CLINICAL RESEARCH PROTOCOL

HRV Biofeedback in Pain Patients: Pilot Intervention for Pain, Fatigue & Sleep

Principal Investigator:

James Burch, MS, PhD, Associate Professor
Health Research Specialist
WJB Dorn Department of Veterans Affairs
Medical Center
6439 Garners Ferry Road
Building 100, Room 3A156
Columbia, SC 29209-1639
Phone: (803) 776-4000 ext. 1-6635
E-mail: james.burch@va.gov
and
Cancer Prevention and Control Program,
Dept. of Epidemiology and Biostatistics,
Arnold School of Public Health, University of
South Carolina, Columbia, SC
915 Greene St., Columbia, SC 29208
Phone: 803 576-5659
E-mail: burch@mailbox.sc.edu

Co-Investigator:

Alexander McLain, PhD
Assistant Professor of Biostatistics
Department of Epidemiology and Biostatistics
Arnold School of Public Health
University of South Carolina, Columbia, SC 29208

Consultants:

Raouf (Ron) Gharbo, DO, PhD
Adjunct Clinical Faculty
Eastern Virginia Medical School
Physical Medicine & Rehabilitation
Eastern Virginia Medical School
and
Staff Physiatrist
Riverside Medical Group
Hampton Roads Riverside Regional Medical Center
Newport News, VA 23601

James R. Hebert, ScD
Health Sciences Distinguished Professor
Director, South Carolina Statewide Cancer Prevention and Control Program,
Dept. of Epidemiology and Biostatistics,
Arnold School of Public Health,
University of South Carolina, Columbia, SC 29208

Consultants (Continued):

Francis G. Spinale, MS, PhD, MD
Distinguished Professor of Personalized Medicine
Professor, Cell Biology Anatomy, and Surgery
School of Medicine
University of South Carolina, Columbia, SC 29208
and
Staff Physician
WJB Dorn Department of Veterans Affairs Medical Center
6439 Garners Ferry Road
Columbia, SC 29209-1639

PROTOCOL ABSTRACT

Pain initiates a stress response that increases sympathetic output and facilitates autonomic imbalance. Heart rate variability (HRV) is a valid, quantitative, noninvasive measure of autonomic function. HRV biofeedback (HRV-B) is a novel biobehavioral procedure whereby patients learn to restore autonomic imbalance by developing 'HRV coherence'. Patients in this state have improved mood, cognition, and executive function, and HRV-B interventions have been used to treat: pain, anxiety, depression, insomnia and symptoms of posttraumatic stress disorder (PTSD) and heart disease. We have found that HRV-B training among Veteran PTSD patients can enhance HRV coherence and improve cognition and sleep relative to controls. Importantly, our pilot study showed that HRV-B can alleviate chronic pain and stress among Veteran Pain Clinic patients. HRV-B thus has a pivotal role in restoring autonomic balance and managing pain and the symptoms that amplify it. The proposed project is a randomized, sham-controlled, biobehavioral intervention that will test the hypotheses that HRV-B increases HRV coherence and reduces pain, stress, fatigue, insomnia and depression and improves sleep, activity, and cognition. Because this is a behavioral intervention, it is difficult to blind the subjects to the active vs sham treatment, and the biofeedback coach who provides the training must know the condition to which the subject is assigned. However, all others in the study are blinded to the training. The specific aims are: 1.) Determine whether HRV-B increases HRV coherence and reduces pain or stress relative to a sham treatment among Veterans with chronic, nonmalignant, axial neuromusculoskeletal pain; 2.) Determine as an exploratory aim whether HRV-B reduces insomnia, fatigue or depressive symptoms, and increases sleep and activity and improves cognition among patents relative to sham treatment. We hypothesize that HRV-B will (1) reduce self-reported pain and stress ratings, (2) improve objective measures of actigraphic sleep parameters (sleep latency, duration, efficiency, fragmentation), rest/activity rhythms (dichotomy index, interdaily stability) and cognitive function (reaction time, attention); and (3) alleviate self-reported fatigue and depression symptoms. Patients from both groups will be randomized to our previously established HRV-B or sham protocol (n=40 each), and will complete a baseline assessment, 6 weekly training sessions, a post-training assessment, a 4-week booster training and assessment, and an 8-week post-training follow-up evaluation, for a total of 10 visits to the research site. Portable, hand-held, data-logging devices will be used to practice attaining HRV coherence at home by the active treatment group, while those in the sham training group will get a 'stress squeeze ball'. Standard methods will quantify HRV coherence and other HRV measures, and validated instruments will be used to assess pain and stress. Wrist actigraphy is a valid, quantitative, and noninvasive procedure that will be used to characterize insomnia via continuous recordings collected 24-hrs/day over three 1-week periods (at the baseline, post-treatment, and 8-week follow-up assessment). A small battery of tests measuring attention and reaction time will assess changes in cognitive performance. Data analyses will apply linear models for repeated measures to evaluate the effect of HRV-B on study outcomes, and on treatment persistence, after adjusting for confounding factors. For each measure we will calculate three estimates of change for each patient (post-training minus pre-training, 4-week booster minus post-training, and 8-week follow-up minus the 4-week booster assessment). Path analyses will examine direct and indirect relationships between HRV-B, the primary and exploratory outcomes, and personal or social factors that may modify these relationships. This study will be the first to examine HRV-B for pain management among Veteran chronic pain patients. This study is directly responsive to a national VHA/DoD Task Force's recommendations to provide complementary, integrative therapies for pain management to Veterans.

1.0 BACKGROUND AND SIGNIFICANCE

Pain is the most common reason for seeking health care in the United States, impacting ~116 million Americans and generating >$600 billion in medical care costs.[1] Chronic pain is a significant issue among Veteran patients and is one of the most common reasons for seeking care among Veterans of all wars.[2,3] Among all Veterans, musculoskeletal pain is the most commonly diagnosed medical problem.[4] Pain patients experience improved quality of life when pain is controlled and they are more likely to successfully manage other aspects of their daily lives.[5] Because the prevalence and intensity of pain increases with disease progression, and since pain impacts multiple domains of quality of life (QoL),[6,7] the ability to manage pain becomes increasingly important over time for medical patients of all types.

Pain initiates a stress response that increases sympathetic output leading to autonomic imbalance and psychological stress; physical and psychological stress in turn exacerbates pain, thus creating a self-perpetuating cycle of autonomic dysregulation that facilitates persistent pain.[8] Stress from chronic pain leads to loss of the glucocorticoid (GC)-mediated negative feedback control of the hypothalamic-pituitary-adrenal (HPA) axis, producing positive HPA drive and down-regulation of central and peripheral GC receptors. In addition, peripheral cytokine pro-inflammatory signals from nociceptive receptor chronic stimulation is sent to the spinal dorsal horn and ascends to brainstem, and gated in thalamus prior to cognitive appraisal in primary somatosensory cortex. This creates pain awareness. As a result, monoaminergic (dopamine, norepinephrine and epinephrine) neurons in the brainstem that normally descend to the spinal cord and 'brake' nociceptive transmission become depleted during chronic pain.[9] This loss of inhibitory monoaminergic tone to the spinal cord leads to enhancement of pain ('central sensitization'). In what is now a feed-forward system, the loss of GC inhibition of pro-inflammatory cytokines leads to proliferation of peripheral inflammatory agents, which are nociceptive transmitters, further contributing to central pain sensitization and an HPA driven stress response.[10] At the same time, down-regulation of GC negative-feedback modulation from chronic stress also promotes depressed mood.[11] Thus, chronic pain can induce both stress and depression (Figure 1, 'Disease Pathway').

Pain Medication & Addiction. Pain remains a major healthcare problem despite advances in treatment of major medical illnesses and illness-related pharmacological pain management.[12] Opioid analgesics such as morphine and hydrocodone and its derivatives effectively produce analgesia through antagonism of the receptors of naturally occurring opioids that are released from the periaqueductal gray region of the central nervous system in response to pain. However, the use of opioid analgesic medication poses an increased risk of physical and psychological addiction, as well as a number of other side effects including intolerance, hyperalgesia, hormonal imbalance, respiratory depression, constipation, nausea, vomiting, drowsiness, and itching[13] Nonetheless, Vicodin is the most prescribed medication in the United States.[14] Overdose deaths from opioid painkillers have more than quadrupled since the turn of the century, approaching 20,000 annually, and opioid overdose is the leading cause of accidental death in several states.[15] Many Veterans with mental health diagnoses receive prescription opioids for pain-related conditions, which can compromise their mental health.[16] An effective non-pharmacological pain therapy that reduces the usage of opioid medications would be a significant benefit to chronic pain patients.

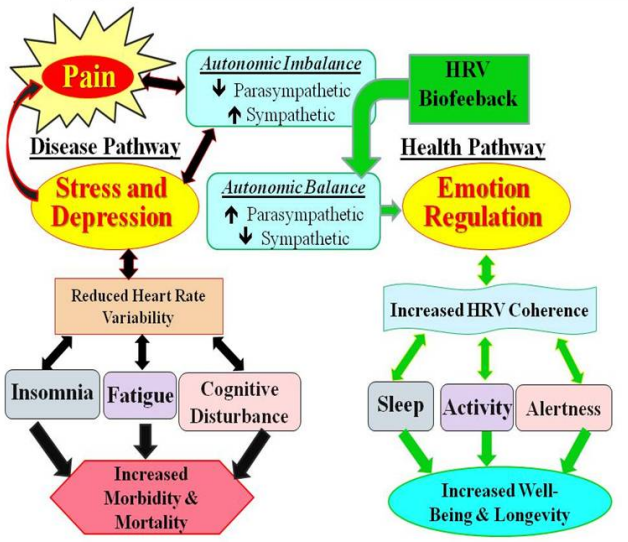
Non-Pharmacological Pain Treatment. Many veterans in chronic pain use avoidance strategies to dampen the intensity of pain in their lives. This avoidance is most clearly seen in behaviors and movement. Painful activities are avoided in an attempt to decrease the overall experience of pain. Though behavioral avoidance can lessen pain in the short term, it can also cause long term problems and decrease QoL.[17] Complementary therapies, such as Heart Rate Variability (HRV) Biofeedback (HRV-B), that can help improve both physical and psychological well-being have been advocated for integration with conventional therapies because many medical patients are not satisfied with mainstream treatments in relieving their pain symptoms and the attendant erosion of their QoL.[18]

Our proposal is designed to test whether HRV-B is feasible and effective in increasing HRV coherence and reducing perceived pain, stress, depression, and insomnia among Veterans suffering from chronic physical pain. Our path analyses will examine both direct and indirect relationships between HRV-B, the primary and exploratory outcomes, and other individual or social factors that may modify this relationship. The relationship

between autonomic dysfunction and disrupted sleep has not been thoroughly examined. Our path analyses are thus important from a practical standpoint because HRV coherence and sleep represent potentially modifiable risk factors that can be targeted to reduce symptom burdens and improve QoL for Veteran patients. In addition, we will implement the use of portable, hand held finger plethysmographs (personal stress relievers) to reinforce the HRV-B training and promote practice outside the clinic. We have obtained encouraging preliminary results by incorporating this technique in our other research. In our path analysis, we will identify factors predicting persistent HRV-B effects at the 8-week post-treatment follow-up. This information will be useful in designing future interventions. The use of HRV-B to normalize autonomic function represents a novel, complementary strategy that engages patients in their own care and is directly responsive to the VHA/DoD Pain Management Task Force's recommendations to adopt complementary pain management strategies (including HRV-B) to alleviate pain among Veterans.[19] This application addresses several CSR&D priority research areas (Pain Management, Complementary and Alternative Medicine, Patient-Centered Care) and coincides with a nationwide VHA/DoD initiative to include complementary, integrative, therapies into pain management for Veterans. To our knowledge, the use of HRV-B to improve chronic pain symptoms in a Veteran population has not previously been studied in a randomized clinical trial.

Chronic Pain, Stress, Depression and Autonomic Imbalance. HRV is a valid, noninvasive measure of autonomic function with both physiological and psychological attributes. Reduced HRV is an established risk factor for mortality due to cardiovascular or other chronic diseases.[20,21] For example, about 80% of advanced cancer patients exhibit autonomic dysregulation,[20,22] and several recent studies report increased mortality risk among patients with reduced HRV.[20-26] Furthermore, emotional disruption caused by a stressful experience is characterized by a chaotic, disordered pattern of inter-beat intervals as well as an increased heart rate, which lowers HRV (Figure 2, top panel). Irregular heart beat rhythm due to disordered inter-beat intervals, reflects sympathetic nervous system over-activity and low HRV coherence. In contrast, emotional balance and a feeling of well-being results in an ordered, sine wave-like pattern of inter-beat interval changes and a narrow peak in the low frequency (LF) range of the power spectrum, centered around 0.1 Hz, which equals a period of 10 seconds, or 6 cycles per minute (Figure 2, bottom panel).[27]

Fig. 1. HRV Biofeedback reduces effects of chronic pain



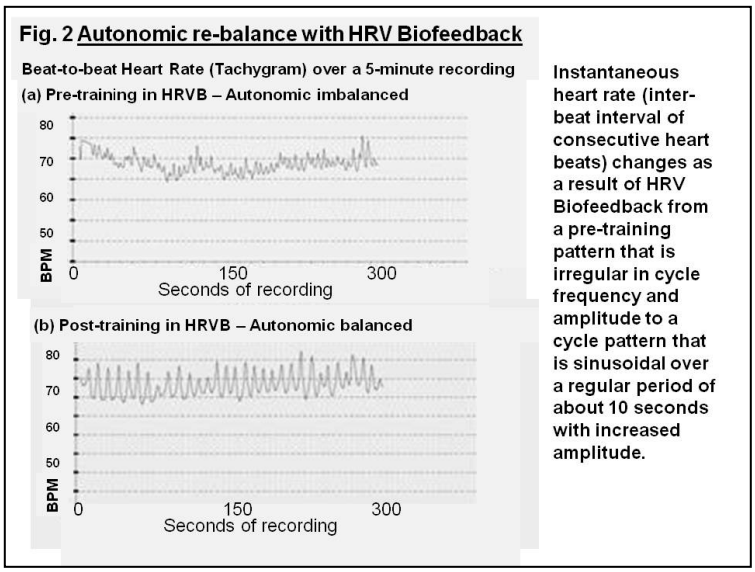
Chronic pain causes central sensitization and loss of negative feedback regulation of the stress response, leading to autonomic imbalance, allostatic stress, and depressed mood (Disease Pathway). When autonomic balance is restored, stress is reduced and emotional regulation is recovered (Health Pathway).

