Published in final edited form as:

Physiol Meas. 2012 June ; 33(6): 1043–1052. doi:10.1088/0967-3334/33/6/1043.

Simultaneously measured pupillary light reflex and heart rate variability in healthy children

C Daluwatte¹, J H Miles², and G Yao¹

G Yao: YaoG@missouri.edu

¹Department of Biological Engineering, University of Missouri, Columbia, MO 65211

²Child Health and Thompson Center for Autism and Neurodevelopmental Disorders, University of Missouri, Columbia, MO 65211

Abstract

We investigated the potential interrelationship between two measures of autonomic nervous system: pupillary light reflex (PLR) and heart rate variability (HRV), in healthy children of 8–16 years old. PLR was measured at both dark- and light-adapted conditions with various stimulation intensities. Simultaneously measured HRV was obtained in five different PLR testing phases: before PLR test, light-adapted PLR test, dark adaptation, dark-adapted PLR test, and after PLR test. The frequency domain HRV parameters measured during PLR test were significantly different from those measured during rest. Both regression analysis and factor analysis indicated that PLR and HRV parameters were not correlated, which suggests that they may provide complementary assessment of different aspects of the overall autonomic nervous system.

Keywords

pupillary light reflex; heart rate variability; children

1. Introduction

The autonomic nervous system (ANS) is a complex and pervasive system that controls the critical visceral functions of the human body and is involved in many psychophysiological responses. Its two divisions, the sympathetic and parasympathetic systems, act in a complementary manner regulated by the central autonomic network (Levy 1997). In such a highly integrated organization, ANS dysfunctions are often widespread with symptoms appearing in multiple subsystems. Hence an interesting question arises: whether an assessment of a specific ANS subsystem may reflect the overall physiological status of the system, and how variations in different measures of ANS correlate with each other (Bär *et al* 2009). This study investigated the possible interrelationship between two specific ANS measures: the pupillary light reflex (PLR) and heart rate variability (HRV).

PLR refers to the change of pupil size in response to luminance changes. The pupil size is controlled by two iris muscles, the sphincter and dilator, which produce pupil constriction and dilation, respectively. The sphincter is mainly innervated by the parasympathetic nervous system (PNS); whereas the dilator is innervated by the sympathetic nervous system (SNS) (Barbur 2004). The parasympathetic nerve fibers synapse in the ciliary ganglion and control the sphincter muscle via the short ciliary nerve (Lowenstein and Loewenfeld 1950). These fibers originate from the pupilloconstrictor neurons at the Edinger-Westphal (EW) nucleus that receives input from the olivary pretectal nucleus (OPN) in the midbrain. The dilator muscles are controlled by post-ganglionic sympathetic fibers from superior cervical ganglion that receives input from the ciliospinal center of Budge (Appenzeller 1999). The

pupil size is determined by the balance between the sympathetic and parasympathetic systems (Fotiou *et al* 2000). Abnormal PLR has been observed in many neurological disorders associated with ANS dysfunction (Bremner 2009) such as panic disorder (Kojima *et al* 2004), autism (Fan *et al* 2009b), and Parkinson's disease (Giza *et al* 2011, Stergiou *et al* 2009).

Heart rate variability (HRV) assesses the beat-to-beat variations of the heart rate. Both parasympathetic and sympathetic systems are involved in cardiovascular system regulation. Stimulation of the parasympathetic fibers (vagus nerves) reduces the heart rate; whereas the sympathetic stimulation increases heart rate through the sinoatrial (SA) node. HRV parameters have been widely applied to evaluate cardiac autonomic functions (Kamath and Fallen, 1993) and have been useful in identifying ANS function in various allostatic systems (Thayer and Sternberg 2006). HRV has also been applied in evaluating ANS dysfunction in disorders such as panic disorder (Yeragani *et al* 1993), schizophrenia (Bär *et al* 2005, Bär *et al* 2007), and sleep disorders (Bonnet and Arand 1998).

The possible association between PLR and HRV has been investigated recently by several authors. Correlations between HRV and PLR parameters were found in adults during exercise (Kaltsatou *et al* 2011) and in patients with acute schizophrenia (Bär *et al* 2008). However, Bär *et al* (2009) found limited correlation between specific PLR and HRV parameters in healthy adults 19–64 years old. It is unclear whether similar relationships may exist in younger subjects as both HRV and PLR are affected by age. Here we report a study that measured simultaneously the PLR and HRV in healthy 8–16 year old children to further investigate the possible association between measures obtained from pupillary light reflex and heart rate variability.

