1 Functional Screen for Neuroprotective Microglial Activation Factors

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Peritoneal injection of lipopolysaccharide (LPS), the gram-negative bacterial endotoxin, elicits a rapid and potent innate immune response. While this systemic inflammatory response can be destructive, tolerable LPS doses of .05 to 1 mg/kg render the heart and brain transiently resistant to subsequent ischemic injury. Microglia, the resident central nervous system (CNS) immune cells, become activated following LPS treatment and can have neuroprotective roles within the brain.

Our lab has established a model for microglial activation induced by a series of four intraperitoneal (IP) LPS injections (1 mg/kg). LPS injection triggers peritoneal macrophages to secrete a barrage of cytokines and other soluble factors into the bloodstream. The purpose of the present study is to identify upstream microglial activation factors.

Enzyme-linked immunoassay of serum tumor necrosis factor (TNF)–α, a cytokine upregulated directly by LPS signaling, revealed that the innate immune response elicited by a single 1-mg/kg IP injection of LPS peaks in the bloodstream after 1 hour. We have isolated large quantities of LPS-stimulated mouse serum from donor mice at this timepoint. Similar to our LPS injection paradigm, a series of three intravenous (IV) injections of 500 μL LPS serum into recipient mice was capable of activating cortical microglia. Flow cytometry revealed a 3.5-fold increase in monocyte CNS infiltration from LPS-treated animals; however, IV injections of 5×10^6 white blood cells purified from LPS donor mice were incapable of initiating microglial activation.

Thus, serum-derived factors appear to be largely responsible for microglial activation within the cerebral cortex. Purification of these upstream serum factors that initiate neuroprotection in LPS-treated mice may provide a unique CNS therapeutic tool that can be administered through the bloodstream without the adverse systemic LPS inflammatory response.

2 Development of Cardiac Hypertrophy and Altered Gene Regulation in Vasopressin-Deficient Rats

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Many organisms display patterns of behavior or physiological responses that are coordinated with environmental cycles of light and dark and are known as circadian rhythms. An important aspect of circadian rhythm biology is the control of output genes by transcription factors. The regulation of circadian genes in the heart is an area of active inquiry, with several hormonal signals proposed to play a role. Vasopressin (VP), a hormone secreted by the posterior pituitary gland in response to hypothalamic stimulation, regulates physiological processes of the excretory and cardiovascular systems. VP is also known to be involved in the mammalian stress response, and may be involved in the regulation of circadian rhythms. This study was designed to assess the effects of VP deficiency on cardiac size and the expression of genes that are involved in circadian regulation.

Long-Evans (LE) rats and VP-deficient Brattleboro (also called diabetes insipidus [DI]) rats were compared. Rats were exposed to a 12-hour/12-hour light/dark cycle with ad libitum feeding. Body weight, food intake, and water intake were monitored. Following sacrifice, hearts were rapidly excised, weighed, and frozen for later analysis. Ventricular tissue was homogenized, and Northern blot analysis with specific cDNA probes was used to quantify expression of atrial natriuretic factor (ANF) and circadian-locomotor output cycles "kaput" (CLOCK).

Data analysis revealed that DI rats consumed more water than LE rats, which was attributed to the VP deficiency. There was no difference in food intake between LE and DI rats, but LE rats had consistently higher body weights, suggesting that VP plays a role in body weight regulation. In spite of increased body weight in LE rats, the ratio of heart weight to body weight was significantly greater in DI rats, demonstrating that lack of VP results in considerable cardiac hypertrophy. ANF expression was also fivefold greater in DI rat hearts than in LE rat hearts. Ventricular expression of ANF is a hallmark of cardiac hypertrophy, and the increased ANF in DI rats verifies the presence of a compensatory and possibly pathological process in VP-deficient animals. The mRNA for CLOCK was not significantly different between DI and LE rat hearts.

Data from this study suggest that lack of VP results in a compensatory cardiac hypertrophy, possibly due to up-regulation of the renin-angiotensin system or altered volume status. Data further suggest that VP is not involved in regulation of CLOCK mRNA. Further study is required to elucidate the potential role of VP in regulating additional genes and transcription factors involved in circadian rhythms.

3 A Cortical Potential Reflecting Cardiac Function

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Emotional trauma and psychological stress can precipitate cardiac arrhythmia and sudden death through arrhythmogenic effects of efferent sympathetic drive. Patients with preexisting heart disease are particularly at risk. Moreover, generation of proarrhythmic activity patterns within cerebral autonomic centers may be amplified by afferent feedback from a dysfunctional myocardium. An electrocortical potential reflecting afferent cardiac information has been described, the magnitude of which reflects individual differences in interoceptive sensitivity (awareness of one's own heartbeats). To inform our understanding of mechanisms underlying arrhythmogenesis, we extended this approach, identifying electrocortical potentials corresponding to the cortical expression of afferent information about the integrity of myocardial function during stress.

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Ten male cardiology patients (mean age [± SD] = 59 ± 11.11 years) with one- to three-vessel heart disease and established ventricular dysfunction **(Table)** were recruited consecutively from two cardiology outpatient clinics (The Heart Hospital, University College London Hospitals Trust, London, UK, and The Whittington Hospital, Hampstead, London, UK). We measured stress-induced changes in cardiac response simultaneously with electroencephalography (EEG), electrocardiography (ECG), and beat-to-beat finger arterial blood pressure measurements (Finometer). Experimentally induced mental stress enhanced cardiovascular indices of sympathetic activity (systolic blood pressure, heart rate, ventricular ejection fraction, and skin conductance) across all patients. However, the functional response of the myocardium varied; some patients increased, while others decreased, cardiac output during stress. Across patients, heartbeat evoked potential amplitude at left temporal and lateral frontal electrode locations correlated with stress-induced changes in cardiac output, consistent with an afferent cortical representation of myocardial function during stress **(Figure)**. Moreover, the amplitude of the heartbeat evoked potential in the left temporal region reflected the proarrhythmic status of the heart (inhomogeneity of left ventricular repolarization).

These novel observations delineate a cortical representation of afferent cardiac information predictive of proarrhythmic abnormalities in cardiac repolarization. Our findings highlight the dynamic interaction of heart and brain in stress-induced cardiovascular morbidity.

TABLE
PATIENT CLINICAL PROFILES

					Current medications		
Pt	Age	Sex	MI	Wall mot	β-blk	Ca-blk	ACE
1	63	M	No	Mod	No	Diltiazem	Ramipril
2	60	M	Yes	Mod	Atenolol	No	Ramipril
3	55	M	Yes	Mild	Atenolol	Diltiazem	Ramipril
4	60	M	Yes	Mod	Atenolol	No	No
5	45	M	Yes	Mod	Atenolol	No	Ramipril
6	77	M	Yes	Mild	Atenolol	Diltiazem	Lisinopril
7	58	M	Uncertain	Mild	Atenolol	No	No
8	42	M	Yes	Mod	Bisoprolol	No	Perindopril
9	71	M	Dilated	Severe	Bisoprolol	No	No
10	58	M	Yes	Severe	Atenolol	Amlodipine	Perindopril

One patient had one-vessel heart disease; all remaining patients had two- or three-vessel heart disease. In addition, patients 2 and 8 had undergone coronary artery surgical procedures; patients 7 and 10 had undergone angioplasty procedures; and patients 2, 9, and 10 had previous heart failure.