As a measure of the interplay between the excitatory sympathetic and the inhibitory parasympathetic nervous systems, HRV has a clear relationship with pain sensitivity and perception. Linkages between HRV alterations and various types of pain have been documented. Appelhans and Luecken found an inverse relationship between HRV and sensitivity to cold thermal pain measured as 'unpleasantness', and 'threshold for barely noticeable pain'. [28] In patients with fibromyalgia, HRV was a predictor of pain perception, pain anxiety, and physical health functioning.[29] The pattern of HRV in patients under general anesthesia changed to reflect decreased vagal tone when a surgical stimulation was painful, even though the patient was not conscious.[30] Alternatively, a reduction in visceral pain during esophageal balloon distension was associated with higher HRV.[31]

The autonomic nervous system exhibits a distinct circadian rhythm as do several cardiac measures including heart rate and blood pressure.[13] The heart rate increases in the morning at or after waking, reaches peak between 10 am to 12 pm, and then gradually begins to decline later in the day, maintaining a low level during night. During sleep, increases in parasympathetic tone and HRV predominate. Despite these associations, few

studies have examined relationships between disrupted sleep and autonomic function.[16,32] In a study of 13 healthy male volunteers randomized to 5 nights of partial sleep deprivation (<5 h), or 5 nights of control sleep (>7 h), those with sleep deprivation had a statistically significant increase in sympathetic activity as indicated by an increase in low frequency (LF) and decrease in high frequency (HF) components of HRV.[33] The results were consistent with another study that reported increased sympathetic and decreased parasympathetic HRV measures after 36-hours of total sleep deprivation.[32]

Depression is relatively common in patients with coronary heart disease (CHD) and its association with autonomic nervous system dysfunction has been investigated among CHD patients. HRV is lower among depressed patients with either stable CHD, or among those with a recent acute coronary event, compared with non-depressed patients.[34] Studies examining HRV and depression have expanded into other patient populations. Another recent meta-analysis concluded that depression in the absence of cardiovascular disease is also associated with reduced HRV, and that HRV decreases with increasing depression severity.[35] Reduced HRV is an established predictor of increased mortality risk both in cardiovascular or other chronic disease patients, and in the general population.[20,21]



Loss of HRV coherence is also associated with PTSD. Our recently published meta-analysis of HRV and PTSD found that HRV variables are associated with PTSD, and these associations can be interpreted in terms of a basic and widespread model of PTSD as a disorder comprising hyper-arousal of the sympathetic nervous system (see Preliminary Studies).[36]

HRV Biofeedback. Improvements in HRV can be therapeutic. Complementary therapies, such as HRV-B that can help improve both physical and psychological well-being have been advocated for integration with conventional therapies. HRV-B is an interactive procedure whereby patients increase HRV in real time by practicing focused attention, positive emotion, and resonant frequency breathing. Previous research suggests that HRV-B interventions may be useful for reducing symptoms of: chronic pain,[28,31,37,38] anxiety,[39-41] depression,[37,42] post-traumatic stress disorder (PTSD),[43,44] heart disease,[45] and insomnia.[46]

HRV-B is a complementary, non-pharmacological technique for improvement of HRV and autonomic function. It is an interactive procedure whereby patients monitor their HRV patterns in real time while practicing focused attention, positive emotion, and resonant frequency breathing. The resonant frequency of breathing (RFB) is the respiratory rate producing the highest HRV in the LF band; RFB is necessary for attainment of HRV coherence.[47] HRV coherence is achieved when beat-to-beat intervals increase and decrease in a smooth rhythm, producing a spike around a 10 second period (0.1 Hz on the fast Fourier transformation to the power spectrum) of the instantaneous heart rate (Figure 2). It is defined according to consensus guidelines as the ratio of power in the LF peak to the remainder of power in the HRV spectrum.[27,44,48] Using HRV-B, patients learn how to produce HRV patterns indicative of higher vagal parasympathetic tone. With practice, patients acquire the ability to enhance their HRV coherence on demand (see Figure 2). High coherence has been associated with a favorable psychological state, including improved affect, cognition, and executive function.[27]

HRV-B had analgesic effects on experimental heat pain threshold and tolerance scores in normal subjects.[49] The HRV-B technique has been used in several clinical settings to improve HRV and reduce pain symptoms of fibromyalgia,[37] back pain,[38] and chronic stress-related neck pain.[41] In our own work[50] with chronic pain patients from our DVAMC Pain Clinic, several pain and stress perception ratings were lowered by HRV-B compared to

a control group of treatment as usual. Thus, the neurobiology underlying analgesia induced by this method of complementary, non-pharmacological, psychophysiological self-regulation appears to be different from other commonly used techniques involving distraction. The potential benefits of improving autonomic function among medical patients has been acknowledged.[20,21,26,51]

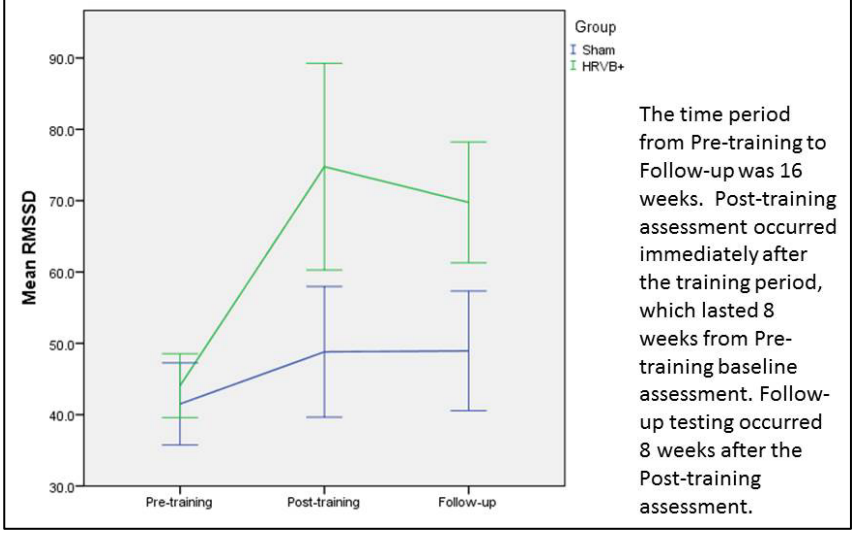
Chronic pain is a significant issue among Veterans, and to our knowledge, this will be the only study other than our pilot study[50] to evaluate HRV-B for the amelioration of pain in this population. The alleviation of persistent pain, fatigue, insomnia or depression via improved HRV coherence may provide a direct clinical benefit to Veteran pain patients. Our study will examine both direct and indirect relationships between HRV-B, the primary and exploratory outcomes, and other individual or social factors that may modify this relationship. We will also develop an integrated statistical model of autonomic dysfunction as a potential underlying psychophysiological process that can be targeted to improve the management of pain. This will include the identification of predictors of disrupted sleep and rest/activity rhythms. If our aims are achieved, our study has importance from a practical standpoint because HRV coherence and sleep represent potentially modifiable risk factors that can be targeted to reduce symptom burdens and improve QoL for pain patients. The integrated path analysis model derived from this study will aid in the development of more effective intervention strategies for addressing symptoms and thus targets for the design of future projects. If this study's hypotheses are supported, we will seek funding for a full-scale intervention to comprehensively examine whether HRV-B therapy alleviates pain in a wider population of Veterans with pain.

1.1. Preliminary Studies

We are now conducting a randomized, single blind, sham-controlled study funded by the Department of Defense (DoD) to examine the effectiveness of HRV-B in alleviating PTSD symptoms. Three groups of combat veterans are being studied: (1) PTSD patients who received active HRV-B training (n=30); (2) PTSD patients who received sham HRV-B training (n=30); and (3) Veterans without PTSD who were assessed only at baseline and received no HRV-B training. Training lasted six weeks. Participants were assessed Pre-training, immediately Post-training, and 8 weeks after training. To date, 73 participants have been enrolled, with 32 assigned to the Sham group, 29 to the active Biofeedback group, and 12 to the Control group. Data analysis is ongoing and results are being prepared for submission for review for publication.

Effect of HRV-B on HRV Coherence. In this ongoing study, the time domain variable RMSSD was used as an indicator of coherence because it correlates very highly with vagal tone and parasympathetic output (r=0.853, p<0.001).[52,53] Linear mixed model analysis of RMSSD for Group (Sham vs. HRVB) x Time Period of Assessment (Pre-, Post-, Follow-up) showed that the interaction effect was significant, F(5, 125)=2.50, p=.034. Follow-up testing of the interaction effect revealed that the Group main effect was statistically significant, HRV-B > Sham, 60.2 (43.6) vs. 46.1 (37.3), F(1,127)=4.66, p=.033. Contrasts within the HRV-B group revealed that Pre-training RMSSD was statistically lower than combined Post-training and Follow-up, 44.1 (22.8) vs. 72.6 (51.4), t(57)=-2.58, p=.013, but Post-training was not statistically different from Follow-up, 74.8 (63.2) vs. 69.7 (32.8), t(57)=0.346, p=.730. In summary, active HRV-B increased HRV coherence and the increase persisted until the Follow-up period (Figure 3).

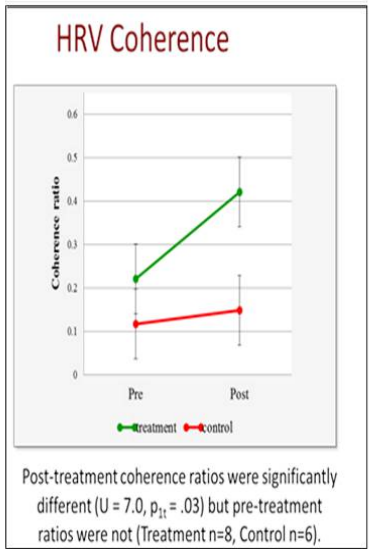
Figure 3. Effect on HRV Coherence of active HRV-B compared to Sham HRV-B training



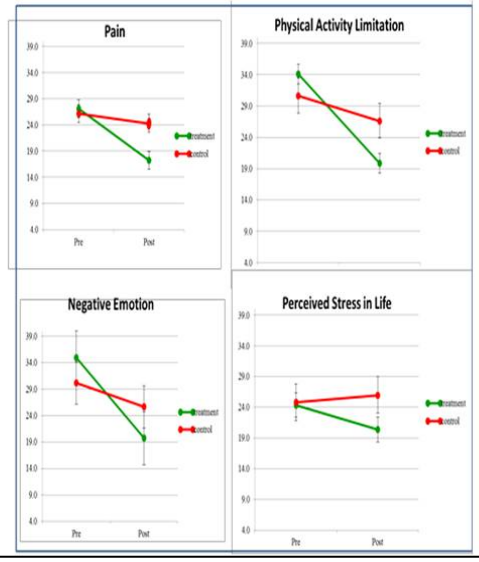
The time period from Pre-training to Follow-up was 16 weeks. Post-training assessment occurred immediately after the training period, which lasted 8 weeks from Pre-training baseline assessment. Follow-up testing occurred 8 weeks after the Post-training assessment.

Effects of HRVB on Veterans with Chronic Pain. This study[50] determined the effectiveness of HRV-B as a pain management intervention for DVAMC Veterans with chronic pain by comparing pre- and post-intervention pain and stress scores among two groups of randomly assigned Veterans that met the chronic pain inclusion criterion. One group received HRV-B plus treatment as usual and the other group received only treatment as usual. The outcomes of this study were HRV coherence and four scale scores (pain, emotional distress, activity limitation, and stress) that were obtained from two previously validated psychometric instruments (BPI and PSS, respectively). Veterans receiving HRV-B training were coached to use constructive processes to engage and manage internal sensations of pain rather than rely on strategies of avoidance. All subjects received a baseline and a follow-up assessment after 6 weeks of HRV-B training or treatment as usual. Retention for this pilot study was 100%. The HRV-B group received five weekly HRV-B training sessions. Treatment effects were analyzed with ANCOVA of follow-up scores by group using baseline scores as covariates. Eight Veterans completed the HRV-B intervention and six Veterans in the usual treatment group returned for a follow-up assessment. The mean baseline values for the two groups were not statistically different for any of the measures. In the HRV-B group, there was a statistically significant increase in HRV coherence and a reduction among each of the four outcome measures for pain or stress post-HRV-B training relative to the group that only received usual treatment (all $p < 0.05$, Figure 4). Results from this study indicate that HRV-B can effectively reduce the negative perceptions of pain. Data obtained from this investigation were used to develop statistical power and sample size estimates since they are most directly relevant to the proposed intervention.

Figure 4. Changes in HRV Coherence and their associated effect on measures of pain, physical activity, negative emotion, and perceived stress in Veterans with chronic pain who received HRV-B + standard care (green lines) vs only standard care (red lines).



Post-treatment coherence ratios were significantly different ($U = 7.0$, $p_{1t} = .03$) but pre-treatment ratios were not (Treatment n=8, Control n=6).



Effect of HRV-B on PTSD among Combat Veterans. In 2010, we examined the effects of HRV-B on combat Veterans with PTSD in a small pilot study at DVAMC (n=10). In our published findings, we reported that HRV-B significantly improved HRV coherence as well as measures of attention and immediate memory (ATTN/IM).[44] In our current DoD-funded research, baseline PTSD symptoms were not significantly different between the active and sham training groups (Table 1). Linear mixed model analysis[54] of PTSD rating (CAPS) for the Training Group by Time interaction was statistically significant, $F(5, 127)=2.60$, $p=.028$; furthermore the Pre- to Follow-up difference within the HRV-B group was significant. Paired t-test within the HRV-B group of Pre- to Follow-up was statistically significant, mean=16.7, $t(12)=4.66$, $p=0.001$ ($\eta_p^2=0.663$). These treatment effect was well beyond the accepted reliable change score of 12 points reduction in CAPS[55]; a reduction of 15 points on the CAPS is considered clinically beneficial.[56] These results indicate that PTSD severity was reduced by HRV-B training compared to the Sham group, and the benefit persisted for 8 weeks after training ended.

