PROCEEDINGS OF THE
3RD HEART-BRAIN SUMMIT
Hosted by the Earl and Doris Bakken Heart-Brain Institute at Cleveland Clinic
JUNE 4–5, 2008

SUPPLEMENT EDITOR:
MARC S. PENN, MD, PhD
CLEVELAND CLINIC

SUPPLEMENT TO CLEVELAND CLINIC JOURNAL OF MEDICINE
SUPPLEMENT 2, VOLUME 76
APRIL 2009
The 3rd Heart-Brain Summit, on which this supplement is based, was supported in part by educational grants from Boston Scientific and Medtronic (silver-level grants) and from Cordis, a Johnson & Johnson Company, and St. Jude Medical (bronze-level grants).
Heart-Brain Medicine Honorees

The Bakken Award and Lecture is named in honor of Dr. Earl Bakken, developer of the first transistorized, battery-operated wearable cardiac pacemaker, founder of Medtronic, Inc., and inspiration behind the evolution of the field of heart-brain medicine.

The Bakken Award recognizes an individual who has conducted innovative research that has a significant impact on the field of heart-brain medicine.

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**Proceedings of the 3rd Heart-Brain Summit**

June 4–5, 2008

Hosted by the Earl and Doris Bakken Heart-Brain Institute at Cleveland Clinic

Supplement 2 to Volume 76, April 2009

www.ccjm.org/content/76/Suppl_2

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* These proceedings represent the large majority of presentations at the 3rd Heart-Brain Summit, but several presentations were not able to be captured for publication here.

Topics and editors for supplements to the Cleveland Clinic Journal of Medicine are determined by the Journal’s editor-in-chief and staff. Supplement editors are chosen for their expertise in the topics discussed and are responsible for the scientific quality of supplements, including the review process. The Journal ensures that supplement editors and authors fully disclose any relationships with industry, including the supplement underwriter. For full guidelines on grant-supported supplements to the Journal, go to www.ccjm.org/site/misc/Supplement_Guidelines.pdf.
I started the study of heart-brain medicine with a symposium in Miami on June 19–20, 1978, entitled “Cerebral Manifestations of Episodic Cardiac Dysrhythmias.” One of the participants, Dr. Shlomo Stern, said, “I believe that this meeting was the first of its kind, focusing as it did on the brain/heart relationship. I do not know of a previous meeting that has been directly devoted to the subject, and I consider this as important as the discussions on the areas that we do not know much about.”

It was a very successful meeting, but little happened after we all went home. Since that time, I have felt strongly that we need to study the whole body—not just the individual “organs”—when someone has a disease. Doctors who look at the whole body (such as internists and general practitioners) do better, most of the time, at being correct in their diagnoses, whereas those who specialize in a single organ tend to be less accurate. This approach of looking at the whole body helps us all with the use of “integrated” medicine, which is more complete at “healing” at much lower cost. Healing is 20% science and 80% alternative (complementary and alternative medicine).

I am pleased with what is happening at the Heart-Brain Summits, and pleased with the great research that is ongoing at Cleveland Clinic and around the world in the area of heart-brain medicine. But I am even more pleased that patients everywhere are receiving better care because better diagnosis is taking place when the whole person is considered—body, brain, heart, spirit, and, very importantly, the mind. (See my “10 points” from the proceedings of the first Heart-Brain Summit, held in 2006.1)

The mind is separate from the brain and yet can have major effects on our health. Professional journals on the mind are becoming more pervasive. More and more, I am encountering doctors, educators, and researchers who want to discuss the mind—how the mind takes over when the brain shuts off. The mind is in us, and much is around us, and quite possibly there is some part of it connected to the cosmos.

It is interesting, and hard to understand, how the mind reacts to a placebo and causes the same change in the brain as a chemical or medication does. We have much to learn about the internal workings of the body. Many of these ideas relating to these complex connections will be covered at the 4th Annual Heart-Brain Summit, to be held in October 2009.

Although I wasn’t able to attend the 2008 Summit, I stay updated and involved with the Bakken Heart-Brain Institute and the world of knowledge that continues to emerge on the significance and importance of these multiple connections—30 years after the first meeting of this kind.

REFERENCES
**INTRODUCTION**

Investigators involved in heart-brain medicine are dedicated to defining the physiology associated with interactions of the neurological and cardiovascular systems. In 2004 the Bakken Heart-Brain Institute was founded at Cleveland Clinic because we believed that furthering our understanding of this physiology could lead to a better understanding of chronic disease, define novel therapies, and improve patient outcomes.

The 2008 Bakken Heart-Brain Summit, held last June in Cleveland, further demonstrated real progress in our understanding of the importance of heart-brain interactions in health and disease. The opening session of the 2008 Summit focused on reviewing the evidence linking psychiatric disorders—specifically depression—to increased inflammation and plaque rupture associated with worsening outcomes in patients with atherosclerotic heart disease. These pathways linking psychiatric disorders to acute coronary syndrome were proposed after the 2007 Bakken Heart-Brain Summit:

- Depression leads to decreased vagal tone
- Decreased vagal tone leads to increased inflammation
- Increased inflammation leads to acute coronary syndrome.

Speakers at the 2008 Summit offered insights into the physiology, clinical measures, and molecular pathways involved in linking the heart and the brain, including:

- Measures of heart rate variability in depression
- The utility of heart rate variability and heart rate recovery in quantifying vagal tone and outcome in patients with and without coronary artery disease
- Pathways of inflammation involved in acute coronary syndrome.

**MOUNTING CLINICAL EVIDENCE LINKING DEPRESSION WITH CARDIAC OUTCOMES**

The 2007 and 2008 Summits highlighted the link between depression and outcomes in patients with atherosclerosis (2007) and the potential associated mechanisms (2008). Just as exciting are the developments since last June: numerous papers have been published demonstrating this link in clinical populations, and depression screening has been included in recommendations from the American Heart Association on the treatment of patients with coronary artery disease—recommendations that are endorsed by the American Psychiatric Association.

The studies published since June 2008 demonstrate clear links between depression and morbidity and mortality from cardiovascular causes. A recent paper from the Nurses’ Health Study showed that individuals with depression had a higher incidence of cardiovascular death. Notably, subjects in the Nurses’ Health Study had no clinical evidence of atherosclerotic heart disease at enrollment. In another recent study, depression was associated with worse outcomes in patients following coronary stenting. Finally, and most interestingly, depression was recently associated with endothelial dysfunction in patients with atypical angina and angiographically normal coronary arteries. Thus, regardless of the degree of underlying atherosclerosis, depression is associated with cardiovascular morbidity or mortality.

Less clear is the relationship between depression and inflammation as measured by surrogate inflammatory markers. An analysis of the Canadian Nova Scotia Health Survey [NSHS95] Prospective Population Study suggested that increased inflammatory markers accounted for only a small portion of the risk of coronary heart disease associated with depression. Conversely, a recent analysis of patients with stable coronary artery disease demonstrated a strong correlation between major depressive disorders and high-sensitivity C-reactive protein.

Clearly, significant work has yet to be done to fully elucidate the molecular pathways that link depression and adverse outcomes in patients at risk for coronary artery disease. That said, it is very encouraging that professional societies are beginning to recognize the value and importance of heart-brain medicine in identifying novel strategies for improving patient outcomes.
INTRODUCTION

STILL ELUSIVE: EVIDENCE THAT DEPRESSION THERAPY IMPROVES CARDIAC OUTCOMES

At the 2008 Summit there was clear enthusiasm among attendees and faculty for advances in our understanding of the pathways discussed above. Since then, as reviewed above, significant publications have furthered the link between heart and brain in the setting of atherosclerotic heart disease. That said, the missing piece—the demonstration that treating depression leads to improved outcomes in patients with coronary artery disease—remains missing.

Some advances in this regard have been made. A recent study from the Enhancing Recovery In Coronary Heart Disease (ENRICHD) clinical trial demonstrated that major depression in any patient who survived myocardial infarction decreased survival over 2.5 years.\(^8\) Interestingly, and perhaps critical for an event-driven treatment trial in the future, this analysis showed an even worse outcome in patients who experienced their initial episode of major depression after their myocardial infarction.\(^8\) The need, ethics, and design of clinical trials to determine whether treatment of depression leads to improved outcomes in patients with coronary artery disease will be a major topic of the 4th Annual Heart-Brain Summit, to be held in Chicago on October 15–16, 2009.

OTHER HIGHLIGHTS, INCLUDING ROLE OF THE HEALING ENVIRONMENT

While much of the early focus of the 2008 Heart-Brain Summit was on the interaction of depression, inflammation, and outcomes in patients with coronary artery disease, a significant portion of the Summit identified other disease states and opportunities. The disease states discussed can be divided into primary cardiac, primary psychiatric, and primary neurologic. Cardiac topics under continued investigation include the role of vagal tone on the inflammatory response that regulates left ventricular remodeling following acute myocardial infarction\(^9\) as well as the role of spinal stimulation for treatment of refractory myocardial ischemia. Psychiatric disorders of interest that have been shown to modulate vagal tone include post-traumatic stress disorder,\(^10\) which has also been shown to increase the risk for coronary heart disease.\(^11,12\) Neurologically, advances concerning the polyvagal theory of autonomic nervous system control and cardiac control were discussed.\(^13,14\)

On the Summit’s final day, the discussions of neuropathways, inflammation, and cardiac control gave way to presentations on the role of the healing environment. Following discussions of how depression can have significant ramifications on systemic inflammation and acute coronary syndrome, it was interesting to review data on how the presence of family and the patient environment can improve patient outcomes.

Many of the topics touched on above are discussed in greater detail in the following pages of this proceedings of the 2008 Bakken Heart-Brain Summit. We are gratified to see the advancements in the field of heart-brain medicine over the past 5 years, and especially to see the recognition the discipline is receiving in our attempt to improve patient outcomes.

FAR MORE QUESTIONS REMAIN

Without a doubt there are more questions than answers at this time. That said, by continuing the rigorous multidisciplinary approach that has served this
field well to date, many questions will be answered. We hope you will join us in Chicago on October 15–16, 2009, for the 4th Annual Heart-Brain Summit, which will be jointly hosted by the Society of Heart-Brain Medicine and the Bakken Heart-Brain Institute.

REFERENCES


Correspondence: Marc S. Penn, MD, PhD, Director, Bakken Heart-Brain Institute, Cleveland Clinic, 9500 Euclid Avenue, J2-4, Cleveland, OH 44195; pennm@ccf.org
ABSTRACT
Therapeutic hypothermia in acute resuscitation medicine has a long history, but its currently recommended use dates back to work in the mid-1960s by the late Dr. Peter Safar and colleagues. Compared with normothermia, mild therapeutic hypothermia, induced right after restoration of spontaneous circulation in comatose survivors of cardiac arrest, leads to 1 additional patient with intact neurological outcome for every 6 patients treated. Demonstrating benefit from therapeutic hypothermia in other acute neurological insults, such as traumatic brain injury, has been more difficult. Current research to optimize the benefits of mild therapeutic hypothermia in cardiac arrest is focused on hypothermia’s profound effects on drug metabolism, determining the best anesthetics and sedatives to use with cooling, and identifying compounds that may promote induction of hypothermia or create a poikilothermic state. Future applications of therapeutic hypothermia may include induction of emergency preservation and resuscitation to buy time for damage-control surgery in patients with exsanguination cardiac arrest.

THERAPEUTIC HYPOTHERMIA: A HISTORICAL PERSPECTIVE
The late Dr. Peter Safar and his colleague, the late Dr. Hubert Rosomoff, played an instrumental role in the use of therapeutic hypothermia in the early 1960s in patients with acute neurological insults. Their classic 1965 publication, “Management of the comatose patient,” contained recommendations that in many ways outline the current use of mild therapeutic hypothermia as recommended by the American Heart Association and the International Liaison Committee on Resuscitation. In addition, in 1964, Dr. Safar recommended in his historic “first ABCs of resuscitation” that hypothermia be used in patients who remain comatose after successful restoration of spontaneous circulation (Figure 1). That recommendation holds true in today’s guidelines. However, therapeutic hypothermia in acute resuscitation medicine has a remarkably long history.

Baron Dominique Jean Larrey, surgeon-in-chief of the Napoleonic armies and the father of modern military medicine, observed in 1814 that the wounded “privileged” soldiers lying closer to the campfire died sooner than those in more remote, colder areas. Similarly, Dr. Charles Phelps, surgeon to the New York City Police Department, in 1897 recommended the use of the “ice cap” for traumatic brain injury. In the 1980s, however, therapeutic hypothermia began to fall out of favor. This resulted, in part, from overzealous application in some patients, who were treated for durations longer than a week and at temperatures in the moderate (28°C to 32°C) rather than mild (33°C to 35°C) range. This led to an increase in complications. Laboratory studies in a rat model of global cerebral ischemia by Busto et al in 1987 and in a canine model of cardiac arrest by Leonov et al in 1990 demonstrated that benefit could be produced using mild cooling after the insult. This and parallel work in neonatology led to the ultimate breakthrough that translated
into improved outcomes with the use of mild therapeutic hypothermia in adults with cardiac arrest and in newborns with hypoxic-ischemic encephalopathy.

Clinicians and scientists familiar with hypothermia might suggest that its potential therapeutic benefit has been known for decades, given the use of hypothermic circulatory arrest for neuroprotection and cardioprotection in open heart surgery. However, one of the most interesting aspects of neuroprotection provided by mild therapeutic hypothermia is that it is not clearly linked to attenuation of energy failure. Unlike the setting of deep hypothermic circulatory arrest—where the induction of hypothermia occurs before the insult, and levels of hypothermia are such that energy failure is prevented—mild cooling, applied after cardiac arrest, appears to confer benefit by other mechanisms. Effects on cell signaling, oxidative and nitritative stress, apoptosis, excitotoxicity, and other mechanisms appear to mediate this benefit.

**THERAPEUTIC HYPOTHERMIA: CONTEMPORARY APPLICATION**

**Use in cardiac arrest**

Compared with normothermia, mild therapeutic hypothermia, induced immediately after restoration of spontaneous circulation in comatose survivors of ventricular fibrillation cardiac arrest, leads to 1 additional patient with intact neurological outcome for every 6 patients treated. This is a remarkable effect given the extremely poor overall outcomes observed after out-of-hospital cardiac arrest. Studies in animal models, however, suggest that the therapeutic potential of mild hypothermia can be maximized with application either during or as early as possible after the insult. However, clinicians in the field of cardiology appropriately have cause for concern about the possibility that even mild cooling could reduce that potential for successful defibrillation or lead to re-arrest. In 2005, an important paper by Boddicker et al explored the impact of mild hypothermia on defibrillation success in experimental ventricular fibrillation in pigs and found, remarkably, that the success rate actually improved with mild or moderate hypothermia! That report opened the door for a number of studies that are now focused on rapid cooling during cardiopulmonary resuscitation (CPR) and on the rapid induction of mild hypothermia using intravenous cooling.

Support for the use of intra-arrest cooling came initially from work in animal models of cardiac arrest—first from the work of Abella et al in a mouse model of potassium-induced cardiac arrest, and later from a canine model in work by Nozari et al. In the latter study, delaying the onset of mild hypothermia during advanced cardiac life support markedly worsened both multisystem organ failure and survival. Cardiovascular function in that model appeared to be substantially improved by early intra-arrest cooling.

The potential for the use of intravenous cooling in patients with a bolus of crystalloid to induce mild hypothermia was pioneered in a seminal paper by Bernard et al. In that report, an approximately 2°C reduction in core temperature could be achieved with infusion of about 30 mL/kg of fluid over 30 minutes. Mean arterial blood pressure increased mildly, and the intervention was well tolerated when applied early after restoration of spontaneous circulation. Kim and colleagues built upon that initial work and demonstrated the feasibility of the use of intravenous iced normal saline to induce mild hypothermia by paramedics in the prehospital setting. This approach, and its impact on neurological
outcome and survival, is currently being evaluated in a randomized controlled trial. Combining intra-arrest cooling with the use of intravenous fluids is the obvious next step. This could facilitate rapid induction, which could then be maintained with commercially available surface cooling devices.21

Cardiac arrest vs traumatic brain injury
One of the interesting aspects of the beneficial effects of mild therapeutic hypothermia in the setting of cardiac arrest relates to the following question: Why is hypothermia effective in improving neurological outcome after cardiac arrest while it has been more difficult to demonstrate benefit in other acute neurological insults, such as traumatic brain injury?22

Application of hypothermia in cardiac arrest may represent something of a “perfect storm.” First, a recent study by Berger et al23 provides some insight into the time course of neuronal death after cardiac arrest versus traumatic brain injury. In that study of children who suffered either cardiac arrest or severe traumatic brain injury requiring management in the intensive care unit, peak levels of the serum biomarker of neuronal death, neuron-specific enolase (NSE), occurred days after cardiac arrest, whereas they occurred generally within a few hours of traumatic brain injury. This suggests a broader therapeutic window for the application of mild hypothermia in cardiac arrest as opposed to traumatic brain injury. In addition, the only neuroprotective therapy used in cardiac arrest is mild hypothermia. In contrast, in traumatic brain injury, myriad therapies are part of standard of care, including intracranial pressure monitoring and cerebrospinal fluid drainage, mannitol, hypertonic saline, barbiturates, and surgical interventions such as decompressive craniectomy.24 These intracranial pressure–directed therapies in traumatic brain injury may confer a variety of neuroprotective actions, thus raising the bar for hypothermia to show benefit. A similar case could be made regarding the surgical treatment of subarachnoid hemorrhage, where hypothermia has been ineffective.25

Efforts to optimize hypothermia
Given the benefit of mild therapeutic hypothermia in cardiac arrest, we and other investigative teams are actively pursuing ways to further optimize its effects beyond the use of a more rapid induction, as discussed above.

One of the most overlooked areas of study relates to hypothermia’s profound effects on drug metabolism; despite the need for many drugs in critically ill patients after cardiac arrest, knowledge of how hypothermia alters drug metabolism and how best to adjust drug doses is limited. Therapeutic hypothermia has recently been shown, during cooling, to directly inhibit binding of drugs to the active site of the key drug-metabolizing enzyme, cytochrome P450.26 In contrast, in the setting of experimental cardiac arrest and resuscitation, mild hypothermia also protects against induction of cytokines such as interleukin-6, which downregulates cytochrome P450. Thus, mild hypothermia reduces drug metabolism during cooling but leads to a better recovery of drug metabolism after rewarming. This dichotomous effect will need to be studied at the bedside. Hypothermia can also reduce drug effects.26 Thus, until we know how to optimally dose various therapies in patients treated with hypothermia, it is probably best to carefully monitor levels (when possible) and also drug effects. The best example of this at the bedside is the use of monitoring neuromuscular blockade in patients treated with vecuronium or pancuronium during mild hypothermia.

Another interesting area of study involves defining the best anesthetics or sedatives to use with cooling. For example, a recent report by Statler et al27 showed that hypothermia was much less effective as a neuroprotectant after experimental traumatic brain injury in rats anesthetized with fentanyl than with isoflurane. In that study, fentanyl was unable to blunt the stress response to cooling. Given the variety of sedatives and analgesics used at the bedside in both neurointensive care units and coronary care units, understanding which agents work best with hypothermia could further enhance hypothermia’s therapeutic benefit.

There is also a search for agents that may promote induction of hypothermia or create a poikilothermic state, thereby facilitating tolerance of the hypothermic state without a stress response. One agent that has shown some promise in the setting of experimental cardiac arrest is the endogenous peptide neurotensin, which has direct effects on temperature regulation at the hypothalamic level. In an experimental model of asphyxial cardiac arrest in rats, Katz et al28 reported that the neurotensin analog NT69L facilitated induction of hypothermia and improved outcome. Another agent that has been touted to induce a state of “hibernation on demand” is hydrogen sulfide gas. A recent experiment by Blackstone et al29 demonstrated induction of deep hypothermia and a hibernation-like state in mice allowed to breathe hydrogen sulfide gas at 80 parts per million. This state was completely reversible upon discontinuation of the agent. Unfortunately, studies in large animal models have not been able to demonstrate induction of hypothermia with this approach.30 Nevertheless, these drugs represent
prototypes for future exploration; if the right agent is found, it could lead, in theory, to markedly enhanced efficacy of cooling.

**FUTURISTIC APPLICATIONS OF THERAPEUTIC HYPOTHERMIA**

**Emergency preservation and resuscitation**

Exsanguination cardiac arrest is one of the most refractory types of cardiac arrest, with mortality rates generally greater than 95%. Obviously, therapies such as CPR are ineffective in the absence of an adequate circulating blood volume.

In 1984, Dr. Peter Safar and military expert Col Ronald Bellamy pioneered a new approach to exsanguination cardiac arrest that they called suspended animation for delayed resuscitation. The concept was a logical one—namely, in the setting of otherwise lethal trauma-induced exsanguination cardiac arrest, a transient state of preservation would be induced to buy time for damage-control surgery, and then a delayed resuscitation would be carried out using cardiopulmonary bypass. Our center has worked on this concept since 1988 in studies funded initially by the US Navy and later by the US Congress. Ultimately, we coined the phrase emergency preservation and resuscitation (EPR) for this method. Using a canine model of exsanguination cardiac arrest, we first demonstrated the feasibility of this approach by inducing a state of preservation via an aortic flush of iced saline. A schematic of the protocol is presented in Figure 2.

In initial reports, we targeted relatively brief insults ranging from 15 to 60 minutes. We determined that for insults at or beyond 60 minutes, profound levels of hypothermia (tympanic temperature of ~10°C) were most effective. In subsequent studies, we demonstrated that pharmacologic adjuncts to hypothermia were relatively ineffective. Indeed, only one agent, the brain-penetrating antioxidant Tempol, enhanced the efficacy of profound hypothermia. We also demonstrated that the prolonged use (36 to 48 hours) of mild hypothermia after the acute application of EPR further enhanced neurological outcomes as compared with more rapid rewarming. Similarly, unlike drugs, the addition of energy substrates (namely, dissolved oxygen and 2.5% dextrose) to the flush facilitated the ability to achieve remarkably long EPR durations in experimental exsanguination cardiac arrest—as long as 3 hours of preservation at approximately 10°C. These findings could also have important implications for optimizing conventional use of deep hypothermia circulatory arrest in cardiac or neurological surgery. We also have recently developed a rat model of EPR using a miniaturized cardiopulmonary bypass system. It is used to screen novel therapeutic adjuncts to EPR and to study mechanisms of neuroprotection in this special paradigm.

Two other investigative teams, one at Harvard University and another at the Vienna General Hospital, have also been exploring the use of EPR-related technologies—and observing similar success. Alam et al have used a low-flow EPR approach in pigs to facilitate damage-control surgery after otherwise lethal traumatic insults. Janata et al have successfully used EPR in the setting of refractory normovolemic cardiac arrest, simulating the typical cardiac arrest victim who cannot be resuscitated in either the field or the emergency department.

Finally, the EPR concept recently received funding to proceed to a clinical trial in civilian trauma. The study, to be led by Dr. Samuel Tisherman, one of the pioneers of this approach at the Safar Center, will include several trauma centers in the United States and target otherwise lethally injured trauma victims with exsanguination cardiac arrest.

**REFERENCES**

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BAKKEN LECTURE: THERAPEUTIC HYPOTHERMIA


Correspondence: 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, 3434 Fifth Avenue, Pittsburgh, PA 15260; kochanekpm@ccm.upmc.edu
Depression and heart rate variability in patients with coronary heart disease

**Abstract**

Depression is common in patients with coronary heart disease (CHD) and is a risk factor for cardiac morbidity and mortality in these patients. Depression is associated with autonomic nervous system dysfunction, which may at least partially explain this increased risk. Low heart rate variability (HRV), which reflects excessive sympathetic and/or inadequate parasympathetic modulation of heart rate, is a strong predictor of mortality in patients with CHD. Most studies—both in patients with stable CHD and in patients with a recent acute coronary event—have found HRV to be lower in depressed patients than in their nondepressed counterparts. This manuscript provides an overview of this literature and concludes that HRV may account for a substantial part of the risk associated with depression in CHD.

Depression is a common psychiatric disorder in patients with coronary heart disease (CHD). Whereas the lifetime prevalence of major depression in the United States is estimated to be about 16%, with an annual rate of about 7%, approximately 20% of patients with CHD have major depression at any point in time. About the same proportion have minor depression. During the 12 months following an acute coronary event, as many as 30% of patients may develop major depression; the prevalence of minor depression during this 12-month period has not been reported but is also estimated to be about 30%. Thus, up to 60% of patients with an acute coronary event experience symptoms of depression within the 12 months following the event.

In addition to being highly comorbid with CHD, depression is also a significant risk factor for cardiac morbidity and mortality in patients with CHD. This risk is present from the time of initial diagnosis of CHD by cardiac catheterization and angiography as well as after an acute myocardial infarction (MI), an episode of unstable angina, or coronary artery bypass graft surgery. A recent meta-analysis of more than 20 studies of depression following acute MI found that major depression more than doubles the risk of mortality in the months following the acute event. Another meta-analysis found that just having symptoms of depression at various times in the course of CHD doubles the risk of death, and that clinical depression is associated with an even higher risk.

Depression has been associated with many behavioral and biological abnormalities that could help explain the increased mortality risk in depressed patients with cardiac disease, including reduced adherence to treatment regimens, increased prevalence of smoking and diabetes, platelet dysfunction and coagulant processes, inflammatory processes, and alterations in hypothalamic-pituitary-adrenal axis and autonomic nervous system (ANS) function. Excessive sympathetic or reduced parasympathetic nervous system activity in patients with CHD may promote myocardial ischemia, ventricular tachycardia, ventricular fibrillation, and even sudden cardiac death.

Studies dating back to the 1960s have found plasma and urinary catecholamine levels and resting heart rate (HR) to be elevated in medically well psychiatric patients with major depression compared with nondepressed controls. Studies of patients with CHD have also found elevated resting and 24-hour HRs in depressed compared with nondepressed patients. Additional evidence of ANS dysfunction in depressed CHD patients includes increased HR response to orthostatic...
challenge, increased QT interval variability, reflecting abnormal ventricular repolarization; abnormal HR response to ventricular arrhythmias (turbulence); and an increased incidence of ventricular tachycardia. All of these factors have been related to ANS dysfunction, and all are predictors of mortality in cardiac patients.

Many, though not all, studies of medically well depressed psychiatric patients have also found reduced HR variability (HRV), reflecting abnormal ANS modulation of HR. Low HRV is an excellent predictor of cardiac-related mortality and thus may further help to explain the relationship of depression to increased risk of mortality.

### Measurement of Heart Rate Variability

Analysis of HRV is a widely used method for studying cardiac autonomic modulation of HR. Low HRV generally reflects excessive sympathetic and/or inadequate parasympathetic modulation of HR and is a strong predictor of mortality in patients with CHD.

#### Three methods of deriving HRV

In large prognostic or epidemiologic studies, HRV is usually measured over a 24-hour period and is derived from electrocardiographic (ECG) data by one of three methods: time domain analysis, frequency domain analysis, and nonlinear statistical models.

**Time domain indices** are based on descriptive statistical analyses of the HR time series. These include the standard deviation of all normal-to-normal intervals (SDNN) and the root mean square of successive N-N differences (rMSSD).

**Frequency domain indices.** Fast Fourier transforms and spectral analyses of ECG data are used to characterize HRV in the frequency domain. Frequency domain indices are defined by specific frequency ranges:

- Ultra low frequency (ULF; < 0.0033 Hz)
- Very low frequency (VLF; 0.0033 to 0.04 Hz)
- Low frequency (LF; 0.04 to 0.15 Hz)
- High frequency (HF; 0.15 to 0.4 Hz).

These indices are usually log-transformed to produce approximately normal distributions. Efferent vagal activity is largely responsible for the HF component, whereas LF power seems to reflect both sympathetic and parasympathetic activity. There is less certainly about the contributions to ULF. While not completely understood, VLF power is known to be unaffected by beta-blockade but nearly abolished by atropine, suggesting that the parasympathetic nervous system is the predominant determinant of VLF.

**Nonlinear statistical models.** HRV has also been characterized by nonlinear mathematical models, such as those based on chaos theory and fractals. Nonlinear methods quantify the structure of the HR time series, including its regularity and self-similarity. These indices include the short-term fractal scaling exponent and approximate entropy.

### Heart Rate Variability and Depression in CHD

Some studies have assessed HRV and depression following acute MI, whereas others have focused on HRV in medically stable patients with CHD. Most of the studies have used frequency domain indices to calculate HRV.

**HRV in post-MI patients with depression**

In the largest study of depressed post-MI patients published to date, 24-hour HRV levels were compared between 380 patients with a recent MI who had either major or minor depression and 425 post-MI patients who were not depressed. In univariate analyses, the four frequency domain indices of HRV (ULF, VLF, LF, and HF) were significantly lower in the depressed than in the nondepressed patients. After adjustment for possible confounders, all the indices except HF remained significantly lower in depressed patients than in nondepressed patients.

**HRV in depressed patients with stable coronary disease**

Most but not all studies have also found HRV to be lower in depressed than in nondepressed patients with stable CHD. The one exception was reported by Gehi et al, who assessed participants from the Heart and Soul Study cohort who had stable CHD at the time HRV was determined. Of the 873 outpatients with stable CHD who received 24-hour ambulatory ECG monitoring, 195 were found to have major depression. No differences between depressed and nondepressed patients were found on any time domain or frequency domain measure of HRV. This is the largest study to date of medically stable CHD patients assessed for depression and HRV, but its results differ from those of most smaller studies. The authors noted that although there was no difference in HRV between depressed and nondepressed patients, HRV in the nondepressed patients was similar to that in depressed patients in other samples. They speculated that the participants in their study, who were largely recruited from a Veterans Affairs hospital, may have been sicker than most participants in other studies and that this might have obscured depression-related differences in HRV.

**What is the clinical significance of HRV differences?**

When evaluating differences in HRV between depressed and nondepressed patients, it is important to look past statistical comparisons and consider the clinical significance of these differences—i.e., whether they are large enough to affect clinical outcomes or to be responsible
for the depressed patients’ increased risk of death.

In the Cardiac Arrhythmia Pilot Study, HRV was assessed 1 year after acute MI in 331 patients. All measured indices of HRV were strong predictors of mortality. Patients with VLF power of less than 600 ms
s averaged higher risk for all-cause mortality, even after adjusting for confounders (hazard ratio = 2.8; 95% CI, 0.9 to 3.8; P = .08). When the LnVLF was added to this model, the adjusted hazard ratio for depression decreased to 1.9 (95% CI, 0.9 to 3.8; P = .18). Thus, the combination of VLF and HR response to VPCs explained about half of the effect of depression on survival in these patients.

### Causality not proven, but further study warranted

Obviously, these results do not prove that there is a causal relationship between depression, low HRV, and mortality. However, they are consistent with the interpretation that HRV, especially when combined with measures of HR response to VPCs, may account for a significant proportion of depression’s association with mortality following an MI. Future studies of these risk markers should explore their potential interrelationships to clarify how they may jointly contribute to the risk of death in patients with depression.

### RELATIONSHIP AMONG HRV AND OTHER POSSIBLE BIOLOGICAL PATHWAYS

As discussed earlier, other biological pathways that may link depression to increased mortality have
been reported. The two that have received the most support are proinflammatory and procoagulant processes. \(^{18,19}\) Studies of medically healthy depressed psychiatric patients and of depressed CHD patients have found depression to be associated with higher levels of the inflammatory risk markers interleukin-6 (IL-6), C-reactive protein (CRP), and tumor necrosis factor–alpha (TNF-\(\alpha\)) and with inflammatory-procoagulant markers such as fibrinogen, \(^{56-60}\) as well as with platelet activation. Low HRV and elevations in proinflammatory cytokine release, thereby reducing systemic inflammation. \(^{62,63}\) Low HRV, reflecting reduced vagal activity, should therefore be associated with higher levels of both proinflammatory and procoagulant markers. Recent studies have found a relationship between HRV activity and increased markers of inflammation in other high-risk patients, including those with heart failure \(^{64,65}\) and with acute coronary syndrome. \(^{66}\)

In a recent study of 44 patients with major depression, moderate negative correlations were found between fibrinogen and four measures of HRV. \(^{67}\) IL-6 was also negatively correlated with one measure of HRV (total power) and was marginally related to two others (VLF and LF power). On the other hand, neither CRP nor TNF-\(\alpha\) was significantly related to any measure of HRV. The finding that fibrinogen and IL-6 are moderately related to HRV suggests a link between these factors in depressed CHD patients. Thus, these risk markers, which are commonly found in patients with depression, may be related and contribute to the increased mortality associated with depression. This possibility should be investigated in larger mechanistic studies of depression and cardiac morbidity and mortality.

## SUMMARY AND FUTURE DIRECTIONS

Low HRV and other markers of cardiac ANS dysfunction in depressed patients are likely to contribute to the elevated risk associated with depression in patients with CHD. More work is needed to clarify the physiologic and behavioral mechanisms underlying depression’s role as a risk factor for mortality in patients with CHD. Work is also needed to identify treatments that improve both depression and HRV, and to determine whether such treatments might also improve survival in these patients. \(^{68}\)

## REFERENCES


44. Maes M, Bosmans E, De Jongh R, et al. Increased serum IL-6 and IL-1 receptor antagonist concentrations in major depression and treatment resistant depression. Cytokine 1997; 9:853–858.


Correspondence: Robert M. Carney, PhD, Behavioral Medicine Center, 4320 Forest Park Boulevard, Suite 301, St. Louis, MO 63108; carney@bmc.wustl.edu
Autonomic nervous system function is assessed in the clinic by measuring resting heart rate, heart rate variability, or heart rate recovery following exercise. Each of these measures is a strong predictor of cardiovascular risk and all-cause mortality in primary and secondary prevention settings. These measures have been used to identify correlates of autonomic nervous system dysfunction at both the patient level (e.g., obesity, diabetes, heart failure) and the environmental level (e.g., smoking, social stress, air pollution). Future research must determine how to exploit the associations between autonomic system dysfunction and poor prognosis to improve patient outcomes.

First-year medical students are well aware that the autonomic nervous system regulates heart rate and blood pressure along with respiratory and digestive functions. The past 10 to 20 years have seen increased appreciation of the medical relevance of the autonomic nervous system beyond first-year physiology examinations; even mild disturbances of autonomic nervous system function predict materially worse prognosis.1,2,3 Researchers have focused on the use of readily available measures, such as heart rate,4 heart rate variability,5 and heart rate recovery,6 to link autonomic nervous system dysfunction with mortality and morbidity.1 In addition, epidemiologists have exploited these tools to identify correlates of autonomic nervous system dysfunction at patient and environmental levels.7 Although it is not yet known how best to incorporate autonomic nervous system measures into routine clinical care, there is increasing excitement about the insights that this work has revealed.

