SAFAR CENTER FOR
RESUSCITATION RESEARCH

2002/2003 ANNUAL REPORT

DEPARTMENT OF CRITICAL CARE MEDICINE

UNIVERSITY OF PITTSBURGH
SCHOOL OF MEDICINE
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Featured on the cover:  From Left to Right, Drs. Paul Shore, Kate Felmet, Yi-Chen Lai, Hülya Bayr, and Trung Nguyen at the 2003 Congress of the Society of Critical Care Medicine. The number of awards that these fellows received at the SCCM congress from a single program highlights the high quality of our trainees and the strong commitment to training by our faculty. Drs. Shore, Lai, Bayr, and Nguyen were fellows in the programs of both the Safar Center and the Children’s Hospital of Pittsburgh division of pediatric critical care medicine. Dr. Felmet worked with Dr. Carcillo in the pediatric critical care medicine program.
MISSION STATEMENT

The global mission of the Safar Center for Resuscitation Research is to improve understanding of the mechanism of secondary injury after trauma and cardiopulmonary arrest, from whatever cause, and to contribute to the development and implementation of novel therapies. The treatment and prevention of secondary injury after these life-threatening catastrophic events is a major goal in each venue of investigation.
A letter from the Safar Center’s Director

As I write this letter summarizing the many accomplishments of investigators and trainees at the Safar Center in the 2002/2003 academic year, it is overshadowed by the recent loss of our good friend, colleague, CPR and acute medicine pioneer and the founder of our Center, Dr. Peter Safar. After a courageous 15-month battle against cancer, Dr. Safar passed away on August 3rd, 2003. Dr. Safar was a genius that inspired everyone that worked with him to search for clinical breakthroughs in resuscitation, never rest until they are implemented, and carry out this mission with elegance and humanism. His loss has been difficult for all of us at the Center, and we extend our deepest sympathy to Eva Safar and the entire Safar family. We are honored to be able to carry his work forward.

Academically, the 2002/2003-year was another strong one for faculty and trainees at the Safar Center. Our multidisciplinary Center continues to produce a unique and exciting environment and the productivity and successes of the investigators and trainees never cease to amaze me. Our multidisciplinary Center continues to grow.

As introduced in last year’s report, we have expanded our efforts into five major areas of research and research training—including research in traumatic brain injury (TBI), training in pediatric neurointensive care and resuscitation research, hemorrhagic shock and suspended animation, CNS rehabilitation research, and most recently we have begun, through the efforts of Drs. Robert Clark and Robert Hickey, the programmatic study of cardiopulmonary arrest in children. We are also fostering an increasing collaboration with Dr. Clifton Callaway of the University of Pittsburgh Center for Emergency Medicine whose research focuses on cardiopulmonary arrest and resuscitation in adults.

Our TBI program is funded by a program project from the National Institute of Neurological Disorders and Stroke (NINDS), five RO-1 awards, two R-21, one KO-8, and K23 awards, and a variety of other grants. Our work in TBI spans a number of areas of study—including evaluation of novel resuscitative therapies targeting neuronal death, unraveling the mechanisms of secondary injury in both experimental models and in brain injured patients, the development of novel tools to facilitate detection of occult cases of child abuse, and the testing of new strategies in brain injury rehabilitation. Accomplishments in 2002/2003 in this program included the successful competitive renewal of the University of Pittsburgh Center for Injury Control and Research (CIRCL) grant by Dr. Hank Weiss in the Department of Neurological Surgery. Dr. Weiss has been a strong leader of this CDC-funded injury prevention grant. Safar Center investigators direct two of the projects in CIRCL. Dr. Amy Wagner, in the Department of Physical Medicine and Rehabilitation (PM&R), has a project entitled “Relationship of female sex hormones to CSF pathophysiology and outcome after TBI.” Amy has a portfolio of projects both at the bench and bedside that address the important issue of the influence of...
gender on outcome in TBI. Similarly, Drs. Rachel Berger and Kochanek have a project in CIRCL entitled “Improving the diagnosis and prognosis of inflicted head trauma in infants”—that is testing the use of a battery of blood tests to aid in diagnosing “silent brain” injury—specifically targeting missed cases of child abuse (i.e., the shaken baby syndrome). It is exciting to see that Drs. Wagner and Berger, both young clinician-scientists supported by K-awards from NIH, are developing into independent investigators. Dr. Wagner also is the assistant director of the Clinical Trials Cooperative Network in TBI—recently funded by the National Center for Medical Rehabilitation Research (NCMRR)/NIH. Dr. Ross Zafonte, Chairman of the Department of PM&R at the University of Pittsburgh, is the local PI for that project. I am also pleased that funding for Dr. Chien Ho’s superb Pittsburgh NMR Center for Biomedical Research was renewed. We have had a longstanding and fruitful collaboration with Dr. Ho’s group. They have provided contemporary magnetic resonance imaging methods to our TBI work and we look forward to continued interaction. Dr. C. Edward Dixon received an R21 award from NIH to apply gene array to study the delayed period of recovery in experimental TBI—work that capitalizes on the unique interaction between acute and rehabilitation medicine in our Center. Dr. Kochanek renewed his RO-1 from the NINDS entitled “Adenosine in TBI.” I would like to thank Dr. Edwin Jackson for the incredible support of his laboratory toward this work. I also wish to thank Drs. Jiang-Fan Chen at Boston University and Dr. Jürgen Schnermann of the NIH for providing the A2a-receptor and A1-receptor knockout mice, respectively, that are instrumental to this project. We are pleased to report on the anticipated funding of the competitive renewal of the RO-1 award of Dr. Larry Jenkins in developmental TBI. Also in the area of pediatric TBI, Dr. David Adelson has been leading a clinical trial of moderate hypothermia in children that is centered at Children’s Hospital of Pittsburgh. Several of our fellows have linked the biochemical and molecular expertise of the Safar Center to this project, resulting in a series of studies that are providing what we believe to be the most comprehensive assessment of the biochemical/molecular effects of hypothermia in any clinical trial. These bench-to-bedside studies (see later) typify the mission of our Center. Another major development in pediatric TBI was the publication of the first “Guidelines for the Management of Severe Traumatic Brain Injury in Infant, Children, and Adolescents.” The document, which was published as a supplement in three journals (Pediatric Critical Care Medicine, Critical Care Medicine, and Journal of Trauma), included substantial contributions from both Drs. Adelson and Kochanek. I know that I speak for the entire guidelines committee in thanking Drs. Mary Ellen Michel at NINDS/NIH and Michael Weinrich at NCMRR/NIH for their efforts toward providing the funding for this document. The guidelines committee owes a debt of gratitude to Drs. Randall Chesnut and Nancy Carney who led the charge on the production of this seminal document.

Research training remains the key priority in our Center—including the development of both postdoctoral fellows (MD and/or PhD) and junior faculty. This also represents the most important and enjoyable part of my own efforts. Postdoctoral clinician-scientist development in the field of pediatric critical care has been greatly facilitated by our T-32 grant from the National Institute of Child Health and Human Development (NICHD) entitled “Training in Pediatric Neurointensive Care and Resuscitation Research.” Related to Dr. Safar’s death, Dr. Clark has assumed the role of co-principal investigator
of this training grant. I wish to thank Drs. Ralph Nitkin, Michael Weinrich, Carol Nicholson, and Beth Ansel at NICHD for their valuable insight and support of this exciting program.

We are also grateful to the Department of Anesthesiology for their support of Dr. Hülya Bayır as the 2002 Charles Schertz Fellow. Dr. Bayır is a rising star in the field of Pediatric Critical Care Medicine who recently joined our faculty. A few additional postdoctoral fellowship positions are supported by individual faculty grants. Research productivity by the trainees continues to be spectacular, including a total of 10 fellow first-author peer-reviewed publications and 21 abstract presentations this academic year.

The highlight of the year was the fact that Safar Center fellows received six awards at the 2003 Congress of the Society of Critical Care Medicine (see cover photo). In addition, Dr. Paul Shore received the Neuroscience Award for a paper entitled “Therapeutic hypothermia does not affect markers of injury, cellular energetics, inflammation, and regeneration in cerebrospinal fluid after severe TBI in infants and children” that he presented at the Congress of the World Federation of Pediatric Intensive and Critical Care Societies, in Boston, in June of 2003. Paul’s work is a perfect example of the powerful link between the Safar Center and Dr. Adelson’s pediatric hypothermia clinical trial at Children’s Hospital. Finally, we are sad to report that this year, Dr. Nancy Caroline, one of Dr. Safar’s early trainees who went on to become the mother of CPR in Israel, and later, the head of the Israeli Red Cross, died on December 12, 2002. In her honor, we have created the Nancy Caroline Fellow Award at the Safar Center. This award is given annually to the fellow working with a Safar Center Scientist who has made the greatest contribution to the field of resuscitation medicine. Dr. Safar presented the first award to Dr. Ala Nozari (see photo) for his work in neuroprotection and preservation that was described above. Congratulations to Dr. Nozari.

Junior faculty development is supported by a number of grants, including KO8 awards to Drs. Robert Hickey in the Division of Pediatric Emergency Medicine (mentored by Dr. Steven Graham) and Amy Wagner in the Department of PM&R (mentored by Dr. Dixon), and most recently, a K-23 award to Dr. Rachel Berger in the Department of Pediatrics (mentored by Dr. Kochanek). Finally, Dr. Kochanek has begun to collaborate with Dr. Sam Poloyac in the School of Pharmacy on the study of the cytochrome-P450 metabolite 20-HETE in brain injury. Dr. Poloyac is a promising young investigator who is developing an RO-1 submission. I am especially proud of our successes in fellow, resident, student, and faculty development, which I feel is the most important facet of our work.
The hemorrhagic shock and suspended animation program thrived in 2002/2003 guided by the late Dr. Peter Safar and by Dr. Samuel Tisherman. This program, which is focused on novel approaches to resuscitation of traumatic hemorrhagic shock and exsanguination cardiac arrest, is supported through congressional plus-up funding via the United States Army. The program is focused on new approaches to the use of hypothermia and other pharmacologic strategies for protection and preservation of the entire organism during circulatory arrest. Studies in 2002/2003 tackled the difficult problem of the combination of multiple trauma and prolonged cardiac arrest using profound hypothermia and with the novel addition of plasma exchange therapy. In 2002/2003, we wish to thank Drs. Joseph Carcillo at Children’s Hospital of Pittsburgh, Dr. Ann Hale of the Midwest Animal Blood Service, Stockbridge, MI, and Dr. Frank Bontempo at the University of Pittsburgh School of Medicine, for their expertise in plasma exchange, blood banking, and coagulation, respectively. Remarkably, we have been able to achieve intact survival after exsanguination cardiac arrests of 2 hours. This work continues to break new frontiers in the area of cerebral and whole-organism preservation and resuscitation. We also thank the investigators of our industrial partner, Ardiem Medical, for their work in the development of cooling devices for this project. We also thank Drs. Ala Nozari and Xianren Wu, two talented fellows working with us on this project. This area of study, with the loss of Dr. Safar, now represents a special challenge for Drs. Tisherman and Kochanek to continue to push into the future.

Consultative and administrative support from Dr. Lyn Yaffe, former director of the United States Naval Medical Research Institute is instrumental to the program. Dr. Yaffe is a resource and a special friend to our Center. We cannot thank him enough for this support. We are also very thankful to Col. Dean Calcagni and Robert Read of the United States Army for their continued encouragement and support at the Telemedicine and Advanced Technology Research Center (TATRC) of the United States Army Medical Research and Materiel Command.

Investigators in the Center published 33 peer-reviewed papers, 21 chapters and editorials, and 60 abstracts in 2002/2003. Included among these reports were publications in the Journal of Biological Chemistry, FASEB Journal, the Journal of Cerebral Blood Flow and Metabolism, Pediatrics, Critical Care Medicine, Pediatric Critical Care Medicine, Brain Research, and the Journal of Neurotrauma. There were several noteworthy publications in 2002/2003. Lina Du, working with Dr. Clark, published an important report in the Journal of Biological Chemistry on the role of intra-mitochondrial PARP in the cascade of neuronal death after brain injury. Their work introduced a novel concept into the PARP cell suicide theory and garnered the cover of the journal. Dr. Rachel Berger authored a manuscript entitled “Neuron-specific enolase and S100B in cerebrospinal fluid after severe TBI in infants and children” that was published in Pediatrics. She described a more delayed pattern of release of the neuronal death marker neuron-specific enolase into the cerebrospinal fluid of infants and children after severe head injury from child abuse than from accidental injuries, supporting a unique mechanism of damage in those victims. Rachel’s paper was voted at the Annual San Diego Conference on Child and Family Maltreatment to be the most important paper of the year in the field of research in child abuse. Dr. Xiaopeng Zhang, working in the group of Dr. Clark, published a bench-to-bedside study of the caspase-8 neuronal death
pathway in the *FASEB Journal*. Dr. Zhang is a talented clinician-scientist who has helped everyone in our Center with his molecular skills. These high-impact publications reflect the outstanding science of the Clark research group. I am pleased to report that Dr. Zhang was just accepted into the Neurosurgery residency program of the Massachusetts General Hospital. Although he will be a big loss to our group, this is a fabulous career opportunity for Xiaopeng, and we wish him well. Dr. Bayir reported, in the *Journal of Cerebral Blood Flow and Metabolism*, on the formation of nitrosothiols in cerebrospinal fluid of infants and children with severe TBI. This was the first report of nitrosylation in ischemic or TBI in humans, and was accomplished through the collaboration between the Safar Center and the outstanding laboratory of Dr. Valerian Kagan and his free radical biology group in the Department of Environmental and Occupational Health. We look forward to continued productive collaboration with Dr. Kagan. Manu Varma and Sumeeta Varma, summer students who worked in our Center, published manuscripts as first authors in *Brain Research* and the *Journal of Neurotrauma*, respectively. This is exemplary productivity for undergraduates, and attests to their hard work and dedication. Fellow, Dr. Wilhelm Behringer published an important paper in the journal *Critical Care Medicine* on the use of profound hypothermia to achieve neuroprotection for up to two hours in our suspended animation project. Also, Dr. Nozari, a talented visiting clinician-scientist in our Center from Uppsala University, is now at the Massachusetts General Hospital as a resident in Anesthesiology. One of our prior reports from 1997 entitled “Expression of Endothelial Adhesion Molecules and Recruitment of Neutrophils Following Traumatic Brain Injury in Rats” that was authored by Dr. Timothy Carlos, was recognized during the 2002/2003 academic year by the *Journal of Leukocyte Biology* as one of the five most highly cited articles in that journal over the last 5 years. Finally, I was honored to author an editorial on hypothermia in brain injury for the *Journal of the American Medical Association* with the late Dr. Safar. Although many subsequent papers will appear over the next few years on which Dr. Safar had an important role as co-author, this editorial in *JAMA* was the final publication that Dr. Safar worked on before his death.

On November 20, 2002, we hosted the first Safar Symposium at the University of Pittsburgh School of Medicine. The symposium featured a morning session on *Breakthroughs in Resuscitation Research* and an afternoon session on the *Role of Human Simulation in Medical Education and Research*. 

