Circadian Rhythm Adaptation in the Intensive Care Unit

By Sandra K. Hanneman, Ph.D., RN, FAAN

Abstract: Acute stress is known to disrupt circadian rhythms, but it is not known if the organism adapts to prolonged acute stress by recovery of circadian rhythms over time. Intensive Care Unit (ICU) patients on a respirator constitute a natural model of prolonged acute stress. Our research in preclinical and clinical ICUs suggests that circadian rhythm adaptation to ICU stress occurs without intervention. Knowing when adaptation occurs in an ICU patient would facilitate timing of medical intervention concordant with the patient’s internal clock timing and testing of time-dependent therapies to accelerate adaptation.

The intensive care unit (ICU) represents the intersection of critical illness; loss of usual daily behavioral cues; and episodic, variable and noxious light, sound and touch intensities. Imagine what you would feel if sedated in the ICU, with tubes coming from your airway, mouth, nose, bladder, rectum, arteries and veins – unable to move, speak, open your eyes at will or control your breathing. Each year, over 4.4 million people in the United States become a patient in an ICU where nurses, physicians and other health care professionals battle the ravages of illness 24 hours a day, 7 days a week to change the biological trajectory from death or disability to as full a recovery as possible. Of the 4.4 million patients in ICUs, an estimated 1.5 million patients have their breathing controlled by a mechanical ventilator (respirator). Although critical to survival, mechanical ventilation is far from innocuous; this therapy is associated with substantial morbidity and mortality, and the pervasive goal is to wean the patient from the ventilator as quickly and safely as possible.

Care of mechanically-ventilated ICU patients focuses on maintaining parameters of vital organ function within normal limits (the homeostatic paradigm). This approach to care, however, ignores emerging paradigms of adaptation to stress based on systemic organization of the individual's biologic processes. The chronobiology paradigm presumes that biological processes have predictable variability as a function of time at every level, from the subcellular to the whole organism. Although each level is presumed to have intrinsic self-regulation, lower levels are influenced by higher level organization, culminating with the master organizing level – the circadian pacemaker, commonly

CONTINUED ON PAGE 4; LEE

Circadian Clocks and Homeostasis

By Cheng Chi Lee, Ph.D.

Abstract: Circadian rhythm is a ubiquitous biological process that governs the behavior of mammals including human beings. Our sleep/wake pattern is a classic feature of circadian rhythm, and disruption of circadian rhythm affects many biological processes. Temporal control of gene expression underlies the physiological and biochemical control of circadian rhythm.

Living organisms have evolved through hundreds of millions of years to adapt to the effects of the light-dark cycle generated by the Earth’s rotation on its axis relative to the sun. A large body of information indicates that the physiology and behavior of living organisms, from bacteria to humans, are controlled by circadian rhythms driven by endogenous oscillators and modulated in response to daily environmental cues. The endogenous clock is characterized by a cycle approximately 24 hours in duration. When organisms are placed under invariant environmental conditions such as constant darkness, this clock is self-sustaining, behaving as a pacemaker. The endogenous clock is further distinguished by its ability to be entrained by environmental cues such as light, temperature, and feeding cycles. Circadian clock control, while a crucial evolutionary adaptation to life on Earth, prevents adaptation of mammals to extreme light-dark cycle regimes, such as the light-dark cycle regime encountered during space travel. Alteration of the circadian rhythm in humans can lead to behavioral and sleep problems, including jet lag and disorders associated with shift work. In addition, circadian rhythms have been linked to pathological effects such as increased frequency of asthma, stroke and heart attacks during the morning period as well as seasonal affective disorders during the winter. Studies in rodents demonstrated that mammals could not be entrained to light-dark regimes that are

CONTINUED ON PAGE 5; HANNEMAN
Neuroscience education for both graduate students and medical students has been given a significant financial boost, both within the University of Texas System as well as from the National Institutes of Health (NIH).