MI = myocardial infarction; Wall mot = ventricular wall motion impairment; β-blk = beta-blocker; Ca-blk = calcium channel blocker; ACE = angiotensin-converting enzyme inhibitor

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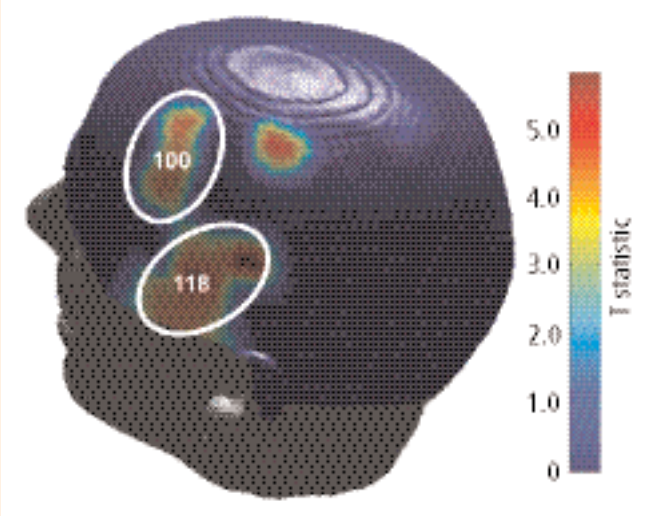
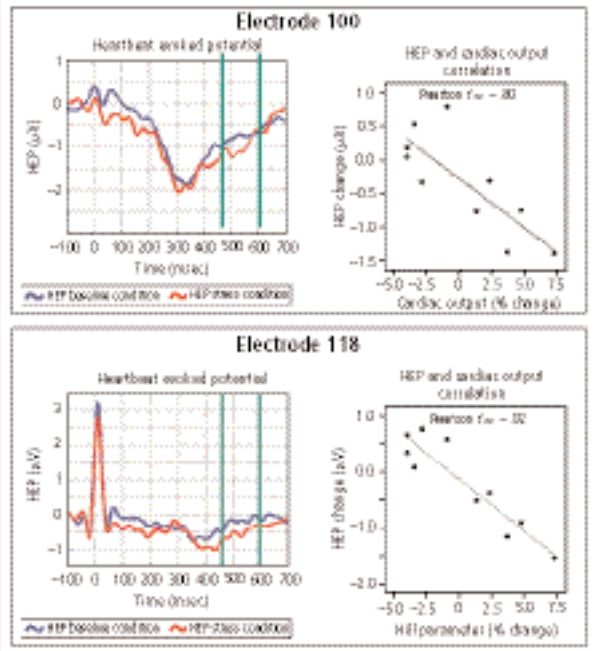
Significant associations of HEP change with cardiac output change



Cluster level significance: $P = .001$



Reprinted from Gray MA, et al. A cortical potential reflecting cardiac function. Proc Natl Acad Sci U S A 2007; 104:6818–6823.

FIGURE. Stress-induced change in cardiac output was significantly correlated with change in heartbeat evoked potential (HEP) amplitude within the left temporal and posterior frontal electrode locations.

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4 Depression Kills: Changes in Depressive Symptoms Predicted Mortality in Community-Dwelling Elderly People

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Purpose: Previous research suggests that depression may be associated with the development of coronary heart disease, myocardial infarction, and increased mortality. In community samples, people with depression may be at risk of higher mortality. However, inconsistent results were found in 32 longitudinal studies on depression and mortality in the literature. Only 14 studies showed positive findings, 11 showed negative findings, and 7 showed positive findings only in subgroups. There are a number of limitations in these studies, including mostly single assessment of depression, relatively short-term follow-up, and inadequate statistical analysis to capture progression of depression. In this study, we examined a group of community-dwelling elderly people to determine the impact of changes in depression on long-term mortality, using a new statistical methodology.

Methods: At study entry, 865 people (mean age of 80.7 years; 65.8% women) underwent comprehensive psychosocial and health assessment, including the Center for Epidemiologic Studies Depression Scale (CES-D, 10-item version). They were then assessed annually for up to 11 years. Mortality was ascertained by the Social Security Death Index up to 15 years. Joint modeling of repeated measures and survival data, as well as separate individual growth curve analysis and Cox regression, was conducted to model the change in depressive symptoms over time in each participant and its impact on mortality. Five classes of covariates were controlled for: demographic (age, sex, education, marital status, income, living situation), health behavior (smoking, alcohol consumption, exercise), chronic disease (body mass index, heart disease, stroke, cancer, diabetes, hypertension, hyperlipidemia), health status (self-rated health, ADL, IADL), and cognitive impairment.

Results: Death occurred in 603 participants (69.7%). The baseline CES-D score predicted mortality after adjusting for age and sex (HR = 1.03, $P < .001$). This predictive power disappeared, however, after adjusting for other covariates. Linear change rates of CES-D scores over time were predictive of mortality after adjusting for covariates (HR = 1.60, $P < .001$). Therefore, an annual increase of 1 point in CES-D score was associated with a 60% higher risk of mortality. To further interpret the results, the sample was divided into three groups based on each individual's change trajectory of depressive symptom scores: "Down," "Stable," or "Up." Compared with the Stable group, the Up group (whose depressive symptom scores increased) had a 70% increase in mortality risk ($P < .001$). On average, they lived almost 4 years less. The Down group was not different from the Stable group. CES-D scores increased by 2.3 points annually, on average, in the Up group, whereas they decreased by 1.1 points annually, on average, in the Down group.

Conclusion: In a longitudinal study, it is important to examine the change trajectory of depressive symptoms and its effects on mortality. Although baseline depression score was not predictive of mortality, a change in depressive symptoms—specifically, an increase in depression over time—was a significant and robust predictor of mortality, even after adjusting for five classes of covariates. Early screening and treatment may stop the progression of depression, which may help prevent excess mortality among the elderly.

5 Endotoxin Preconditioning of the Brain: A Neuroprotective Role of Microglia

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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 (IP) 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. In this study, we investigated the mechanisms by which the central nervous system (CNS) is protected by preconditioning with LPS.

C57BL/6 mice were injected with four IP injections of LPS (1 mg/kg), 24 hours apart, and were sacrificed 1, 7, and 14 days later. One day after LPS treatment, cortical microglia became activated and ensheathed neuronal cell bodies and proximal dendrites. Electron microscopy analysis demonstrated that these activated microglia separated pre- and postsynaptic components of axosomatic synapses. A reduction of 30% in the neuronal area occupied by presynaptic terminals was also observed by confocal microscopy analysis. In addition, a significant decrease in GABA receptor transcripts was obtained 1 day after LPS treatment. mRNA and protein levels of the anti-apoptotic molecule Bcl-2 were increased in LPS-treated animals, indicating a Bcl-2-mediated neuroprotective response. Microglial targeting of neurons and Bcl-2 upregulation were transient and returned to control levels at 14 days postinjection.