Drs. Peter Safar (left) and Max Weil at the first Safar Symposium at the University of Pittsburgh School of Medicine. Drs. Safar and Weil were two of the pioneers of the field of Critical Care Medicine in the United States.
Two hundred clinicians, scientists, and allied faculty, fellows, paramedics, and students attended the symposium. The program was opened by Chancellor Mark Nordenberg of the University of Pittsburgh and was held at the new Peterson Events Center. The keynote address was given by Dr. Max Harry Weil, of the Institute of Critical Care Medicine, one of the three founding fathers of the field of Critical Care Medicine, a resuscitation pioneer, and a long-time friend of Dr. Safar. Invited speakers also included Drs. Mark Angelos and Larry Katz from the Departments of Emergency Medicine at the Ohio State University, and the University of North Carolina Chapel Hill, respectively.

On November 21, 2002, Dr. Lyn Yaffe, of the Illinois Institute of Technology, and former director of the United States Naval Medical Research Institute was the 23rd Peter and Eva Safar Lecturer in Medical Sciences and the Humanities at the University of Pittsburgh School of Medicine. Dr. Yaffe’s lecture was entitled “Future Medicine: Biomedical Technology Systems for Victims of Combat and Terrorism” outlined a futuristic view of combat casualty care needs and novel potential innovations in the field for military and civilian trauma care, ranging from smart catheters to robotics. During 2002/2003, Dr. Yaffe spearheaded a group of investigators and companies in the development of novel devices for field resuscitation and continues to be an outstanding collaborator with our Center and its efforts in hypothermic protection.

A special visiting professor in 2002/2003 to the Safar Center was Dr. Heinz Redl of the Ludwig Boltzman Institute in Vienna, Austria. Dr. Redl is the scientific director of that renowned institute which shares many overlapping missions with our own work. He is one of the true gentlemen in academics and his comments to our trainees were greatly appreciated.

Once again, I would like to thank everyone working at the
Safar Center for a terrific job this year. I am personally indebted to Linda Amick, Marci Provins, and Fran Mistrick for their administrative and secretarial excellence. Linda and Marci are extremely dedicated to the Safar Center and its success. Linda continues to take on an increasingly greater administrative role on the business end of the Center while Marci serves as our key secretarial resource for the academic programs in our Center—along with her dedicated work as my local editorial assistant for the journal *Pediatric Critical Care Medicine*. Fran Mistrick was the devoted personal secretary of Dr. Safar for 23 years. I owe her a tremendous gratitude for her help this year. Fran, along with Drs. Ake Grenvik, John Schaefer, did a remarkable job on the Festschrift to Dr. Safar that was published in the journal *Critical Care Medicine*, as we were preparing this report. I will say more about that in next year’s annual report. I cannot tell you how pleased I am that Fran is staying on at the Safar Center. I would also like to thank Julian Smith and Val Sabo for their continued dedication and hard work I would also like to personally thank Henry Alexander, John Melick, Keri Janesko, Vincent Vagni, Xiecheng Ma, Lina Du, Paula Nathaniel, Ray Griffith, Jackie Pantazes, Grant Peters, and S. William Stezoski, who were senior administrative and technical staff members during the 2002/2003 academic year for their spectacular contributions to the individual missions of the Center. A special word or thanks is in order to Bill Stezoski. Bill has the lab coordinator for Dr. Safar for over 30 years, and has been an important reason for the success of the work of Dr. Safar. Bill, I know that Dr. Safar would have wanted me to personally thank you and each of the technicians in the team that you directed for him for an outstanding job. I am similarly pleased that you are staying on to continue with the hemorrhagic shock and suspended animation program; your work is indispensable. I continue to be amazed by the work ethic of all of the technical and secretarial staff at our Center.

I would like to thank Dr. Mitchell Fink for his support as the Chairman of the Department of Critical Care Medicine and Susan Stokes, departmental administrator. I am grateful to them for their support with the renovation of our Center. I would like to thank Drs. Clark, Dixon, Jenkins, Zafonte, Callaway, Adelson, Zhang, Hong Qu Yan, and of course the late Peter Safar for their camaraderie and guidance with the continued development of the Safar Center and its programs. They have been instrumental in its success. I would also like to thank Dr. John Williams, Chairman of the Department of Anesthesiology, for supporting the Safar Symposium and the Peter and Eva Safar Lecture.

Special thanks are also due to Dr. Ho and Kristy Hendrich at the Pittsburgh NMR Center for Biomedical Research, Dr. Edwin Jackson in the Center for Clinical Pharmacology, Dr. Valerian Kagan in the Department of Environmental and Occupational Health, Dr. Stephen Wisniewski in the Department of Epidemiology, Dr. Timothy Carlos in the Department of Medicine, Dr. Simon Watkins in the Department of Cell Biology and Physiology, Dr. Timothy Billiar in the Department of Surgery, Dr. Paul Paris in the Department of Emergency Medicine, Dr. David Perlmutter in the Department of Pediatrics, and Dr. Melyvn Heyes at the Curagen Corporation for outstanding collaborative expertise that raises the level of the research at the Safar Center.

I also owe a debt of gratitude to Mr. Tore Laerdal of Laerdal Medical and to Hans Dahl of the Laerdal Foundation. Their generous support for the publication of the Festschrift
to Dr. Safar was deeply appreciated. We also thank them for their continued support of grants to our Center through the Laerdal Foundation. We also congratulate Dr. John Schaefer, Mr. Tore Laerdal, Dr. Ake Grenvik, and of course, Dr. Peter Winter, for the recent completion of the new Winter Institute for Simulation Education and Research in McKee Place, on the campus of the University of Pittsburgh. This spectacular new state-of-the-art simulation center will be a unique resource for education and research far into the future.

As this academic year comes to a close, we are about to embark on a new expansion of the Safar Center, to include nearly 8,000 additional square feet of space, increasing the size of the center to about 20,000 square feet. This new space is badly needed and will provide important upgrades for the laboratories of several very productive faculty in our Center, in particular a new cell culture and wet lab facility for Dr. Clark, and a new functional outcome suite for Dr. Dixon, along with several additional upgrades. We also plan on building a historical exhibit outlining the many accomplishments of Dr. Safar during his illustrious career. We thank Dean Arthur Levine for facilitating this expansion. We also thank Frank Adams, Doug Schlach, and Julie Polleta, architects involved in this project, for their dedication to our new facility.

Finally, with the help of Chancellor Nordenberg, we have begun a fundraising campaign to ensure the Safar Center for Resuscitation Research will continue in perpetuity. Based on Dr. Safar’s wishes before his death, we have established a fundraising committee and three funds, including a “Safar Legacy Fund,” to provide a core budget for the center, along with funds to support the “Nancy Caroline Fellowship Award” and, of course, the “Safar Symposium.” We have enclosed a pledge card describing those funds in this year’s report and thank you in advance for your support. I also thank each of the members of the fundraising committee for their ideas and hard work. I would also like to personally thank each of you who have already donated to these efforts. Our total goal for these three programs is an endowment of two million dollars toward Dr. Safar’s goal of the resuscitation of “brains and hearts too good to die.”

I once again look forward to success in 2003-2004 in our investigative efforts to develop new therapies in the field of resuscitation medicine, and thank you for your continued support our work.

Respectfully submitted,

Patrick M. Kochanek, MD
Patrick M. Kochanek, M.D., Director, Safar Center for Resuscitation Research
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Clifton Callaway, MD, PhD
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Funding

During the 2002/2003 academic year, Safar Center investigators had a total of 41 active grants. 35 of these grants were extramural. The direct and indirect costs for the full award period of these grants totaled $15,985,736 and this is plotted for the current and preceding eight academic years on the following page. The specific sources of this grant support are shown on the subsequent page. Remarkably, the Safar Center is continuing to grow and maintain a high level of extramural support. This has required a huge effort by our faculty since our support is almost completely derived from extramural grants. Congratulations to the faculty for their remarkable funding successes.

The portion of the budget for use in each academic year (July 1 through June 30) is also plotted for the current and preceding four academic years on the pages following. This represents direct and indirect costs and is shown for total, extramural, and intramural grant support.

Extramural funding sources included the National Institutes of Health, the United States Army, the United States Navy, the Centers for Disease Control and Prevention, the Laerdal Foundation, and a variety of other sources, including contributions made to the Safar Center in memory of Eric Bundy.

Intramural funding was provided by the Departments of Critical Care Medicine, Anesthesiology, the Children’s Hospital of Pittsburgh, and the Pittsburgh Mercy Foundation, Mercy Hospital of Pittsburgh.

We are deeply grateful for the prior and current support from all of these granting agencies and donors.
Direct and Indirect Costs for the Full Award Period of SCRR Grants
TRAUMATIC BRAIN INJURY (TBI) PROGRAM

Traumatic brain injury (TBI) affects 1.5 to 2 million people in the United States each year, making it one of the more prevalent and debilitating of all neurological disorders. Approximately 300,000 of the cases are severe enough to warrant hospitalization. Of the 250,000 survivors of severe TBI, 100,000 endure long-term disabilities that require rigorous, lengthy, and costly medical and rehabilitative care. In addition to the medical expenses associated with TBI, societal costs are also significant in terms of loss wages due to the inability to resume employment. While the true cost of TBI is incalculable, it is estimated at $100,000 annually per patient or about $48.3 billion per year. TBI is a serious and survivable medical problem with no acknowledged treatment. Therefore, investigation of therapeutic strategies that may facilitate the recovery process after TBI at the Safar Center are essential. Equally important are studies identifying mechanisms involved in the evolution of secondary damage after TBI and determining if pharmacological agents are detrimental to the recovery process.

TBI Investigation by Safar Center Director and Associate Directors

1. Studies directed by Patrick M. Kochanek, MD

Patrick M Kochanek, MD, Director, Safar Center for Resuscitation Research, Professor and Vice Chairman, Department of Critical Care Medicine, University of Pittsburgh School of Medicine. Professor of Anesthesiology and Pediatrics.

Dr. Kochanek’s Research at the Safar Center is accomplished through a collaborative effort between a number of investigators, fellows, students and staff located principally in the Department of Critical Care Medicine (CCM), including the Clinical Research, Investigation, and Systems Modeling of Acute Illness (CRISMA), Neurosurgery, PM&R, and Neurology at the University of Pittsburgh School of Medicine. A large number of collaborations are also ongoing with investigators in other University of Pittsburgh Departments including the Center for Clinical Pharmacology, Environmental and Occupational Health Medicine, Pediatrics, Epidemiology, Anesthesiology, and Surgery. In addition, a long-standing collaboration is in place with the Pittsburgh NMR Center for Biomedical Research at Carnegie Mellon University. We have also had a number of important extramural collaborators, Dr. M Heyes at the Curagen Corporation, Dr. N Minamino at the National Cardiovascular Center Research Institute in Osaka, Japan, Dr JF Chen at Boston University, and Dr. J Schnermann at the NIH. Taken together, these collaborations have allowed us to investigate a broad spectrum of mechanisms that may be important to the evolution of secondary damage after TBI. Our most important work continues to be in the area of defining the mechanisms important to secondary brain injury both after experimental TBI and in the human condition. Our studies of mechanism of secondary damage and repair in human materials (cerebrospinal fluid [CSF], brain tissue samples from resected contusions, and microdialysis samples) have generated new insight into the biochemistry and molecular biology of human head injury. Based on this mechanistic work, we are currently testing novel therapies in our experimental models. Our goal is to develop new therapies that can be successfully
translated to clinical application. Our clinical research of taking the bench to the bedside—particularly as it relates to child abuse—has been featured many times in the lay press.

A. Biochemical Assessment of Secondary Mechanisms of Injury and/or Repair after Severe TBI in Infants and Children: The Role of Child Abuse.

This continues to be an important area of research for our group and, as indicated above, continues to generate considerable publicity. We are using samples of CSF and blood collected from infants and children suffering severe TBI to study a variety of biochemical mediators of secondary damage and/or repair. These samples are collected by Dr. Rachel Berger in the Department of Pediatrics and member of our critical care team including Drs. Clark, Bayr, Shore, Ruppel, Lai, Chadha, and Fink in the division of Critical Care Medicine, Dr. Berger, in the Department of Pediatrics, and Dr. Adelson in the division of Neurosurgery at Children’s Hospital of Pittsburgh. To generate a CSF bank for this purpose, Dr. Kochanek is funded by the CDC (University of Pittsburgh Center for Injury Control and Research [CIRCL]). We have now over 1000 samples from nearly 100 infants and children who have suffered a severe TBI—including over 20 victims of inflicted TBI (shaken baby syndrome). In addition, we continue to collaborate with Dr. Neal Thomas at the Hershey Medical Center, Hershey, PA, who is also collecting samples.

Studies using the pediatric CSF bank at the Safar Center

The pediatric CSF bank and related clinical projects have produced some of the most interesting findings in the area of TBI at the Safar Center in the 2001-2002 academic year. Work has progressed in seven major areas including 1) oxidative stress, 2) detection of “silent” inflicted childhood neurotrauma, 3) adenosine and related metabolites in TBI, 4) markers of neuronal death study, 5) growth factors and markers of regeneration and repair, 6) studies of the effect of hypothermia on markers of secondary damage after TBI, and 7) assessment of the effect of the mode of CSF drainage in pediatric TBI.

The potential use of CSF in understanding the pathophysiology of child abuse and potentially serving as a diagnostic adjunct originated from a small grant awarded to Dr. Kochanek within the University of Pittsburgh CIRCL focused on the use of inflammatory markers in CSF as a biological clock to provide insight into the timing of injury in infants who were victims of the shaken baby syndrome. The first report by our group in that area was by Dr. Michael Bell (see prior annual reports). This area of investigation has been broadened by Safar Center Scientist, child abuse specialist, and general pediatrician Dr. Rachel Berger to include the use of serum to assess for the possibility of “silent” brain injury in infants who are victims of inflicted childhood neurotrauma (abuse). Her exciting program of work in this area is described latter in her section within the TBI program.

Oxidative stress in TBI
This is an exciting area of research spearheaded by Dr. Hülya Bayır, a senior PICU fellow and the 2002 Charles Schertz Fellow in the Department of Anesthesiology. Dr. Bayır’s work entitled “Assessment of antioxidant reserve and oxidative stress in CSF after severe TBI in infants and children” was published as a full paper in Pediatric Research. That work, done in collaboration with Dr. Kagan, provided substantial evidence for oxidative stress in brain after severe TBI in infants and children. Dr. Bayır followed up on that study an equally important report entitled “Effect of hypothermia on oxidative stress after TBI in humans: a preliminary report” that she presented at the 2001 meeting of the National Neurotrauma Society and will present at the 2002 meeting of the SCCM. She has begun to use the battery of markers of oxidative stress and damage that she has developed with Dr. Kagan, to evaluate the effect of therapies—including moderate hypothermia in adults. Current studies by Dr. Bayır of the effect of hypothermia on oxidative stress in pediatric TBI are also underway—done in conjunction with the RCT being carried out by Dr. Adelson at Children’s Hospital of Pittsburgh. Dr. Bayır has also worked under the direction of Dr. Kagan to study a novel marker of nitrosative stress in pediatric TBI, namely, S-nitrosylation. Their work in this novel area was presented at the 2001 meeting of the Society for Neuroscience and a full manuscript is in press in the Journal of Cerebral Blood Flow and Metabolism. Dr. Bayır will join our faculty next year in Pittsburgh and will be a welcome addition to the growing list of Scientists at the Safar Center.

**Adenosine in TBI**

Dr. Kochanek is beginning year-4 on an RO-1 from NINDS focused on adenosine in TBI. Translational work is an important part of this effort and the CSF bank represents a key resource. Dr. Courtney Robertson’s article on CSF adenosine in pediatric TBI (see last year’s report) was published this year in the journal Critical Care Medicine. Investigation of the effect of hypothermia on adenosine and purine related markers of energy failure are ongoing in collaboration with Dr. Edwin Jackson. Similarly, Ava Puccio is evaluating the relationship between CSF adenosine and tissue oxygen levels in adults with severe TBI, in work done with Dr. Marion at Presbyterian Hospital.

