Report article

An open-source R-R interval analyzer with a graphical user interface for electrophysiological studies

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Abstract

Heart rate variability (HRV) is an established measure for evaluating changes in human autonomic nervous functions, including reactions to mental stresses. In particular, the ratio of the low-frequency components (low-frequency (LF), 0.04–0.15 Hz) to the high-frequency components (HF, 0.15–0.40 Hz) in the R-R interval fluctuation (the LF/HF ratio) reflects the sympathetic tonus of the subject. This study introduces an easy-to-use R-R interval analyzer with a graphical user interface (GUI) for electrophysiological studies of HRV. The GUI framework and the analyzer itself are open source and the complete source code is freely accessible via the Internet, allowing their modification or customization by users. This application was tested in subjects under oral enquiries on the national examination which they were preparing for. Besides the low-cutoff frequency was low (0.08 Hz), more than 95% QRS complexes were automatically detected, indicating the usefulness of the application.

Introduction

The heart rate (HR) constantly fluctuates. This reflects changes in the body's internal condition, and the heart rate variability (HRV) provides a reliable indication of many physiological factors that affect the HR. Various indexes can be derived from HRV; among these, frequency-domain analysis is widely applied to evaluate the autonomic nervous functions [1, 2]. In this, the spectrum of HRV is divided into its high-frequency components (HF, 0.15–0.40 Hz) and low-frequency components (LF, 0.04–0.15 Hz). HF reflects the respiratory rhythms driven by the parasympathetic nervous system, whereas low-frequency (LF) reflects the activity of both the sympathetic and the parasympathetic nervous systems; thus, the LF/HF ratio is considered to be a measure of the sympathetic nervous activity [3]. The LF/HF ratio has been used to evaluate the mental stress of nuclear power plant operators [4], health care workers [5], computer workers [6], elderly subjects with orthostatic hypotension [7], young subjects under sleep restriction [8], and blood donors [9]. Other examples of the application of the LF/HF ratio include evaluating the effect of touching wood [10] and predicting risk of cardiovascular diseases [11].

The immediate HR is calculated from the electrocardiogram (ECG) as the reciprocal of the time interval between two adjacent QRS complexes (the R-R interval). It can be difficult to

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manually detect QRS complexes from an entire ECG chart, so a number of computer-based R-R interval analyzers have been developed. However, most are commercial or proprietary, or are based on UNIX commands without a graphical user interface (GUI), such as the WFDB Software Package [12].

Since the 1980's, the field of computer programming has seen movements, such as the Free Software [13, 14] and Open-Source [15] movements, that have aimed to improve computer programs through sharing source code among many programmers. Free access to the source code also allows users to customize applications for their own environments, for example by changing the structure and size of the data set, which can vary according to recording systems used in each laboratory.

This report introduces an open-source, multi-platform, off-line R-R interval analyzer with a GUI for physiological studies. It has been fully developed with an open-source programming environment.

Materials and Methods

1. GUI framework

The application was created using Lazarus (version 1.8.2; http://www.lazarus-ide.org/), an open-source rapid application developing environment (RAD) that is highly compatible with Borland Delphi and its predecessor, Turbo Pascal. Lazarus and the applications built with it run on many operating systems, including Linux, Macintosh, and Windows (both 32-bit and 64-bit).

A RAD is a combination of a resource editor and a class library that includes an encapsulated call-back loop. It allows programmers to develop applications with a GUI without needing experience of complex application program interfaces (APIs). Unlike Eclipse, another open-source RAD based on the Java language, Lazarus creates native machine code for various target central processing units, including Power PC, Intel 80386, Intel x86-64, and ARM, and for the GUI subsystems of the operating systems; this results in more effective and faster object code.

