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Is Heart Rate Variability Related to Memory Performance in Middle Aged Men?

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Is Heart Rate Variability Related to Memory Performance in Middle Aged Men?

By

Amit Shah

BA, Princeton University, 2002

MD, University of Pennsylvania School of Medicine, 2006

Thesis

The Master of Science in Clinical Research Program

Advisor: Viola Vaccarino, MD, PhD

An Abstract of

A thesis submitted to the Faculty of the James T. Lane School of Graduate Studies Emory University in partial fulfillment of the requirements for the degree of Master of Science in Clinical Research

2011

ABSTRACT

Is Heart Rate Variability Related to Memory Performance in Middle Aged Men?
By Amit Shah

OBJECTIVE: Heart rate variability (HRV), a measure of autonomic function, has been associated with cognitive function, but studies are conflicting. Previous studies have also not controlled for familial and genetic influences.

METHODS: We performed power spectral analysis on 24-hour ambulatory ECG's in 416 middle-aged male twins from the Vietnam Era Twin Registry. Memory and learning were measured by verbal and visual selective reminding tests (SRT). Mixed-effect regression models were used to calculate associations between and within twin pairs, while adjusting for covariates.

RESULTS: The mean age ± SD was 55 ± 2.9 years. A statistically significant positive association was found between measures of HRV and verbal, but not visual, SRT scores. The most statistically significant unadjusted association was found between very low frequency (VLF) HRV and verbal total recall SRT, such that each natural logarithm of increase in VLF was associated with an increased verbal SRT score of 4.85 points ($p=0.002$). The association persisted despite adjustment for demographic and cardiovascular risk factors, and after accounting for familial, and genetic factors by comparing twins within pairs. A significant interaction was found between post-traumatic stress disorder (PTSD) and HRV, such that total power and ultra low frequency were associated with SRT in twins ($n=362$) without PTSD, but not in those with PTSD.

CONCLUSION: In conclusion, lower frequency spectra of HRV are associated with verbal, but not visual, learning and memory, particularly in subjects without PTSD. This association may highlight mechanisms of cognitive dysfunction and have implications on prevention and therapy of cognitive decline.

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INTRODUCTION

The incidence and prevalence of Alzheimer's dementia (AD) are increasing at alarming rates as the baby boomer population ages; by 2050, the number of people with dementia in the United States is expected to almost triple (1). Given the lack of effective treatment strategies, further work is needed to establish mechanisms of disease that may help in diagnostic, prevention, and treatment strategies. Traditional cardiovascular risk factors such as diabetes and hypertension have been established through epidemiologic studies as risk factors for AD, which is known to be comorbid with small vessel vascular disease (2, 3). Autonomic dysfunction is a recently emerging cardiovascular risk factor that may also associate with cognitive function, and may indicate poor neurological health in addition to high cardiovascular risk (4, 5). Further understanding of the connection of the autonomic nervous system with cognitive function may support the use of novel pharmacologic and behavioral interventions in risk reduction of dementia, as well as help to identify those at higher physiologic risk of developing cognitive decline in the future (6).

Current studies of autonomic dysfunction and cognitive function are conflicting (7). Multiple factors may influence both, including exercise, smoking, genetics, depression, familial, and sociodemographic factors (1, 8). Therefore, studies in which such confounders may be controlled are necessary to further explore this possible association. If no confounding is seen despite controlling for environmental, genetic, and familial risk factors, then this would support the direct role of autonomic dysfunction in the pathophysiology of cognitive impairment. If confounding is found, then HRV may only be an index of cognitive performance, but not have a direct physiologic impact on cognitive performance.

In order to investigate the association of autonomic dysfunction with cognitive function while controlling for multiple possible confounders, we conducted a cross-sectional study involving 524 middle-aged veteran male twins who were recruited from the Vietnam Era Twin Registry as part of the Emory Twin Studies, which is a composite of two companions studies, the Twins Heart Study (THS), and the Stress and Vascular Evaluation in Twins (SAVEIT). The purpose of these studies was to elucidate the role of psychological, behavioral, and biologic risk factors for subclinical cardiovascular disease in twins. As part of this evaluation, autonomic dysfunction was measured by heart rate variability (HRV), which is the beat to beat change in heart rate over time (9). Cognitive testing, via the Selective Reminding Test, was also done to measure verbal and visual learning and memory (10). By using twins, we were able to control for familial and genetic confounding by comparing each twin with his brother (within-pair analysis). If dizygotic (DZ) twins, who are 50% genetically similar, show more of an association than monozygotic (MZ) twins (100% genetically identical), then this may imply genetic confounding. Also, if associations are seen in individual twins, but not seen within MZ nor DZ pairs, then this implies familial confounding.

We hypothesized that in this population of middle-aged men, autonomic function and cognitive function are associated despite controlling for cardiovascular, genetic, and familial confounding, implying that autonomic dysfunction is a direct predisposing factor for cognitive decline.

BACKGROUND

The sympathetic and parasympathetic nerves are two opposing forces that primarily regulate the cardiovascular and respiratory systems, but also are involved in multiple other organ systems. The behavior of these systems, which together comprise the autonomic nervous system, help the body to adapt to internal and external changes, and maintain perfusion to critical organs. The sympathetic nervous system is involved in the stress response, and can cause an increase in the heart rate, increased strength of cardiac contraction, and constriction of the peripheral blood vessels, amongst other physiologic changes in multiple organ systems. From an evolutionary perspective, the sympathetic nervous system is an adaptive response to an acute stressor that enables the person to run from danger, labeled as the “fight or flight response.” The parasympathetic nervous system, which antagonizes the sympathetic nervous system, generally exerts the opposite effect, and is increased in states of relaxation (11).

Neurovisceral integration theory, which was first proposed by Thayer and Lane, describes a neuronal network that integrates the autonomic, attentional, and affective systems (8). They describe multiple areas in the brain that influence the activity of the autonomic nervous system, and area also involved with emotion and attention. One important aspect of this theory is the central autonomic network (CAN), which is a functional unit of the brain that supports goal-directed behavior and adaptability. This includes the anterior cingulate, insular, orbitofrontal, and ventromedial prefrontal cortices. These structures may both inhibit impulses as well as aspects of the nervous system that are responsible for excitation, such as the amygdala, and eventually (through several downstream connections) cause sympathetic augmentation and

parasympathetic suppression. Figure 1 outlines the general structures involved in this network.

The rostral limbic system (RLS) is another neuronal network that shares many structures with the CAN, and is associated with cognitive functions. The cingulate cortex, in addition to autonomic regulation, also affects executive function (12). Additionally, the caudal aspect of the anterior cingulated cortex regulates memory functions. Multiple additional neural circuits overlap between eh RLS and the CAN as well, including the prefrontal cortex, which is known to be dysfunctional in attention deficit syndromes, as well as affective disorders such as bipolar (13, 14). Therefore, common neural structures in both RLS and CAN may explain the association found between autonomic function and executive cognitive function.

In addition to neurovisceral integration theory, however, an additional hypothesis exists behind the potential association between cognitive and autonomic function. While common neural structures may control both systems, it is also possible that the poor performance of the autonomic nervous system may directly reduce performance and functionality of the cognitive structures in the brain as well. One of the main reflexes that help to regulate blood flow in the body through the autonomic nervous system is the baroreceptor reflex (15). This reflex is important in regulation of blood flow to the brain during activities such as standing, and decreased functionality of this reflex may be a risk factor for transient cerebral ischemia. If repeated ischemic shocks occur frequently, this may eventually lead to cognitive dysfunction. Although such mechanisms have been proposed, no direct evidence has been found (16).

Thus far, evidence in favor of either of these theories has been conflicting. Kim *et al.* found that in 311 physically disabled, community-dwelling women over 65 years of

age, those in the lowest quartile of HRV had a higher prevalence of cognitive impairment (mini-mental state exam score < 24) than those in the other quartiles (4). Additionally, Hansen *et al.* found that in 65 participants from the Royal Norwegian Navy, high HRV associated with superior performance on a continuous performance task and a working memory task (17). Zulli *et al.* also found lower HRV in 33 subjects with AD than 29 controls (18). On the other hand, multiple negative studies also exist. Allan *et al.* did not find any associations in HRV between 14 subjects with AD and 80 controls (19). Briton *et al.* measured cognitive function in two different trials in 5,375 people of mean age 55 (trial 1) and 61 (trial 2); while some associations were found, they were not consistent across trials and cognitive tests (7). Overall, despite several proposed mechanisms with substantial evidence, the association of autonomic and cognitive function is still debated.

One of the main limitations of previous studies relates to the type of data obtained from their measures of autonomic function. Most of the studies measure HRV from short-range measures, which are usually obtained while the subject is supine, and measured over the course of 5 minutes (9). These data allow measurement of heart rate changes and autonomic activity in response to breathing, but otherwise do not provide much physiologic data. On the other hand, HRV data obtained from ambulatory 24 hours of recording, as done in the study by Zulli *et al*, include long-range measures that indicate autonomic responses to position, physical activity, sleeping, and slower neuro-hormonal changes, such as fluctuations in cortisol and renin-angiotensin levels (20). Such data are also highly prognostic for future events in patients with coronary disease, and because they are more descriptive, may be more sensitive to detecting cognitive dysfunction. Because the small study by Zulli *et al.* is the only one thus far to use long-range data for this analysis, further such studies are needed.