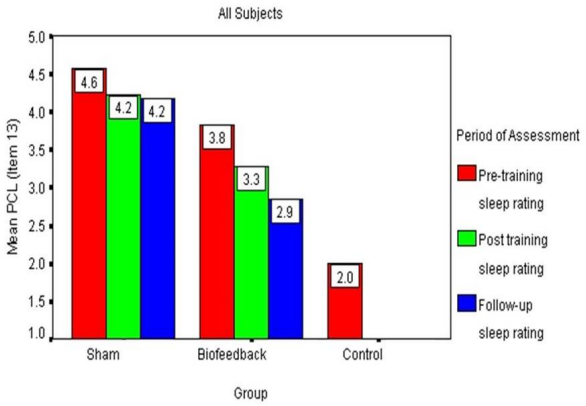
Table 1. Effect of HRVB on PTSD (CAPS)

Training	CAPS	Pre-	Post-	8-week Follow-up
Sham	Mean	76.1	69.9	72.9
	SE	4.3	5.2	7.1
	n	32	23	18
HRV-B	Mean	77.5	53.1	60.8
	SE	3.7	7.7	7.7
	n	29	16	12

Effect of HRV-B on Depression in Combat Veterans with PTSD. Depression (BDI-II) was also measured in our current DoD-funded research. The repeated measures contrast of Group x Time for the effect of HRV-B on Depression for Pre- to Post-Training was statistically significant, (F (1,28)=6.29, p=0.02 (η_p^2=0.173)). The reduction in BDI-II in the HRV-B group compared to Pre-training was statistically significant, (mean=5.9, t(16)=1.82, p(1-tail)=.045 (η_p^2=0.180)), while the BDI-II change in the Sham training group was not, p(1-tail)=.23. These results indicate that depression was reduced by the HRV-B treatment compared to the Sham training at the Post-Training assessment. A change of 5 points in the BDI is considered to be a reliable and clinically significant change.[57]

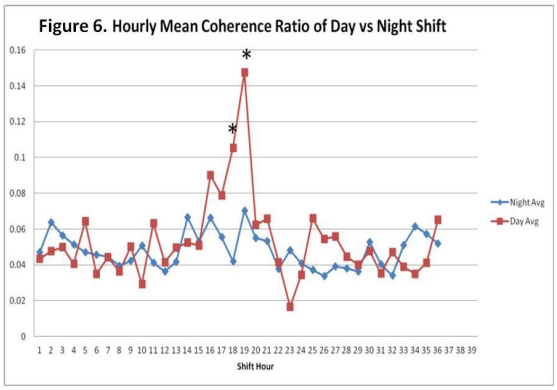
Effect of HRV-B on Sleep in Combat Veterans with PTSD. To evaluate the effect of active versus sham HRV-B on sleep in our on-going DoD-funded PTSD study, we examined the self-rating of sleep difficulty from the PCL-M. This rating is on a scale of 1 to 5, where 1 means no difficulty and 5 corresponds to extreme sleep difficulty. Participant's scores on sleep difficulty, analyzed using a linear mixed model, indicated a statistically significant Group by Time interaction (F(5, 127)=2.41, p=.028) with better post-training sleep ratings in the HRV-B group (Figure 5). Thus, there is preliminary evidence from our study among PTSD patients to suggest that active HRV-B training reduces self-reported sleep difficulties. Data from more sources measuring sleep quantity and quality is required to fully evaluate this possibility. This study of HRV-B effectiveness for PTSD is still in progress and no conclusions can be drawn at this time. However, the results are encouraging and suggest that HRV-B may improve the negative impact of PTSD on sleep among combat Veterans.

Figure 5. Effect of HRV Biofeedback Training on Self-reported Sleep Difficulty



HRV & Shift Work among DVAMC Nurses. Few studies have examined autonomic dysregulation or the circadian characteristics of HRV in relation to shift work. Dr. Burch recently completeda study of the relationship between shift work and HRV among DVAMC nurses. Participants were studied over one 36-hour period coinciding with the last two shifts of their work week. The night nurse's schedule consisted of non-rotating 12-hour shifts from 7:00 PM to 7:00 AM for 3 or 4 consecutive days in a 2-week period. Day nurses worked a similar schedule from 7:00 AM to 7:00 PM. Each participant wore a portable, data-logging HRV monitor (Firstbeat Bodyguard, Jyväskylä, Finland) starting at the beginning of one 12-hour work shift and stopping at the end of the final shift of a given work week. This light weight heart rate monitor attaches directly to the skin with two chest electrodes and continuously logs the R-R heart beat interval in milliseconds. Data were processed using KUBIOS software. Hourly averages of frequency- (HRV coherence, VLF, LF, HF power, peak frequency, peak frequency power) and time-domain variables (heart rate mean and standard deviation, NN50, pNN50, RMSSD) were compared among two groups of nurses working either permanent day (n=7) or night (n=11) shifts using repeated measures ANOVA. Results indicate that nurses working permanent day shifts had a clear circadian pattern of HRV coherence coinciding with their sleep period (hours ~15-23), whereas those working permanent night shifts had no apparent overnight amplitude in the circadian rhythm of their HRV coherence (Figure 6). These results suggest a pattern of autonomic dysregulation among nurses working permanent night shifts.

Figure 6. Hourly Mean Coherence Ratio of Day vs Night Shift



Sleep Actigraphy. The value of wrist actigraphy for sleep characterization is recognized in both clinical and research settings as an alternative to polysomnography (PSG), which is the most definitive method but can be

more expensive, labor-intensive, and burdensome to participants. We have studied actigraphic sleep profiles among: shiftworkers, patients with sleep apnea or cancer, and in the general population.[58-60,74] We studied shiftwork tolerance and melatonin metabolite excretion among manufacturing workers on permanent day, evening or night shifts, and used wrist actigraphy to document greater sleep disruption among night workers relative to day workers.[58] Recently, we compared the accuracy of sleep measures obtained via wrist- or hip-mounted actigraphs with PSG recordings. We also attempted to improve the correspondence between actigraphic and PSG sleep using a novel sleep scoring algorithm (spline regression). Compared to original wrist actigraphy data, spline-modified wrist actigraphy had better agreement with PSG-defined sleep. Thus, spline regression has the potential to improve sleep estimates obtained via wrist actigraphy.[61]

1.2. Specific Aims

This study fulfills the national VHA/DoD Task Force recommendation that complementary, integrative therapies for pain management be provided to Veterans. Chronic pain elicits stress which increases sympathetic output fostering autonomic nervous system imbalance, an overextended stress response, fatigue, depression and insomnia. Heart Rate Variability (HRV) is a measure of the interplay between the sympathetic and parasympathetic nervous systems, and thus is a useful and easily-measured index of autonomic balance that has a relationship to chronic pain effects. HRV biofeedback (HRV-B) is a novel, biobehavioral procedure that restores normal autonomic balance. Through HRV-B, patients increase parasympathetic cardiac output and restore autonomic balance via induction of 'HRV coherence'. Our pilot study indicated that HRV-B produced coherence and alleviated self-reported ratings of chronic pain and stress among Veterans attending our Pain Clinic.[50] The proposed clinical intervention will further test hypotheses that HRV-B increases HRV coherence, reduces self-reported pain, stress, depression, fatigue, and insomnia, and improves cognition among Veterans with chronic pain.

The **specific aims** are to: (1) conduct a randomized, sham-controlled, pilot intervention trial to determine whether HRV-B increases HRV coherence among chronic pain patients (n=40 each for the HRV-B and sham treatment groups; total N=80 patients); (2) determine whether HRV-B reduces self-reported pain and stress among chronic pain patients. The primary endpoints include HRV coherence, pain (Brief Pain Inventory or BPI), and stress (Perceived Stress Scale or PSS).

Furthermore, chronic stress is associated with disrupted circadian rest/activity rhythms and domains of quality of life (QoL) including fatigue, insomnia, and reduced physical and social functioning.[62,63] Interventions that relieve pain thus represent a novel therapeutic target for normalizing dysfunctional rest/activity rhythms and these QoL domains among pain patients. We are also interested in assessing the effects of HRV-B on cognitive function in pain patients. Thus, our **secondary exploratory objectives** are to determine if HRV-B: (1) improves sleep and rest/activity rhythms; (2) alleviates self-reported fatigue and depression; and (3) improves cognitive function (reaction time, attention). Circadian endpoints will be measured as actigraphic parameters of sleep (e.g., duration, efficiency), and rest/activity (e.g., dichotomy index, interdaily stability), and with the Insomnia Symptom Questionnaire (ISQ); fatigue will be assessed using the Multi-Dimensional Fatigue Inventory (MFI), which assesses general physical and mental fatigue and motivation. Depression will be assessed via the Beck Depression Inventory II (BDI-II). Cognitive function will be measured with a cognitive battery comprised of the Paced Auditory Serial Addition Test (PASAT), the Rey Auditory Verbal Learning Test (RAVLT), and the Psychomotor Vigilance Test (PVT).

2.0 STUDY DESIGN AND METHODS

Overview. The proposed intervention will use a previously approved, standardized HRV-B training protocol.[44] The primary (HRV coherence, pain, stress) and exploratory outcomes (insomnia, fatigue, depression, cognition) will be assessed at baseline, after the 6 weekly treatments, at the 4-week post-training booster, and at the 8-week post-training follow-up. Standardized procedures will characterize HRV coherence and other frequency- or time-domain HRV measures, and validated instruments will be used to assess pain[64,65] and

stress.[66] Sham intervention subjects will have pulse and respiration monitored but not receive active training; instead they will view a static, relaxing nature picture on a computer screen. We will provide portable data-logging devices for practicing HRV-B at home, and sham devices. Wrist actigraphy will characterize insomnia via continuous, 24-hour/day personal monitoring of rest/activity rhythms at 1-week intervals coinciding with the baseline, post-training, and 8-week follow-up assessments only.[58,67-69]

Data analyses will test for differences in primary and secondary endpoints at baseline, after 6 weeks of active versus sham HRV-B treatment, at the 4-week post-training booster/assessment, and 8 weeks after training. Analyses will be based on intent to treat with two-sided tests. The effect of HRV-B on coherence and other HRV variables will be analyzed using linear mixed models for repeated measures data from sequential time assessments, and a between-subjects factor to evaluate the intervention after adjusting for potential confounding factors (e.g., age, standard therapy, medications, co-morbid disease). Baseline relationships between HRV coherence and endpoints will be examined using multiple regression models. Few studies have examined the multivariate relationship between pain, autonomic dysfunction, stress, depression, sleep, rest/activity rhythms, fatigue, and cognition. Path analyses will be used to model interrelationships between HRV-B, primary and exploratory outcomes, and behavioral or social factors that facilitate the persistence of HRV coherence and pain management effectiveness.

2.1 Study Population and Recruitment

Subjects from the DVAMC Pain Clinic who are: English literate, ≥18 years old, of any race, ethnicity, or sex meeting inclusion and exclusion criteria will be recruited. An additional inclusion criterion is that the patient reports having chronic pain as defined by our screening questions. Our initial recruitment effort will target patients in the Dorn VAMC Pain Clinic. The Pain Clinic runs an active Outpatient Interdisciplinary Pain Program, which consists of weekly sessions of Nursing Education, Nurse Triage, Behavioral Medicine (ACT, or Acceptance and Commitment Therapy), Physical Therapy, Pharmacy Education, Biofeedback, and Acupuncture. This patient population is appropriate for the proposed HRV-B intervention. If our accrual milestones are lagging recruitment goals, we will expand our search for eligible patients to include the Rehabilitative Medicine, Rheumatology, or Primary Care departments at the DVAMC. Our approved poster (Section 2.1) will be used to advertise the study at the DVAMC, the Columbia Veterans Center, the University of South Carolina Veterans Association office, or similar Veteran venues. Permission from local offices will be obtained prior to posting the advertisement.

Inclusion criteria for the Pain Clinic includes patients with: chronic, non-malignant, axial neuromusculoskeletal pain (e.g., osteoarthritis, degenerative discs or joints, scoliosis, spinal stenosis, spondylolisthesis, compression fractures, or other musculoskeletal conditions with primarily nociceptive pain, ICD-9: 715.x, 721.x, 722.x, 723.x, 724.x, 729.x, 733.x, 738.x, 756.x). A Recruitment Coordinator will be responsible for screening and applying inclusion and exclusion criteria to potential research subjects.

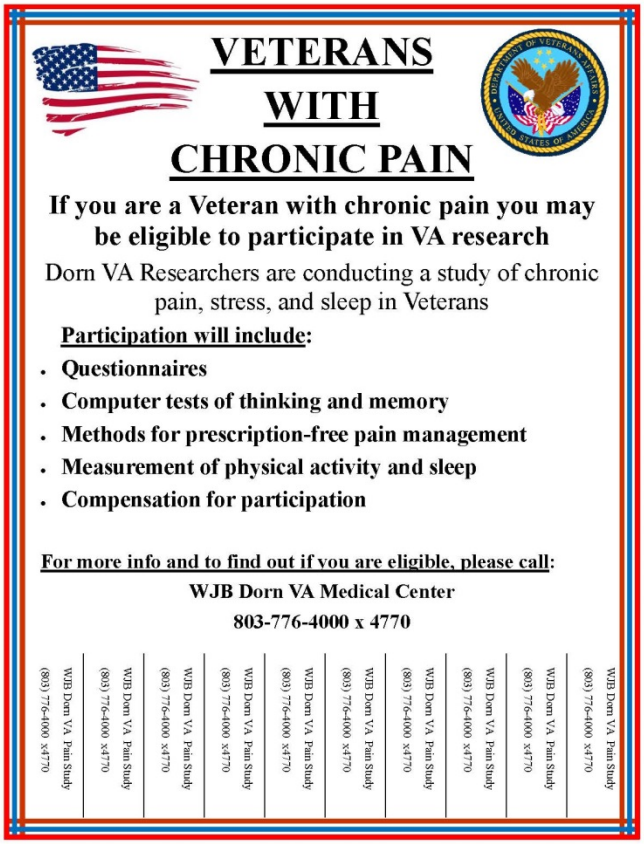
We will exclude patients who are: not appropriate for the intervention from a safety standpoint; have a condition that can bias HRV measures; or those not able to cognitively complete the protocol. We will exclude patients with: a) history of arrhythmias requiring medication and/or hospitalization, including supraventricular tachycardia or atrial arrhythmias (e.g., atrial fibrillation); b) patients with a pacemaker or automatic implantable cardioverter-defibrillator; c) history of an acute coronary syndrome, revascularization, thrombolytic or other therapy related to ischemic heart disease; d) uncontrolled hypertension (systolic blood pressure <140 mmHg, diastolic blood pressure <90 mmHg), although patients with well-controlled hypertension (no change in medications for 6 months) will not be excluded; e) history of heart transplant or cardiovascular surgery within 1 year; f) patients receiving beta-adrenergic antagonists; g) patients receiving non-dihydropyridine calcium channel blockers; h) patients receiving an antagonist of the renin-angiotensin-aldosterone system are eligible if their medication profile is stable; i) patients with New York Heart Association class 3 or 4 congestive heart failure; j) history of seizure disorder or use of antiseizure or anticonvulsant medication; k) cognitive impairment (e.g., dementia), or a history of acquired neurocognitive deficit, or central nervous system or neurological

disorder (e.g., Gulf War Syndrome); l) moderate or severe head injury or stroke; m) evidence of active substance abuse or dependence (alcohol or tobacco use will not be an exclusion, patients will be asked to report their use via questionnaire); n) life history of bipolar, psychotic, panic or obsessive-compulsive disorder (history of depression will not be an exclusion); o) previous experience with HRV biofeedback. Eighty participants will be recruited into the study. All participants will be Veterans being treated at the DVAMC.

