, I.V., Conti, B., Behrens, M., Korn, H., Bartfai, T. Electrophysiological properties and thermosensitivity of mouse preoptic and anterior hypothalamic neurons in culture. *Neuroscience* 135:433, 2005.

Wirz, S.A., Davis, C.N., Lu, X., Zal, T., Bartfai, T. Homodimerization and internalization of galanin type 1 receptor in living CHO cells. *Neuropeptides* 39:535, 2005.

Wirz, S.A., Tobias, P.S., Ulevitch, R.J., Aribibe, L., Bartfai, T. TLR2 is required for the altered transcription of p75NGF receptors in gram positive infection. *Neurochem. Res.* 31:297, 2006.