An additional limitation to previous studies is that they do not take into account genetic and familial influences. Multiple studies have shown that HRV is a highly hereditary phenotype, with approximately 40% of the variation thought to be from genetic contributions (21). Similar estimates were found for cognitive abilities as well (22). Because a significant proportion of both phenotypes are genetically determined, it is possible that some of the causative genotypes may be shared. Examples may include genes related to acetylcholine, for example, which functions in both memory formation (23) and the autonomic nervous system (24). Another example is catechol O-methyltransferase (COMT), a dopamine gene which has been found to influence cognitive function (25), may also influence autonomic function (26).

Early environmental and gestational influences may also play a critical role in both autonomic and cognitive function. Socioeconomic status, race, family income, and home environment, can have profound influences on both HRV and cognitive performance (27, 28). Maternal nutrition during fetal development, which affects fetal birth weight and fetal head circumference, is related to offspring's risk for cardiovascular disease as well as cognitive performance (29-31). Maternal behaviors and other familial factors have also been found to affect the on/off switching of offspring genes during fetal development, affecting MZ and DZ twins equally (32). One example of this is the epigenetic gene regulation of melatonin production in the rostral ventrolateral medulla, which is a part of the central autonomic network (8, 33). Serum concentration of melatonin is associated with autonomic function, heart disease risk and dementia risk (34-38). Additionally, melatonin production has a similar concordance in both MZ and DZ twin pairs, which further supports the influence of familial epigenetic mechanisms (39). A summary of the possible causal pathways involving familial, genetic, and environmental factors is highlighted in figure 2.

In summary, previous studies have supported a possible mechanisms relating autonomic and cognitive function. However, epidemiologic studies are limited and conflicting. In addition, there are also possibly common genetic and familial influences on both autonomic and cognitive function. Further study is therefore needed to investigate these and other possible confounders, which can help explore understand this hypothesized relationship and its utility in treatment/prevention of dementia.

METHODS

Null Hypothesis

No association (independent of genetic, familial, and environmental confounders) exists between spectral measures of HRV and measures of cognitive function, as measured by the verbal and visual selective reminding tests, in our sample of middle aged veteran twins.

Characteristics of Study Design

Cross-sectional study. Subjects were not characterized as cases and controls because the "exposure," HRV, and the "outcome," cognitive function, were both measured as continuous variables.

Characteristics of Study Population, Inclusion/Exclusion Criteria

The Emory Twin Studies (ETS) includes samples recruited in two companion studies: the Twins Heart Study (THS) and the Stress and Vascular Evaluation in Twins (SAVEIT). The purpose of these studies was to elucidate the role of psychological, behavioral and biological risk factors for subclinical cardiovascular disease in twins. Both projects recruited middle-aged, male monozygotic and dizygotic twin pairs (who were raised in the same household) from the Vietnam Era Twin (VET) Registry, one of the largest twin registries in the United States (40). Both studies followed identical procedures, measurements and protocols. THS enrolled 180 twin pairs between 2002 and 2006 (41) and SAVEIT included 82 twin pairs enrolled between 2005 and 2008.

Twins included in ETS, most of whom were Caucasian, were randomly selected from the VET Registry among those born between 1946 and 1956. In addition, a random sample of twin pairs discordant for major depression was included in THS and a random sample of twin pairs discordant for posttraumatic stress disorder (PTSD) was included in SAVEIT. Pairs of twins were examined at the same time at the Emory University General Clinical Research Center, and all data collection, including ambulatory electrocardiogram (ECG) monitoring, occurred during a 24-hour admission under controlled conditions. Both studies were approved by the Emory Institutional Review Board, and all twins signed an informed consent.

The two twins maintained an identical schedule while in the study. Activity was limited to leisurely ambulation within the Emory facilities, and all assessment, including the ambulatory ECG monitoring, began and ended at the same time. Zygosity information by means of DNA typing was available for all but 31 twin pairs. The zygosity of these remaining 31 pairs was based on questionnaires supplemented by blood group typing abstracted from military records, a method that in our sample had an accuracy of 94% (42).

Outcome Variable

The outcome was cognitive function, which was measured by the total recall scores of the verbal and visual selective reminding tests. These tests measure both short-term memory as well as long-term learning. Of the two tests, the verbal test was the more important test and primary outcome, given it has been better validated as a measure of future cognitive decline and executive function (43). Additionally, while the verbal test is

thought to measure performance of the dominant lobe, the visual test is more thought to measure performance of the non-dominant lobe (44).

Predictor Variables

Primary exposure of interest and measurement of confounding/interaction: The primary predictor of interest was HRV, a measure of autonomic function, which was composed of five correlated measures including total power (TP), ultra low frequency (ULF), very low frequency (VLF), low frequency (LF), and high frequency (HF). Each frequency measures a slightly different aspect of autonomic function (45). HF is known to reflect parasympathetic influence, whereas LF is thought to indicate more of both sympathetic and parasympathetic acitivity. VLF is known to be influences by renin-angiotensin system acitivty as well. ULF is highly prognostic, but its significance is less well-known (46). Total power is the sum of all individual frequency measures, and although nonspecific, may be regarded as the primary predictor. All of these measures were included, however, because they may give a more descriptive measure of the physiology underlying any associations found.

For each HRV variable, a core model was first built based on basic sociodemographic factors, including age and education, and traditional cardiovascular risk factors, including hypertension, LDL cholesterol, current/past smoking, and diabetes. These were chosen based on previous research that identified these variables as having a high probability for causing confounding (1, 47). Additional cardiovascular, sociodemographic, and psychological factors were deemed a-priori to be possible confounders, and where therefore added individually in a serial manner to the model to

test for further confounding, which we defined as >10% change in the coefficient of the main predictor (HRV variables) (48). These variables included beck depression score, lifetime/current depression, lifetime/current PTSD, Armed Forces Quotient Test score percentile (1990 baseline intelligence test), employment, body mass index, waist-hip ratio, fasting glucose, hemoglobin A1c, history of drug abuse, history of heart disease, medication use (antidepressants, beta-blockers, statins, aspirin, vitamin D, omega 3 consumption, alcohol intake, and caffeine intake. Only beck depression score was found to be a significant confounder. Given the known neurologic mechanisms linking affective disorders and cognitive function, we also tested for interaction with depression and PTSD, and in cases in which interaction was found, we conducted stratified analysis (49).

Measurement of Variables

Measurement of Heart Rate Variability. Twins wore an ambulatory ECG (Holter) monitor (GE Marquette SEER digital system) for 24 hours. Both twins in a pair were studied at the same time and their recording times, schedule, and activity level during the recording were matched. Activity was restricted to quiet walking around the campus, and participants were instructed to refrain from smoking and drinking alcohol or coffee during the recording. HRV data were analyzed following published methodology (50, 51). Each tape was manually processed, edited, and analyzed with customized software that used methods described in the literature (28, 52). The heart rate spectrum was computed using a fast Fourier transform (FFT) with a Parzen window. Because long-term autonomic function was the goal of this study, the FFT was performed on the 24-hour R-R interval file. The power spectrum was integrated over four discrete frequency bands

(51): ultra low frequency (ULF) <0.0033 Hz; very low frequency (VLF) 0.0033 to <0.04 Hz; low frequency (LF) 0.04 to <0.15 Hz; and high frequency (HF) 0.15 to <0.40 Hz. Total power (TP), incorporating the full spectrum <0.40 Hz, was also measured. Twins whose recordings showed >20% interpolation or <18 recorded hours were excluded from the analysis. None of the twins were found to have atrial fibrillation or flutter.

Measurement of Cognitive Function: Verbal and visual learning and memory were assessed using the verbal Selective Reminding test (SRT) and the visual SRT (53). In the verbal SRT, each twin was read a list of twelve unrelated words, and asked to immediately recall as many words as possible. Each twin was then reminded only of the words that were not recalled, and was asked to recall all twelve words again. Twelve total recall trials were conducted, except in cases with 2 consecutive trials of perfect recall. Total recall (TR) was defined as the total number of words recalled on all 12 trials, representing a sum of long-term and short-term storage and learning of words. The maximum score for total recall is 144, and the average score for ages 50-59 is 121.6, with standard deviation 10.5 (54). A TR score of less than 2 standard deviations below the population-specific mean has been found to be 47% sensitive and 86% specific for future (1-2 years later) dementia in an elderly population (mean age 80 years) (43). While other parameters were measured, including consistent long-term recall (CLTR) and delayed recall (DR), these were not included in the analysis because of the high degree of correlation with TR. The visual SRT was similar to the verbal test. Twelve designs were presented one at a time for 3 seconds each, followed by an opportunity to draw all from memory. Each design that was not accurately reproduced on a given trial was shown again until perfect recall was obtained or 12 trials.

Assessment of Depression, PTSD, and Other Psychiatric Diagnoses. We administered the Beck Depression Inventory-II (BDI-II) (55), a standardized scale providing a measure of depressive symptoms, with higher scores indicating more severe depression (56). We also administered the Structured Clinical Interview for DSM IV (SCID) (57) to classify twins based on a lifetime history of major depressive disorder (MDD) and PTSD. The SCID also provided a diagnosis of other psychiatric disorders, including a lifetime history of alcohol and of drug abuse or dependence.