Recruitment will follow a multi-step process of: 1.) obtaining a HIPPA wavier to identify active patients who are eligible for the study based on inclusion and exclusion criteria; 2.) informing potential participants of the nature of the study by letter, and excluding those who decline from the potential participant list; 3.) for those who do not decline, making a follow-up telephone call to ascertain interest in study, and if the patient expresses interest in the study; 4.) screening them during the same telephone call for chronic pain and any other inclusions and exclusions not ascertained from the medical record; and 5.) obtaining informed consent from patients eligible and willing to participate. The PIs will obtain a HIPPA wavier to access the docket of patients with a new or current pain diagnosis (within 6 months). This list will be narrowed to eligible patients based on medical record review. A staff recruitment coordinator will be responsible for patient screening and recruitment.

Patient contact information will be obtained for this refined list of patients, and each patient will receive a packet of information about the study that includes a cover letter from the DVAMC Pain Clinic Director co-signed by the study PIs, and a study brochure. The letter and brochure will briefly describe: the nature of this study in lay terms, the extent of participation, confidentiality requirements, and contact information for the recruitment specialist and PIs. The letter will be written at no more than a sixth grade reading level using plain language. The letter will contain a coded, pre-addressed, postage-paid envelope that provides a ‘no contact’ response. The letter will also state that if a negative response is not received by the patient within two weeks, then a recruitment specialist will contact the individual by telephone to ascertain their willingness to participate. Patients returning postcards who decline further contact will be removed from the eligible contact list. As an alternative, potentially eligible patients will be referred to a study coordinator by the nursing staff attending patients in the Pain Clinic. A study brochure has been developed to provide information about the study to patients in the Pain Clinic. It may be provided to potentially eligible patients by medical or research staff. The study brochure was developed based on information in the consent form, and it is presented in Appendix VI. A poster that will be used to advertise the study is presented below.

The study investigators will meet with staff in each clinic to provide information on the study protocol, design, and eligibility requirements. The recruitment procedure for referred patients will be the same as that described above. Eligibility will be checked, patients will then receive the packets and will be telephoned to ascertain interest in the study and perform the chronic pain eligibility screening (Pain Screening Questionnaire, Vanderbilt University Medical Center, Center for Quality Aging, Nashville, TN). The pain screening instrument assesses pain using these questions: 1.) Do you have pain anywhere right now? 2.) Does pain ever keep you from sleeping at night? 3.) Does your pain ever keep you from participating in activities/doing things you enjoy? 4.) Do you have pain every day? Chronic pain is present if the patient answers yes to questions 1-3, or to question 4 alone. During the study, patients will be asked to self-report the duration of their chronic pain. In addition, the patient's medical record will be examined (via the CPRS) to determine the duration of time between their first pain clinic appointment and enrollment in the study. These variables will be included as covariates in the data analysis. Once enrolled in the study, we will record the pain treatments received by participants from the medical record for use in data analysis.



2.2 Intervention and Data Collection

An appointment for the baseline assessment will be made among patients with chronic pain who meet other eligibility criteria and express an interest in participation; scheduled at a mutually agreed upon date and time. Study staff will meet participants at the DVAMC in order to obtain informed consent, conduct an interview to include baseline ascertainment of primary outcomes, and initiate baseline actigraphy. Participants will receive a $30 reimbursement for each of the sessions they attend. A total of 10 sessions is planned: baseline (pre-training), six weekly training sessions, post-training evaluation, a 'booster session' 4 weeks after post-training, and an 8-week post-treatment follow-up session (4 weeks after the booster session) for a total of 10 sessions. (= $300 for protocol completion, Figure 7). Patients attending the DVAMC Pain Clinic will be recruited over ~36 months of the 48 month period funding for randomization to an active HRV-B (n=40) or sham (n=40) protocol. Given the purpose of the study (pain and stress reduction) and the accessibility of chronic non-malignant neuromusculoskeletal pain patients, we anticipate a recruitment rate of ~75%. Research personnel will annotate each participant's CPRS medical record that the Veteran has enrolled in this research. Participants will be requested not to change their ongoing pain treatment for the duration of the study unless it is urgently indicated. The Veteran's pain clinical provider will be advised to forego changes in the Veteran's pain treatment

plan during the Veteran's participation in the study unless a change in the pain treatment plan is imperative. Determination of the need to change the pain treatment plan will be made by the Pain Clinic Director (Dr. Katz) or his designee. When a change in pain treatment is made, the participant will remain in the study and specifics of the change in treatment will be recorded for later evaluation in the statistical analysis. Non-participants will be asked to provide the following information: age, race/ethnicity, education, sex, and pain rating. This information will be compared among participants and non-participants to assess potential selection bias. Reasons for nonparticipation or noncompliance will be summarized and we will evaluate whether this information may suggest selection bias and protocol modifications in future research. The Rehabilitative Medicine and Rheumatology departments at the DVAMC will serve as alternative recruitment sites, if needed, to expand the pool of eligible patients. The study brochure and poster may be used to provide information about the study to patients attending these clinics. A project schedule is presented in Table 2. A subject sign-in sheet for tracking site visits is presented below. A list of frequently asked questions with answers is presented in Appendix VI.

Figure 7. Intervention Timeline

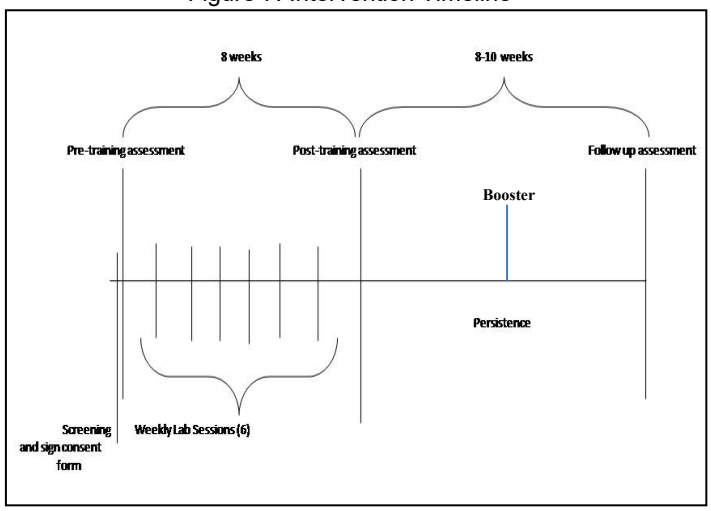


Table 2. Project Schedule					
Project Month	1-6	6-9	10-36	37-42	42-48
Obtain Institutional Review Board and Research & Development Committee approvals	X				
Hire Research Associate (Project Coordinator) and Graduate Research Assistant	X				
Finalize clinical protocol for HRV-B and sham treatments, subject recruitment and retention, actigraphic data collection, and database development, validation and quality control	X				
Purchase equipment and supplies for data collection and storage	X				
Data management and quality control review	X				
Initiate recruitment, data collection, and database development		X			
Continue and complete subject enrollment		X	X	X	
Complete database; Data management and quality control review				X	
Perform statistical data analyses				X	
Data management and quality control review					X
Prepare and submit manuscripts for publication					X

Subject ID#:_____

HRV Biofeedback in Pain Patients
Pilot Intervention for Pain, Fatigue & Sleep
Subject Sign-In Sheet

Subject Name:		**SSN:**	
Date	**Sessions**	**Subject Signature**	**Research Personnel Initials**
	Baseline (Pre-Training)		
	Training (Week 1)		
	Training (Week 2)		
	Training (Week 3)		
	Training (Week 4)		
	Training (Week 5)		
	Training (Week 6)		
	Post Training Evaluation		
	Post Training Booster		
	Post Treatment Follow Up		

Baseline HRV Assessment. Resting physiological measures to be recorded for 20 minutes at baseline. We will use our autonomic testing system (budgeted start-up equipment) to precisely determine HRV at the four time periods (pre-testing, post-testing, booster, 8-week follow-up). Testing will be performed in a standardized, recognized, and quantified manner that is non-invasive. By monitoring the amplitude changes of the peak frequency in a power spectrum of the inter-beat intervals in synchrony with respiration, it is possible to detect when a specific respiration rate is causing an increase in the peak frequency output, which defines the

resonant frequency of breathing. Resonance frequency breathing produces the highest HRV in the LF band. Cardiovagal (parasympathetic) function is monitored and HRV is derived using heart rate response to deep breathing and transformation of tachygram recordings, procedures that the investigator (JPG) is experienced with, using easy-to-perform, and time efficient measurements. Heart rate will be recorded via dry electrode wrist straps and respiration will be monitored via a Piezo-respiratory transducer. Software integrated into the HRV physiological monitoring system will run on a laptop computer and be used to de-artifact raw data and calculate the FFT power spectrum of HRV for each patient (Appendix I). This will provide data for frequency-(HRV coherence, VLF, LF, HF power, peak frequency, peak frequency power) and time-domain HRV measures (heart rate mean and standard deviation, NN50, pNN50, RMSSD). HRV coherence is defined in accordance with consensus guidelines.[27,44,47,48] It is estimated by calculating the ratio of power in the LF peak to the remainder of power in the spectrum.

Random Assignment to the 6-week HRV-B treatment or sham intervention group will occur following the baseline assessment. We will use a permuted block randomization procedure, with a block size of 4 and no stratification. Thus, for example, assuming 20 patients are to be assigned either to Sham (S) or Biofeedback, there would be 20 blocks of 4 patients, and two patients will be assigned each to the (S) treatment and two to the biofeedback by randomly selecting one of six possible permutations of the two treatments in blocks of four. The order of the blocks will be randomly generated. The treatment assignment will be determined before any patient is enrolled into the study and the documentation and assignment of patients will be kept confidential and known only to the PI (JPG). Only the Biofeedback professional, who delivers the treatment, will know the treatment status of the participant; other study personnel will be blinded to treatment status.

HRV-B Training. HRV-B training will follow our previously established, standardized protocol that is consistent with recommendations of the Biofeedback Certification Institute of America (BCIA), consisting of a 15-minute resting period that includes HRV recording, and a 25-minute biofeedback training and coaching or sham period. The resting periods are the same for both the HRV-B treatment and sham groups. Participants will be affixed with HRV monitoring equipment and will be instructed to sit quietly for 15 minutes in a relaxed posture, viewing a static nature scene on a computer monitor, without any instruction, coaching or biofeedback. Coaching or instruction will start after the 15-minute resting period. Patients in the sham condition will be asked to ‘just relax’ or ‘take it easy’ for the remainder of the 25-minute session. Patients in the HRV-B group will be coached on how to achieve HRV coherence.

HRV-B training is conducted in an interactive manner using a computerized system to monitor and display the individuals’ HRV patterns in real time while their heart rate, measured as blood volume pulse (BVP), is recorded with a fingertip photoplethysmograph (PPG). Visual HRV feedback (either a quantitative display or animated challenge games) is provided as participants practice attention focusing, resonant frequency breathing, imagery, and the induction of positive emotion. The resulting changes in HRV patterns are readily observed by the subject. The visual feedback enables associations to be formed between these techniques and HRV patterns indicative of higher vagal parasympathetic output. Before HRV-B training, the biofeedback trainer discusses individual issues and the training goals of the participant. The physiological connection between resonant frequency breathing and heart rate is introduced. This is reinforced with corrective breathing instruction and help finding the resonant frequency (~6 respirations/min). Dual monitors also display the HRV power spectrum in real-time so that the participant can visualize the impact of proper breathing on the coherence peak a 0.10 Hz. Positive emotion and interactive visual images are then introduced. This interactive process allows the participant to customize a sustainable system, with occasional shifts back to the power spectrum screen to observe the 0.10 Hz HRV peak. During the recording period after coaching, subjects view a static nature scene on the computer while their heart rate is measured with the plethysmograph and recorded (Appendix III).

Home Practice. The biofeedback coach will instruct participants in both groups to practice their training at home a minimum of 10 minutes a day, and participants will be given a log to record their daily home practice. Home practice self-report data will be incorporated into the analysis to examine the potential dose-response

effects of home practice. Information will be solicited in the questionnaire regarding the factors that contributed to or detracted from home practice for inclusion in the analysis. One goal of this intervention is to spontaneously and consistently achieve HRV coherence on demand outside a clinical environment. Therefore, participants will be provided with a portable colorimetric finger PPG (emWave® hand held personal stress reliever; dimensions similar to a cell phone; weight: 2.2 ounces) for home practice and use between weekly HRV-B training sessions. Home HRV-B practice period data from the device can be compared to self-report and further analyzed with regard to the outcomes (see Appendix III, IV).

Intervention Integrity. Several procedures will be used to evaluate treatment integrity. The intervention will follow the HRV-B protocol adopted by the BCIA, and BCIA certification for HRV-B or its equivalent training will be required for trainers in this study. Protocol compliance integrity will be monitored and supervised by research staff and Dr. Burch , and via communication between them and the certified biofeedback professional (Jane Areve). We will ask patients about whether they thought they were enrolled in the active or control condition. We will then summarize these data along with the number and duration of participant visits over time, and compare these values between the sham and treatment groups. Contrasts between the sham and treatment condition for outcomes measured at baseline, post-treatment, booster, and at the 8-week follow-up will allow us to evaluate the accuracy and delivery of the intervention. Measures of HRV coherence will allow for direct, quantitative assessment of participant performance and receipt of intervention.

Sham Treatment. To control for the laboratory environment or other placebo effects, participants assigned to the sham HRV-B training group will be scheduled by the biofeedback professional for a weekly clinic meeting during the 6-week period of the project that matches the timing and duration of the active HRV-B sessions. The sham procedure has been used successfully in our ongoing study of HRV-B among PTSD patients for the past three years. During these lab visits, sham training participants will have their heart rate, respiration, blood pressure and blood pressure volume recorded during a 15-minute resting period, but no active training or coaching will be provided.

During the ~25 minute coaching period, the Biofeedback professional has the participant sit quietly in front of the computer screen with the fingertip PPG in place, but the recording input is switched off so no information on heart rate is delivered to the recording equipment. No active biofeedback coaching is delivered regarding resonant frequency breathing, imagery, or positive emotion induction. The Biofeedback professional uses passive relaxation statements such as: ‘try to relax’ and ‘please sit quietly’ during the coaching period. During the passive 15-minute HRV recording period after the coaching session, the recording input of the PPG is switched on, and the subjects view the same static relaxing nature scene on the monitor as is presented to the HRV-B group. For home practice, participants in the sham group are given a ‘squeeze stress ball’ and asked to use it throughout the normal activities of the day. Subjects in the sham group will be asked to self-report the frequency and duration of squeeze ball use.