In summary, preconditioning doses of LPS lead to microglial activation that targets neurons and strips inhibitory synapses in the cortex. Our data suggest that this interaction may be neuroprotective since activated microglia preferentially remove GABAergic inhibitory axosomatic synapses, thereby transiently favoring neurotrophic activity of excitatory NMDA agonist.

6 Axonal Conduction Block Using High-Frequency Pulse Trains

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Current literature suggests that pulse train high-frequency stimulation (HFS) affects axons by driving them at the stimulation frequency, disrupting abnormal activity in neural networks. Yet recent work has shown that sinusoidal HFS suppresses axonal conduction in vitro.[1] Therefore, we tested the hypothesis that pulse train HFS of fiber tracts blocks axonal conduction.

HFS (monophasic, 0.5 to 200 Hz, 100 μsec, 1 to 2 min) was applied via a monopolar tungsten electrode to either the alveus of transverse hippocampal slices in vitro (rat) or the commissural fiber pathway in vivo (rat). Antidromic field potentials were recorded in the CA1 alveus in vitro or the CA3 region of both hippocampi in vivo. Field potential amplitude, width, and latency were analyzed

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prior to, during, and after HFS. Pulse trains were applied at 100%, 75%, and 50% of the stimulation amplitude required to produce a maximal evoked potential. For block experiments, a second tungsten electrode provided an evoked test pulse through the site of HFS.

The data show that HFS failed to drive axonal activity in vitro (n = 5) or in vivo (n = 4). The number of cycles required for failure was frequency-dependent but independent of stimulus amplitude ($P < .001$ and $P > .5$, respectively, ANOVA). Axons were unable to follow extracellular pulse trains above 80 Hz in vitro, while axons in vivo were unable to follow stimulation above 125 Hz. Axons were unable to follow HFS even after isolation of the alvear axon field in vitro (n = 5). Pulse train HFS above 150 Hz reversibly blocked axonal conduction in an amplitude-dependent manner in vitro and in vivo. These data indicate that HFS of fiber tracts has the potential for controlling abnormal propagating activity within the brain.

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Supported by NIH R01-NS-40894, NIH R01-NS-46556, the Epilepsy Foundation, and a Neural Engineering Training Grant (GAANN) from the US Department of Education.

7 Prevention of Depression and Anxiety in Patients with Acute Coronary Syndrome (DECARD): A Double-Blind, Placebo-Controlled Study of Escitalopram

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Background and Aims: The prevalence of depression and anxiety is higher in patients recovering from acute coronary syndrome (ACS), ie, myocardial infarction (MI) and unstable angina, than in the general population, and both depression and anxiety are associated with poor cardiac outcomes and higher mortality. Despite the prognostic role of depression in ischemic heart disease, no clinical trials have been undertaken to assess prevention of depression and anxiety in this population of patients. The aim of this study is to evaluate the efficacy of preventive treatment with a selective serotonin reuptake inhibitor (escitalopram) in the first year after ACS.

Methods: Two hundred thirty-four (234) nondepressed patients with STEMI, non-STEMI, or unstable angina will be enrolled within 8 weeks after ACS and randomly assigned to treatment with escitalopram (5 to 20 mg) or placebo for 52 weeks. There will be nine psychiatric and three cardiologic assessments during the year of the study. The primary outcome measures are the diagnosis of depression and Hamilton Depression Scale score. Psychiatric measurements: Schedules for Clinical Assessment in Neuropsychiatry, Hamilton Depression Scale, Hamilton Anxiety Rating Scale, UKU Side Effect Rating Scale, ENRICHD Social Support Instrument, Short Form-36 Health Survey, SCL-92 (Symptom Check List), and Beck Depression Inventory. Cardiologic measurements are blood pressure, electrocardiography, echocardiography (left ventricular ejection fraction), heart rate variability, and use of medicine.

Conclusion: ACS patients with mental illness often remain untreated and have an increased risk of somatic comorbidity and mortality. DECARD is the first study evaluating the effect of prophylactic treatment of depression in patients with ACS. The study is ongoing and had enrolled 182 patients as of April 15, 2007.

8 Knowledge Discovery in Databases and Other Techniques in Biomedical Informatics: New Contributions to Heart-Brain Biology and Medicine, with a Focus on Traditional Cognitive-Behavioral Practices

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This presentation describes the development of a new, state-of-the-art database resource in heart-brain biology and medicine focused on the potential contribution to heart-brain health of cognitive-behavioral practices, especially traditional ones such as meditation, yoga, and related practices. Developed by Massachusetts Institute of Techology researchers in collaboration with colleagues from other leading research institutions (Columbia and Cornell Universities), along with His Holiness the Dalai Lama (in the role of nonsectarian representative of expertise in traditional meditation/yoga practice), this database resource is derived from principles in biological and medical informatics that are currently revolutionizing many fields in the human life sciences and medicine. In particular, the resource, or database-based "knowledge discovery system,"[1] has been designed utilizing principles of "knowledge discovery in databases" (KDD)[1,2] through searches designed according to "an expert-guided decision tree construction strategy."[3–5]

In this original investigation in the biomedical informatics of heart-brain medicine utilizing KDD and expert-guided decision tree search strategies, four components of the traditional yoga meditation regimen (cognitive-meditational, respiratory, postural, dietary) were identified as possessing robust and highly significant enhancing effects on cardiac vagal tone, as reflected in heart rate variability (HRV) and baroreflex sensitivity, in agreement with published findings from several studies (Bushell et al, in preparation). A second set of components of the traditional cognitive-behavioral regimen (meditational, respiratory) was identified which preliminary evidence suggests may be associated with protection against an ischemic/reperfusion event (Bushell et al, in preparation). A third set of components of the traditional regimen (respiratory, meditational, postural) was identified which is suggestive of the potential capacity to stimulate regenerative rather than scar-forming responses to an insult in cardiac tissue (Bushell et al, in preparation). Analysis of this bioinformatics evidence was conducted within the framework provided by colleagues' experimentally derived models (see proceedings of the conference, "Longevity and Optimal Health: Integrating Eastern and Western Perspectives," forthcoming in the *Annals of the New York Academy of Sciences*, Bushell and Olivo, editors): Tracey's anti-inflammatory vagal pathway; Blackburn's stress-induced telomere attrition associated with cardiovascular disease; and Heber-Katz's mammalian cardiac regeneration model. Detailed explanations of results will be provided in the presentation.