The Neuroscience Program of the Graduate School of Biomedical Sciences (GSBS) was recently reorganized into four neuroscience tracks: the cognitive track; the systems track; the cell and molecular track; and the theoretical and computational track. Each track has its own development plan and requirements, and the purpose of the specialization within the neurosciences is to produce more highly trained neuroscientists for the future.

Following this reorganization, Dr. Harel Shouval of the Department of Neurobiology and Anatomy submitted an application to the University of Texas System Board of Regents for an Initiative on Theoretical and Computational Neuroscience. This included a detailed plan for attracting both undergraduates to neuroscience summer research as well as graduate students to the Theoretical and Computational Track in the Neuroscience Program. Out of 37 applications from the different campuses of the University of Texas, this initiative was one of seven funded for a five-year period. Because it is an interdisciplinary field, unifying mathematical models with observational data, the first challenge is to present neuroscience as a viable field of study for engineers, mathematicians, statisticians, as well as to students in the fields of psychology and biology with strong mathematical backgrounds.

The “seed” aspect of this program is to increase the number of students who are brought in as undergraduates to do theoretical and neuroscience projects over the summer in conjunction with the ongoing National Science Foundation funded REU program of the Gulf Coast Consortia. The Initiative is a plan to expose undergraduates to both theoretical models and experimental research, and the unique exchange between modeling with known variables and testing with unknown variables, in order to generate student interest in continuing their careers at the graduate level in Theoretical and Computational Neuroscience. The first group of undergraduates in this program arrived in late May, 2009 for 10 weeks of research experience.

The Initiative then provides additional support to increase the number of graduate students in the Theoretical and Computational Track in the Neuroscience Program. Students have an almost unique opportunity at UTHSC-Houston because of the partnership with the Gulf Coast Consortium in Theoretical and Computational Neuroscience, which includes Rice University, Baylor College of Medicine, The University of Houston, the UT M.D. Anderson Cancer Center, the UT Medical Branch at Galveston, and the UT Medical School at Houston. In addition, the Department of Neurobiology and Anatomy has a newly formed Center for Theoretical and Computational Neuroscience which will be an additional resource for neuroscience graduate students.

Dr. David Marshak, also of the Department of Neurobiology and Anatomy, submitted an application and received a training grant for medical students. Here, support was requested for short-term research training in neuroscience for first-year medical students at The University of Texas Medical School at Houston (UTMS-H). This training activity will be a new component of the existing UTMS-H Summer Research Program that also includes undergraduate students from universities and colleges throughout the United States and medical students from Latin America and Asia. The need for this kind of training arose from a concern that a dwindling number of neurologists and neurosurgeons are trained as research scientists, and the goal of this training program is to provide training opportunities for prospective physician-scientists as early as possible in their careers.

Also with the physician-scientist in mind, Dr. Ian Butler of the Division of Pediatric and Adolescent Neurology in the Department of Pediatrics is in the process of developing a “Scholarly Concentration in Neuroscience for Medical Students”. This would be built on a number of courses and events already developed by the Neuroscience Research Center, including the Poster Session, Distinguished Lecturer and Topics in Neurobiology Disease course given through the Graduate School. In addition, attendance at the Department of Neurobiology and Anatomy Seminar series, teaching in the Medical Neuroscience Laboratory, and attending relevant Grand Rounds in Pediatric Neurology, Adult Neurology, Psychiatry, and Neurosurgery would be required.

Dr. Gary Rosenfeld, Assistant Dean for Educational Affairs, is in the process of developing a similar program for the Medical School with a required research focus that would also build on Neuroscience Research Center activities. This Neuroscience Track in the Medical School would require significant research efforts from the medical students, particularly in their first and fourth year. This program will dovetail nicely with Dr. Marshak’s training grant for first-year medical students, and it will be a capstone for the development of the physician-scientist in a world where translational research (i.e., bringing the findings of basic science to the bedside) has become a vital element of medical care’s future in the United States.

The Neuroscience Research Center is proud that our established programs and activities can contribute significantly to the education of graduate students and medical students and can contribute to the development of the neuroscientists and physician-neuroscientists of the future.
Grants

Ananth Annapragada, University of Iowa, Effects of Metabolic Disorders on Bone Marrow-Derived Cell Function.