2. Methods

2.1. Participants

A total of 54 healthy 8–16 years old children participated in this study including 27 boys (138.2±28.2 months) and 27 girls (age 136.1±28.1 months) without any known vision, neurological and cardiovascular problems. All were tested at least 1 hour after their last meal. Among the participants, 23 children (9 girls and 14 boys) were tested in the morning (8am–12pm) and the remaining 31 (18 girls and 13 boys) were tested in the afternoon (12pm–5pm). All participants and their legal guardians were thoroughly informed of the procedure and consented with a written informed consent as approved by the Institutional Review Board of the University of Missouri.

2.2. Instrument

A binocular pupilogram recording system was used to measure the PLR. This system is similar to that reported previously (Fan *et al* 2009a, 2009b) except faster cameras were used to record pupil images at 115 fps. The spatial resolution of the system was 35µm/pixel. A 530nm green LED provided the light stimulus. The stimulus pulse width was 100 ms. Neutral density filters and LED current were used to control the stimulation intensity.

To obtain heart rate variability, real-time QRS intervals were recorded by using a remote heart rate measuring device (Polar RS800CX, Polar Electro Oy, Finland). A chest strap with enclosed heart rate sensor and wireless transmitter was wrapped around the participant's chest. The system acquires the ECG at 1kHz rate. The heart beat QRS signals transmitted from the chest strap were received and recorded by a watch-like device. Multiple studies (Gamelin *et al* 2006, Gamelin *et al* 2008, Goodie *et al* 2000, Nunan *et al* 2009, Porto and Junqueira Jr 2009) have shown this device to be reliable for short term R-R interval measurement and to provide results consistent with traditional ECG.

2.3. Test procedure

Heart rate recording was started 5 minutes before the PLR test to acquire a baseline reference. The participant remained in a sitting position during the entire test. PLR was first measured in a light adapted (LA) condition (30 cd/m² room illumination). Three optical stimulation intensities were used in LA tests: 69.3 cd/m², 872.1 cd/m² and 8721.1 cd/m² to induce different amounts of pupillary constriction. Following a 15 min dark adaptation (<0.02 cd/m² room illumination), dark-adapted (DA) PLR was then measured at a stimulation intensity of 63.1 cd/m². At each test condition, the left eye was stimulated first and the right eye was stimulated next. Images of both pupils were recorded for analysis. The measurements were repeated four times for each condition with a 20 sec interval between measurements. Pupil imaging was started 1 sec before the onset of the 100 ms optical stimulation to obtain baseline pupil size, and was recorded continuously for 4.5 sec. Heart rate was continuously recorded during the PLR test and stopped 5 minutes after completing the PLR test. The entire test procedure is illustrated in figure 1.

2.4 Data analysis

The pupilogram was constructed by extracting the pupil size from acquired pupil images as described in detail previously (Fan *et al* 2009a, 2009b). As in our previous studies, the PLR parameters listed in table 1 were measured in this study to characterize PLR responses. PLR parameters obtained from both eyes during the four repeat measurements were averaged to calculate the mean value at any given condition.

HRV was assessed using both time domain and frequency domain methods (Malik *et al* 1996). As shown in table 2, two time domain parameters were calculated: SDNN and rMSSD. To be consistent with most recent studies on HRV, two frequency domain parameters were analyzed: the normalized HF and the LF/HF ratio. Frequency domain power spectrum was analyzed by using Fast Fourier Transform (FFT) as described by Malik *et al* (1996). To study potential effect of the PLR tests on HRV parameters, HRV was analyzed in five testing phases: before PLR test (5 min), LA test (10 min), dark adaptation (15 min), DA test (5 min), after PLR test (5 min).