**MEASURES OF AUTONOMIC NERVOUS SYSTEM FUNCTION**

Although many measures of autonomic nervous system function have been described, three relatively straightforward approaches are based on heart rate.1

**Resting heart rate** is the simplest to obtain, as it does not require any special technology. People with high levels of parasympathetic nervous system tone have lower resting heart rates, as is typically seen in world-class athletes. Conversely, conditions characterized by increased levels of sympathetic tone manifest as sinus tachycardia; classic examples include congestive heart failure, anemia, and hypovolemia.

**Heart rate variability.** Even before the advent of the electrocardiogram, it was known that heart rate normally varies with respiration.8 Physiologic sinus arrhythmia can be easily demonstrated by plotting heart rate over time in resting supine subjects, typically yielding a tracing characterized by high-frequency, low-amplitude waves. When a normal subject assumes an upright position, the resting heart rate increases and there is an increased absolute amount of variability, but the frequency of the variability wave decreases. Using Fourier transform techniques, one can translate the amplitude and frequency of the waves shown in the top plot of **Figure 1** into power domain functions, as shown in the bottom plot of **Figure 1.**8 Heart rate variability functions can be divided into high-frequency, low-frequency, and very-low-frequency domains.8,9 The high-frequency peak, which is reflective of the very fine variability seen with respiration at rest, is thought to reflect parasympathetic nervous system tone. The low-frequency and very-low-frequency peaks are thought to reflect mixed effects of parasympathetic tone and sympathetic tone.

**Heart rate recovery.** Heart rate variability measures require continuous Holter monitoring as well as sophisticated software. The numerous types of heart rate variability measures are not intuitive for most clinicians. Exercise heart rate recovery is an arguably more straightforward method of assessing parasympathetic tone.1 During a graded exercise test, heart rate increases as a result of withdrawal of parasympathetic...

Dr. Lauer reported that he has no financial interests or relationships that pose a potential conflict of interest with this article.

doi:10.3949/ccjm.76.s2.04
tone and increased sympathetic tone. During the first
30 seconds after exercise, heart rate decreases quickly,
mainly because of rapid reactivation of the parasym-
pathetic nervous system.10
The association between early heart rate recovery
and parasympathetic nervous system function was
demonstrated elegantly by Imai and colleagues in a
study of three groups of subjects—athletes, normal
subjects, and patients with heart failure.10 Among
athletes and normal subjects there was a biexpo-
nential pattern of heart rate during early recovery,
with a steep log-linear decrease during the first 30
seconds followed by a more shallow decline
(Figure 2A). When the same subjects were given atropine
and exercise testing was repeated, the initial steep
decrease in heart rate observed among athletes and
normal subjects disappeared (Figure 2B). The authors
concluded that early heart rate recovery is primarily a
manifestation of parasympathetic reactivation.

AUTONOMIC NERVOUS SYSTEM FUNCTION
AND MORTALITY

Resting heart rate
There is a remarkably strong association between
heart rate and survival, an association that transcends
species.4 Small mammals that have rapid heart rates
have short life expectancies. Larger mammals that
have slower heart rates have correspondingly higher
life expectancies. Among nearly all mammals, life
expectancy is close to 1 billion heartbeats.
Investigators have been able to increase survival
in animal models by deliberate slowing of heart rate.
An experiment performed in mice more than 30 years
ago showed that life expectancy increases with low-
dose digoxin, a parasympathomimetic agent.11 More
recently, a mouse model has been used to show that
ivabradine, a sinus node ion channel blocking agent
that specifically reduces heart rate without affecting
vascular tone, inhibits development of atherosclerosis in genetically susceptible knockout mice.12

There is an extensive epidemiological literature linking heart rate to mortality in large human populations.4,13 As heart rate increases to 75 to 80 beats per minute, there are marked increases in total mortality and mortality due to coronary heart disease. As is well known, administration of beta-blockers reduces mortality in survivors of myocardial infarction. What is particularly remarkable is that the magnitude of reduction in mortality with beta-blocker therapy is directly proportional to the magnitude of heart rate decrease.14 In a recent analysis of hypertensive patients enrolled in a large-scale randomized trial, a strong association was noted between mortality and increasing heart rate at the time of randomization as well as after treatment with either verapamil or a beta-blocker.15

Heart rate variability

Just as with resting heart rate, there is a robust literature linking decreased heart rate variability to cardiac events and mortality. Among healthy elderly subjects enrolled in the Framingham Heart Study, decreased heart rate variability was associated with a substantially increased likelihood of major cardiac events (Figure 3).16 The Framingham investigators measured the standard deviation of R-R intervals of normal beats (SDNN) as well as time-domain measures. Lower values of the ratio of low-frequency power to high-frequency power, which would correspond to lower levels of parasympathetic tone, were also associated with increased mortality. Similarly, among survivors of myocardial infarction, especially those with low ejection fractions, decreased heart rate variability predicted substantially higher mortality rates.5,17,18

Heart rate recovery

In 1999, Cole and colleagues reported on the association between heart rate recovery during the first minute after exercise and all-cause mortality in approximately 2,400 patients who were candidates for first-time coronary angiography.6 An abnormal heart rate recovery was defined as a reduction from the peak heart rate of 12 beats per minute or less, which corresponded to the lowest quartile. Thus, a patient achieving a peak heart rate of 160 beats per minute would be considered to have an abnormal heart rate recovery if 1 minute later the heart rate was 148 beats per minute or higher. Patients who had an abnormal heart rate recovery had a nearly fourfold increased risk of all-cause death; even after adjusting for numerous confounders, including exercise capacity, there was still a twofold independent increased risk of death. This initial observation has since been confirmed in other cohorts.19,20 The link between heart rate recovery, mortality, and cardiovascular prognosis appears to be independent of symptom status,19 type of recovery protocol,21 left ventricular ejection fraction,22 and angiographic severity of coronary artery disease.23

The mechanism by which an abnormal heart rate recovery predicts increased mortality is unclear. Given that heart rate recovery is thought to reflect parasympathetic nervous system function, and given that increased parasympathetic tone is believed to have antiarrhythmic effects, one might hypothesize that lower heart rate recovery would predict sudden cardiac death. In 2005, investigators from the Paris Civil Service Study reported on the association of exercise heart rate recovery and type of mortality; low heart rate recovery was strongly predictive of sudden cardiac death but not of non-sudden cardiac myocardial infarction death.20 A separate study from the Cleveland Clinic showed that among more than 29,000 patients, frequent ventricular ectopy during early recovery was strongly predictive of death, whereas frequent ventricular ectopy during exercise was not.24 These two studies together suggest that the link between heart rate recovery and mortality may be a reflection of the antiarrhythmic properties of the parasympathetic nervous system.

It is well known that there is an exceptionally powerful link between functional capacity and cardiovascular risk.25,26 People who are in excellent physical shape have high levels of parasympathetic tone. Among patients with suspected coronary artery disease, there is a strong dose-response relationship
between heart rate recovery and physical fitness. While the link between functional capacity and prognosis is complex, it is conceivable that parasympathetic protection against arrhythmias and shear-induced plaque rupture may play a role.

Both heart rate recovery and functional capacity are easy to measure using standard exercise test equipment. Recently, investigators from the Cleveland Clinic and Kaiser Permanente derived and externally validated a simple multivariable instrument by which all-cause mortality can be predicted in subjects with a normal electrocardiogram and no history of coronary disease. This instrument includes measures of functional capacity, heart rate recovery, and frequent ventricular ectopy during recovery. An example of the user interface is shown in Figure 4.

**FIGURE 4.** User interface of an externally validated mortality prediction model for primary prevention patients with normal electrocardiograms undergoing exercise testing.27 This prediction model includes easily obtained cardiovascular risk factors as well as measures of autonomic function.

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**DETERMINANTS OF AUTONOMIC NERVOUS SYSTEM FUNCTION**

There is an extensive literature documenting a number of determinants of autonomic tone. On a patient level, decreased levels of parasympathetic tone or increased levels of sympathetic tone have been linked to obesity, insulin resistance, diabetes, hypertension, hypercholesterolemia, depression, anxiety, heart failure, and peripheral vascular disease.

The association between diabetes and autonomic nervous system dysfunction is well known to clinicians caring for patients with clinically manifest autonomic neuropathy. What is less appreciated is that even minor degrees of glucose intolerance are associated with abnormalities of autonomic balance. For example, among patients enrolled in a population-based cohort, the likelihood of an abnormal heart rate recovery increased in a steady fashion as fasting plasma glucose increased from 70 to 80 to 90 mg/dL and above (Figure 5). Even at levels of plasma glucose that would be considered normal, the likelihood of an abnormal heart rate recovery increased as plasma glucose increased.

Perturbations of autonomic nervous system function have also been associated with environmental exposures. People who have lower levels of education, live in neighborhoods characterized by lower socio-
economy status, or are exposed to small-particulate air pollution have been shown to manifest abnormal heart rate recovery or decreased heart rate variability.

**CONCLUSIONS**

Autonomic nervous system function can be measured in the clinic by recording resting heart rate, heart rate variability, or exercise heart rate recovery. All three of these measures are strong predictors of cardiovascular risk and all-cause mortality in both primary and secondary prevention settings. A number of determinants of autonomic nervous system function have been identified, including patient-level factors like obesity, diabetes, and heart failure as well as environmental correlates like smoking, social stress, and air pollution. It is not yet known, however, how best to take advantage of the associations between abnormal autonomic nervous system function and poor prognosis to improve patient outcomes. Future research will be needed to identify strategies of favorably modulating autonomic function that improve outcomes in the clinic and among large populations.

**REFERENCES**


**Correspondence:** Michael S. Lauer, MD, Director, Division of Prevention and Population Sciences, National Heart, Lung, and Blood Institute, 6701 Rockledge Drive, Rockledge Center II, Room 10021, Bethesda, MD 20892; lauerm@nhlbi.nih.gov
Vagal tone and the inflammatory reflex

ABSTRACT
Inhibition of sympathoexcitatory circuits is influenced by cerebral structures and mediated via vagal mechanisms. Studies of pharmacologic blockade of the prefrontal cortex together with neuroimaging studies support the role of the right hemisphere in parasympathetic control of the heart via its connection with the right vagus nerve. Neural mechanisms also regulate inflammation; vagus nerve activity inhibits macrophage activation and the synthesis of tumor necrosis factor in the reticuloendothelial system through the release of acetylcholine. Data suggest an association between heart rate variability and inflammation that may support the concept of a cholinergic anti-inflammatory pathway.

The neurovisceral integration model of cardiac vagal tone integrates autonomic, attentional, and affective systems into a functional and structural network. This neural network can be indexed by heart rate variability (HRV). High HRV is associated with greater prefrontal inhibitory tone. A lack of inhibition leads to undifferentiated threat responses to environmental challenges.

THE CENTRAL AUTONOMIC NETWORK
Some of the structures involved in this model, called the central autonomic network, are illustrated in Figure 1. Communication is bidirectional between these neural structures, which include the medial prefrontal cortex, insular cortex, central nucleus of the amygdala, periaqueductal gray region, and parabrachial region. At the medullary level are the autonomic output regions, including the nucleus ambiguus and the nucleus tractus solitarius. The primary outputs for this set of neural structures are from the stellate ganglia and the vagus nerve to the sinoatrial node of the heart.

Activity of the heart permits us to infer activity in this set of neural structures. Excitatory and inhibitory pathways form the connections between the prefrontal cortex and the autonomic output regions in the medullary area, with further connections to heart rate (HR) and HRV.

Central, respiratory, cardiopulmonary, and arterial baroreflex influences on the brainstem signal the sinoatrial node of the heart. Autonomic inputs at the heart have a differential influence. The sympathetic inputs to the sinoatrial node of the heart are relatively slow, such that a burst of sympathetic outflow from the brain produces an effect on the heart several seconds later. In contrast, inputs to the cholinergic or vagal pathway are relatively fast, on the order of milliseconds. The interplay of sympathetic and vagal neural control of the heart produces a complex variability in heart rhythm that characterizes a healthy system.

PARASYMPATHETIC CONTROL AND THE RIGHT VAGUS NERVE
Pharmacologic blockade of prefrontal cortex
The effect of pharmacologic blockade of the prefrontal cortex on HR and HRV was investigated in patients undergoing preoperative evaluation for epilepsy surgery. The hypothesis was that inactivation of the prefrontal cortex (using an injection of intracarotid sodium amobarbital) would be associated with an increase in HR and a decrease in vagally mediated HRV.

During 10 minutes of inactivation, an increase in HR was observed in both the left and right hemispheres. HR peaked 3 to 4 minutes postinjection and decreased gradually, returning to preinjection baseline at about 10 minutes. The increase was larger in the right hemisphere, a finding that is consistent with the known neuroanatomy in which the right-sided neural inputs selectively signal the sinoatrial node, and the left-sided inputs signal the atrioventricular node. The pronounced effect on HR in the right hemisphere was related specifically to the vagally mediated (high-
VAGAL TONE AND INFLAMMATORY REFLEX

frequency) component of HRV. This experiment strongly suggests that cerebral structures tonically inhibit sympathoexcitatory circuits, and that the inhibition is mediated via vagal mechanisms.

Further analysis, in which the subjects were divided into tertiles based on age, revealed disinhibition of brainstem sympathoexcitatory circuits, resulting in an increase in HR of approximately 9 beats per minute in the youngest individuals (mean age, 20 years), but an absence of a laterality effect, which suggests that the prefrontal cortex is not fully developed in this young age group. Disinhibition of sympathoexcitatory circuits as indicated by a HR increase of 11 beats per minute and a right-sided laterality effect occurred in subjects in the second tertile (mean age, 33 years). In the oldest age group (mean age, 45 years), the disinhibition effect on HR was only 3 beats per minute, consistent with the known changes in prefrontal inhibitory tone and prefrontal activity that occur with age.

Confirmation from neuroimaging studies

Neuroimaging studies support the predominant role of the right hemisphere in the regulation of vagal tone during emotion. Twelve healthy females underwent measurements of cerebral blood flow and the high-frequency component of HRV during two stimulus modalities (film, recall) and six stimulus conditions (happiness, sadness, disgust, and three neutral conditions), for a total of 12 conditions. Significant covariation (increased activity associated with increased HRV) was found for four brain areas: the right superior prefrontal cortex, the right dorsal lateral prefrontal cortex, the right parietal cortex, and the left anterior cingulate.

THE INFLAMMATORY REFLEX

The cholinergic anti-inflammatory pathway

Acetylcholine and parasympathetic tone inhibit pro-inflammatory cytokines such as interleukin (IL)-6. These proinflammatory cytokines are under tonic inhibitory control via the vagus nerve, and this function may have important implications for health and disease.

The cholinergic anti-inflammatory pathway is associated with efferent activity in the vagus nerve, leading to acetylcholine release in the reticuloendothelial system that includes the liver, heart, spleen, and gastrointestinal tract. Acetylcholine interacts with the alpha-7 nicotinic receptor on tissue macrophages to inhibit the release of proinflammatory cytokines, but not anti-inflammatory cytokines such as IL-10.

Approximately 80% of the fibers of the vagus nerve are sensory; ie, they sense the presence of proinflammatory cytokines and convey the signal to the brain. Efferent vagus nerve activity leads to the release of acetylcholine, which inhibits tumor necrosis factor (TNF)-alpha on the macrophages. Cytokine regulation also involves the sympathetic nervous system and the endocrine system (the hypothalamic-pituitary axis).

The sympathetic system has both pro- and anti-inflammatory influences. The inflammatory response is a cascade of cytokines, such that it may begin with the release of TNF-alpha, leading to the production of IL-1 and IL-6. IL-6 has both pro- and anti-inflammatory properties and represents a negative feedback mechanism. Expression of IL-6 in the liver promotes the production of the acute-phase reactant C-reactive protein (CRP). Therefore, activation of the cholinergic receptor to induce acetylcholine release may be an early intervention to short-circuit this inflammatory cascade, a potential therapeutic strategy to blunt inflammatory-mediated disease.

Inverse relationship between HRV and CRP

In a study of 613 airplane factory workers in southern Germany, vagally mediated HRV was inversely related to high-sensitivity CRP in men and premeno-
pausal women, even after controlling for urinary norepinephrine as an index of sympathetic activity. Most previous studies in which the relationship between HRV and CRP (or other inflammatory markers) was assessed failed to control for sympathetic nervous system activity. In the total sample and in men, the parasympathetic effect on CRP was comparable with that of smoking; in women, the effect was 4 times larger and comparable with that of high body mass index. A negative association was again found between vagally mediated HRV and white blood cell count.

**Inverse relationship between HRV and fibrinogen**

In a related report from the same study, vagal modulation of fibrinogen was investigated. Fibrinogen is a large glycoprotein that is synthesized by the liver. Plasma fibrinogen is a measure of systemic inflammation crucially involved in atherosclerosis. Meta-analyses have shown a prospective association between elevated plasma fibrinogen levels even in the normal range and an increased risk of coronary artery disease in different populations. We investigated the relationship between nighttime HRV, assessed by root mean square of successive R-R interval differences (RMSSD), and fibrinogen in 559 mostly male workers from southern Germany. Among all workers, there was a mean ± SEM increase of 0.41 ± 0.13 mg/dL fibrinogen for each ms decrease in nighttime RMSSD, even after controlling for established cardiovascular risk factors. The increase in men was 0.28 ± 0.13 mg/dL and, in women, 1.16 ± 0.41 mg/dL for each ms decrease in nighttime RMSSD. Such an autonomic mechanism might contribute to the atherosclerotic process and its thrombotic complications.

**Vagal regulation of allostatic systems**

Whereas the role of the autonomic nervous system, and the vagus nerve in particular, in the regulation of the cardiovascular system seems clear, the role of the vagus nerve in the regulation of other systems associated with allostatics is less evident. In addition to the regulation of inflammatory markers as discussed thus far, decreased vagal function and HRV have been associated with increased fasting glucose and glycated hemoglobin (HbA1c) levels, and with increased overnight urinary cortisol. These factors have been associated with increased allostatic load and poor health. Thus, vagal activity seems to have an inhibitory function in the regulation of allostatic systems. The prefrontal cortex and the amygdala are important central nervous system structures linked to the regulation of these allostatic systems, including inflammation via the vagus nerve. The next section describes evidence for the prefrontal regulation of inflammation.

**Prefrontal cortical activity and immune indices**

Ohira et al used neuroimaging to explore the association between the brain and immune function. Their study examined the neural basis of the top-down modulation accompanying cognitive appraisal during a controllable or uncontrollable acute stressor. HR and blood pressure increased significantly during a mental arithmetic task and returned to baseline soon after termination of the task. HR increased to a greater extent in the controllable versus the uncontrollable condition; blood pressure was unaffected by controllability. Endocrine and immune indices were also affected by the acute stress task: the proportions of natural killer cells increased and helper T cells decreased acutely during the stressor.

Importantly, cerebral blood flow measurements demonstrated that the areas of the prefrontal cortex that we have found to be associated with HRV, including the medial prefrontal cortex and the insula, were also associated with immune indices (medial and lateral orbitofrontal cortices and insula), suggesting prefrontal or frontal modulation of immune responses possibly via the same vagal pathways.

**Vagal activity and cardiovascular risk factors**

The regulation of physiologic systems that are important for health and disease has been linked to vagal function and HRV. We have recently reviewed the literature on the relationship between vagal function and the risk for cardiovascular disease and stroke. The National Heart, Lung, and Blood Institute lists eight risk factors for heart disease and stroke. Six are considered modifiable. Of the six modifiable factors, three are associated with what could be called biologic factors: high blood pressure (hypertension), diabetes, and abnormal cholesterol; the other three could be considered lifestyle factors: tobacco use (smoking), physical inactivity (exercise), and overweight (obesity). Two factors, age and family history of early heart disease or stroke, are considered nonmodifiable. At least some data suggest that each of these risk factors is associated with decreased vagal function as indexed by HRV.

Interventions to modify HRV include exercise, ingestion of omega-3 fatty acids, stress reduction (eg, mediation), pharmacologic manipulations, and vagus nerve stimulation, suggesting that methods that increase vagus nerve activity might favorably modify an individual’s risk profile.
CONCLUSION

The brain and the heart are intimately connected. Both epidemiologic and experimental data suggest an association between HRV and inflammation, including similar neural mechanisms. Evidence of an association between HRV and inflammation supports the concept of a cholinergic anti-inflammatory pathway.

REFERENCES


Correspondence: Julian F. Thayer, PhD, Department of Psychology, 133 Psychology Building, 1835 Neil Ave., Columbus, OH 43210; thayer.39@osu.edu
Inflammation, atherosclerosis, and arterial thrombosis: Role of the scavenger receptor CD36

ABSTRACT

The CD36 scavenger receptor recognizes oxidized low-density lipoprotein (LDL) and cell-derived microparticles. It is expressed on macrophages and platelets and is a mediator of both atherogenesis and thrombosis. Macrophages from CD36-null mice have a defect in foam cell formation in response to exposure to oxidized LDL, and CD36-null mice fed an atherogenic Western diet have significantly less atherosclerosis than their wild-type counterparts. On platelets, CD36 recognition of oxidized LDL contributes to their activation and provides a mechanistic link between hyperlipidemia, oxidant stress, and the prothrombotic state. Cell-derived microparticles are also major ligands for CD36 and contribute to thrombus formation in a CD36-dependent manner even in the absence of hyperlipidemia. CD36 deficiency in mice is associated with inhibition of thrombus formation and with a reduction in microparticle accumulation in thrombi. Targeting CD36 is a promising avenue for the treatment of atheroinflammatory disorders.

Atherosclerosis is recognized as a chronic inflammatory disorder of the vessel wall. Four categories of evidence support the model of atherosclerosis as an inflammatory disease:

- Biomarkers of inflammation are clearly associated with risk and prognosis of atherosclerosis. Three that have been linked conclusively are: C-reactive protein, myeloperoxidase (a marker of leukocyte activation), and antibodies to oxidative modifications of low-density lipoprotein (LDL).
- Tissue studies demonstrate that leukocytes and products of the inflammatory system are prevalent in atherosclerotic plaque.
- Animal models show an absence of atherosclerosis in the absence of monocytes or monocyte recruitment as well as a crucial role for T-cell–derived proinflammatory cytokines.
- It is becoming apparent that patients with chronic systemic inflammatory disorders (eg, systemic lupus erythematosus, Wegener granulomatosis, chronic obesity, and aging) have increased risk of atherosclerosis.

This article examines the mechanisms by which inflammation promotes the development of atherosclerosis and coronary artery disease, with particular attention to the role of CD36, a scavenger receptor for oxidized LDL.

OXIDATION IN PLAQUE FORMATION

Prevailing models that link inflammation to plaque formation suggest that inflammatory stimuli (eg, cigarette smoke, hypertension) provoke changes in the phenotype of the cells of the arterial vessel wall that allow penetration of leukocytes and LDL particles across the endothelial barrier, trapping them in the subendothelial space. An inflammatory reaction then occurs in the subendothelial space involving monocytes/macrophages and lymphocytes (especially T cells). Ultimately, through the production and release of oxidizing enzymes such as myeloperoxidase and nitric oxide synthase, the reaction leads to generation of reactive oxygen and nitrogen species. In this setting, LDL particles become modified to a form known as oxidized LDL (oxLDL). OxLDL loses its ability to bind to LDL receptors, which interferes with its normal processing; perhaps more important, oxLDL gains an affinity for a family of proteins called scavenger receptors. Scavenger receptors on macrophages bind and internalize the oxLDL particles, leading to accumulation of cholesterol and other lipids in the cells. Over prolonged periods, increasing...
quantities of oxLDL become internalized, leading to formation of foam cells (lipid-laden macrophages), the precursor to atherosclerotic plaque. These lipid-laden cells are more prone to apoptosis, which further contributes to plaque growth and rupture.

**CD36: A CRITICAL SCAVENGER RECEPTOR**

One of the most critical scavenger receptors on macrophages is CD36, which is a transmembrane glycoprotein that crosses the membrane twice. CD36 is expressed heavily on monocytes, macrophages, dendritic cells, fat, muscle, capillary endothelial cells, and platelets. It has multiple physiologic functions, including acting as a high-affinity receptor for specific oxidized phospholipids that are found within oxLDL. It is also a receptor for phosphatidyl serine (PS) and oxidized PS (oxPS) that is expressed on the surface of apoptotic cells. CD36 is highly conserved in evolution; orthologs are even found in flies, worms, and sponges. Evidence suggests that CD36 and other scavenger receptors probably evolved as part of the innate immune system as recognition molecules for pathogens and pathogen-infected cells.

An interesting aspect of CD36 biology is that its expression on macrophages is increased when the cells are exposed to oxLDL. Among the changes that occur in the lipid components of LDL when it is oxidized is the formation of oxidized fatty acids such as 9- and 13-hydroxy octadecadienoic acid (HODE). These oxidized fatty acids are ligands for the nuclear hormone receptor peroxisome proliferator–activated receptor (PPAR) gamma, a transcription factor that regulates expression of many genes, including CD36. Thus, oxLDL promotes increased expression of CD36 and further cellular uptake of oxLDL. This feed-forward loop presumably accelerates foam cell formation in the arterial neointima. Furthermore, CD36 expression is upregulated at the transcriptional level by inflammatory cytokines such as granulocyte macrophage colony-stimulating factor (GM-CSF), macrophage colony-stimulating factor (M-CSF), and interleukin-4. Hyperglycemia increases CD36 expression through a nontranscriptional mechanism and may contribute to the proatherosclerotic state associated with diabetes.

**CD36 mediates atherogenesis**

The pathogenic role of oxLDL in atherosclerosis is largely dependent on CD36. Studies using macrophages from genetically altered mice developed in our laboratory that do not express CD36 demonstrated that absence of CD36 expression was associated with a lack of foam cell formation in vitro when cells were exposed to oxLDL. Wild-type mice, in contrast, showed foam cell formation after 12 to 24 hours.

To demonstrate in vivo relevance of these findings, we crossed CD36-null mice with proatherogenic apoE-null mice. When fed an atherogenic Western diet, apoE-null mice develop aortic atherosclerosis within several weeks, in a pattern and histology that closely resembles the human disease. In our experiment, the mice that lacked both CD36 and apoE had a dramatic decrease in the volume of atherosclerosis. Further studies showed that the proatherogenic role of CD36 was highly mediated by the CD36 on macrophages, since transplantation of bone marrow from CD36-null mice into apoE-null mice had the same effect on atherosclerosis as seen in the apoE/CD36-double-null mice.

Scavenger receptor–dependent formation and progression of atherosclerosis is supported by findings of an abundance of oxidized phospholipids that serve as binding partners for CD36 in the plaque region of blood vessels, along with an absence of oxidized phospholipids in the nonplaque region of blood. The enrichment of oxidized phospholipid in the plaque allows CD36 to penetrate the plaque, whereas removal of CD36 drastically decreases the progression of atherosclerosis.

**Platelet activation in the setting of hyperlipidemia**

In addition to the formation and progression of plaque, CD36 may be involved in the terminal phases of atherosclerosis (ie, the thrombosis that occurs on a plaque) as a result of abundant CD36 expression on platelets. CD36, in fact, was discovered as a platelet protein and named platelet glycoprotein IV, although for many years the function of CD36 on platelets was not known.

Recent studies by Podrez and colleagues, along with our group, revealed that oxLDL binds to the surface of platelets in a concentration-dependent manner, whereas normal LDL does not. The binding of oxLDL to platelets can be blocked almost completely by inhibiting CD36 with an antibody; binding did not occur with platelets obtained from CD36-deficient mice or people. Importantly, exposure to oxLDL caused platelets to be activated via a highly specific cell-signaling pathway; low concentrations of oxLDL, such as those found in plasma of individuals with even modest hyperlipidemia, made platelets more sensitive to low doses of “classic” platelet agonists such as collagen and adenosine diphosphate (ADP). These studies suggest that platelet CD36 could serve as a mechanistic link between inflammation, oxidant stress, and hyperlipidemia to create a prothrombotic state.
It has been known for some time through the work of Eitzman and others that apoE-null mice fed a Western diet are “hypercoagulable”; ie, they have shortened thrombosis times.9 This observation led us to investigate the role of CD36 in the hyperlipidemia-associated prothrombotic state. In one experiment, tail-vein bleeding times were measured in apoE-null and apoE/CD36-double-null mice fed a high-fat diet. Whereas the apoE-null animals had markedly shortened bleeding times (~ 2 minutes), the double CD36/apoE-null animals were normal (~ 6–8 minutes).

To examine a model more reflective of pathologic thrombus formation (eg, heart attack, stroke), we induced carotid artery injury in mice by topical application of ferric chloride. This method induces oxidant injury to the endothelium and causes platelet-dependent carotid occlusion. With this model, thrombosis can be monitored in “real time” with a Doppler flow probe and video microscope. As with tail-vein bleeding time, we found that time to carotid occlusion was much shorter in apoE-null mice fed a high-fat diet than in mice fed a normal chow diet or in wild-type mice; further, this prothrombotic state was rescued by genetic ablation of CD36 expression.7

Possible role in thrombus formation
More recent experiments from our lab have shown that CD36-null mice fed a normal chow diet have a subtle defect in thrombus formation when arteries or veins are subjected to relatively mild injury.10 This finding implies a potential role for CD36 in “normal” platelet function and perhaps the existence of an endogenous ligand for CD36 that is unrelated to hyperlipidemia. Since we know that CD36-null mice and CD36-deficient people do not have a bleeding disorder and have normal bleeding times, it is possible that pharmacologic targeting of CD36 may provide a means to inhibit thrombosis without having a major impact on hemostasis.

The possibility that mice or humans can be protected from developing thrombi by blocking CD36 function is supported by initial data obtained from the carotid artery injury model in mice. In the laboratory, an antithrombotic state can be created by blocking the specific CD36-signaling pathway described below. Thrombocytopenic wild-type mice transfused with platelets from wild-type mice exhibit a dramatic increase in thrombosis time when the donor platelets are pretreated with a CD36-signaling inhibitor.8 This protective effect vanishes when the same experiment is performed in CD36-null mice.

### MICROPARTICLES: MAJOR LIGAND FOR CD36

Based on the experiments described above, we hypothesized the existence of endogenous CD36 ligands involved in thrombosis and proposed that cell-derived microparticles (MPs) were likely candidates. MPs are small (200–1,000 nm) phospholipid vesicles that “bud” off from cells as a result of stimulation or

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**FIGURE 1.** A mouse carotid artery (in cross section) injured with ferric chloride and then analyzed using immunofluorescent microscopy and an antibody to the endothelial cell–specific antigen CD105. The blue dots represent the nuclei in the vessel (stained with DAPI). In the top panel, a carotid thrombus in the lumen of a wild-type mouse is heavily enriched with CD105 (stained red), implying microparticle incorporation. In the CD36-null mouse (bottom panel), CD105 staining is decreased dramatically. The green staining represents an antibody to the platelet-specific antigen CD61.10

apoptosis. MPs can be derived from endothelial cells, leukocytes, cancer cells, and platelets; they contain selected membrane receptors as well as other proteins inherent to their parental cell (eg, MPs derived from a white cell contain tissue factor that can activate the coagulation cascade). MPs are known to circulate in patients with chronic inflammatory disorders, including acute coronary syndromes, lupus erythematousus, Wegener granulomatosis, and rheumatoid arthritis, and their number probably increases with aging.

Our hypothesis is based on the well-known observation that a major feature of MP generation is a loss of membrane asymmetry; that is, the PS normally expressed on the inner limit of the membrane instead is expressed on the surface. Previous studies from our lab and others had shown that PS and oxPS can be a ligand for CD36.11

To test our hypothesis we developed a rapid flow cytometry assay using an antibody to CD105, an antigen expressed only on endothelial cells, to detect an interaction between endothelial cell–derived MPs and platelets. This interaction is CD36-dependent in that it can be blocked with antibodies to CD36 and does not occur if platelets are taken from mice or humans who are CD36-deficient.10 MPs behaved like oxLDL in that platelets pretreated with MPs undergo a dramatic augmentation of aggregation in response to low doses of classic agonists. This augmentation of platelet activation does not occur in platelets from CD36-null donors or CD36-null mice.10

**Microparticle accumulation in thrombi**

To confirm a role for the platelet-MP interaction during thrombus formation, we examined CD105 antibody staining in thrombi induced in carotid arteries in our mouse models. We found that the endothelial cell–specific CD105 antigen accumulated in the thrombi formed in wild-type mice, but that staining was dramatically reduced in the thrombi formed in CD36-null animals (Figure 1).10

**Specific cytoplasmic signaling cascade**

When CD36 binds to its ligands (oxLDL or MPs), it transmits a signal to the cell. In macrophages this signal leads to oxLDL internalization and foam cell formation, while in platelets it contributes to platelet activation and aggregation. In a series of studies from our laboratory and others, it has been shown that these signals are relayed by a series of molecular interactions that involve specific tyrosine kinases from the Src family and serine/threonine kinases from the mitogen-activated protein (MAP) kinase family.12 The signal to the platelet is mediated by a MAP kinase called c-Jun N-terminal kinase (JNK). Carotid thrombi in wild-type mice stain for the presence of the activated, phosphorylated form of JNK, whereas phospho-JNK expression is decreased by 50% to 60% in carotid thrombi in CD36-null mice,13 similar to the decrease in MP mass in thrombi from CD36-null mice.

**CONCLUSION**

These experiments suggest that CD36 has both a proatherogenic and a prothrombotic role in the vascular system. Macrophage CD36 promotes foam cell formation and plaque formation. Platelet CD36 promotes thrombosis by signaling in response to oxLDL and by phospholipids present in cell-derived MP. Therefore, targeting CD36 or CD36-signaling pathways could be a strategy in the treatment of athero-inflammatory disorders and deserves exploration.