**CSF markers of neuronal death in TBI**

As part of the impressive work of Dr. Robert Clark’s group on mechanism of neuronal death, including studies on caspases, apoptosis inducing factor (AIF), and poly ADP ribose polymerase (PARP), translational studies are similarly taking advantage of our CSF bank and brain tissue samples. Drs. Margaret Satchell and Xiao Peng Zhang have published a series of abstracts on PARP activation and protein kinase B signaling after TBI in humans. Some of Dr. Satchell’s work was discussed in last year’s annual report. This translational approach is providing important human data on contemporary and intensively investigated mechanisms of neuronal death in experimental TBI—and has the potential to help guide the development of novel therapies.

**Growth factors and markers of regeneration and repair**

Building on the prior work of both Dr. Steven DeKosky on nerve growth factor, and Edwin Jackson on the relationship between adenosine A2β receptor activation and elaboration of vascular endothelial growth factor (VEGF), Dr. Paul Shore presented a
paper at the 31st Congress of the SCCM reporting marked increases in VEGF after severe TBI in infants and children. At the same meeting, Dr. Erica Fink, a senior pediatric resident working with Dr. Clark reported increases in hepatocyte growth factor in CSF after injury. We have been struck by the robust and rapid regenerative response that occurs after TBI and by the fact that this is readily detected using CSF.

**Effect of modes of CSF drainage**
Dr. Paul Shore is carrying out a comparative study (in collaboration with Dr. Neal Thomas at Hershey Medical Center) assessing the effect of continuous versus intermittent CSF draining on mediator levels and pathophysiology after severe TBI in infants and children. We are pleased to collaborate with Dr. Thomas, a former fellow in our program, in this study that addresses a basic treatment approach (CSF drainage) that has been subjected to remarkably little investigation.

Our pediatric CSF bank continues to represent a key research tool of our trainees to help bring the bench to bedside in the study of secondary injury mechanism in clinical TBI research.

Support: Quinolinic Acid in CSF Early after Severe Head Injury in Victims of Child Abuse R49/CCR310285-03, (9/1/01-8/31/02), $45,110, P Kochanek, PI, M Heyes, PhD, [Curagen Corporation], R Berger, S Wisniewski, PhD, D Marion, MD, and P David Adelson, MD, Co-investigators); collaborators. CDC, CIRCL (D Marion, MD, PI); Adenosine and TBI, NS38037, (8/2/01-7/31/02) $263,910, P Kochanek, PI; iNOS and TBI, NS30318 (P Kochanek, PI), Project 3 within the University of Pittsburgh Brain Trauma Research Center (BTRC), D Marion, PI. Protocol #3480500 (5/1/00-4/30/01), $11,215, R Berger, PI, CHP GCRC. Oxidative Stress after Severe Head Injury in Infants and Children: Effect of Therapeutic Hypothermia, Laerdal Foundation, H Bayır, PI.

**B. Adenosine and TBI**

Adenosine is produced during the breakdown of adenosine triphosphate (ATP) after TBI. Its powerful vasodilator, anti-excitotoxic, and anti-inflammatory effects may represent an important endogenous defense mechanism in injured brain. The role of adenosine as an endogenous neuroprotectant molecule, particularly early after TBI, and its potential participation in delayed cerebral swelling are being pursued both in the rat TBI model and in patients after TBI. We are beginning the 4th year of this RO-1-funded project. This project continues to be the most active area of research in Dr. Kochanek’s laboratory this year and has produced a number of reports of studies in both patients and experimental models of brain injury. This work is being carried out in collaboration with Dr. Edwin Jackson in the Center for Clinical Pharmacology. In laboratory aspects of the research on this project, we have continued to evaluate the effect of local injection of adenosine receptor agonist and antagonists on cerebral blood flow. That work is carried out in collaboration with Dr. Chien Ho and Kristy Hendrich at the Pittsburgh NMR Center. A recent study was presented at the 2001 meeting of the National Neurotrauma Society—and demonstrated that adenosine receptor agonist mediated cerebrovasodilatory effects are mediated by the A2a receptor and can increase cerebral blood flow in both the
normal and traumatically injured rat brain. A number of outcome studies of adenosine agonists are ongoing in collaboration with Dr. C. Edward Dixon in our center using the controlled cortical impact (CCI) model. Manu Varma, an undergraduate from the University of Michigan who worked on that project in our laboratory again this summer, was just informed that his manuscript on this work is accepted for publication in the journal *Brain Research*. Using our CCI model, we are currently studying the A2a-receptor knockout mouse, obtained from Dr. Jiang-Fan Chen at the Massachusetts General Hospital and the A1-receptor knockout mouse obtained from Dr. Jurgen Schnermann at the NIH to begin to unravel the role of specific adenosine receptors in the mechanisms of secondary damage and repair after experimental TBI. Key collaborators on the RO-1 are Drs. E Jackson, CE Dixon, C Ho, S Graham, D Marion, and Ms. K Hendrich.

Support: NIH RO-1, Adenosine and TBI, ($1,593,730, 08/02/99-07/31/03, P Kochanek, MD, PI).

C. Role of Inducible Nitric Oxide Synthase (iNOS) in the Inflammatory Response after TBI

iNOS is induced by cytokines and NF-κB is suggested to play an important role in the pathophysiology of sepsis outside of the central nervous system. Both beneficial and detrimental actions of iNOS have been reported. Using both inhibitors of iNOS and knockout mice, Dr. Elizabeth Sinz (1996-97 Charles Schertz Fellow) reported a powerful endogenous neuroprotectant effect of iNOS in experimental TBI. This area of study is carried out as part of our funded project within the University of Pittsburgh Brain Trauma Research Center (BTRC) Program Project. In collaboration with Drs. Kagan and Timothy Billiar, Hülya Bayır has been studying protein nitration and nitrosylation after experimental TBI using iNOS knockout mice. Nitrosothiols may represent a nitric oxide reservoir and could play important roles in signal transduction, immunomodulation, vascular regulation, and neurotransmission.

Support: NIH 2P50 NS30318, iNOS and TBI, ($582,986), P Kochanek, MD, PI, Key Collaborators: RSB Clark, MD, CE Dixon, PhD, T Billiar, MD, V Kagan, PhD, L Jenkins, PhD, X Zhang, PhD, H Yan, MD, and T Carlos, MD, collaborators.

D. Emergency Interventions after TBI: Effect on Secondary Damage

Studies in this area of investigation were funded, this year, by both the Laerdal Foundation and the Curagen Corporation. Dr. Kimberly Statler (one of our T-32 fellows) has been the leading investigator on this work. Dr. Statler presented a surprising paper showing that moderate hypothermia, applied after experimental TBI, expands lesion volume at 72 h after injury in rats anesthetized with the narcotic fentanyl. That work was presented at the National Neurotrauma Society meeting and is *in press* as a full manuscript in the journal *Critical Care Medicine*. In that study, Dr. Statler discovered that hypothermia after TBI produces an enhanced stress response—reflected by higher serum catecholamine levels—compared to the normothermic condition. These studies
are in contrast to the remarkable neuroprotection that others and we have consistently observed with hypothermia in rats anesthetized with isoflurane. The importance of this work lies in the fact that patients are sedated with narcotics after TBI. It may be that to maximize the potential benefit of therapeutic hypothermia after TBI, sedation must be optimized. To further understand the mechanism underlying the effect of hypothermia on experimental TBI, we are carrying out studies evaluating the effect of hypothermia on gene expression using our mouse model of controlled cortical impact. This work is being carried out in collaboration with Dr. Melvin Heyes at the Curagen Corporation, a leader in gene culling technology. In an initial report, two summer students, Becky Sullivan and Gilna Alce published an abstract of work in Critical Care Medicine showing a robust beneficial effect of the resuscitative application of transient, moderate hypothermia in this model. This, to our knowledge, is the first report of the beneficial effects of hypothermia in a mouse model—and sets the stage for studying the combined effects of hypothermia in genetically modified mice. Finally, Dr. Statler published an invited review on this area of work in the Journal of Neurotrauma that was based on a plenary talk by Dr. Kochanek, entitled “The Simple Model Versus the Super Model: Translating Experimental TBI Research to the Bedside.” We hope to also soon apply proteomics approaches to the study of hypothermia in TBI in collaboration with Dr. Larry Jenkins in our Center.

Support: Laerdal Foundation, MRI Assessment of cerebral blood flow and calcium accumulation after TBI in rats: Effect of isoflurane versus Fentanyl, ($7,500, 1/1/00 – 6/30/01, K Statler, PI). Training in Pediatric Neurointensive Care and Resuscitation Research, T32-HD40686, National Center for Medical Rehabilitation Research (NCMRR), National Institute of Child Health and Development (NICHD), P Kochanek, PI 9/25/00-4/30/01.

E. Magnetic Resonance Imaging (MRI) Assessment of Experimental TBI

Contemporary and novel MRI methods are being used to characterize our injury model and facilitate the testing of novel therapies in experimental TBI in rats. The goal of this work is to use non-invasive NMR methods to access acute physiologic derangements early after injury and to couple these to assessment of functional outcome at more delayed times after TBI. MRI methods were used to augment investigation in our study of both adenosine and anesthetics in experimental TBI. We have begun to expand the use of MRI to our mouse model of experimental TBI with the help of Kevin Hitchens and Lesley Foley. Dr. Ho’s outstanding multidisciplinary NMR center for biomedical research continues to be a key collaboration for our work in experimental TBI.

Support: NIH-NINDS 2P50 NS3031809 A1, Rat/Surgery/Imaging Core C, ($470,095 over 5 years, P Kochanek, MD, PI, C Ho, PhD, Co-PI, K Hendrich, D Williams, PhD, and S DeKosky, MD, Co-investigators). NIH Grants RR-03631 and RR-10962, (C Ho, PI) support the Multidisciplinary Pittsburgh NMR Center at Carnegie Mellon University. NIH PAR00-031, In-Vivo MR Microscopy Instrumentation at 11.7 Tesla ($500,000, C Ho, PhD).
2. Studies directed by C. Edward Dixon, PhD

C. Edward Dixon, PhD, Professor of Neurological Surgery, Anesthesiology, Neurobiology, and PM&R, University of Pittsburgh School of Medicine. Director, University of Pittsburgh Brain Trauma Research Center

Research Interests

Research in Dr. Dixon’s laboratory is directed towards understanding the molecular mechanisms of cognitive deficits following TBI. Current studies are evaluating the effects of brain injury on dopaminergic and cholinergic systems and the relationship between these changes and the induction and recovery cognitive deficits. Experimental neurotherapeutic studies are ongoing to evaluate the effects of neurotrophic growth factors and neurotransmitter receptor activation on recovery of function. Clinical studies include measuring CSF and extracellular levels of catecholamines and markers of oxidative injury in humans acutely after brain trauma.

A. Dopaminergic/Cholinergic Mechanisms of TBI

Recovery of cognitive function after TBI is a dynamic process in which alterations in neurotransmitter systems do not likely occur in isolation. During the prior funding period we noted that substantial cholinergic neurotransmission deficits occur without a chronic (4-wk post injury) loss of cholinergic cell bodies. We also have extensive data that TBI causes chronic changes in key dopaminergic proteins that occur concomitantly with these cholinergic changes. Numerous studies have shown that the dopaminergic innervation of medial septum and diagonal band of broca (medial septal area [MSA]) regions that are dense with cholinergic neurons, can affect hippocampal acetylcholine (ACh) release, especially via D1 receptor agonists. Furthermore, we have compelling preliminary data that dopaminergic innervation of cholinergic nuclei is reduced after TBI. In this project, we propose to extend our previous findings to hypothesize that cognitive deficits after TBI may be, at least partially, attributable to decreased dopamine (DA) modulation of septohippocampal cholinergic function. A systematic series of studies are proposed to test this hypothesis. We will focus on DA modulation of the selectively vulnerable septohippocampal cholinergic system. To better grade an effect of TBI on these systems, we will compare in the MSA the effects of TBI to an established model of DA deafferentation effects; 6-hydroxydopamine (6-OHDA)-induced DA denervation. We will examine the effects of TBI and 6-OHDA lesions on DA modulated ACh release in the hippocampus and DA release in the medial septum. We will also determine whether changes in hippocampal ACh release are associated with altered D1 receptors in the MSA. Dr Dixon’s group will determine the effect of exogeneous administration of neurotrophic factors on DA biochemical markers, cognitive deficits, as well as hippocampal ACh release and MSA DA release after TBI. Lastly, we will determine the effects of clinically relevant DA agonist therapies on cognitive deficits, as well as hippocampal ACh release and MSA DA release after TBI. Our long-term goal is to develop new therapies to accelerate cognitive recovery following TBI.
During this year, we have found that TBI can produce chronic changes in proteins necessary for DA neurotransmission. We have also found that TBI can produce a reduction in DA release in the MSA at 2-wks postinjury and that the number of TH-positive fibers with the medial septum and diagonal band are decreased after TBI. Immunohistochemical and Western blot studies have revealed a distributed up-regulation of TH and downregulation of DAT protein levels. Western blot studies have found decreases in D2 receptor protein levels in the striatum at 4-wks postinjury. We have also demonstrated that DA agonists can enhance recovery of cognitive function after TBI. Overall, there is new evidence that ACh and DA systems are altered chronically after TBI. We also have preliminary data that markers of DA innervation of the septal region are chronically diminished after TBI.

Support: NIH-NINDS, Chronic Changes in Neurotransmission Following TBI, R01 NS-33150-06 ($1,000,000/$484,819 over 5 years, 4/1/00-3/31/05, CE Dixon, PhD, PI).

B. Functional Outcome Core

During this year, the Functional Outcome Core has evaluated post-injury function in several hundred rats and mice for seven different Principal Investigators associated with the Safar Center.

The Functional Outcome Laboratory Core Facility provides a centralized site and highly standardized procedural control for all animal experiments employing functional outcome as an endpoint following TBI to rats. The Functional Outcome Laboratory Core gives the investigators of the University of Pittsburgh BTRC the capability to assess the effects of physiological manipulations and therapeutic interventions of recovery of function after experimental brain injury.

Support: NIH, BTRC Supplement—Functional Core to P50 NS-30318-041A ($274,583 over 4 years, 4/1/96-3/31/00, CE Dixon, PhD, PI).

C. Examination of the Cellular Mechanisms of Mesocortical Dopaminergic Deficits after TBI in a Rodent Model Using Biochemical Indices of DA Autoxidation and Biochemical, Molecular Biological and Immunohistochemical Indices of DA Metabolism and Neurotransmission.

The goal of this project is to examine the cellular mechanisms of mesocortical dopaminergic deficits after TBI in a rodent model using biochemical indices of DA autoxidation and biochemical, molecular biological and immunohistochemical indices of DA metabolism and neurotransmission. Neurochemical and immunohistochemical markers of DA neurotransmission in the dopaminergic ventral tegmental/forebrain systems, as well as functional deficits, will be assessed after injury. The effects of therapies that either reduce oxidative damage of DA terminals and/or chronically stimulate DA activity on neurochemical and immunohistologic markers, and on functional performance will be assessed following TBI. Lastly, the relationship between
early biochemical markers of DA activity to neuropsychological outcome measures specific to frontal lobe function will be evaluated in severe TBI patients. This project represents the first systematic examination of the mechanisms of induction and recovery of catecholaminergic cognitive deficits after TBI. Our long-term goal is to develop new therapies to attenuate the induction and enhance the recovery of DA-mediated neurobehavioral deficits after TBI.