Primary & Exploratory Questionnaire Outcomes. In addition to HRV coherence, the primary outcomes (pain, stress), as well as the related exploratory measure, will be assessed at baseline, after the 6-week intervention period, during the booster session and at the follow-up session. Validated instruments will be used to administer a structured questionnaire focusing on: 1). pain (BPI[64,65]) and stress (PSS[66]); 2). the exploratory outcomes (sleep, fatigue, depressive symptoms, cognitive performance); 3). well-being (coping, social support); 4). lifestyle (chronotype, social jetlag); 5). socio-demographic and personal characteristics (age, height, weight, education, income, physical activity; 6). occupation (current or previous job title, combat history, shift work); 7). supplemental use of pain medications including over the counter anti-inflammatory agents [e.g., NSAIDs], as well as alcohol and tobacco consumption; and 8). whether they have a pending worker compensation claim or personal injury litigation related to their pain or other symptoms. Some items from the medical record (e.g., age, body weight) will be compared with questionnaire data to assess reporting validity. Questions focusing on the duration of symptoms (e.g., sleep disruption, fatigue) will be integrated into the study instrument (e.g., the duration of: sadness, fatigue, stress, or sleep disruption). Co-morbid diagnoses of: hypertension, diabetes, cardiovascular disease, stroke, obstructive sleep apnea or other sleep disorders, or

post-traumatic stress disorder (PTSD), and use of prescription medications (hypnotics and other sleep aids, antidepressants, anxiolytics), and will be assessed by chart review after obtaining a HIPAA release of medical information as part of the informed consent process. Questionnaires are presented in Appendix V.

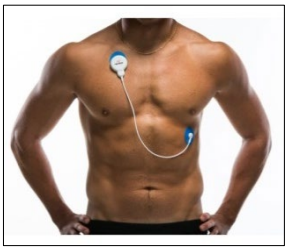
Pain. The BPI was developed by the World Health Organization (WHO) specifically for use among cancer patients, and it has since been widely adopted for assessment of clinical pain and pain treatment effectiveness in a variety of clinical and research settings.[64,70] Among the barriers that result in under treatment of cancer pain, one key consideration is the adequate pain characterization. The BPI is an assessment tool pain that measures both pain intensity (sensory dimension) and the interference of pain in the patient's life (reactive dimension). The BPI has demonstrated reliability and validity across different cultures and languages, and it has been applied to studies of clinical pain assessment, as well as epidemiological investigations and research on the effectiveness of pain treatment.[64,70]

Pain Catastrophizing Scale (PCS). The PCS was developed to facilitate research on the mechanisms by which catastrophizing impacts the pain experience. Items on the PCS were drawn from previous experimental and clinical research on catastrophic thinking in relation to pain experience (http://sullivan-painresearch.mcgill.ca/).[71] The factor structure has been replicated in several investigations. The PCS total score is computed by summing responses to all 13 items. PCS total scores range from 0-52. The PCS requires a reading level of approximately Grade 6 and can be completed and scored in less than 5 minutes. It is a 13-item instrument derived from definitions of catastrophizing described in the literature. The PCS instructions ask participants to reflect on past painful experiences, and to indicate the degree to which they experienced each of 13 thoughts or feelings when experiencing pain, on 5-point scales with the end points (0) not at all and (4) all the time. The PCS yields a total score and three subscale scores assessing rumination, magnification and helplessness. The PCS has been shown to have adequate to excellent internal consistency (coefficient alphas: total PCS = .87, rumination = .87, magnification = .66, and helplessness = .78).[71]
Stress. The PSS is the most widely used psychological instrument for measuring the perception of stress. The original version, published in 1983,[72,73] has been refined over the years into its current form and is available a public domain instrument. It measures the degree to which situations in one's life are appraised as stressful. Items were designed to tap how unpredictable, uncontrollable, and overloaded respondents find their lives. The items are easy to understand and general in nature, and hence relatively free of content specific to any subpopulation group. PSS scores are correlated with self-reported health, health behaviors, smoking status, and help seeking behavior.[73] The PSS exhibits excellent construct validity and reliability.[66]

Sleep & Rest/Activity Rhythms. Wrist actigraphy is a non-invasive, quantitative, and validated method that will provide a primary means by which circadian disruption is assessed in this study. Weekly actigraphic monitoring will be performed once each at baseline, post-treatment, and at the 8-weeek follow-up using a standardized protocol.[14,74-76] Actiwatch-Spectrum monitors (with built-in ambient multiple spectrum light sensor, event marker, digital watch, and off-wrist detection capability, Respironics, Bend, OR) will be used in this study. They are similar in size, weight, and appearance to a wristwatch. Monitors will be worn on the non-dominant wrist, recording activity and light exposures at 1-minute intervals. Subjects will wear the monitors 24-hours per day for three 1-week intervals (baseline, post-treatment, and at the 8-week post-training follow-up). Participants will be asked to follow their usual sleep-wake schedules and maintain a log of when they go to bed and awaken on days when they wear the actiwatch. At the end of each 1-week actigraphic monitoring period, a study technician will retrieve the monitor from the subject during their weekly training session. Alternatively, participants can mail the actiwatch to research staff using a pre-labeled, postage-paid envelope that will be provided to the participants, or a study investigator can retrieve the monitor from the individual's residence. These methods have all been successfully implemented in previous studies among DVAMC patients and elsewhere.[14,77] Actigraphic sleep parameters have been previously validated,[67,68,78,79] and 1-week intervals provide for ample sleep or rest/activity rhythm assessment.[79] A weekly average of sleep metrics (latency, duration, efficiency, fragmentation, wake time after sleep onset [WASO], daytime nap duration) will be quantified using the manufacturer's accompanying software (Appendix II).

Sleep disruption will be ascertained using the Pittsburgh Sleep Quality Index (PSQI), which is a self-administered 19 question instrument, categorized into 7 components, that assesses sleep duration as well as latency, frequency, and severity of sleep problems with a possible range of 0-21. The 7 components are subjective sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disturbances, use of sleep medication, and daytime dysfunction. The Cronbach's alpha coefficient for all component scores was 0.83 which indicates high internal consistency. Those with disorders of initiating and maintaining sleep and with disorders of excessive somnolence had statistically significantly higher scores compared to control subjects, which indicates good construct validity. A cut-point score of 5.0 indicated a sensitivity of 90% and a specificity of 87% in identifying those with a sleep disorder.[80] One advantage of the PSQI over other instruments is that it provides information on overall subjective sleep quality and includes several items related to snoring and the induction of sleep apnea or hypopneas, which can influence heart rate, a primary study outcome. We will modify the time scale for reporting on sleep quality to the previous week to coincide with planned wrist actigraphy measures. We will augment the use of this instrument by performing CPRS queries for each participant (with a HIPAA release) to identify diagnostic codes for insomnia or other sleep disorders.

Using actigraphy data, insomnia may be defined using the following cut-off values: sleep onset latency ≥12 minutes, total sleep time ≤440 minutes, WASO ≥25 minutes, sleep efficiency ≤92%, or the number of 5-minute wake episodes ≥2.[69] The phase and amplitude of circadian rest/activity rhythms will be characterized via previously described non-parametric (interdaily stability, intradaily variability, relative amplitude, 24-hour autocorrelation coefficient) and cosinor statistics (acrophase, mesor, amplitude, amplitude/mesor ratio, goodness of fit).[67,68,81] Ambient light exposures collected via actiwatches will be summarized using the same descriptive, cosinor, and nonparametric statistics.



Ambulatory Heart Rate Variability Monitoring. In a limited number of participants, a convenience sample (n=15-30 total) will be asked to wear a data-logging ambulatory heart rate monitor (Firstbeat Bodyguard 2; Firstbeat Technologies, Jyväskylä, Finland) for two 1-week periods that coincide with data collection for the wrist activity monitor (i.e., at baseline and during the post-training week). The heart monitor is lightweight, portable and noninvasive (see photo). It attaches to the skin with a snap electrode and does not require a belt around the body. There is no need for the participant to operate or adjust the monitor in any way, they only need to wear it. Note: it should be removed when taking a shower, or while bathing or swimming (extra skin patch electrodes will be provided to re-attach the device). Participants will wear this device 24 hours per day. The data record allows for time- (mean and std HR, NN50, pNN50, and RMSSD) and frequency-domain variables (VLF, LF and HF power, peak frequency, peak frequency power, and coherence) to be generated for comparison with actigraphic sleep data and other exploratory research. Research staff will hand out and collect bot the heart rates and wrist actigraphy monitors at the same time for each participant. Data are downloaded for processing with a cable attached to the computer's USB port, and the battery is recharged in a similar manner. Participants will be provided with written and verbal instructions on appropriate use (no showering, bathing or swimming) and with extra leads for re-attaching the monitor after showering, etc.

Depression & Fatigue will be ascertained using several valid instruments. Fatigue will be assessed using the Multi-Dimensional Fatigue Inventory (MFI), comprised of 20 questions that assess general, physical, and mental fatigue, and reduced motivation and activity. It also has good internal consistency (Cronbach alpha: >0.75) and high convergent validity confirmed via strong correlations with several other validated instruments.[82,83] Depressive symptoms will be ascertained using the Beck Depression Inventory II (BDI-II).[84-86] The BDI-II is a 21-item self-report instrument that asses the existence and severity of symptoms of depression as listed in the American Psychiatric Association's Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV). The BDI-II has excellent clinical sensitivity (Cronbach alpha: 0.92).[84] Its construct validity has been established, and it readily differentiates depressed from non-depressed patients.[87]

Cognitive Performance. A short and simple battery consisting of three validated neuropsychological tests will be administered at the 4 test periods: the Paced Auditory Serial Addition Test (PASAT), the Hopkins Verbal Learning Test (HVLT), and the Psychomotor Vigilance Test (PVT). These PASAT and HVLT have alternate forms and the PVT has no significant practice effect. This battery of assessment instruments has sufficient reliability to detect changes over repeated test sessions. Brief descriptions are provided below.

Paced Auditory Serial Addition Test (PASAT). The PASAT is a measure of cognitive function that assesses auditory information processing speed and flexibility, as well as calculation ability. It was developed in 1977 and is now very widely used in clinical and research applications to assess cognitive function. In the PASAT, single digits are presented every 3 seconds and the patient must add each new digit to the one immediately prior to it. Several alternate forms have been developed that are publically available to minimize familiarity with the stimulus items when the PASAT is repeated on more than one occasion. Administration time is approximately 5 minutes. The score for the PASAT is the total number correct out of 30 possible answers. The PASAT has demonstrated high split-half reliability and evidence for convergent and divergent validity with good sensitivity for deficits in the areas of auditory information processing speed and flexibility.

Hopkins Verbal Learning Test. The HVLT is a measure of verbal learning and memory, in which the participant is presented with a list of 12 semantically categorized words and is asked to recall as many as he/she can. This process is repeated twice more, and the score is equal to the total number of words recalled across all three trials. The test takes approximately 5-10 minutes to administer. There are several alternate versions to eliminate practice effects across repeat test sessions. The norms are in the public domain and available without cost.

Psychomotor Vigilance Task (PVT). The psychomotor vigilance task (PVT) is a widely used and well-validated test of sustained-attention and reaction-time that measures the speed with which subjects respond to a visual stimulus. The PVT correlates with lowered alertness, slower problem-solving, decreased psycho-motor skills, and increased rate of false responding. The PVT is popular because of its ease of administration and scoring, simple metrics, and convergent validity. The PVT is a simple task where the subject presses a button as soon as a light appears on the computer screen. The light will turn on randomly every few seconds for 5 minutes. The main measurement of this task is to assess reaction time and how many times the button is not pressed when the light is on. The purpose of the PVT is to measure sustained attention, and give a numerical measure of level of arousal. The program is publically available at no cost.

Lifestyle and sociodemographic characteristics will be assessed using instruments developed in our previous studies among Veterans.[44,77,88] Chronotype refers to the self-reported characterization of individuals with inherent morning or evening preference (or neither).[89,90] This trait has been associated with intrinsic circadian period,[91] and the circadian timing of numerous psychophysiological processes,[92-95] including sleep,[96,97] arousal and alertness,[98] mood,[99] and the secretion of catecholamines,[100] and cortisol.[101] The Munich Chronotype Questionnaire (MCTQ)[102] will be used to ascertain self-reported chronotype and sleep/wake timing on free and work days. Disparities between chronotype and social schedules can result in chronically desynchronized sleep-wake timing or 'Social Jetlag',[103,104] which will be quantified as the absolute difference between mid-sleep on weekdays and weekends, after adjusting for weekend make-up sleep.[104] Other potential modifiers of the HRV-B treatment that will be assessed include: social support,[105] and coping.[106]

Saliva Specimens. Saliva samples (~1/2 tablespoon) will be collected by each participant; once at baseline, once at the post-treatment assessment, and another at the final follow-up visit. One ~2 mL saliva sample will be collected each time using the simple, noninvasive passive drool method with Oragene® DNA collection kits (model: OGR-500, DNA Genotek, Ontario, Canada, http://www.dnagenotek.com).[107] The vial has a fill line for precise volume collection, and salivary DNA is immediately stabilized by a preserving reagent. Samples will be frozen at -80°C for long term archive. DNA can be purified from the archived specimens using a standard ethanol precipitation method from commercially available kits. The median yield for a 2 mL sample using this device is 110 µg DNA. Good quality DNA will be defined as a 260/280 UV absorbance ratio of 1.7-2.0. These

specimens will serve as a valuable resource for future research targeting biological processes that may mediate of modify the treatment effect. Another ~2 mL native saliva sample (without added reagent) will be collected for recovery of proteins or peptides using the same collection procedure.

The samples will be archived in frozen storage for future research. Samples will be coded with a unique number that will not include participant's name or any other personal information. Only the Principal Investigators, or a study Co-Investigator, will have access to the code that links the sample to their identity. The samples will be stored indefinitely in a -80C freezer for future research purposes at the DVAMC Building 9A, basement. Analyses that are under consideration include the study of genes involved in pain or other study endpoints, or responses to the HRV-B treatment. This may include candidate gene or genomic sequences, gene expression, or genetic modifications (e.g., epigenetic changes, sequencing). Analyses may also include immune system markers, hormones, or other compounds or microbes (e.g., bacteria) in saliva. Some sample analyses will be performed in the laboratory of Dr. Angela Murphy, 6439 Garners Ferry Rd., School of Medicine Building 1, Room C27, Columbia SC 29209. Sample analyses also will be performed by EpigenDx, 96 South St, Hopkinton, MA 01748. These measures will be for exploratory research and not for clinical use. Therefore, individual results will not be provided to participants. Use of these samples for future research is noted on the consent document.

2.3 Data Analysis Plan

Statistical Power & Sample Size. Preliminary data for HRV coherence and the pain (BPI), and stress (PSS) scales from our HRV-B pilot study among DVAMC chronic pain patients were used to estimate statistical power for the proposed primary study outcomes (HRV coherence, pain rating, negative emotion, activity limitation, perceived stress scale). Two-sided, two sample t-tests were used for the statistical power calculations. The analysis for the proposed study will use more sophisticated methods that tend to reduce variance in the dependent variables and thus increase power, thus what is presented here is likely conservative. An overall significance level of 0.05 was used with correction for multiple testing via the Bonferroni method for the 5 outcomes (adjusted significance level = 0.01; 0.05/5). Our pilot data indicate that at baseline, no statistical differences were observed between treatment groups for any outcome. The detectable effect differences between treatment and sham groups with corresponding standard deviations are presented in Table 3. We assumed equal variances in the differences between pre- and post-treatment comparisons for both groups. A sample size of 25 in each group will attain a statistical power of at least 84.5% to detect the expected effect differences between treatment groups, and groups sizes of 40 patients will provide statistical power exceeding 95% for comparisons between treatment groups for each outcome. Thus, a sample size of 40 patients per group would achieve ample statistical power even with retentions on of 55-60%.