**REFERENCES**


Correspondence: Roy L. Silverstein, MD, Department of Cell Biology, Cleveland Clinic, 9500 Euclid Avenue, NC10, Cleveland, OH 44195; silverrr2@ccf.org
ABSTRACT

Researchers ordinarily work by deriving testable hypotheses from theories using a deductive process. Hypothesis testing is inherently biased, however, because of the practical requirements of finding and publishing positive results. In contrast, ignorance isn't biased. The combination of relevant new technology, sufficient mastery of the topic to know what is not yet known, and access to patients with rare but informative disorders sets the stage for discoveries about disease mechanisms based on induction from observations. Patient-oriented research is a strength of heart-brain medicine. Patients are a unique scientific resource because they tell us the truth. We experience the joy and thrill of a “sparkle of insight” when we realize what they teach.

This is a momentous occasion for me, for the extraordinary people in the Clinical Neurocardiology Section at the National Institutes of Health (NIH), and for my family—my wife Minka and son Joey drove all the way from Maryland late last night and early this morning to be here. I thank them publicly here.

THE ‘SPARKLE OF INSIGHT’ FROM ENLIGHTENED INDUCTION

In these brief comments, as I look back on the road I have taken over the past 40 years carrying out patient-oriented research in heart-brain medicine, I would like to convey a viewpoint instead of dwelling on the presentation of research data.

The idea I wish to convey is that ignorance isn’t biased. If you have a hypothesis you want to test, you are inherently biased to find something positive—and, if you are in academic medicine, publishable—in the data you obtain. But if you have the technical capability to measure something no one else can measure, and you have sufficient mastery of the topic to know what is not yet known, then if you make an observation that you did not predict and if you recognize its significance, you have made a discovery. You have revealed a bit of the truth. You experience the highest joy and thrill a scientist can feel—a “sparkle of insight.” When this happens, if you have sense, you stop what you have been doing to pursue that discovery.

Hardly anyone has received a Nobel Prize for testing a theory, but many Nobel Prizes have been awarded for technological advances and for discoveries based on those advances. In my view, discoverers use an enlightened inductive approach at least as much as deduction. They develop new technology that enables key novel measurements, and they keep in mind gaps in knowledge, so that they are ready to appreciate the significance of their observations.

A PERSONAL EXAMPLE

‘You have to measure something’

Let me share an example of this process by relating a sparkle of insight I had several years ago. When I began working at the NIH, I met with the chief of the Hypertension-Endocrine Branch of the National Heart, Lung, and Blood Institute about the research program I would pursue. After listening patiently to me for many minutes as I spouted about how I was going to test hypotheses derived from the concepts that people with hypertension are “hyper-tense,” and that stress causes heart disease, the chief responded, “Well, these ideas are all well and good. But what are you going to measure? You can measure whatever you want, but you have to measure something.”

Measure something. I wanted to see if there was hyperactivity of the sympathetic nervous system or excessive sympathetic innervation in hypertension, and I started working on ways to measure sympathetic activity.

The sympathetic nervous system at a glance

First I should introduce you to the sympathetic nervous system, which is one of the main effectors by
which the brain regulates the heart and blood vessels. It is a key link between the brain and heart. The sympathetic nerves to the heart and other organs do not come directly from the brain but from ganglia, which are clumps of nerve cell bodies strung like pearls on a necklace on each side of the spinal column. This origin outside the central nervous system will be an important fact to keep in mind.

In the heart, the sympathetic nerves travel with the coronary arteries and then dive into the heart muscle from the outside. Sympathetic nerves also enmesh the walls of arteries and arterioles. The arterioles constitute the main determinant of total peripheral resistance to blood flow in the body and therefore figure prominently in the control of blood pressure. The architectural association between sympathetic nerves and the muscle in the heart and arteriolar walls has enticed hypertension researchers for many decades.

A false start with plasma norepinephrine measurement
I developed novel methods for measuring plasma levels of norepinephrine, which is the chemical messenger that the sympathetic nervous system uses in regulation of the circulation, and of adrenaline (epinephrine), which is the well-known and potent “fight-or-flight” hormone.\(^1\) Applying this technology to patients with high blood pressure led to several publications\(^2\)–\(^9\) but actually shed more heat than light on the hypothesis of sympathetic hyperactivity as a cause of or contributor to hypertension. In the face of negative data, the theory was qualified—sympathetic hyperactivity might be apparent only in the young, or the thin, or the Caucasian, or the male—but not abandoned.

Insights from visualizing sympathetic nerves in the heart
Then I embarked on a project to visualize sympathetic nerves in the heart, by a new technology called positron emission tomographic (PET) scanning. With several colleagues—including Irwin J. Kopin, Graeme Eisenhofer, Peter Chang, David Hovevey-Zion, Ehud Grossman, and Courtney Holmes—to whom I will always be grateful, I developed a PET imaging agent called 6-\(^{18}\text{F}\)fluorodopamine.\(^10\)–\(^13\)

After injection of 6-\(^{18}\text{F}\)fluorodopamine into a person’s vein, PET scan slices of the chest reveal the sympathetic nerves in the heart (Figure 1). The top row of Figure 1 shows where the blood is going—perfusion—in four people, and the bottom row shows the 6-\(^{18}\text{F}\)fluorodopamine scans in the same people. The horseshoe-shaped structure is the main pumping muscle of the heart, the left ventricular myocardium. The “blob” on the patient’s right is the liver.
Normally, PET scans using 6-[18F]fluorodopamine look remarkably similar to scans using 13N-labeled ammonia, a perfusion imaging agent. The first patient I studied with this new technology was a patient with a rare disease called pure autonomic failure (PAF). In PAF, there was already good evidence for a loss of sympathetic nerves throughout the body. Myocardial perfusion in this patient was normal, but there was much less than normal 6-[18F]fluorodopamine-derived radioactivity in the heart muscle. In another uncommon disease, multiple system atrophy (MSA), the perfusion was also normal, and the cardiac sympathetic nerves seemed intact, in line with what was already known about this progressive neurodegenerative disease.

Then I tested a patient who had been thought to have MSA but actually had Parkinson disease (PD) with orthostatic hypotension (a fall in blood pressure each time the person stands up). PD with orthostatic hypotension can be very difficult to distinguish from the parkinsonian form of MSA. To my complete surprise, the patient with PD had a remarkable decrease in 6-[18F]fluorodopamine-derived radioactivity in the heart muscle. There was normal blood flow to the heart muscle, so the 6-[18F]fluorodopamine was being delivered, but there was no evidence of sympathetic nerves in the heart. The scans resembled those in the PAF patient, not the MSA patient.

This finding did not arise from a prediction to test a hypothesis. It wasn’t long before I tested additional PD patients and found the same unexpected results.13,14 Because I was ignorant, I wasn’t biased. I felt I had put my finger on a piece of the truth, and I had to stop and think about the implications of this discovery. I never did come to test the hypotheses that I had sought out originally to test. Instead, I followed a totally new path, based on the discovery of cardiac sympathetic denervation in PD.

### Beyond a brain disease: Seeing PD as a heart-brain disorder

More than 50 neuroimaging studies since our original report have agreed remarkably consistently on the association between PD and loss of sympathetic nerves in the heart; moreover, postmortem pathology studies have amply confirmed that a profound loss of cardiac sympathetic nerves is characteristic of PD.16 I have yet to come across a single patient with PD and orthostatic hypotension who has not had cardiac sympathetic denervation, and virtually all patients with PD who do not have orthostatic hypotension seem to have at least partial loss of cardiac sympathetic nerves.

Considering that the source of those nerves is the ganglia, which lie outside the central nervous system, PD must be more than a brain disease and more than a movement disorder. It must also be a disease of the sympathetic nerves in the heart, a form of a dysautonomia, and a heart-brain disorder.

### The role of catecholamines

#### Another discovery born of unbiased ignorance

To appreciate fully the significance of this finding, I must mention my favorite chemical family, the catecholamines, whose chemical structures resemble cats (Figure 2).15-17 PD results from a loss of a particular chemical, dopamine, in a particular pathway in the brain; dopamine is a catecholamine. The other catecholamines in humans are norepinephrine and adrenaline. As noted above, norepinephrine is the chemical messenger of the sympathetic nerves, and adrenaline is the well-known hormone that produces many of the signs of emotional distress.

Almost a half century ago, Hornykiewicz and colleagues made the pivotal discovery that PD features loss of dopamine in the nigrostriatal system in the brain.18 Given the cardiac sympathetic denervation, PD might be a disease of catecholamine systems both inside and outside the central nervous system—dopamine in the nigrostriatal system, and norepinephrine in the sympathetic nerves of the heart.

Then what of the third catecholamine, adrenaline, in PD? Plasma levels of adrenaline and of its metabolite, metanephrine, are normal in PD, even in patients who have PD and orthostatic hypotension, which involves loss of norepinephrine-producing nerves not only in the heart but in other organs.19 What is different about the adrenaline-producing cells in the...
medulla (from the Latin for “marrow”) of the adrenal glands atop each kidney? Why aren’t these catecholamine-producing cells also lost in PD?

I have some ideas in mind but won’t go into them here. The point is that the discovery of normal adrenomedullary cells in PD, despite loss of cells producing the other catecholamines, was not based on my testing a hypothesis. It was a discovery born of ignorance, and because ignorance isn’t biased, that discovery points to the truth. Whatever the eventual explanation for the specific pattern of catecholamine cell loss in PD, it cannot refute the discovery itself.

**HOW DISCOVERIES ARISE: AN APPLIED EXERCISE FOR READERS**

Now let’s have you, the reader, make a discovery and induce its significance based on what I have tried to teach so far, that discoveries arise from the application of relevant technology and from insights of the prepared mind. Take a look at the scans in the left panels of Figures 3 and 4. The large red structures in Figure 3, which look like sad clown’s eyes, correspond to the striatum. The striatum is made up of the putamen, which is like the mascara on the side of the sad clown’s eyes, and the caudate, which is like the beady eyes themselves. In the left panel of Figure 4, the small spots in the midbrain correspond to the substantia nigra, a major site of dopamine-producing neurons in the human brain.

We can see in the right panel of Figure 3 that in PD there is a loss of the ability to store dopamine in the striatum—especially in the putamen, the mascara of the sad clown’s eyes. In the right panel of Figure 4 we see that in the brainstem there is a loss of the dopamine-containing nerve cells in the substantia nigra. These scans therefore demonstrate graphically the nigrostriatal lesion characteristic of PD. There is a loss of the nerve cells in the substantia nigra in the midbrain and a loss of the dopamine-containing terminals in the striatum.

Now take a look at the scans of these areas in a patient with PAF in Figure 5. Remember that PAF involves a loss of sympathetic nerves in the heart, just like in PD, but that PAF does not involve parkinsonism. Look at the sad clown’s eyes. The mascara is there, of course, because the patient does not have parkinsonism. But now look for the spots in the substantia nigra—they are missing, just as in PD.

PAF is a rare disease, and I have only studied several cases with high-resolution PET scanning of the brain, but so far they have all had this unexpected, unpredicted finding of loss of dopaminergic neurons in the substantia nigra.

What does this pattern mean? If PAF patients have just as much loss of nigral neurons as PD patients do, and if PAF patients do not have parkinsonism, then the movement disorder in PD cannot result from loss of the dopamine neurons in the substantia nigra per se. Instead, the movement disorder in PD seems to come from loss of the dopaminergic terminals in the striatum.

How can PAF patients have normal dopamine terminals in the putamen when the number of dopaminergic...
cell bodies is severely reduced? Somehow, PAF patients must be able to sprout new terminals, even as they lose the cell bodies. Maybe if we knew how PAF patients do this, we would have a way to treat or even prevent PD.

How do PAF patients maintain normal dopamine terminals as the cell bodies die off? No one knows. Until now, no one thought of asking such a question. No one hypothesized that this discovery would be made, but it was. And because ignorance isn’t biased, we have put our finger on the truth. By keeping in mind what isn’t known, we could see what wasn’t there. Now we can begin to think of what to look for next.

**SUMMARY AND CONCLUSIONS**

Because ignorance isn’t biased, if you have the tools to make relevant measurements, if you have sufficient mastery of the subject to know what isn’t known, and if you have access to patients with rare but informative disorders, you can make important discoveries based on inductions from observations.

The discoveries that cardiac sympathetic denervation characterizes PD and that parkinsonism does not result from loss of dopamine neurons per se depended crucially on studying patients with a rare disease, PAF. In 1657, William Harvey—the same William Harvey who first described the circulation of the blood and who first pointed out the effects of emotions on the heart—wrote eloquently about the extraordinary power of studying patients with rare diseases:

Nature is nowhere accustomed more openly to display her secret mysteries than in cases where she shows traces of her workings apart from the beaten path; nor is there any better way to advance the proper practice of medicine than to give our minds to the discovery of the usual law of nature, by the careful investigation of cases of rarer forms of disease. For it has been found in almost all things, that what they contain of use or of application, is hardly perceived unless we are deprived of them, or they become deranged in some way.

I hope I have convinced you of the importance of seeing what isn’t there. My thanks go out again to the Earl and Doris Bakken Heart-Brain Institute for this prestigious award, to my family, to my colleagues and friends, and to my patients. As I have written in Adrenalin and the Inner World: An Introduction to Scientific Integrative Medicine, patients serve as a unique scientific resource. They report what is wrong; they tell us the truth. We have to make sense of what they teach.

**REFERENCES**


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**FIGURE 5.** High-resolution positron emission tomographic scans, superimposed over magnetic resonance images, at the levels of the basal ganglia and midbrain, after intravenous administration of 6-[18F]fluorodopa in four subjects:
- a normal volunteer (upper left)
- a control patient without parkinsonism or autonomic failure (upper right)
- a patient with Parkinson disease (PD) (lower left)
- a patient with pure autonomic failure (PAF) (lower right).

Red indicates the maximum amount of radioactivity. Note the severely decreased 6-[18F]fluorodopa-derived radioactivity bilaterally in the region corresponding to the substantia nigra in both PD and PAF. Adapted from Goldstein et al.20


Correspondence: David S. Goldstein, MD, PhD, Building 10, Room 6N252, 10 Center Drive, MSC-1620, Bethesda, MD 20892-1620; goldsteind@ninds.nih.gov
Heart rate variability with deep breathing as a clinical test of cardiovagal function

ABSTRACT

Research into heart rate variability (HRV) and respiration over the past 150 years has led to the insight that HRV with deep breathing (HRVdb) is a highly sensitive measure of cardiovagal or parasympathetic cardiac function. This sensitivity makes HRVdb an important part of the battery of cardiovascular autonomic function tests used in clinical autonomic laboratories. HRVdb is a reliable and sensitive clinical test for early detection of cardiovagal dysfunction in a wide range of autonomic disorders.

Heart rate variability (HRV) has been a focus of interest in cardiovascular physiology for more than 150 years. This review will briefly survey the history of research linking HRV to respiration and then explore the clinical significance of this linkage, with a focus on HRV with deep breathing.

HRV AND RESPIRATION: THE EARLY RESEARCH

The first report linking HRV to respiration has been credited to Karl Ludwig, who in 1847 noted that heart rate increased with inspiration and decreased with expiration.1,2 The precise origin of this variability has been studied extensively, but a single unifying mechanism defining the determinants of HRV with respiration has not been established. However, several mechanisms have been identified that may be contributing to HRV. Hering in 1871 noted in dog experiments that inflation of the lungs was associated with a tachycardia and that additional higher-pressure insufflation resulted in a bradycardia. He concluded that HRV was determined by pulmonary reflexes.2,3 Bainbridge observed in dog experiments in 1915 that the heart rate increased during the diastolic filling of the heart that occurred during inspiration.4 In a subsequent article, published in 1920, Bainbridge attributed HRV to this reflex, which now carries his name.5

There is also evidence that HRV may be caused by central nervous system mechanisms. Canine experiments have revealed that rhythmic variations in the heart rate and ventricular pressure waves may coincide with rib cage movements in innervated, isovolumetric, left ventricular preparations.6 These data are consistent with radiation of respiratory center activity to the cardiovascular autonomic centers in the medulla resulting in HRV. There is also evidence that stretch of the right atrium and sinus node region may produce HRV via cardiac reflexes.7 It is likely that all of these mechanisms are contributing at some level to the HRV that is observed with respiration.

INSIGHTS INTO CLINICAL IMPLICATIONS

Clinical interest in HRV was sparked by the 1973 report of Wheeler and Watkins, who first drew attention to cardiac vagal innervation as the mediator of HRV and its potential value as a clinical test of cardiovagal function.8 These investigators studied HRVdb in normal subjects and diabetic subjects, some with and some without evidence of autonomic neuropathy. They noted that HRVdb was abolished by atropine, implying that the efferent component of the reflex is vagally mediated (Figure 1). They also noted that HRVdb was reduced or abolished in diabetic subjects with autonomic neuropathy. They concluded that HRVdb was a clinically useful test for autonomic neuropathy in diabetic patients.

The relationship between vagal tone of the heart and HRV was further explored by Katona and Jih, who in 1975 reported on their experiments in a canine model.9 They found a linear relationship between HRV as assessed by variations in heart period and parasympathetic control of the heart, defined as the difference in the average heart rate before and after complete abolishment of vagal innervation (Figure 2). They concluded that the magnitude of the respiratory HRV is a measure of parasympathetic cardiac control. Fouad and colleagues duplicated this experiment in humans and found a similar linear relation-

Dr. Shields reported that he has no financial interests or relationships that pose a potential conflict of interest with this article.

doi:10.3949/ccjm.76.s2.08
HEART RATE VARIABILITY WITH DEEP BREATHING

Ship between HRVdb and parasympathetic cardiac control, leading them to conclude that HRVdb is an accurate index of cardiac vagal tone (Figure 3).10

METHODS OF MEASURING HRV

A wide variety of methods have been developed to measure HRV.11,12 Some of the methods employ statistical analysis, typically of prolonged recordings of 24 hours or longer. These methods include simple statistics such as the standard deviation of the heart rate or the R-R interval as well as more complex statistical measures such as the mean squared successive difference of the R-R intervals. These methods have been applied mostly to the analysis of prognosis following acute myocardial infarction. Reduced HRV has been established as a powerful predictor of mortality and arrhythmic complications following acute myocardial infarction.11 The methods developed for clinical tests of cardiovagal function typically involve measuring HRVdb over short intervals (< 90 sec). Deep breathing magnifies HRV with respiration, allowing for methods to assess HRV with respiratory cycles.

The two most widely used methods are the mean heart rate range (MHRR) and the expiratory-to-inspiratory ratio (E:I). The MHRR method is typically measured from a series of successive deep breaths, usually at least 6 breaths at a rate of 5 or 6 breaths per minute. The MHRR is calculated by subtracting the maximum heart rate during inspiration from the minimum heart rate during expiration for each cycle of breathing, and then determining the mean of these differences (Figure 4).12 The MHRR can also be measured from a single breath.13

The E:I ratio assesses the ratio of the longest R-R interval during expiration to the shortest R-R interval during inspiration.12 The E:I ratio may also be assessed from a

FIGURE 1. Heart rate variability with deep breathing in a healthy 30-year-old man under normal conditions (top panel) and after administration of intravenous propranolol (middle panel) and atropine (bottom panel).8 Note how atropine abolishes the heart rate variability. Arrows indicate periods of deep breathing. Reprinted from British Medical Journal (Wheeler T, Watkins PJ. Cardiac denervation in diabetes. Br Med J 1973;4:584–586) with permission from the BMJ Publishing Group.

FIGURE 2. There is a linear relationship (correlation coefficient = 0.986) between respiratory variations in heart period and parasympathetic control, defined as the difference in the heart period before and after parasympathetic block. Data are from a series of experimental states in the canine; control (cross), propranolol block (triangle), propranolol block with phenylephrine HCl (square), and atropine (diamond).9 Reprinted, with permission, from Journal of Applied Physiology (Katona PG, Jih F. Respiratory sinus arrhythmia: noninvasive measure of parasympathetic cardiac control. J Appl Physiol 1975;39:801–805).
single breath or the mean of successive breaths.14 Analysis of HRV has also been studied in the frequency domain by using Fourier transformation and converting heart rate to a power spectrum.15,16 The peak power at the highest frequencies (> 0.15 Hz) reflects respiratory sinus arrhythmia, while the lower frequencies reflect both sympathetic and parasympathetic influences. In a comparison of low-frequency power, high-frequency power, and total power to standard methods of measuring HRVdb, all of these spectral measures were proven to be strong predictors of the results from the standard methods.16 Marked reduction in the power spectrum was noted in patients with diabetic autonomic neuropathy (Figure 5).16

**FACTORS THAT AFFECT HRV WITH DEEP BREATHING**

Many variables may affect HRVdb.12 HRVdb is influenced by age, as the variability decreases with advancing age, so it is essential to use methods with well-defined age-stratified normal values. HRVdb is maximal when the patient is lying supine and breathing at a rate of 5 to 6 breaths per minute. The depth of breathing for a maximum result requires a tidal volume of approximately 1.2 L for an average adult. Protocols that involve breathing for more than 90 seconds may induce hypocapnea, which can reduce HRVdb. Most importantly, numerous medications can affect HRVdb. Medications with anticholinergic activity, including over-the-counter cold medications, tricyclic antidepressants, and antispasmodics, should be discontinued at least 48 hours prior to testing, if possible. Patients are also instructed to not drink caffeinated beverages, use nicotine, or drink alcohol 3 hours prior to testing.

**CLINICAL APPLICATIONS**

HRVdb represents a very sensitive measure of cardiovascular or parasympathetic cardiac function and thus is an important component of the battery of cardiovascular autonomic function tests used in clinical autonomic laboratories. In most autonomic disorders, parasympathetic function is affected before sympathetic function, so HRVdb provides a sensitive screening measure for parasympathetic dysfunction in many autonomic disorders. HRVdb has proven to be a sensitive and reliable clinical test for the early detection of cardiovagal dysfunction in a wide spectrum of autonomic disorders, including diabetic autonomic neuropathy,14 uremic neuropathy,17 familial autonomic neuropathies,18 and various small fiber neuropathies.19,20 HRVdb has also been valuable in assessing patients with pure autonomic failure,21 multisystem atrophy,22 and other central neurodegenerative disorders.23
HEART RATE VARIABILITY WITH DEEP BREATHING

FIGURE 5. Power spectrum of (A) the normal resting heart rate and (B) the resting heart rate of a diabetic patient with severe autonomic dysfunction. Note the severe loss of power at all frequencies for the patient with severe autonomic dysfunction (note the lower y-axis scale of the power spectrum for this patient).

References


Correspondence: Robert W. Shields, Jr, MD, Neuromuscular Center, Cleveland Clinic, 9500 Euclid Avenue, S90, Cleveland, OH 44195; shieldr@ccf.org
Basic research models for the study of underlying mechanisms of electrical neuromodulation and ischemic heart-brain interactions

- ABSTRACT

The study of mechanisms of action underlying the use of electrical neuromodulation for angina and myocardial ischemia may illuminate heart-brain interactions that influence these conditions. To investigate these mechanisms of action, we initiated a neurocardiology program in the 1990s. This review discusses the experimental models we have studied to unravel the heart-brain interactions involved in the use of electrical neuromodulation for ischemic disease.

- RATIONALE

In the industrialized world, average life expectancy has nearly doubled since the 19th century. One of the consequences of this increase in life span is that the sequelae of diseases also have increased. For coronary artery disease (CAD), one of the most prevalent diseases in the western world, this has resulted in an amplification of the number of patients suffering from heart failure, arrhythmias, and refractory angina. Much progress has recently been made in nonpharmacologic therapies for these deleterious consequences of CAD, such as cardiac resynchronization for heart failure, implantable defibrillators for ventricular arrhythmias, and electrical neuromodulation by means of spinal cord stimulation for chronic angina that is refractory to conventional strategies.

For patients suffering from severe angina secondary to end-stage CAD who have no other options to alleviate their complaints, electrical neuromodulation may be the preferred adjunctive treatment. Although spinal cord stimulation is still not approved by the US Food and Drug Administration for treatment of refractory angina, it is is accepted in the American College of Cardiology/American Heart Association guidelines for chronic stable angina, with a class II indication, and is frequently used for this indication in Europe.

However, to understand underlying mechanisms of therapies such as electrical neuromodulation—executed through either transcutaneous electrical nerve stimulation or spinal cord stimulation—for angina pectoris and to improve the effect and safety of these therapies, clinical questions concerning neuromodulation must be evaluated in experimental models. The outcomes of these preclinical experimental studies subsequently need to be assessed in humans.

Although therapeutic improvements from implantable devices would not have been possible without experimental work, any experimentation must be avoided if it is not approved by the relevant ethics committee(s) or is not conducted in keeping with standard guidelines. For this reason it is sometimes more feasible, when appropriate, to make use of simulation models—for instance, to study regularization of atrial fibrillation by means of a device.

So, on the one hand it is challenging to use electrical neuromodulation as a tool to study heart-brain interactions in general; on the other hand, electrical neuromodulation may be used to study its own underlying mechanisms of action, more specifically on characteristics of angina and myocardial ischemia. To investigate these mechanisms of action of electrical neuromodulation, we initiated a neurocardiology program in the 1990s (Figure 1). This article will discuss the experimental models we have studied to unravel the heart-brain interactions involved. We studied electrical neuromodulation both in patients and in experimental animals. However, the lack of knowledge about fundamental aspects of cardiovascular regulating circuitry and cardiac pain, as well as the lack of an animal model for angina pectoris, is the background for the various projects we have conducted concerning heart-brain interactions.
In 1772, Heberden described to physicians in England the clinical symptoms of exercise-induced chest discomfort, with its emotional component and vaguely distributed projection on the chest, as follows: “The seat of it, and sense of strangling and anxiety with which it is attended, may make it not improperly be called angina pectoris.” Since then, it has been demonstrated repeatedly that strong emotional distress frequently precedes or is associated with complaints of pain in the chest. Further, emotional suffering has been associated with increased mortality in patients with CAD. We and others, unfortunately, were confronted with very limited knowledge of the precise locations of the origin of emotions in the limbic structures of the forebrain. Even less was known about the relationship of these brain structures and the heart, owing to technical limitations in the field of neuroanatomical tract tracing, among other reasons. As a result, the nervous pathways from the heart, through which signals are propagated to the brain in order to activate emotional components, were not accurately identified. We therefore initiated Project 1 to study, in a rat model, neuroanatomical characterization of the neuronal circuitry controlling cardiac activity, specifically during cardiac distress.

In the area of identifying efferent neural pathways from the heart, we were the first to publish an experimental setup making use of a neurotropic herpesvirus from the Bartha strain of the pseudorabies virus (PRV). Following injections of PRV into the left and right myocardium or into atrial tissue, PRV infects the neurons that innervate the injection site and is then transported in the neural network, where the virus may cross at least four synapses. This transneuronal retrograde viral pathway labeling method with PRV provided us the opportunity to study cardiovascular controlling networks. The distribution of the PRV-infected cells was studied immunocytochemically after survival times of 3 to 6 days. Right ventricular infection showed labeling in the same nuclei as left ventricular labeling, but the number of PRV-positive cells was always higher and the localization of PRV within the nuclei differed. These obvious signs for differentiation within the nuclei suggest differential neuronal pathways to various parts of the heart.

Following injection of PRV at different cardiac sites, differences in density and localization of PRV-positive cells were found predominantly in higher-order neurons that are known to be involved in cardiac control. Transection of the spinal cord at Th1, performed to reveal selectively the parasympathetic neuronal networks, reduced the number of labeled cells, specifically in the periaqueductal gray matter. Virus-labeled sympathetic preganglionic cells were found in the Th1–Th7 thoracic intermediolateral cell groups, with some additional infections at Th8–Th11 after inoculations of the ventricular myocardium. The rostral parts of the insular cortex appeared to be linked selectively to sympathetic innervation of the heart.

From the experiments we hypothesized that, according to the type of lesion, the pattern of cardiac innervation may account for a specific malfunctioning. Subsequently, the subendocardial clustered parasympathetic nerves make these nerves more vulnerable for myocardial damage than the superficial spread of sympathetic nerves. In this respect, the identification of three preganglionic parasympathetic nuclei in cardiac control—ie, the dorsal motor nucleus of the vagus (20% labeling), the nucleus ambiguus, and the peri-ambiguus—constituted the most striking findings.

PROJECTS 2 AND 3: CARDIAC NOCICEPTOR ACTIVATION

The cortical structures and their related output pathways also serve as effector systems for initiation of autonomic and behavioral responses by forebrain neuronal networks that make us aware of cardiac pain. However, these cortical and subcortical structures...
involved in cardiac pain perception were more or less terra incognita. In addition, we studied fundamental aspects of cardiac nociceptor activation (Project 2) and transduction of cardiac pain (Project 3). Unfortunately, there was no experimental animal model for angina pectoris. The aim of these projects was to obtain, both in patients and in animals, knowledge about cardiac nociceptor activation mechanisms, the transmission and perception of cardiac pain, and behavioral and autonomic responses.

To enable the study of mechanisms of neurostimulation during episodes of acute cardiac pain, we worked out an animal model for angina pectoris. For that reason we experimented with models in which we created an acute myocardial infarction. We had to reject this model since surgery and, more importantly, anesthesia interfered with the patterns of cerebral expression of immediate early genes (c-fos, c-jun) triggered by cardiac pain and/or neurostimulation. However, a spinoff from this project was the observation that cardiac tissue damage causes a reproducible and selective cerebral endothelial leakage of immunoglobulin G (IgG) molecules. Follow-up experiments showed that proinflammatory cytokines, which are released into the circulation after cardiac tissue damage, can generate the same pattern of blood-brain barrier dysfunction7 (see Project 4).

We then experimented with infusions of capsaicin into the pericardial space of unrestrained and unanesthetized rats to induce acute cardiac pain. This model appeared to be very promising and allows visualization of the behavioral and autonomic responses to cardiac pain. Cerebral c-fos expression patterns, a marker for structures involved in cardiac pain transmission and perception, were studied and validated with positron emission tomography (PET) imaging.8

Quantification of data makes it possible to study the behavioral and autonomic responses to cardiac pain. Cerebral c-fos expression patterns, a marker for structures involved in cardiac pain transmission and perception, were studied and validated with positron emission tomography (PET) imaging in patients.8

Project 2: Nociception of cardiac pain in patients
To study relationships between neurotransmitters and other molecules that contribute to pain and psychological variables, we studied cardiac tissues obtained from 22 patients with angina during coronary artery bypass graft surgery (CABG). Cardiac nociceptor activation mechanisms were investigated in heart biopsies from these 22 CABG patients; reverse transcriptase polymerase chain reaction analysis (RT-PCR) was conducted for adenosine and bradykinin receptor mRNA.9,10

An age-related decrease was observed in the adenosine A1 mRNA density but not in the bradykinin receptor mRNA levels. The adenosine A1 receptor density also correlated with pain characteristics reported in a questionnaire. Making use of semi-quantitative RT-PCR, cardiac tissue substrates were assessed to determine the expression of adenosine A1 and bradykinin B1/2 receptor mRNA densities. The outcomes were associated with the quality of pain, age, gender, medication, and duration of disease.9,10

For evaluation of pain characteristics, we used questionnaires and objective pain scores. We found that qualitative age-related alterations in angina perception correlated with the development of the more “strangling” component of angina at older age. This observation may be explained, in part, by a reduction in adenosine A1 receptor mRNA expression in the heart, since bradykinin B1/2 receptor densities remain the same.9,10

Project 3: Nociception of cardiac pain in unrestrained rats
Having identified neural pathways, we studied neurons that were activated during electrical neuromodulation.11 In search of a putative mechanism of action of electrical neuromodulation, we hypothesized that neuromodulation affects processing of nociceptive information within the central nervous system (CNS). To characterize neural activity we used expression of both the immediate early gene c-fos and the “late gene” or stress protein known as heat shock protein 72 (HSP72). c-fos was used to identify structures in the CNS affected by spinal cord stimulation. HSP72 was applied to ascertain whether spinal cord stimulation might operate as a stressor.12

Animal experiments were conducted on unrestrained unanesthetized rats implanted with a permanent catheter in the pericardial space; acute cardiac pain was triggered in this space using capsaicin as the algogenic substance.13 The autonomic cardiovascular responses were recorded with implantable telemetric devices. Behavioral responses were recorded on videotapes taken from the same animals in which the involved cerebral structures were characterized by analyzing cerebral immediate early gene expression. Quantification of data makes it possible to study the effects of electrical neuromodulation and analgesic drugs on perception of cardiac pain. To apply electrical neuromodulation, two electrodes were positioned and sutured epidurally at the spinal cord of the rats. One electrode was fixated at spinal nerve C7 and the other at T2. Furthermore, we studied the effect of spinal cord stimulation on behavior. Three hours after stimulation, the rats were sacrificed and their brains and spinal cords were removed.

The treated group showed regional increased c-fos expression in a select group of regions of the limbic...
system—periaqueductal gray, paraventricular hypothalamic nucleus, paraventricular thalamic nucleus, central amygdala, agranular and dysgranular insular cortex, (peri)ambiguus, nucleus tractus solitarius, and spinal cord—involves in the processing of pain and cardiovascular regulation, among other functions. Moreover, in both treated rats and controls, HSP72 expression was found in the endothelium of the enthorhinal cortex, the amygdala, and the ventral hypothalamus, but not in the neurons. The treated animals were significantly more alert and active than were the controls.

Thus, the rat model we developed appears to be suitable for studying potential mechanisms through which neuromodulation may act. Moreover, neuromodulation affects c-fos expression in specific parts of the brain known to be involved in regulation of pain and emotions. HSP72 expression is limited to the endothelium of certain parts of the CNS, and thus physical stress effects were excluded as a potential mechanism of neuromodulation. Finally, our experimental model identified regions corresponding with regional cerebral blood flow changes during neurostimulation in patients.8

PROJECT 4: BIDIRECTIONAL HUMORAL AND NERVOUS HEART-BRAIN INTERACTIONS

With respect to the emotional component of angina, we thought to study alternative pathways of communication between the heart and the brain. This idea occurred as a consequence of observations that many patients who suffer serious cardiac events, such as CABG or myocardial infarction, are confronted with a period of emotional problems following these events. So, from our experimental projects, the question became relevant as to whether emotional alterations in behavior following a cardiac life event may be executed by a humoral pathway from the heart to the brain, since, vice versa, the brain controls the heart through both nervous and humoral pathways. In other words, is it feasible that both humoral and neural pathways are involved, bidirectionally, in interactions between the brain and the heart?