The distributions of PLR and HRV parameters were verified to conform to normal distributions using the Kolmogorov-Smirnov test. For each PLR and HRV parameter, the Analysis of Covariance (ANCOVA) using the PROC MIXED procedure in SAS (Version 9.2, SAS Institute Inc., Cary, NC, USA) was applied to examine the effects of test conditions, gender and age. *P* values were Bonferroni-corrected appropriately using the number of stimulus conditions for PLR and the number of testing phases for HRV. To study the association between PLR and HRV parameters, linear correlations between them were first analyzed with Pearson product moment correlation (PROC CORR procedure in SAS). To investigate whether variations in PLR parameters can be explained by a combination of HRV parameters or vice versa, multilinear regression (PROC REG procedure in SAS) was applied with HRV (or PLR) parameters treated as dependent variables and PLR (or HRV) parameters treated as independent variables. Moreover, explanatory factor analysis (PROC FACTOR procedure in SAS) was performed on the overall data set to study the potential relationships among PLR and HRV parameters using oblique matrix rotation. Factor analysis reduces a multivariate system to a system with a fewer number of dimensions by categorizing highly correlated dependent variables into a single factor.

3. Results

3.1 Pupillary Light Reflex

Table 3 shows the mean values and standard deviations of all measured PLR parameters. The initial pupil diameter D_0 increased from 6.6 ± 0.6 mm in LA to 7.5 ± 0.8 mm in DA. The relative constriction amplitude $\Delta A_{\%}$ increased from $12.8\% \pm 6.0\%$ at LA 69.3 cd/m^2 to $40.6\% \pm 7.3\%$ at LA 8721.1 cd/m^2 . The PLR latency was between 200 to 300 ms. In LA tests, PLR latency decreased with stimulus intensity (F value = 236.0, $p < 0.0001$ in ANOVA test for a linear trend). $\Delta A_{\%}$ was similar at LA 8721.1 cd/m^2 and DA 63.1 cd/m^2 , but the DA latency was ~ 30 ms longer. Average constriction velocity v_C ranged from 1.0 to 2.8 mm/s at the four stimulation conditions; whereas the average redilation velocity v_R was 0.5–1.0 mm/s.

3.2 Heart Rate Variability

The HRV parameters are shown in table 4. The average heart rate was around ~ 90 bpm in our study population. The time domain HRV parameter SDNN was ~ 65 –72 ms on average and the rMSSD ranged between 33–39 ms. The frequency domain HRV parameters were: HF normalized power (~ 22 –32) and LF/HF ratio (~ 2 –4).

3.3 Interrelationship between PLR and HRV

As shown in table 4, the average heart rate, SDNN and rMSSD measured during different PLR testing phases were not significantly different (ANCOVA F value = 1.06, 0.52 and 0.86, $p = 0.38$, 0.72 and 0.48, respectively). In fact, the HRV parameters obtained during different testing phases were highly correlated with each other. The heart rate had the highest correlation coefficient among all parameters. Table 5 shows that the correlation weakened as the measurement phase became distinct in time, i.e. the r value decreased from 0.95 between phase 1 & 2 (5 min interval) to 0.88 between phase 1 & 5 (25 min interval).

However, the correlation of frequency domain HRV among different testing phases was not as high as the time domain parameters. The LF/HF ratio had the least correlation r values (0.54–0.87) among different testing phases. In fact, the ANCOVA model indicated that the PLR testing phase had a significant fixed effect on LF/HF ratio and HF normalized power (ANCOVA F value = 6.74 and 8.52, $p < 0.0001$ and $p < 0.0001$). As shown in figure 2, HF normalized power was significantly lower during the two PLR testing phases (both LA and DA tests) than during the three resting periods (before PLR test, dark adaptation and after PLR test); while LF/HF ratio was significantly higher.

The Pearson product moment correlation did not show any significant correlation between simultaneously measured PLR parameters and HRV parameters ($p > 0.05$, $r < 0.5$ for all the correlations). Further multilinear regression analysis concluded that no significant model could be developed to explain variations in PLR parameters by using HRV parameters, nor vice versa.