1. *Fuller SS, Revere D, Bugni PF, Martin GM. A knowledgebase system to enhance scientific discovery: Telemarkus. Biomed Digit Libr 2004; 1:2–16.*
2. *Crasto CJ, Morse TM, Migliore M, et al. Creating knowledgebases to text-mine PUBMED articles using clustering techniques. AMIA Annu*

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Symp Proc 2003; 821–829.
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9 P-Selectin–Targeted Liposomes for Potential Applications in Thrombolytic Therapy

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Atherosclerosis-related vascular thrombosis and occlusion are principal causes of acute cardiovascular and cerebrovascular events leading to morbidity and mortality. Thrombosis and occlusion stem from dysregulated hemostatic phenomena involving platelet and endothelial cell activation, platelet adhesion and aggregation, and triggering of the subsequent coagulation cascade to ultimately form the fibrin "clot." Clot growth leads to arterial occlusion and reduction of antegrade blood flow, which in turn results in ischemia and related acute events. Intravenous or transcatheter systemic infusion of thrombolytic agents is highly effective in revascularization and prevention of acute outcomes. However, this therapy has a very narrow treatment window (hours to minutes after the event, depending on location and severity) and also suffers from limitations such as high cost, short circulation half-life of the drugs (requiring continuous or recurring administration), and potential systemic hemorrhage. If thrombolytics could be maintained in circulation in a "protected state" for longer periods, and then rapidly yet selectively localized only at sites of thrombogenesis, the aforementioned problems could be eliminated. Recent advances in the area of long-circulating "stealth" nanoparticles, coupled with the ever-expanding library of cell-targeted receptor-specific ligands, could potentially provide a way to achieve prophylactic, target-specific action of thrombolytics.

Based on the cellular/molecular mechanisms in thrombosis, we postulated that the P-selectin molecule on activated platelets and endothelial cells would be an ideal receptor for nanoparticle targeting. P-selectin is stored at relatively high concentration within alpha-granules and Weibel-Palade bodies of quiescent platelets and endothelial cells, respectively, and is rapidly translocated to the cell surface for expression upon activation by thrombogenic or inflammatory events. P-selectin is a quantitative marker for platelet activation and forms the surface adhesion receptor for leukocyte interaction with platelets and endothelial cells. Hence, a nanoparticle selectively targeted to sites of P-selectin upregulation can essentially localize specifically at thrombotic sites and deliver an encapsulated thrombolytic drug at that site for sustained release. A similar strategy has demonstrated a promising pharmacokinetic profile and therapeutic efficacy in cancer treatment and hence forms the rationale for this approach.

We have chosen the peptide sequence CDVEWVDVS [Molenaar et al], with specificity and high affinity for P-selectin, as our targeting ligand. We have developed the peptide through solid-phase synthesis, conjugated the peptide to a lipid molecule, and used the lipid-peptide conjugate to form spherical lipid vesicles (liposomes) with the peptide displayed on the liposome surface in multiple copies **(Figure)**. We have tested the peptide-modified liposomes for their platelet P-selectin targeting efficacy in fluorescence microscopy and flow cytometry assays using human whole blood and plasma. We have also performed preliminary formulation and release studies with the liposomes by encapsulating a model drug. Optimization of encapsulation and purification procedure and manipulation of targeting efficacy renders a thrombus-targeted nanoparticle that can potentially carry a variety of therapeutics for cardiovascular applications. Since activated platelets and inflamed endothelium are also involved in the developmental and progressive stages of vascular diseases, P-selectin–targeted nanoparticles may also provide a selective way to deliver optimum payloads of imaging probes to diseased vascular areas for sensitive molecular imaging and diagnostics.

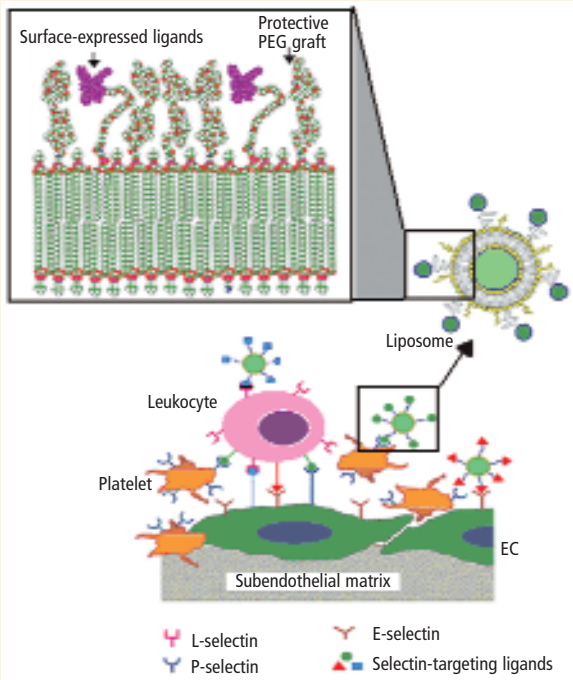

FIGURE. Schematic of selectin-targeted liposome.

10 The Heart-Brain Link: Neural Concomitants of Heart Rate Variability During Emotion

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The vagal (high-frequency [HF]) component of heart rate variability (HRV) is known to predict morbidity and mortality. Previous studies of the neural correlates of vagal tone involved cognitive and mental stress tasks. To explore the neural substrates of vagal tone during emotion, we correlated HF-HRV with measures of cerebral blood flow (rCBF) derived from positron emission tomography (PET) and ^{15}O-water in 12 healthy women.

Happiness, sadness, disgust, and three neutral conditions were each induced by film clips and recall of personal experiences (12 conditions). Inter-beat intervals derived from electrocardio-

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graphic (lead II) recordings during 60-second scans were spectrally analyzed, generating 12 separate measures of HF-HRV in each subject. The six emotion (E) and six neutral (N) conditions were grouped together. We report the correlations between HF-HRV and rCBF specifically attributable to emotion (E minus N). This random-effects analysis in SPM2 updates a previous preliminary report of our findings from a fixed-effects analysis in SPM98.

Four brain areas survived correction for multiple comparisons at the cluster level ($P < .05$) when the significance threshold for the second-level contrast was set at $P < .001$ ($z = 3.11$). Three frontal areas—the right superior (26 43 35: BA 9, 8), the right dorsolateral prefrontal (50 47 5: BA 46), and the bilateral medial prefrontal cortices (−6 47 5: BA 24, 32)—were significant at P values of .025, .025, and .014, respectively. The right parietal cortex (44 −29 47: BA 40) was significant at $P < .043$.

These new random-effects findings, which generalize to the population, demonstrate a predominant role of the right hemisphere, as well as the bilateral medial frontal area, in the regulation of vagal tone during emotion. These results are consistent with the neurovisceral integration model, which posits a role for the prefrontal cortex in the inhibition of sympathoexcitatory circuits in the regulation of emotion.

11 The Unknown Face of Epilepsy and Cardiovascular Care*

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Rationale: The industrial nations face a gray future. The proportion of the population that is 65 years or older has increased from 4.1% in 1900 to 13.0% in 2000. In 2000, Americans enjoyed the longest life expectancy in US history—almost 77 years. Life expectancy was 74 years for men and almost 80 years for women. Accordingly, there is an increased need for updated and tailored patient care congruent with specific age- and gender-related morbidities.