Terri S. Armstrong, MDACC, Gauging Impact of Treatment on Symptoms, Health Related Quality of Life and Neurocognitive Function in Patients with Primary Brain Tumors.

Michael R. Blackburn, National Institutes of Health (NIH), Adenosine Metabolism and Signaling in Patients with COPD and Pulmonary Fibrosis.

Guy L. Clifton, NIH, National Acute Brain Injury Study: Hypothermia II.

Vittorio Cristini, National Science Foundation (NSF), Collaborative Research: Multiscale Modeling of Solid Tumor Growth.

Stephen P. Daiger, NIH, DNA Linkage Studies of Degenerative Retinal Diseases.

Myriam Fornage, NIH, GWAS (genomewide association studies) of Longitudinal Blood Pressure Profiles from Young Adulthood to Middle-Age.

James C. Grotta, NIH, UT Specialized Program in Acute Stroke.

Jianping Jin, Pew Charitable Trusts, Dissection of UBA6-Dependent Ubiquitin Signaling Pathway Using System Biology Approaches.

Susan Landry, Texas Education Agency, Texas Early Education Model (TEEM).

Maureen D. Mayes, NIH, Two-Stage Genomewide Association Study in Systemic Sclerosis (SSC, Scleroderma).

Hope Northrup, Tuberous Sclerosis Alliance, Tuberous Sclerosis Complex Natural History Database Project.

Sean I. Savitz, NIH, Preclinical Development of Bone Marrow Mononuclear Cell Therapy for Ischemic Stroke. ATHERSYS Inc., Multipotent Adult Progenitor Cell Therapy for Acute Stroke.

Eva M. Sevick, NIH, Functional Lymph Angiography with NIR Imaging. NIH, Fluorescence Enhanced Optical Tomography in Small Animals. NIH, Diagnostic Nodal Staging with Nuclear and NIR Molecular Optical Imaging.

Kazim A. Sheikh, NIH, Pathogenesis of Anti-Ganglioside Antibody-Mediated Neuropathies.


Jerry S. Wolinsky, Clayton Foundation for Research, Viral Mimicry and Multiple Sclerosis.

Awards

Dr. Pedro Mancias, associate professor of pediatric neurology, is the winner of the Benjy F. Brooks Teaching Award which is presented by the alumni of the Medical School to recognize individuals “who complement and enhance the education program by serving as role models for students.”

Dr. Mancias is also the recipient of the John P. McGovern Award which is given annually to the outstanding clinical faculty member as chosen by the senior class.

Dr. Francisco Fuentes, professor of internal medicine in the Division of Cardiovascular Medicine, is the recipient of the 2009 Herbert L. and Margaret W. DuPont Master Clinical Teaching Award.

Dr. James “Red” Duke, professor of surgery, is this year’s winner of the Leonard Tow Humanism in Medicine Award.

We welcome new Chairs

Dr. Jair Soares is the new chair of the Department of Psychiatry and Behavioral Sciences as of June 1, 2009. He was a distinguished professor of psychiatry and director of the Center of Excellence in Research and Treatment for Bipolar Disorders at the University of North Carolina at Chapel Hill.

Dr. Robert Feldman, an expert in the treatment of glaucoma and other degenerative eye disorders, has been named chair of the Richard S. Ruiz, M.D. Department of Ophthalmology and Visual Science as of January 2009. He had been acting chair since the end of August 2008.
less than 18 hours, or with a light phase that is less than 4 hours. These biological constraints on the circadian clock are essential for normal cellular and physiological processes.

Studies in the 1970s that tracked the uptake of radioactive deoxyglucose in the brain demonstrated that a structure in the hypothalamus known as the suprachiasmatic nucleus (SCN) is the site of the master clock. SCN ablation and transplantation studies confirmed that the master circadian clock structure in mammals resides primarily in the SCN. Cell culture studies further demonstrated that individual cells of the SCN display an independent circadian rhythm. Later studies revealed that the circadian clock is present in all peripheral tissues and even in individual fibroblast cells in culture, suggesting that clock control is pervasive and is part of normal cellular function. Although individual clocks are found in many cell types, the SCN remains the central pacemaker. Explant culture studies revealed that these peripheral clocks are unable to maintain precise phase with each other, further supporting the role of the SCN as the central pacemaker. However, food restriction studies revealed that the peripheral clock can be uncoupled from the SCN clock in vivo.