Support: NIH-NINDS, Mechanisms of Prefrontal Dysfunction Following Brain Trauma, R01 NS-40125-01 ($800,000/$376,775 over 4 years, 3/1/00-3/31/04, CE Dixon, PhD, PI).

D. Transcriptomic Analysis of Therapeutics in Brain Trauma

Recovery of cognitive function after TBI is a dynamic process that likely involves multiple neural systems. Several studies by our laboratory and others indicate that cognitive recovery can be enhanced by post injury activation of dopaminergic systems or exposure to an enriched environment. The effectors of such therapeutic activation are likely to involve simultaneous gene expression changes in numerous neural systems. The recent development of DNA microarrays has allowed scientists for the first time the ability to observe thousands of gene expression changes in parallel. While there are limitations, DNA microarrays provide a new systemic view to study brain injury and the treatments that stimulate and enhance recovery of function. We have evaluated a number of DA agonists that are clinically used “off label” for their ability to enhance recovery of cognitive function in our experimental model of TBI and found three to be beneficial: amantadine hydrochloride, bromocriptine, and methylphenidate. While all are putative DA agonists, they have varying degrees of specificity. We have also observed that bromocriptine treatment, when initiated 24 h after TBI, can attenuate hippocampal cell death and lipid peroxidation. This suggests that DA agonists may have mechanisms of action beyond just being DA replacement therapies (e.g. cell survival effects). Supporting this concept, we have new pilot microarray data indicating that relative to a vehicle treatment, the DA agonist methylphenidate can enhance the gene expression of DA receptors and alter injury-induced inflammatory responses. DNA microarrays are well suited to investigate the effects of DA agonists on multiple pathways. The overall goal of the project is to determine common genes that are changed by these therapies and whether these gene expression changes can be further enhanced by the addition of enriched environment therapy. This project will obtain the preliminary information needed for a larger-scale R01 study to increase the number of cases, refine and increase the number of genes analyzed, and to more comprehensively study those genes whose expression are related to recovery of function after TBI.

Support: NIH-NINDS, R21 NS47919, Transcriptomic Analysis of Therapeutics in Brain Trauma. CE Dixon as PI, 10% effort. 07/01/03–06/30/06. $95,000-annual direct costs.

Grant Support

NIH, R21 NS47919, Transcriptomic Analysis of Therapeutics in Brain Trauma. CE
Dixon as PI, 10% effort. 07/01/03 – 06/30/06. $95,000-annual direct costs; $138,000 total indirect costs; $285,000 total direct costs; NIH, R01 NS40125, Mechanisms of Prefrontal Dysfunction Following Brain Trauma. CE Dixon PI, 22.5% effort. 03/01/00-03/31/04. $200,000-annual direct costs; $472,499 total indirect costs; $1,000,000 total direct costs; NIH, R01 NS33150, Chronic Changes in Neurotransmission Following TBI. CE Dixon PI, 26% effort. 04/01/00-03/31/05. $200,000-annual direct costs; $804,064 total indirect costs; $1,645,223 total direct costs; CDC, R49 CCR312296, CIRCL: Acute Care Core Project 1–Effects of Amantadine Hydrochloride on Functional Outcome After TBI: a Randomized, Multi-Center, Placebo-Controlled Clinical Trial; and Acute Care Core Project 2-Relationship Between Amantadine Hydrochloride Efficacy and Brain Function Using PET Imaging, CE Dixon PI, 15% effort. 09/01/98-08/31/02. $106,000-annual direct costs; $1,261,222 total indirect costs; $2,709,778 total direct costs; USAMRMC, 00-451-4360, Novel Resuscitation from Lethal Hemorrhage. P Safar PI, CE Dixon Co-I, 5% effort. 09/15/02-09/14/03. $347,418 annual indirect costs; $712,336 annual direct costs; NIH, R21 NS40049, Protein Synthesis, Memory and Pediatric Brain Injury. LW Jenkins PI, CE Dixon Co-PI, 15% effort. 04/01/00-03/31/03. $125,000-annual direct costs; $187,031 total indirect costs; $375,000 total direct costs; NIH, R01 NS38087, Adenosine and TBI. P Kochanek PI; CE Dixon Co-PI, 7% effort. 08/02/99-07/31/03. $747,440 total direct costs; NIH, R03 HD41399, Gender Differences in DA Function after TBI. AK Wagner PI, CE Dixon Co-PI, 5% effort. 02/06/02 – 01/31/04. $50,000 annual direct costs; $45,535 total indirect costs; $100,000 total direct costs; NIH, K08 HD40833, DA Function in TBI and Effects of Therapeutic Intervention. AK Wagner PI, CE Dixon Primary Sponsor. 09/01/01-09/30/06. $114,365 annual direct costs; $576,165 total direct costs; NIH, R03 HD043851, Interaction of Serotonin and Cholinergic Systems after TBI. AE Kline PI, CE Dixon Co-I, 5% effort. 04/01/03–03/31/05. $50,000 annual direct costs; $44,233 total indirect costs; $100,000 total direct costs.

3. Studies by Robert S. B. Clark, MD

Robert SB Clark, Associate Professor of Critical Care Medicine and Pediatrics, University of Pittsburgh School of Medicine, Fellowship Director, Pediatric Critical Care Medicine Program, Children’s Hospital of Pittsburgh.

A. Endogenous Neuroprotectant Gene Expression after TBI

This research focuses on the genetic regulation and execution of delayed neuronal death in selectively vulnerable neurons after TBI. We have now characterized the expression of several potential cell death-suppressor genes and their translated proteins including bcl-2 gene family members and heat shock protein 72 (endogenous neuroprotectants), as well as potential cell death-effector genes including the pro-apoptotic bcl-2 gene family member bax. These genes appear to be up-regulated and/or activated after TBI in both our experimental model (CCI injury with secondary hypoxic insult followed by resuscitation in rats) and in humans. Studies documenting that bcl-2 family genes may be important in both adult and pediatric patients after TBI were reported previously in the
FASEB Journal and the Journal of Pediatrics, respectively. A role for heat shock proteins after human head injury is also being investigated. Regulation of some of these proteins is via post-translational modification, including the bcl-2 family members bad and bag-1. Bag-1 regulates the chaperone function of heat shock proteins, pointing to a direct interaction between these two classes of endogenous neuroprotectants. As pictured on the cover of this report, PCCM fellow Yi-Chen Lai received a research award form the SCCM for his work on two papers in this area of research including “Mitochondrial over-expression of HSP-70 protects neurons from oxidative stress” and “Age-related increases in protein kinase B after pediatric TBI.” In both of these studies, Dr. Clark mentored Lai.

B. Caspase-Mediated Neuronal Death after Head Injury

Increasing evidence suggests that activation of caspases regulate and execute programmed cell death after TBI in experimental models and in humans. Accordingly, the objective of this research is to develop pharmacological and molecular treatment strategies that reduce caspase-mediated programmed-cell death after TBI. We previously described potential roles for caspase-1 and –3 after severe TBI in humans in a paper published in the FASEB Journal. Studies examining other more potent caspase inhibitors, and combination treatment strategies targeting multiple points in the programmed cell death cascade are ongoing.

C. Divergent Pathways of Cell Death after Brain Injury

It is clear that both apoptotic and necrotic cell death contribute to neuronal cell loss after acute brain injury; however, recent data suggest that this is in fact over simplistic, and that multiple, interrelated pathways exist. A key regulator in this regard is the mitochondrial protein AIF. Work by Dr. Xiaopeng Zhang under the direction of Dr. Clark has clearly demonstrated that AIF-mediated cell death occurs after experimental TBI. That work was published last year in the Journal of Neurochemistry. This year Drs. Zhang and Clark demonstrated an important role for an additional pathway of delayed neuronal death after experimental and clinical TBI—namely—the Fas/Fas ligand pathway. Specifically, they reported, in the FASEB Journal, caspase-8 expression and proteolysis in human brain after severe TBI. This work suggests the need for additional experimental and clinical investigation of this pathway in TBI, and the possibility of novel avenues for therapy. Ongoing studies are determining the contribution of these divergent pathways of cell death to secondary damage in TBI using multiple strategies in collaboration with Drs. Jun Chen, Steven Graham, Patrick Kochanek, Csaba Szabo (Inotek Corp., Beverly, MA), Simon Watkins, Hector Wong (Cincinnati Children’s Medical Center), and Ian Reynolds.

D. PARP Activation after TBI

The study of PARP in experimental TBI is an expanding area of investigation at our center. PARP is an abundant nuclear enzyme with a role in DNA repair pathways. However, in the setting of energy failure, it is suggested that excessive ADP-ribosylation
of proteins resulting from activation of PARP leads to marked nicotine adenine dinucleotide (NAD) depletion and exacerbation of energy failure. Drs. Whalen, Clark, and Kochanek collaborated with Dr. Csaba Szabo (an expert in the area of PARP and sepsis at the Inotek corporation) to study the PARP knockout mouse in our model of experimental TBI. We previously reported highly significant level of protection against functional deficits after TBI in PARP knockout vs wild-type mice, and a role for PARP inhibitors in improving outcome in experimental TBI in mice. However, we also noted deleterious effects of PARP inhibitors on memory acquisition in normal mice—supporting a role for PARP in memory acquisition. This year, we also published a report showing that intra-mitochondrial PARP activation contributes to NAD depletion and cell death both in neuronal culture and in experimental TBI. That work was published by Lina Du in the *Journal of Biological Chemistry* and was featured on the cover of the journal. This work provides novel and valuable insight into the cascade of cell death in the setting of PARP activation—a mechanism that is believed to contribute importantly to a number of important diseases in critical care medicine including CNS injury, stroke, cardiac arrest, sepsis, shock and MOF. In addition, this work further establishes the presence of PARP in mitochondria.

Support: RO1-NS38620-03, Caspase-Mediated Neuronal Death After Head Injury ($584,022 total direct costs over 4 years beginning 2/1/99, R Clark, MD, PI); KO8-NS01946-05, Role of Neuroprotective Genes After TBI ($455,960 total direct costs over 5 years beginning 2/1/99, R Clark, MD, PI; S Graham, MD, PhD. and P Kochanek, MD, Sponsors); P01-NS30318, PARP Activation After TBI, Project 4 of the BTRC Program Project ($595,000 total direct costs over 5 years beginning 6/1/00, R Clark, MD, PI).

4. Studies directed by Larry W. Jenkins, PhD

Larry Jenkins, PhD. Associate Professor of Neurological Surgery, University of Pittsburgh School of Medicine

A. Protein Synthesis, Memory and Pediatric Brain Injury

We have further examined the potential role of impaired protein synthesis in memory deficits after experimental pediatric TBI. There is extensive data suggesting that protein synthesis is critical for the consolidation of hippocampal dependent learning and memory. Protein synthesis is involved in developmental synapse formation, long-term potentiation (LTP) and memory consolidation. Our initial study employing 2-D gel electrophoresis to examine global protein expression during the consolidation of spatial memory acquisition has been submitted for publication. Proteomic studies have potential to expand our understanding of neural injury and therapy but have yet to be applied to TBI. The purpose of this study was to examine global hippocampal protein changes in 17 PND rats 24 h after moderate CCI. Analysis was limited to a wide pH range (nonlinear pH 3-10) for isoelectric focusing with immobilized pH gradients (IPG strips) and large format (22 x 22 mm) SDS slab gels. We evaluated only the most soluble cellular protein fraction using hippocampal tissue protein lysates from sham and injured rats. About 1500 proteins spots were found in each gel with 40% spot matching. Of these 600 matched
proteins 50% showed a 2-fold increase or decrease, 20%, a 5-fold increase or decrease, and 10%, a 10-fold decrease or increase. Limited spot matching with protein databases showed changes in some important cytoskeletal (actin, tubulin), and cell signaling (phosphatidylinositol transfer protein, superoxide dismutase) proteins suggesting that this approach is feasible and informative in the study of protein changes after pediatric TBI.

Our long-term goals are to also characterize some of the most important changes in neuronal signaling known to influence cognitive dysfunction after injury and determine if these changes can be normalized by delayed treatment with trophic factors. Protein synthesis may be altered after TBI by changes in the phosphorylation state of PKB. Phosphorylated PKB (p-PKB) alters protein synthesis by phosphorylating the target of rapamycin protein kinase (mTOR/FRAP) that in turn phosphorylates 4EBP (p-4EBP) the repressor binding protein (4EBP) of eukaryotic initiation factor 4E (eIF4E). p-PKB also activates eukaryotic initiation factor 2α (eIF2α) indirectly by phosphorylating glycogen synthase kinase 3 (GSK-3) reducing the phosphorylation of eIF2α and activating p-eIF2α. Thus, PKB phosphorylation modulates the selection of translated mRNA by eIF4E and the global rate of protein synthesis by increasing p-eIF2α activity. We evaluated the level and distribution of brain p-PKB, p-4EBP, p-eIF4E, and p-eIF2α activity in injured or sham 17 PND rats at 6, 24 or 72 h after moderate CCI using immunohistochemistry. TBI increased the levels of all impacted hippocampal p-proteins at only 6 h except p-eIF4E suggesting an early but unsustained up-regulation of PKB linked protein synthesis activators after pediatric CCI. We are further expanding these studies.

Support: NIH-NINDS, Protein Synthesis, Memory and Pediatric Brain Injury, R21 NS-40049, ($186,250/yr, 5/1/00-4/30/04, Larry W. Jenkins, PhD, PI).

B. Protein Kinase B and C in Head Injury

The PKB and PKC enzyme families participate in many cellular functions including protein synthesis. Hippocampal protein synthesis after TBI is critical for neuronal survival, learning and memory, and synaptic plasticity. TBI alters hippocampal protein synthesis and while improved protein synthesis enhances recovery after cerebral ischemia, this has not been examined after TBI. Pathological changes in protein synthesis mediated by dysfunction of eIF2 and eIF4 pathways after TBI may impair the initiation and fidelity of protein synthesis and injury related restorative and growth responses. Pathological changes in the phosphoinositide 3-kinase-protein kinase B (PI3K-PKB), PKC, GSK-3, mitogen activated protein kinase (MAPK) and mTOR pathways may all be involved in abnormal protein synthesis after TBI. Protein synthesis can be modified by cap-dependent (eIF4E), cap-independent (internal ribosome entry segment [IRES]), and 5'TOP-5' oligopyrimidine tract (mTOR) protein synthesis initiation. This project tests the hypothesis that improved functional recovery following TBI can occur by therapeutically activating beneficial stress related IRES protein synthesis after injury causing stress induced tolerance to secondary injury processes.
Thus, the aims of this proposal are to determine fundamental kinase and chaperone protein pathways that regulate protein synthesis in relation to hypothermia treatment after TBI by examining the control of three major initiation pathways, namely, cap-dependent, cap-independent (IRES) and 5’ terminal oligopyrimidine tract (5’TOP) translation. We will further examine the expression of key protein products representative of these pathways involved in recovery from injury. Protein synthesis regulation is fundamental to most cellular processes. Recent advances in understanding the complexities of protein synthesis regulation contribute to the potential for therapeutic manipulation of protein synthesis. However, the manipulation of signals controlling protein synthesis after TBI may not only affect regional injury and restorative responses, but the normal function of relatively uninjured brain regions after TBI.