Other Measurements. A medical history and a physical exam were obtained by a research nurse. Abdominal and hip circumferences were measured to derive the waist to hip ratio. Hypertension was defined by a measured systolic blood pressure > 140 mm Hg or current treatment with anti-hypertensive medications.

Diabetes was defined as having a fasting glucose level > 126 mg/dl or current treatment with anti-diabetic medications. Venous blood samples were drawn for the measurement of glucose and lipid profile after an overnight fast. Glucose was measured on the Beckman CX7 chemistry autoanalyzer. Direct high-density lipoprotein (HDL) and low density lipoprotein (LDL) cholesterol were measured with homogeneous assays (Equal Diagnostics, Exton, PA). Serum Vitamin 25(OH)D levels were measured by using an enzyme linked immunsorbent assay (IDS Inc, Foundations Hills, AZ). Dietary intake of omega 3 fatty acids, alcohol, and caffeine were measured using the Willett food frequency questionnaire (58). Physical activity was assessed with a modified version of the Baecke Questionnaire of Habitual Physical Activity (used in the Atherosclerosis Risk

in Communities (ARIC) Study (59)) that documented physical activity at work, during sports and non-sports activities. The global physical activity score was used in the analysis. Cigarette smoking was classified into current versus never or past smoker. A history of coronary heart disease was defined as a previous diagnosis of myocardial infarction or angina pectoris, or previous coronary revascularization procedures. Information on current use of other medications was also collected. Baseline intelligence, measured via the Armed Forces Qualification Test (AFQT), was abstracted from military records (42).

Sample Size Calculation

The primary consideration for the sample sizes of the ETS studies were for the purposes of detecting differences in myocardial ischemia due to depression (THS) and PTSD (SAVEIT). Nonetheless, we calculated that for our population, which had a mean +/- standard deviation (SD) verbal SRT score of 110 +/- 20, a difference of at least 5 words (which is ½ of the standard deviation found in normal population studies) would be clinically significant (54). In order to find such a difference in score with $\alpha=0.05$ and $\beta=0.80$ between 2 twins separated by 1 natural log of HRV, we would need at least a sample size of 252.

Analysis, Statistical Approach

Analysis was conducted using SAS 9.2 for Windows (©2008, Cary, NC). In initial analyses, we compared means and percents of study factors between twins with verbal memory score above and below the median. P values were corrected for the correlation

between co-twins using generalized estimating equations for categorical variables and mixed-models for continuous variables.

To examine the association between cognitive function and HRV, two sets of analyses were performed: 1) amongst individuals, in which all twins were eligible for inclusion regardless of whether their brother was available for analysis; and 2) between and within twin pairs, comparing one twin brother with the other (within-pair effect), as well as analyzing the association amongst pairs of brothers (between-pair effect). To do this, we used mixed effects regression models and accounted for the twin pairs using a random effect term for each pair. The mixed model is shown in equation (1); Y is the outcome, β_0 is the intercept, β_S is the coefficient for the independent variable, α_i is the pair-level random effect, and ε_{ij} is the individual random effect.

$$Y = \beta_0 + \beta_S X_i + \alpha_i + \varepsilon_{ij} \quad (1)$$

Verbal and visual selective reminding test scores were used as continuous outcomes, and log-transformed HRV measures were used as predictors. Twin pairs were considered discordant for HRV unless their HRV was exactly the same, up to 2 decimal places of ms^2. Statistical significance was determined if $p<0.05$, two-sided. For each unit increase in log (HRV), the β coefficient corresponds to the associated increase in cognitive function score. Model goodness-of-fit was assessed using the Akaike information criterion (AIC) before and after addition of HRV to the base model. Distributions and residuals of the models were examined for outliers with the use of the Cook's D statistic.

Within-pair Analysis: We performed within-pair analyses which examined differences in HRV between twins that were discordant for heart rate variability. The within-pair effects inherently control for demographic, shared familial and early environmental influences; in addition, daily activities and other environmental factors during the ambulatory ECG recording are controlled in this analysis since co-twins were examined at the same time and under nearly identical conditions. We fitted mixed effects models adapted for twin research, which allow for partitioning within and between pair differences in the dependent variable (Figure 3, equation 2), cognitive function, as a function of the independent variables (60).

$$Y = \beta_0 + \beta_B \overline{X}_i + \beta_W (X_{ij} - \overline{X}_i) + \alpha_i + \varepsilon_{ij} \quad (2)$$

This included the average HRV of the twin pair (average= $\overline{X}_i$ "between effect" coefficient= β_B), as well as the difference in HRV between the individual twin (X_{ij}) and the pair average ($\overline{X}_i$) (difference= $X_{ij} - \overline{X}_i$, "within effect" coefficient= β_W). This coefficient for the within effect is identical to the coefficient from a model that fits the absolute difference between the co-twins (60). The analysis allowed the inclusion of unpaired twins, who contribute to the between-pair analysis, without substantially affecting the within-pair results, thus allowing full use of the dataset.

Genetic Influence: The last set of analyses involved testing for a possible genetic influence underlying the association between cognitive testing scores and HRV, by adding to the model a term for the interaction between zygosity and the within-pair difference in HRV. If the DZ effect is larger than the MZ effect and the interaction term is

significant ($p < .05$), this may suggest a role for genetic factors in the association. If the MZ and DZ effects are not significantly different, then this suggests that genetics do not play a major role in the relationship.

RESULTS

Group and Subset Characteristics

Of the 524 twins in the sample, 416 (79%) had usable HRV and cognitive data, including 169 twin pairs (61% monozygotic) and 78 singletons. The twins excluded due to missing HRV data (n=107) were similar to the rest of the twins with respect to demographic, socioeconomic, psychiatric, and cardiovascular risk profiles. They had a similar verbal SRT score, and a slightly higher (131 vs. 127, p=0.04) visual SRT score than those with complete HRV data. One twin was excluded because of incomplete cognitive data.

For the included subjects, the mean age was 55.1 (SD=2.9) years, 54 (13%) had a lifetime history of PTSD, 98 (24%) had a lifetime history of depression, and they completed on average 14 years of education. The mean verbal SRT total recall score was 110 of 144 maximum, with median=114, standard deviation = 20 and skewness = -0.81. For the visual SRT, the mean score was 127 of 144 maximum, with median=132, standard deviation=16 and skewness=-2.54. The twins with higher verbal total recall were more educated and less likely to have a history of alcohol abuse (Table 1); otherwise, there were no statistically significant differences in cognitive function, physical health, mental health, and health behaviors between those above and below the median verbal SRT score.

Association of HRV and Selective Reminding Test Scores Amongst Twins

Overall, significant associations were found between HRV and verbal total recall both before and after multivariable adjustment for potential confounding factors (Table

2). Visual inspection (figure 4) of the jackknife residuals, frequency plot of residuals, and residuals versus leverage scatterplot showed the assumptions of linearity, homoscedasticity, and normality were met for the primary association between total power and verbal total recall. Upon inspection for interaction with major depression and PTSD, a significant interaction was found between TP and ULF HRV and lifetime PTSD. In the group without PTSD, each unit increase in ln(TP) was associated with an adjusted increase in verbal total recall score of approximately 5 words (p=0.01). Similar adjusted associations were found with ULF and VLF HRV, although the effect sizes were not as large. With regards to model fit, the AIC decreased from 3917 to 3129 when ln(TP) was added to the base multivariate model (p<0.001); similar model improvements were seen with the other frequency spectra as well. An approximately linear trend was observed when plotting the mean verbal SRT score per quartile of total power HRV, and in the subgroup without PTSD (n=362), the trend appeared slightly more consistently increasing, particularly in the middle quartiles (figure 5). Additionally, no trend was seen in the group with PTSD (n=54). LF and HF HRV were also significantly associated with verbal memory in unadjusted analysis, but the associations did not persist after multivariate adjustment. Additionally, no associations were found in the entire sample between HRV and visual SRT, and no interaction with depression or PTSD was found (table 3).

Association of HRV and Selective Reminding Test Scores Within Pairs

Within-pair analysis included 169 complete pairs and 78 unpaired twins. All pairs were discordant for HRV except for 1 pair in the TP and ULF spectra, and 2 pairs in the LF and HF spectra. In general, adjusted within-pair analysis showed similar results to

adjusted analysis of individual twins (table 4). A significant interaction was found between TP and ULF spectra and PTSD, and the subgroup without PTSD showed the most significant associations between TP and ULF spectra and verbal total recall; each ln(TP) increase in discordance between twins was positively associated with an approximately 7 word difference in verbal memory score. No associations were found between LF and HF spectra and verbal memory. The between pair coefficients, which represent the association of the average HRV of the pair and cognitive function, were less than the within-pair coefficients, and were not statistically significant. No significant between and within-pair associations were found with visual total recall score, nor with verbal recall score in the PTSD subgroup.

Analysis of within-pair effects within MZ and DZ subgroups (Table 5) showed no significant difference between MZ and DZ groups in the unadjusted and adjusted associations between HRV and verbal SRT score. Analysis was also performed in the subgroup without PTSD because of the interaction between PTSD and total power/ULF HRV (Table 6) and shows larger associations for total power and ULF than in the overall group. In this subgroup, the MZ group showed a larger association than the DZ group for VLF, LF, and HF that was statistically significant. No significant associations were found in the PTSD only group.