Cardiac disease, proinflammatory cytokines, and blood-brain barrier damage

Cardiac ischemia, the underlying cause of cardiac pain in angina pectoris, triggers a cascade of events that release numerous substances in the myocardium and circulation, all of which are potential candidates for nociceptor activation and initiation of behavioral and autonomic responses to cardiac pain. Some of the substances that are released into the circulation may play a role in the humoral communication between heart and brain, but when released chronically, these substances may induce neuropathological modifications. Anxiety disorders and depression are cerebral disorders that are frequently comorbid with ischemic heart diseases. The latter are attributed to noncoping behavior, but our own experiments (as part of the program) showed that immune activation after tissue damage in the heart generates regional blood-brain barrier damage (Project 4) that could be an underlying organic basis for comorbid neuropsychiatric disorders. The incentive for this project in general was the observation that myocardial infarction is accompanied by behavioral and neuronal abnormalities.

In this project we established whether release of proinflammatory cytokines after tissue damage in the heart is a possible inducer of comorbid neuropsychiatric diseases.

As a model for immune activation, we studied the effects of intravenous injections of the proinflammatory recombinant tumor necrosis factor–alpha (TNF-α) on cerebral endothelial leakage, induction of neuronal damage, and motor and cognitive function in rats. Determinants of selectivity of blood-brain barrier damage were assessed with a molecular biological approach in which we studied regional differences of TNF-α–induced expression in the cerebral endothelial cells of the immediate early gene c-fos and proteins involved in leukocyte docking (intercellular adhesion molecules [ICAMs]) and TNF-α receptors.

To examine the mechanisms by which this interaction occurs, we induced myocardial infarction in a group of rats and then performed immunohistochemistry of the brain. This experiment revealed regional serum protein extravasation, pointing to leakage of the blood-brain barrier. This process occurred in certain cortical, subcortical, and hindbrain areas in discrete patches. The leakage was colocalized with expression of the immune activation marker ICAM-1. To assess the involvement of the immune system in the effects shown, a second group of rats was injected with TNF-α, as the major proinflammatory cytokine. This procedure rendered the same results. It was concluded that myocardial infarction may interfere with the integrity of the blood-brain barrier and possibly with brain functioning through activation of the immune system. The relevance for pathophysiological processes may provide a substrate for further research in unraveling the emotional consequences of serious cardiac events.

In the state of immune activation that follows myocardial ischemic events, various cytokines are released from the myocardium into the plasma. These cyto-
kines potentiate the cytotoxicity of TNF-α. In the next experiment we were able to demonstrate that intravenous injection of TNF-α induces a selective and regional neural IgG and endothelial ICAM-1 immunoreactivity. The expression of TNF-α-induced changes in the brain suggests that TNF-α is capable of inducing blood-brain barrier dysfunction. It is hypothesized that through dysfunction of the blood-brain barrier, the released cytokines bind to specific cognitive centers in the brain and thus may lead to emotional disturbances following cardiac events.

Having identified some specific centers involved in cardiovascular control, we further studied the effects of electrical and chemical stimulation of a specific brain center on the heart.

■ PROJECT 5: EFFECT OF BRAIN STIMULATION ON CORONARY FLOW

From a clinical PET study performed in patients with end-stage CAD during active spinal cord stimulation therapy, as well as from our PRV experiments and the literature, we concluded that the periaqueductal gray plays a central role in the regulation of different cardiovascular responses and in the integration of motor output from the limbic system. Subsequently, the periaqueductal gray has been thought to be one of the pivotal cerebral centers involved in executing electrical neuromodulation effects.

We investigated the function of the periaqueductal gray in regulation of the coronary flow of the heart. Depending on the stimulation site, electrical stimulation in the periaqueductal gray resulted in increases and decreases in coronary flow and conductance. These effects were organized topographically. The sites producing increases in coronary flow and conductance were found in both the dorsolateral and the ventrolateral periaqueductal gray. The sites producing decreases were restricted mainly to the ventrolateral portion. Similar topographic distributions were observed for the sites producing changes in carotid conductance and heart rate, but not for those producing changes in blood pressure and carotid flow. It is hypothesized that the topographic distribution of coronary vasoconstrictive and vasodilatory responses from the periaqueductal gray may enable optimal adjustments of the coronary perfusion. These optimal adjustments can then accommodate variations in myocardial oxygen demands accompanying different behavioral modes.

■ CONCLUSION

From all our experiments, mainly performed in rats (but sometimes also in a cat model due to the existence of a stereotactic brain atlas for the cat), we have learned about heart-brain communication through the use of electrical neuromodulation. In the last decade we have further studied heart-brain interactions in the International Working Group on Neurocardiology (IWGN), making use of canine and rabbit models. The main focus of the IWGN is on neural hierarchy in cardiac control. These projects are discussed by one of us (R.D.F.) elsewhere in these proceedings. In brief, the importance of the intracardiac neuron system and controlling centers at the C1 spinal level, in conjunction with the induction of myocardial ischemia, will be highlighted. For a more extensive overview of recent work performed by the IWGN, see the reviews by Foreman et al and Wu et al.

■ REFERENCES


Correspondence: Mike J.L. DeJongste, MD, PhD, Department of Cardiology, Thoraxcenter, University Medical Center Groningen, PO Box 30.001, 9700 RB Groningen, The Netherlands; M.J.L.de.Jongste@Thorax.UMCG.nl
Cardiac sympathetic denervation preceding motor signs in Parkinson disease*

ABSTRACT

There is substantial interest in identifying biomarkers to detect early Parkinson disease (PD). Cardiac noradrenergic denervation and attenuated baroreflex-cardiovagal function occur in de novo PD, but whether these abnormalities can precede PD has been unknown. Here we report the case of a patient who had profoundly decreased left ventricular myocardial $6\text-[^{18}\text{F}]$fluorodopamine-derived radioactivity and low baroreflex-cardiovagal gain, 4 years before the onset of symptoms and signs of PD. The results lead us to hypothesize that cardiac noradrenergic denervation and decreased baroreflex-cardiovagal function may occur early in the pathogenesis of PD.

In Parkinson disease (PD), by the time the movement disorder develops, most of the nigrostriatal dopamine terminals have been lost. Identification of biomarkers of PD should improve early diagnosis and spur development of effective treatments.

Braak has proposed a pathogenetic sequence beginning outside the brain, with invasion of peripheral, vulnerable autonomic neurons, followed by alpha-synucleinopathy in lower brainstem nuclei and then by alpha-synucleinopathy in the midbrain substantia nigra and then finally in the cerebral cortex.3,4 Consistent with early involvement of peripheral autonomic or lower brainstem centers, several studies of de novo PD have reported evidence of cardiac noradrenergic denervation5,8,14,22 or of decreased baroreflex-cardiovagal function.1,2,6,14,18

Whether these abnormalities can actually precede symptomatic PD has been unknown. Here we report the case of a patient who had cardiac noradrenergic denervation, detected by $6\text-[^{18}\text{F}]$fluorodopamine positron emission tomography, and decreased baroreflex-cardiovagal gain, detected by abnormal beat-to-beat blood pressure and heart rate responses to the Valsalva maneuver, 4 years before the clinical onset of PD.

CASE REPORT

A 56-year-old man was referred for possible pheochromocytoma, based on episodic hypertensive episodes and symptoms suggesting excessive catecholamine effects.

He had no serious health problems until about 1998, when he began to experience malaise and exercise intolerance and episodes of hypertension or hypotension, palpitations, and chest tightness. He also had a long history of constipation and dyspepsia, a tendency to urinary retention, and complained of a sense of fullness in the left neck. The patient’s career was in marketing and business development, until he quit work due to his symptoms. His mother had died of PD. Cardiac catheterization showed normal coronary arteries. Gastrointestinal endoscopy was unrevealing. Biochemical testing showed elevated plasma levels and urinary excretion of epinephrine. Thyroid function was normal.

Because of the hypertensive paroxysms, pheochromocytoma was suspected. In April 2000, the patient had a plasma epinephrine level about twice the upper limit of normal and a plasma metanephrine level about 50% above normal. In July 2001, he was evaluated at the National Institutes of Health (NIH). Normal follow-up plasma metanephrine, and failure of $6\text-[^{18}\text{F}]$fluorodopamine PET to detect an adrenal or extra-adrenal focus of radioactivity, excluded pheochromocytoma.17 At that time the concentration of $6\text-[^{18}\text{F}]$fluorodopamine-derived radioactivity was found to be markedly decreased in the left ventricular myocardium (Figure 1).

Autonomic function testing included measure-
ments of beat-to-beat blood pressure and heart rate during and after performance of the Valsalva maneuver. Blood pressure decreased early in Phase II and then leveled off, and there was an overshoot in pressure during Phase IV (dashed line in Figure 2), which are normal findings. Baroreflex-cardiovagal gain, calculated from the slope of the relationship between cardiac interbeat interval (with one beat delay) and systolic blood pressure during Phase II of the maneuver, was decreased at 3.2 msec/mm Hg; baroreflex-cardiovagal gain calculated from the data in Phase IV after release of the maneuver was also decreased at 3.1 msec/mm Hg).11,14,15

Over several months in 2005 the patient noted progressive slowing of movement and inability to relax the arms, small handwriting, decreased facial expression, and decreased voice volume. The patient returned to the NIH in November 2005, to participate in a protocol on pseudopheochromocytoma, the evaluation again including 6-[18F]fluorodopamine positron emission tomographic scanning and beat-to-beat blood pressure and heart rate associated with the Valsalva maneuver. 6-[18F]fluorodopamine PET again revealed severely decreased 6-[18F]fluorodopamine-derived radioactivity throughout the left ventricular myocardium (Figure 1). In the interventricular septum, radioactivity at the midpoint of the scanning frame between 5 and 10 minutes after initiation of injection of 6-[18F]fluorodopamine was 1,286 nCi-kg/cc-mCi, more than 2 standard deviations below the normal mean and one of the lowest values we have recorded so far (Figure 3). Blood pressure decreased progressively in Phase II of the Valsalva maneuver, to a greater extent than in 2001, there was no overshoot of pressure after release of the maneuver, and the return of pressure toward baseline was prolonged, findings pointing to failure of sympathetically mediated reflexive vasoconstriction.12,23 Baroreflex-cardiovagal gain was also lower than in 2001 (1.2 msec/mm Hg from the results in Phase II, 2.6 msec/mm Hg from those in Phase IV), both because the range of heart was smaller and the extent of change in systolic pressure larger in 2005 than in 2001.

As a test of the status of the adrenomedullary hormonal system, blood was obtained via an indwelling arm catheter during supine rest and after bolus i.v. injection of 1 mg of glucagon and assayed for plasma catecholamines in our laboratory. Both in July 2001 and November 2005, the ratio of plasma epinephrine (in pg/mL) to norepinephrine (in pg/mL) was relatively high during supine rest (76:99 and 101:234), and the patient had large increases of plasma epinephrine levels in response to glucagon (peak values more than 250 pg/mL, more than six times the normal peak value).

Neurological consultation in November 2005 noted stooped posture and axial instability, cogwheel rigidity in all four extremities, paucity of spontaneous movements, masked face with infrequent blinking, and monotone voice, but with normal speed of gait and no resting tremor. The patient was diagnosed with mild PD.

DISCUSSION

In this patient, results of 6-[18F]fluorodopamine PET scanning indicated cardiac sympathetic denervation 4 years before the clinical onset of PD. Considering that in PD loss of cardiac noradrenergic innervation progresses slowly over years,13 and that the patient
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already had evidence for markedly decreased cardiac noradrenergic innervation at the time of initial evaluation, loss of cardiac sympathetic nerves probably preceded the movement disorder by many more than the 4 years between initial testing and the onset of PD. The findings in this case fit with previous reports of cardiac noradrenergic denervation in de novo PD and with the concept of a peripheral-to-central and caudal-to-rostral pathogenetic sequence. Orimo and co-workers have noted loss of noradrenergic terminal innervation of the myocardium before loss of cell bodies in sympathetic ganglia in PD.16

Our patient also had evidence for decreased baroreflex-cardiovagal function 4 years before the movement disorder. The baroreflex is a homeostatic arc, and abnormalities of afferent neurotransmission, central integration by brainstem centers, or vagal efferent pre-ganglionic or post-ganglionic fibers could result in the same clinical laboratory finding of low baroreflex-cardiovagal gain. In particular, the extent to which baroreflex-cardiovagal failure in PD reflects a brainstem lesion, as opposed to an afferent lesion or loss of parasympathetic cholinergic efferents, remains unknown. The results in our patient are consistent with the view that baroreflex-cardiovagal function worsens over years before the onset of PD.

Chronic constipation, which also preceded parkinsonism in our case, would be consistent with early dysregulation of gastrointestinal autonomic function. Accumulations of alpha-synuclein in enteric neurons and in the dorsal motor nucleus of the vagus nerve, the central neural site of origin of parasympathetic innervation of much of the gastrointestinal tract, has been reported to be an early pathological finding.3 As noted above, however, the occurrence of central neural pathology would not exclude a concurrent afferent or efferent lesion, and studies have found Lewy bodies in the myenteric plexus of both the esophagus and colon,9 as well as loss of enteric dopaminergic neurons in PD with chronic constipation.19

Evidence for abnormalities of the sympathetic noradrenergic and parasympathetic cholinergic components of the autonomic nervous system in our patient occurred without evidence for compromised adrenergic adrenergic function. On the contrary, the patient had augmented plasma epinephrine responses to glucagon injection, both upon initial evaluation and at follow-up. The patient therefore did not appear to have diffuse loss of catecholaminergic cells. Although studies have noted decreased adrenergic adrenergic function

FIGURE 2. Beat-to-beat heart rate and blood pressure responses to the Valsalva maneuver (12-second duration, 30 mm Hg) in July 2001 and November 2005. In the latter recording, note progressive decline in blood pressure during Phase II, smaller pressure overshoot, and delayed return of pressure toward baseline in Phase IV, consistent with worsening baroreflex-sympathoneural function. Heart rate responses during and after the maneuver were also smaller in 2005 than in 2001, despite larger changes in blood pressure, consistent with worsening baroreflex-cardiovagal function.

FIGURE 3. Individual values for septal myocardial 6-{[18F]fluorodopa}mine-derived radioactivity, in normal control subjects (white circles), patients with Parkinson disease without sympathetic neurocirculatory failure (PD no SNF, green circles), patients with Parkinson disease and sympathetic neurocirculatory failure (PD + SNF, blue circles), and the case reported here (large green circle). Dashed line shows the normal mean value and light green shaded area 2 standard deviations from the normal mean. Note markedly decreased 6-{[18F]fluorodopamine-derived radioactivity in the current case.
concentrations in patients with severe PD, plasma levels of epinephrine and its O-methylated metabolite, metanephrine, have been reported to be normal.

Combined cardiac sympathetic denervation (with attendant denervation supersensitivity), baroreflex-cardiovagal hypofunction, and adrenomedullary hyperresponsiveness might explain the symptoms and signs of cardiovascular instability, such as episodic hypertensive paroxysms, tachycardia, palpitations, and chest pain despite normal coronary arteries, that led to clinical suspicion of pheochromocytoma in this patient.

The results in this case lead us to propose that cardiac sympathetic denervation and decreased baroreflex-cardiovagal gain may be biomarkers of early autonomic involvement in PD. Studies in progress about autonomic function in relatives of patients with familial PD should help test this hypothesis.

Acknowledgments
This research was supported by the Intramural Research Program of the NIH, National Institute of Neurological Disorders and Stroke.

REFERENCES

Correspondence: David S. Goldstein, MD, PhD, Clinical Neurocardiology Section, NINDS, NIH, 10 Center Drive MSC-1620, Building 10, Room 6N252, Bethesda, MD 20892-1620; goldstein@ninds.nih.gov
Supine low-frequency power of heart rate variability reflects baroreflex function, not cardiac sympathetic innervation*

ABSTRACT

Background. Power spectral analysis of heart rate variability (HRV) has been used to indicate cardiac autonomic function. High-frequency power relates to respiratory sinus arrhythmia and therefore to parasympathetic cardiovagal tone; however, the relationship of low-frequency (LF) power to cardiac sympathetic innervation and function has been controversial. Alternatively, LF power might reflect baroreflexive modulation of autonomic outflows. Objective. We studied normal volunteers and chronic autonomic failure syndrome patients with and without loss of cardiac noradrenergic nerves to examine the relationships of LF power with cardiac sympathetic innervation and baroreflex function. Methods. We compared LF power of HRV in patients with cardiac sympathetic denervation, as indicated by low myocardial concentrations of 6-[18F]fluorodopamine-derived radioactivity or low rates of norepinephrine entry into coronary sinus plasma (cardiac norepinephrine spillover) to values in patients with intact innervation, at baseline, during infusion of yohimbine, which increases exocytotic norepinephrine release from sympathetic nerves, or during infusion of tyramine, which increases non-exocytotic release. Baroreflex-cardiovagal slope (BRS) was calculated from the cardiac interbeat interval and systolic pressure during the Valsalva maneuver. Results. LF power was unrelated to myocardial 6-[18F]fluorodopamine-derived radioactivity or cardiac norepinephrine spillover. In contrast, the log of LF power correlated positively with the log of BRS ($r = 0.72, P < 0.0001$). Patients with a low BRS ($\ll 3$ msec/mm Hg) had low LF power, regardless of cardiac innervation. Tyramine and yohimbine increased LF power in subjects with normal BRS but not in those with low BRS. BRS at baseline predicted LF responses to tyramine and yohimbine. Conclusion. LF power reflects baroreflex function, not cardiac sympathetic innervation.

Spectral analysis of heart rate variability (HRV) has been used widely as a noninvasive technique for examining sympathetic and parasympathetic nervous outflows to the heart. Low-frequency (LF) and high-frequency (HF) power have been used most commonly. Human and animal experiments have repeatedly confirmed the dependence of HF power on respiration-related alterations in parasympathetic cardiovagal outflow–respiratory sinus arrhythmia; however, whether LF power provides an indirect measure of cardiac sympathetic activity has been contentious. Pagani et al$^1$ reported that LF power (normalized to total spectral power) increased during states associated with sympathetic noradrenergic activation and that bilateral stellectomy in dogs reduced LF power. Alvarenga et al,$^2$ however, reported that LF power was unrelated to all measures of norepinephrine kinetics in the heart; and in congestive heart failure, which is associated with a high rate of entry of norepinephrine into coronary sinus plasma (cardiac norepinephrine spillover),$^3$ LF power is decreased, not increased as might be expected if LF power reflected sympathetic activity.$^4$–$^7$

Sleight et al$^8$ proposed an alternative explanation for the origin of LF power. In a small group of human subjects, power spectral analysis of HRV showed that the amplitude of LF power was related to baroreflex...
gain and not to the level of sympathetic activity. Carotid sinus stimulation increased LF power only in individuals with normal baroreflex sensitivity and did not do so in those with depressed baroreflex gain. Therefore, results of power spectral analysis of LF power might reflect baroreflex-cardiovagal function.9

Studies of patients with dysautonomias provide an unusual opportunity to examine neurocirculatory correlates of LF power. Some chronic autonomic failure syndromes feature cardiac sympathetic denervation, whereas others do not. Parkinson disease with neurogenic orthostatic hypotension and pure autonomic failure feature cardiac sympathetic denervation, whereas multiple system atrophy does not.10 All 3 diseases involve baroreflex-cardiovagal and baroreflex-sympathovagal failure.11 Chronic orthostatic intolerance syndromes (postural tachycardia syndrome, neurocardiogenic syncope) do not entail either cardiac sympathetic denervation or baroreflex failure.12

For this article, we carried out power spectral analyses of HRV on digitized electrocardiographic recordings from dysautonomia patients and normal volunteers during supine rest, measurement of cardiac norepinephrine spillover, and intravenous infusion of yohimbine and tyramine, 2 drugs that are known to release norepinephrine from cardiac sympathetic nerves.13,14 Cardiac sympathetic innervation was assessed by 6-[18F]fluorodopamine positron emission tomographic scanning.15 We hypothesized that if LF power indicated cardiac sympathetic innervation and function, then patients with neuroimaging or neurochemical evidence of cardiac sympathetic denervation would have low LF power and attenuated increments in LF power in response to yohimbine and tyramine. Alternatively, if LF power was reflective of baroreflex function, alterations of LF power would be independent of cardiac sympathetic innervation status and correlate with changes in baroreflex gain.

**METHODS**

The study protocols were approved by the Intramural Research Board of the National Institute of Neurological Disorders and Stroke. All subjects were studied at the National Institutes of Health Clinical Center after giving informed, written consent.

**Subjects**

The study population consisted of a total of 98 subjects who participated in research protocols studying chronic orthostatic intolerance and chronic autonomic failure (Table 1). The subjects underwent autonomic function testing and had reviewable, digitized electrocardiographic data enabling retrospective power spectral analysis of HRV. ECG and blood pressure data were sampled at 1 kHz.

The study subjects were separated into 4 groups, depending on their state of cardiac sympathetic innervation and baroreflex-cardiovagal slope (BRS; see below). There were 40 subjects with intact sympathetic innervation and normal BRS (Innervated-Normal BRS), 24 with intact sympathetic innervation and low BRS (Innervated-Low BRS), 4 with sympathetic denervation and normal BRS (Denervated-Normal BRS), and 30 with sympathetic denervation and low BRS (Denervated-Low BRS).

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**TABLE 1**

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Yohimbine

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COI = chronic orthostatic intolerance; Denerv = denervated; Innerv = innervated; MSA = multiple system atrophy; NI BRS = normal baroreflex-cardiovagal slope; NOH = neurogenic orthostatic hypotension; PAF = pure autonomic failure; PD = Parkinson disease; r/o CAF = rule out chronic autonomic failure.
Autonomic function testing
Each subject was studied while supine with head on pillow after an overnight fast. Each patient had monitoring of the electrocardiogram and beat-to-beat blood pressure using either noninvasive devices (Finometer, Finapres Medical Systems, Amsterdam, the Netherlands; Portapres, Finapres Medical Systems; or Colin tonometer, Colin Medical Instruments, San Antonio, TX) or a brachial intra-arterial catheter. We previously studied formally and reported excellent agreement between intra-arterial and these noninvasively obtained measures of beat-to-beat blood pressure. Continuous vital signs data were digitized and recorded using a PowerLab (AD Instruments Pty Ltd, Castle Hill, Australia) data acquisition system and stored for later analysis on an Apple PowerBook G4 computer (Apple, Cupertino, CA).

After about a 10-min baseline period, each subject performed a Valsalva maneuver (30 mm Hg for 12 sec) at least 3 times.

Baroreflex function
As an index of baroreflex function, we used the slope of the relationship between cardiac interbeat interval and systolic blood pressure during phase II of the Valsalva maneuver. BRS, in units of msec/mm Hg, was calculated from the linear regression equation for the relationship between interbeat interval (with 1-beat delay) and systolic pressure. A BRS value of $\leq$ 3 msec/mm Hg was considered low.

Pharmacologic testing
Pharmacologic testing was performed on completion of the autonomic evaluation, using either tyramine or yohimbine. If a subject received both drugs, each drug administration was on a separate day. The durations of drug infusion were sufficient for heart rate and blood pressure to reach plateau values.

In a total of 22 subjects (Table 1), yohimbine was infused intravenously at 62.5 $\mu$g/kg over 3 min and then at 0.5 $\mu$g/kg/min for 12 min. In a total of 50 subjects, tyramine was infused at a rate of 1 mg/min for 10 min. In patients with severe supine hypertension (systolic pressure more than 200 mm Hg) and orthostatic hypotension, the test drugs were infused during head-up tilting (15$^\circ$ to 30$^\circ$), to decrease baseline pressure, or else the drugs were not given.

HRV analysis
LF power (0.04 to 0.15 Hz), HF power (0.16 to 0.4 Hz), and total power (TP, 0.0 to 0.4 Hz) were calculated using Chart 5.4.2 and the HRV module version 1.03 (PowerLab, AD Instruments Pty Ltd, Castle Hill, Australia). Stable heart rate epochs 3 to 5 min in duration were chosen for analysis. One epoch was sampled immediately before initiation of drug testing; the second followed attainment of steady-state hemodynamic effects. Interbeat interval data were reviewed carefully to eliminate artifacts from noise and T waves, using segments with little to no premature beats. LF power and HF power were calculated as absolute power (msec²), with or without normalization for total power (0.04 to 0.4 Hz). Reported LF or HF power was integrated within their defined frequency bands.

Cardiac sympathetic neuroimaging
For cardiac sympathetic neuroimaging the subject was positioned supine, feet-first in a GE Advance scanner (General Electric, Milwaukee, WI), with the thorax in the gantry. After positioning the patient with the thorax in the scanner and transmission scanning for attenuation correction, 6-[$^{18}$F]fluorodopamine (usual dose 1 mCi, specific activity 1.0 to 4.0 Ci/m mole, in about 10 mL normal saline) was infused intravenously at a constant rate for 3 min, and dynamic scanning data were obtained for thoracic radioactivity, with the midpoint of the scanning interval at 7.5 min after injection of the tracer (data collection interval between 5 and 10 min). Cardiac sympathetic denervation was defined by low concentrations of 6-[$^{18}$F] fluorodopamine-derived radioactivity in the interventricular septum (< 5,000 nCi/kg/cc-mCi) or left ventricular free wall (< 4,000 nCi/kg/cc-mCi) corresponding to about 2 SD below the normal means.
Low-frequency power reflects baroreflex function

Subgroups of subjects (3 PD + NOH, 3 MSA, 3 PAF, 5 normal volunteers) underwent right heart catheterization for measurement of cardiac norepinephrine spillover. 3H-norepinephrine was infused intravenously, and arterial and coronary sinus blood was sampled and coronary sinus flow was measured by thermodilution for measurements of cardiac norepinephrine spillover as described previously. In some subjects, yohimbine was infused during cardiac catheterization. Patients with chronic autonomic failure received the doses described above; normal volunteers and patients with chronic orthostatic intolerance received twice the doses described above.

Data analysis

Statistical analyses were performed using StatView version 5.0.1. (SAS Institute, Cary, NC). Mean values in the baseline condition for the several subject groups were compared using single-factor ANOVA. Responses to drugs were analyzed by dependent-means t-tests. Differences in response to pharmacologic tests among subject groups were compared using repeated measures analyses of variance. Relationships between individual hemodynamic values were assessed by linear regression and calculation of Pearson correlation coefficients. Post-hoc testing consisted of the Fisher PLSD test. Multiple regression analysis was done on the individual data, with the log of LF power as the dependent measure and the log of baroreflex slope and septal 6-[18F]fluorodopamine-derived radioactivity as independent measures. Mean values were expressed ± SEM.

Results

Baseline

Across the 7 subject groups (N = 98), LF power was unrelated to subject group (F = 1.2). When individual subjects were stratified in terms of cardiac sympathetic denervation or innervation, based on concentrations of 6-[18F]fluorodopamine-derived radioactivity more than 2 SD below the normal mean, then LF power was lower in the Denervated group (mean 221 ± 55 msec²/Hz, N = 34) than in the Innervated group (516 ± 93 msec²/Hz, N = 64, F = 4.8, P = 0.03). LF power normalized for total power, HF normalized for total power, and the ratio of LF:HF were not related to 6-[18F]fluorodopamine-derived radioactivity.

When subjects were stratified in terms of BRS, then LF power was lower in the Low BRS group (223 ± 105 msec²/Hz, N = 46) than in the Normal BRS group (617 ± 97 msec²/Hz, N = 25, F = 6.1, P = 0.02). The Low BRS group did not differ from the Normal BRS group in normalized LF power (F = 0.8).

When individual subjects were stratified into 4 groups, based on both cardiac 6-[18F]fluorodopamine-derived radioactivity (Innervated or Denervated) and on baroreflex-cardiovagal slope (Normal BRS or Low BRS), then both LF power and the log of LF power varied highly significantly as a function of subject group (F = 9.5, P < 0.0001; F = 4.6, P = 0.0004). The Denervated-Low BRS group had lower LF power than did the Denervated-Normal BRS group (P = 0.05), and the Innervated-Low BRS group had lower LF power than did the Innervated-Normal BRS group (P < 0.0001). When level of baroreflex function was taken into account, the Innervated and Denervated groups did not differ in LF power (Figure 1).

Values for HF power also varied with subject group when individual subjects were stratified in terms of both cardiac sympathetic innervation and BRS (F = 4.8, P = 0.004; Table 2). The Innervated-Low BRS group had

Cardiac norepinephrine spillover

Subgroups of subjects (3 PD + NOH, 3 MSA, 3 PAF, 5 normal volunteers) underwent right heart catheterization for measurement of cardiac norepinephrine spillover. 3H-Norepinephrine was infused intravenously, and arterial and coronary sinus blood was sampled and coronary sinus blood flow was measured by thermodilution for measurements of cardiac norepinephrine spillover as described previously. In some subjects, yohimbine was infused during cardiac catheterization. Patients with chronic autonomic failure received the doses described above; normal volunteers and patients with chronic orthostatic intolerance received twice the doses described above.
lower HF power than did the Innervated-Normal BRS group ($P = 0.003$); however, the Denervated-Low BRS group did not differ from the Denervated-Normal BRS group in HF power. Normalization of LF and HF power for total power, and the ratio of low-to-high frequency did not reveal additional group differences (Table 2). In particular, the LF:HF ratio did not vary with the subject group ($F = 0.6$).

Analysis of data from subjects during cardiac catheterization showed that LF power varied as a function of subject group ($F = 5.3, P = 0.03$, Figure 2). The Innervated-Low BRS group had lower LF power than did the Innervated-Normal BRS group ($P = 0.04$), whereas the Denervated-Low BRS and Innervated-Low BRS groups did not differ in LF power. As expected, the Denervated-Low BRS group had lower cardiac norepinephrine spillover than the Innervated-Low BRS group.

Individual values for LF power were positively correlated with BRS. When values for both variables were log-transformed, the log of LF power correlated positively with the log of BRS slope ($r = 0.72, P < 0.0001$, Figure 3). Individual values for the log of LF power were also correlated with the magnitude of decrease in systolic pressure during performance of the Valsalva maneuver ($r = -0.60, P < 0.001$) and with the orthostatic change in systolic pressure ($r = 0.58, P < 0.001$). In contrast, the log of LF power was unrelated to the septal myocardial concentration of 6-[18F]fluorodopamine-derived radioactivity, the plasma norepinephrine concentration, or cardiac norepinephrine spillover.

From multiple regression analysis for the log of LF power as the dependent measure and the log of baroreflex slope and septal 6-[18F]fluorodopamine-derived radioactivity as independent measures, the regression coefficient for the log of baroreflex slope was $0.92 (P < 0.0001)$, whereas the regression coefficient for 6-[18F] fluorodopamine-derived radioactivity was $3 \times 10^{-6}$. At baseline, the log of HF power correlated positively with the log of LF power ($r = 0.77, P < 0.0001$). HF power varied with the subject group ($F = 4.9, P = 0.004$). As with LF power, HF power was greater in
LOW-FREQUENCY POWER REFLECTS BAROREFLEX FUNCTION

the Innervated-Normal BRS than in the Innervated-Low BRS (P = 0.001, Table 2). As expected, the log of HF power correlated positively with the log of BRS (r = 0.60, P < 0.0001). The log of HF power also correlated negatively with the magnitude of decrease in systolic pressure during the Valsalva maneuver (r = −0.24, P = 0.02) and positively with the orthostatic change in systolic pressure (r = 0.40, P = 0.004).

Yohimbine

Yohimbine infusion increased LF power (t = 2.9, P = 0.007). The group with cardiac sympathetic denervation did not differ from the group with intact cardiac innervation in terms of the change in LF power during yohimbine infusion (F = 0.7). Yohimbine infusion increased LF power in the Innervated-Normal BRS group (t = 2.8, P = 0.01), but not in the innervated or denervated groups with low BRS (Figure 4). The Innervated-Normal BRS group had a larger increase in LF power during yohimbine infusion than did the Innervated-Low BRS group (P = 0.02). Too few patients with cardiac denervation and normal BRS were studied to include in the ANOVA. The log of the change in LF power during yohimbine administration was positively correlated with the log of BRS at baseline (Figure 5).

Yohimbine increased HF power in the Innervated-Normal BRS group (t = 2.1, P = 0.05) but not in the innervated or denervated groups with low BRS.

The change in LF power in response to yohimbine during cardiac catheterization was unrelated to the change in norepinephrine spillover (r = −0.09, N = 12).

Tyramine

Overall, tyramine infusion increased LF power (t = 2.9, P = 0.008). The group with cardiac sympathetic denervation did not differ from the group with intact cardiac innervation in terms of the change in LF power during tyramine infusion (F = 1.7). Tyramine increased LF power in the Innervated-Normal BRS group but not in the Innervated-Low BRS or Denervated-Low BRS groups (Figure 4; data for 2 outliers excluded). The log of the change in LF power during tyramine administration was positively correlated with the log of BRS at baseline (Figure 5; data for 2 outliers excluded).

DISCUSSION

In this study, patients with neuroimaging evidence of cardiac sympathetic denervation had low LF power of heart rate variability. At first glance, this finding would seem to support the view that LF power can provide an index of cardiac sympathetic outflow. As explained below, several lines of evidence from the present study led us to infer that the association between low LF power and cardiac sympathetic innervation is determined mainly by concurrent baroreflex function.

Patients with low BRS had low LF power, and patients with normal BRS had normal LF power, regardless of the status of cardiac sympathetic innervation as assessed by 6-[18F]fluorodopamine scanning. Neither normalization of LF and HF power for total power nor use of the LF:HF ratio improved the association with indices of cardiac sympathetic innervation.

Neurochemical findings during cardiac catheterization supported the above results based on cardiac

FIGURE 4. Mean (± SEM) values for the change in low-frequency power (∆LF power) of heart rate variability during (A) intravenous infusion of yohimbine or (B) tyramine in groups with innervated (Innerv) or denervated (Denerv) hearts, as indicated by low 6-[18F]fluorodopamine-derived radioactivity, and normal (NI) or low baroreflex-cardiovagal slope (BRS), as indicated by slope ≤ 3 mssec/mm Hg during the Valsalva maneuver. *Significant difference, P < 0.05. ***Significant difference, P < 0.001.
sympathetic neuroimaging. Among patients with innervated hearts who had normal cardiac norepinephrine spillover, LF power was decreased only in the group with low BRS and was normal in the group with normal BRS. As expected, cardiac norepinephrine spillover was decreased in patients with neuroimaging evidence of cardiac sympathetic denervation.

Effects of pharmacological manipulations that increase norepinephrine release from sympathetic nerves provided further support for an association between baroreflex failure and low LF power, independent of cardiac sympathetic function. Both tyramine and yohimbine increased LF power only in the subjects with normal BRS. In subjects with low BRS, neither drug increased LF power, even in the group with intact cardiac sympathetic innervation. Moreover, individual values for responses of the log of LF power to both drugs were correlated positively with the log of BRS at baseline.