Control of protein synthesis primarily occurs at the rate-limiting step of initiation. Pathological changes in protein synthesis mediated by dysfunction of eIF2 (eIF2 - rate of translation - quantitative) and eIF4 (eIF4- mRNA selection-qualitative) pathways after TBI may impair the rate and fidelity of protein synthesis and injury repair. Protein kinases and phosphatases modulate many critical control steps in the initiation and fidelity of protein synthesis, especially the initiation steps mediated by eIF2 and eIF4 protein pathways and thus the activity of these kinase and eIF pathways can be determined in part by their phosphorylation status. Using a reproducible and clinically relevant model of controlled cortical impact (CCI) in the rat, (resulting in spatial memory dysfunction as occurs in humans, we have identified a number of important hippocampal signaling changes that affect protein synthesis initiation. Time dependent changes in PKB, PKC zeta, GSK-3B, 4E-BP, mTOR, p70S6K, eIF4E, and eIF2a phosphorylations after TBI have been documented and will be explored further in this project.

Support: NIH-NINDS, PKB and PKC in Head Injury, R01 NS42648-, ($ 231,250) yearly direct cost, 02/15/04-01/31/08, LW Jenkins, PhD, PI).

5. Studies directed by Anthony E. Kline, PhD

Anthony E. Kline, PhD, Assistant Professor, Department of PM&R, University of Pittsburgh School of Medicine

A. Protective Effects of Serotonin1a (5-HT1A) Receptor Agonists Against TBI-Induced Cognitive Deficits and Histopathology

Serotonergic pathways originating in the raphe nuclei have extensive projections to brain areas involved in cognition and 5-HT receptor agonists and antagonists alter these processes. Of all the 5-HT receptors characterized thus far, the 5-HT1A is the most widely studied. 5-HT1A receptors (5-HT1AR) are abundantly expressed in brain regions, such as the cortex and hippocampus, that play key roles in learning and memory and that are susceptible to neuronal damage by TBI. During the past three years, our laboratory has been investigating the effects of 5-HT1A receptor agonists on neurobehavioral, cognitive, and histological outcome. We first evaluated the high affinity 5-HT1AR agonist
Repinitan HCL (BAY x 3702), which was given (iv) as a 4-h continuous infusion commencing 5-min after TBI or sham injury. The data revealed that repinotan significantly attenuated spatial learning deficits as demonstrated by decreased latencies to locate a submerged (hidden) platform in a water maze task compared to the injured vehicle-treated group. Repinotan also attenuated histopathology as evidenced by more hippocampal CA1/CA3 neurons and smaller cortical lesion volumes vs. the vehicle group. This study, which was published in the journal *Neuroscience* in 2001, was the first investigation of 5-HT1AR agonist interventions in any model of TBI. We then investigated whether the widely used 5-HT1AR agonist 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT) would produce similar beneficial effects. Using our standard injury paradigm, we found that 8-OH-DPAT-treated rats exhibited significantly reduced latencies in locating the hidden platform vs. the vehicle-treated group over time, which is indicative of improved learning and memory. Significantly more CA3 surviving neurons were observed in the group treated with 8-OH-DPAT vs. vehicle. This study was published in *Neuroscience Letters* in 2002. Because 5-HT1AR agonists produce mild hypothermia, which may have contributed to the benefits observed, we are currently conducting temperature controlled studies in an effort to clarify this issue. We are also testing the potential efficacy of a delayed and chronic 5-HT1AR agonist treatment paradigm. Our published and ongoing studies lend support for continued investigation of this therapeutic strategy. Collaborators include Drs. C. Edward Dixon, Amy Wagner, and Ross Zafonte from the Departments of Neurological Surgery, and PM&R.

**B. Role of Environmental Enrichment (EE) after TBI**

Enriched housing, which provides a complex, stimulatory, and social environment, and may be considered a rodent correlate of physiotherapeutic intervention, has been extensively studied in numerous experimental conditions. EE has been reported to increase brain weight, dendritic arborization, synaptogenesis, and to decrease apoptosis of neuronal precursor cells in the hippocampal dentate gyrus. Rats housed in EE for 30 days exhibit significantly higher levels of nerve growth factor mRNA in the rat visual cortex and hippocampus than rats housed in standard conditions. EE has also been shown to increase the expression of brain-derived neurotrophic factor mRNA in the rodent hippocampus. Furthermore, EE has been shown to improve spatial memory and reduce contusion lesion volume. EE has also been demonstrated to improve motor performance on a beam walk task or sensory neglect after cortical lesions. In our laboratory we are comparing the effect of 28 days of EE with standard living conditions on functional and histological outcome after TBI. The data suggest that EE is superior to standard housing in facilitating functional recovery and suggests that this interventional strategy may be useful in a rehabilitative setting by augmenting pharmacotherapies. On-going studies in our laboratory are examining the role of EE coupled with the 5-HT1A receptor agonists 8-OH-DPAT and buspirone on neurobehavioral and histological outcome after TBI. An R01 grant entitled “Novel Rehabilitative Approaches for Recovery from TBI” has been submitted to further examine the relationship between EE and 5-HT1A receptor agonists on the recovery process after TBI. Collaborators include Drs. A Wagner, R Zafonte, and CE Dixon from the Departments of PM&R and Neurological Surgery.
C. Effects of Atypical Antipsychotics on Functional Outcome after TBI

Over 1 million survivors of TBI receive maintenance pharmacotherapy, of which a substantial number receive antipsychotic agents for the treatment of psychoses, agitation and aggression, and other maladaptive behaviors. The incidence of agitation after severe TBI varies from 11% to 50%. In spite of the common clinical use of antipsychotics, the motor and cognitive risks vs. benefits are unclear. Seminal studies by Feeney and colleagues have shown that treatment with antipsychotics (e.g., haloperidol) after TBI retard functional recovery. Moreover, the administration of such agents reinstates deficits in subjects appearing to be “recovered.” More recent work has shown similar detrimental effects on motor function with haloperidol and clozapine after ablation-induced brain injury. Our laboratory is currently evaluating the effects of single (24 h after TBI or sham injury) and/or chronic (24 h–28 d) administrations of the atypical antipsychotic risperidone on motor (beam-balance and beam-walk) and cognitive (spatial learning and memory) functioning in rats. Additionally, risperidone is being compared to the classical antipsychotic, haloperidol. The results from these studies should provide a clearer understanding of the effects of antipsychotic treatments in the recovering brain. An NIH grant is being prepared to further explore this important avenue. These studies are being conducted in collaboration with Drs. R Zafonte and CE Dixon.

Support: NIH-NICHD R03 HD043851-01, Interaction of serotonin and cholinergic systems after TBI, $144,233 for two years (04/01/03 – 03/31/05). AE Kline, PhD, PI. The Pittsburgh Foundation, Evaluation of the serotonergic \( \text{c}_{1\text{A}} \) receptor agonist, 8-OH-DPAT, on biochemical, functional, and histological outcome following TBI in rats, $19,096 for one year (2001-2002). AE Kline, PhD, PI. The Pittsburgh Foundation.

6. Studies Conducted by Amy K. Wagner, MD

Amy K Wagner, MD, Assistant Professor, Department of PM&R, University of Pittsburgh School of Medicine

A. Clinical Gender Differences in TBI Pathophysiology

There is conflicting evidence as to whether there are gender differences with TBI pathophysiology and outcomes. Some studies have reported that with brain injuries of equal magnitude, women sometimes fair worse. Previous work by Dr. Wagner shows that one year after hospitalization with TBI, women have more disability. Yet several animal studies show that female hormones are neuroprotective in attenuating aspects of secondary injury such as excitotoxicity, ischemia, and oxidative stress. We completed a retrospective clinical study using the NIH funded BTRC database identify if there are gender differences cerebrospinal fluid markers of traumatic brain injury and if hypothermia affects these markers in a gender specific manner. Multivariate regression modeling techniques were used to show that there are gender differences with the production and time-course of a cerebrospinal fluid marker of excitotoxic injury and a marker of ischemia early after injury. Females appear to have some neuroprotection against excitotoxic and ischemic injury. However, based on this study, hypothermia
appeared to reduce excitotoxic injury primarily in males. This finding may be due to an apparent “floor effect” with hypothermia in reducing excitotoxic injury in females. Ischemic injury and excitotoxicity were also linked to a marker of oxidative stress. Again there were significant gender differences in the relationship of ischemia/oxidative stress & excitotoxicity/oxidative stress. Females have much lower oxidative stress loads than males for a given excitotoxic or ischemic insult. These findings indicate that there may be acute clinical correlates to the early neuroprotection previously reported in studies on experimental brain trauma. A manuscript reporting this work is currently submitted for review. Another manuscript has recently been published in *Journal of Neurotrauma*. Dr. Wagner has recently been funded by and R01 grant in the successful competitive renewal of the CDC center grant, the CIRCL. This grant is focusing on the role of sex hormones in mediating gender differences in CSF markers of TBI and evaluating the role of acute and chronic hormone levels on neuropsychological and functional outcome, and quality of life. Collaborators include the NIH funded Brain Trauma Research Center CSF Bank [(CE Dixon (Neurosurgery), Mary Kerr (Nursing), Ava Puccio (Neurosurgery)], Anthony Fabio (CIRCL), Ross Zafonte (PM&R, Hülya Bayir (CCM), and Sarah Berga (OB/GYN Emory University).

B. Gender Specific Effects of Environmental Enrichment on Dopamine (DA) Markers and Neurotrophin Production after Experimental TBI.

Environmental enrichment has been shown in a variety of animal models to improve behavioral performance and impact neural substrates affecting plasticity such as angiogenesis, neurotrophin production, gliogenesis, and dendritic sprouting. Enrichment of the housing environment has also been shown to improve spatial memory after experimental TBI in male rat models. Recently we reported that 3 weeks of environmental enrichment after experimental TBI improved cognitive recovery in male but not female rats. We then investigated the effects of gender and an enriched environment on dopaminergic markers and neurotrophin production after TBI. Using Western Blot techniques, we evaluated dopamine transporter (DAT) levels in the striatum and frontal cortex. Results showed significant injury related reductions in DAT protein levels both in frontal cortex and striatum for males. Females did not have significant injury related reductions, with the exception of one striatal region. However, enriched housing post-injury did result in significant reductions in two additional regions for injured females. In the second experiment, we used western blot to evaluate brain derived neurotrophic factor (BDNF) levels in the frontal cortex and hippocampus after TBI and housing in an enriched environment. In males, no significant enrichment or injury effects were observed with hippocampal BDNF expression, but there was a significant post-injury increase in frontal cortex BDNF expression that was not significantly augmented by EE. Neither injury nor EE significantly altered frontal cortical BDNF expression in females, but there was a trend for decreased BDNF expression in the hippocampus of injured females vs. sham. In contrast, there were robust increases in hippocampal BDNF expression for EE injured females compared to both sham and injured animals placed in standard housing. These results reveal significant, region-specific gender differences in
chronic BDNF expression with both injury and EE that may impact enrichment-mediated improvements in cognitive recovery and responses to therapeutic interventions. Portions of the work were funded through Dr. Wagner’s NIH K08 award. Portions of the work are going to be submitted to the journal *Neuroscience* for review. Future work will focus on the role of sex hormones on these findings as well as continuing to explore relevant neurotransmitter systems affecting a dimorphic response to environmental enrichment with cognitive recovery. This work was presented at the 2003 National Neurotrauma Society. Collaborators include Xiangbai Chen (PM&R), CE Dixon (Neurosurgery), A Kline (PM&R), and R Zafonte (PM&R).

C. **DA Kinetics and TBI**

Altered DA neurotransmission is hypothesized to play a role in neurobehavioral deficits after traumatic brain injury. DA enhancing agents (DA agonists) have been shown clinically to improve aspects of mental functioning after traumatic brain injury, and have been shown in multiple animal studies to improve behavioral performance. This laboratory has demonstrated reductions in striatal dopamine transporter (DAT) protein and increases in tyrosine hydroxylase (TH) chronically after TBI. These proteins play a critical role in DA release and reuptake. However, the effects of DAT reduction and TH increases on DA neurotransmission is unknown. Fast scan cyclic voltammetry (FSCV) permits real time in vivo evaluation of DAergic kinetics. The goal of this project was to assess striatal DA neurotransmission by evaluating presynaptic striatal DA kinetics in conjunction with neuropeptide and neurobehavioral correlates after experimental traumatic brain injury. We evaluated electrically evoked DA release and DA clearance kinetics 2 weeks after injury. Striatal dopamine release during bilateral electrical stimulation of the medial forebrain bundle was monitored in anesthetized rats by FSCV in conjunction with Nafion-coated carbon fiber microelectrodes. Prior to FSCV, we also evaluated rotational behavior. After FSCV, we evaluated a variety of striatal DA markers, including DAT, TH, Dopamine type 2 receptors (DRD2), and Vesicular Monoamine Transporter (VMAT). Striatal evoked overflow of DA was lower in injured animals, compared to naïve. We also showed significant differences in zero and first order DA clearance for injured animals as well as an increase in DAT efficiency (function) after TBI. Decreases in DAT expression were noted post-injury, despite no changes in VMAT expression, indicating a regulatory change in DAT concentration. Behavioral data suggested a low incidence of rotational behavior in this injury model and correlated well with bilateral changes in presynaptic kinetics and DA marker expression. Increases in DAT efficiency post-TBI provide one explanation for the potential efficacy of DAT inhibitors (DA agonists) with improving cognitive recovery. A manuscript for this work has recently been submitted. In future work, we will investigate regional and post-injury time course differences in DA kinetics as well as response to acute and chronic pharmacotherapies. This work is being conducted in conjunction with Dr. A Michael in the Dept. of Chemistry, whose research focuses on electrochemical techniques and the measurement of neurotransmitters using microsensor technology. Other collaborators and students include CE Dixon (Neurosurgery), R Zafonte (PM&R), Joshua Sokoloski, (PM&R/Chemistry) and Zachary Repanshek (PM&R/Chemistry). This and other pilot work (see genetics section) were used to submit an NIH R01 application.
evaluating the role of DAT genotype in striatal neurotransmission and responsiveness to treatment with methylphenidate in a clinical population with TBI

D. The Impact of Gender & Hormonal Status after Experimental TBI

Some studies have shown that sex hormones have neuroprotective qualities in the setting of acute traumatic brain injury. However, less is known about endogenously circulating sex hormones or particular hormone levels at the time of injury effect behavioral recovery. Recently, we reported that females appear to have a neuroprotective advantage with behavioral recovery on motor tasks performed early after injury. However, no gender differences were noted with spatial learning later after injury. A manuscript on this work was recently published in *Brain Research*. Currently, we are beginning to evaluate the role of physiological hormone replacement in female rats on behavioral recovery after TBI. Additional work will focus on how hormone manipulations affect histochemical markers of injury. Students and Collaborators include Xiangbai Chen (PM&R), Michael Wenger (PM&R/Neuroscience), Lauren Willard (PM&R/Neuroscience), CE Dixon (Neurosurgery), A Kline (PM&R) and R Zafonte (PM&R).