Table 3. Estimated Power for Differences Between HRV-B Treatment & Sham Interventions

Sample Size per Group	Negative Emotion (4.5±10; 15.5±10)*	Activity limitation (4± 9.5; 14± 9.5)*	Pain Rating (1.8±5.0; 10±5.0)*	Perceived Stress (-1.2±4.5; 4±4.5)*	HRV Coherence (-0.03±0.15; -0.20±0.15)*
22	82.3%	78.1%	99.6%	86.5%	84.9%
25	88.1%	84.5%	99.9%	91.4%	90.2%
30	94.1%	91.7%	99.9%	96.2%	95.4%
40	98.7%	97.9%	99.9%	99.3%	99.1%

*Average pre-post change ± SD in the control group; and average pre-post change ± SD in the treatment group, respectively

Data Quality Control. Data collected by physiological sensors will be encoded and recorded without any identifying information. Analysis and interpretation of data files also will be conducted without reference to any identifying information. Questionnaire data will be labeled using the participant's identification number and will not have personal identifying information anywhere on the form. Once collated, coded questionnaire data will

be entered into a password protected computer for further processing and analysis. We will use the double key entry method of data verification (or equivalent) to help ensure data entry accuracy.

Data Security. Multiple steps will be taken to ensure complete confidentiality of the study data, and confidentiality will be maintained to the fullest extent allowed by law. Data files will be stripped of personal identifiers and replaced with coded participant identification numbers that will be used to link data files from multiple sources (e.g., questionnaires). Once collated, coded data will be entered into encrypted VA workstation computers for further analysis and processing. Data files will be stored on an access-controlled data server (network access to the server is by password for a limited set of study personnel: Building 106, Room E111; \\v07.med.va.gov\cms\vhacms\vhacmsburchj for Dr. Burch.. To minimize risk of loss of confidentiality, only authorized personnel will have access to research data. Participants will be informed that the local IRB may review de-identified or identified study data. The key linking participant identifiers to the coded data will be maintained by the Contact PI in a locked file cabinet at the DVAMC. Hard copy records with patient identifiers (e.g., signed consent forms) will be maintained in the Contact PI's office at the DVAMC in a locked file cabinet, accessible only to authorized study personnel (Building 106, Hard copies with patient identifiers will be stored in Room E111A). Consultants will not have access to any identifiable data. Data analyses will be performed at the DVAMC using coded data with personal identifiers removed, using coded data via an approved data use agreement with the Department of Epidemiology and Biostatistics, Arnold School of Public Health, University of South Carolina, or using coded data without identifiers via a secure VA Virtual Private Network (VPN) workspace (see below). All study personnel will maintain current VA training requirements (e.g., data security, human subjects protection). If an investigator or research staff member terminates their work on the project, their access to the data will become restricted, and the Information Security Officer will be notified. Data will be maintained in a secure manner until it is no longer needed or until its maintenance is no longer legally required. Data destruction will be performed by deleting electronic files and by shredding paper files.

VINCI Workspace. The VA Informatics and Computing Infrastructure (VINCI) is a Department of Veterans Affairs (VA) Health Services Research & Development (HSR&D) resource center that provides a secure, central analytic platform for performing research and supporting clinical operations activities. It is a partnership between the VA Office of Information Technology (OI&T) and the Veterans Health Administration Office of Research and Development (VHA ORD). VINCI includes a cluster of servers for securely hosting suites of databases integrated from select national VA data sources, as well as statistical software packages for data analysis. VINCI servers for data, applications and virtual sessions are physically located at the VA Austin Information Technology Center (AITC) in Austin, Texas. This secure enclave with 105 high-performance servers and 1.5 petabytes of high-speed data storage has multiple layers of security and disaster recovery to prevent data loss. To ensure the protection of Veteran data, VINCI maintains compliance with the guidelines set forth by Veterans Health Administration (VHA) Handbook 1200.12, Use of Data and Data Repositories in VHA Research, and other applicable VA and VHA policies and regulations. In addition, VINCI has undergone security certification activities in support of obtaining an Authorization to Operate (ATO). Access to VINCI resources are approved in accordance with the requirements of National Data Systems (NDS), VHA Handbook 1200.12, Use of Data and Data Repositories in VHA Research, and all other applicable VA and VHA policies and regulations. All data transferred from VINCI is subject to audit for compliance.

VA-credentialed investigators are granted access to study-specific data along with tools for analysis and reporting in the secure, virtual working environment through a certified VHA network computer within the VA. If not working within a VA or VHA hosted office environment containing VA network access, researchers may apply for and then access VINCI through an approved VPN and Remote Desktop application. The remote computing environment enables data analysis to be performed directly on VINCI servers, offering a number of advantages: uniform security standards for access; a common point of entry for all investigators who use the data; tools for analysis and reporting; tighter and more consistent control of data quality; and the ability to standardize and update terminology and format as technology and methodology improve. Software used for

data analysis in the VINCI workspace, whether provided by VINCI or developed by the study team, will run in remote virtual desktop sessions on VINCI servers within the Austin Information Technology Center.

For this study, coded project specific data without personal identifiers may be uploaded to VINCI servers by secure file transfer protocol as directed by VINCI data managers. Study data maintained on VINCI servers will be kept in accordance with the Department of Veterans Affairs Record Control Schedule 10-1 (RCS 10-1). Data transferred to VINCI servers will be coded using a participant ID number as described above (see Data Security section) so that none of the data will be personally identifiable. Upon completion of the research project, the study Principal Investigators in conjunction with the VA Information Security Officer (ISO), and in accordance with VA policy, will ensure that, study data will be returned to the DVAMC. The study Principal Investigators have the responsibility for security of study data. Only explicitly authorized study personnel will have access to project data. When study personnel are no longer part of the research team, the study Principal Investigators will amend the data access request to terminate that person's access to all study data and notify the VA Information Security Officer of such action. VINCI data managers and VA OI&T personnel not under the purview of the study Principal Investigators control the servers, network, processors, firewall and software in the VINCI environment, including access rights granted to study personnel. Study specific data stored on VINCI servers will be located at the Austin Information Technology Center, 1615 Woodward St., Austin, TX 78772-0001. The specific server where the data are stored within the VINCI environment will be chosen by VINCI personnel. The server name and location within the Austin Information Technology Center may be changed at any time at the discretion of VINCI personnel.

Statistical Analyses. Analysis and interpretation of data files will be conducted without reference to any personal identifying information. We will begin all analyses with an inspection of all variables for completeness, any missing values will be tabulated and range checks will be used to identify observations that need to be validated. Once the data set has been finalized, a locked version will be created to use for all analyses. Descriptive statistics will be applied to questionnaire scores, their potential predictors, demographic and lifestyle variables to evaluate their characteristics and assess their general inter-relationships (e.g., via correlations for numeric measures, or by frequencies among treatment groups for categorical variables). These analyses will provide an understanding of relationships among variables and will aid in planning subsequent analyses. The main analyses pertaining to aims 1 and 2 will consist of linear mixed models for repeated measures data to evaluate the treatment effect on outcomes of interest. We will also implement secondary exploratory path analyses to evaluate direct and indirect effects of the treatment on the primary (HRV coherence, pain, stress), and secondary (insomnia, fatigue, depression, cognition) outcomes.

For each of the outcomes, there will be three measurements per subject (post-training minus pre-training, 4-week booster minus post-training, and 8-week follow-up minus the 4-week booster assessment). To test the effect of HRV-B relative to controls, we will apply linear mixed models for repeated measures data. Models will include the group (intervention or sham), time, and the interaction between group and time as the main independent variables. The effect of the intervention on the outcome could be non-linear in time, thus we will evaluate the inclusion of time as a nominal or ordinal variable and choose the specification that has the best fit in terms of the model assumptions and the BIC statistic. Covariates to be considered in the models include (but are not limited to: age, type and duration of usual pain treatment or medication, or other outcomes that may modify the effect (e.g., stress, pain, sleep). We will use a variable selection procedure to screen and identify all variables that differ among the treatment and control groups (initial selection set at $p<0.15$), and we will include them in the final statistical model if their inclusion alters the effect estimate for HRV-B by ≥10%. Linear mixed effect models will be implemented using the PROC MIXED procedure in SAS (v9.3, SAS Institute). The conceptual model of each linear mixed model is:

$$Y_{ij} = \beta_0 + \beta_1 X_{ij} + \beta_2 t_j + \beta_3 X_{ij} \times t_j + s_i + \varepsilon_{ij},$$

where Y is the dependent variable, X is the independent variable of interest (or a vector of covariates), s_i is a subject-specific random effect, and t represents the outcome assessment time points (baseline, follow-up). The β coefficients measure each independent variable's effect. If time is included as an ordinal variable,

significance between treatment and control groups will be tested using Wald tests for appropriate β coefficients. If time is included as an nominal variable, we will use least squared means to test for significant differences between treatment and control groups at each of the post-intervention time points (post-training, booster, 8-week follow-up). In this situation we will use a Bonferroni adjusted alpha level = 0.005 (0.05/10), which results in at least 78% power for all of the outcomes with 25 per group to account for attrition (data not shown).

We will use "intention to treat" (ITT) analyses on all subjects without regard to level of compliance. ITT analysis is based on the assumption that once a subject is randomized to a group, data for that subject will enter into the final analysis. ITT assumes unrelated treatment events are equally likely across groups and that outcome data are complete. All data from non-adherent and dropout subjects are included; therefore, ITT provides conservative effect estimates. ITT is most appropriate for randomized, controlled clinical trials involving lengthy treatment protocols when an effective treatment arrests progression of disease. Because recommendations for treatment of primary and secondary outcomes will likely continue to include care (i.e., controls still receive usual care), ITT analysis will better reflect what will be observed if HRV-B is introduced into clinical practice. This analysis answers the question: What is the effect of HRV-B on outcome measures in a situation where individuals range from fully compliant to non-compliant? In this pilot intervention, however, we also are interested in the actual effect of HRV-B on the outcomes. In ancillary analyses, we will perform multiple imputations for subjects with missing data (see below). These *post hoc* analyses will answer the question: What is the effect of full HRV-B training on the outcomes? This will provide a more accurate picture of HRV-B effects because it focuses only on subjects who completed the training.

Missing Data. Inevitably, missing data will arise and a variety of approaches will be considered to address this issue. First, our integrated team will meet and discuss the hypothesized nature of the missingness mechanism.[108] For example, if the subjects have data that are missing completely at random or missing at random (MAR) then multiple imputation (MI) procedures can be used (e.g., “PROC MI”).[109] When missingness is suspected to be non-ignorable, then a model for the missingness mechanism must be employed.[110,111] In all models, sensitivity analyses will be performed to test the robustness of the estimates to the missing data assumption (if any). We will create a number of locked datasets (e.g., 10) with imputed values that will be used in all analyses that require MI. This will ensure that all MI analyses are based on the same imputed data.

Path analysis is a useful statistical tool for modeling how multifaceted processes combine to influence specific outcomes. We will apply path analysis to evaluate the direct and indirect effects of HRV-B on the pain-related study outcomes (pain, stress, fatigue, insomnia, depression) via changes in HRV coherence (Figure 8). This analysis will estimate the direct effect of the intervention on the post-training minus pre-training measurements of the study outcomes. There are three paths that are depicted by the arrows in Figure 8. The path model will determine the path coefficients, variance explained, and statistical significance of direct and indirect (modifier) effects. Modifiers may include: race, income, lifestyle, duration or quality of hand held stress reliever use, social jetlag, family or work history, physical activity, major life events, coping strategies, or social support. Other paths that consider the influence of modifiers or confounders will be explored to determine if they improve model fit. PROC CALIS in SAS will be used; satisfactory model parameters will be identified using a Bentler comparative fit index >0.93. This integrated model with appropriate modifiers and mediators will identify variables that contribute to, or detract from, the treatment effect on HRV coherence and pain, stress, fatigue, depression and insomnia. The above SEM is a relatively simple structure with only 6 baseline parameters per outcome, thus with a sample of 60 (30 per

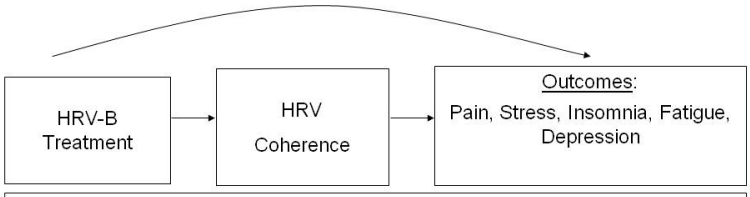


Figure 8.

Proposed Paths to Evaluate Direct and Indirect Effects of HRV-B Treatment on Primary and Exploratory Outcomes

group) the study will have the required minimum number of observations based on the 10:1 rule-of-thumb for sample size with SEMs.[112]

2.4. Data and Safety Monitoring Plan

The PIs and research staff will conduct ongoing monitoring the progress of this pilot intervention trial and the participant safety. This Data and Safety Monitoring Plan will be used to assess on-going participant safety. No invasive procedure will be used in the study. The participants will be fully informed of the procedures, including notification that their medical records from the DVAMC will be examined. Participants will voluntarily sign an Informed Consent and HIPAA Authorization approved by the DVAMC IRB before participation begins.

Data will be collected from the participants by face-to-face interview, having them complete objective psychometric instruments such as questionnaires and validated tests, and *via* the widely used procedure of HRV Biofeedback. In addition, data will be collected by record review of medical and service history obtainable from the Dorn VAMC Electronic Medical Record (CPRS), after authorization has been signed by the patient. Data collection will be specifically for the proposed research. Data collection time points for HRV and questionnaire will be at pre-training (T-1), post-training (T-2), 4-week post-training booster (T-3), and 8-week post-training follow-up (T-4). Baseline measurements will occur after the patient has been screened for eligibility, and consented. Randomization will occur after baseline assessment. All variables will be measured at each time point except demographics (T-1 only), and saliva (T-1, T-2, T-4). Measurements will be the same for both the active and sham intervention groups.