Methods: An 88-year-old female without lung disease was admitted to the Department of Cardiology, Sentara CarePlex Hospital, located in Hampton, Virginia, for new onset of convulsive seizures following a cardiac catheterization procedure. The cardiac catheterization was performed as part of a preoperative cardiovascular risk assessment prior to the patient undergoing surgical removal of a 3.4-cm tumor of the anterior wall of the gastric fundus. Before the catheterization, the patient had received a single 25-mg dose of metoprolol. In addition to mitral valve prolapse, the cardiac catheterization revealed normal coronary arteries, including those supplying the sinus node.

Results: A wandering atrial pacemaker (WAP) abnormality was observed upon admission of the patient to the hospital after her seizure and was also documented following another nocturnal epileptic seizure during hospitalization. The diagnosis of epileptic seizures was supported by the admission electroencephalogram showing a focal disturbance with right-side hemisphere slowing in the presence of a brain MRI revealing mild general atrophy and small chronic basal ganglia lacunes.

The patient was successfully treated with phenytoin with full remission of the cardiac rhythm abnormality and cessation of her epileptic activity.

Conclusions: To the best of our knowledge, this is the first time that a WAP abnormality has been described in a patient experiencing de novo epileptic seizures. Importantly, this patient had no evidence of lung disease and she underwent a cardiologic work-up that excluded an underlying cardiac cause for this abnormality.

Generally, WAP is a benign cardiac dysrhythmia that occurs in normal hearts as a result of fluctuations in vagal tone, although more hazardous conditions, such as hypoxia and chronic obstructive pulmonary disease, can cause WAP. Significantly, WAP may be a precursor to a potentially dangerous cardiac condition, such as atrial fibrillation, that can lead to death from stroke or heart failure. In fact, the death rate among patients with atrial fibrillation is about double that among patients who do not have it.

An important consideration in the context of this case report is the abundant neurological literature describing the association between epileptic seizures and cardiac abnormalities caused by parasympathetic fluctuations and hypoxia, leading to severe atrioventricular blocks and even death. Accordingly, the present case brings us to consider ictal hypoxia along with the vagal fluctuations as the underlying mechanism that triggered this postictal abnormality.

Our case report warrants further studies to look into the specific association between cardiovascular rhythm abnormalities and epileptic seizures, especially in the elderly. Accordingly, better-tailored patient care should be provided by such means as simultaneous electrocardiographic-electroencephalographic monitoring in order to optimize diagnosis and subsequent treatment.

** Finalist for Young Investigator Award.*

12 Does an Acute Inflammatory Response Temporarily Attenuate Autonomic Nervous System Function?*

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Background: Although recent observational studies suggest that inflammatory markers are associated with autonomic function, the causal relationship of this is not clear. Because inflammatory parameters could influence the autonomic nervous system by affecting the hypothalamic-pituitary-adrenal axis, we tested the hypothesis that an acute inflammation is associated with temporarily attenuated autonomic nervous system activity.

Methods: Using a randomized sham placebo-controlled, double-blind design, 24 healthy subjects (age 24.8 ± 3.5 years) were injected with an influenza vaccine (0.5 m/L, Influenza Split Vaccine) or a sham vaccine (0.5 m/L, normal saline) as a model to generate a systemic inflammatory response. Heart rate recovery (HRR) after maximal treadmill exercise was used as an index of autonomic nervous system function and was calculated as the difference between maximal heart rate during the test and heart rate 1 minute (HRR 1) and 2 minutes (HRR 2) after cessation of exercise. Blood samples were taken and HRR was measured before each vaccination and 48 hours after each vaccination.

Results: Log C-reactive protein was significantly increased after influenza vaccination (from 1.87 ± 1.2 to 2.75 ± 1.3, $P <$

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.05), but log tumor necrosis factor-α was not (from 2.01 ± 0.1 to 2.00 ± 0.19, *P* = NS). HRR 1 was significantly attenuated after influenza vaccination but not after sham vaccination **(Table)**. However, HRR 2 was not significantly attenuated after influenza vaccination.

Conclusions: These findings show that an acute inflammation caused a temporary deterioration of autonomic nervous system function. This suggests that inflammation alters autonomic function consistent with an increase in cardiovascular risk.

** Finalist for Young Investigator Award.*

TABLE
EFFECT OF VACCINES ON HEART RATE RECOVERY (HRR)

	Influenza vaccine (n = 15)		Sham vaccine (n = 9)	
Variable	Pre	Post	Pre	Post
HRR 1 (bpm)	23.4 ± 6.4	20.5 ± 4.9*	26.8 ± 10.1	26.2 ± 9.8
HRR 2 (bpm)	46.0 ± 9.3	43.9 ± 10.5	52.0 ± 14.9	51.4 ± 15.2

Values are means ± SD.
* *P* < .05, pre vs post

13 Effects of Guided Imagery on Heart Rate Variability*

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Background: Numerous prospective studies have shown that psychological stressors such as depression, anger, and anxiety are important and major risk factors contributing to the development and progression of cardiovascular disease. These findings have led to an increased interest in elucidating mechanisms linking the brain and the cardiovascular system. Brain imaging studies have shown that various psychological states are associated with changes in the activity of specific regions of the brain. These regions include those that regulate the autonomic nervous system and the hypothalamic-pituitary-adrenal (HPA) axis, providing mechanistic explanations for the altered heart rate variability (HRV), immune system activation, and platelet activation that are associated with psychological stress. Given the effect that emotions have on health, it is also not surprising that there is a growing interest in incorporating stress-reduction practices into an overall wellness approach to medical care. One such practice, guided imagery, has been used, for example, to reduce postsurgical anxiety and pain, yet nothing is known about its mechanism of action. We have thus conducted an exploratory study to assess the protective effect of guided imagery on downstream pathways affected by psychological stress.

Questions/Hypotheses: *Primary outcomes:* (1) Guided imagery will show decreased sympathetic and increased parasympathetic responses as assessed by HRV measures and (2) decreased HPA activity as measured by salivary cortisol. (3) Guided imagery will show improved well-being as measured by psychometric instruments.

Secondary outcomes: (4) Guided imagery will show improved well-being as measured by elevated melatonin and DHEA.

Methods: The intervention was performed in two group sessions of approximately 30 participants each. Blood, saliva, and psychometric questionnaires were obtained before and after the intervention. Heart rate was recorded throughout the session.

Psychometric instruments: A 25-item state-anxiety version of the Profile of Mood States (POMS) questionnaire was used, as was the Smith Relaxation States Inventory (SRSI), which addresses positive emotions associated with relaxation.

Salivary cortisol and melatonin: Saliva was collected by passive drool and stored at −80°C. Assays were performed en masse by enzyme-linked immunoassay (ELISA) using commercial kits.