DISCUSSION

In this cross-sectional study of predominantly healthy, asymptomatic middle-aged men, we found a positive association between verbal, but not visual, memory performance and HRV. This association remained when controlling for cardiovascular risk factors, education, depressive symptoms, and familial factors. Because the within-pair associations in the DZ twins were not greater than in the MZ twins, genetic confounding is also unlikely. Furthermore, HRV was selectively associated with verbal, and not visual, working memory, implying that the neurobiology of this association may be specific to verbal learning and memory only. Given the known association between poor performance on verbal SRT and dementia risk, these results suggest that autonomic dysfunction may be a marker of early cognitive decline, and it may also have direct physiologic effects on certain neural circuits (43). Our study expands upon the findings of previous smaller studies by finding an association between verbal learning and memory with more prognostic, long-term HRV measures that are independent of cardiovascular comorbidities, depression, familial factors, and genetic factors.

Visual memory was not associated with HRV. There are several possible explanations for this finding. First, the mean score was higher (127 vs. 110) and variance was lower (286 vs. 441) for visual SRT compared to verbal SRT, which may lead to a ceiling effect in our data and reduce our ability to detect an association. Additionally, there is debate as to whether visual memory utilizes the prefrontal cortex (61-63). Many known regions involved in visual function, including parietal, temporal, and occipital lobes, are all outside of the central autonomic network (62, 64). These other regions may help compensate for a possible loss of prefrontal function. Visual memory also utilizes the non-dominant temporal lobe, whereas verbal memory utilizes

the temporal lobe, and may preferentially receive input from the central autonomic network (49, 65). Therefore, HRV may correlate with verbal, but not visual, working memory performance because verbal memory more specifically involves the central autonomic network than visual memory.

Although our primary analysis showed consistent associations, spectral analysis showed not all frequency bands were consistently associated with verbal memory. Low frequency, 24-hour HRV indices such as ULF and VLF, which best predict mortality after myocardial infarction, demonstrated the most significant and consistent association with verbal memory performance (51). Although much is yet to be learned about these frequency measures, they are known to vary with physical activity (ULF), temperature regulation (ULF), and hormonal activity in the renin-angiotensin-aldosterone system (RAAS) (VLF) (45, 66, 67). The higher frequency bands (HF, LF), however, were not as significantly associated with cognitive function; they are known to vary with position, respiration, and blood pressure (9, 68, 69). Although it is unclear why lower frequency spectra were more significantly associated with verbal memory than high frequency spectra, it is known that RAAS system activity may influence both VLF and cognitive function (70-73), and thus partially explain this association.

Interaction with PTSD. Subjects with PTSD were found not to have an association between TP and ULF HRV and verbal/visual SRT, which may be due to independent effects of PTSD on memory. In our sample, those with lifetime PTSD scored lower on the verbal SRT (105 vs. 111, p=0.20), although this was not statistically significant; no difference was found with visual SRT, p=0.99. PTSD is known to be associated with poor memory performance, which may be a consequence of damaging effects of traumatic stress on areas of the brain such as the hippocampus that are

important for memory (74, 75). In subjects with PTSD, it is possible that stress-related changes to the hippocampus that reduce memory performance override any possible effects due to other risk factors for memory performance, or otherwise create a floor effect that limits the ability to find an association with HRV. Further research is needed to understand this interaction.

Implications. Our results support a possible mechanistic link between autonomic and cognitive function, and provide important insight to advance the field of cognitive therapies. Exercise, for example, affects both autonomic function and cognitive status, particularly executive function (6, 66, 76-78). In the study of sailors by Hansen and coworkers, those who underwent a 4-week exercise training program had higher HF HRV and better cognitive performance scores on working memory tests compared to sailors who did not undergo the training program (79). Eisenberg et al. showed the efficacy of biofeedback on HRV and symptoms of attention-deficit-hyperactivity-disorder (ADHD) in children, and also found a positive correlation between HRV and ADHD improvement (80). Multiple drug and non-drug interventions have been found to increase HRV, highlighting the possibility of developing interventions that may improve cognitive function through enhancement of the central autonomic network (6).

Limitations. Our population included primarily Caucasian middle-aged male veteran twins, and our findings may not generalize to other races, women, and other age groups. Performance on cognitive tests and HRV measures may have been subject to circumstances faced by individual twins, such as prolonged travel or undetected medical conditions. While such circumstances were not taken into account, outliers were carefully examined for extraneous circumstances that may have affected cognitive performance. Also, this is a cross-sectional study, and therefore direction of the

association and causation cannot be proven. Unmeasured confounders, such as differences in quality of education environments, may play a role, although they were minimized by our within-pair analysis, in which we compared each twin to his brother, thus controlling for unmeasured common childhood environmental factors.

Conclusion. In a sample of middle-aged male veteran twins, we found an association between HRV and verbal (but not visual) memory performance that was independent of demographic and cardiovascular risk factors, as well as familial and genetic factors. This association was particularly robust for twins without PTSD. These findings indicate that cortical neurologic mechanisms relevant to cognitive performance are linked to autonomic regulation pathways, and therefore treatments, such as exercise, that improve autonomic function may also reduce the risk of cognitive impairment.

REFERENCES

1. Middleton LE, Yaffe K. Promising strategies for the prevention of dementia. *Arch Neurol* 2009;66(10):1210-5.
2. Function MC, Study A, Yip AG, et al. Risk factors for incident dementia in England and Wales: The Medical Research Council Cognitive Function and Ageing Study. A population-based nested case–control study. *Age and Ageing* 2006;35(2):154-60.
3. Launer LJ. Demonstrating the case that AD is a vascular disease: epidemiologic evidence. *Ageing Res Rev* 2002;1(1):61-77.
4. Kim DH, Lipsitz LA, Ferrucci L, et al. Association between reduced heart rate variability and cognitive impairment in older disabled women in the community: Women's Health and Aging Study I. *J Am Geriatr Soc* 2006;54(11):1751-7.
5. Thayer JF, Hansen AL, Saus-Rose E, et al. Heart rate variability, prefrontal neural function, and cognitive performance: the neurovisceral integration perspective on self-regulation, adaptation, and health. *Ann Behav Med* 2009;37(2):141-53.
6. Nolan RP, Jong P, Barry-Bianchi SM, et al. Effects of drug, biobehavioral and exercise therapies on heart rate variability in coronary artery disease: a systematic review. *Eur J Cardiovasc Prev Rehabil* 2008;15(4):386-96.
7. Britton A, Singh-Manoux A, Hnatkova K, et al. The association between heart rate variability and cognitive impairment in middle-aged men and women. The Whitehall II cohort study. *Neuroepidemiology* 2008;31(2):115-21.
8. Thayer JF, Lane RD. A model of neurovisceral integration in emotion regulation and dysregulation. *J Affect Disord* 2000;61(3):201-16.
9. Heart rate variability. Standards of measurement, physiological interpretation, and clinical use. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. *Eur Heart J* 1996;17(3):354-81.
10. Buschke F. Updated Hodgkin's disease. C. Advanced disease and special problems. Conservative radiation therapy for advanced disease. *JAMA* 1973;223(2):170-1.
11. Guyton AC. *Anatomy and physiology*. Philadelphia: Saunders College Pub.; 1985.
12. Devinsky O, Morrell MJ, Vogt BA. Contributions of anterior cingulate cortex to behaviour. *Brain* 1995;118 (Pt 1):279-306.
13. Adler CM, Delbello MP, Mills NP, et al. Comorbid ADHD is associated with altered patterns of neuronal activation in adolescents with bipolar disorder performing a simple attention task. *Bipolar Disord* 2005;7(6):577-88.
14. Rajkowska G, Halaris A, Selemon LD. Reductions in neuronal and glial density characterize the dorsolateral prefrontal cortex in bipolar disorder. *Biol Psychiatry* 2001;49(9):741-52.
15. Cowley AW, Jr., Liard JF, Guyton AC. Role of baroreceptor reflex in daily control of arterial blood pressure and other variables in dogs. *Circ Res* 1973;32(5):564-76.
16. Viramo P, Luukinen H, Koski K, et al. Orthostatic hypotension and cognitive decline in older people. *J Am Geriatr Soc* 1999;47(5):600-4.
17. Hansen AL, Johnsen BH, Thayer JF. Relationship between heart rate variability and cognitive function during threat of shock. *Anxiety Stress Coping* 2009;22(1):77-89.
18. Zulli R, Nicosia F, Borroni B, et al. QT dispersion and heart rate variability abnormalities in Alzheimer's disease and in mild cognitive impairment. *J Am Geriatr Soc* 2005;53(12):2135-9.