Using exploratory factor analysis with oblique matrix rotation (OBVARIMAX in SAS), the multivariate PLR and HRV dataset was reduced to a system of four factors which were uncorrelated with each other (inter-factor correlations $r < 0.3$). These four factors were determined such that the total variance of the dataset (proportion = 1) can be explained by the resulting system with a reduced dimension and also using the scree plot to identify the last substantial drop which is after factor 4 (Fabrigar *et al* 1999). The factor loading after oblique matrix rotation (OBVARIMAX in SAS) is shown in table 6, where each factor loading represents the correlation between a PLR or HRV parameter with the identified factor. The factors 1 and 2 were mainly attributed to loadings from HRV parameters. The heart rate and

time domain HRV parameters (SDNN and rMSSD) had the highest correlation with factor 1; whereas the two frequency domain HRVs, LF/HF and HF_n, had the highest correlation with factor 2. PLR parameters were the major contributors to factor 3 and 4. Factor 3 had a high loading from t_C and t_R, while factor 4 had moderately high correlations with t_L and D₀. The reliability of each variable was evaluated by analyzing “Cronbach’s Coefficient of Alpha When Deleted” with “PROC CORR”. The results indicated that heart rate should be removed from factor 1. In addition, a “Cronbach’s Coefficient of Alpha” of 0.7 was used as the acceptable threshold to evaluate the reliability of the entire factor system. The results indicated that the final solution should only consist of factor 1 and 3, where factor 1 represented HRV system and factor 3 represented PLR system. Communalities of selected parameters over the final factor solution were above 0.8 for all the parameters selected in the factor solution. Such consistently high communalities suggested that the sample size of the design had minimal effect on the factor solution (MacCallum *et al* 1999). The numbers shown in table 6 were calculated by using PLR data obtained at LA 8721.1 cd/m² and HRV data obtained during the LA testing phase. However, the same conclusion was reached for all other testing conditions.

4. Discussion

The PLR parameters obtained in this study are similar to those reported previously (Fan *et al* 2009b) in a similar age group at similar stimuli conditions. Similarly, the time domain HRV parameters obtained in this study are consistent with those reported in previous studies (Umetani *et al* 1998). Our mean HF_n (%) is smaller than that reported by Gamelin *et al* (2008) in boys younger than 11 years in supine position. We think these differences can be attributed to the seated position used in our study. It is known that HRV measured at supine position has higher HF_n (%) and smaller LF/HF than that measured at standing position (Montano *et al* 1994). The HRV parameters obtained in seated position are in between of those obtained at supine and standing positions (Chan *et al* 2007).

Because 43% of participants were tested in the morning and the rest were tested in the afternoon, we examined the potential effect of different time-of-day of the test. The results showed no significant difference between the PLR and HRV measurements obtained in children tested in the morning and those tested in the afternoon (t test $p > 0.15$). The HRV results (table 4) measured in phases 2 and 3 (figure 1) had longer measurement time (10 min and 15 min respectively) than the other three phases (5 min each). To investigate whether such a difference may lead to different HRV values, we divided the 10 min LA test period into two 5 min segments and calculated HRV parameters in each segment. The obtained HRV parameters in the two 5 min segments were similar to each other (paired t test $p > 0.28$) and were also similar to the one calculated over the entire 10 min period. The same results were obtained for the HRV parameters obtained in the 15 min DA period.

The frequency domain HRV parameters were clearly affected by the PLR testing phases. The mean results (table 4) indicate that HF_n decreased 27.3% and LF/HF increased 45.9% when going from “before test” to PLR test. This trend is similar to posture induced HRV changes that are related to sympathetic activity caused by orthostatic stress (Mukai and Hayano 1995, Montano *et al* 1994, Yeragani *et al* 1993). Because the participant slightly inclined forward (~15°) during the PLR tests, it is possible that such posture change led to elevated sympathetic activity due to increased muscle stress. In addition, the PLR test itself may induce some task-related stress. Delaney and Brodie (2000) reported that psychological stress can decrease high frequency HRV and increase low frequency HRV. Vagal tone and respiratory sinus arrhythmia (RSA) are the principle contributors to the high frequency power of HRV while both vagal and sympathetic tones influence the low frequency component (Berntson *et al* 1997). Overall the observed changes in frequency domain HRV

parameters suggest lower vagal tone and increased sympathetic modulation during the PLR test.