Serum DHEA: Serum was prepared and DHEA assayed using a commercial ELISA kit.

Results: The intervention was effective in decreasing anxiety and increasing well-being, as determined by inventories. It decreased HPA activity as assessed by cortisol level. Finally, it had a relaxing effect on the autonomic nervous system as reflected by decreased heart rate and increased total and high-frequency HRV power.

Conclusions: The relaxation response of guided imagery may exert its beneficial effects by decreasing HPA axis activation, decreasing sympathetic nervous system activity, and improving vagal tone.

This research was supported in part by the National Institutes of Health, National Center for Complementary and Alternative Medicine Grant AT-001884; and in part by the National Institutes of Health, National Center for Research Resources, General Clinical Research Center Grant M01 RR-018390.

** Finalist for Young Investigator Award.*

Winner of the Young Investigator Award

14 High-Sensitivity C-Reactive Protein in Patients with Coronary Heart Disease and Depression

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Background: Large prospective epidemiological studies indicate that a high plasma level of C-reactive protein (CRP) is a significant predictor of cardiovascular events in individuals with or without known coronary heart disease (CHD). Also, recent findings indicate that depression is associated with an activation of the inflammatory response system expressed in increased levels of proinflammatory cytokines (IL-2, IL-6, interferon gamma) and CRP. Individuals diagnosed with major depression had been shown to be at increased risk of developing CHD and having worse outcomes compared with nondepressed counterparts. On the other side, 20% of patients with CHD are also diagnosed with depression. Successful treatment of depression in CHD patients improves outcomes.

Objective: To explore retrospectively the level of CRP in patients with CHD with and without depression.

Methods: *Study design:* Retrospective review of medical records between January 2002 and November 2006 of patients who had been diagnosed with CHD and had high-sensitivity CRP (hs-CRP) level measured in our institution. Patients were excluded

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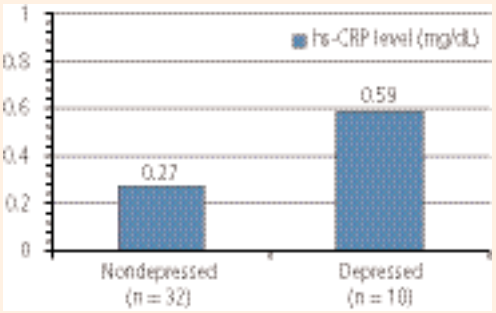

FIGURE 1. Median levels of high-sensitivity C-reactive protein (hs-CRP).

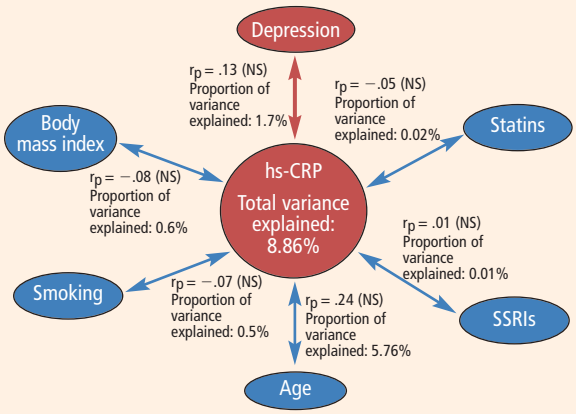

FIGURE 2. Factors influencing levels of high-sensitivity C-reactive protein (hs-CRP).

if they had acute or chronic inflammatory conditions at the time of measurement, history of bipolar disorder, history of alcoholism or drug abuse, history of recent myocardial infarction (< 3 months), or any recent change in their statin dose (< 6 weeks). *Patients:* Forty-two patients with CHD (32 males, 10 females) were identified, 10 of them with a diagnosis of depression (8 males, 2 females). Mean age (± SD) = 67.1 ± 10.46 years; range, 47 to 88 years (mean for males, 68.03 ± 10.51; mean for females, 64.1 + 10.21). *Statistical methods:* Levels of hs-CRP were compared between the depressed and nondepressed groups using the Mann-Whitney test with partial correlation coefficients between depression status (dummy coded: 0 = nondepressed, 1 = depressed) and the level of hs-CRP, controlling for factors known to influence hs-CRP levels. Analyses were conducted using SPSS-14.0.

Results: Depressed patients with CHD had higher (statistically nonsignificant) hs-CRP levels than nondepressed patients with CHD **(Figure 1)**. When controlling for other factors influencing the level of hs-CRP, a small percentage (1.7%) of the variance was explained by depression status alone **(Figure 2)**.

Discussion: *Study limitations:* Retrospective study design, accuracy of the medical records reviewed, accuracy of depression diagnosis by history, small number of subjects, poor documentation of diet/exercise, and concomitant medication effects. *Implications/future directions:* Inflammatory processes may be involved in the link between CHD and depression, and hs-CRP may be a useful indicator of such processes. Interventions (selective serotonin reuptake inhibitors [SSRIs]/statins) that lower CRP may be useful for such patients. *Future plans:* Collection of further retrospective data, prospective study on the effect of SSRIs on CRP in CHD patients with depression.

Abstracts of Research Funded by the Bakken Heart-Brain Institute at Cleveland Clinic

15 Long-Term Cardiac Complications of Subarachnoid Hemorrhage (SAH)

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While the influence of the central nervous system on cardiac rhythm and function has been studied, the mechanisms of this control and neurogenic cardiac dysfunction are poorly understood. Cardiac complications in the acute setting of subarachnoid hemorrhage (SAH) provide evidence of the impact of the central nervous system on cardiac function. These complications range from arrhythmias to changes on electrocardiogram (ECG), such as ST changes or QTc prolongation, to myocardial necrosis. Approximately two thirds of patients with SAH will have ECG abnormalities in the acute phase.

The average age of patients with acute SAH is 45 years, and in all but the most minor cases, these patients are left with irreversible brain damage. The long-term risk of cardiac death in these patients has never been studied. While several studies have examined the results of Holter monitoring or serial ECGs in the acute phase, only one study has examined these changes in the chronic setting, and none has examined the relationship between SAH severity and the persistence of these changes. Understanding the long-term effects of SAH on the heart is important, as it will provide further insight into the mechanisms of the central nervous system's influence on cardiac rhythm and function. In addition, since SAH survivors are relatively young, predicting who will have long-term arrhythmias may have important implications for monitoring these patients in the future. The long-range goal is to investigate the long-term cardiac manifestations of acute aneurysmal SAH. This will have the dual purpose of elucidating the mechanisms of cardiac control in the brain and determining the long-term risk of serious cardiac complications. The central hypothesis of this study is that at 3-month and 6-month follow-up, patients with severe SAH will have persistence of QTc prolongation, decreased heart rate variability, and increased frequency of supraventricular and ventricular arrhythmias compared to patients with less severe SAH.