E. Associations between DA Transporter Genotype, Outcome, & Cerebrospinal Fluid Dopamine Levels after Severe TBI: A Follow-up Analysis

DA pathways have been implicated in cognitive deficits after TBI. While not associated with alterations in protein structure, the DAT genotype is associated with differences in DAT protein density and development of DA mediated pathophysiological conditions. For instance, the DAT 10/10 genotype is associated with higher DAT protein levels and is implicated in the development of attention deficit disorder. Differential DAT expression presumably also affects both pre-synaptic DA release, via reverse transport, and DA reuptake. DAT regulation may have a role in DA mediated neurotoxicity acutely after TBI and play a compensatory role with DA neurotransmission chronically after TBI. Catecholamines, including DA and its metabolites, are subject to auto-oxidation, resulting in the formation of reactive oxygen species that can contribute to oxidative stress associated with secondary injury. Prior work from this laboratory has shown reductions in DAT protein after experimental traumatic brain injury. The role of DAT genotype on injury and outcome has not been studied. We hypothesized that genetic & gender related differences in DAT density would affect CSF DA production & metabolism post-TBI, through reverse transport of DA via DAT. We genotyped & collected CSF for DA & metabolite (DOPAC & HVA) analysis via HPLC for 73 patients with acute severe TBI. Mixed effects multivariate regression analyses showed a significant impact of DAT genotype & a trend for female gender to increase CSF DA levels. Gender impacted CSF DOPAC & HVA production without affecting DA turnover, while DAT genotype impacted DA turnover. Further, preliminary analyses suggest acute CSF DA levels are linked to functional recovery curves. Data from this project was used as pilot data to submit an NIH R01 application evaluating the role of DAT genotype in striatal neurotransmission and responsiveness to treatment with methylphenidate in a clinical population with TBI. This work is being done in collaboration with the University of Pittsburgh BTRC, Dianxu Ren (Public Health), CE
Dixon (Neurosurgery), Yvette Conley (Health Promotion and Development), Robert Ferrell (Human Genetics), Sue Beers (Psychiatry), R Zafonte (PM&R), and Mary Kerr (Nursing).

Support: NIH K08HD40833, AK Wagner, MD PI, Dopamine Function and the Effects of Therapeutic Intervention $622,258 beginning 2001 for 5 years (Sponsors: CE Dixon, PhD, AC Michael PhD, and RD Zafonte, DO); NIH R03HD41399, AK Wagner PI Gender Differences in Dopamine Function after TBI $145,535 beginning 2002; CDC R49/CCR323155-01-1---CIRCL, AK Wagner, MD Project PI (H Weiss PhD PI Center Grant), Evaluating the Impact of Neuroendocrine Hormones on Pathophysiology and Outcomes after Traumatic Brain Injury $772,948; CDC CCR310285-07---CIRCL, Small Grants Program AK Wagner, MD Project PI (H Weiss PhD PI Center Grant) $10,000 beginning 2002 for Characterization of Alterations in the Female Rat Estrous Cycle after Experimental TBI; NIH P50NS30318 Clinical Core--University of Pittsburgh BTRC, CE Dixon PI; NIH Loan Repayment Program; Department PM&R, University of Pittsburgh.

TBI Investigation by Safar Center Scientists and Visiting Scientists

7. Studies by P. David Adelson, MD

A. Severe TBI in Immature Rats

Dr. Adelson’s laboratory has been examining the role of excitotoxicity and anti-excitotoxic therapies in experimental TBI in developing rats. He has studied both PND 7 and PND 17 rats and demonstrated important age-related differences in this pathway. He is also studying the impact of therapeutic hypothermia on outcome as it relates to age at injury in these same developmental paradigms. Dr. Adelson and his investigative team presented work in both of these areas at the annual meeting of the National Neurotrauma Society. He has focused his recent efforts on the controlled cortical impact model of TBI.

Support: NIH Grant No. 1 R01 NS42298, Efficacy of Hypothermia in Pediatric TBI

B. Hypothermia for Severe TBI in Children

The major goal of this project is to test the safety and efficacy of therapeutic hypothermia in children after severe head injury. This program has been funded at an R01 level by the NIH/NINDS and seeks to investigate hypothermia as a treatment of TBI in children, with a special emphasis on the development of novel methods for the initial and outcome assessment. Dr. Adelson is the principal investigator of this important multi-center study that includes 8 centers. Dr. Harvey Levin, at the Baylor College of Medicine, is a co-investigator on that study, along with Drs Sue Beers and Tom Campbell, at the University of Pittsburgh, that is assessing long-term functional outcomes including language and speech acquisition, long-term effects of mild to moderate head injury, and a number of other collaborative and related efforts. Studies of the effect of therapeutic hypothermia on a variety of biochemical and molecular mediators of secondary injury
and repair are ongoing from CSF samples obtained from patients enrolled at the Children’s Hospital of Pittsburgh. PICU fellows H Bayırı and P Shore carried out those studies as previously described in Dr. Kochanek’s report.

Support: NIH Grant No. 1 R21 NS043293, Hypothermia for Severe TBI in Children (planning grant) and NIH Grant NO. 1 R01 NS38448, Hypothermia for Severe TBI in Children.

Relevant to the area of child abuse, Dr. Adelson was the co-editor of an issue of Neurosurgical Clinics of North America that was devoted to child abuse and several Safar Center faculty and fellows were authors on review papers in that important issue that addressed a very underserved subgroup of pediatric TBI—inflicted childhood neurotrauma.

8. Studies by Rachel Berger, MD, MPH

Rachel Berger, MD, MPH. Assistant Professor of Pediatric, University of Pittsburgh School of Medicine and Children’s Hospital of Pittsburgh

A. Use of Serum Biomarkers in the Detection of Silent Inflicted Childhood Neurotrauma

Infants who are victims of inflicted traumatic brain injury are often injured on multiple occasions or brought to care many hours to days after their injury. In addition, their injury is often not recognized since caretakers rarely provide a history of trauma and the infants often do not have any external signs of trauma. In the past year, Dr. Rachel Berger, a general pediatrician working in the area of child abuse at Children’s Hospital of Pittsburgh, has broadened the potential relevance of this project by studying the potential use of serum markers of brain injury with the hope of detecting otherwise unidentified brain injury in possible victims of child abuse. Rachel first showed that CSF levels of markers of neuronal (neuron specific enolase [NSE]) and glial (S-100B) death were massively increased versus control after severe TBI in infants and children—including child abuse victims. That work was published this year in the journal Pediatrics. That paper was selected as the most important paper in the field of child abuse at the Annual San Diego Conference on Child and Family Maltreatment. Dr. Berger also published an important report in the Journal of Neurotrauma showing that these markers of brain injury are increased in the serum in over one-third of infants and children with mild TBI—children that are often sent home from the emergency department. This study has set the stage for an assessment of the use of these biomarkers in a target population of infants in diagnostic categories that occasionally represent missed cases of inflicted traumatic brain injury - such as vomiting without diarrhea, a seizure without fever, unexplained bruising, etc. A positive serum test for such biomarkers would not confirm trauma as etiology of the increase, rather it would “point to the head” and suggest to the health care provider, the need to either obtain additional history, perform a careful fundoscopic examination, or perform a cranial imaging study. That important prospective study is the centerpiece of Dr. Berger’s recently funded K-23 award—from NICHD, and
the project of Drs. Berger and Kochanek that was funded in the renewal of the CDC-University of Pittsburgh CIRCL.

Support: 1K23HD43843-01 “Using Biochemical Markers to Detect Abusive Head Trauma,” General Clinical Research Center (GCRC) M01RR00084 “Using Biochemical Markers to Detect Silent Brain Injury” and “Can We Detect Brain Injury by Looking in the Blood?” Children’s Hospital of Pittsburgh of the UPMC Health System–Faculty Start-up Grant – “The Use of Biochemical Markers to Assess Accidental and Abusive Head Trauma in Infants and Young Children.”

Collaborators: P Kochanek, Critical Care Medicine; P David Adelson, Neurosurgery; Mary Clyde Pierce, Emergency Medicine, John Leventhal, Department of Pediatrics, Yale University.

9. **Studies by Steven DeKosky, MD**

Steven T. DeKosky, MD. Professor and Chairman of the Department of Neurology and Director of the Alzheimer’s Disease Research Center, University of Pittsburgh School of Medicine.

**A. Antioxidant and Neurotrophic Response after TBI**

Dr. DeKosky’s laboratory studies the role of neural cells and their products in the brain’s attempt at repair following TBI. The laboratory is particularly interested in the cytokine and antioxidant cascades that occur over the course of days to weeks after injury (secondary injury processes), and their relationship with the upregulation of neuroprotective proteins such as neurotrophins. The goal is to elucidate the brain’s injury response and provide insight into possible therapeutic interventions that could be used in clinical settings to treat human TBI patients.

Dr. DeKosky’s group has examined the timecourse of changes in antioxidant activities (catalase, glutathione and superoxide dismutase) and neurotrophins (such as NGF) expression after experimental TBI. Close temporal relationships were observed between the upregulation of NGF protein and complex changes in antioxidant enzyme activities. To further investigate the relationship between NGF and the antioxidant enzyme response, Dr. DeKosky’s group examined the effect of hypothermia on the post-injury level of NGF and on antioxidant enzyme activity, and demonstrated that in rats subjected to post-traumatic hypothermia, both NGF protein levels and catalase and glutathione peroxidase activity levels are suppressed. In an attempt to restore post-injury antioxidant enzyme activities in hypothermia-treated animals, NGF protein was infused immediately after injury, and during the course of hypothermia treatment. The study showed that NGF infusion was ineffective in restoring enzymes activities to post-injury levels. These results suggest that the low percentage of improved outcome in hypothermia-treated TBI patients may be due to blunted antioxidant response, and that infusion of exogenous NGF may not be sufficient to restore normal antioxidant enzyme activity after injury. The
results of these studies have been accepted for publication in the *Journal of Neurotrauma* and the *Journal of Neurochemistry*.

**B. Effects of TBI on Amyloid Precursor Protein (APP) Metabolism**

Alzheimer’s disease (AD) is a progressive neurodegenerative disorder characterized by neuronal loss in discrete brain regions and by formation of neurofibrillary tangles and beta-amyloid associated neuritic plaques. A major component of these plaques is the 42-43 amino acid amyloid beta (Aβ) peptide that is cleaved from the transmembrane region of amyloid precursor protein (APP).

One of the known risk factors for AD is TBI. Therefore, alterations in APP processing may play an important role in the pathogenesis of both TBI and AD. To better understand the relationship between TBI and AD, Dr. DeKosky's laboratory is conducting experiments using both mouse models and surgically excised tissue and CSF samples from patients with severe head injury. Collectively, these studies center on the cytokine-related molecular cascades involved in pathological alterations in APP and Aβ production and metabolism after TBI, and the effect of therapies designed to interrupt these cascades.

*The humanized Aβ mouse model of TBI*

In collaboration with Cephalon Inc., Dr. DeKosky’s lab has developed a colony of mice that produce detectable levels of human Aβ (the “hAβ mouse”). This mouse represents a significant advance of previous transgenic mouse models of AD in that the APP gene is under its endogenous promoter, and APP itself is produced at normal levels. This mouse is therefore particularly important for the studies of Aβ changes after TBI because 1) unlike in rats or wildtype mice that produce rodent Aβ, we are able to employ well characterized biochemical assays to detect human Aβ and 2) the continuous over-expression of APP as seen in transgenic mice is avoided, which is particularly important in our injury and intervention paradigms. Dr. DeKosky's laboratory is currently examining post-injury changes in APP and Aβ proteins, as well as components of a molecular cascade involving interleukin-1β, nuclear factor κB, and caspase-3 that are involved in post-injury upregulation and amyloidogenic processing of APP. Ultimately, the goals are twofold; 1) to define the pathways causing, and pathological effects of, Aβ overproduction after TBI, and 2) to assess the effect of therapies designed to prevent Aβ overproduction after TBI in mouse models, that could potentially be translated into therapeutic strategies to treat TBI patients.

*Studies in TBI patients*

To better understand the relationship between TBI and AD, Dr. DeKosky's laboratory is examining the distribution and levels of APP and Aβ protein in surgically resected temporal cortical tissue and serial CSF samples obtained from head-injured patients. This study is the first to demonstrate AD-like Aβ plaques in freshly resected brain tissue after severe TBI. Furthermore, within hours after TBI, human temporal cortex reacts to injury with a robust up-regulation of APP in pyramidal neurons, which are likely the main
source of Aβ. This process is paralleled by increased neuronal accumulation of amyloidogenic APP fragments, as well as a marked up-regulation of apolipoprotein E in both neurons and glial cells. These observations are of particular importance for our understanding of TBI as a potential risk factor for later development of AD, suggesting a pathological cascade that involves neuronal overproduction of APP and Aβ, and glial upregulation of apoE, the latter of which has been known to facilitate Aβ deposition in AD brains. Of additional importance, the development of acute Aβ pathology after TBI is not paralleled by formation of neurofibrillary tangles (another pathological hallmark of AD), indicating that intracellular neurofibrillary changes and progression to dementia of AD can occur only after extended survival periods (i.e., months to years). This indicates that there is a large window of opportunity for therapeutic interventions after TBI before the onset of cellular pathology that could lead to AD dementia. Collectively, these studies convincingly demonstrate that increases in Aβ after injury result in acute AD-like pathological alterations that could be an important target for therapies that are being developed in our humanized Aβ mouse model. Co-investigators on these studies include Drs. Milos Ikonomovic, C Edward Dixon, Robert Clark and Patrick M Kochanek.

Support: Core C of 2 P50 NS30318-04A21, Project #3 in the University of Pittsburgh Head Injury Research Center (S DeKosky, MD, PI).

10. Studies by Steven Graham, MD, PhD

Steven Graham, MD, PhD. Associate Chief of Staff for Research, Geriatric Research Educational and Research Center, V.A. Pittsburgh Health System Professor and Vice-chairman, Department of Neurology, University of Pittsburgh School of Medicine

A. Bcl-2 Family genes in TBI

Dr. Graham's laboratory studies the molecular and cellular mechanisms of neuronal cell death. In collaboration with the Safar Center, Dr. Graham's laboratory investigates neuronal death in TBI. This work is part of the University of Pittsburgh BTRC funded by NINDS. The recent emphasis of the laboratory has been the genetic mechanisms that regulate neuronal cell death. In particular, the role of genes that regulate programmed cell death, the bcl-2 and the cysteine protease family of genes, is being investigated in trauma.

B. Role of COX-2 in TBI

Cyclooxygenase-2 (COX-2), the inducible isoform of the enzyme catalyzes the formation of prostaglandins. Dr. Graham’s laboratory is investigating the role of COX-2 in both experimental cerebral ischemia and TBI. Expression of COX-2 is induced by neuronal excitation and COX-2 activity produces free radicals, so COX-2 may be an important mechanism whereby excitotoxicity is expressed. As discussed latter in this report, Dr. Graham also serves as a research mentor to Dr. Robert Hickey who is studying the age-related effects of COX-2 in cerebral ischemia and excitotoxicity.
Support: Core C of 2 P50 NS30318-04A21, Project #1 in the University of Pittsburgh BTRC (Steven Graham, MD, PhD, PI). Department of Veterans Affairs/Department of Defense Brain Trauma Initiative-Merit Review (SH Graham, MD, PhD, PI, 10/1/00 - 10/1/03, 20% of VA Time. Department of Veterans Affairs, The Role of Inducible Cyclooxygenase in Delayed Neuronal Death. Current Year Direct Costs: $143,000.) Technician: Marie Rose
Peer-Reviewed Manuscripts: TBI Program


Chapters, Editorials and Invited Papers: TBI Program


**Abstracts: TBI Program**


26. Shore PM, Thomas NJ, Clark RSB, Adelson PD, Wisniewski SR, Janesko KL, Bayır H, Marion DW, Kochanek PM: Continuous vs intermittent cerebrospinal


CARDIOPULMONARY ARREST PROGRAM

Introduction to the Cardiopulmonary arrest program by Patrick M. Kochanek, MD

Last year, I named Dr. Clifton Callaway, Director of the Cardiopulmonary Arrest Program at the Safar Center. Although Dr. Callaway’s bench and clinical research is carried out outside of the Safar Center, he is a disciple of the Safar Center, is conducting state-of-the-art work in this area at the University of Pittsburgh Center for Emergency Medicine, and graciously serves in that role. Dr. Callaway holds a cardiac arrest investigators meeting each month at the Safar Center that includes a broad spectrum of faculty and trainees interested in this area of research. I am optimistic that through Clif’s role as director of the cardiopulmonary arrest program, a substantial body of research in this area will be unified at the University of Pittsburgh School of Medicine and even greater collaboration will develop between the Safar Center and the Center for Emergency Medicine.