Potential Benefits. This study is designed to elicit relief from pain and related symptoms, although the study it is an experimental intervention and subjects may not obtain direct personal benefit. However, benefit to future patients is possible. Patients will receive an acknowledgement of their time and effort in participating in the study and they will receive financial compensation for each assessment and training session according to the schedule of incentives. The knowledge generated by this study will provide an understanding of the potential benefit of HRV-B on pain, stress, fatigue, sleep and quality of life for Veterans with chronic pain, and may inform clinical practice and programmatic standards concerning appropriate ways to assist with pain management, sleep disturbance and fatigue among DVAMC patients.

Potential Risks. No significant physical, social, or legal risk is anticipated with this study. All proposed procedures have been widely used over a long period of time by the investigators and/or others. HRV-B is a noninvasive procedure. There have been a few cases of relaxation-induced anxiety noted (i.e., with progressive muscle relaxation), but these effects are not associated with HRV-B or controlled breathing. Other negative reactions that have been associated with other modalities of biofeedback-assisted relaxation (such as sensation of heaviness, feelings of sadness, disturbing thoughts, or mind wandering) are rare, mild, and brief. All these reactions can be prevented by an experienced biofeedback professional with precautions, patient education, and careful monitoring.

Risks to confidentiality are minimal since all data will be coded with a study ID. Study ID and patient list will be kept separate, and the file linking study IDs with personal identifiers will be maintained in a locked file cabinet of password protected electronic file in one or both of the PI's offices. Data safety and monitoring is described below.

No adverse events were observed during other HRV-B studies. . Subjects will be informed that the experimental protocol will be provided in addition to their treatment as usual, will not affect their usual care, and no other intervention is available as an alternative intervention at the DVAMC. Minimal risks are anticipated for the subjects.

Protection Against Risks. Veterans seeking care at the DVAMC who meet the eligibility criteria will be recruited for participation according to applicable laws, regulations, guidelines, and policies protecting privacy and

confidentiality, and not until after a HIPAA Waiver of Authorization is approved by the IRB and R&D Committees. Following IRB and R&D Committee approvals, recruitment will be initiated among patients in the DVAMC Pain Clinic. If accrual milestones are not met, we will expand our search for eligible patients to include the DVAMC Rehabilitative Medicine and Rheumatology departments.

A waiver for release of protected health information will be requested to obtain the name, address, phone number, and screening information for patients with scheduled appointments in the Pain Clinic, or Rehabilitative Medicine or Rheumatology departments. The use and destruction of this data will follow the policies and procedures of VA privacy regulations. After receiving the authorization, the recruitment plan described in Section 2.1 will be used. Confidentiality will be maintained to the fullest extent allowed by law. Only authorized personnel will have access to research data. Participants will receive a unique study ID number that will be used to track all data. The cross-reference file linking subject identities to the study ID number will be maintained in a locked file cabinet or password-protected VA computer by the Co-PIs or a designated staff member. Psychophysiological data collected by sensors will be stored electronically without any identifying information. Analysis and interpretation of data files also will be conducted without the use of personal identifying information. Questionnaire data will not have identifying information on the forms. Once collated, coded data will be entered into password-protected VA computer for further analysis and processing.

If patients exhibit excessive anxiety, research staff will check with the patient to ascertain if data collection needs to be temporarily discontinued or rescheduled. Adverse physical or psychological events sometimes occur even if they are not be directly related to the study protocol. If a participant experiences a major or minor physical or psychological adverse event while enrolled in this study, supportive measures described in the section of the consent form labeled ‘possible risks’ in will be implemented. The Co-PIs will be contacted immediately. The PI will consult with clinical support staff to refer the patient for appropriate follow-up care, if needed..

Adverse Event Reporting. If patients develop a severe affective disorder or psychosis, these will be considered a serious adverse experience. The PIs will send an adverse events report to the VA IRB as soon as they are apprised that an adverse event has occurred. If the VA IRB or either of the PI’s recommend that the study be temporarily or permanently suspended, this action will be reported to the VA grant program officer or director.

Data and Safety Monitoring. The PIs and research staff will routinely monitor the progress of the trial and the safety of participants. The PIs, in collaboration with study staff, will check accrual and attrition on a weekly basis. Subject eligibility forms will be completed on all participants to assure appropriate subjects are recruited. None of the study procedures are considered invasive and the risks to subjects are expected to be minimal. Subjects will be informed that the experimental protocol will be provided in addition to their usual care, will not affect their treatment as usual, and no other intervention is available as an alternative intervention. HRV-B training is a simple, non-invasive procedure and no major adverse reactions to the protocol are anticipated. The proposed procedures have been widely used; no adverse events were experienced by Dr.Burch in previous studies using HRV-B or actigraphy. Questionnaire data will be collected from the participants by face-to-face interview using validated instruments and questions that are similar to those that might be encountered in a clinical setting. Participants will be informed that if they don’t want to answer a question, they can skip it. An actiwatch is similar in size, weight, and configuration as a wristwatch. Saliva collection also is simple and non-invasive. Saliva biospecimens will be labeled in a manner that ensures participant confidentiality without personal identifiers. The participants will be fully informed of the procedures, including notification that their medical records will be examined. Participants will voluntarily sign an Informed Consent document and HIPAA Authorization approved by the IRB before participation begins.

We are planning a follow-up HRV-B session plus questionnaire and cognitive evaluation at 4 weeks and 8 weeks post-training (Figure 7). Subjects will be told that they may contact either PI for any reason for 6 months after this final follow-up session. Subjects will be encouraged to make contact during this 6 month post-follow-up period if they have questions, or if any problem arises that the subject believes may be related to this research. If, during the course of the protocol, any adverse reaction develops or a subject wishes to terminate

his or her participation, the assigned treatment will be stopped. We will encourage any patients who have their assigned treatment stopped to maintain attendance at their training visits even without treatment, and attempt to collect the evaluation data on the appropriate schedule. Follow-up would be stopped if the subject advises us that he or she wishes to terminate all participation in the research. During the consent process, the subject will be told that cessation of follow-up can be voluntarily obtained by signing a form requesting that no further contact be made. They also will be informed that termination of their participation will not impact their usual care at the DVAMC.

The DVAMC uses a Research Compliance Officer (RCO) as the clinical trial monitor who performs audits of consent forms, reporting and regulatory compliance, and documentation according to Standard Operating Procedures of the VA Office of Research and Development, and the local DVAMC Research office. The monitor reports separately from the IRB structure. The monitor will also perform random audits on any trial including the proposed study.

Data Accuracy and Protocol Compliance. Subject eligibility forms will be completed on all subjects to assure appropriate subjects are recruited. Biofeedback Certification Institute of America (BCIA) certification of training and skill in HRV-B or its equivalent will be required of the HRV-B professional. This research will follow the HRV-B training protocol adopted by the BCIA. Protocol compliance integrity will be monitored by supervision and communication between the biofeedback professional, research staff, and the Co-PIs.

Literature Cited

1. Simon LS. Relieving pain in America: A blueprint for transforming prevention, care, education, and research. *Journal of Pain and Palliative Care Pharmacotherapy.* 2012;26(2):197-198.
2. Beckham JC, Crawford AL, Feldman ME, et al. Chronic posttraumatic stress disorder and chronic pain in Vietnam combat veterans. *Journal of Psychosomatic Research* 1997;43(4):379-389.
3. Gironda RJ, Clark ME, Massengate JP, Walker RL. Pain among veterans of Operations Enduring Freedom and Iraqi Freedom. *Pain Medicine.* 2006;7(4):339-343.
4. Kerns RD, Dobscha SK. Pain among veterans returning from deployment in Iraq and Afghanistan: Update on the Veterans Health Administration Pain Research Program. *Pain Medicine.* 2009;10(7):1161-1164.
5. Gatlin C, Schulmeister L. When Medicine is not enough: Nonpharmacological Management of Pain. *Clinical Journal of Oncology Nursing.* 2007;11(5):699-504.
6. Patrick DL, Ferketich SL, Frame PS, et al. National Institutes of Health State-of-the-Science Conference Statement: Symptom management in cancer: pain, depression, and fatigue, July 15-17, 2002. *Journal of the National Cancer Institute. Monographs.* 2004(32):9-16.
7. Gauthier LR, Rodin G, Zimmermann C, et al. Acceptance of pain: a study in patients with advanced cancer. *Pain.* May 2009;143(1-2):147-154.
8. Pain Management Task Force OoTASG. *Final Report*2010.
9. Blackburn-Munro G, Blackburn-Munro R. Chronic pain, chronic stress and depression: coincidence or consequence? *Journal of neuroendocrinology.* 2001;13(12):1009-1023.
10. Turnbull AV, Rivier CL. Regulation of the hypothalamic-pituitary-adrenal axis by cytokines: actions and mechanisms of action. *Physiological reviews.* 1999;79(1):1-71.
11. Pittenger C, Duman RS. Stress, depression, and neuroplasticity: a convergence of mechanisms. *Neuropsychopharmacology.* 2007;33(1):88-109.
12. Caraceni A, Portenoy RK. An international survey of cancer pain characteristics and syndromes. IASP Task Force on Cancer Pain. International Association for the Study of Pain. *Pain.* Sep 1999;82(3):263-274.
13. Trescot AM, Helm S, Hansen H, et al. Opioids in the management of chronic non-cancer pain: an update of American Society of the Interventional Pain Physicians'(ASIPP) Guidelines. *Pain physician.* 2008;11(2 Suppl):S5-S62.
14. Institute of Medicine . Committee on Advancing Pain Research C, Education. *Relieving pain in America: A blueprint for transforming prevention, care, education, and research*: National Academies Press; 2011.
15. Centers for Disease Control and Prevention. Vital signs: risk for overdose from methadone used for pain relief-United States, 1999-2010. *MMWR. Morbidity and mortality weekly report.* 2012;61(26):493.
16. Meerlo P, Sgoifo A, Suchecki D. Restricted and disrupted sleep: effects on autonomic function, neuroendocrine stress systems and stress responsivity. *Sleep medicine reviews.* Jun 2008;12(3):197-210.
17. Asmundson GJ, Norton PJ, Norton GR. Beyond pain: The role of fear and avoidance in chronicity. *Clinical Psychology Review.* 1999;19(1):97-119.
18. Deng G, Cassileth BR. Integrative oncology: complementary therapies for pain, anxiety, and mood disturbance. *CA: a cancer journal for clinicians.* Mar-Apr 2005;55(2):109-116.
19. *Pain Management Task Force Final Report*: Office of The Army Surgeon General;2010.
20. Fadul N, Strasser F, Palmer JL, et al. The Association Between Autonomic Dysfunction and Survival in Male Patients with Advanced Cancer: A Preliminary Report. *J Pain Symptom Manag.* Feb 2010;39(2):283-290.
21. Kim do H, Kim JA, Choi YS, Kim SH, Lee JY, Kim YE. Heart rate variability and length of survival in hospice cancer patients. *J Korean Med Sci.* Aug 2010;25(8):1140-1145.

22. Walsh D, Nelson KA. Autonomic nervous system dysfunction in advanced cancer. *Supportive care in cancer : official journal of the Multinational Association of Supportive Care in Cancer.* Oct 2002;10(7):523-528.

23. Chiang JK, Koo M, Kuo TBJ, Fu CH. Association Between Cardiovascular Autonomic Functions and Time to Death in Patients With Terminal Hepatocellular Carcinoma. *J Pain Symptom Manag.* Apr 2010;39(4):673-679.

24. Hoffmann J, Grimm W, Menz V, et al. Prognostic value of heart rate variability analysis in patients with carcinoid syndrome. *Digestion.* 2001;63(1):35-42.

25. de Miguel Sanchez C, Elustondo SG, Estirado A, et al. Palliative performance status, heart rate and respiratory rate as predictive factors of survival time in terminally ill cancer patients. *Journal of Pain and Symptom Management.* Jun 2006;31(6):485-492.

26. Mouton C, Ronson A, Razavi D, et al. The relationship between heart rate variability and time-course of carcinoembryonic antigen in colorectal cancer. *Autonomic neuroscience : basic & clinical.* Jan 26 2012;166(1-2):96-99.

27. McCraty R, Childre D. Coherence: bridging personal, social, and global health. *Altern Ther Health Med.* Jul-Aug 2010;16(4):10-24.

28. Appelhans BM, Luecken LJ. Heart rate variability and pain: associations of two interrelated homeostatic processes. *Biological psychology.* Feb 2008;77(2):174-182.

29. Mostoufi SM, Afari N, Ahumada SM, Reis V, Wetherell JL. Health and distress predictors of heart rate variability in fibromyalgia and other forms of chronic pain. *Journal of psychosomatic research.* Jan 2012;72(1):39-44.

30. Logier R, Jeanne M, Tavernier B, De Jonckheere J. Pain/analgesia evaluation using heart rate variability analysis. *Conference proceedings : ... Annual International Conference of the IEEE Engineering in Medicine and Biology Society. IEEE Engineering in Medicine and Biology Society. Conference.* 2006;1:4303-4306.

31. Paine P, Kishor J, Worthen SF, Gregory LJ, Aziz Q. Exploring relationships for visceral and somatic pain with autonomic control and personality. *Pain.* Aug 2009;144(3):236-244.

32. Zhong X, Hilton HJ, Gates GJ, et al. Increased sympathetic and decreased parasympathetic cardiovascular modulation in normal humans with acute sleep deprivation. *Journal of applied physiology.* Jun 2005;98(6):2024-2032.

33. Dettoni JL, Consolim-Colombo FM, Drager LF, et al. Cardiovascular effects of partial sleep deprivation in healthy volunteers. *J Appl Physiol.* Jul 2012;113(2):232-236.

34. Carney RM, Freedland KE. Depression and heart rate variability in patients with coronary heart disease. *Cleve Clin J Med.* Apr 2009;76 Suppl 2:S13-17.

35. Kemp AH, Quintana DS, Gray MA, Felmingham KL, Brown K, Gatt JM. Impact of depression and antidepressant treatment on heart rate variability: a review and meta-analysis. *Biological Psychiatry.* Jun 1 2010;67(11):1067-1074.

36. Nagpal M, Gleichauf K, Ginsberg JP. Meta-analysis of Heart Rate Variability as a psychophysiological indicator of Posttraumatic Stress Disorder. *Journal of Trauma and Treatment.* in press.

37. Hassett AL, Radvanski DC, Vaschillo EG, et al. A pilot study of the efficacy of heart rate variability (HRV) biofeedback in patients with fibromyalgia. *Applied psychophysiology and biofeedback.* Mar 2007;32(1):1-10.

38. Kapitza KP, Passie T, Bernateck M, Karst M. First non-contingent respiratory biofeedback placebo versus contingent biofeedback in patients with chronic low back pain: a randomized, controlled, double-blind trial. *Applied psychophysiology and biofeedback.* Sep 2010;35(3):207-217.

39. Henriques G, Keffer S, Abrahamson C, Horst SJ. Exploring the effectiveness of a computer-based heart rate variability biofeedback program in reducing anxiety in college students. *Appl Psychophys Biof.* Jun 2011;36(2):101-112.