The fact that HF power was positively correlated with LF power could also fit with the notion of baroreflex function acting as a common determinant of values of both variables. We cannot exclude concurrent parasympathetic-cardiovagal and sympathetic denervation as an explanation for the association between HF and LF power. Inhibition of the effects of parasympathetic activity after atropine administration results in the almost complete absence of both LF and HF HRV, further suggesting a common determinant.19

Several previous investigations have cast doubt on the validity of LF power as a measure of sympathetic activity because of dissociations between LF power and cardiac norepinephrine spillover, directly recorded sympathetic nerve traffic, and plasma norepinephrine levels.4,6,20 Such dissociations are especially glaring in patients with congestive heart failure, which is characterized by decreased LF power9 despite marked cardiac sympathetic activation.1

Other pathophysiologic states do result in both decreased sympathetic nervous system activity and decreased LF power. In these pathophysiologic states, the possibility remains that low LF power might reflect failure of baroreflexive modulation of sympathetic neuronal outflows, rather than sympathoinhibition itself. For instance, Wiklund et al21 noted low LF power in patients with palmar hyperhidrosis undergoing bilateral transthoracic sympathectomy; however, baroreflex-cardiovagal sensitivity also declines after thoracic sympathectomy.22

Sleight et al8 suggested dependence of LF power on baroreflex function, based on effects of carotid baroreceptor stimulation in 3 patients: 1 with normal BRS; 1 with ischemic heart disease, congestive heart failure, and normal BRS; and 1 with ischemic heart disease, congestive heart failure, and initially low BRS who subsequently had an improved clinical state and BRS. In the baseline state, both congestive heart failure patients had low LF power, despite a presumably hypernoradrenergic state. Direct baroreceptor stimulation at 0.1 Hz increased LF power in the normal subject and in the patient with congestive heart failure and normal BRS. The congestive heart failure patient with low BRS did not have an increase in LF power until BRS normalized. These data revealed an initial dissociation between cardiac noradrenergic state in the patients with congestive heart failure and LF power. During carotid sinus stimulation, LF power increased only when BRS was normal. Low BRS obviated this effect.

Because congestive heart failure is well known to be associated with baroreflex-cardiovagal inhibition,23–25 the finding of low LF power in heart failure also supports an association between LF power and BRS, inde-
pendently of increased tonic release of norepinephrine from sympathetic nerves in the heart. Cevese et al\textsuperscript{26} inhibited noradrenergic vasomotor tone using an alpha-adrenoceptor blocker in human subjects while maintaining mean blood pressure at control levels using angiotensin II. This drug combination, which would be expected to attenuate sympathetically mediated vasomotor tone and thereby decrease arterial baroreceptor input, markedly decreased or abolished LF power of HRV, suggesting that, at least under resting supine conditions, a baroreflex mechanism accounts almost entirely for LF power of HRV.

deBoer et al\textsuperscript{27} developed a beat-to-beat model of the human circulation using a set of differential equations and the following principles of operation: (1) the baroreflex regulates heart rate and peripheral vascular resistance; (2) Windkessel properties characterize the systemic arterial tree; (3) contractile properties of the ventricular myocardium follow the Starling law; and (4) respiration exerts mechanical effects on blood pressure. The model attributes LF power to a resonance in the circulatory control system, produced by a slow time constant for reflexive sympathetically mediated responses to beat-to-beat blood pressure changes. The resonance can be upregulated or downregulated by the state of baroreflex activity. The model of deBoer et al predicts that changes in blood pressure would lead heart rate changes at 0.1 Hz through a delayed sympathetic response. Changes in HR would depend on summed effects of sympathetic and vagal effects, with the sympathetic response delaying the overall response. At the respiratory frequency (0.2 to 0.3 Hz), blood pressure and HR changes would occur with little delay because of fast parasympathetic control. In essence, the response of the sympathetic nervous system behaves as a low band pass filter, with a peak response at 0.1 Hz and little response at frequencies above 0.2 Hz. Systolic blood pressure would lead to changes in heart rate via the baroreflex. In general the results of this study fit with the deBoer model.

In conclusion, LF power derived from the interbeat interval spectrogram predominantly reflects baroreflex-mediated, phasic changes in cardiacovagal and sympathetic noradrenergic outflows. In the setting of baroreflex failure, baseline LF power is reduced, regardless of the status of cardiac sympathetic innervation.

\section*{LIMITATIONS}

The combination of cardiac sympathetic denervation and normal baroreflex function seems quite rare. One must exercise caution in drawing inferences from the findings in the Denervated-Normal BRS group, which contained only 4 subjects, even though the difference in mean log-transformed LF power from the Denervated-Low BRS group was highly statistically significant.

All of the testing in our study was done with the subjects supine. LF power measured in other positions might have different sources and meaning.

\section*{REFERENCES}


Correspondence: Jeffrey P. Moak, MD, Building 10, Room 6N252, 10 Center Drive, MSC-1620, Bethesda, MD 20892-1620; moakj@mail.nih.gov
Is posttraumatic stress disorder related to development of heart disease? An update*

**ABSTRACT**

It has long been hypothesized that posttraumatic stress disorder (PTSD) increases coronary heart disease (CHD) risk; however, empirical evidence is limited. In the first prospective study to date, individuals with higher PTSD symptom levels had a significantly increased risk for CHD, after controlling for known coronary risk factors. PTSD indicates a chronic stress reaction and is hypothesized to influence CHD either by causing biological alterations that lead to cardiovascular damage, or by leading to adverse health behaviors that increase CHD risk. A key issue is whether PTSD contributes to the development of CHD, if PTSD and CHD share common pathways, or if CHD causes PTSD. Research combined across different disciplines suggests that prolonged or chronic stress does influence the development of CHD. A better understanding of the relationship will increase prevention and intervention efforts. Cardiologists may be most effective when they can recognize and manage emotional distress in practice.

We recently published the first prospective test of the hypothesis that individuals with higher levels of posttraumatic stress disorder (PTSD) symptoms are at higher risk of developing coronary heart disease (CHD). With colleagues from the Normative Aging Study, we used a questionnaire-based measure to assess PTSD in a sample of men who had served in the military and did not have CHD at the start of the study. All CHD end points were confirmed by a board-certified cardiologist. Over an average of 10 years of follow-up, for each standard deviation increase in symptom level, men had age-adjusted relative risks of 1.26 (95% confidence interval [CI], 1.05–1.51) for non-fatal myocardial infarction (MI) and fatal CHD combined. Results were maintained after controlling for all known coronary risk factors and replicated when considering an alternative measure of PTSD.1 Several aspects of the findings were particularly interesting. Cardiotoxic effects of PTSD symptoms were evident even though PTSD symptom levels were low to moderate in this group. In fact, few of the men would have met criteria for a PTSD diagnosis. There was also a dose-response relation between levels of symptoms and CHD risk, suggesting individuals with significantly higher levels of distress would be at considerably greater risk. Moreover, effects of PTSD symptoms on angina were significantly weaker than effects on MI and fatal CHD, each an objectively verified outcome. These results suggest that individuals with PTSD do not merely appear to be ill because they report more pain. Effects were also maintained even after accounting for potentially damaging health behaviors that have often been linked with PTSD. Finally, because PTSD and depression often occur together and since depression has been identified as a risk factor for CHD, an ongoing debate has considered whether PTSD per se may have cardiotoxic effects, or if effects can be explained by its association with depression. Findings from this study indicated that PTSD symptoms were associated with CHD, independent of depression.

**NATURE OF PTSD**

PTSD has been identified as a marker of extreme distress in response to a potentially traumatic event and may also be indicative of a chronic stress reaction. Diagnosis of PTSD is often difficult because PTSD symptoms overlap with those of anxiety and affective disorders, both of which are generally more recognized. However, unlike depressive and anxiety disorders, PTSD is defined by the combination of exposure to a potentially...
traumatic event (eg, combat, sexual assault, or serious natural disaster) and the occurrence of three types of symptoms: reexperiencing the traumatic event, avoidance of traumatic reminders and emotional numbing, and hyperarousal. The time course of PTS can follow one of several patterns, where high levels of symptoms after traumatic exposure are followed by recovery, chronic symptoms persist over time, or symptoms relapse and remit. Since the disorder reflects dysregulation of the stress-response system, which is associated with potentially atherogenic processes, a link between PTSD and CHD has long been speculated.

**PATHWAYS BETWEEN PTSD AND CHD**

Numerous studies have found that cardiovascular disease and its risk factors are more prevalent among individuals with PTSD. PTSD is hypothesized to contribute to the development of CHD, but because these studies have examined concurrent PTSD and cardiovascular disease or risk, they cannot determine the direction of causality. The causal relationship between PTSD and CHD has been hypothesized based on a model of prolonged stress reaction that posits that stress leads to impaired adaptation and increased wear and tear on the body. These processes may ultimately lead to atherosclerosis and cardiovascular system damage. Adults with PTSD exhibit neuroendocrinologic alterations characterized by enhanced negative feedback sensitivity of glucocorticoid receptors in the stress-response system and lower than normal urinary and plasma cortisol levels. Exaggerated catecholamine responses to trauma-related stimuli have also been found in adults diagnosed with PTSD. Higher concentrations of circulating catecholamines and increased total body sympathetic activity may eventually lead to autonomic nervous system dysfunction, including diminished heart rate variability, baroreflex dysfunction, and increased QT variability. Chronic stress and emotional arousal may also lead to or exacerbate endothelial damage and promote the development of atherosclerosis.

Another hypothesized pathway by which PTSD may influence CHD is through behavior. Studies have consistently demonstrated that individuals with PTSD are more likely to engage in adverse behaviors, which are themselves risk factors for CHD. For example, individuals with PTSD are more likely to smoke and to abuse alcohol. Interestingly, although these behaviors are generally believed to be on the causal pathway between PTSD and CHD, epidemiologic studies generally control for them. As a result, the magnitude of the association between PTSD and CHD may well be underestimated.

**COULD THE ASSOCIATION BETWEEN PTSD AND CHD BE SPURIOUS?**

At the heart of the endeavor to understand the relationship between PTSD and CHD is the question of whether PTSD actually leads to CHD through behavioral or biological alterations or if PTSD and CHD simply share common pathways. Another possibility is that the development of CHD (which itself can be a traumatic event) may cause PTSD. Biomedicine has generally been somewhat skeptical of the notion that feelings or psychological stress may lead to physical health outcomes, with three primary objections typically identified:

- A third underlying factor (eg, one or more genes or toxic environmental exposures) may cause both PTSD and CHD
- Most studies to date have been cross-sectional, leaving the question of causality unresolved
- Even with prospective studies, findings may be explained by either unmeasured potential confounds (ie, physical activity) or residual confounding by inadequately measured factors.

Moreover, since PTSD can develop in response to physical trauma, it may be difficult to distinguish whether effects on CHD are due to physical harm or psychological stress reactions.

**CONCLUSION**

Support for the theory that PTSD is causally related to CHD is provided by the recent prospective findings and the fact that they are highly consistent with findings from work in related areas. Several studies have reported that exposure to trauma and adverse events increases the risk of CHD. Other research has suggested that trauma increases the risk of adverse health outcomes only when PTSD develops in response to the trauma. Moreover, there is a growing body of evidence that chronic stress in various forms (eg, work stress), as well as high levels of emotional distress, may increase risk of CHD. Findings from this body of work are less susceptible to the concern that physical trauma rather than psychological stress reaction is driving the effects. Other work has also linked PTSD with reduced vagal tone and hypercoagulability. Taken together, research to date suggests that prolonged or chronic stress does play a role in the development of CHD (Table 1).

**FUTURE PERSPECTIVE**

PTSD is common in the general population. Approximately 7% of Americans will meet diagnostic criteria for PTSD in their lifetime. Prevalence rates are much
higher among war veterans; in a recent study, 13% of US military personnel who served in Iraq screened positive for PTSD. If PTSD is demonstrated to have significant cardiotoxic effects, there are numerous implications for both prevention and treatment.

More conclusive evidence of the association may be obtained using a variety of approaches. A first step will be to obtain longitudinal data in more diverse samples. This will include considering other groups (ie, women), individuals with clinically significant PTSD, and individuals with non-combat-related PTSD. Additional work will further examine exactly the duration or chronicity of PTSD necessary to initiate pathophysiological processes. Moreover, it is unknown whether the cardiotoxic effects of PTSD can be reversed if PTSD is successfully treated. Future work may compare long-term cardiac outcomes between individuals with PTSD who were successfully treated and those whose PTSD was refractory to treatment. A more careful examination of biological mechanisms is also required. Numerous studies have linked other types of chronic emotional distress with altered vagal tone, increased rate of atherosclerosis, and inflammation, suggesting these as likely pathways. However, the possibility of acute effects of PTSD should also be considered in light of recent work that found evidence of myocardial stunning in response to extreme emotional distress.

More conclusive evidence and a better understanding of the mechanisms will increase our ability to identify effective forms of prevention and intervention. Emotional distress may be more effective if they can recognize and manage emotional distress in practice.
disease. With improved prevention and more effective treatment strategies, we have the potential to significantly improve patient outcomes.

**Addendum**

Since the original publication of this review, several new studies have been published that uniformly provide additional empirical support for the hypothesis that individuals with higher levels of PTSD symptoms are at increased risk of developing CHD.

### ADDITIONAL PROSPECTIVE STUDIES

**Population-based study of military veterans**

A second prospective study was conducted using a random sample of men less than 65 years of age at follow-up who served in the US Army during the Vietnam War. Two measures of PTSD were obtained, one based on criteria from the *Diagnostic and Statistical Manual of Mental Disorders, 3rd edition* (DSM-III), and a second one using the Keane PTSD scale. After excluding any men with a history of heart disease at baseline and controlling for known coronary risk factors, the researchers found that a diagnosis of PTSD (using the DSM-III measure) more than doubled the risk for early-age heart disease mortality (hazard ratio = 2.25; 95% CI, 1.02–4.95). These results were maintained after controlling for depression and whether or not men actually served in Vietnam or elsewhere, and results were similar when the Keane PTSD measure was used. Compared with the men participating in the Normative Aging Study, this study’s population had generally higher PTSD symptom levels and had a significantly younger average age. Thus, findings from this population-based study of US veterans are highly consistent with earlier findings from a more limited sample within the Normative Aging Study.

**Community-based study of civilian women**

To address the question of whether effects are constrained to men with military experience (and likely combat exposure) or to older individuals, we recently examined the association between PTSD and CHD in civilian women, again using a prospective study design. Past-year trauma and associated PTSD symptoms were assessed using the National Institute of Mental Health Diagnostic Interview Schedule and considered in relation to incident CHD during the 14-year follow-up. After excluding individuals with heart disease at baseline and controlling for known coronary risk factors as well as depression and trait anxiety, we found that women with 5 or more PTSD symptoms had a threefold increase in the risk of incident CHD (odds ratio = 3.21; 95% CI, 1.29–7.98) compared with women with no PTSD symptoms. These findings were unchanged after women with angina were excluded and after known coronary risk factors were controlled for. Women in this study were even younger than the men in the prior prospective studies, with a mean age at baseline of 44.4 years. This study provides evidence that that damaging effects of PTSD symptoms are not limited to military men but are also evident among initially healthy community-dwelling civilian women exposed to non-combat-related trauma.

Together, these studies suggest that PTSD may be involved in the etiology of CHD, as all were meticulous in excluding individuals who might have already had heart disease at baseline.

### MORE STUDIES FOCUSING ON POTENTIAL MECHANISMS

As empirical evidence emerges that consistently suggests that PTSD is involved in the etiology of CHD, more studies are focusing on potential mechanisms and biological alterations related to PTSD that may help to explain its association with CHD. It has long been observed that individuals with PTSD often exhibit hypocortisolism and corresponding alterations in hypothalamic-pituitary-adrenal axis regulation that seem to be linked with reduced responsiveness to glucocorticoids. Moreover, several studies have suggested an association of PTSD with inflammatory and autoimmune diseases, leading investigators to speculate that PTSD causes chronic low-level inflammation.

As a result, studies focusing directly on the relationship between PTSD and inflammation or consequences of inflammation are beginning to appear. For example, one recent study compared levels of both proinflammatory and anti-inflammatory activity across patients with PTSD and age- and gender-matched controls without PTSD. Findings indicated the presence of a low-grade systemic proinflammatory state among patients with PTSD, and levels of proinflammatory activity were associated in a dose-response fashion with PTSD symptom levels. In another study, patients with PTSD were found to have more endothelial dysfunction, as measured by plasma concentrations of soluble tissue factor, compared with age- and gender-matched controls without PTSD.

Another line of research has considered whether links between PTSD and CHD may be explained in part by alterations in vagal function. Various studies in small samples have found reduced heart rate vari-
ability and increased sympathetic activity at rest, with parasympathetic activity blunted in response to challenge or trauma reminder among PTSD patients compared with healthy individuals without PTSD. \cite{16,29,31} A similar line of work has considered the effect of PTSD on parasympathetic nervous system functioning by examining effects on baroreflex sensitivity. Arterial baroreflex responses contribute to parasympathetic tone and have been linked with psychosocial stress, carotid atherosclerosis, and increased risk of cardiovascular disease. \cite{32-34} Two studies have considered whether baroreflex sensitivity is reduced among individuals with PTSD relative to those without PTSD. \cite{35,36} One study, conducted among smokers, found reduced baroreceptor sensitivity among women but not men after controlling for demographics, medications, diagnostic characteristics, and smoking variables. \cite{36} A second study, conducted among women only, found baroreceptor sensitivity to again be reduced among women with PTSD after controlling for a range of potential confounders, including comorbid psychiatric disorders. \cite{35} These women also appeared to have attenuated parasympathetic withdrawal response during a stressful challenge condition, similar to findings from studies of heart rate variability. Results from this small number of studies are somewhat preliminary, but given the consistency across these initial findings and other work linking related disorders (such as depression and anxiety) with reduced heart rate variability, ongoing work in this area is recommended. \cite{27}

**CAN IT BE SHOWN THAN PTSD PRECEDES CHD DEVELOPMENT?**

One of the challenges for studying potential mechanisms and biological alterations that explain how PTSD might influence the development of CHD is establishing that PTSD actually precedes the biological change under study. Much of the research to date compares individuals with PTSD or high levels of PTSD symptoms to individuals without PTSD or its symptoms. As a result, these studies cannot definitively determine whether PTSD caused the biological alteration or if presence of the biological alteration preceded PTSD and in fact increased susceptibility to the disorder. \cite{15} Convincing evidence that PTSD is involved in the etiology of CHD will include demonstrating that PTSD precedes the biological changes posited to contribute to the development of CHD.

**Suggestive evidence from an animal model**

One recent study in animals provides some reassurance that the posited direction of effects for the research reviewed above is plausible. Using an animal model of PTSD, rats were randomly assigned to exposure to a severe stress (a predator) for 10 minutes or to a control group. \cite{37} Behavioral reactions were tested 7 days after the stress exposure, and measures of ACTH, prolactin, and heart rate variability were obtained. Rats exposed to extreme stress demonstrated behavioral and biological changes commensurate with disruptions expected with PTSD. For example, stressed rats exhibited increased plasma ACTH, higher heart rate, lower heart rate variability, and many more maladaptive behaviors when compared with control rats. Since the animals were randomly assigned to exposure to severe stress, it is unlikely that these effects could be attributed to biological differences between the groups at baseline. Moreover, clear biological changes were evident as a result of exposure to severe stress. Taken together, these findings provide some reassurance that PTSD may have biological sequelae that in turn influence the risk of CHD, although prospective studies of PTSD and biological alterations in human populations clearly are needed.

**AS PTSD PREVALENCE RISES, URGENCY FOR INSIGHTS INCREASES**

PTSD occurs commonly in the general population but is of particular concern for individuals working in high-risk service occupations and in the military. With ongoing conflicts we may expect to see significant increases in the population prevalence of PTSD. Giving due consideration to the burden of illness associated with PTSD has added urgency, as recent studies have highlighted problems with access to and quality of mental health care. \cite{18,38} Thus, understanding the relationship between PTSD and CHD remains critical. Insights obtained from this work may increase our understanding of how biological susceptibility to heart disease develops and may aid in identifying strategies for disease prevention and intervention.

**REFERENCES**

Papers of special note have been highlighted as either of interest (*) or of considerable interest (**) to readers.


**Describes the first prospective test of the hypothesis that post-traumatic stress disorder increases the incident risk of coronary heart disease.**


3. Koenen KC, Stellman JM, Stellman SD, Sommer JE. Risk factors for course of posttraumatic stress disorder among Vietnam veter-
Creating a healing environment: Rationale and research overview

ABSTRACT

The environment of care has been shown to reduce the burden of disease and shorten healing time across multiple medical conditions. Given that many diseases respond to the unexplained regulation of the autonomic nervous system, the mechanism of interplay between the environment and this regulation needs to be explored and addressed as part of health care delivery. Complementary and alternative medical practices can be blended with traditional evidence-based medicine to optimally promote a healing environment and overall patient well-being. This review outlines the rationale behinded “blended medicine” and healing-oriented design of health care facilities, featuring examples and lessons learned from the North Hawaii Community Hospital.

It has long been known that the physical environment has important implications for the disease process. One of the first instances where the impact of one’s environmental surroundings on disease was appreciated was the discovery that hand washing and linen changes reduced rates of puerperal fever. At the time, it must have seemed strange that the “bad humours” of childbed fever could be removed by bathing the physician’s hands and changing the linens of the mother. Now, however, we routinely accept that infection is a battle between ever-present microbes and the human immune system’s exposure to them via the physical environment.

Traditional medicine is only now recognizing the effect on the disease process of less measurable, nonphysical factors such as stress. Many disease processes have a well-established relationship with stress; examples include the relation between psychosocial stress and more rapid progression of Parkinson disease, as well as the “broken heart syndrome.” Studies of inner city children under stress due to violence or socioeconomic factors show that they have greater disease burdens and worse disease outcomes compared with less-stressed children. Many stressors, such as physical or emotional abuse, lifetime traumas, turmoil in the childhood family, and recent stressful life events, have implications for both disease and healing. Similarly, the spiritual component of healing cannot be ignored, nor can the effect of a patient’s environment and aesthetic surroundings.

For these reasons, it makes sense to view health care as a comprehensive approach to combat all factors contributing to the disease process. The integration of all therapies—peaceful and comforting surroundings, stress reducers, caring health care providers, together with evidence-based medicine—creates a healing environment. This article presents an overview of this concept of comprehensively integrated therapies, with a focus on the role of the “healing environment,” or healing-oriented design and architecture, and provides examples and lessons from my institution, the North Hawaii Community Hospital.

‘BLENDED MEDICINE’ AND HEALING

Many people refer to traditional medicine as “Western medicine.” Western medicine in the United States is evidence-based and, in most circumstances, validated by clinical trials. These therapies have either stood the test of time or been shown to have superior effectiveness in treating a given disease. Introducing and validating a new treatment, either via the US Food and Drug Administration (as is the case with pharmaceuticals) or within the medical community, can take considerable time and money.

“Blended medicine” involves the use of complementary and alternative medicine together with traditional medicine. Blended medicine techniques are not necessarily validated in large clinical trials, but...
blended medicine has been found to promote stress reduction, faster healing, decreased infection rates, staff and patient satisfaction, and the economic benefit of lower hospital operating costs.6,8

Blended medicine recognizes the practical reality that healing usually relies on both traditional medicine and other components of care. It has been argued that high-tech treatment (eg, subspecialty care and advanced imaging) accounts for 20% of healing while “high-touch” treatment (complementary and alternative medical therapies) and a healing environment account for the remaining 80% (and that most treatment centers leave out this 80%).9 This third component—the environment—completes the triad of blended medicine.

Potential for improved outcomes
As early as the late 1980s, the treatment of heart disease came to recognize the beneficial effects of stress management, as demonstrated by recognition of the association between heart disease and the “type A” personality and its role in emotional expression.10 Back then, one of the few “alternative therapies” widely known in the West was meditation. Pharmacologic advances in the treatment of heart disease have improved outcomes exponentially. In preliminary studies, alternative therapies such as meditation have been shown to impact blood pressure and may prove effective in the treatment of hypertension and heart disease.11.12 Considering the outcomes of achieving the same treatment targets with blended medicine has provocative implications. For instance, if transcendental meditation results in a blood pressure goal of less than 130/80 mm Hg and a low-density lipoprotein cholesterol level of less than 70 mg/dL, what reason is there to believe that the outcomes would not match those of comparable pharmacologic manipulations of blood pressure and lipid levels?

HOLISTIC APPROACHES TO HEALING
For many acute illnesses, holistic approaches to healing are being used to augment traditional hospital care; such approaches exemplify the concept of blended medicine. Our experience at the North Hawaii Community Hospital has been that effective treatment of patients must include the ideology of holistic medicine: treating the body, mind, and spirit in the context of the patient’s culture and natural surroundings. We have found that complementary treatments that embody this holistic ideology yield benefits in terms of patient satisfaction. These therapies, some of which are covered by insurance,13 include the following:

- Manipulation/massage—pressing, rubbing, and moving muscles and other soft tissues, primarily using the hands and fingers. The aim is to increase the flow of blood and oxygen to the massaged area. The use of therapeutic massage has demonstrated benefit in both adult and pediatric conditions.14,15
- Acupuncture therapy—a family of procedures that originated in traditional Chinese medicine. Acupuncture is the stimulation of specific points on the body by a variety of techniques, including the insertion of thin metal needles though the skin. It is intended to remove blockages in the flow of qi—a traditional Chinese concept that roughly translates to “energy flow” or “vitality”—and restore and maintain health.
- Biofeedback—the use of electronic devices to help people learn to control body functions that are normally not consciously controlled (such as breathing or heart rate). The intent is to promote relaxation and improve health. One particular program, known as HeartMath®, is a systematized program developed for heart patients.
- Guided imagery—a gentle but powerful technique that focuses and directs the imagination. Although guided imagery has been called “visualization” and “mental imagery,” these terms are misleading, as the technique involves far more than just visual sense. Guided imagery involves all of the senses, and almost anyone can do it. It involves the whole body, the emotions, and all the senses, and it is precisely this body-based focus that makes for its powerful impact.
- Naturopathy—a comprehensive medical system that originated in Europe and aims to support the body’s ability to heal itself through dietary and lifestyle changes together with other therapies such as herbs, massage, and joint manipulation. An example of its application in the hospital would be the use of ginger root for the treatment of nausea.
- Healing touch or healing energy—a relaxing, nurturing energy therapy. Gentle touch assists in balancing physical, mental, emotional, and spiritual well-being. Healing touch works with the body’s energy field to support its natural ability to heal. It is safe for all ages and works in harmony with standard medical care.
- Aroma therapy—the use of pure and natural essential oils, absolutes, floral waters, resins, carrier oils, infused oils, herbs, and other natural substances. The natural ingredients used in aromatherapy have specific medicinal uses; for example, ginger and peppermint can treat nausea.
- Pet therapy. The comforting effects of animals have been noted through the years. For instance, Florence Nightingale recommended “a small pet animal”
as an “excellent companion for the sick.” A growing number of studies provide supportive evidence that these “huggable health care workers” truly help the healing process.16

Music therapy—the clinical and evidence-based use of music interventions to accomplish individualized goals (eg, stress management) within a therapeutic relationship. Programs exist for credentialing professional music therapists.

THE ROLE OF THE HEALING ENVIRONMENT

As noted above, part of holistic healing and blended medicine is the environment of care. Stress is an inherent part of the hospital experience and can serve to complicate a patient’s disease. The general appearance of a hospital’s rooms, grounds, and environment has important effects on patients.

Creating a patient-friendly environment is a challenge, especially since patients come in all sizes and from all cultures. A patient-friendly therapeutic environment for children arguably will be different from one designed for seniors. One unifying concept, however, is low-stress, high-comfort design. Research from the Center for Health Design has shown that the more attractive the environment, the higher the perceived quality of care and the lower the anxiety of patients. For example, there is a significant relationship between perceived wait times (which are affected by the pleasantness and aesthetics of waiting areas) and perceived quality/perceived anxiety.17 Patients underestimated longer (>30 minutes) actual wait times and overestimated short (0 to 5 minutes) actual wait times. There was no significant relationship between actual wait times and perceived quality or perceived anxiety,17 suggesting that perceived wait times, which are influenced strongly by the physical design of the environment of care, are a more important determinant of patient satisfaction.

Research on the healing environment is proliferating

Research and industry efforts to promote healing through design are ongoing in a number of centers. The Pebble Project is a joint research effort between the Center for Health Design, a nonprofit research and advocacy organization, and selected health care providers.17 The project, launched in 2000, is charged with creating a ripple effect in the health care community to provide research and documented examples of health care facilities whose design has made a difference in the quality of care. Such design-related improvements in care also can translate into improved financial performance of the institution.17

The North Hawaii Community Hospital experience

The North Hawaii Community Hospital, built in 1996, has incorporated the healing environment into many aspects of its design. We had the advantage of being able to build the hospital with a therapeutic design that includes elements such as wide corridors that deliberately do not trigger the “fight or flight” response. The use of natural lighting, floor-to-ceiling windows, and skylights throughout the hospital helps to keep the patient in sync with respect to chronobiologic principles. Against the backdrop of architectural and design elements like these, care is delivered in a restorative, therapeutic environment based on holistic principles and cultural wisdom to create a total healing environment.18

Hospital building boom presents an opportunity

As our nation’s population ages, the US health care system is anticipating a hospital construction boom worth $200 billion over the next decade.19 In California alone, new spending for hospital buildings was projected to exceed $14 billion between 2002 and 2010.8 This represents a great opportunity: at this pivotal moment, hospitals leaders are discovering the role of complementary medicine and healing design in improving patient and community health. Evidence suggests that hospital adoption of design approaches that minimize ecological harm and maximize patient healing and staff satisfaction leads to measurable outcomes such as reductions in length of stay, use of pain medication, medical mistakes, and cost of care.7,20,21

These findings should remind us that patient satisfaction is defined not only by clinical outcomes but also by the aesthetics of the hospital experience. Patients want a healthful, healing environment. It is not hard to predict patients’ preferences. They are similar to those that all of us share—for a comfortable environment and respect for our preferences and culture together with evidenced-based, high-tech diagnostics.

REMAINING QUESTIONS AND CONCLUSIONS

As the study of blended medicine and the healing environment advances, a number of questions loom before us:

- Will we find that hospitals are just warehouses for sick bodies and that the ideal healing environment may in fact be a spa, the patient’s home, or some yet-to-be-discovered variation on the current hospital system?
- Are there some disease processes that are solely caused by stress, or rather by an exaggerated process of normal injury?
• Why do we not study the biochemical makeup of healthy individuals involved in the complementary and alternative medicine practices mentioned above?
• What are the mechanisms of recovery in stress-induced injury?

The answers to these questions will unquestionably be complex, but as the study of heart-brain medicine grows more widespread, research to provide insight into the intricacies of alternative therapies will increase. No doubt there will be evidence against some accepted modalities, as well as discovery of new ones. The key lies in the heart-brain relationship.

Given that many diseases respond to the unexplained regulation of the autonomic nervous system, the mechanism of interplay between environment and this regulation needs to be explored and addressed as part of health care delivery. Systematic documentation of findings and clinical trials on the supposed mechanisms are needed. Once complementary and alternative therapies are validated, they must be implemented into treatment in much the same way as we now use as-needed medications. Instruction in the role and implementation of blended medicine and the healing environment should be part of the curriculum in medical and nursing schools.

REFERENCES


18. Bakken E. Presentation at an American College of Cardiology meeting on integrated medicine. October 2003; Mauna Lani Resort, HI.

Correspondence: Jone Geimer-Flanders, DO, Kona Community Hospital, Division of Cardiology, Ali‘i Health, 79-1019 Haukapila Street, Kealakekua, HI 96750; jgflanders@earthlink.net
Redesigning the neurocritical care unit to enhance family participation and improve outcomes

**ABSTRACT**

Emory University Hospital recently converted its neurocritical care unit into an environment that enhances involvement of the patient’s family. Each patient room now has an adjacent family area with comfortable accommodations for daytime and nighttime use. The new unit design, which drew from evidence on the impact of the physical environment on patient outcomes, facilitates better interactions between families and the medical team, and early studies show that patient satisfaction and staff satisfaction have increased. This article describes the impetus for and process of the unit redesign, as well as initial results and lessons learned.

Although my medical training prepared me well for treating brain injuries, I learned very little about caring for the enormous emotional needs of patients in neurocritical care units and their families. Having a physical environment that encourages the participation of the patient’s family is extremely important. Not only can having loved ones nearby give great comfort to the patient, but it helps provide a critically ill patient with an identity, which affects quality of care in fundamental ways. Having an identity is an anchor for everything, ultimately influencing not only clinical care but research as well.

This article describes our experience in developing a new neurocritical care environment at Emory University Hospital over the last 10 years using an evidence-based design centered on caring for patients and their families.

**STARTING POINT: A RAPIDLY GROWING PATIENT POPULATION**

Emory University Hospital, part of the Emory Healthcare health system, is the largest medical center in Georgia, with 43 neuroscience floor beds, 27 dedicated neurocritical care beds, and 10 intermediate neurocritical care beds. We have experienced rapid growth, with neurocritical care admissions rising from 587 in 1999 to more than 1,400 in 2007. We treat patients with meningitis, brain aneurysms, tumors, massive strokes, Guillain-Barré syndrome, myasthenia gravis, and other severe problems.

When we proposed building a replacement neurocritical care unit, we first appealed to the bottom line: if we had more beds and could attract more patients, we would generate more revenue. The hospital’s mission stated that we had to take care of patients with neurological emergencies because no one else in town could.

The administration countered with predictable restrictions: because Emory University was at that time considering building an expensive replacement hospital, they did not want to spend a lot of money improving a single unit. They agreed only to meet the state and federal requirements so that we could quickly open up and receive additional patients.

The initial design was for a 24-bed intensive care unit (ICU) with a “track” around it: visitors would enter patient rooms from the back so as not to disrupt the central area used by the doctors and nurses. The rooms measured 200 square feet, as required by the state of Georgia, with no dedicated space for family members. This design actually duplicated the system we already had in many ways.