19. Allan LM, Kerr SR, Ballard CG, et al. Autonomic function assessed by heart rate variability is normal in Alzheimer's disease and vascular dementia. *Dement Geriatr Cogn Disord* 2005;19(2-3):140-4.
20. Akselrod S, Gordon D, Madwed J, et al. Hemodynamic regulation: investigation by spectral analysis. *American Journal of Physiology- Heart and Circulatory Physiology* 1985;249(4):H867.
21. Kupper NHM, Willemsen G, van den Berg M, et al. Heritability of ambulatory heart rate variability. *Circulation* 2004;110(18):2792-6.
22. Ando J, Ono Y, Wright MJ. Genetic structure of spatial and verbal working memory. *Behav Genet* 2001;31(6):615-24.
23. Hasselmo ME. Neuromodulation: acetylcholine and memory consolidation. *Trends Cogn Sci* 1999;3(9):351-9.
24. Burnstock G. Review lecture. Neurotransmitters and trophic factors in the autonomic nervous system. *J Physiol* 1981;313:1-35.
25. Malhotra AK, Kestler LJ, Mazzanti C, et al. A functional polymorphism in the COMT gene and performance on a test of prefrontal cognition. *Am J Psychiatry* 2002;159(4):652-4.
26. Lindvall O, Bjorklund A, Skagerberg G. Dopamine-containing neurons in the spinal cord: anatomy and some functional aspects. *Ann Neurol* 1983;14(3):255-60.
27. Tong S, Baghurst P, Vimpani G, et al. Socioeconomic position, maternal IQ, home environment, and cognitive development. *J Pediatr* 2007;151(3):284-8, 8 e1.
28. Lampert R, Ickovics J, Horwitz R, et al. Depressed autonomic nervous system function in African Americans and individuals of lower social class: a potential mechanism of race- and class-related disparities in health outcomes. *Am Heart J* 2005;150(1):153-60.
29. Barker DJ. Fetal programming of coronary heart disease. *Trends Endocrinol Metab* 2002;13(9):364-8.
30. Raikkonen K, Forsen T, Henriksson M, et al. Growth trajectories and intellectual abilities in young adulthood: The Helsinki Birth Cohort study. *Am J Epidemiol* 2009;170(4):447-55.
31. Barker DJ, Eriksson JG, Forsen T, et al. Fetal origins of adult disease: strength of effects and biological basis. *Int J Epidemiol* 2002;31(6):1235-9.
32. Bird A. Perceptions of epigenetics. *Nature* 2007;447(7143):396-8.
33. Irmak MK, Sizlan A. Essential hypertension seems to result from melatonin-induced epigenetic modifications in area postrema. *Med Hypotheses* 2006;66(5):1000-7.
34. Rechcinski T, Trzos E, Wierzbowska-Drabik K, et al. Melatonin for nondippers with coronary artery disease: assessment of blood pressure profile and heart rate variability. *Hypertens Res* 2010;33(1):56-61.
35. Nishiyama K, Yasue H, Moriyama Y, et al. Acute effects of melatonin administration on cardiovascular autonomic regulation in healthy men. *Am Heart J* 2001;141(5):E9.
36. Ferrari E, Magri F. Role of neuroendocrine pathways in cognitive decline during aging. *Ageing Res Rev* 2008;7(3):225-33.
37. Zhu LQ, Wang SH, Ling ZQ, et al. Inhibition of melatonin biosynthesis activates protein kinase a and induces Alzheimer-like tau hyperphosphorylation in rats. *Chin Med Sci J* 2005;20(2):83-7.
38. Zhu LQ, Wang SH, Ling ZQ, et al. Effect of inhibiting melatonin biosynthesis on spatial memory retention and tau phosphorylation in rat. *J Pineal Res* 2004;37(2):71-7.

39. Hallam KT, Olver JS, Chambers V, et al. The heritability of melatonin secretion and sensitivity to bright nocturnal light in twins. *Psychoneuroendocrinology* 2006;31(7):867-75.
40. Goldberg J, Curran B, Vitek ME, et al. The Vietnam Era Twin Registry. *Twin Res* 2002;5(5):476-81.
41. Vaccarino V, Brennan ML, Miller AH, et al. Association of major depressive disorder with serum myeloperoxidase and other markers of inflammation: a twin study. *Biol Psychiatry* 2008;64(6):476-83.
42. Henderson WG, Eisen S, Goldberg J, et al. The Vietnam Era Twin Registry: a resource for medical research. *Public Health Rep* 1990;105(4):368-73.
43. Masur DM, Fuld PA, Blau AD, et al. Predicting development of dementia in the elderly with the Selective Reminding Test. *J Clin Exp Neuropsychol* 1990;12(4):529-38.
44. Giovagnoli AR, Casazza M, Avanzini G. Visual learning on a selective reminding procedure and delayed recall in patients with temporal lobe epilepsy. *Epilepsia* 1995;36(7):704-11.
45. Akselrod S, Gordon D, Ubel FA, et al. Power spectrum analysis of heart rate fluctuation: a quantitative probe of beat-to-beat cardiovascular control. *Science* 1981;213(4504):220-2.
46. Bigger JT, Jr., Fleiss JL, Steinman RC, et al. Frequency domain measures of heart period variability and mortality after myocardial infarction. *Circulation* 1992;85(1):164-71.
47. Tsuji H, Venditti FJ, Jr., Manders ES, et al. Determinants of heart rate variability. *J Am Coll Cardiol* 1996;28(6):1539-46.
48. Kleinbaum DG. *Applied regression analysis and other multivariable methods*. 4th ed. Australia ; Belmont, CA: Brooks/Cole; 2007.
49. Thayer JF, Lane RD. Claude Bernard and the heart-brain connection: further elaboration of a model of neurovisceral integration. *Neurosci Biobehav Rev* 2009;33(2):81-8.
50. Lampert R, Ickovics JR, Viscoli CJ, et al. Effects of propranolol on recovery of heart rate variability following acute myocardial infarction and relation to outcome in the Beta-Blocker Heart Attack Trial. *Am J Cardiol* 2003;91(2):137-42.
51. Bigger J, Fleiss J, Steinman R, et al. Frequency domain measures of heart period variability and mortality after myocardial infarction. *Circulation* 1992;85:164-71.
52. Pagani M, Lombardi F, Guzzetti S, et al. Power spectral analysis of heart rate and arterial pressure variability as a marker of sympatho-vagal interaction in man and conscious dog. *Circ Res* 1986;59:178-93.
53. Buschke H, Fuld PA. Evaluating storage, retention, and retrieval in disordered memory and learning. *Neurology* 1974;24(11):1019-25.
54. Larrabee G, Trahan D, Curtiss G, et al. Normative data for the Verbal Selective Reminding Test. *Neuropsychology* 1988;2(3-4):173.
55. Beck AT, Steer RA, Brown GK. *BDI-II. Beck Depression Inventory: Second Edition.* San Antonio, TX: The Psychological Corporation; 1996.
56. Osman A, Kopper BA, Barrios F, et al. Reliability and validity of the Beck depression inventory--II with adolescent psychiatric inpatients. *Psychol Assess* 2004;16(2):120-32.
57. First MB, Spitzer RL, Williams JBW, et al. *Structured Clinical Interview for DSM IV-Patient Edition (SCID-P)*. Washington, D.C.: American Psychiatric Press; 1995.
58. Willett WC, Sampson L, Stampfer MJ, et al. Reproducibility and validity of a semiquantitative food frequency questionnaire. *Am J Epidemiol* 1985;122(1):51-65.

59. Richardson MT, Ainsworth BE, Wu H, et al. Ability of the Atherosclerosis Risk in Communities (ARIC)/Baecke Questionnaire to assess leisure-time physical activity. *Int J Epidemiol* 1995;24:685-93.
60. Carlin JB, Gurrin LC, Sterne JAC, et al. Regression models for twin studies: a critical review. *Int J Epidemiol* 2005;34(5):1089-99.
61. Desimone R. Neural mechanisms for visual memory and their role in attention. *Proc Natl Acad Sci U S A* 1996;93(24):13494-9.
62. Pessoa L, Gutierrez E, Bandettini P, et al. Neural correlates of visual working memory: fMRI amplitude predicts task performance. *Neuron* 2002;35(5):975-87.
63. Baxter MG, Gaffan D, Kyriazis DA, et al. Dorsolateral prefrontal lesions do not impair tests of scene learning and decision-making that require frontal-temporal interaction. *Eur J Neurosci* 2008;28(3):491-9.
64. Roland PE, Gulyas B. Visual imagery and visual representation. *Trends Neurosci* 1994;17(7):281-7; discussion 94-7.
65. Milner B. Interhemispheric differences in the localization of psychological processes in man. *Br Med Bull* 1971;27(3):272-7.
66. Serrador JM, Finlayson HC, Hughson RL. Physical activity is a major contributor to the ultra low frequency components of heart rate variability. *Heart* 1999;82(6):e9.
67. Fleisher LA, Frank SM, Sessler DI, et al. Thermoregulation and heart rate variability. *Clin Sci (Lond)* 1996;90(2):97-103.
68. Cooke WH, Cox JF, Diedrich AM, et al. Controlled breathing protocols probe human autonomic cardiovascular rhythms. *Am J Physiol* 1998;274(2 Pt 2):H709-18.
69. Taylor JA, Eckberg DL. Fundamental relations between short-term RR interval and arterial pressure oscillations in humans. *Circulation* 1996;93(8):1527-32.
70. Basso N, Paglia N, Stella I, et al. Protective effect of the inhibition of the renin-angiotensin system on aging. *Regul Pept* 2005;128(3):247-52.
71. Gard PR, Rusted JM. Angiotensin and Alzheimer's disease: therapeutic prospects. *Expert Rev Neurother* 2004;4(1):87-96.
72. Amouyel P, Richard F, Berr C, et al. The renin angiotensin system and Alzheimer's disease. *Ann N Y Acad Sci* 2000;903:437-41.
73. Li NC, Lee A, Whitmer RA, et al. Use of angiotensin receptor blockers and risk of dementia in a predominantly male population: prospective cohort analysis. *BMJ* 2010;340:b5465.
74. Bremner JD, Scott TM, Delaney RC, et al. Deficits in short-term memory in posttraumatic stress disorder. *Am J Psychiatry* 1993;150(7):1015-9.
75. Bremner JD. Does stress damage the brain? *Biol Psychiatry* 1999;45(7):797-805.
76. Erickson KI, Kramer AF. Aerobic exercise effects on cognitive and neural plasticity in older adults. *Br J Sports Med* 2009;43(1):22-4.
77. Aberg MA, Pedersen NL, Toren K, et al. Cardiovascular fitness is associated with cognition in young adulthood. *Proc Natl Acad Sci U S A* 2009.
78. Dishman RK, Berthoud HR, Booth FW, et al. Neurobiology of exercise. *Obesity (Silver Spring)* 2006;14(3):345-56.
79. Hansen AL, Johnsen BH, Sollers JJ, 3rd, et al. Heart rate variability and its relation to prefrontal cognitive function: the effects of training and detraining. *Eur J Appl Physiol* 2004;93(3):263-72.