To complete the objectives of this proposal, clinical data on the severity of cardiac risk factors, as well as CT scans of patients with acute SAH, will be graded using previously validated severity scales. We will obtain serial ECGs and Holter monitoring on these patients during their acute phase to assess for QTc prolongation, arrhythmia, ischemic changes, and QTc dispersion and heart rate variability. We will evaluate transthoracic echocardiograms done

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during patients' acute hospital stay to assess for left ventricular systolic function and regional wall motion abnormalities. We will compare findings in the acute setting to 12-lead ECG and Holter monitor data obtained from these patients at 3- and 6-month follow-up. A subset of 20 patients in whom arrhythmia is noted at 3 months will have transthoracic echocardiography at 6 months.

Our expectation is that at the end of this study we will have preliminary data on the long-term cardiac manifestations of SAH. The role of the central nervous system in modulating cardiac rhythm and neurogenic cardiac dysfunction will allow us to develop better human studies and animal models to investigate the mechanisms involved. We expect that, in the future, this understanding will not only lead to improved treatment of patients with severe SAH but also suggest novel therapies for the treatment of cardiac arrhythmias.

16 Cardiac Damage in Subarachnoid Hemorrhage: The Role of Cerebral Control of Inflammation

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Introduction: Cardiac ischemic changes have been reported in patients who experience subarachnoid hemorrhage (SAH). The specific mechanism for these changes remains unclear, but clinical data suggest that catecholamine release alone is unlikely to be responsible for the cardiac muscle injury seen in SAH. There is evidence from animal models of sepsis that the central nervous system directly controls the systemic inflammatory response. We hypothesize that the brain injury caused by SAH may lead to a dysregulation of the inflammatory system mediated through both the sympathetic and parasympathetic systems, which in turn leads to cardiac injury.

Methods: We studied adult C57BL/6J mice with either experimental subarachnoid hemorrhage (ESH) or saline injection sham (SH). In addition, a subset of mice were treated for 48 hours with a mouse monoclonal antibody against the neutrophil receptor Lys-6g (Gr1) to deplete neutrophils. Prior to the procedure and again 16 to 20 hours after the procedure, some animals were evaluated with echocardiography. Heart sections were analyzed by hemotoxylin-eosin (H&E) staining for the presence of inflammation.

Results: H&E staining showed areas of inflammation in the pericardium and in the perivascular spaces in the ESH animals that were not present in the SH animals. In addition, Gr1-treated ESH animals showed no inflammation. The echocardiographic findings are presented.

17 Heart Rate Variability Biofeedback in the Treatment of Cardiovascular Disease

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Many forms of cardiovascular disease are characterized by autonomic nervous system hyperactivation. Pharmacologic agents such as beta-adrenergic blocking drugs are effective in treating these disorders, but there are also nonpharmacologic ways to inactivate an overactive sympathetic drive. Nonpharmacologic methods are appropriate in patients who cannot tolerate beta-blocking drugs and may also be used in a wide range of patients to provide a greater feeling of control over the disease state and its consequences. Studies have demonstrated that cardiac patients who are able to experience a greater sense of control over their disease have a better prognosis.

Biofeedback has been used to increase patient understanding of the psychophysiology that links mental arousal to the consequences of sympathetic nervous system overactivity. Biofeedback refers to the use of equipment to monitor and display parameters of physiologic arousal in order to train the patient to control that arousal. Physiologic parameters that have been used successfully in treating cardiovascular patients include skin conductance, digital peripheral temperature, and heart rate. In each case, the patient is taught to decrease arousal of the sympathetic nervous system, resulting in an improvement in physiologic parameters. Biofeedback therapy has been demonstrated to be effective in hypertension and ischemic heart disease. In patients with heart failure, one session of biofeedback has been shown to improve cardiac output and overall quality of life.

Recently, studies have demonstrated that heart rate variability (HRV) is a physiologic parameter that accurately reflects the balance between the two components of the autonomic nervous system—the sympathetic nervous system and the parasympathetic nervous system. Decreased HRV has been shown to correlate with worse outcome in patients with cardiovascular disease. Therapies that increase HRV have been shown to be effective in decreasing mortality. HRV biofeedback methods have recently been developed in which the patient is taught to maximize the variability in heart rate by optimizing a visual signal. HRV biofeedback has been successfully used to treat conditions such as asthma, emphysema, and ventricular fibrillation.

We are involved in a study of HRV biofeedback in patients who have newly diagnosed heart failure with early onset of sympathetic nervous system overactivation. We hypothesize that training these patients utilizing HRV biofeedback will result in slowed progression to overt heart failure. This presentation describes HRV biofeedback and the training method employed.

18 Mindfulness, Yoga, and Cardiovascular Disease

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There is increasing evidence that acute and chronic stressors and negative emotions such as anxiety and depression play a major role in the development and progression of cardiovascular disease. Imbalance of autonomic nervous system tone and stress-related hormones, with resulting dysregulation of the function of endothelial cells, platelets, and immune cells, have been proposed as key molecular pathways linking psychological distress and the development and progression of coronary artery disease (CAD). However, direct evidence for any of these proposed pathways is still lacking.

Practices such as tai chi, yoga, and mindfulness meditation are becoming increasingly popular as ways of releasing stress and improving well-being. For some of these practices, there is evidence for beneficial modulation of (1) activity of the autonomic

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nervous system and (2) levels of stress-related hormones. However, studies providing insights into whether such changes can have effects on downstream cardiovascular risk factors, including inflammatory markers, are lacking.

In this study, we will evaluate the efficacy and potential mechanisms of action of mindfulness (a practice that originated in Buddhism focusing on enhancing awareness and acceptance of the present moment) and yoga as compared with an exercise- and education-based stress-reduction program.

Specific Aims: *Primary objectives:* To evaluate the effectiveness of a regular practice of mindfulness meditation or yoga in (1) improving mood and decreasing blood pressure, and (2) modulating cardiovascular risk factors such as (a) autonomic nervous system activity, (b) inflammatory mediators of CAD, (c) stress-related hormones, and (d) platelet activity.

Secondary objective: To determine whether there is an association between improved mood and any of the physiological markers of psychological stress and cardiovascular risk factors.

Design and Method: One hundred five otherwise healthy individuals who have moderate cardiovascular risk factors, including elevated blood pressure and mild to moderate anxiety, will be randomly assigned to one of the three intervention groups: an 8-week program of mindfulness, a 12-week program of yoga practice, or a 12-week exercise- and education-based group program. All groups will meet weekly; subjects will also perform daily practice that will continue after the weekly sessions end, allowing a follow-up assessment at 24 weeks. Mood and psychological distress (determined with psychometric instruments), stress hormones, blood pressure, inflammatory markers, and autonomic nervous system activity (as assessed by heart rate variability, plasma catecholamines, and inflammatory markers) will be determined immediately before and after the intervention.

We anticipate that the study will provide information on the efficacy of the mindfulness and yoga interventions as stress-reduction practices, their effects on cardiovascular risk factors, and potential pathways mediating the brain/cardiovascular system connections. The study will also provide the data needed to design a future study that will rigorously address these questions in a larger, randomized trial of mindfulness and yoga in patients with cardiovascular risk factors.