**TRADITIONAL SYSTEM: PATIENTS SURROUNDED BY EQUIPMENT, NOT FAMILY**

In our old unit, the typical patient room was so crowded with specialized equipment that it was virtually impossible to get to the patient without tripping over cords and knocking out catheters. It took some time to respond to an emergency, and maintaining...
sterility in such an environment was obviously difficult. During rounds, residents, fellows, and the multidisciplinary team practically fell over one another, and actually seeing the patient in the midst of all this was a challenge. In the central area, nurses were crowded around desks with charts spread all over tables, increasing the potential for mistakes in record-keeping and medications.

Where were the families? We previously had a dark, dingy common space in the outside hallway, well away from patients. Families were prohibited from being in patient rooms during rounds for fear they should misinterpret or be alarmed by something they heard. Discussions between doctors and families took place either in the cluttered patient room or in a public area. Imagine this in situations in which a patient’s prognosis was poor and discussion was needed regarding brain death and organ donation. The new space promised little more than some new converter chair/beds in the common areas.

I did not have a clear idea of exactly what we needed, but I knew that the proposed design was not it.

**EVIDENCE FOR A BETTER WAY**

To convince the administration that we should pursue a completely new concept, we focused on key people: the chief executive officer of Emory Healthcare and the chief nursing officer of the neurosciences critical care unit. We told them that the current ICU was terrible for families and was inherently dangerous. The potential for medical mistakes was enormous and probably largely unrecognized. Staff burnout was also a potential issue: we reminded them of the tremendous nursing turnover, especially with our aging nursing population. We also told them that we believed there was a better way.

The medical community bases clinical decisions, such as choosing a drug to treat infection, on evidence from the literature. Shouldn’t such evidence also inform how we design hospitals and ICUs? I rapidly learned that convincing scientific evidence exists that the physical environment affects outcome. The literature shows that we can empower families and staff and significantly reduce cost.

We proposed a new design founded on an evidence-based approach for patient- and family-centered care. We were confident that a better design could reduce staff stress and enhance performance, and we hoped it could also reduce costs and improve effectiveness. As an academic institution, we wanted to measure such factors and continue to study this issue by building a living laboratory of a new type of family-centered ICU. We also wanted every treatment tool available while remaining flexible enough that we could continue to change in the future. Most importantly, we wanted to keep patients the center of our focus.

**EMPHASIS ON FAMILY INVOLVEMENT**

We sometimes fool ourselves into thinking that technology improves outcomes when, in fact, many other factors may be more beneficial. When we designed the ICU we had several goals or “design drivers” in mind, with accompanying measurable outcomes to be tracked (Table 1).

Our primary driver was support for families. We proposed completely eliminating all the signs restricting visitors to the ICU, such as those reading, “No visiting: Physician rounds in progress” (we were tempted to rewrite that sign as, “Physician rounds in progress: Visitor presence mandatory”). Rarely is the family actually required to participate in the care of a patient; we have no contract with the family delineating what the health care system provides and what we expect the family to do.

We planned for a family zone in the patient room, a children’s room, lockers and showers, and a family quiet room. Outcome measures would be patient/family satisfaction and provider satisfaction based on surveys, as well as the number of patient/family complaints and the number of litigation filings.

Other important drivers were the desire to support more procedures at the bedside, reduce infection, reduce medical errors, and increase patient safety. Every goal had measurable outcomes to be tracked.

**DESIGN PROCESS WAS DYNAMIC**

To help determine factors such as patient room size and configuration as well as the design of family spaces, we analyzed best practices of the prior 10 years’ winners of the ICU Design Citation Award, which is given jointly by the Society of Critical Care Medicine, the American Association of Critical Care Nurses, and the American Institute of Architects Academy on Architecture for Health. We partnered with the division of health care design at Georgia Institute of Technology’s College of Architecture as well as with a psychologist specializing in the role of the physical environment and with numerous graduate students. Several architectural design brainstorming meetings were held.

We then created a simulation that consisted of a large mock-up of the proposed ICU, including a nurses’ station, patient rooms, booms, and family areas. We spent an entire day role-playing a variety
of procedures, including resuscitation, intubation, implantation of a brain monitor, handoffs with nurses inside and outside the room, and interactions between families and staff. Videographers recorded everything for later analysis.

We changed designs as we learned from such experiences. We originally planned to distribute the nurses’ stations throughout the ICU but later decided to keep a communal area as well, recognizing that nurses and doctors like to be with each other and need to support one another.

About 50 family members of patients who had graduated from or were still in the critical care unit were involved with the unit’s design throughout the process.

### PROPOSAL BECOMES REALITY

The new unit opened February 2007. The new rooms range from 345 to 450 square feet, compared with 120 to 200 square feet for the old rooms. Each room is a suite, consisting of the patient room and a family area separated by a curved wall with large glass-block windows that let in light and create a cocoon-like effect (Figure 1). The family area has a table, chairs, comfortable sleeping arrangements, a flat-screen television, wireless Internet access, music, and a white-noise system to blunt surrounding noises.

The new unit allows us to do things we could not do before. I can now easily hold a private conversation with a family member when I visit a patient. Family members can leave the room for some respite and still be just a stone’s throw away from their loved one.

Patient rooms are much bigger than before, and the booms lift a lot of equipment off the floor. The beds and doors are configured so that patients who are awake have a direct line of sight to the nurse’s station.

### MEDIA ATTENTION AND REACTIONS

Our new unit was featured in both an article and a health care blog by the Wall Street Journal. The article opened as follows: “For decades, hospitals tried to keep visitors out of intensive-care units for more than a few minutes at a time. This year, Emory University Hospital here went the other way: It began inviting family members to move into the ward and take a hand in the patient’s care.” I think the reporter captured the key idea well, but I would change the word “visitors” to “participants” to indicate that patients’ family members really have a degree of responsibility.

There were interesting comments from readers in response to the article. Many were positive, but not

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**TABLE 1**

Redesign principles: drivers, responses, measurable outcomes

<table>
<thead>
<tr>
<th>Design drivers</th>
<th>Design response</th>
<th>Outcome measures</th>
</tr>
</thead>
<tbody>
<tr>
<td>Support families</td>
<td>Family zone in patient room</td>
<td>Greater satisfaction on Press-Ganey and Emory ICU surveys</td>
</tr>
<tr>
<td></td>
<td>Kids’ room</td>
<td>Fewer complaints and litigation</td>
</tr>
<tr>
<td></td>
<td>Lockers and showers</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Family quiet room</td>
<td></td>
</tr>
<tr>
<td>Support more procedures at the bedside</td>
<td>Medical gas booms</td>
<td>Fewer patient transfer complications and lower costs</td>
</tr>
<tr>
<td></td>
<td>Larger patient zone</td>
<td>Fewer errors</td>
</tr>
<tr>
<td></td>
<td>Improved ergonomics</td>
<td>Shorter stays</td>
</tr>
<tr>
<td>Reduce infection</td>
<td>Numerous rubs and handwashing stations</td>
<td>More time spent by ICU staff in the ICU area</td>
</tr>
<tr>
<td>Reduce medical errors and increase patient safety</td>
<td>Improved ceiling tiles</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Carpet where appropriate</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Charting niches</td>
<td></td>
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<tr>
<td></td>
<td>Zoned caregiver area</td>
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</tbody>
</table>

ICU = intensive care unit; MRSA = methicillin-resistant Staphylococcus aureus
everyone felt the changes were a good idea. One reader wrote, “Pandering to a half-dozen relatives is rarely beneficial to anyone. When we realize that hospitals are there to heal and not to entertain, we’ll cut down the excess costs of treating critical care patients. A close relative is entitled to know what’s happening on a timely basis. Any involvement beyond that should be limited to what is medically beneficial to the patient.”

Another comment, probably from someone who works in an ICU, was, “This sounds more like a marketing ploy by hospital administrators than a plan developed by the nurses and physicians in the trenches.” Interestingly, administrators at Emory resisted the changes because of the high expense. Although the tone of this comment seems cynical, the writer brings up a valid danger—that limited health care resources potentially could be diverted from the patient to the family. But although care that fosters family participation costs more money and takes more energy, what matters is that we are doing a better job for patients and their families.

**BENEFITS OF FAMILY-CENTERED UNITS:**

**A CASE STUDY**

The following case study illustrates some of the advantages of our new family-centered unit.

David was a 31-year-old computer programmer, the father of a 3-year-old girl, and about to be married. He came in with a grade 3 subarachnoid hemorrhage from a severe carotid intracranial aneurysm. He was in the old neurocritical care unit for 4 or 5 days, and then was moved to the new unit when it opened.

The family—David’s parents and his fiancée—kept a rotating vigil. The *Wall Street Journal* article described how they always felt that they were in the way in the old ICU, whereas they felt welcome in the new facility. The family often stood at David’s bedside as the team explained the purpose of the complex monitors and instruments. The mother said, “This was our home for a month, and it got so that the nurses could tell when we needed a hug.”

After 2 weeks, David developed neurogenic pulmonary edema, severe pneumonia, acute respiratory distress syndrome, and heart failure. We induced a coma to protect his brain from high intracranial pressure and placed hypothermia catheters to lower his core temperature in an attempt to better oxygenate him. Just as he was getting better, the aneurysm ruptured again, and we knew that recovery was hopeless.

The family was by his bedside 24 hours a day and knew that the medical team was as well. They witnessed the whole situation and understood when we ran out of options. As David’s parents and fiancée gathered at the bedside, I told them that David had progressed to brain death. Shortly after that, the team that arranged organ donation came to speak with David’s parents, and they elected to donate. They were grateful for the time they had with him and for the way they were treated. David’s father said, “No one ever misled us or told us anything but the truth...and most importantly, we were there for everything.”

We did everything we could for David, and nothing could change his ultimate outcome. But I think that the way someone dies is incredibly important. The circumstances of how he was treated probably helped allow the family to donate David’s organs and better come to terms with his death. They later generously donated their time to help the neurocritical care unit develop the family-centered approach we wanted by participating in many discussions about their experiences.

**FAMILY-CENTERED UNITS POSE CHALLENGES**

Units that are designed for both patients and their families bring to the fore enormous issues that arise in the ICU daily. How does one care for patients and their families simultaneously? Our challenges have included the following, among others:

- **Team rounding.** Nobody was happy about inviting families to rounds. Training medical students and fellows with families in the room is a real paradigm shift and raises many controversial issues. Yet I feel that the family needs to be aware of what is going on, particularly because our patients often are intubated and sedated and cannot act as their own advocates.
- **Nursing handoffs.** Imagine a nurse operating...
six or seven intravenous pumps and trying to figure out medications while having a family member—or three or four members—“in her face” 24 hours a day.

- **Urgent or frightening treatment.** How do you deal with resuscitation? What if the family is right by the bedside: do you ask them to leave? What kind of support do they need?

  We do not have all the answers to such problems. We are currently studying them and trying to figure out best practices.

### SUCCESSES AND FUTURE DIRECTIONS

Emory’s neurosciences critical care unit won the 2008 ICU Design Citation Award from the Society of Critical Care Medicine, the American Association of Critical Care Nurses, and the American Institute of Architects Academy on Architecture for Health.

We are now beginning to look at outcomes resulting from the unit redesign, and they all are going in the right direction. ICU patient satisfaction and staff satisfaction have increased, according to self-assessments. Other outcomes being assessed are length of stay and benchmark parameters of quality.

We are currently piloting a staff-family simulation workshop that will train all 80 members of our ICU nursing staff, including fellows, residents, and other faculty, in the fundamentals of communication. Using a one-way mirror, a team of psychologists and experts in grief and posttraumatic stress will watch simulated conversations among staff and actors role-playing situations involving brain death, organ donation, and diagnoses involving high mortality.

Although the concept of care centered around the patient and his or her family seems as acceptable as motherhood and apple pie, there is enormous resistance to it, even from the most dedicated health care workers. The process was long and laborious: we spent about a year and a half preparing for it with a family-centered team and involved all sorts of charters and directors along the way. Starting the changes is the real challenge.

### REFERENCES


**Correspondence:** Owen Samuels, MD, Department of Neurosurgery, The Emory Clinic, 1364 Clifton Road NE, F324, Atlanta, GA 30322; owen.samuels@emoryhealthcare.org
Neuromodulation of cardiac pain and cerebral vasculature: Neural mechanisms

ABSTRACT

Research using animal models has helped elucidate the neural mechanisms of angina pectoris, sensitization of cardiac nociceptive stimuli, and neuromodulation of cardiac pain and cardiovascular function. Findings over the last 2 decades include evidence of convergence of visceral-somatic input to spinothalamic cells and a major role for the vagus nerve in spinal cord processing. Stress-related glucocorticoids may manipulate amygdala function, inducing hypersensitivity to nociceptive input from the heart via central sensitization of upper thoracic spinal neuronal activity. Spinal cord stimulation may have therapeutic effects, although the underlying mechanism is unclear.

The cardinal symptoms of angina pectoris—chest pain and pain that may radiate to either arm or the neck and jaw—are well recognized. The visceral characteristics of anginal pain are also familiar; for example, referral to somatic structures, pain that is diffuse and poorly localized, skin and deep tissue tenderness, enhanced autonomic reflexes such as sweating and vasomotor symptoms, and muscular rigidity.

The neurologic mechanisms that explain the manifestations of angina pectoris are less well clarified, and are targets of active research. Our research into the neuromodulation of cardiovascular function over the last 2 decades has produced results that may have clinical implications and others that have raised new questions. This article summarizes some of our key findings from studies of neural mechanisms of angina pectoris, central sensitization of cardiac nociceptive stimuli, and the neuromodulation of cardiac pain, with a focus on processing in the spinal cord.

NEURAL MECHANISMS OF ANGINA PECTORIS

Cells of the spinothalamic tract form a sensory pathway that transmits afferent information to the thalamus. One of our research objectives was to examine how these cells process information when the heart is exposed to noxious stimuli.

Thoracic spinal processing

The animal model for our early studies was an anesthetized primate. The afferent nerves were activated in one of two ways: either the coronary artery was occluded or bradykinin and algesic chemicals were injected into the pericardial sac or left atrial appendage. Recorded activity was then made from the spinothalamic tract cells in the T1-T5 and C5-C6 segments. We found convergence of visceral and somatic input, generally to the chest and upper arm. The finding was consistent with the observation that pain from angina commonly occurs in proximal somatic fields. No visceral input was evident in cells in C7-C8, where the somatic effects are primarily distal—to the hand, for example.

Upper cervical processing

It is known that some patients experience angina pectoris as neck and jaw pain. The dental literature has shown that what is initially considered to be a toothache occasionally turns out to be angina and coronary artery disease. Clinical literature from the late 1940s observed that despite the use of sympathectomy to relieve angina pectoris, neck and jaw pain continued or developed. This pain was attributed to transmission of nociceptive information in vagal afferent fibers, commonly thought to transmit innocuous cardiac sensory information.

When we recorded activity from spinothalamic tract cells in the C1-C2 region to observe the effect
of cardiac nociceptive stimulation, we demonstrated a major role for the vagus nerve. Injection of saline into the heart had no effect in the C1-C2 region, but injection of algesic chemicals into the pericardial sac caused significant activity that disappeared after transection of the vagus nerve. This finding suggested that vagal afferent fibers ascend into the nucleus tractus solitarius of the medulla and either directly or indirectly modulate the C1-C2 neurons, which also receive converging somatic information from the neck and jaw region.

**CENTRAL SENSITIZATION OF CARDIAC NOCICEPTIVE STIMULI**

Clinical studies suggest that anxiety and depression are prevalent in patients suffering from chest pain with and without underlying cardiac disease. Anxiety and/or stress increases circulating levels of corticosteroids, which can act on the glucocorticoid receptors in the amygdala, particularly in the central area. The amygdala plays a pivotal role in transforming chronic stressful stimuli into behavioral, visceral, and autonomic responses.

Previous studies have shown that corticosteroids upregulate expression of corticotropin-releasing factor in the central nucleus of the amygdala and increase indices of anxiety. They are also associated with hypersensitivity in visceromotor responses to colorectal distention and sensitize lumbosacral spinal neurons to colorectal and urinary bladder distention. We therefore hypothesized that glucocorticoids manipulate amygdala function, inducing hypersensitivity to nociceptive input from the heart through the modulation of upper thoracic spinal neuronal activity.

To examine the impact of stress on the nervous system when the heart is exposed to noxious stimuli, we assessed the effect of chronic activation of the amygdala on the T3-T4 spinal neurons and on C1-C2 propriospinal neurons. Fisher 344 rats were selected for this study because of their relatively low level of anxiety-related behavior. Micropellets of crystalline corticosterone or cholesterol (30 μg, used as a control) were implanted in the central nucleus of the amygdala. After 7 days, the corticosterone-implanted, but not the cholesterol-implanted, animals displayed high-anxiety behavior, as determined with an elevated plus maze.

The responses of T3-T4 spinal neurons to intrapericardial injections of the algesic chemical bradykinin were compared in the corticosterone- and cholesterol-implanted rats. Compared with cholesterol-implanted animals, the duration of activity in response to the noxious cardiac stimulus was significantly longer in the corticosterone-implanted rats; in addition, activity shifted from the short-lasting (the response lasts only as long as the stimulus is applied) to long-lasting excitatory (the response lasts well beyond the period the stimulus is applied) neurons. Long-lasting excitatory neuronal activity is associated with intense pain and hypersensitivity, while short-lasting neurons are associated with a more acute response. The number of neurons with large field sizes in the corticosterone-implanted animals also increased, which is another indication of sensitization.

To study the role of the propriospinal pathway from C1-C2 segments in transmitting information from the amygdala to the thoracic spinal cord, we stimulated the central nucleus of the amygdala, which created a burst activity in T2-T4 spinal neurons that ended when the stimulus was removed. We then exposed the C1-C2 and C5-C6 spinal cord segments to ibotenic acid, which disrupts cell function but does not affect axons, and repeated the amygdala stimulation. Overall, the responses of 65% of the T2-T4 cells tested by amygdala stimulation were eliminated after C1-C2 cell disruption, but none of the neuronal responses to amygdala stimulation were eliminated after ibotenic acid was applied to the C5-C6 segments. The results suggest that C1-C2 plays a role in transmitting information from the amygdala to the T3-T4 neurons, and that there is a small direct pathway between the two areas (Figure 1).

**NEUROMODULATION OF CEREBROVASCU LATURE AND CARDIAC PAIN**

**Neuromodulation of cerebral blood flow**

Spinal cord stimulation is used to treat several cerebrovascular disorders, including cerebral ischemia, focal cerebral ischemia, stroke, postapoplectic spastic hemiplegia, and prolonged coma (see Yang et al for citations that address these pathologies). There is no clear explanation for its therapeutic effect; mechanisms being investigated include changes in cerebral blood flow and processing of nociceptive information.

To assess the effect of spinal cord stimulation on cerebral blood flow, we exposed the C1-C2 area of an anesthetized rat, stimulated the area with a ball electrode, and used laser Doppler flow probes to measure the blood flow on the surface of the cortex bilaterally. The stimulus parameters were 30%, 60%, and 90% of motor threshold; the threshold was determined by gradually increasing the intensity of spinal
cord stimulation until the neck muscles contracted. Blood flow increased on both sides with increasing stimulation intensities.\textsuperscript{13}

Other studies have evaluated cerebral blood flow but did not measure change in cerebrovascular resistance. We observed that spinal cord stimulation—particularly at 60\% and 90\% of motor threshold—increased blood flow and reduced resistance to spinal cord stimulation on the dorsal columns at C1, both ipsilaterally and contralaterally.

In other tests, cerebral blood flow and vascular resistance to spinal cord stimulation were not changed after transection of the spinal cord at the C6-C7 segments. These results suggested that information was not being transmitted to the sympathetic nervous system via the thoracic spinal cord. We applied ibotenic acid to C1-C2 to assess whether the underlying stimulated neurons affected cerebral blood flow; there was no significant change. On the other hand, a small cut in the dorsal column rostral to the stimulation site caused significantly reduced cerebral blood flow and vascular resistance, indicating that the dorsal columns function in an ascending manner to produce the vasodilation in the cerebral cortex.\textsuperscript{13}

Capsaicin-sensitive sensory nerves, which contain transient receptor potential vanilloid-1 (TRPV1) receptors, may have a role in spinal cord stimulation–induced vasodilation. TRPV1 receptors are nonselective cation channels activated by capsaicin, heat, and hydrogen ions.\textsuperscript{14} Activation, which causes an influx of cations and release of calcitonin gene-related peptide (CGRP) and substance P, is related to the pathogenesis of inflammation and hypertension. To examine the potential role played by capsaicin-sensitive sensory nerves, we administered resiniferatoxin (RTX), an ultrapotent capsaicin agonist; RTX specifically targets and desensitizes TRPV1-containing sensory fibers.\textsuperscript{13,15} Administration either intravenously or by direct application to the spinal cord results in a 15- to 20-minute period of sensitization followed by several hours of desensitization; if exposure lasts for several days, the nerves are destroyed.

Intrathecal administration of RTX to the spinal cord resulted in no significant change in cerebral blood flow. However, intravenous administration resulted in significantly decreased cerebral blood flow and decreased resistance, suggesting a role for TRPV1 receptors in cerebral blood flow.\textsuperscript{13}

There may be a connection between spinal cord stimulation at C1 and vasodilation of the cortex. The literature suggests that spinal cord stimulation activates the dorsal column nuclei\textsuperscript{16}, and we found evidence of this in our laboratory when we recorded activity from cells in the cuneate and gracilus nuclei after spinal cord stimulation. There is also a possible pathway between the dorsal column, the rostral ventrolateral medulla, and the sphenopalatine ganglion that influences vasodilation.\textsuperscript{17-20} Although not yet clearly defined, evidence suggests a connection between spinal cord stimulation and transmission of this information through the dorsal columns to influence vasodilation.\textsuperscript{17-20}

**Neuromodulation of thoracic spinal processing of cardiac nociceptive information**

Stimulating the dorsal columns activates the large afferent fibers, which in turn activate neuronal mechanisms in the spinal cord gray matter. These mecha-
nisms may be partly attributed to “gate control,” in which large afferent fibers can decrease the amount of information coming from the nociceptive afferent nerves to reduce the nociceptive sensation.  

González-Darder et al.  

considered this mechanism in a study of 12 patients with unstable angina (Table 1). Upper cervical spinal cord stimulation resulted in a decreased number of anginal episodes per week and an improved rate-pressure product (heart rate × systolic blood pressure). Their findings suggest that stimulating the upper cervical region could achieve effects similar to those seen after stimulating the spinal cord at T2.

Using a rat model to assess the effects of spinal stimulation, we recorded T3 activity during dorsal column stimulation of either C8-T1 or C1-C2 segments. Activity was almost completely suppressed with C1-C2 stimulation during bradykinin injection into the pericardial sac. The results suggest that spinal cord stimulation suppresses the processing of nociceptive information.  

Stimulating the spinal cord at C8-T1 also suppresses the effect of bradykinin. One possible mechanism for this effect is that spinal cord stimulation activates large afferent fibers; GABAergic connections in the superficial dorsal horn may suppress the processing of information in the spinothalamic tract neurons.  

**SUMMARY**

Our investigations have generated information about afferent input to the spinothalamic tract cells, the effects of glucocorticoids on amygdala function, and possible therapeutic mechanisms of spinal cord stimulation.

We have demonstrated convergence of visceral somatic input in spinothalamic cells. There is virtually no viscerocardiac input at the C7-C8 region, but there is input at C5-C6. Vagal afferent activity is the major source of input at the C1-C2 region; vagal stimulation also affects propriospinal neurons in this region. Vagal nerve stimulation may have a major role in processing in the upper cervical spinal cord and may change the balance of processing in the supraspinal nuclei.

Glucocorticoids manipulate amygdala function by inducing hypersensitivity to nociceptive input from the heart through central sensitization of upper thoracic spinal neuronal activity. Descending information from the amygdala depends, in part, on the C1-C2 propriospinal pathway.

Spinal cord stimulation at C1-C2 or C8-T1 can activate inner neuronal mechanisms that may involve GABA, modulating the wide dynamic range of neurons that are part of the spinothalamic tract.

**REFERENCES**


16. Sagher O, Huang DL. Effects of cervical spinal cord stimulation...


Correspondence: Robert D. Foreman, PhD, Department of Physiology, University of Oklahoma Health Sciences Center, 940 S.L. Young Blvd., Room 653, Oklahoma City, OK 73104; robert-foreman@ouhsc.edu
ABSTRACT

Vascular and neural systems are highly interdependent, as evidenced by the wealth of intrinsic modulators shared by the two systems. We tested the hypothesis that pinacidil, a selective agonist for the SUR2B receptor found on smooth muscles, could serve as an independent means of inducing vasodilation and increased local blood volume to emulate functional hyperemia. Application of pinacidil induced vasodilation and increased blood volume in the in vivo neocortex in anesthetized rats and awake mice. Direct application of this agent to the in vitro neocortical slice had no direct impact on biophysical properties of neurons or astrocytes assessed with whole-cell recording. These findings suggest that pinacidil provides an effective and selective means for inducing hyperemia in vivo, and may provide a useful tool in directly testing the impact of hemodynamics on neural activity, as recently predicted by the hemo-neural hypothesis.

Interactions between the brain and blood are essential to health. Metabolic supply of the brain is provided through the vasculature, and disruptions of this relationship, in extreme cases such as stroke, is a key characteristic of neurologic disease. Neuro-hemodynamic coupling is also demonstrated in healthy individuals on faster time scales in functional hyperemia, the local increase in blood flow and volume that accompanies neural activity.1,2

We have recently proposed a further level of interdependence between the two systems—ie, the hemo-neural hypothesis—which predicts that hemodynamic events such as functional hyperemia will modulate neural activity.3 An impact of hemodynamics on neurons could occur through a number of mechanisms, including the activation of mechanoreceptors on astrocytes or neurons, a thermal impact of increased blood flow on ion channels and vesicle release, and the local increase and diffusion of blood-borne factors such as nitric oxide.4,5 Astrocytes are predicted to play a key role in hemo-neural modulation, as they are tightly coupled to the vascular system and participate in a number of neural functions.6,7 Through these mechanisms and others, hemodynamics could shift the “state” of the local neural circuit, thereby impacting information processing. This regulation of neural dynamics could also provide a homeostatic mechanism for promoting healthy brain function (eg, prevention of kindling).

To study the impact of hyperemia on neural and astrocytic activity in vivo, it is essential to independently control blood flow in the brain with means that do not directly impact neurons or astrocytes. Pinacidil is a sulfonylurea receptor agonist that opens the SUR2B potassium-sensitive ATP channel.8 In the telencephalon, SUR1 receptors are localized to neurons and glia.9,10 In contrast, SUR2 receptors are localized to vasculature, with SUR2A in cardiac and skeletal muscle, and SUR2B in vascular smooth muscle, with primary expression in smaller arteries, arterioles, and capillaries.11 By opening the SUR2B channel, pinacidil hyperpolarizes and relaxes smooth muscle, causing vasodilation. Pinacidil is a potent and selective SUR2B agonist, with a dissociation constant of 135 nM and a
half maximal effective concentration (EC₅₀) value of
680 nM. This agonist is approximately 5 times more specific for SUR2B than for SUR2A and shows approximately 5 orders of magnitude lower affinity for SUR1 (in the mM range). Previous studies have demonstrated the efficacy of this agent as a vasodilator.

In the present study, we systematically examined the utility of pinacidil for the selective induction of hyperemia. First, we quantified the vasodilation induced by pinacidil in vivo, and examined local increases in blood volume in the parenchyma. These studies were conducted in anesthetized rats and awake mice. Second, we used in vitro slice recordings to examine whether direct application of relatively high concentrations of pinacidil would have any impact on the physiology of neurons and astrocytes. We found that (1) in vivo, pinacidil induces a level of vasodilation and increased local blood volume consistent with natural functional hyperemia across a variety of preparations, and (2) in vitro, pinacidil has no detectable impact on intrinsic biophysical measures in neurons and astrocytes.

METHODS

Animal preparation in vivo
To probe the impact of pinacidil on arterial diameter and parenchymal blood volume in vivo, we measured the effects of topical application to the primary somatosensory cortex (SI) of rats and mice. Sprague-Dawley rats (250–500 g) and C57BL/6 mice (~25 g) were anesthetized with pentobarbital (50 mg/kg intraperitoneal dose, followed by 5-mg supplements as needed for maintenance). Animals were maintained at approximately 37°C by a heating blanket. Craniotomy (diameter of ~2 mm in rats, ~1 mm in mice) and durotomy were performed over SI, and the cortex was protected with Kwik-Cast silicone elastomer sealant (WPI, Sarasota, FL) while an imaging chamber was attached with dental cement. Kwik-Cast was removed, and the chamber filled with 0.9% saline and sealed with a round cover glass (avoiding bubbles) secured with cyanoacrylate.

Controlling visualization during drug delivery in vivo
To minimize brain motion and flow artifacts during visualization of hemodynamics in the rat preparation, we constructed a customized pressurized chamber with inflow and outflow for constant perfusion. The volume of the chamber was approximately 0.3 mL, and the flow through the system averaged about 2 mL/min. The chamber consisted of a plastic ring 1 cm in diameter and 3 mm high with a flat-top profile and a base shaped to the angle of the lateral skull edge over SI. In the wall of this chamber, three large holes were drilled and patched with pieces cut from rubber NMR septa (VWR International, West Chester, PA) to create resealable ports for drug application and bubble removal. Three additional permanent holes were drilled in the chamber walls, through which blunted 1-cm lengths of 18-gauge stainless steel needles were wedged and affixed with Super glue: one for artificial cerebrospinal fluid (ACSF) inflow, one for combined outflow, and the third for pressure regulation. The overall pressure of the chamber was regulated by a small vertical tube whose height (and thus fluid level) could be adjusted on a manipulator stand, and whose other end was open to the atmosphere. Inflow and outflow were controlled via regulators on a gravity feed system. In the mouse preparation, the need to control visualization was addressed by maintaining a constant rate of wicking in a smaller-profile open chamber, and a microfluidic switch with 12 μL of dead space was added to minimize propulsive impact and delay due to switching between solutions. Drug and ethanol solutions were delivered to rat and mouse chambers after being heated to physiological temperature (37°C).

Optical measurement of hemodynamics in vivo
We used a charge-coupled device camera (the Roper 512B, Princeton Instruments, Trenton, NJ) to image the cortical surface at a frame rate of approximately 4 Hz, with illumination from a voltage-regulated xenon arc lamp. A green band-pass filter (550 nm) was used to maximize imaging near the isosbestic point of hemoglobin, providing optimal vessel contrast and a surrogate measure for blood volume change in the parenchyma. Lenses (50 and 125 mm) were arranged in series to form a macroscope.

We measured the impact of pinacidil on the diameter of the middle cerebral artery (MCA) and on parenchymal blood volume. Arteries were distinguished from veins by their lighter color, lower tortuosity, and/or inability to follow individual movement of red blood cells (indicating high flow rate). To measure arterial diameter, we took a cross section. We calculated the borders in each frame as the point 50% between the pixel brightness of the lightest part of the profile (over parenchymal tissue) and the darkest part (over the vessel). In Figure 1A, the green bar bisecting an artery indicates the point at which we obtained data that went into the width plot at the top of Figure 1B, which shows a line scan of darkness around the artery as a function of time. For parenchymal measurements, we summed all pixels in a region without detectable vessels (such as the red enclosed region in Figure 1A) and measured the change in darkness over successive frames.
Pinacidil administration in vivo

Pinacidil is hydrophobic and was therefore dissolved in ethanol at approximately 12 mg/kg and then diluted 1:100 in ACSF to achieve a 400-μM solution in 1% ethanol. Stock solutions were stored at –20°C and diluted in fresh ACSF for each experiment. For each run, the cortex was imaged for 3 minutes to establish baseline. For the pressurized rat chamber, at the end of the baseline period, 0.1 to 0.3 mL of 400-μM pinacidil in 1% ethanol in ACSF or saline would be pumped into the 0.3-mL chamber (taking about 1 second). Simultaneously, an equivalent volume was drawn out to balance pressure by a push-pull pump with access through two of the resealable rubber ports.

Pinacidil administration in vitro

Coronal slices were prepared from Sprague-Dawley rats at postnatal day 14 to 40 and maintained in a submersion chamber at 27°C for recording. Solutions were prepared in ACSF: 125 mM NaCl, 2.5 mM KCl, 1 mM MgCl₂, 1.25 mM NaH₂PO₄, 2 mM CaCl₂, 25 mM NaHCO₃, and 25 mM d-glucose). The applied solutions were 1% ethanol, ~400 μM pinacidil in 1% ethanol, and ACSF, all perfused with 5% CO₂ in 95% O₂ (carbogen).

For astrocyte recordings, slices were incubated immediately after cutting for 20 minutes in ACSF containing 50 μM sulforhodamine 101 (SR101), a water-soluble fluorescent dye specifically taken up by astrocytes. The slices were then allowed to rest for an hour before recording as usual. For fluorescence, the light source was a 100-W mercury arc lamp, with excitation and barrier filters and dichroic mirrors tailored to the spectral characteristics of SR101 (excitation ~586 nm, emission ~605 nm).

Slices were imaged under differential interference contrast optics with infrared illumination. Cells in layers 2/3 and in the same field and plane of view as a blood vessel greater than 20 μm in diameter were targeted, and vessel expansion was monitored during intracellular recording at approximately 4 Hz with a cooled charge-coupled device camera (Retiga EX, QImaging, Surrey, BC, Canada) connected to the microscope via...
CAO AND COLLEAGUES

This configuration also enabled imaging of neurons and astrocytes in the slice. Drug/control solutions were switched every 90 to 180 seconds. Recording pipettes were filled with 120 mM Kglu, 10 mM NaCl, 20 mM KCl, 10 mM HEPES, 2mM Mg-ATP, 0.3 mM Na-GTP, 0.5 mM EGTA, and 0.3% to 1% biocytin (wt/vol) for subsequent visualization of the neurons.

RESULTS

Pinacidil induces vasodilation in anesthetized rats and awake mice

Figure 1A shows the cortical surface over SI. The green bisection line over the MCA shows the point of sampling for the darkness plot in the rectangular box in Figure 1B. The width of this dark band is the width of the MCA over time, showing expansion after pinacidil addition (gray bar). The point at which dilation is observed corresponds to a darkening in a parenchymal region (red triangle in Figure 1A) and over the MCA and surrounding cortex (purple rectangle in Figure 1A, reflecting expansion). Vasodilation and parenchymal signal increases were consistently observed on the first trial in all experiments (4 first runs from 4 anesthetized rats, Figure 1C and 1D). Dilation began less than 10 seconds after drug arrival, with maximal dilation and parenchymal darkening at an approximately 50- to 60-second latency. Ethanol in a 1:100 solution with ACSF under the same conditions evoked a nonsignificant reduction in vessel diameter and no change in parenchymal darkening. Following the first presentation, subsequent pinacidil effects were less reliable.