80. Eisenberg J, Ben-Daniel N, Mei-Tal G, et al. An autonomic nervous system biofeedback modality for the treatment of attention deficit hyperactivity disorder--an open pilot study. *Isr J Psychiatry Relat Sci* 2004;41(1):45-53.

FIGURES

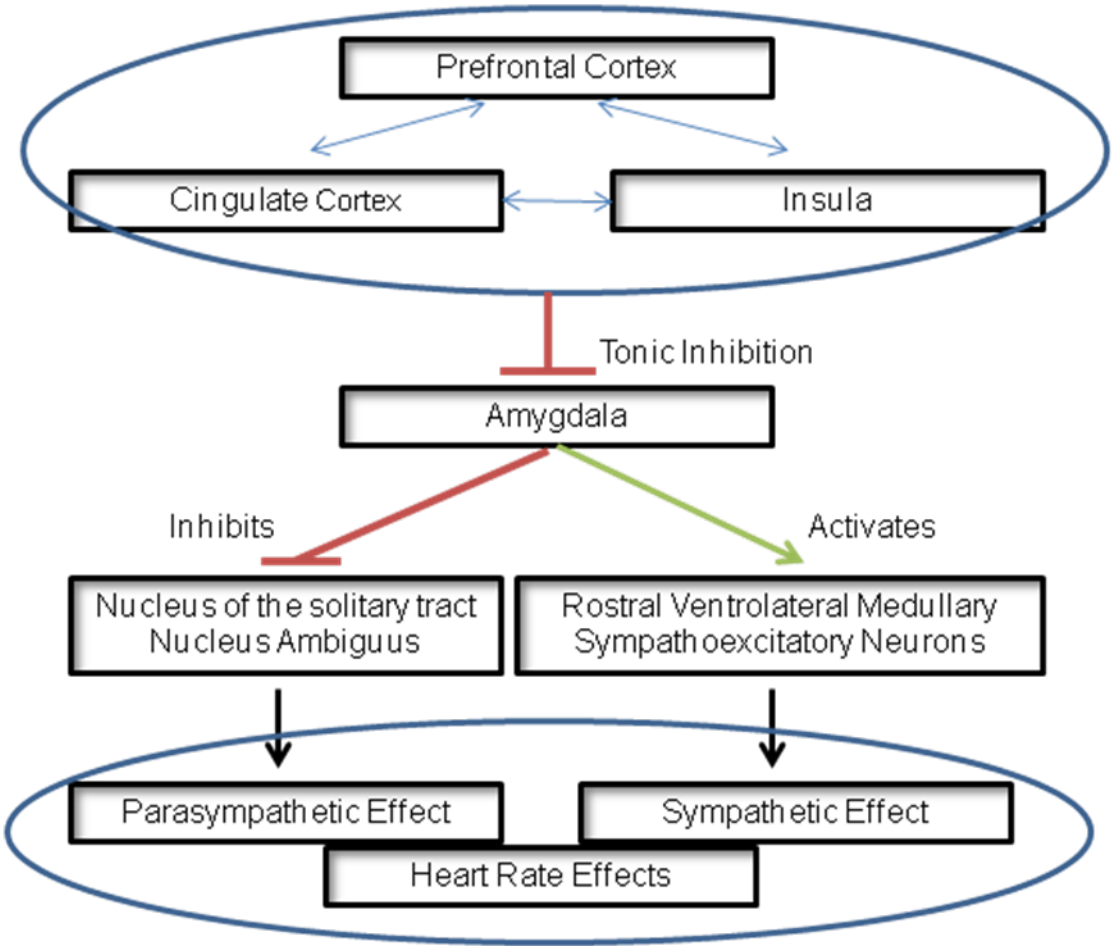

Figure 1. Association of Neural Structures involved in Neurovisceral Integration Theory Associating Executive Function and Autonomic Function. This diagram highlights connection between cortical structures and autonomic nervous system. The prefrontal, anterior cingulated, and insular cortices form an interconnected network that communicate bi-directionally with the amygdala. The prefrontal cortex tonically inhibits the amygdala. When active, the amygdala inhibits parasympathetic neurons, including the nucleus of the solitary tract and nucleus ambiguous, as well as activates the sympathetic neurons of the rostral ventrolateral medulla. These inputs, which are also involved in executive function (eg. prefrontal cortex) and emotion (eg. amygdala) are therefore able to modulate heart rhythm through their autonomic effects. (49)

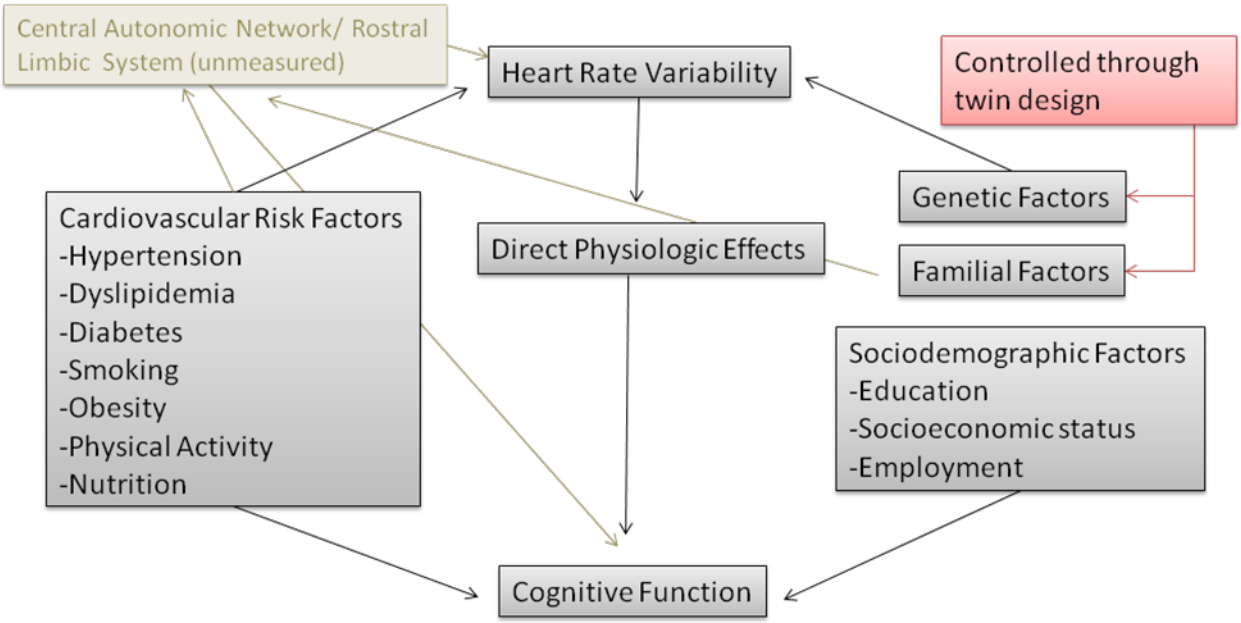

Figure 2. Causal Diagram of Possible Influences of Both Heart Rate Variability and Cognitive Function. This diagram outlines several possible influences on both autonomic function, measured through heart rate variability, and cognitive function. Traditional cardiovascular risk factors, familial factors, genetic factors, and Sociodemographic factors are all known to influence both cognitive function and heart rate variability. Additionally, hemodynamic effects of poor autonomic function may also affect cognitive function. The shared cortical structures central autonomic network and rostral limbic system are not measured directly, but likely mediate the effects of the possible confounders.