The molecular components that constitute these oscillators in mammals were unknown until about 10 years ago. In the early 70’s, Drosophila (fruit flies) that lost circadian rhythm behavior were identified from mutagenic screens. Studies on these mutant fruit flies laid the foundation for the molecular basis of the clock. By the mid 80’s, the first gene encoding a molecular player of the circadian clock, period (Per), was cloned in fruit flies and characterized. In the mid 90’s, a mouse circadian mutant, Clock, was identified through mutagenic screens. The cloning of the Clock gene was carried out in parallel with several independent studies to identify the mammalian homologs of PER. My laboratory was among the first to identify the two mammalian period genes, mPer1 and mPer2. Mouse genetic studies in my laboratory demonstrated that mPer1 and mPer2 are key players that control the mouse circadian clock. Loss of function mutations of the mPer2 gene result in mice with severely shortened transient periods that lead to a complete breakdown of circadian rhythm. The loss of the mPer1 gene only affected the precision of the clock period, but the mutant animals retained circadian rhythmicity. Moreover, the loss of both mPer1 and mPer2 resulted in a complete absence of circadian rhythmic activity when the animals were placed in invariant conditions such as constant darkness. In the absence of circadian clock function, the mPER1 and mPER2 deficient animals can be entrained to extreme light-dark regimes that cannot be undertaken by normal mice (see Figure 1).

The identification of PER1 and PER2 together with CLOCK began a period of many successful investigations by research groups on other components that made up the mouse and fruit fly clock mechanism. Many of the genes found in Drosophila have counterparts in mammals, including humans, and vice versa. These findings suggest that, to a large extent, the clock mechanism is evolutionarily preserved.

In addition, the cryptochromes (CRY1 and CRY2) and BMAL1 were also identified as central regulators in the mammalian clock mechanism. BMAL1 is a transcription factor that forms an active transcriptional heterodimer with CLOCK. This heterodimer is the core transcriptional unit in clock mechanism in both mammals and fruit flies. With the exception of BMAL1, each of the identified mammalian clock components has a redundant homolog. The homolog of CLOCK is NPAS2, which can also form an active transcriptional heterodimer with BMAL1. Interestingly, the binding of promoter DNA by CLOCK/BMAL1 and NPAS2/BMAL1 transcriptional heterodimer complex is modulated by the NAD/NADH redox ratio. A shared feature of BMAL1, CLOCK, NPAS2, PER1 and PER2 is a protein domain known as PAS. At least for PER2 and NPAS2, their respective PAS domains bind to heme, an important co-factor for electron transport. Another circadian regulator Rev-erb, which inhibits Bmal1 transcription, is also a heme binding protein. Our studies show that ALAS1, the rate-limiting enzyme of heme biosynthesis, is under the regulation of the circadian clock. Together, these observations suggest a functional connection between the circadian rhythm and metabolic homeostasis.

Proteins modified after translation are important players in the mammalian clock mechanism. Among these are proteins that regulate the phosphorylation of clock proteins, such as casein kinase-1 epsilon and casein kinase-2. For example, the stability of PER2 is determined by its phosphorylation state. Other post-translational modifications include acetylation and sumoylation. Sumoylation of BMAL1 is induced by CLOCK and is carried out in a temporally regulated fashion. Recently it was demonstrated that BMAL1 and PER2 undergo acetylation and deacetylation during a