As shown in Figure 1E and 1F, the hemodynamic impact of pinacidil in anesthetized rats was replicated in awake, head-posted mice (2 mice, 2 runs each). Presentation of 220 or 440 µM pinacidil evoked comparable mean increases in arterial diameter (peak diameter increase of ~20%) and parenchymal darkening (peak increase of ~2%), effects that were repeatable within subjects in a single session (N = 2).

Pinacidil does not have direct effect on nonvascular tissue

Vessels in slice only rarely responded to pinacidil application, with no significant changes in vessel diameter over 14 vessels in 14 distinct slices in vitro (Figure 2A). Presumably, pinacidil succeeds...
in inducing smooth muscle hyperpolarization under these conditions, but because unpressurized vessels in slice do not have a source of dilatory force against vessel walls, no expansion is observed.

Pinacidil does not impact spiking probability or input resistance in neurons or membrane potential in neurons and astrocytes

In recordings from regular-spiking neurons of pyramidal shape (N = 30), we saw no change in any metric measured. At 50 seconds after application, approximately the time of peak vasodilatory effects in vivo, the resting membrane potential did not change (variation of 0.5 ± 0.9 mV standard deviation; Figure 2E), the spike rate induced by current injection did not change (variation of 0.1 ± 1.1 spikes/stimulation; Figure 2F), and input resistance did not change (variation of 0.7 ± 6.5 MΩ; Figure 2D). Similarly, in a limited subset of recordings from fast-spiking interneurons (N = 3), we did not observe any impact of pinacidil application. All significance tests were paired t tests (P > .10).

Astrocytes (N = 35) also showed no significant effects of pinacidil application, demonstrating only a slow depolarization during application of ethanol (1.3 ± 1.7 mV at 50 seconds after drug application) and pinacidil with ethanol (0.5 ± 2.1 mV) (Figure 2B). When we plotted the change in membrane potential at 10-second intervals since drug application (0 to 80 seconds post-drug), we found no trends in astrocytic response to either ACSF or pinacidil (Figure 2C).

During recordings at double our typical application dose (800 μM), we observed 2 pyramidal cells (out of 8) that showed depolarization (peak of 10 to 15 mV) and a loss of spike initiation capability. Following washout, these cells recovered membrane potential but spiking responses to current injection remained impaired. We did not evaluate the impact of this dose on vascular tone or rhythmic vasomotion.

In contrast to the absence of a detectable impact of pinacidil, we found that the membrane potentials of neurons and astrocytes were sensitive to flow rate. Decreasing flow rate caused a consistent depolarization of up to approximately 10 mV that showed an immediate onset, reaching a new baseline within 2 to 5 seconds; increasing flow rate had the opposite effect. In preliminary experiments, we observed two astrocytes that depolarized on switching to the pinacidil solution. These two recordings were obtained prior to placement of an inline pressure meter in the flow pathway that allowed us to monitor and exclude trials that showed flow changes. In the 35 subsequent recordings that did not have flow changes, we never observed a detectable impact of either ethanol or pinacidil on astrocytes or neurons. We also noted that neurons and astrocytes were more likely to die and/or to lose recording quality during a cycle of ethanol or pinacidil presentation, as opposed to ACSF presentation.

DISCUSSION

Pinacidil provides an effective means of inducing vasodilation in vivo. At concentrations less than 400 μM, pinacidil is also selective for cortical vascular smooth muscle, exhibiting no direct effect on intrinsic properties of neurons or astrocytes. As an independent means to induce increased vasodilation and blood volume in a manner analogous to that seen in functional hyperemia, pinacidil provides a viable method for testing the impact of hyperemic events on neural or astrocytic activity. Pinacidil may also be a selective means of emulating other normal hemodynamic phenomena and could have therapeutic applications, such as targeted administration of pinacidil in response to acute vessel obstruction to maintain sufficient perfusion.

The hemodynamic effects induced by pinacidil are similar to natural functional hyperemia. In SI during sensory stimulation in rodents, increases in total oxygenated hemoglobin during sensory stimulation—analogous to our measurement of cortical darkening at 550 nm—peak in a range of 2% to 5%, and arteries/arterioles dilate 10% to 20%.

The time course of pinacidil’s effects also parallels the sustained response to continued sensory drive. Arterial diameter in rodent SI and the blood oxygen level—dependent response on functional magnetic resonance imaging in humans and rodents remain high when tactile input is sustained for periods lasting tens of seconds, as they do under pinacidil application.

Although pinacidil represents an important step forward in our ability to control blood flow while probing the impact of hemodynamics in cortex, it has limitations. The drug is only capable of producing vasodilation; drugs in the same family that block the SUR2B channels to create vasoconstriction (such as diazoxide or glibenclamide) or thromboxane receptor agonists are unfortunately known to be non-specific, affecting neurons as well as blood vessels. Pinacidil is also not water-soluble, requiring its dissolution in ethanol or DMSO, agents that can have confounding impacts on the system. Applied in vivo, pinacidil also does not appear to wash out fully, or its impact on smooth muscles persists, so that the first trial in each animal is the most consistent and effective one. These limitations stated, this pharmacological approach nevertheless represents a unique means of selective hyperemia induction in vivo.
REFERENCES

15. Correspondence: Christopher I. Moore, PhD, Massachusetts Institute of Technology, Department of Brain and Cognitive Sciences, Building 46–2171, 77 Massachusetts Avenue, Cambridge, MA 02139 (cim@mit.edu) and Joshua C. Brumberg, PhD, Neuropsychology PhD Subprogram, Queens College and The Graduate Center, CUNY, 65–30 Kissena Blvd., Flushing, NY 11367 (joshua.brumberg@qc.cuny.edu)
The polyvagal theory: New insights into adaptive reactions of the autonomic nervous system

**ABSTRACT**

The polyvagal theory describes an autonomic nervous system that is influenced by the central nervous system, sensitive to afferent influences, characterized by an adaptive reactivity dependent on the phylogeny of the neural circuits, and interactive with source nuclei in the brainstem regulating the striated muscles of the face and head. The theory is dependent on accumulated knowledge describing the phylogenetic transitions in the vertebrate autonomic nervous system. Its specific focus is on the phylogenetic shift between reptiles and mammals that resulted in specific changes to the vagal pathways regulating the heart. As the source nuclei of the primary vagal efferent pathways regulating the heart shifted from the dorsal motor nucleus of the vagus in reptiles to the nucleus ambiguus in mammals, a face–heart connection evolved with emergent properties of a social engagement system that would enable social interactions to regulate visceral state.

**HISTORICAL PERSPECTIVES ON THE AUTONOMIC NERVOUS SYSTEM**

Central nervous system regulation of visceral organs is the focus of several historic publications that have shaped the texture of physiological inquiry. For example, in 1872 Darwin acknowledged the dynamic neural relationship between the heart and the brain:

> . . . when the heart is affected it reacts on the brain; and the state of the brain again reacts through the pneumo-gastric [vagus] nerve on the heart; so that under any excitement there will be much mutual action and reaction between these, the two most important organs of the body.1

Although Darwin acknowledged the bidirectional communication between the viscera and the brain, subsequent formal description of the autonomic nervous system (eg, by Langley) minimized the importance of central regulatory structures and afferents. Following Langley, medical and physiological research tended to focus on the peripheral motor nerves of the autonomic nervous system, with a conceptual emphasis on the paired antagonism between sympathetic and parasympathetic efferent pathways on the target visceral organs. This focus minimized interest in both afferent pathways and the brainstem areas that regulate specific efferent pathways.

The early conceptualization of the vagus focused on an undifferentiated efferent pathway that was assumed to modulate “tone” concurrently to several target organs. Thus, brainstem areas regulating the supradiaphragmatic (eg, myelinated vagal pathways originating in the nucleus ambiguus and terminating primarily above the diaphragm) were not functionally distinguished from those regulating the subdiaphragmatic (eg, unmyelinated vagal pathways originating in the dorsal motor nucleus of the vagus and terminating primarily below the diaphragm). Without this distinction, research and theory focused on the paired antagonism between the parasympathetic and sympathetic innervation to target organs. The consequence of an emphasis on paired antagonism was an acceptance in physiology and medicine of global constructs such as autonomic balance, sympathetic tone, and vagal tone.

More than 50 years ago, Hess proposed that the autonomic nervous system was not solely vegetative and automatic but was instead an integrated system with both peripheral and central neurons. By emphasizing the central mechanisms that mediate the dynamic regulation of peripheral organs, Hess anticipated the need for technologies to continuously monitor peripheral and central neural circuits involved in the regulation of visceral function.

**THE VAGAL PARADOX**

In 1992, I proposed that an estimate of vagal tone, derived from measuring respiratory sinus arrhythmia, could be used in clinical medicine as an index of stress vulnerabil-
The polyvagal theory articulates how each of three phylogenetic stages in the development of the vertebrate autonomic nervous system is associated with a distinct autonomic subsystem that is retained and expressed in mammals. These autonomic subsystems are phylogenetically ordered and behaviorally linked to social communication (e.g., facial expression, vocalization, listening), mobilization (e.g., fight-flight behaviors), and immobilization (e.g., feigning death, vasovagal syncope, and behavioral shutdown).

The social communication system (i.e., social engagement system; see below) involves the myelinated vagus, which serves to foster calm behavioral states by inhibiting sympathetic influences to the heart and dampening the hypothalamic-pituitary-adrenal (HPA) axis. The mobilization system is dependent on the functioning of the sympathetic nervous system. The most phylogenetically primitive component, the immobilization system, is dependent on the unmyelinated vagus, which is shared with most vertebrates. With increased neural complexity resulting from phylogenetic development, the organism's behavioral and affective repertoire is enriched. The three circuits can be conceptualized as dynamic, providing adaptive responses to safe, dangerous, and life-threatening events and contexts.

Only mammals have a myelinated vagus. Unlike the unmyelinated vagus, originating in the dorsal motor nucleus, there are situations in which both measures covary (e.g., during exercise and cholinergic blockade), there are other situations in which the measures appear to reflect independent sources of neural control (e.g., bradycardic episodes associated with hypoxia, vasovagal syncope, and fetal distress). In contrast to these observable phenomena, researchers continue to argue for a covariation between these two parameters. This inconsistency, based on an assumption of a single central vagal source, is what I have labeled the vagal paradox.

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THE POLYVAGAL THEORY

The social engagement system

![Diagram of the social engagement system](https://www.sciencedirect.com/science/journal/03010511)

**FIGURE 1.** The social engagement system consists of a somatomotor component (solid blocks) and a visceromotor component (dashed blocks). The somatomotor component involves special visceral efferent pathways that regulate the striated muscles of the face and head, while the visceromotor component involves the myelinated vagus that regulates the heart and bronchi.7


of the vagus with pre- and postganglionic muscarinic receptors, the mammalian myelinated vagus originates in the nucleus ambiguus and has preganglionic nicotinic receptors and postganglionic muscarinic receptors. The unmyelinated vagus is shared with other vertebrates, including reptiles, amphibians, teleosts, and elasmobranchs.

We are now investigating the possibility of extracting different features of the heart rate pattern to dynamically monitor the two vagal systems. Preliminary studies in our laboratory support this possibility. In these studies we have blocked the nicotinic preganglionic receptors with hexamethonium and the muscarinic receptors with atropine. The data were collected from the prairie vole,17 which has a very high ambient vagal tone. These preliminary data demonstrated that, in several animals, nicotinic blockade selectively removes respiratory sinus arrhythmia without dampening the amplitude of the lower frequencies in heart rate variability. In contrast, blocking the muscarinic receptors with atropine removes both the low and respiratory frequencies.

■ CONSISTENCY WITH JACKSONIAN DISSOLUTION

The three circuits are organized and respond to challenges in a phylogenetically determined hierarchy consistent with the Jacksonian principle of dissolution. Jackson proposed that in the brain, higher (ie, phylogenetically newer) neural circuits inhibit lower (ie, phylogenetically older) neural circuits and “when the higher are suddenly rendered functionless, the lower rise in activity.”18 Although Jackson proposed dissolution to explain changes in brain function due to damage and illness, the polyvagal theory proposes a similar phylogenetically ordered hierarchical model to describe the sequence of autonomic response strategies to challenges.

Functionally, when the environment is perceived as safe, two important features are expressed. First, bodily state is regulated in an efficient manner to promote growth and restoration (eg, visceral homeostasis). This is done through an increase in the influence of mammalian myelinated vagal motor pathways on the cardiac pacemaker that slows the heart, inhibits the fight–flight mechanisms of the sympathetic nervous system, dampens the stress response system of the HPA axis (eg, cortisol), and reduces inflammation by modulating immune reactions (eg, cytokines). Second, through the process of evolution, the brainstem nuclei that regulate the myelinated vagus became integrated with the nuclei that regulate the muscles of the face and head. This link results in the bidirectional coupling between spontaneous social engagement behaviors and bodily states. Specifically, an integrated social engagement system emerged in mammals when the neural regulation of visceral states that promote growth and restoration (via the myelinated vagus) was linked neuroanatomically and neurophysiologically with the neural regulation of the muscles controlling eye gaze, facial expression, listening, and prosody (Figure 1; see Porges7 for review).

The human nervous system, similar to that of other mammals, evolved not solely to survive in safe environments but also to promote survival in dangerous and life-threatening contexts. To accomplish this adaptive flexibility, the human nervous system retained two more primitive neural circuits to regulate defensive strategies (ie, fight–flight and death-feigning behaviors). It is important to note that social behavior, social communication, and visceral homeostasis are incompatible with the neurophysiological states and behaviors promoted by the two neural circuits that support defense strategies. Thus, via evolution, the human nervous system retains three neural circuits, which are in a phylogenetically organized hierarchy. In this hierarchy of adaptive responses, the newest circuit
is used first; if that circuit fails to provide safety, the older circuits are recruited sequentially.

Investigation of the phylogeny of regulation of the vertebrate heart\textsuperscript{11,12,19,20} has led to extraction of four principles that provide a basis for testing of hypotheses relating specific neural mechanisms to social engagement, fight-flight, and death-feigning behaviors:

- There is a phylogenetic shift in the regulation of the heart from endocrine communication to unmyelinated nerves and finally to myelinated nerves.
- There is a development of opposing neural mechanisms of excitation and inhibition to provide rapid regulation of graded metabolic output.
- A face–heart connection evolved as source nuclei of vagal pathways shifted ventrally from the older dorsal motor nucleus to the nucleus ambiguous. This resulted in an anatomical and neurophysiological linkage between neural regulation of the heart via the myelinated vagus and the special visceral efferent pathways that regulate the striated muscles of the face and head, forming an integrated social engagement system (Figure 1; for more details, see Porges\textsuperscript{7,15}).
- With increased cortical development, the cortex exhibits greater control over the brainstem via direct (eg, corticobulbar) and indirect (eg, corticoreticular) neural pathways originating in motor cortex and terminating in the source nuclei of the myelinated motor nerves emerging from the brainstem (eg, specific neural pathways embedded within cranial nerves V, VII, IX, X, and XI), controlling visceromotor structures (ie, heart, bronchi) as well as somatomotor structures (muscles of the face and head).

**NEUROCEPTION: CONTEXTUAL CUEING OF ADAPTIVE, MALADAPTIVE PHYSIOLOGICAL STATES**

To effectively switch from defensive to social engagement strategies, the mammalian nervous system needs to perform two important adaptive tasks: (1) assess risk, and (2) if the environment is perceived as safe, inhibit the more primitive limbic structures that control fight, flight, or freeze behaviors.

Any stimulus that has the potential for increasing an organism’s experience of safety has the potential of recruiting the evolutionarily more advanced neural circuits that support the prosocial behaviors of the social engagement system.

The nervous system, through the processing of sensory information from the environment and from the viscera, continuously evaluates risk. Since the neural evaluation of risk does not require conscious awareness and may involve subcortical limbic structures,\textsuperscript{21} the term neuroception\textsuperscript{22} was introduced to emphasize a neural process, distinct from perception, that is capable of distinguishing environmental (and visceral) features that are safe, dangerous, or life-threatening. In safe environments, autonomic state is adaptively regulated to dampen sympathetic activation and to protect the oxygen-dependent central nervous system, especially the cortex, from the metabolically conservative reactions of the dorsal vagal complex. However, how does the nervous system know when the environment is safe, dangerous, or life-threatening, and which neural mechanisms evaluate this risk?

**Environmental components of neuroception**

Neuroception represents a neural process that enables humans and other mammals to engage in social behaviors by distinguishing safe from dangerous contexts. Neuroception is proposed as a plausible mechanism mediating both the expression and the disruption of positive social behavior, emotion regulation, and visceral homeostasis.\textsuperscript{7,22} Neuroception might be triggered by feature detectors involving areas of temporal cortex that communicate with the central nucleus of the amygdala and the periaqueductal gray, since limbic reactivity is modulated by temporal cortex responses to the intention of voices, faces, and hand movements. Thus, the neuroception of familiar individuals and individuals with appropriately prosodic voices and warm, expressive faces translates into a social interaction promoting a sense of safety.

In most individuals (ie, those without a psychiatric disorder or neuropathology), the nervous system evaluates risk and matches neurophysiological state with the actual risk of the environment. When the environment is appraised as being safe, the defensive limbic structures are inhibited, enabling social engagement and calm visceral states to emerge. In contrast, some individuals experience a mismatch and the nervous system appraises the environment as being dangerous even when it is safe. This mismatch results in physiological states that support fight, flight, or freeze behaviors, but not social engagement behaviors. According to the theory, social communication can be expressed efficiently through the social engagement system only when these defensive circuits are inhibited.

**Other contributors to neuroception**

The features of risk in the environment do not solely drive neuroception. Afferent feedback from the viscera provides a major mediator of the accessibility of prosocial circuits associated with social engagement behaviors. For example, the polyvagal theory predicts that states of mobilization would compromise our ability to detect positive social cues. Functionally, visceral states color our perception of objects and others. Thus, the same
features of one person engaging another may result in a range of outcomes, depending on the physiological state of the target individual. If the person being engaged is in a state in which the social engagement system is easily accessible, the reciprocal prosocial interactions are likely to occur. However, if the individual is in a state of mobilization, the same engaging response might be responded to with the asocial features of withdrawal or aggression. In such a state, it might be very difficult to dampen the mobilization circuit and enable the social engagement system to come back on line.

The insula may be involved in the mediation of neuroception, since it has been proposed as a brain structure involved in conveying the diffuse feedback from the viscera into cognitive awareness. Functional imaging experiments have demonstrated that the insula plays an important role in the experience of pain and the experience of several emotions, including anger, fear, disgust, happiness, and sadness. Critchley proposes that internal body states are represented in the insula and contribute to states of subjective feeling, and he has demonstrated that activity in the insula correlates with interoceptive accuracy.23

■ SUMMARY

The polyvagal theory proposes that the evolution of the mammalian autonomic nervous system provides the neurophysiological substrates for adaptive behavioral strategies. It further proposes that physiological state limits the range of behavior and psychological experience. The theory links the evolution of the autonomic nervous system to affective experience, emotional expression, facial gestures, vocal communication, and contingent social behavior. In this way, the theory provides a plausible explanation for the reported covariation between atypical autonomic regulation (eg, reduced vagal and increased sympathetic influences to the heart) and psychiatric and behavioral disorders that involve difficulties in regulating appropriate social, emotional, and communication behaviors.

The polyvagal theory provides several insights into the adaptive nature of physiological state. First, the theory emphasizes that physiological states support different classes of behavior. For example, a physiological state characterized by a vagal withdrawal would support the mobilization behaviors of fight and flight. In contrast, a physiological state characterized by increased vagal tone on the heart (via myelinated vagal pathways originating in the nucleus ambiguous) would support spontaneous social engagement behaviors. Second, the theory emphasizes the formation of an integrated social engagement system through functional and structural links between neural control of the striated muscles of the face and the smooth muscles of the viscera. Third, the polyvagal theory proposes a mechanism—neuroception—to trigger or to inhibit defense strategies.

■ REFERENCES


Correspondence: Stephen W. Porges, PhD, Brain-Body Center, Department of Psychiatry (MC 912), 1601 W. Taylor Street, Chicago, IL 60612; sporges@uic.edu
**Poster Abstracts**

1. **Insulin Use Does Not Protect Against Restenosis in Diabetic Patients Presenting with Acute Coronary Syndrome or Symptomatic Angina**

Matthew C. Becker, MD; John M. Galla, MD; Saif Anwaruddin, MD; Samir Kapadia, MD; and Richard A. Krasuski, MD

Department of Cardiovascular Medicine, Cleveland Clinic, Cleveland, OH

**Background:** Percutaneous coronary intervention (PCI) in diabetic patients is associated with increased rates of cardiovascular morbidity and a higher incidence of restenosis (ISR) due to neointimal hyperplasia. While prior work has shown that intensive glycemic control may reduce the incidence of ISR, the influence of insulin versus oral therapy on the rate of ISR remains undefined.

**Methods:** Of 5,239 consecutive diabetic patients undergoing diagnostic angiography, we identified 256 previously stented patients who presented with acute coronary syndrome (ACS) or anginal symptoms and subsequently underwent diagnostic angiography. The cohort included 126 patients with target vessel restenosis (> 50%) and 130 controls matched by age, sex, gender, and stent type (drug eluting vs bare metal) and dimensions. Diabetic therapy and laboratory data at the time of initial intervention were prospectively collected for both groups.

**Results:** The mean age was 64 ± 10; 64% of patients were men, 91% were type 2 diabetics, 84% were treated with statins, and 35% received drug-eluting stents. Post-PCI stent dimensions were similar between the groups. Patients treated with insulin developed ISR at a rate similar to those treated with oral medications (70% vs 58%, *P* = .211) despite a similar degree of glycemic control (proportion of patients with A1c > 7%: 81% vs 75%, *P* = .30). There were no significant differences in LDL cholesterol concentration or frequency of statin use between the groups.

**Conclusions:** In a population of diabetic patients undergoing PCI due to ACS or anginal symptoms, the use of insulin therapy was not associated with an increased risk of ISR. These data suggest that the use of insulin therapy to achieve optimal glycemic control in a high-risk population is not accompanied by an increased risk of target vessel restenosis.

2. **Postoperative Statin Use and Lower LDL Cholesterol Concentration Are Associated with Reduced Incidence of Stroke**


Cleveland Clinic, Cleveland, OH

**Background:** Postoperative stroke remains a catastrophic and costly complication of coronary artery bypass grafting (CABG). Prior work has demonstrated a significant reduction in the rate of stroke associated with statin use in the nonoperative setting. We evaluated the effect of postoperative statin use and LDL cholesterol concentration (LDL-C) on the incidence of stroke following CABG.

**Methods:** The Cleveland Clinic cardiothoracic surgery database was used to identify 5,205 consecutive patients who underwent first-time, isolated CABG from 1/1993 to 12/2005. Patients with a prior history of atrial fibrillation, known clotting disorder, or requirement for anticoagulation were excluded from analysis. Discharge medications, including statins, were prospectively collected. Patients were divided into groups based upon serum LDL-C: < 70 mg/dL, 70 to 100, 101 to 130, or > 130.

**Results:** The overall incidence of postoperative stroke at 1 year was 3.3% (181 events). Patients discharged on statin therapy were more likely to have a lower LDL-C and were significantly less likely to suffer a postoperative stroke at 1 year (Table). Multivariate logistic regression identified age (HR 1.05 [1.024, 1.075]; *P* < .001), peripheral vascular disease (1.89 [1.233, 2.891]; *P* < .001), and antiplatelet therapy (2.3 [1.352, 3.962]; *P* < .001) as independent predictors of stroke.

**Conclusions:** Postoperative stroke is a significant complication of CABG. Use of statins and lower LDL-C are associated with a lower risk of postoperative stroke. These data suggest that aggressive lipid management may be an important strategy to reduce the risk of stroke following CABG.

**Table**

<table>
<thead>
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<th>LDL-C</th>
<th>&lt; 70 mg/dL</th>
<th>71–100 mg/dL</th>
<th>101–130 mg/dL</th>
<th>&gt; 130 mg/dL</th>
<th><em>P</em> value</th>
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<td>Beta-blocker</td>
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<td>97/171 (56.7)</td>
<td>163/308 (52.9)</td>
<td>257/510 (50.4)</td>
<td>.218</td>
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<td>Statin</td>
<td>*37/75 (49.3)</td>
<td>*52/171 (30.4)</td>
<td>76/308 (24.7)</td>
<td>108/510 (21.2)</td>
<td>&lt; .001</td>
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<tr>
<td>ACE inhibitor</td>
<td>34/75 (45.3)</td>
<td>66/171 (38.6)</td>
<td>78/308 (25.3)</td>
<td>105/510 (20.6)</td>
<td>&lt; .001</td>
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<td>Aspirin</td>
<td>66/75 (88.0)</td>
<td>145/171 (84.8)</td>
<td>268/308 (87.0)</td>
<td>451/510 (88.4)</td>
<td>.656</td>
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<td>Calcium channel blocker</td>
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<td>44/171 (25.7)</td>
<td>71/308 (23.1)</td>
<td>121/510 (23.7)</td>
<td>.665</td>
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<td>15/307 (4.9)</td>
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<td>Antiplatelet</td>
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<td>4/171 (2.3)</td>
<td>5/306 (1.6)</td>
<td>11/507 (2.2)</td>
<td>.853</td>
</tr>
</tbody>
</table>

**Outcome at 1 year**

| Stroke | 2/139 (1.4) | 6/388 (1.5) | 22/706 (3.1) | 54/1,272 (4.2) | **.033** |
| Death/MI | 20/139 (14.4) | 70/388 (18.0) | 122/706 (17.3) | 218/1,272 (17.1) | **.808** |
| Death/stroke/MI | 22/139 (15.8) | 74/388 (19.1) | 139/706 (19.7) | 270/1,272 (21.2) | **.407** |

Values inside parentheses are percentages.

* Significant difference between groups (*P* < .01).
POSTER ABSTRACTS

3 Brain Edema and Blood-Brain Barrier Leakage
Influence Antiepileptic Drug Levels
Giulia Betto, Vincent Fazio, Damir Janigro, and Chaitali Ghosh
Cerebrovascular Research Center, Cleveland Clinic, Cleveland, OH

Purpose: Cerebrovascular dysfunction can result from cardiac events (eg, cardiac arrest or surgical interventions (eg, coronary artery grafting). Cerebrovascular disease is a common cause of acute seizures and is characterized by loss of blood-brain barrier (BBB) permeability and extravasation of serum protein. While brain penetration of antiepileptic drugs (AEDs) in chronic epilepsy has been extensively studied, comparably little is known about AED pharmacokinetics under conditions of pronounced BBB leakage. We studied the effect of BBB disruption (BBBD) on brain-to-plasma distribution of hydrophilic (deoxyglucose and sucrose) and lipophilic (phenytoin, doxorubicin, and phenobarbital) molecules. Specifically, we wished to test the hypothesis that lipophilic and hydrophilic drug distribution is differentially affected by BBBD.

Methods: In vivo BBBD was performed in rats by intracarotid injection of hyperosmotic mannitol. Radiolabeled drugs or unlabeled phenytoin were measured and correlated to brain water content and protein extravasation. In vitro hippocampal slices were exposed to different osmolalities; drug penetration and water content were assessed by analytical and densitometric methods, respectively.

Results: BBBD resulted in a rapid extravasation of serum protein and radiolabeled drugs independently from brain edema. In contrast, large shifts in water content in in vitro brain slices had a small effect on drug penetration. In both cases, the total drug permeability increase was greater for lipophilic than hydrophilic compounds. BBBD reduced the amount of free lipophilic drug in the brain parenchyma.

Discussion: Our data show that damage of the BBB as seen after cerebrovascular failure due to cardiac arrest or extracorporeal circulation results in a dramatic increase in serum protein extravasation and reduced free AED levels. This may represent a new mechanism contributing to poor efficacy of AEDs in acute patients affected by postoperative seizures or seizures following cardiac arrest or stroke.

4 CPAP Treatment vs Conservative Treatment in Mild Obstructive Sleep Apnea: Implications on Cardiovascular Morbidity
Kumar Budur, MD, and Nattapon Jaicharityam, MD
Sleep Disorders Center and Department of Psychiatry and Psychology, Cleveland Clinic, Cleveland, OH

Background: Obstructive sleep apnea (OSA) is associated with significant cardiovascular morbidity and an increase in overall mortality. The apnea-hypopnea index (AHI), defined as the number of apnea and hypopnea episodes per hour of sleep, defines disease severity (5 to 14.9 mild; 15 to 29.9 moderate; ≥ 30 severe). Continuous positive airway pressure (CPAP) is the treatment of choice for moderate to severe OSA as it results in improved daytime functioning and decreased cardiovascular morbidity. No studies have compared outcomes between CPAP and conservative therapy in mild OSA.

Objective: To determine if CPAP treatment of mild OSA is associated with a lower incidence of cardiovascular morbidity (hypertension, angina, and stroke). This is part of an ongoing larger study. Here we focus on mean arterial blood pressure (MBP), as the other outcomes (stroke, angina, death) require a longer follow-up.

Research Questions: (1) Will treating mild OSA with CPAP lead to reduced or stable MBP 2 years after the diagnosis of OSA? (2) If so, are the differences in outcomes between these groups significant enough to recommend CPAP therapy for mild OSA?

Classification: Retrospective cohort study.

Setting: Cleveland Clinic Sleep Disorders Center.

Participants: Subjects with mild OSA diagnosed between November 2004 and March 2006.

Inclusion Criteria: Age > 18 years and < 65 years; diagnosis of mild OSA by polysomnography; subject’s primary care physician was within Cleveland Clinic Health System.

Exclusion Criteria: Hypertension (>130/90 mmHg), angina, stroke, cigarette smoking, alcohol abuse/dependence, licit drug abuse.

Intervention: CPAP therapy.

Primary Outcome Measure: MBP 2 years after the diagnosis.

Results: Unmatched for covariates (age, sex, BMI, neck circumference, AHI, arousal index, and family history of cardiovascular problems), subjects with mild OSA on CPAP treatment had a 1.97-point drop in MBP while subjects who did not receive CPAP treatment had a 9.61-point elevation in MBP (P < .0001). Analysis of data after propensity score matching for covariates showed a mean difference in MBP of −11.97 (95% CI: −14.03 to −9.92; P < .0001) with a sensitivity analysis result of 2.646. Furthermore, stratification of propensity scores and quintile analysis revealed a similar result, although the magnitude of the net treatment effect was smaller: −3.83 (95% CI: −1.92 to −5.74).

Conclusions: OSA is a common disorder associated with significant morbidity and an increase in overall mortality. Although benefits of CPAP treatment are well established in moderate and severe OSA, there is a paucity of knowledge regarding the long-term morbidity and treatment benefits associated with mild OSA. This study revealed worsening of MBP in subjects with mild OSA who did not receive CPAP treatment. Also, CPAP treatment effectively stabilized or decreased MBP over a 2-year period. Further research with a large sample and a longer follow-up is recommended to determine if mild OSA is also associated with other cardiovascular and cerebrovascular complications and to determine the effectiveness of CPAP in alleviating these complications.

LDL-C concentration, which significantly reduced the risk of stroke as well as the composite end point of death, MI, or stroke. These data suggest that a discharge regimen including statin therapy may reduce postoperative morbidity and warrants prospective validation.

New Bioinformatics Program Identifies Behavioral Medicine Interventions for Epidemic Cardiovascular Disease in the Developing World: Analysis of Multidisciplinary Findings for Launching a New Global Public Health Initiative in Heart-Brain Medicine

William C. Bushell, PhD
Medical Anthropology Program, Massachusetts Institute of Technology, Cambridge, MA

The Institute of Medicine of the National Academy of Sciences recently reported that cardiovascular disease has become “an emerging epidemic” and one of several principle causes of morbidity and mortality in the developing world.1,2 The Institute further recommends behavioral medicine as one of the interventional components with “huge potential” for combatting this epidemic.3,5

Behavioral medicine is central to the basic scientific, theoretical, and clinical core of heart-brain biology and medicine, and recent research has identified contemporary (eg, biofeedback) and traditional (eg, meditation, yoga) cognitive-behavioral practices which appear to possess significant cardiovascular health-enhancing properties. In fact, more specifically, a recently developed state-of-the-art bioinformatics program in heart-brain biology and medicine (see Bushell and Bushell et al, in preparation), deriving from advances in “knowledge discovery in databases,”6 “literature-related discovery,”6 and “expert-guided search construction,”7 has identified a regimen of cardiovascular health-enhancing cognitive-behavioral practices (particular forms of meditation, yogic breath control, special physical exercises, and dietary practices) which, according to recent evidence (reviewed in Bushell et al, in preparation) can be particularly well suited for dissemination, training, and practice in the context of the developing world. Preliminary evidence demonstrates significant efficacy of this regimen with respect to enhancements in cardiac vagal tone as reflected in heart rate variability; blood pressure; urinary sodium excretion rate; recovery from acute myocardial infarction, as reflected in the Short Physical Performance Battery and other measures; treatment of symptoms of advanced heart failure, including systemic vascular resistance and cardiac output; and, according to a recent study,8 a 30% reduction in the rate of cardiovascular mortality (all reviewed in Bushell et al, in preparation).

The above data will be reviewed in this presentation in the context of further database development and other plans in behavioral medicine for a new global public health initiative which is in the process of bringing together experts from both “traditional behavioral medicine” (including the Dalai Lama) and the field of heart-brain medicine. The emerging field of heart-brain medicine should follow the admirable lead of the Institute of Medicine (and many others) and include in its mission a “heart-centered,” compassionate commitment to helping as much as possible those struggling against terrible odds with disease in the developing world.


Do Systemic Inflammation and Blood-Brain Barrier Failure Play a Role in Pediatric Psychosis?

Erin Carlton,1 Tatiana Falcone,1 Ayush Batra,1 Vince Fazio,1
Kathleen Franco,1 and Damir Janjgro1,2,4
1Cerebrovascular Research Center, Department of Neurosurgery, and Department of Psychiatry and Psychology, Cleveland Clinic; 2Department of Molecular Medicine, Cleveland Clinic Lerner College of Medicine, Cleveland, OH

Context: Blood-brain barrier (BBB) failure occurring down-stream of inflammatory processes plays a role in seizure disorders and other neurological diseases. Human and animal studies have suggested an underlying inflammatory mechanism for a variety of neurological disorders, including schizophrenia. To date, all available reports focused on adult patients with chronic schizophrenia. No studies have evaluated a possible link between inflammation and BBB leakage.