PLR parameters and HRV parameters are categorized as independent and uncorrelated factors according to the factor analysis. The SDNN and RMSSD were closely aligned only with factor 1 and had very low loading on other factors (table 6). Similarly, the constriction time and redilation time were aligned only with factor 3. LF/HF and HF_n were highly aligned with factor 2 with high loading. Though with a smaller loading, PLR latency and initial pupil diameter were best aligned with factor 4. Therefore, the data clearly indicated a lack of association between PLR and HRV. This observation may be attributed to the fact that healthy typically developing children were tested in this study. In this group, many PLR parameters were quite consistent with very small between-subjects variation. Noticeably, the coefficient of variation of PLR latency was <10%, in agreement with the fact that latency did not contribute to the four-factor system derived from common factor analysis.

The lack of association between PLR and HRV can be further corroborated by the different gender effect in PLR and HRV parameters. In agreement with a previous report by Krishnan *et al* (2009) in a large group of children of similar age range, our data showed that girls had a significantly higher heart rate than boys. A higher heart rate indicates stronger sympathetic influence (Malik *et al* 1996). We observed a trend of smaller resting pupil size in girls although the difference didn't reach statistical significance. The same difference was reported by Fan *et al* (2009a) as statistically significant in individuals with a tightly controlled age range. A smaller resting pupil size may suggest a stronger parasympathetic modulation or a weaker sympathetic modulation (Barbur 2004). Summarizing the above comparisons, it is clear that gender effects in PLR and HRV do not completely corroborate with each other.

Although PLR and HRV are part of an integrated ANS, individual neurological pathways behave differently. The natural variations in an individual system may not affect other subsystems. However, this conclusion may not hold true for some disorders associated with ANS dysfunctions where pervasive ANS changes exist in multiple subsystems. For example, significant correlations between PLR and HRV were found in patients with acute schizophrenia (Bär *et al* 2008).

5. Conclusion

We measured PLR and HRV simultaneously in age- and gender-matched healthy children 8–16 years old. Gender effect was observed in both HRV and PLR parameters. However, significant age effect was observed in HRV parameters but not in PLR. We found the frequency domain HRV parameters were significantly different in different PLR testing phases, likely due to the psychological or/and physiological stress induced by the PLR tests. Using both regression analysis and factor analysis, we conclude that variations of PLR and HRV are not associated in healthy children. However, this conclusion might be altered in case of ANS disorders or other situations when pervasive changes might appear in multiple different ANS subsystems. Nevertheless, PLR and HRV may provide complementary assessment of different aspects of the overall autonomic nervous system.

Acknowledgments

This study was supported by National Institute of Neurological Disorders and Stroke (1R21NS070299-01) and U. S. Army Medical Research Materiel Command (DoD W81XWH-10-1-0474). We thank Andrew Lofgreen and Jill Akers for their help in recruiting participants, and Deborah Ratliff for proofreading this manuscript.