A. Clifton Callaway and the Department of Emergency Medicine

Clifton W. Callaway, MD, PhD, Assistant Professor, Department of Emergency Medicine, University of Pittsburgh School of Medicine

We continue to emphasize the fact that resuscitation from cardiac arrest must attend to both heart and brain. About one-third of attempted resuscitations result in restoration of spontaneous circulation. Therefore, improved approaches to cardiac resuscitation are needed. Only about one-quarter of patients with restoration of circulation will regain consciousness. Therefore, therapies to improve neurological recovery are also required. Without attention to both of these organ systems, overall survival from cardiac arrest is unlikely to increase.

Work this year was devoted largely to expanding our understanding of the molecular mechanisms of neurological recovery after cardiac arrest using our rat model, and to expanding our commitment to clinical cardiac arrest research. Additional funding was secured for the molecular studies, and an out-of-hospital investigation was initiated with the City of Pittsburgh Emergency Medical Services. Because induced hypothermia after cardiac arrest appears to be an effective therapy for improving brain recovery, we have advocated its acceptance as therapy rather than as research.

1. Altered Intracellular Signaling in Brain after Resuscitation

As previously described, the activity of two mitogen-activated protein kinases (MAPKs) increase in hippocampus over the 24 hrs period after resuscitation from cardiac arrest: the p42/p44 MAPK (extracellular-signal regulated kinase, ERK) and the Jun-N-terminal kinase (JNK). Induction of mild hypothermia (33°C) between 1 and 23 hrs after reperfusion, further increases activity of ERK relative to normothermic (37°C) controls. The same regimen of hypothermia also decreases histological and behavioral signs of
brain damage, prompting us to speculate that some of the beneficial effects of induced hypothermia are mediated via increased ERK activation.

As planned last year, we established tools for manipulating ERK activation after cardiac arrest. We conducted a dose-response and time course study of the ERK kinase inhibitor U0126. This drug prevents ERK phosphorylation and activation. We have previously found that this drug does not cross the blood-brain barrier and produced no appreciable inhibition of ERK activation when injected intravenously at doses up to 2 mg/kg. Therefore, naïve rats (n=2-3 per group) were anesthetized with halothane, placed in a stereotaxic device and injected over 5 minutes into one lateral ventricle with doses of U0126 ranging from 10 µg to 500 µg dissolved in 50%:50% DMSO:phosphate-buffered saline.

This experiment revealed partial reduction of ERK activation after 10 µg U0126, with robust reductions after 100 µg U0126. There was no inhibition or perhaps activation of ERK after 500 µg U0126, probably reflecting nonspecific effects or the effects of the large volume of injection required for this dose. The reduction of ERK activation was evident in both the left and right hippocampus, indicating good distribution of the drug from the injection site. Based upon this series of experiments, we selected 100 µg of U0126 for further study.

When rats (n=2-3 per group) were sacrificed at 2, 4, 9, 12 and 24 hours after injection of 100 µg U0126, immunoblots of hippocampal tissue revealed near complete inhibition of ERK activity bilaterally for 12 hours. ERK activity had returned to near normal levels by 24 hours. This study confirmed that we could use a single injection of U0126 to block ERK activation in bilateral forebrain structures for at least 12 hours, with partial inhibition lasting almost 24 hours.

Using the information derived in the dose-response and time-course studies described above, we have examined the effect of inhibiting ERK activation on neurological recovery after cardiac arrest. Rats (n=32) were subjected to eight minutes of asphyxia. Thirty minutes after resuscitation, each rat was randomly assigned to receive an intracerebroventricular injection of either U0126 (100 µg/40 µl) or vehicle. Sixty minutes after resuscitation rats were randomly assigned to either normothermia (37ºC x 23 hours) or hypothermia (33ºC x 23 hours).

This study confirmed that induced hypothermia improved neurological recovery after cardiac arrest and resuscitation. Neurological deficit scores were persistently better in the hypothermia group, with all rats achieving a grossly normal status (score=26) by day 4 (Table 2). Most normothermia rats achieved a normal status only after day 7. All rats exhibited a weight loss over the first 5 days after resuscitation, with weight gain resuming thereafter (Table 3). There was no significant difference between the U0126-treated and vehicle-treated rats on these measures. These data suggest that the gross improvement in neurobehavioral recovery afforded by induced hypothermia is not dependent upon forebrain ERK activation.

2. Altered Neurotrophic Factor Signaling in Brain after Resuscitation
We have previously reported that brain-derived neurotrophic factor (BDNF) increases in hippocampus at 24 hours after resuscitation from cardiac arrest. BDNF levels are further increased by the beneficial regimen of induced hypothermia. We did not detect any increase in neurotrophin-3 or in nerve growth factor. These data were published in the *Journal of Cerebral Blood Flow and Metabolism*. The association between induced hypothermia and increased BDNF levels suggests that this neurotrophic factor may participate in the beneficial effects of post-cardiac arrest, induced hypothermia. It is also tempting to speculate that BDNF is the upstream signal that triggers increased ERK activation after cardiac arrest and hypothermia.

To supplement these observations, we have also examined the expression of glial-cell derived neurotrophic factor (GDNF) in brain after resuscitation. Immunoblots were used to measure GDNF in brain tissue from rats resuscitated from 8 minutes of asphyxial cardiac arrest. Sixty minutes after resuscitation rats were randomly assigned to either normothermia (37°C x 23 hours) or hypothermia (33°C x 23 hours). These experiments revealed that GDNF increased in hippocampus and cortex 24 hours after resuscitation. This increase was less evident in the cerebellum. Induced hypothermia appears to accelerate the expression of GDNF in hippocampus and cerebellum. These data suggest that induced hypothermia increases GDNF levels as well as BDNF levels in vulnerable brain regions.

Our immediate plans are to continue assessing the role of ERK and BDNF signaling in the beneficial effects of induced hypothermia. (1) We will develop a pharmacological strategy to block BDNF and GDNF expression in brain using either intracranial injections of neutralizing antibodies or antisense oligonucleotides. The most successful regimen will be used to assess the contribution of these factors on neurobehavioral outcome after resuscitation with or without hypothermia. (2) We will examine the pattern of new gene expression after resuscitation and hypothermia using DNA arrays. The influence of ERK-dependent pathways on the patterns of gene expression will be assessed using U0126 injections. We anticipate that these approaches will better elucidate the temperature-dependent molecular events after global brain ischemia.

3. **Vasopressin in Cardiac Arrest**

We believe that chest compressions, augmented by vasoactive drugs, are one of the most important determinants of restoration of circulation after cardiac arrest. With Dr. Menegazzi, we have noted that vasopressin is superior to epinephrine for increasing coronary perfusion pressure during chest compressions in swine. Also, several case series from Europe suggest that vasopressin is a superior vasoconstrictor for treating human cardiac arrest. Based on this background, we initiated the necessary steps to conduct a clinical trial of vasopressin use by paramedics for resuscitation of cardiac arrest.

Vasopressin is a generic drug with no corporate backing. It is packaged in a manner that does not resemble epinephrine. Furthermore, we are aware that there is a large
multicenter trial in Europe that is examining the use of vasopressin versus epinephrine as the first-line drug for treatment of cardiac arrest. For these reasons, we designed a study to examine the effect of adding vasopressin to standard treatment for cardiac arrest. Subjects in cardiac arrest receive all standard care. If epinephrine is required, these subjects receive also a vial of study drug that is either vasopressin (40 IU) or saline placebo.

This study employs an Exception from the Requirement from Informed Consent for Emergency Research. During the year, we consulted with our own Institutional Review Board, and set in motion the steps to meet the requirements for this waiver. An Investigation New Drug application was submitted to the Food and Drug Administration. The protocol was reviewed by the Local and State Department of Health. We made press releases, and conducted interviews with local media to publicize the trial. A public forum was held to solicit comments from the community. Groups representing the community were also consulted. All approval was obtained, and the first subjects were enrolled in May 2003.

We hope that this prospective, blinded trial of a drug therapy administered by paramedics will pave the way for more clinical research to improve resuscitation. The clinical data acquired during this study will provide an invaluable baseline against which to gauge future progress.

Support: Brain Ischemia and MAP Kinase Activation, (#K02 NS02112) National Institute of Neurological Disorders and Stroke (07/99 – 06/04) total award $573,480 ($101,854 direct costs + $8,148 indirect costs per year) Clifton W. Callaway, MD, PhD, PI. Hypothermia and Gene Expression after Cardiac Arrest, (#R01 NS046073) National Institute of Neurological Disorders and Stroke (07/99 – 06/04) total award $848,032 ($166,250 direct costs + $80,631 indirect costs per year) Clifton W. Callaway, MD, PhD, PI. Vasopressin in Cardiac Arrest, Pittsburgh Emergency Medicine Foundation, total award $1525, Clifton W. Callaway, MD, PhD, PI.

B. Pediatric Cardiopulmonary Resuscitation

There is an expanding pediatric cardiac arrest program at the Safar Center that now has both bench and clinical components. Dr. Robert Clark (see prior report in TBI), Associate Professor in the Department of Critical Care Medicine, Associate Director of the Safar Center and Pediatric Critical Care Medicine specialist at Children’s Hospital, has received funding from Children’s Hospital of Pittsburgh to initiate laboratory studies in a new model of asphyxial cardiopulmonary arrest in rats. This research is off to a spectacular start. In addition, Dr. Robert Hickey in the Department of Pediatrics, Division of Emergency Medicine at Children’s Hospital of Pittsburgh has ongoing mechanistic studies in the area of developmental brain injury and has played a key role in the national guidelines committees in resuscitation. Finally, Dr. Howard Ferimer of the Mercy Hospital Department of Pediatrics is carrying out studies in asphyxial cardiopulmonary arrest.
1. Laboratory Research in Pediatric Resuscitation

A. Asphyxial Cardiopulmonary Arrest in the Developing Rat (Robert Clark, MD)

In 1995, Drs. Larry Katz and Peter Safar published a seminal paper in the *Journal of Cerebral Blood Flow and Metabolism* describing a clinically relevant model of asphyxial cardiopulmonary arrest in adult rats. Based on that work, and with special talents of senior laboratory technician, Henry Alexander, Dr. Robert Clark developed an important pediatric analog of that asphyxial cardiopulmonary arrest model using post-natal-day (PND) 17 rats. This is an important development in that the PND 17 rat models a toddler or young child—the population most commonly afflicted by cardiopulmonary arrests resulting from asphyxiation (i.e., near drowning, trauma, child abuse, choking, SIDS). Although there are models of perinatal ischemia, there are no small animal models mimicking cardiopulmonary arrest. Equally important is the fact that this is a clinically relevant model that includes a global insult to the entire organism and all of the standard clinical components of resuscitation as guided by contemporary pediatric advanced life support (i.e., mechanical ventilation, chest compressions, epinephrine). PICU fellow Dr. Ericka Fink presented the initial report of the successful development of this model at the 2003 meeting of the Society for Pediatric Research. The full manuscript of this work is in press in *Pediatric Critical Care Medicine*. Important therapeutic trials including the use of mild hypothermia and novel mechanism-based pharmacological approaches are underway under the direction of Dr. Clark at the Safar Center. In addition, Dr. Clark is pursuing studies of the effect of gender on histopathological and functional outcome in this model.


B. Developmental aspects of COX-2-mediated brain injury (Robert Hickey, MD)

Dr. Robert Hickey continued work on his KO-8 award from NICHD to study developmental aspects of the role of COX-2 in brain injury. This research is being carried out under the mentorship of Dr. Steven Graham in the department of Neurology at the VA Hospital. Dr. Kochanek is a co-sponsor of the grant. COX-2 plays an important role in secondary injury in models of stroke, trauma, and cardiac arrest in adult investigation. Its role in pediatric brain injury remains to be defined. Dr. Hickey presented a paper at the 2003 meeting of the National Neurotrauma Society on Differential age at injury effect on COX-2 expression after TBI in immature rats. Studies are planned to evaluate the effects of COX-2 inhibitors in the developing rat subjected to asphyxial cardiopulmonary arrest. Drs Graham and Hickey also co-authored a review article on COX2 published in *Archives of Neurology*. Finally, Dr. Hickey published a paper in the journal *Critical Care Medicine* entitled “Induced hyperthermia exacerbates neurological neuronal histological damage after asphyxial cardiac arrest in rats” which provided further insight into the importance of avoiding fever after cardiopulmonary arrest. This study embellishes upon a prior clinical report by Dr. Hickey and associates.
(Pediatrics, 2000) demonstrating that fever is remarkably common after cardiopulmonary arrest in infants and children.

Support: COX-2 and Injury in the Immature Brain, KO-8 (#HD40848) National Institute of Health, National Institute of Child Health and Development, (7/01-7/06), total award $623,430 ($115,450 direct + $9,236 indirect per year), Robert W. Hickey, MD, PI, Steven Graham, MD, Patrick Kochanek, MD, Co-Investigators; Robert Clark, MD, C. Edward Dixon, PhD, Peter Safar, MD, Consultants. COX-2 and Excitotoxicity in Developing Rat Brain, Competitive Medical Research Fund (CMRF), University of Pittsburgh, (7/1/03-6/30/05), total award $25,000. Robert W. Hickey MD, PI: Steven H Graham MD, PhD, Co-investigator.

C. Role of Adenosine after Asphyxial Cardiac Arrest (Howard Ferimer, MD)

Adenosine is produced by the breakdown of ATP during ischemia. It is neuroprotective via multiple mechanisms; reduced free radical production, associated hypothermia, improved cerebral blood flow, and reduced metabolic demands. Systemic administration of adenosine is limited by its short half-life, inability to cross the blood brain barrier and adverse cardiovascular side effects (hypotension, bradycardia). The beneficial effects of augmenting adenosine levels locally in the brain have been documented in experimental TBI and stroke. Augmenting adenosine levels in the brain after asphyxial cardiac arrest is the focus of our work. In collaboration with Dr. Edwin Jackson, Dr. Ferimer is continuing to study the effects of adenosine modulating drugs on interstitial levels of purines in the brain of rats subjected to asphyxial cardiac arrest using microdialysis and HPLC. This work is also tests pharmacological strategies to increase brain adenosine levels early after resuscitation. Ultimately, our studies may improve therapy in this all-to-common problem in the pediatric population.

2. Public Education and National Guidelines Committee

Dr. Robert Hickey is the current Vice-Chair of the American Heart Association Emergency Cardiovascular Care Committee (ECC) and the immediate past-chair of the American Heart Association subcommittee on Pediatric Resuscitation. The ECC is responsible for overseeing the American Heart Association’s pediatric advanced life support (PALS), advanced cardiovascular life support (ACLS) and basic life support (BLS) courses. The AHA has approximately 250,000 instructors that train over 7 million people annually. In his capacity as Vice-Chair of the ECC, Dr. Hickey also serves as a representative to the international liaison committee on resuscitation (ILCOR) and has recently participated in meetings in Australia, Italy and Brazil to develop international consensus on new developments in resuscitation science. Dr. Hickey was co-author on important guidelines manuscripts that addressed therapeutic hypothermia and automated external defibrillators in children (see publication list below).