Objective: We wished to test the hypothesis that first-episode psychosis, a prodromic event often leading to chronic schizophrenia, is associated with inflammation and BBB leakage.

Patients: We studied patients admitted to a pediatric inpa-
tient psychiatric unit. Patients (n = 86) had new-onset psychosis diagnosed using DSM-IV TR criteria for Psychosis NOS, schizophreniaform disorder, or schizoaffective disorder. Patients were matched for age, race, and gender with nonpsychotic inpatient controls within the same unit (n = 86). We also compared these values to normal control ranges. An additional 10 psychotic patients and as many normal controls were used for cytokine and S100B serum level analysis.

Main Outcome Measures: In this study, we measured cellular and serum markers of systemic inflammation and BBB leakage.

Results: White blood cell values revealed a significant increase in absolute monocytes (0.62 ± 0.29; P < .01) and lymphocytes (2.51 ± 0.8; P < .05) in psychotic patients compared to nonpsychotic controls (0.47 ± 0.16 and 2.21 ± 0.69, respectively). All other hematologic values were similar between the groups. In addition, psychosis was characterized by increased serum levels of S100B, a peripheral marker of BBB damage. Several inflammatory mediators (eg, TNF-α, IL-1-β, IL-6) were elevated in psychotic children.

Conclusions: These results strongly support a link between systemic inflammation, subsequent BBB failure, and first-episode psychosis in pediatric patients.
Brain, Heart, and Education

Linda Bryant Caviness, PhD
La Sierra University, Riverside, CA

Connectivity asserts itself today as a universal principle. Well-informed minds recognize integral connections within all life processes. Connectivity’s verity reconfirms the value of holistic perspective as a research method. Predicated on this value, heart-brain connections become a justifiable consideration for integrated studies.

This presentation reports on research which suggests that a fractal-like triad pattern of form and function repetitiously typifies brain, body, and heart function at micro and macro levels. This triad pattern offers new perspective relating heart-brain science to educational practice—learning abilities/disabilities, emotional intelligence, states of resilience, motivational considerations, heart-brain-friendly instruction, etc. The triad construct further substantiates the need for interdisciplinary collaboration.

More than any other profession, education shapes brains and patterns social/emotional behavior, yet teacher education currently provides little or no emphasis on the science of learning, relating, and thriving. There exists a need to include this study—including the heart’s involvement—in teacher training.

Gradually forming now is a consortium recognizing this need. This evolution resembles the development of “neuroeducation,” an initiative that relates brain science to classroom practice. Harvard University’s Graduate School of Education leads this movement with their Mind, Brain and Education program.

Developing now at La Sierra University’s School of Education is a graduate concentration called Brain, Heart, and Education (BHE). Established on the postulate that in order to understand the brain, we must also understand its connections to the heart, this 20-unit concentration emphasizes study and research on educational implications of heart-brain connections. Related research already under way includes “Effect of Media Violence on HRV/GSR/RR” and “Baseline HRV Comparisons Among Varied K-6 School Populations Across North America.”

This session reports on education-related heart-brain science developments. Additionally, it describes interdisciplinary affiliations, including mentorship provided by Earl Bakken in support of the concept of “blended education.”

Tobacco Smoke Mediates a Monocytic and Endothelial Proinflammatory Activation that Synergistically Affects BBB Integrity

L. Cucullo,1,2 T. Sathe,1 M. Hossain,1 and D. Janigro1,2
1Cerebrovascular Research Center, Cleveland Clinic, and 2Flocel Inc., Cleveland, OH

Cigarette smoke is known to contain high concentrations of free radicals and oxidants. However, virtually nothing is known about the oxidative damage associated with cigarette smoke on the human brain microvasculature and, more specifically, on the cellular components of the blood-brain barrier (BBB). In this study we assessed whether exposure to tobacco smoke (TS) affects the BBB integrity and the specific effect on BBB endothelial cells.

Our results clearly show that chronic exposure to CSSE induced the proinflammatory activation of the microvascular endothelium demonstrated by increased levels of locally secreted proinflammatory cytokines (interleukin-6 [IL-6], tumor necrosis factor-alpha [TNF-α], and interleukin-1 beta [IL-1β]). In addition, the expression level of relevant vascular adhesion molecules such as VCAM-1, P-selectin, and E-selectin was also increased. Exposure to TS facilitated the differentiation of a well-known human acute monocytic leukemia cell line (THP-1) into mature and activated macrophages. This differentiation process was accompanied by a TS dose-dependent MMP-2 and MMP-9 activation. MMP activity was also detected by zymography in the culture medium of endothelial cells following the exposure to TS. These data strongly suggest that cigarette smoke synergistically modulates WBC differentiation as well as WBC and endothelial cell proinflammatory activation, thus ultimately hampering BBB integrity. BBB integrity was monitored by real-time measurements of transendothelial electrical resistance (TEER) while the levels of adenylate kinase released in the culture medium were used to assess for cell viability.

TS exposure also caused a cellular shift toward a more anaerobic and therefore less efficient metabolism. This was determined by significant (P < .01) increase in lactate production. Interestingly, antioxidant supplementation with vitamins C and E reduced or fully prevented the oxidation and the inflammatory damage induced by cigarette smoke.

This work was supported by Philip Morris USA and Philip Morris International external research awards to Dr. Luca Cucullo and by NIH-2RO1 HL51614, NIH-RO1 NS43284, and NIH-RO1 NS38195 to Damir Janigro.

Dynamic Changes in ECG Predict Poor Outcome After Aneurysmal Subarachnoid Hemorrhage (aSAH)

H.A. Elsharkawy, MD;1 S.M. El Hadi, MD, PhD;2 J.E. Tetlaff, MD; and J.J. Provencio, MD, FCCM1
1Departments of General Anesthesiology, Neurology, and Neurosciences, Cleveland Clinic, Cleveland, OH, and 2Department of Anesthesiology, Alexandria University Hospital, Alexandria, Egypt

Electrocardiographic (ECG) abnormalities following a SAH have been well documented. New evidence suggests that ECG changes and cardiopulmonary dysfunction worsen outcome, but determining which patients are at most risk is unclear and important.

To address this issue, we prospectively studied clinical markers, cardiac abnormalities, and clinical outcome in 20 patients (12 women and 8 men) admitted to the neurosurgical ICU of a large academic hospital within 48 hours of SAH due to ruptured cerebral aneurysm. All patients had ECGs performed prior to surgical clipping, during the clipping surgery, and during the subsequent postoperative period. Their ages ranged between 18 and 70 years (mean = 47.21). The aneurysm was located in the anterior circulation in 17/20 patients (85%) and in the posterior circulation in 3/20 patients (15%). Seven patients (35%) were Hunt and Hess grade I, 5 (25%) were grade II, 2 (10%) were grade III, 3 (15%) were grade IV, and 3 (15%) were grade V.
Patients were grouped according to the presence or absence of ECG abnormalities during the study period. Seven patients had normal ECGs and 13 had abnormalities at some time during the study period. Four patients (30.7%) with ECG changes showed dynamic ECG abnormalities (an abnormality that presented and disappeared during the study period or changed in character). A good outcome was achieved in 6/7 patients (86%) who had no ECG abnormalities compared with 8/13 patients (62%) with ECG abnormalities (not statistically significant, P = .277). All 4 patients who had fluctuating ECG changes had a poor outcome.

In conclusion, the presence of an abnormal ECG alone did not help predict the postoperative neurological outcome, but fluctuating ECG changes did predict a worse outcome. This has implications as a predictor of poor outcome, as well as in defining the pathophysiology of cardiac abnormalities after acute brain injuries. Further research is needed to determine the significance of these dynamic ECG changes and the optimal treatment of cardiac injury in patients with SAH.

### 10 Mechanisms Studies of Malformation of Cortical Development by Prenatal Exposure of Combined Methylazoxymethanol and Thalidomide*

Q. Fan,1 S. Ramakrishna,1 N. Marchi,1 V. Fazio,1 K. Hallene,1 and D. Janigro1,2

1Cerebrovascular Research Center and 2Department of Molecular Medicine, Cleveland Clinic, Cleveland, OH

Malformations of cortical development (MCD) represent a common CNS pathology associated with epilepsy. Animal models of MCD include the prenatal exposure to toxins interfering with neuronal migration (methylazoxymethanol, MAM) or vascular formation (thalidomide, THAL). We have recently evaluated the effect of the combination of such toxins. The offspring showed gross anatomical alterations including ectopic neurons, abnormal ventricular size, and edema. We evaluated the molecular correlate underlying such changes. We performed immunohistochemical studies using the neuronal cell marker NeuN and glial cell marker GFAP on brain sections of MAM-THAL-treated and control rats. Brain density was evaluated by gravimetric-densitometric assay. Western blot analysis of aquaporin-1 (AQP1), AQP4, vasculogenesis marker VEGF, and GFAP was performed. TIMM staining was used to visualize mossy fibers of the dentate gyrus. Our results showed ectopic neurons associated with focal leakage of the blood-brain barrier and islets of GFAP-positive cells in early MAM-THAL postnatal rats (P1-P4). Brain water content was significantly higher in MAM-THAL rats in early postnatal stage (P2 and P9), but significantly lower in adult stage (P29), compared with controls. AQP1 and AQP4 levels were significantly higher in MAM-THAL rats throughout the early postnatal and adult stage. VEGF and GFAP levels were downregulated in the early postnatal stage and back to normal in the adult stage. The adult MAM-THAL rats showed abnormal hippocampus and robust mossy fiber sprouting in dentate gyrus and CA3 of hippocampus even though the pathophysiological phenomenon was minimal in cortex. Treatment with MAM-THAL provokes changes in the neurovascular architecture resembling some of the features observed in animals exposed to a single toxin. However, a more dramatic effect on brain water content and significant changes in the levels of expression of channels associated with water parenchymal homeostasis were observed in MAM-THAL-treated animals.

* Finalist for Young Investigator Award.

### 11 Proapolipoprotein A1 Demonstrates Improved Potential as a Serum Marker for Brain Metastases Without Vascular Disease Interference

Vince Fazio,1 Peter Mazzone,1 Nicola Marchi,1 Thomas Masaryk,2 and Damir Janigro1

1Cerebrovascular Research Center and Department of Cell Biology; 2Department of Pulmonary, Allergy, and Critical Care Medicine and Department of Hematology/Oncology; and 3Department of Radiology, Cleveland Clinic, Cleveland, OH

There is controversy about how the brain should be staged in individuals with lung cancer. In addition, continuous monitoring of the brain for the development of brain metastases after definitive treatment is not usually performed. A serum marker of brain metastases would thus be useful. The development of brain metastases is accompanied by disruption of the blood-brain barrier (BBB). Proteomic markers from the brain may appear in the blood when metastases develop. S100β is a well-established marker of BBB opening that has a high rate of false positives for detecting brain metastases in recovering lung cancer patients. This is due frequently to minor leakage of the BBB resulting from small vessel ischemic disease (SVID), a common cerebrovascular finding in patients suffering from hypertension, diabetes, and/or hyperlipidemia, as well as in the elderly. This work discusses a new marker that appears to have a higher positive predictive value for brain metastases compared with S100β.

Based upon initial findings produced by 2D protein electrophoretic analysis of serum from a single lung tumor patient collected both before and after the detection of brain metastases by MRI, we identified strong upregulation of a protein for brain metastases. By LC-tandem MS this protein was identified to be proapolipoprotein A1. This was investigated further by selecting several lung tumor patients screened as positive or negative by MRI with SVID present and absent, as well as control CSF as a brain protein reference. Proapolipoprotein A1 was analyzed in these samples on a 2D gel with an expanded pH range (4.7 to 5.9) by conventional staining and Western blot analysis.

2D protein staining and Western blot analysis confirmed the upregulation of proapolipoprotein in the MRI positive samples and a reference CSF sample. This protein also does not appear to be elevated by the presence of SVID.

Proapolipoprotein and its related variants appear to be potential markers for the presence of brain metastases in lung cancer patients and, unlike S100β and transthyretin, it does not appear to be elevated by the presence of SVID. Detailed findings from this research work can be found in Cancer (Marchi N, Mazzone P, Fazio V, et al. Cancer 2008 [March]; 112[6]:1313–1324).

This work was supported by NIH-RO1 HL51614, NIH-RO1 NS43284, and NIH-RO1 NS38195 to Damir Janigro.
90% Oxygen Saturation—Which Is Important?

Heather Henrickson, PhD; Michael G. McKee, PhD; and Christine S. Moravec, PhD

Heart failure is the leading cause of death in American men and women, and for years this disease was believed to be irreversible. Pharmacological therapies were provided to control disease symptoms, but cardiac transplantation was viewed as the only truly successful therapy. Recent studies, however, have illustrated marked recovery in end-stage heart failure patients who have been hemodynamically supported by a left ventricular assist device (LVAD) prior to transplant. Cardiac tissue removed from these patients before and after LVAD support has shown a reversal of the maladaptive changes to both muscular and cellular function as well as gene and protein expression, suggesting that the failing heart is capable of recovery. This project will test the hypothesis that biofeedback-assisted stress management (BFSM) training can cause a similar reversal of myocardial remodeling in end-stage heart failure patients. The hypothesis will be tested using end-stage heart failure patients who are listed for heart transplantation at the Cleveland Clinic Foundation over a 2-year period. Patients will be divided into three groups: (1) patients who receive BFSM training, (2) patients who do not receive BFSM training, and (3) patients who do not receive BFSM training but require LVAD support while waiting for cardiac transplantation. These three groups will be compared in order to achieve the following specific aims: (1) to measure the efficacy of BFSM on cellular and molecular myocardial remodeling, (2) to measure the clinical efficacy of BFSM on cardiac function, and (3) to assess the effects of BFSM on quality of life, perceived stress, and coping strategies.


Nitric Oxide and Arginine Metabolism in Depression: Effect of a Serotonin-Norepinephrine Reuptake Inhibitor

Angelos Halaris, John Piletz, Omer Iqbal, Debra Hoppensteadt, Jawed Fareed, He Zhu, James Sinacore, and C. Lindsay DeVane

Depressed mood is known to be an independent risk factor for cardiovascular disease. Nitric oxide (NO)-mediated oxidative stress has been linked to endothelial dysfunction preceding cardiovascular disease (CVD).

In this study, we sought to determine if the metabolic conversion of l-arginine to NO might be altered in the plasma of depressed patients as a possible early-warning sign of future CVD, and whether treating depression might affect this pathway favorably. To do this, the following five plasma biomarkers were measured: (1) asymmetric-dimethylarginine (ADMA; an endogenous inhibitor of the NO synthases), (2) total nitrite (a biomarker of endogenous NO production), (3) arginine (an alternative metabolite of arginine and putative stress-related transmitter), (4) myeloperoxidase (an enzyme leading to peroxynitrite production), or (5) nitrotyrosine (an index of peroxynitrite levels). No baseline differences were observed for any of these biomarkers between depressed patients (n = 23) and matched healthy controls (n = 17). An ancillary finding was that nitrotyrosine covaried with body mass index (P = .03). Fourteen of the depressed patients were then treated for 8 weeks with venlafaxine, a serotonin-norepinephrine reuptake inhibitor (SNRI). All 14 patients responded by achieving improved Hamilton Depression rating scores < 10. After 8 weeks on venlafaxine, lowered plasma levels of agmatine (P = .02) and myeloperoxidase (P = .02) were observed and there was a trend for elevated nitrite to be correlated with antidepressant drug levels (P = .07). None of the other biomarkers were affected by venlafaxine treatment.

Thus, the l-arginine to NO pathway appeared normal in depressed patients lacking symptoms of CVD, yet the antidepressant venlafaxine appeared to affect certain components of this pathway. The possible long-term clinical consequences of these changes in the l-arginine to NO pathway are unknown but may warrant further study of the cardiovascular effects of venlafaxine and other SNRIs.

Association Between Excessive Daytime Sleepiness and Oxygen Desaturation in Obstructive Sleep Apnea Syndrome: Nadir Oxygen Saturation vs Mean Oxygen Saturation vs Time Spent Below 90% Oxygen Saturation—Which Is Important?

Nattapong Jaimchariyatam, MD, and Kumar Budur, MD

Introduction: Excessive daytime sleepiness (EDS), one of the most common symptoms associated with obstructive sleep apnea syndrome (OSAS), is thought to result from sleep fragmentation and/or hypoxemia during sleep. Multiple oxygen saturation values have been used to indicate the severity of hypoxemia secondary to respiratory events. Previous studies have shown some evidence linking either nadir or mean oxygen saturation with EDS. To date, none of the studies has systematically investigated the importance of time spent below 90% oxygen saturation nor compared the relative value of each of these three measures.

Methods: 300 polysomnograms of OSA patients (apnea-hypopnea index [AHl] > 5) were reviewed (150 cases with > 5% of total sleep time spent below 90% oxygen saturation; 150 cases with nadir oxygen saturation of 90%) over the period from 2003 to present. Daytime sleepiness was defined using the Epworth sleepiness scale (ESS).

Results: ESS score was significantly correlated with neck circumference (r = 0.343, P < .01), BMI (r = 0.350, P < .01), AHI (r = 0.301, P < .01), arousal index (r = 0.178, P < .05), mean oxygen saturation (r = −0.293, P < .01), nadir oxygen saturation (r = −0.491, P < .01), and spending > 5% of total sleep time below 90% oxygen saturation (r = −0.615, P < .01). Logistic regression modeling and multivariate analysis were used to determine the role of these variables and significant sleepiness (ESS > 10). After adjustment for covariates, only spending > 5% of total sleep time below 90% oxygen saturation was statistically significant for the high ESS in the multivariate analysis, with an odds ratio of 20.176 (P < .001; 95% CI, 10.758 to 37.745).

Conclusion: The results of this study show that the severity of oxygen desaturation, defined as spending > 5% of total sleep time below 90% oxygen saturation, may be the most important factor in EDS in patients with OSAS.
15 Endotoxin Preconditioning of the CNS: Microglia Activation and Neuroprotection

Walid Jalabi, Ranjan Dutta, Yongming Jin, Gerson Criste, Xinghua Yin, Grahame J. Kidd, and Bruce D. Trapp
Department of Neurosciences, Cleveland Clinic, Cleveland, OH

Preconditioning by subthreshold stress can protect the brain from subsequent injury. Preconditioning can be induced by a number of mechanisms including hypoxia, ischemia, heat shock and intraperitoneal injection of the endotoxin lipopolysaccharide (LPS). While global preconditioning with low doses of LPS provides protection against injurious focal ischemia in the brain, the cellular mechanisms involved in LPS neuroprotection are incompletely understood. C57BL/6 mice were injected with four intraperitoneal injections of LPS, 24 hours apart. This LPS paradigm reduced the size of cortical cryoinjury by 60%. In this study, we examined the response of activated microglia to intraperitoneal injection of LPS and investigated the mechanisms by which the CNS is protected. One day after LPS treatment, cortical microglia expressed activation markers and ensheathed neuronal cell bodies and proximal dendrites. Electron microscopy analysis demonstrated that activated microglia directly apposed neuronal plasma membranes. Quantification of confocal microscopy images immunostained for neurons and GAD67-positive presynaptic terminals shows a 27% reduction (P < .001) in the neuronal circumference occupied by inhibitory GABAergic synapses. In addition, GABA receptor transcripts were significantly reduced 1 day after LPS treatment. mRNA and protein levels of the anti-apoptotic molecule Bcl-2 were increased in LPS-treated animals and highly enriched in neurons. Furthermore, LPS treatment inhibits the pro-apoptotic protein BAD. These data support the hypothesis that LPS induces an anti-apoptotic pathway in cortical neurons. Similar to the neuroprotective effects of LPS, microglia activation, reductions in inhibitory innervation of cortical neurons, and cortical Bcl-2 upregulation were transient and returned to control levels at 14 days post-LPS treatment. In summary, microglia activation is a surrogate marker for LPS-induced CNS protection. It remains to be determined if these activated microglia are actively stripping synapses from cortical neurons. These data support microglia activation as part of a CNS neuroprotective response that involves preferential reductions in GABAergic axosomatic synapses. Reductions in inhibitory innervation may transiently favor neurotrophic activity of excitatory NMDA agonists and induction of anti-apoptotic pathways in neurons.

This investigation was supported by a postdoctoral fellowship from the National Multiple Sclerosis Society.

16 Pilot of Stress Reduction Strategies for Patients After a Coronary Event

R. Lindquist,1,2 D. Windenburg,2 K. Savik,1 and U. Bronas1
1University of Minnesota School of Nursing and 2Women’s Heart Health Program of Abbott Northwestern Hospital/Minneapolis Heart Institute Foundation, Minneapolis, MN

Background: Chronic psychosocial stress has become increasingly recognized as a significant risk factor for coronary artery disease (CAD). Interest in stress as a risk factor for cardiovascular disease has surged following the INTERHEART Study findings comprising results from over 52 countries and over 30,000 patients revealing that psychosocial stress ranked second only to lifetime smoking as a risk factor for major cardiac events.

Purpose: This study was designed to assess the effects of two interventions selected for their potential to impact the stress responses of men and women with documented heart disease and to improve subclinical markers of CAD. The effects of these interventions on markers, and the potential benefits of a Web site to facilitate stress reduction, were evaluated to build a foundation for a planned randomized clinical trial (RCT) submission to NHLBI.

Methods: From the cardiac clinic at a large tertiary care institution in the Midwest, 21 patients were recruited and assigned to one of three groups of 8-week intervention:

1. a mindfulness-based stress reduction (MBSR) program (4 men and 7 women)
2. a women-only weekly psychoeducational support group (SG) (6 women)
3. a stress reduction Web site (4 men) with no meetings except an introduction and weekly site-use logs.

The following measures were assessed at baseline and after 8 weeks of intervention participation: subjects’ stress reactivity (NHLBI protocol), including measures of salivary cortisol and amylase; psychosocial measures (Spielberger’s state anxiety, ENRICHID emotional support, PSS for perceived stress, SF-12 PCS and MCS, CES-D for depression, and Cantril Ladder); and serum biomarkers including BNP, HS-CRP, endothelin, cortisol, catecholamines, interleukin-6, and platelet reactivity (BL only).

Patients’ perceptions of program effectiveness and quality were assessed at 8 weeks and psychosocial measures were again assessed at 6 months (data not included). The feasibility, safety, and efficacy of obtaining laboratory measures of endothelial function and structure were also assessed in a small subset of participants; measures included flow-mediated dilation, reactive hyperemia-peripheral arterial tone, and pulse-wave velocity and analysis.

Results: The intervention programs were completed by 90% of subjects. One male patient died of noncardiac causes (MBSR group), and one other man dropped out of the MBSR group. Analysis of baseline to 8-week follow-up data was done across the whole sample for stress reactivity and by the whole sample and by group for the other variables. Overall, for all participating subjects, depression, physical function, perceived quality of life, and perceived stress improved; however, there were no significant pre-to-post changes across groups in the serum biomarkers. The SG had 84% attendance over time; in this group, the psychological and biological variables showing pre-to-post improvement included the ENRICHID support scale, SF-12 PCS, and serum cortisol (P = .026, .043, and .043, respectively). In the MBSR group, HS-CRP, PSS, and the SF-12 physical component improved from baseline (P = .017, .046, and .021, respectively). Two men dropped out of the Web-based group; in this group there were pre-to-post improvements in PSS, CES-D, STAI, and ENRICHID, but no statistical analyses were done since only 2 subjects remained in the group. Across all groups, the quality and helpfulness of the intervention programs were assessed uniformly positively. The stress reactivity protocol was successful in inducing stress, with elevations in blood pressure and heart rate as well as an increase in salivary amylase in 50% of subjects.

Conclusions and Recommendations: Participation in the SG was associated with improvement in emotional support and physical functioning and reductions in serum cortisol. The MBSR program was helpful in improving perceived stress, physical functioning, and inflammatory markers. The stress reactivity protocol was effective in inducing the stress response and elevations in heart rate, blood pressure, and salivary amylase, despite
the use of beta-blockers by some patients. Laboratory evaluations were assessed to be feasible and safe, and they generated fully meaningful/interpretable data. Survey, serum, and laboratory measures that were judged sensitive to stress reduction interventions in this small pilot will be employed in a larger pilot, and strategies to increase the use and effectiveness of the Web site will be employed in preparation for a larger pilot and subsequent RCT. 

Supported by the Women's Heart Health Program of Abbott Northwestern Hospital/Minneapolis Heart Institute Foundation.

17 Cerebrovascular Substrates of Seizures After Cardiopulmonary Bypass
Rebecca O'Dwyer, Tim Wehner, Dileep Nair, Giulia Betto, Nicola Marchi, and Damir Janigro
Cerebrovascular Research Center, Cleveland Clinic, Cleveland, OH

One of the main interests for the Cerebrovascular Research Center has been to test the hypothesis that blood-brain barrier (BBB) failure is implicated in the etiology of a variety of neurological disorders. A limiting factor has been a lack of a reproducible, inexpensive, noninvasive, and easy-to-perform means to measure BBB integrity in humans. The gold standard for these studies is Gd-enhanced MRI. We have recently shown the equivalence between positive Gd-MRI scans and the BBB marker S100B. This has allowed us to perform several studies investigating the BBB in patients affected by a variety of diseases, including brain metastases, epilepsy, psychosis, and a variety of surgical procedures (eg, cardiopulmonary bypass). Our recent published experiments show that acute BBB failure can lead to focal motor seizures in patients undergoing intra-arterial chemotherapy and that blockade of inflammatory events occurring prior to seizures can abort their onset. We have also shown that in animal models of seizures, BBB failure is an important and preventable etiologic event that can lead to epilepsy. Brain injury is a major adverse event after cardiac surgery, especially when extracorporeal circuits are used. It is also well established that frequent undetected seizures contribute to poor outcome after such procedures, particularly cardiac surgery with normo- or hypothermic bypass. We collected data from 116 patients who developed EEG or behavioral seizures following cardiopulmonary bypass procedures ranging from CABG to other reconstructive procedures. Statistical analysis revealed that the highest predictor of seizure duration (but not, surprisingly, of the number of seizures) was time on pump and a preoperative history of seizures. Seizure duration and frequency also correlated with postoperative complications and mortality. Our data suggest that intra- and postoperative seizures are a potentially significant prognostic factor in cardiothoracic patients.

18 Depression and Whole Blood Serotonin in Patients with Coronary Heart Disease from the Heart and Soul Study
Lawson Wulsin,1 Dominique Musselman,2 Christian Otte,3 Erica Bruce,4 Sadia Ali,4 and Mary Whooley4
1University of Cincinnati, Cincinnati, OH; 2Emory University School of Medicine, Atlanta, GA; 3University Medical Center Hamburg-Eppendorf, Hamburg, Germany; and 4Veterans Affairs Medical Center, San Francisco, CA

Objective: Depression is associated with incident coronary heart disease (CHD) and with adverse cardiovascular outcomes. Dysregulation of peripheral serotonin, common to both depression and CHD, may contribute to this association. However, it is unclear whether depression is associated with serotonin in outpatients with stable CHD.

Methods: We performed a cross-sectional study of 791 participants with stable CHD enrolled in the Heart and Soul Study and not taking antidepressant medication. We assessed major depression using the Computerized Diagnostic Interview Schedule (CDIS-IV) and measured whole blood serotonin (WBS) from fasting venous samples.

Results: Of the 791 participants, 114 (14%) had current (past month) major depression, 186 (24%) had past (but not current) major depression, and 491 (62%) had no history of depression. Age-adjusted mean WBS was higher in participants with current major depression (139 ± 6.5 ng/mL) than in those with past depression (120 ± 5.0 ng/mL) or no history of depression (119 ± 3.1 ng/mL) (P = .02). The strength of this association was unchanged after adjustment for demographic characteristics, medical comorbidities, medication use, and cardiac disease severity (P = .2). When serotonin was analyzed as a dichotomous variable, current depression was associated with a 70% greater odds of having WBS in the highest quartile (adjusted OR = 1.7; 95% CI, 1.01 to 2.8; P = .04).

Conclusions: In this sample of patients with stable CHD, current major depression was independently associated with higher mean WBS levels. Future studies should examine whether elevated WBS may contribute to adverse outcomes in patients with depression and CHD.

19 Gender Differences Prominent in Linking Anxiety to Long-Term Mortality Among the Elderly*
Jianping Zhang, MD, PhD;1 Boaz Kahana, PhD;2 Eva Kahana, PhD;3 Bo Hu, PhD;1 and Leo Pozuelo, MD1
1Department of Psychiatry and Psychology, Cleveland Clinic; 2Department of Psychology, Cleveland State University; 3Department of Sociology, Case Western Reserve University; and 4Department of Biostatistics, Cleveland Clinic, Cleveland, OH

Purpose: Previous findings on anxiety predicting mortality were inconsistent. Limitations of previous studies include single anxiety assessment, short follow-ups, and disregard for gender difference. Few studies examined the change in anxiety over time. To address these limitations, we explored gender differences in the association of changes of anxiety and long-term mortality among community-dwelling elderly.

Methods: At baseline, 1,000 people (M age = 79.8 years; 65.8% women) had psychosocial assessment, including an anxiety scale from the Positive and Negative Affect Scale. They were then assessed annually up to 12 years. Trajectories of changes in anxiety were modeled by a joint modeling method of repeated measures and survival data. Cox regression and individual growth curve analysis to predict mortality at follow-up. We controlled for demographics, health behavior, health problems, functional status, and cognitive impairment.

Results: Total mortality rate was 71.2% at 15-year follow-up. In the whole sample, both lower baseline anxiety and increasing anxiety over time were predictive of higher mortality. For men, baseline anxiety was not predictive of anxiety, but increases in...
anxiety scores over time were associated with 45% higher risks of mortality (HR = 1.45, P < .001) after adjusting for covariates. For women, lower baseline anxiety was predictive of higher mortality (HR = .91, P < .001); in contrast, the change in anxiety scores over time was not a significant predictor of mortality.

Conclusion: The association between anxiety and mortality may depend on gender. Anxiety may be protective for women, potentially through increased health care utilization. In contrast, increasing anxiety over time is more detrimental to men. More research is needed to understand the mechanisms.

* Finalist for Young Investigator Award.

**20 Temporal Lobe and Sinus Node: A Case Report Provides Evidence for Bidirectional Effects**

Rebecca O’Dwyer, MD;1 Andreas Alexopoulos, MD, MPH;1 Walid Saliba, MD;2 Imad Najm, MD;1 and Richard Burgess, MD, PhD1

1Epilepsy Center, Department of Neurology, Neurological Institute, and 2Department of Cardiovascular Medicine, Cleveland Clinic, Cleveland, OH

Introduction: Epileptic seizures have been associated with changes in autonomic nervous system (ANS) function, such as changes in blood pressure and in heart and respiratory rate, as well as cardiac arrhythmias. A relationship between temporal lobe epilepsy (TLE) and autonomic dysfunction has been recognized. Electrical stimulation of limbic structures and insula may elicit changes in the cardiovascular regulatory system by exciting the central autonomic network. Likewise, increased activity of the ANS may be associated with increased frequency of epileptic seizures. Often cardiac rhythm changes may precede electroencephalographic or clinical changes at the onset of seizures by more than a few seconds. Tachycardia is more frequently seen with seizures arising from the temporal lobe; however, significant ictal bradycardia that rarely leads to asystole may also been seen. Resective epilepsy surgery (temporal lobectomy) is an established treatment modality for intractable TLE, rendering two-thirds of patients seizure free, on average. Whether surgical resection has an effect on the accompanying cardiac dysautonomia is less clear.

Case Report: A healthy 20-year-old male presented to our clinic with a history of seizures starting at the age of 18 years. Seizures were characterized by a rising abdominal sensation, followed by loss of awareness associated with staring, lip smacking, and purposeless hand fumbling. Interestingly, some seizures were associated with an abrupt loss of axial tone, producing a precipitous fall. Other than one isolated febrile seizure as an infant, the patient had no risk factors for epilepsy. As seizures were not controlled despite several antiepileptic medication (AED) trials, the patient underwent an inpatient video-scalp EEG evaluation at the age of 21 years that suggested that seizures were arising from the right mesial temporal lobe. A long QT interval during the ictus was noted at this time. He was found to be a suitable candidate for a standard right temporal lobectomy. This intervention resulted in cessation of seizures for almost 2.5 years (while on lamotrigine 200 mg qd and levetiracetam 3,000 mg qd). Seizures—semiologically unchanged—returned when the patient independently reduced his AEDs. Despite resumption of AED therapy, seizures occurring 2 to 4 times a week again proved intractable to AEDs. During a repeat video-EEG evaluation at the age of 27 years, a seizure associated with prolonged ictal asystole (> 20 sec) was recorded. A three-chamber DDI pacemaker (PM) was placed and the patient was discharged. His seizure frequency decreased to once a month, and on his annual PM follow-up he reported that he remained conscious throughout his seizures. A previously undetected lesion was noted on MRI involving part of the remaining basal temporal lobe. A third (invasive) video-EEG evaluation, with placement of subdural electrodes to map the remaining epileptogenic zone, led to extension of the previous resection with excision of the MRI lesion, found to be a developmental tumor (DNET) on pathological examination. The patient has remained seizure free postoperatively. PM reports < 1% pacing subsequent to the second (curative) epilepsy surgery.

Discussion: Electrical stimulation studies in both animals and humans have shown effects of both cortical and subcortical structures on cardiac rhythm and their potential role in seizure-induced arrhythmias. However, there is little evidence to show an “antidromic” effect from firing of the sinus node on cerebral dysfunction. The patient’s first resection clearly failed to remove the entire epileptogenic zone, as the seizures recurred. However, after placement of the PM, there was a change in seizure semiology and a reduction in seizure frequency, raising the possibility that ictal preservation of consciousness and decreased seizure frequency were due to the firing of the PM. Some AEDs, such as carbamazepine and phenytoin, are known to have modulatory effects on the ANS, but this patient received neither. Likewise, after resection of the residual epileptogenic zone, an improvement in cardiac function, as manifested by a decreased necessity for PM firing, was seen. This case report illustrates the intricate relationship between the heart and brain in TLE, and provides the impetus to further investigate this relationship and its therapeutic potential.

FIGURE. Timeline of events presented in case report.