References

- Appenzeller, O. The Autonomic Nervous System Part I. Normal Functions. Amsterdam: Elsevier; 1999.
- Bär KJ, Boettger MK, Koschke M, Schulz S, Chokka P, Yeragani VK, Voss A. Non-linear complexity measures of heart rate variability in acute schizophrenia. *Clinical Neurophysiology*. 2007; 118:2009–2015.
- Bär KJ, Boettger MK, Schulz S, Harzendorf C, Agelink MW, Yeragani VK, Chokka P, Voss A. The interaction between pupil function and cardiovascular regulation in patients with acute schizophrenia. *Clinical Neurophysiology*. 2008; 119:2209–2213. [PubMed: 18722810]
- Bär KJ, Letsch A, Jochum T, Wagner G, Greiner W, Sauer H. Loss of efferent vagal activity in acute schizophrenia. *J Psychiatric Research*. 2005; 39:519–527.
- Bär KJ, Schulz S, Koschke M, Harzendorf C, Gayde S, Berg W, Voss A, Yeragani VK, Boettger MK. Correlations between the autonomic modulation of heart rate, blood pressure and the pupillary light reflex in healthy subjects. *J Neurological Sci*. 2009; 279:9–13.
- Barbur, JL. Learning from the pupil - Studies of basic mechanisms and clinical applications. In: Chalupa, LM.; Werner, JS., editors. *The Visual Neurosciences*. MIT Press; Cambridge, MA: 2004.
- Berntson GG, Thomas Bigger J Jr, Eckberg DL, Grossman P, Kaufmann PG, Malik M, Nagaraja HN, Porges SW, Saul JP, Stone PH, Van Der Molen MW. Heart rate variability: Origins methods, and interpretive caveats. *Psychophysiology*. 1997; 34:623–648. [PubMed: 9401419]
- Bonnet MH, Arand DL. Heart rate variability in insomniacs and matched normal sleepers. *Psychosomatic Med*. 1998; 60:610–615.
- Bremner F. Pupil evaluation as a test for autonomic disorders. *Clinical Autonomic Res*. 2009; 19:88–101.
- Chan HL, Lin MA, Chao PK, Lin CH. Correlates of the shift in heart rate variability with postures and walking by time-frequency analysis. *Computer Methods and Programs in Biomed*. 2007; 86:124–130.
- Delaney JPA, Brodie DA. Effects of short-term psychological stress on the time and frequency domains of heart-rate variability. *Perceptual and Motor Skills*. 2000; 91:515–524. [PubMed: 11065312]
- Fabrigar LR, Maccallum RC, Wegener DT, Strahan EJ. Evaluating the use of exploratory factor analysis in psychological research. *Psychological Methods*. 1999; 4:272–299.
- Fan X, Hearne L, Lei B, Miles JH, Takahashi N, Yao G. Weak gender effects on transient pupillary light reflex. *Autonomic Neuroscience: Basic and Clinical*. 2009a; 147:9–13.
- Fan X, Miles JH, Takahashi N, Yao G. Abnormal transient pupillary light reflex in individuals with autism spectrum disorders. *J Autism and Developmental Disorders*. 2009b; 39:1499–1508.
- Fotiou F, Fountoulakis KN, Goulas A, Alexopoulos L, Palikaras A. Automated standardized pupillometry with optical method for purposes of clinical practice and research. *Clinical Physiology*. 2000; 20:336–347. [PubMed: 10971544]
- Gamelin FX, Baquet G, Berthoin S, Bosquet L. Validity of the polar S810 to measure R-R intervals in children. *International Journal of Sports Medicine*. 2008; 29:134–138. [PubMed: 17614016]
- Gamelin FX, Berthoin S, Bosquet L. Validity of the polar S810 Heart rate monitor to measure R-R intervals at rest. *Medicine and Science in Sports and Exercise*. 2006; 38:887–893. [PubMed: 16672842]
- Giza E, Fotiou D, Bostantjopoulou S, Katsarou Z, Karlovasitou A. Pupil light reflex in Parkinson's disease: Evaluation with pupillometry. *International Journal of Neuroscience*. 2011; 121:37–43. [PubMed: 21034369]
- Goodie JL, Larkin KT, Schauss S. Validation of the Polar heart rate monitor for assessing heart rate during physical and mental stress. *Journal of Psychophysiology*. 2000; 14:159–164.
- Kaltsatou A, Kouidi E, Fotiou D, Deligiannis P. The use of pupillometry in the assessment of cardiac autonomic function in elite diVerent type trained athletes. *European Journal of Applied Physiology*. 2011; 111:2079–2087. [PubMed: 21259023]