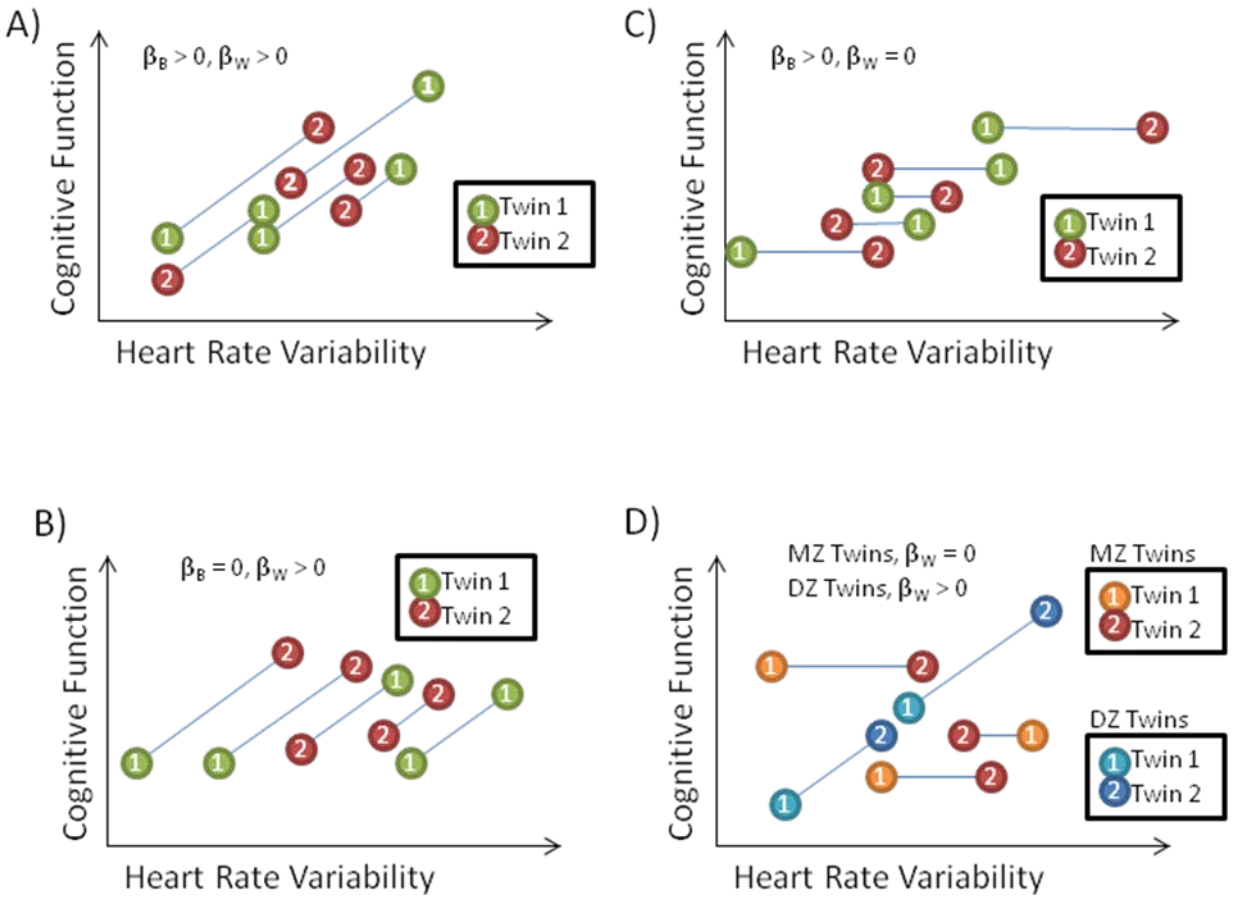

Figure 3. Explanation of Analysis Genetic and Familial Confounding in Twin Models Using Mixed Models. This diagram shows the various relationships that can occur between HRV and cognitive function amongst pairs of twins, with β_B as the coefficient for the association between the twin pairs' average exposure, and β_w as the coefficient of the association within twin pairs, comparing twin 1 with twin 2. In (A), as association exists both between and within twin pairs, implying that the association is not confounded by familial or genetic factors. In (B), an association exists only within twin pairs, but not between twin pairs, implying the association only occurs within twin pairs, but not in the between unrelated pairs. In (C), an association exists between twin pairs, but not within pairs, implying at least familial confounding exists. In (D), a within-pair association is found for DZ pairs only, but not MZ pairs, implying genetic confounding.

Abbreviations: MZ=monozygotic, DZ=dizygotic

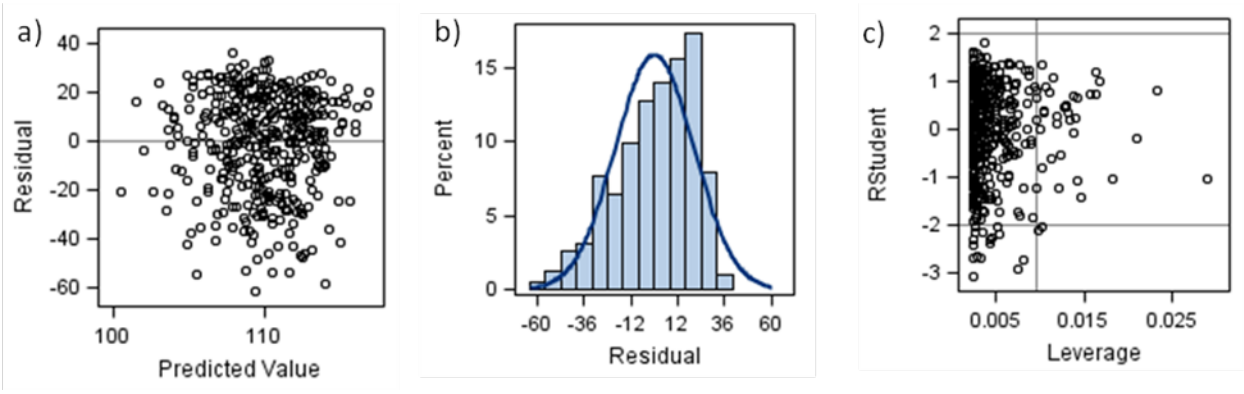

Figure 4. Graphical Representations of Residuals and Tests for Normality and Outliers. Graph (a) shows the residual values plotted against the predicted values; graph B shows the distribution of residuals under a normal curve; graph C shows the jackknife residuals ("RStudent") versus cook's distance ("Leverage"), with the lines showing standardized cutoffs for outliers.

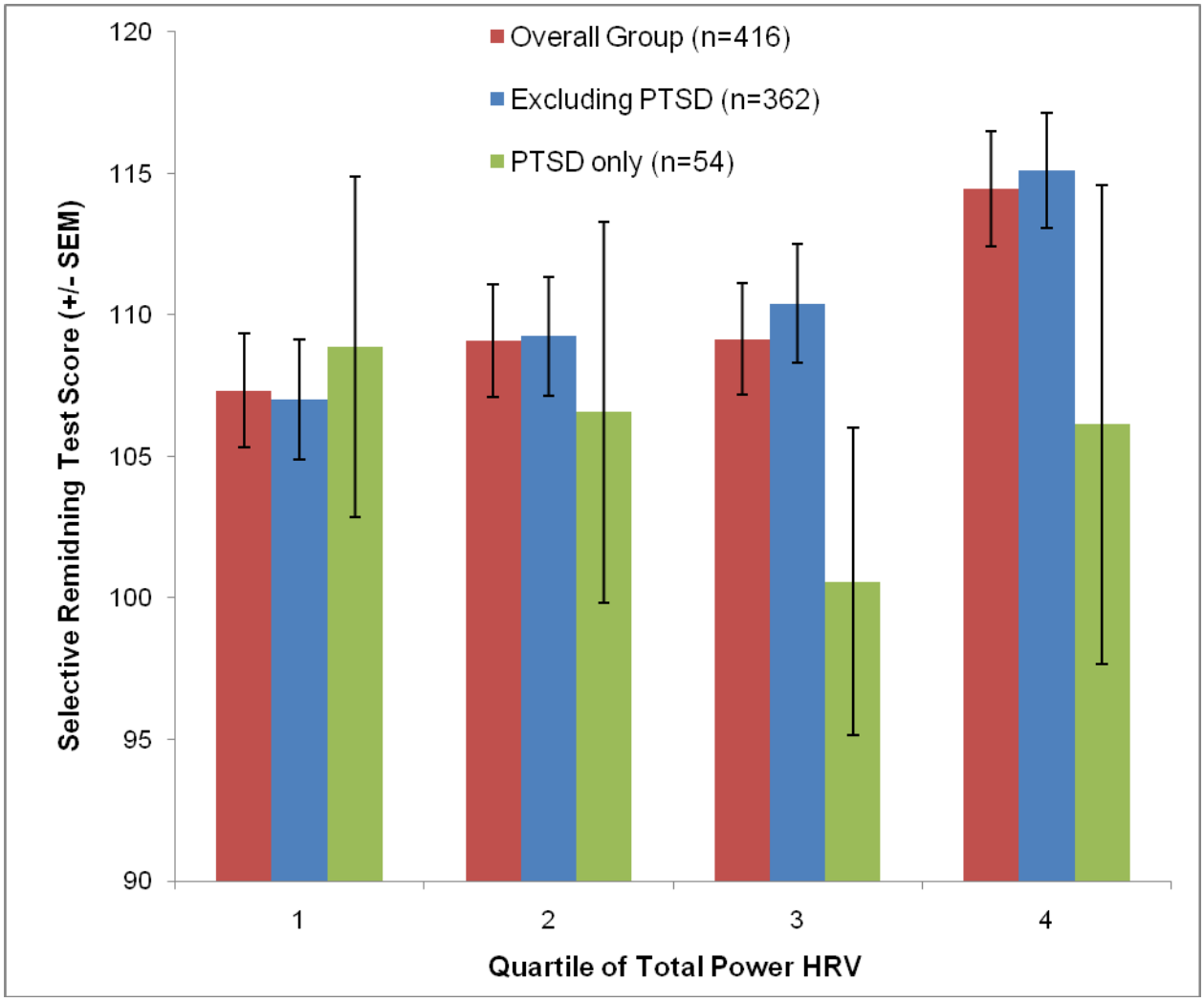

Figure 5. Verbal Total Recall Score versus Quartile of Total Power Heart Rate Variability, Unadjusted. Includes overall group, as well as subgroups stratified by lifetime history of PTSD.