19 Intravascular Electrical Stimulation of the Peripulmonary Artery Sympathetic Nervous System Fibers Increases Cardiac Contractility

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Effective inotropic agents characteristically increase contractility at the expense of significant increments in oxygen consumption secondary to elevations in heart rate. Animal studies have shown the feasibility of selective enhancement of contractility by electrical stimulation of postganglionic sympathetic fibers around the superior, dorsal, and rostral areas of the common pulmonary artery.[1–3] We present an intravascular method for selective stimulation of these fibers.

Methods: Six open-chest dogs were instrumented with left ventricle conductance catheter and aortic flow probe. Modified electrode catheters were placed inside the pulmonary artery under echocardiographic and fluoroscopic guidance in 5 dogs; in the sixth animal, a stent-delivered electrode was used. Stimulation was applied at 20 Hz, 0.4 msec, and 15 to 25 mA. The corresponding hemodynamic effects are reported as averages of 30-second periods of continuous recording.

Results: Pressure variation in the left ventricle over time increased in all dogs. The average increment was 25.7% (± 11.8%), and the average of maximum increase variation was 28.3% (± 8.9%). Emax was measured in the last animal, showing a 45% increase. The average reduction of R-R interval during stimulation was 3.3% (± 10.4%). Electrical stimulation via a pulmonary artery catheter can produce positive inotropic effects with minimal changes in heart rate. The current study demonstrates the feasibility of this novel intravascular approach to selective neuromodulation of the heart.

Learning Objectives: (1) Understand the fibers around the pulmonary artery as a potential neuromodulatory target for improvement of cardiac contractility. (2) Show the importance of selective fiber stimulation in the reduction of myocardium consumption of oxygen. (3) Learn the benefits of a quick and established intravascular access to the specific and direct-acting method of neurostimulation.

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20 Lipopolysaccharide-Induced Ischemic Tolerance in the Heart and Brain of C57BL/6J Mice

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Experimental animal models of cerebral and myocardial infarction are crucial for understanding mechanisms of neuronal and myocardial muscle survival and for developing potential therapeutics that will prevent or reduce ischemic brain and heart injury. It is well established that prior exposure to sublethal stimuli such as ischemia, heat shock, or intraperitoneal injections of lipopolysaccharide (LPS) can transiently protect the heart and brain from ischemic insults. This study compared and contrasted ischemic tolerance mechanisms induced in the brain and heart. In the first stage of this study, acute myocardial infarction and cerebral stroke models were established in C57BL/6J mice. Left anterior descending coronary artery ligation and direct middle cerebral artery occlusion were used for heart and brain ischemic models, respectively. Our data indicated that LPS preconditioning led to ischemic tolerance. Mice preconditioned with LPS had significantly decreased infarct area following cardiac ischemia compared with mice treated with saline. Preconditioning with LPS also had a profound effect in the mouse stroke model. These findings suggest that ischemic tolerance can be achieved in the heart and brain simultaneously through LPS preconditioning. Studies are under way to uncover the cellular and molecular mechanisms that underlie ischemic tolerance in heart and brain.

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21 Mechanisms of LPS-Induced Preconditioning in Murine Heart and Brain

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Preconditioning is a process in which an organ experiences stress near, but not above, the threshold of damage. This stress induces an endogenous response that transiently protects the organ from future insults. Preconditioning can be thought of as the priming of an organ, and can be achieved through various means. Intraperitoneal injections of lipopolysaccharide (LPS) induce a systemic preconditioning response. The focus of this study was to determine genetic components and mechanisms of LPS-induced preconditioning in heart and brain.

Three adult C57BL/6 mice received three intraperitoneal injections daily of LPS; control mice received injections of Hank's balanced salt solution. One day after the injections, animals were anesthetized and sacrificed. Heart and brain tissues were harvested and homogenized using a Dounce homogenizer, and total RNA was isolated using Trizol reagent. Total RNA was labeled and hybridized to Affymetrix 430 2.0 mouse arrays containing 45,101 transcripts covering almost the entire mouse genome. No sample outliers were detected using principal component analysis. Heart and brain profiles were analyzed separately.

Gene profiles of heart and brain from control and LPS-preconditioned animals were compared; 229 transcripts in heart and 309 transcripts in cerebral cortex were altered ($P < .05$ after Benjamini-Hochberg False Discovery Rate correction) in LPS-preconditioned animals. The majority of altered transcripts in heart (198) and brain (287) were increased following preconditioning. Various biological categories, such as immune response, cytoskeletal reorganization, ion transport, and transcriptional regulation, were affected by LPS preconditioning. Forty-three transcripts were increased in both organs, including the inflammatory response proteins like serum amyloid A3, lipocalin 2, calgranulin, complement component 3, and defensin β. A unique EST (mapped to mouse chromosome 7) was decreased 6.17-fold in LPS-preconditioned heart.

Verification of novel transcripts altered in these organs is currently in progress, which will help us to understand the mechanism behind global preconditioning of heart and brain.

22 Psychosocial Factors and Mortality in CABG Surgery: A PreCIS Database Study

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This study examined the existence of life stress, time pressure, anger, or sadness prior to coronary artery bypass graft surgery (CABG) and the predictive value of these four psychosocial variables on post-CABG mortality.

Study population was 4,166 patients admitted to the Cleveland Clinic for CABG from March 2000 through September 2006. Mean patient age was 66.5 years; 76% of patients were men. All patients completed a questionnaire about the presence of the four psychosocial variables prior to admission. Patient mortality, measured up to 6 years after CABG, was assessed via the Social Security Death Index. Kaplan-Meier methods were used to compare the history (or lack of history) of the psychosocial variables.

There were 274 reported deaths. Patients who indicated the presence of any one psychosocial variable demonstrated less mortality ($P = .01$) than those who did not endorse any variable. This trend was equal in men and women. In examining each variable separately, life stress was associated with a protective effect against mortality ($P < .001$), with a more robust effect in men. Time urgency also had a protective mortality effect ($P < .01$), but only in males. A history of anger did not show any effect on subsequent mortality in the overall population ($P = .53$) or in either gender. A history of sadness conferred no overall increase in mortality over a 6-year follow-up period ($P = .16$) but was statistically predictive for mortality between 30 days and 3 years, with peak significance at 1-year follow-up ($P < .001$). By gender, a relation between sadness and mortality was notable for men at 30 days ($P < .001$) and at 6 months ($P = .03$), while for women it was notable at 1 year ($P = .002$).

This study showed that the presence of life stress and time urgency prior to CABG tended to have a protective effect against follow-up mortality, while the presence of anger had no effect. Conversely, the presence of sadness appeared to have an early detrimental effect on mortality. Our findings may validate previous characterizations of "Type A" personality as not being detrimental to coronary artery disease patients while underscoring the mortality effect of depression in patients undergoing CABG.

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