3. Pediatric Cardiopulmonary Arrest: Clinical Studies
Dr. Hickey has initiated the assembly of a multidisciplinary team to evaluate children resuscitated from cardiac arrest. The team has representatives from the entire continuum of care including pre-hospital, emergency medicine, critical care, neurology, neuroimaging, behavioral pediatrics, and rehabilitation medicine. The team will, 1) characterize early molecular markers of HI brain injury, 2) evaluate strategies for prognosis of neurologic recovery, 3) identify patterns of functional deficits in long-term survivors, and 4) develop targeted strategies for rehabilitation of patients with HI brain injuries. This information will facilitate comprehensive evaluation and treatment for individuals suffering from HI brain injury and also develop a profile of the natural history of injury and recovery that can be used for evaluation of anticipated neuroprotective therapies.
Peer-Reviewed Manuscripts: Cardiopulmonary Arrest Program


Chapters, Editorials and Invited Papers: Cardiopulmonary Arrest Program


Abstracts: Cardiopulmonary Arrest Program


SHOCK AND SUSPENDED ANIMATION PROGRAM

The hemorrhagic shock (HS) and suspended animation program consists of project I on hemorrhagic shock (HS) in rats and pigs (P.I., Dr. Tisherman; Co-P.I., Dr. Safar); and project II on suspended animation (SA) in dogs (P.I., Dr. Safar; Co-P.I., Dr. Tisherman). The funding since 1997 was made possible through special “plus-up” funds from Congress initiated by former Navy Commander Lyn Yaffe, M.D. During 2001/2003, the HS studies were funded separately by the Office of Naval Research. For the two programs combined, we received total funds (including 50% institutional “indirect costs”) of approximately $1,182,649 during 2002/2003.

Our research ICU for large animals, initiated in the 1970s, is still considered a unique resource for the documentation of novel CPR methods. It must be maintained continuously to be cost-effective, with at least four technicians, two full-time MD research fellows with CCM experience, and about 80 long-term dog experiments per year. Maintaining this ICU program alone requires over $0.5M per year. In 2002/2003, the research fellows were Dr. Nozari (in his second year) and Dr. Wu (in his fourth year); Mr. William Stezoski has continued as lab coordinator. The co-investigators or consultants included Drs. Kochanek, Klain, Jackson, Dixon, Clark, Kagan, Jenkins, and Radovsky (pathologist).

The objective of the HS-SA program has been to help maximize the reversibility of presently lethal traumatic hemorrhage. The HS studies in rats and pigs were designed to extend the golden hour of HS tolerance; HS (low blood flow), with viscera as the most vulnerable organs, is the prevalent cause of death in soldiers “dying of wounds” (DOW). Exsanguination cardiac arrest (CA) (no blood flow), with the brain as the most vulnerable organ, is the prevalent cause of death in soldiers “killed in action” (KIA). SA is a totally new approach for presently unresuscitable conditions. While SA has been considered science fiction, colleagues are now increasingly using this term seriously, as representing rapidly induced preservation of the organism for delayed resuscitation. This idea was initiated in the 1980s by Drs. Bellamy and Safar. For HS and SA we have explored mainly hypothermic strategies – specifically mild hypothermia (33-36°C) for HS and profound hypothermia (5-15°C) for SA. Dr. Tisherman is planning clinical feasibility studies for both in selected trauma centers. Devices needed for such studies are being developed concurrently with additional laboratory studies and preliminary plans for clinical trials.

The HS models in rats and SA models in dogs used in 2002/2003 had been initiated and further developed over the years by our group. They have several unique features, the most important being clinical relevance in terms of insult, resuscitation strategy, ICU management, and outcome.
1. Hemorrhagic Shock (HS) Studies (Tisherman)

The HS studies of academic year 2002/2003 were completed under year 6 of funding by the Office of Naval Research (PI: Samuel A. Tisherman, MD; Co-PI: Peter Safar, MD). Fellow Xianren Wu, MD supervised all studies. The rat studies were completed by technician Jason Stezoski. The pig studies utilized the ICU team led by S. William Stezoski, with technicians Jeremy Henchir, Sherman Culver, Alan Abraham, Jason Stezoski, Scott Kostelnik, and Murugan Subramanian. Fellow Ala Nozari, MD, also assisted with the pig studies.

Hypothermia and Prolonged HS
Previously, we had demonstrated, using models of uncontrolled HS or pressure-controlled HS, that a mean arterial pressure (MAP) of 50 mmHg was insufficient to allow long-term survival after very prolonged (6 h) HS. Even a MAP of 60 did not consistently allow survival. With this background, we explored the potential for mild hypothermia (34°C) to improve survival after prolonged HS. Mild hypothermia, started at 10 min HS or 1 h HS, improved survival time compared to normothermia after 6 h HS at MAP 60 mmHg. Survival to 72 h was achieved in 2 of 14 rats at normothermia, 7 of 14 if cooling began at 1 h, 9 of 14 if cooling began at 10 min. This study was presented as a poster at the 26th Annual Conference of the Shock Society in Phoenix, Arizona 2003.

Solutions and Prolonged HS
The optimal fluid for hypotensive (limited) resuscitation during very prolonged HS has not been explored. Hypertonic saline (7.2%) has potential immunologic and practical (less volume) advantages over isotonic fluids, such as lactated Ringer’s (LR). Hetastarch (6%) may have similar advantages in terms of less volume requirement. We tested these fluids using a model of volume controlled HS (30 ml/kg) followed by limited resuscitation (with the test fluid) for 6 h. Compared to LR, both 6% Hetastarch and 7.2% Saline reduced the volume requirement for hypotensive fluid resuscitation during prolonged HS. Hypertonic saline decreased survival compared to both LR and Hetastarch. Hetastarch may be the superior fluid for trauma victims with long transport times, such as in military and rural trauma. This study was presented as a poster at America Association for the Surgery of Trauma 2003 Annual Meeting in Minneapolis, Minnesota.

Hypothermia and Poikilothermia
In previous animal studies that demonstrated improved outcome from HS with mild hypothermia, anesthesia was utilized to produce poikilothermia and prevent shivering. Clinically, particularly in the prehospital setting, anesthesia may not be available. Pharmacologic induction of poikilothermia may facilitate induction of hypothermia while preventing deleterious side effects such as shivering and catecholamine surge. Suggested by a study by former fellow Larry Katz, MD, we tested a new neurotensin analog (NT-69L), which he had found to induce rapid and sustained mild hypothermia in rats following asphyxial cardiac arrest. Using a model of volume-controlled HS and limited fluid resuscitation with Hetastarch in awake rats, we compared 1) normothermia, 2 spontaneous cooling to 34°C, and 3) spontaneous cooling with NT-69L. Survival was
significantly prolonged in both hypothermia groups compared to normothermia. NT had no further beneficial effect on survival. This suggests that, unlike asphyxial cardiac arrest, amelioration of stress with NT-69L during the induction of hypothermia in HS (i.e., production of a more poikilothermic state than the use of hypothermia alone) did not confer additional benefit. This highlights the fact that one must carefully determine the optimal application conditions for hypothermia (target temperature, duration, re-warming rate, sedation/analgesia, and associated pharmacological agents) in each disorder that it used. This study was presented as a poster in the Society of Critical Care Medicine 33rd Annual Meeting in Orlando, Florida.

Large Animal Outcome with Mild Hypothermia
Previous studies of mild hypothermia during HS have been performed in small animals. Clinically, there is great concern that hypothermia is associated with worse outcomes in trauma patients. Prior to making final plans for clinical studies of mild hypothermia during HS in trauma patients, we felt that a large animal study using a clinically-relevant model of HS plus trauma, with prolonged life support, was needed. Also, we wanted to test the safety and efficacy of ice-cold fluid infusion for induction of hypothermia during HS. Studies have suggested benefit in patients after cardiac arrest. Consequently, we developed a pig HS model with controlled continuous bleeding (75 ml/kg over 3 h) and trauma induced by laparotomy and splenic transection (delayed splenectomy). At HS 40 min (simulating arrival of paramedics) pigs were randomized into 3 groups: Group-1, normothermia (38°C) with warmed saline, Group-2, hypothermia (34°C ) induced with 2°C i.v. saline and surface cooling, and Group-3, hypothermia (34°C) with 24°C i.v. saline and surface cooling. Resuscitation fluids were given when MAP was <30 mmHg until HS 3 h. Use of ice-cold fluid increased blood pressure and lactate. This study was initiated in the spring of 2003, but not completed until the fall.

Dr. Wu presented posters on the effect of temperatures and NT-69L on outcome from very prolonged HS at the Society of Critical Care Medicine meeting and the study on hypothermia during prolonged HS at the Shock Society meeting.

Dr. Tisherman presented “When is it Enough? Endpoints of Resuscitation” at the American College of Surgeons Spring Meeting, and the effects of Hetastarch, hypertonic saline and LR in the prolonged HS model at the American Association for Surgery of Trauma 2003 annual meeting.

2. Suspended Animation (SA) in Dogs (Safar et al)

Addition of tissue trauma to the SA model: Effect of plasma exchange
In previous SA experiments, we have demonstrated that induction of profound cerebral hypothermia ( tympanic membrane temperature 10°C) can allow intact survival after 90 min exsanguination cardiac arrest. The potential benefits of drugs or specialized solutions have been disappointing. The anti-oxidant Tempol and a specially developed fluid (by Michael Taylor, PhD) for organ preservation with hypothermia (Unisol) seem promising. The addition of tissue trauma (thoracic incision, laparotomy, splenectomy) causes extracerebral organ system dysfunction, although brain histopathology is normal after 60 min
SA. In children with multiple organ system dysfunction and thrombotic microangiopathic anemia, use of plasma exchange has resulted in significant clinical improvement. Thus, we hypothesized that plasma exchange might help alleviate some of the extra-cerebral complications seen after trauma and SA. After 120 min SA in dogs, plasma exchange decreased the coagulopathy and improved overall performance, without affecting neurologic deficits and brain histopathology. These studies support the potential use of 2 h of SA even in the setting of exsanguination cardiac arrest that is accompanied by considerable tissue trauma. This is an important study toward the potential clinical application of this agent. Plasma exchange may be needed as a clinical adjunct. However it must be recognized that our studies in dogs have been carried out without the resources of a canine blood bank (i.e., we are limited by lack of therapies such as platelets, cryoprecipitate, and fresh-frozen plasma).

Dr. Nozari presented posters at the Society of Critical Care Medicine meeting on coagulopathy and multiple organ failure after traumatic exsanguination and suspended animation, as well as on the use of mild hypothermia induction during cardiopulmonary resuscitation during 40 min cardiac arrest.

3. Suspended Animation: Proteomic Studies in Rats (Jenkins and Safar)

In the 2002/2003 academic year, Drs. Larry Jenkins and Peter Safar initiated a project linked to the SA program that seeks to probe into the mechanisms of cellular (neuronal) degradation at prolonged global cerebral ischemia during profound hypothermia—at levels of cooling that are successfully used in our SA experiments. An intriguing question is—during prolonged (1-2 h or more) complete global cerebral ischemia (at profound hypothermia, 10°C), what cellular derangements occur. Is degradation during prolonged periods of hypothermia occurring, does it set the stage for damage during reperfusion, or is reperfusion and re-warming the key? Although mechanisms involving lipid degradation, or DNA or RNA damage, may be important, a key initial focus of our work in this area has been on proteolytic damage. Dr. Jenkins has used proteomics to begin to study protein degradation and post-translational modification in TBI (please see the 2001/2002 annual report and the TBI section of this annual report). This year, Dr. Jenkins, and PICU fellow Dr. Mandeep Chadha, used 2-D gel electrophoresis to examine the effect of 30 min of complete global brain ischemia (at either normothermia or profound hypothermia, 10°C) on the proteome of the isolated rat hippocampus. In these initial studies, ischemia was produced by decapitation. Their initial work suggests that 30 min of complete global brain ischemia produced only modest changes in the proteome of the rat hippocampus. Obviously, the limitations of the sensitivity of 2-D gel electrophoresis for low copy proteins and protein fragments must be taken into consideration. Nevertheless, these initial studies are provocative. Longer ischemia durations and the effect of reperfusion will be studied in future experiments in the next funding year. The latter (reperfusion) will require the development of a complete SA model in rats, including miniaturized cardiopulmonary bypass in our center. This will be required to resuscitation rats from prolonged cardiopulmonary arrest at profound hypothermia. Finally, Dr. Chadha presented an abstract of this initial work at the SCCM meeting.
4. Device Development (Safar et al)

During the 2002/2003 academic year, Safar Center investigators (Drs. Safar, Tisherman, and Kochanek, and Mr. William Stezoski) working on the SA project provided consultation to Dr. Lyn Yaffe and his “Smart Catheter” group working on the development of novel catheters for the clinical and experimental implementation of SA. We evaluated several catheter prototypes for aortic insertion. In addition, this same group of Safar Center investigators provided consultation to the Ardiem Medical Company in the development of cooling devices for use in induction of hypothermia both in SA and HS paradigms. We met with the development team of Ardiem Medical at the Safar Center on several occasions to aid them in formulating plans for the initial prototypes for both laboratory and clinical use.

5. Emergency Hypothermia, Clinical planning

At the annual meeting of the American Association for the Surgery of Trauma meeting in Orlando (9/26/02), Dr. Tisherman held a meeting with representatives from several other centers to begin discussions on clinical trials of therapeutic hypothermia in exsanguination cardiopulmonary arrest and hemorrhagic shock. Future meetings of this consortium group of potential investigators are planned.

Peer-reviewed Manuscripts: Shock and Suspended Animation Program


Chapters, Monographs, and Editorials: Shock and Suspended Animation Program


Abstracts: Shock and Suspended Animation Program


5. Wu X, Safar P, Stezoski J, Nozari A, and Tisherman SA. Hypertonic Saline (7.2%) Worsens Survival Compared to Lactated Ringer’s (LR) and 6% Hetastarch after 6h Hemorrhagic Shock (HS) and Hypotensive Fluid Resuscitation in Rats American Association for the Surgery of Trauma Annual Meeting, 2003. http://www.aast.org/03abstracts/03absPoster_090.html


A Fond Farewell

On August 3, 2003, after a 15-month fight against cancer, the Safar Center for Resuscitation Research lost its Founding Director, a collegial colleague, and a loyal friend -- Peter J. Safar, MD. Now that we can no longer walk into his office and ask for his advice, we must accept the challenge to continue his work and preserve his legacy for future generations of researchers and scientists.

Dr. Safar received his MD degree in 1948 from the University of Vienna. He came to the United States permanently in 1954 along with his wife, Eva Kyzivat Safar. In 1961 at age 37, he became the founding chairman of the Department of Anesthesiology and Critical Care Medicine at the University of Pittsburgh, until 1979 when he founded the International Resuscitation Research Center (later renamed Safar Center for Resuscitation Research). Over the course of his career Dr. Safar earned many awards and honors, as well as the trust and respect of his colleagues. You can read about his many accomplishments and view his extensive publication list on the Safar Center website.

Not only was Dr. Safar our leader, mentor, and colleague for many years, but he was also a personal friend to many of us. Though he is greatly missed, the way he lived life to its fullest continues to inspire us to make the world a better place for all.
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