Abbreviations: SEM=standard error of the mean

TABLES

Table 1 – Distribution of Demographic, Behavioral, and Coronary Risk Factors in Twins Stratified by Verbal Memory Score

	Below Median Verbal SRT Score (n=205)	Above Median Verbal SRT Score (n=211)	p*
Age, years, mean ± SD	55.2 ± 2.9	55.1 ± 2.8	0.59
Greater than high school education, %	20.0	46.0	<0.001
Mean AFQT score Percentile	56.1 ± 1.9	65.4 ± 1.7	<0.001
Systolic blood pressure, mm Hg, mean ± SD	129.4 ± 14.6	128.7 ± 16.6	0.75
Diastolic blood pressure, mm Hg, mean ± SD	81.2 ± 10.2	80.5 ± 10.7	0.59
LDL-cholesterol, mg/dL, mean ± SD	124.7 ± 35.8	119.5 ± 34.5	0.13
HDL-cholesterol, mg/dL, mean ± SD	38.3 ± 11.0	39.1 ± 9.9	0.41
Diabetes, %	13.1	10.4	0.41
Body mass index, mean ± SD	30.0 ± 5.4	29.2 ± 4.2	0.10
Waist-to-hip ratio, mean ± SD	0.95 ± 0.07	0.94 ± 0.06	0.06
Physical activity (Baecke) score, mean ± SD	7.1 ± 1.8	7.4 ± 1.8	0.08
Prior coronary heart disease, %	9.3	9.9	0.69
Lifetime history of PTSD, %	13.7	12.3	0.81
Lifetime history of MDD, %	25.4	21.8	0.41
Beck Depression Inventory, mean ± SD	6.5 ± 7.5	5.2 ± 7.2	0.12
Lifetime history of alcohol abuse or dependence, %	55.1	41.7	0.02
Lifetime history of drug abuse or dependence, %	23.9	17.5	0.24
Taking antidepressant medications, %	18.0	15.6	0.50
Taking beta-blocker medications, %	4.9	9.9	0.07

Abbreviations: SRT: selective reminding test; AFQT=Armed Forces Qualification Test; SD=standard deviation; PTSD=post-traumatic stress disorder; MDD=major depression. *P values are obtained from mixed models for continuous variables or generalized estimating equations for categorical variables

Table 2 - Association of Verbal Selective Reminding Test Score with Heart Rate Variability (HRV) in Individual Twins

HRV Frequency*	Entire Sample (n=416)				Twins without PTSD, adjusted (n=362)		Interaction between PTSD and HRV
	Unadjusted		Adjusted‡				
	β†	p	β	p	β	p	p
Total Power, ms^2	5.14	0.01	3.72	0.048	4.93	0.01	0.04
ULF, ms^2	4.47	0.01	3.25	0.06	4.52	0.01	0.03
VLF, ms^2	4.85	0.002	3.65	0.02	3.91	0.01	0.32
LF, ms^2	3.16	0.01	2.23	0.07	2.01	0.12	0.91
HF, ms^2	2.23	0.05	1.76	0.11	2.15	0.05	0.44

Abbreviations: ULF=ultra low frequency; VLF=very low frequency; LF=low frequency; HF=high frequency; *each frequency domain measure was log-transformed. †β corresponds to increase in selective reminding test score per unit increase in ln(HRV). ‡adjusted for age, hypertension, education, low density lipoprotein cholesterol, current/previous tobacco use, diabetes, and depressive symptoms

Table 3 - Association of Visual Selective Reminding Test Score with Heart Rate Variability (HRV) in Individual Twins

	Entire Sample (n=416)			
HRV Frequency	**Unadjusted**		**Adjusted**	
	β	p	β	p
Total Power, ms^2	1.12	0.45	-0.04	0.98
ULF, ms^2	0.95	0.49	0.01	0.99
VLF, ms^2	1.03	0.40	0.01	1.00
LF, ms^2	0.43	0.66	-0.34	0.75
HF, ms^2	0.93	0.30	0.73	0.42

Abbreviations: ULF=ultra low frequency; VLF=very low frequency; LF=low frequency; HF=high frequency; *each frequency domain measure was log-transformed. †β corresponds to increase in selective reminding test score per unit increase in ln(HRV). ‡adjusted for age, hypertension, education, low density lipoprotein cholesterol, current/previous tobacco use, diabetes, and depressive symptoms

Table 4 – Adjusted* Between and Within-pair Associations of Verbal Selective Reminding Test Score and Heart Rate Variability (HRV)

	Total group (169 pairs, 78 unpaired twins)				Excluding PTSD (133 pairs, 96 unpaired twins)				
HRV Frequency†	**Between Pairs‡**		**Within Pairs**		**Between Pairs**		**Within Pairs**		**PTSD interaction**
	β	p	β	p	β	p	β	p	p
Total Power, ms^2	3.58	0.12	3.98	0.19	3.71	0.11	7.20	0.02	0.01
ULF, ms^2	3.56	0.10	2.75	0.32	3.20	0.14	6.86	0.02	<0.001
VLF, ms^2	2.64	0.16	5.44	0.03	3.20	0.09	5.02	0.05	0.72
LF, ms^2	1.37	0.37	3.66	0.06	1.41	0.35	2.64	0.18	0.11
HF, ms^2	1.56	0.22	2.22	0.25	1.88	0.14	2.18	0.26	0.67

Abbreviations: ULF=ultra low frequency; VLF=very low frequency; LF=low frequency; HF=high frequency; *Adjusted for age, hypertension, education, low density lipoprotein cholesterol, current/previous tobacco use, diabetes, and depressive symptoms. †Each frequency domain measure was log-transformed; for total power and ULF, ‡β corresponds to increase in selective reminding test score per unit increase in ln(HRV).

Table 5 – Unadjusted and Adjusted* Within-pair Associations of Verbal Selective Reminding Test Score and Heart Rate Variability (HRV) in Monozygotic and Dizygotic Twins

	Monozygotic Twins (104 pairs, 44 singletons)				Dizygotic Twins (65 pairs, 34 singletons)				HRV-Zygosity Interaction	
	Unadjusted‡		**Adjusted**		**Unadjusted**		**Adjusted**		**Unadjusted**	**Adjusted**
HRV†	β	p	β	p	β	p	β	p	p	p
TP	5.60	0.20	5.00	0.24	3.19	0.45	4.92	0.27	0.69	0.65
ULF	2.58	0.51	2.35	0.53	2.94	0.46	4.58	0.26	0.95	0.95
VLF	9.32	0.004	8.41	0.01	2.54	0.49	3.45	0.38	0.15	0.12
LF	6.31	0.02	6.38	0.01	1.55	0.59	1.43	0.65	0.21	0.07
HF	4.36	0.09	4.37	0.08	0.64	0.83	0.34	0.91	0.32	0.13

Abbreviations: TP=total power; ULF=ultra low frequency; VLF=very low frequency; LF=low frequency; HF=high frequency; *Adjusted for age, hypertension, education, low density lipoprotein cholesterol, current/previous tobacco use, diabetes, and depressive symptoms. †Each frequency domain measure was log-transformed; for total power and ULF, ‡β corresponds to increase in selective reminding test score per unit increase in ln(HRV).

Table 6 – Unadjusted and Adjusted* Within-pair Associations of Verbal Selective Reminding Test Score and Heart Rate Variability (HRV) in Monozygotic and Dizygotic Twins, Excluding Those with PTSD

	Monozygotic Twins (80 pairs, 56 singletons)				Dizygotic Twins (53 pairs, 40 singletons)				Zygosity Interaction	
	Unadjusted		**Adjusted**		**Unadjusted**		**Adjusted**		**Unadjusted**	**Adjusted**
HRV†	β	p	β	p	β	p	β	p	p	p
TP	11.29	0.02	11.16	0.02	1.58	0.71	5.19	0.13	0.13	0.12
ULF	8.95	0.04	9.13	0.03	2.41	0.55	5.58	0.18	0.26	0.39
VLF	10.32	0.005	9.26	0.01	-0.50	0.89	2.18	0.59	0.04	0.04
LF	5.91	0.03	6.18	0.02	-0.91	0.76	0.32	0.92	0.09	0.03
HF	5.28	0.05	5.52	0.04	-1.65	0.58	-0.65	0.84	0.08	0.04

Abbreviations: PTSD=post-traumatic stress disorder; TP=total power; ULF=ultra low frequency; VLF=very low frequency; LF=low frequency; HF=high frequency; *Adjusted for age, hypertension, education, low density lipoprotein cholesterol, current/previous tobacco use, diabetes, and depressive symptoms. †Each frequency domain measure was natural log-transformed; for total power and ULF, ‡β corresponds to increase in selective reminding test score per unit increase in ln(HRV).