Figure 1 shows the circadian rhythm of wild type and circadian deficient mice based on wheel running activity. The animals were initially entrained in 12h:12h light-dark (LD) cycle. This is followed by a release into 12h:12h dark-dark (DD) cycle. Next 4h:4h light-dark (4:4) cycle regime was imposed on these animals. Note the entrainment of circadian deficient mouse to 4h:4h light-dark regime and a complete absence of circadian rhythm in DD cycle.
circular clock. At least for BMAL1, the acetylation is carried out by CLOCK, which has histone acetyl-transferase (HAT) activity. The deacetylations of BMAL1 and PER2 are carried out in a temporal specific manner by SIRT1, a member of the sirtuin family. SIRT1 is the mammalian ortholog of SIR2 of yeast. Increased dosage of SIR2 in yeast and its corresponding homologs in Droso phila and Caenorhabditis elegans have been shown to extend life spans of both organisms. The deacetylation activity mediated by SIRT1 is under circadian control in vivo. SIRT1 is a NAD+ dependent deacetylase. Thus, the level of NAD not only affects the core clock heterodimer binding of DNA, it also regulates SIRT1 activity. The molecular linkage of SIRT1 in the posttranslational control of PER2 and BMAL1 function integrates circadian function with metabolism and the biological process of aging in mammals.

Interestingly, mice without functional mPER1 and mPER2 display morphological differences that distinguish them from their wild type siblings at 1 year of age. These morphological changes include gradual but significant loss in body weight and the obvious development of kyphosis (abnormal curvature of the spine) of various degrees. Kyphosis is normally associated with aging and is a result of degeneration of connective tissue and bone associated with the spine. X-ray analysis revealed that the loss in body weight of PER deficient mice is primarily due to a significant decrease in soft tissue mass (muscle, fat, and connective tissue). The loss of soft tissue mass and the appearance of kyphosis are consistent with similar pathological changes associated with aging in elderly humans. Therefore, the identification of SIRT1's role in the circadian clock mechanism could provide a molecular basis to explain why the loss of PER protein function results in an apparently accelerated aging process. In addition, studies have also implicated the circadian clock in the gating of cell division and in DNA damage response. Although a number of clock regulators have been identified, it remains unclear how they are integrated with regulators of pathways necessary for organismal homeostasis.

In summary, the identification of key regulators of the circadian clock has revealed that fundamental behaviors such as the sleep – wake cycle are controlled by temporal expression of clock-specific genes. Understanding the mechanism of the molecular clock will shed light on different human disorders that display temporal vulnerability features such as heart attack, asthma attack, seasonal depression and many others.

About the author
Cheng Chi Lee, Ph.D. Professor, Department of Biochemistry and Molecular Biology University of Texas Health Science Center at Houston.
Cheng Chi Lee received his Bachelor's degree in 1982 and his Ph.D. in 1986 both in Biochemistry from the University of Otago, New Zealand. Following a postdoctoral fellowship in the laboratory of Dr. C. Thomas Caskey at Baylor, Dr. Lee joined the faculty in the Institute for Molecular Genetics Baylor College of Medicine in 1994. Dr. Lee moved to the Medical School at University of Texas Health Science Center at Houston in 2003. Dr. Lee's research has made an impact in multiple areas of life science research from disease gene discoveries and circadian biology to hypothermia biology of mammals. Dr. Lee has received multiple grants from the NIH, a private foundation and another US government agency. Dr. Lee became a recipient of the prestigious NIH Director's Pioneer Award in 2006. Dr. Lee currently holds four issued patents from US Patent and Trademark Office. Early in his career, Dr. Lee was involved in developing novel technology for gene cloning. His work also led to the identification of the Spinocerebellar Ataxia Type 6 gene. During the past decade, Dr. Lee's laboratory has carried out innovative research on the identification and characterization of two genes, mPer1 and mPer2, that regulate circadian rhythm in mammals. In addition, his studies have shown the important role of circadian rhythm in metabolism and DNA damage repair.
What is known about circadian rhythms has largely been learned from controlled laboratory experiments; however, disruptions in circadian rhythms of core body temperature, cortisol and melatonin have been reported from observational studies of ICU patients. Our research on circadian rhythm adaptation in the ICU uses preclinical and clinical parallel models: the preclinical model uses mechanically ventilated swine in an experimental porcine adult ICU, and the clinical model consists of mechanically ventilated patients in medical-surgical ICUs.