- Kamath MV, Fallen EL. Power spectral analysis of heart rate variability: A noninvasive signature of cardiac autonomic function. *Critical Reviews in Biomedical Engineering*. 1993; 21:245–311. [PubMed: 8243093]
- Kojima M, Shioiri T, Hosoki T, Kitamura H, Bando T, Someya T. Pupillary light reflex in panic disorder: A trial using audiovisual stimulation. *European Archives of Psychiatry and Clinical Neuroscience*. 2004; 254:242–244. [PubMed: 15309394]
- Krishnan B, Jeffery A, Metcalf B, Hosking J, Voss L, Wilkin T, Flanagan DE. Gender differences in the relationship between heart rate control and adiposity in young children: A cross-sectional study (EarlyBird 33). *Pediatric Diabetes*. 2009; 10:127–134. [PubMed: 19017283]
- Levy MN. Neural control of cardiac function. *Bailliere's Clinical Neurology*. 1997; 6:227–244. [PubMed: 9483290]
- Lowenstein O, Loewenfeld IE. Mutual role of sympathetic and parasympathetic in shaping of the pupillary reflex to light; pupillographic studies. *Archives of neurology and psychiatry*. 1950; 64:341–377.
- Maccallum RC, Widaman KF, Zhang S, Hong S. Sample size in factor analysis. *Psychological Methods*. 1999; 4:84–99.
- Malik M, Camm AJ, Bigger JT Jr, Breithardt G, Cerutti S, Cohen RJ, Coumel P, Fallen EL, Kennedy HL, Kleiger RE, Lombardi F, Malliani A, Moss AJ, Rottman JN, Schmidt G, Schwartz PJ, Singer DH. Heart rate variability. Standards of measurement, physiological interpretation, and clinical use. *European Heart Journal*. 1996; 17:354–381. [PubMed: 8737210]
- Montano N, Ruscone T, Porta A, Lombardi F, Pagani M, Malliani A. Power spectrum analysis of heart rate variability to assess the changes in sympathovagal balance during graded orthostatic tilt. *Circulation*. 1994; 90:1826–1831. [PubMed: 7923668]
- Mukai S, Hayano J. Heart rate and blood pressure variabilities during graded head-up tilt. *Journal of Applied Physiology*. 1995; 78:212–216. [PubMed: 7713814]
- Nunan D, Gay D, Jakovljevic DG, Hodges LD, Sandercock GRH, Brodie DA. Validity and reliability of short-term heart-rate variability from the Polar S810. *Medicine and Science in Sports and Exercise*. 2009; 41:243–250. [PubMed: 19092682]
- Porto LGG, Junqueira LF Jr. Comparison of time-domain short-term heart interval variability analysis using a wrist-worn heart rate monitor and the conventional electrocardiogram. *PACE - Pacing and Clinical Electrophysiology*. 2009; 32:43–51.
- Stergiou V, Fotiou D, Tsiptsios D, Haidich B, Nakou M, Giantselidis C, Karlovasitou A. Pupillometric findings in patients with Parkinson's disease and cognitive disorder. *International Journal of Psychophysiology*. 2009; 72:97–101. [PubMed: 19047001]
- Thayer JF, Sternberg E. Beyond heart rate variability: Vagal regulation of allostatic systems. *Ann NY Acad Sci*. 2006; 1088:361–72. [PubMed: 17192580]
- Umetani K, Singer DH, McCraty R, Atkinson M. Twenty-four hour time domain heart rate variability and heart rate: Relations to age and gender over nine decades. *Journal of the American College of Cardiology*. 1998; 31:593–601. [PubMed: 9502641]
- Yeragani VK, Pohl R, Berger R, Balon R, Ramesh C, Glitz D, Srinivasan K, Weinberg P. Decreased heart rate variability in panic disorder patients: A study of power-spectral analysis of heart rate. *Psychiatry Research*. 1993; 46:89–103. [PubMed: 8464959]

Figure 1.

An illustration of the test procedure used in this study.

Figure 2.

A comparison of (a) normalized HF power and (b) LF/HF ratio during different testing phases. P -values shown were obtained from paired t-test. The testing phases are numbered as 1: before PLR test, 2: during LA PLR, 3: during dark adaptation, 4: during DA PLR, 5: after PLR test. The error bars indicate the standard deviation.

Table 1

PLR parameters measured in this study.

Symbol (unit)	Definition
D_0 (mm)	“Base pupil diameter”: pupil diameter before stimulus onset
$D_{\min}$ (mm)	“Minimal pupil diameter”: pupil diameter before stimulus onset
$\Delta A_{\%}$	“Relative constriction amplitude”: $D_0^2 - D_{\min}^2 / D_0^2$
t_L (ms)	“Constriction latency”: the elapsed time between light stimulus and beginning of constriction
t_C (ms)	“Constriction time”: the time interval from beginning of constriction to the maximal constriction
t_R (ms)	“Redilation time”: the time interval between maximal constriction and recovery to half of the constriction $D_0^2 - D_{\min}^2 / 2$
v_C (mm/s)	“Constriction velocity”: average velocity of the relative constriction $D_0 - D_{\min} / t_C$
v_R (mm/s)	“Recovery velocity”: average velocity of the relative recovery $D_0 - D_{\min} / (2t_R)$

Table 2

HRV parameters measured in this study.