40. Reiner R. Integrating a portable biofeedback device into clinical practice for patients with anxiety disorders: results of a pilot study. *Appl Psychophys Biof.* Mar 2008;33(1):55-61.

41. Hallman DM, Olsson EM, von Scheele B, Melin L, Lyskov E. Effects of heart rate variability biofeedback in subjects with stress-related chronic neck pain: a pilot study. *Applied psychophysiology and biofeedback.* Jun 2011;36(2):71-80.

42. Siepmann M, Aykac V, Unterdorfer J, Petrowski K, Mueck-Weymann M. A pilot study on the effects of heart rate variability biofeedback in patients with depression and in healthy subjects. *Applied Psychophysiology and Biofeedback.* Dec 2008;33(4):195-201.

43. Zucker TL, Samuelson KW, Muench F, Greenberg MA, Gevirtz RN. The effects of respiratory sinus arrhythmia biofeedback on heart rate variability and posttraumatic stress disorder symptoms: a pilot study. *Appl Psychophys Biof.* Jun 2009;34(2):135-143.

44. Ginsberg JP, Berry ME, Powell DA. Cardiac coherence and posttraumatic stress disorder in combat veterans. *Alternative therapies in health and medicine.* Jul-Aug 2010;16(4):52-60.

45. Nolan RP, Kamath MV, Floras JS, et al. Heart rate variability biofeedback as a behavioral neurocardiac intervention to enhance vagal heart rate control. *American heart journal.* Jun 2005;149(6):1137.

46. McLay RN, Spira JL. Use of a portable biofeedback device to improve insomnia in a combat zone, a case report. *Appl Psychophys Biof.* Dec 2009;34(4):319-321.

47. Lehrer PM, Vaschillo E, Vaschillo B. Resonant frequency biofeedback training to increase cardiac variability: rationale and manual for training. *Appl Psychophys Biof.* Sep 2000;25(3):177-191.

48. Camm AJ, Malik M, Bigger JT, et al. Heart rate variability - Standards of measurement, physiological interpretation, and clinical use. *Circulation.* Mar 1 1996;93(5):1043-1065.

49. Chalaye P, Goffaux P, Lafrenaye S, Marchand S. Respiratory effects on experimental heat pain and cardiac activity. *Pain Med.* Nov 2009;10(8):1334-1340.

50. Berry ME, Chapple IT, Ginsberg JP, Gleichauf K, Meyer JA, Nagpal ML. Non-Pharmacological Intervention for Chronic Pain in Veterans: A Pilot Study of Heart Rate Variability Biofeedback. *Global Advances in Health and Medicine.* 2014;3(2):28-33.

51. De Couck M, Mravec B, Gidron Y. You may need the vagus nerve to understand pathophysiology and to treat diseases. *Clinical science.* Apr 2012;122(7):323-328.

52. Berntson GG, Lozano DL, Chen YJ. Filter properties of root mean square successive difference (RMSSD) for heart rate. *Psychophysiology.* 2005;42(2):246-252.

53. Wang H-M, Huang S-C. SDNN/RMSSD as a surrogate for LF/HF: a revised investigation. *Modelling and Simulation in Engineering.* 2012;2012:16.

54. Singer JD, Willet JB. *Applied Longitudinal data analysis: Modeling change and event occurence*: Oxford University Press; 2003.

55. Monson CM, Gradus JL, Young-Xu Y, Schnurr PP, Price JL, Schumm JA. Change in posttraumatic stress disorder symptoms: Do clinicians and patients agree? *Psychological Assessment.* 2008;20(2):131-138.

56. National Center for PTSD. Instruction manual for CAPS. https://www.istss.org/AM/Template.cfm?Section=ResourcesforClinicians&Template=/CM/ContentDisplay.cfm&ContentID=1551.

57. Brouwer D, Meijer RR, Zevalkink J. Measuring individual significant change on the Beck Depression Inventory-II through IRT-based statistics. *Psychotherapy Research.* 2013;23(5):489-501.

58. Burch JB, Yost MG, Johnson W, Allen E. Melatonin, sleep, and shift work adaptation. Sep 2005;47(9):893-901.

59. Ray M, Youngstedt SD, Zhang H, et al. Examination of wrist and hip actigraphy using a novel sleep estimation procedure. *Sleep Science.* in press.

60. Kline CE, Crowley EP, Ewing GB, et al. The effect of exercise training on obstructive sleep apnea and sleep quality: a randomized controlled trial. *Sleep.* Dec 2011;34(12):1631-1640.

61. Ray M, Youngstedt SD, Zhang H, et al. Examination of wrist and hip actigraphy using a novel sleep estimation procedure. *Sleep Science.* 2014;In Press.

62. Innominato PF, Focan C, Gorlia T, et al. Circadian rhythm in rest and activity: a biological correlate of quality of life and a predictor of survival in patients with metastatic colorectal cancer. *Cancer research.* Jun 1 2009;69(11):4700-4707.

63. Mormont MC, Waterhouse J, Bleuzen P, et al. Marked 24-h rest/activity rhythms are associated with better quality of life, better response, and longer survival in patients with metastatic colorectal cancer and good performance status. *Clinical cancer research : an official journal of the American Association for Cancer Research.* Aug 2000;6(8):3038-3045.
64. Cleeland CS, Ryan KM. Pain assessment: global use of the Brief Pain Inventory. *Ann Acad Med Singapore.* Mar 1994;23(2):129-138.
65. Keller S, Bann CM, Dodd SL, Schein J, Mendoza TR, Cleeland CS. Validity of the brief pain inventory for use in documenting the outcomes of patients with noncancer pain. *The Clinical journal of pain.* Sep-Oct 2004;20(5):309-318.
66. Hewitt PL, Flett GL, Mosher SW. The Perceived Stress Scale: Factor structure and relation to depression symptoms in a psychiatric sample. *Journal of Psychopathology and Behavioral Assessment.* 1992;14(3):247-257.
67. Ancoli-Israel S, Cole R, Alessi C, Chambers M, Moorcraft W, Pollak CP. The role of actigraphy in the study of sleep and circadian rhythms. *Sleep.* 2003;26:342-392.
68. de Souza L, Benedito AA, Nogueira ML, Poyares D, Tufik S, Calil HM. Further validation of actigraphy for sleep studies. *Sleep.* 2003;26:81-85.
69. Natale V, Plazzi G, Martoni M. Actigraphy in the assessment of insomnia: a quantitative approach. *Sleep.* Jun 2009;32(6):767-771.
70. Caraceni A. Evaluation and assessment of cancer pain and cancer pain treatment. *Acta Anaesthesiol Scand.* Oct 2001;45(9):1067-1075.
71. Sullivan MJL, Bishop S, Pivik J. The Pain Catastrophizing Scale: development and validation. *Psychol Assessment.* 1995;7:432-524.
72. Cohen S, Kamarck T, Mermelstein R. A global measure of perceived stress. *Journal of health and social behavior.* Dec 1983;24(4):385-396.
73. Cohen S, Williamson G. Perceived stress in a probability sample of the United States. In: Spacapan SO, S., ed. *The social psychology of health*. Newbury Park, CA: Sage; 1988:31-68.
74. Van Someren EJW, Hagebeuk E, Lijzenga C, et al. Circadian rest-activity rhythm disturbances in alzheimer's disease. *Biological Psychiatry.* 1996;40 259-270.
75. Van Someren EJW, Kessler A, Mirmiran M, Swaab DF. Indirect bright light improves circadian rest-activity rhythm disturbances in demented patients. *Biological Psychiatry.* 1997;41:955-963.
76. Rich T, Innominato PF, Boerner J, et al. Elevated serum cytokines correlated with altered behavior, serum cortisol rhythm, and dampened 24-hour rest-activity patterns in patients with metastatic colorectal cancer. *Clin Cancer Res.* 2005;11(5):1757-1764.
77. Guess J, Burch JB, Ogoussan K, et al. Circadian disruption, Per3, and human cytokine secretion. *Integrative Cancer Therapies.* 2009;8(4):329-336.
78. Kushida CA, Chang A, Gadkary C, Guilleminault C, Carrillo O, Dement WC. Comparison of actigraphic, polysomnographic, and subjective assessment of sleep parameters in sleep-disordered patients. *Sleep Medicine.* 2001;2:389-396.
79. Jean-Louis G, Kripke DF, Mason WJ, Elliott JA, Youngstedt SD. Sleep estimation from wrist movement quantified by different actigraphic modalities. *J Neurosci Methods.* 2001;105:185-191.
80. Buysse DJ, Reynolds CF, 3rd, Monk TH, Berman SR, Kupfer DJ. The Pittsburgh Sleep Quality Index: a new instrument for psychiatric practice and research. *Psychiatry Res.* May 1989;28(2):193-213.
81. Van Someren EJ, Swaab DF, Colenda CC, Cohen W, McCall WV, Rosenquist PB. Bright light therapy: improved sensitivity to its effects on rest-activity rhythms in Alzheimer patients by application of nonparametric methods. *Chronobiol Int.* Jul 1999;16(4):505-518.
82. Smets EM, Grassen B, Bonke B, De Haes J. The Multidimensional Fatigue Inventory (MFI) psychometric qualities of an instrument to assess fatigue. *J Psychosom Res.* 1995;39:315-325.
83. Smets EM, Garssen B, Cull A, de Haes JC. Application of the multidimensional fatigue inventory (MFI-20) in cancer patients receiving radiotherapy. *Br J Cancer.* 1996;73:241-245.
84. Beck AT, Steer RA, Brown GG. *Manual for Beck Depression Inventory-II*. San Antonio, TX: Psychological Corporation; 1996.

85. Vandeputte M, Weerd A. Sleep disorders and depressive feelings; a global survey with the Beck depression scale. *Sleep Med Rev.* 2003;4:343-345.
86. Warmenhoven F, van Rijswijk E, Engels Y, et al. The Beck Depression Inventory (BDI-II) and a single screening question as screening tools for depressive disorder in Dutch advanced cancer patients. *Support Care Cancer.* Feb 2012;20(2):319-324.
87. Sharp LK, Lipsky MS. Screening for Depression across the lifespan: A review of measures for use in primary care settings. *Am. Fam. Physician.* 2002;66(6):1001-1008.
88. Ginsberg JP, Ayers E, Burriss L, Powell DA. Disruption of bradycardia associated with discriminative conditioning in combat veterans with PTSD. *Neuropsychiatric disease and treatment.* Jun 2008;4(3):635-646.
89. Roenneberg T, Kuehnle T, Juda M, et al. Epidemiology of the human circadian clock. *Sleep medicine reviews.* Dec 2007;11(6):429-438.
90. Chelminski I, Petros TV, Plaud JJ, Ferraro FR. Psychometric properties of the reduced Horne and Ostberg questionnaire. *Pers Indiv Differ.* Sep 2000;29(3):469-478.
91. Duffy JF, Rimmer DW, Czeisler CA. Association of intrinsic circadian period with morningness-eveningness, usual wake time, and circadian phase. *Behav Neurosci.* Aug 2001;115(4):895-899.
92. Duffy JF, Dijk DJ, Hall EF, Czeisler CA. Relationship of endogenous circadian melatonin and temperature rhythms to self-reported preference for morning or evening activity in young and older people. *J Investig Med.* Mar 1999;47(3):141-150.
93. Roeser K, Obergfell F, Meule A, Vogele C, Schlarb AA, Kubler A. Of larks and hearts - morningness/eveningness, heart rate variability and cardiovascular stress response at different times of day. *Physiol Behav.* Feb 5 2012;106(2):151-157.
94. Nebel LE, Howell RH, Krantz DS, Falconer JJ, Gottdiener JS, Gabbay FH. The circadian variation of cardiovascular stress levels and reactivity: relationship to individual differences in morningness/eveningness. *Psychophysiol.* 1996;33:273-281.
95. Costa G, Lievore F, Ferrari P, Gaffuri E. Usual meal times in relation to age, sex, work activity and morningness-eveningness. *Chronobiologia.* 1987;14(4):383-391.
96. Mongrain V, Dumont M. Increased homeostatic response to behavioral sleep fragmentation in morning types compared to evening types. *Sleep Med Rev.* 2007;30(6):773-780.
97. Taillard J, Philip P, Coste O, Sagaspe P, Bioulac B. The circadian and homeostatic modulation of sleep pressure during wakefulness differs between morning and evening chronotypes. *Journal of sleep research.* Dec 2003;12(4):275-282.
98. Adan A, Guardia J, (), pp. . Circadian variations of self-reported activation: a multidimensional approach. *Chronobiologia.* 1993;20:233-244.
99. Hasler BP, Allen JJ, Sbarra DA, Bootzin RR, Bernert RA. Morningness-eveningness and depression: preliminary evidence for the role of the behavioral activation system and positive affect. *Psychiatry Res.* Apr 30 2010;176(2-3):166-173.
100. Akerstedt T, Fröberg JE. Interindividual differences in circadian patterns of catecholamine excretion, body temperature, performance, and subjective arousal. *Biol Psychol.* 1976;4:277-292.
101. Kudielka BM, Federenko IS, Hellhammer DH, Wüst S. Morningness and eveningness: the free cortisol rise after awakening in "early birds" and "night owls". *Biol Psychol.* 2006;72(2):141-146.
102. Roenneberg T, Wirz-Justice A, Merrow M. Life between clocks: daily temporal patterns of human chronotypes. *Journal of biological rhythms.* Feb 2003;18(1):80-90.
103. Roenneberg T, Allebrandt KV, Merrow M, Vetter C. Social jetlag and obesity. *Current biology : CB.* May 22 2012;22(10):939-943.
104. Wittmann M, Dinich J, Merrow M, Roenneberg T. Social jetlag: misalignment of biological and social time. *Chronobiology International.* 2006;23(1-2):497-509.
105. Zimet GD, Dahlem NW, Zimet SG, Farley GK. The Multidimensional Scale of Perceived Social Support. Journal of Personality Assessment. *Journal of Personality Assessment;.* Spring88 1988;52(1): 30-41.
106. Carver CS. You want to measure coping but your protocol's too long: consider the brief COPE. *Int J Behav Med.* 1997;4(1):92-100.

107. Hofman LF. Human saliva as a diagnostic specimen. *The Journal of nutrition.* May 2001;131(5):1621S-1625S.
108. Little RJA, Rubin DB. *Statistical Analysis with Missing Data (2nd ed.)*. New York, NY: John Wiley; 2002.
109. Rubin DB. Inference and missing data. *Biometrika.* 1976;63:581-592.
110. Albert PS, Follmann DA. Modelling repeated count data subject to informative dropout. *Biometrics.* 2000;56:667-677.
111. Diggle PJ, Kenward MG. Informative dropout in longitudinal data analysis (with discussion). *Applied Statistics.* 1994;43:49-94.
112. Kline RB. *Principles and Practice of Structural Equation modeling (3rd ed).* New York, NY: The Guilford Press; 2010.