We developed a preclinical critical care model that closely mimics the clinical ICU. We instrument 70-kg domestic farm pigs — as is done with patients — with indwelling oral endotracheal tube, arterial, central venous and bladder catheters. The animals are placed in clinical ICU beds, on mechanical ventilation, sedated with continuous intravenous infusion of barbiturates, and cared for 24/7 by advanced practice ICU nurses using clinical ICU protocols. Multiple physiologic and biochemical parameters are continuously monitored and frequently sampled during the ICU stay. The subjects develop complications commonly seen in the clinical ICU, and, to date, our unplanned mortality rate (25%) is equivalent to that in clinical ICUs (26%).

Despite complications of hemorrhage, pneumonia and acute respiratory distress syndrome, we have attained ICU lengths of stay of up to 7 days. Preliminary data show that the subjects recover circadian rhythm in circulating cortisol concentration between days 4 and 7 of the ICU stay. The recovery of cortisol circadian rhythm suggests circadian adaptation to the ICU, even though the porcine ICU stress paradigm exceeds in intensity and duration other stress paradigms applied to a porcine model.

Data collection is in progress for parallel observations in the clinical ICU. Preliminary data suggest that mechanically ventilated patients also recover biomarker circadian rhythm within 7 days of the ICU stay. To our knowledge, we are the first to show recovery of circadian rhythms longitudinally in animal and human ICU models. Our within-subject approach, sampling density and data analysis methods facilitate understanding the natural trajectory of pacemaker adaptation to prolonged acute stress.

An individual’s rhythms are influenced by many endogenous and exogenous conditions, which complicate the assessment of circadian rhythm. For example, the circadian temperature rhythm varies across the menstrual cycle; thus, the menstrual cycle rhythm is layered on top of the circadian cycle during measurement of body temperature in women. Because a myriad of personal contextual conditions influence circadian rhythms, intra-individual rhythms are more stable than inter-individual rhythms. Group-determined rhythms will not necessarily reflect the health status of a given individual in the group, and may obscure the interaction of illness with internal and external environmental conditions. For this reason, our research largely focuses on within-subject changes to determine state of the individual’s well-being.

In both our preclinical and clinical models, we use a longitudinal time-series design. We collect core body temperature data every minute and blood for cortisol and melatonin assay every 2 hours around the clock for up to 7 days to evaluate circadian variation and stability of the rhythm from day to day. Frequent sampling allows smoothing of the continuous temperature signal to improve resolution of the data graphically and to reduce unwanted noise in the data. The sampling frequency for cortisol and melatonin represent a balance between capture of changes in concentration and welfare concerns from excessive blood withdrawal. Cosinor models (i.e., models that fit one or more cosine curves of a given time period to the data) are used to analyze rhythmic data; however, application of cosinor models to long time-series data suffers from noise and drifts in rhythm parameters from cycle to cycle. Thus, we apply cosinor analysis to serial sections of the time series to improve model fit and track changes in the rhythm parameters.

The within-subject approach, frequent sampling over consecutive days and serial-section cosinor analysis explain a large fraction of variability and permit examination of change in circadian rhythm parameters over time. While continued research is in progress, we have preliminary proof-of-principle that organisms habituate to prolonged stress in the ICU over time as demonstrated by recovery of circadian rhythms in reliable biomarkers of circadian pacemaker function.
The first NRC Outstanding Medical Student in Neuroscience Award was presented to Justin Jordan, a fourth year medical student who did research on brain tumors and immunology.

Mayor Bill White proclaimed March 2009 as “Brain Awareness Month” in Houston, Texas. An editorial on neuroscience written by our director also appeared that month in the Houston Chronicle.

The Huffington Lecture was given on April 21, 2009 by Allen Roses of Duke University. Pictured left to right are Dr. John Byrne, Dr. Frank Yatsu, Dr. Roses and Ms. Midge Yatsu.
Questions? Comments? Contact us at 713-500-5540 or E-mail: nba-nrc@uth.tmc.edu