Symbol (unit)	Definition
HR (bpm)	Average heart rate
SDNN (ms)	Standard deviation of normal RR intervals
rMSSD (ms)	Root mean square successive difference of the RR intervals
HF _n	Normalized Powers of HF band (HF, 0.15 – 0.4 Hz), i.e., the relative powers of HF band when removing Very Low frequency (VLF, 0 – 0.04 Hz) band power from total power. HF power/(Total power – VLF power)×100%
LF/HF	The ratio of LF/HF power, (LF, 0.04 – 0.15 Hz)

Table 3

Summary of PLR results. The results are represented as group mean $\pm$ standard deviation.

	Stimulation intensity (cd/m ²)			
	LA 69.3	LA 872.1	LA 8721.1	DA 63.1
Resting pupil diameter (mm)		6.6 $\pm$ 0.6		7.5 $\pm$ 0.8
Relative constriction amplitude (%)	12.8 $\pm$ 6.0	27.0 $\pm$ 7.4	40.8 $\pm$ 7.89	44.6 $\pm$ 7.3
Latency (ms)	269.3 $\pm$ 26.6	236.1 $\pm$ 15.8	211.8 $\pm$ 13.5	239.3 $\pm$ 15.9
Constriction Time (ms)	368.6 $\pm$ 71.8	398.5 $\pm$ 56.6	463.4 $\pm$ 52.8	575.6 $\pm$ 52.3
Redilation Time (ms)	417.6 $\pm$ 74.7	501.7 $\pm$ 109.9	610.7 $\pm$ 135.8	817.8 $\pm$ 172.0
Constriction velocity (mm/s)	1.0 $\pm$ 0.5	2.0 $\pm$ 0.7	2.8 $\pm$ 0.8	2.8 $\pm$ 0.8
Redilation velocity (mm/s)	0.5 $\pm$ 0.2	0.8 $\pm$ 0.3	1.1 $\pm$ 0.3	1.0 $\pm$ 0.0

Table 4

Summary of HRV results. The results are represented as group mean $\pm$ standard deviation.

	Before PLR test	Testing phase			After PLR test
		During LA PLR test	During DA period	During DA PLR test	
Heart rate (bpm)	89.9 $\pm$ 12.1	90.1 $\pm$ 12.1	92.7 $\pm$ 12.1	91.0 $\pm$ 13.2	93.5 $\pm$ 13.2
SDNN (ms)	67.6 $\pm$ 27.0	70.2 $\pm$ 23.9	67.9 $\pm$ 29.1	71.5 $\pm$ 28.5	65.2 $\pm$ 25.0
rMSSD (ms)	38.9 $\pm$ 20.3	36.7 $\pm$ 16.9	34.1 $\pm$ 17.7	36.4 $\pm$ 18.6	33.2 $\pm$ 16.9
HF _n (%)	31.8 $\pm$ 11.0	23.1 $\pm$ 7.1	26.9 $\pm$ 10.1	22.3 $\pm$ 8.2	24.9 $\pm$ 10.1
LF/HF ratio	2.6 $\pm$ 1.4	3.8 $\pm$ 1.6	3.2 $\pm$ 1.4	4.1 $\pm$ 2.1	3.7 $\pm$ 2.3

Table 5

Correlations between average heart rate measured in different testing phases. The results are represented as Pearson's r-value.

	Before Test	LA Test	DA	DA Test	After Test
Before Test	1.00 ^a	0.95 ^a	0.91 ^a	0.92 ^a	0.88 ^a
LA Test		1.00 ^a	0.94 ^a	0.95 ^a	0.90 ^a
DA			1.00 ^a	0.93 ^a	0.91 ^a
DA Test				1.00 ^a	0.90 ^a
After Test					1.00 ^a

^ap<0.0001

Table 6

Factors loading for the entire data set by using factor analysis. The numbers represent correlation between PLR/HRV parameters and the identified factors.

	Factor 1	Factor 2	Factor 3	Factor 4
RMSSD	0.93	0.19	0.03	-0.02
SDNN	0.95	-0.07	0.00	-0.02
HRV HR	-0.81	-0.02	-0.06	0.02
LF/HF ratio	-0.02	-0.90	0.10	0.01
HF _n	0.01	0.94	0.03	0.01
Constriction Time	-0.01	0.00	0.93	-0.07
Redialtion Time	0.02	-0.05	0.91	0.09
PLR Latency	0.02	-0.03	0.04	0.42
Initial Pupil Diameter	-0.02	0.02	-0.02	0.34