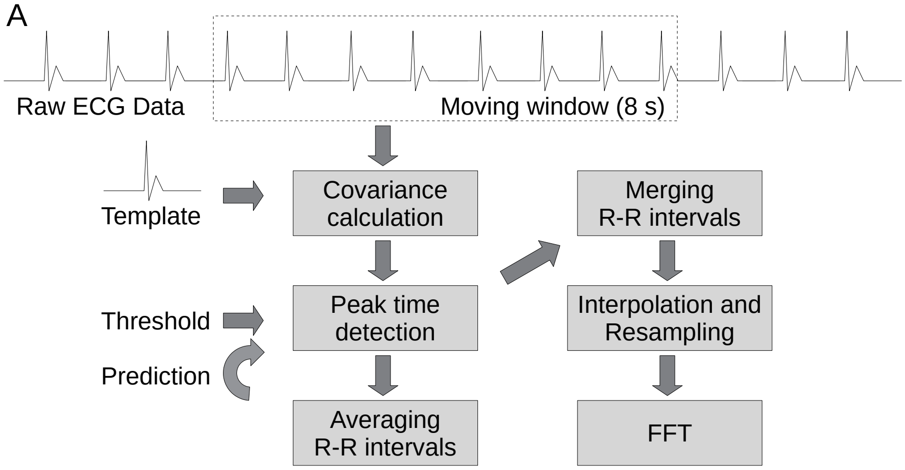
In this study, native machine code was generated for a Macintosh build, a 64-bit Windows build, and a 32-bit Windows build. Two Macintosh computers were used (Mac Pro and iMac; Apple, CA, USA), both running OS X El Capitan, as well as a two-in-one tablet (T101HA; ASUS, ROC) running Windows 10 Home 64 bit, and a tablet (W3-810fp; Acer, ROC) running Windows 10 Home 32 bit.

2. QRS complex detection

The algorithm for the R-R interval analyzer is illustrated in Figure 1. The ECG data are processed using an 8-s time window that is moved through the data, overlapping the previous window by 0.5 s (Figure 1A). The user first indicates a typical QRS waveform as a 0.54-s template using the ECG Waveform Window (Figure 2A and B), and the selected template is automatically corrected for the direct current (DC) offset. The covariance between the ECG data and this template is then repeatedly calculated for each sampling point by a three-step process (Figure 1B). Step 1: A data segment of the same length as the template is extracted from the ECG data. Step 2: The DC offset is removed from the extracted data segment by subtracting the average value. Step 3: The covariance between the data segment and the template is calculated. The next data segment, beginning at the following sampling time, is extracted from the ECG data in the similar way as Step 1 and the process is continued until the whole 8-s time window has been scanned. These steps provide a time course of the covariance between ECG and the template over the current 8-s time window (the Covariance Window in Figure 2A and B).

The QRS complexes are detected by dividing the covariance data into slices with a user-defined threshold (indicated by the horizontal white line in

A



B

```
procedure TForm1.CalcCorrelation;
var data: template_data;
    mm, m, c, d: int64;
    i, ii: integer;
begin
  for ii := succ(template_t1) to data_len - template_t1 do begin
    for i := -template_t1 to template_t1 do data[i] := EEGdata[i + ii];
    m := 0;
    for i := -template_t1 to template_t1 do inc(m, data[i]);
    m := m div data_len;                                             (1)
    for i := -template_t1 to template_t1 do dec(data[i], m);
    c := 0; mm := 0;
    for i := -template_t1 to template_t1 do begin
      inc(c, data[i] * template[i]);                                   (2)
      inc(mm, sqr(data[i]))
    end;
    CorData[ii] := c; AvrData[ii] := m; VarData[ii] := mm
  end
end;
```

C

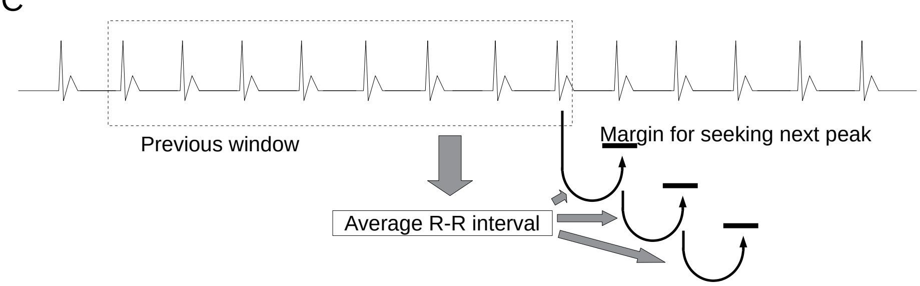


Figure 1. Structure of the analyzer.
A: A schematic diagram of the R-R interval analyzer.
B: A section of the Pascal source code for the covariance calculation: (1) direct current removal; (2) covariance calculation (data for variance are also collected).
C: A schematic diagram of the application's Prediction Mode.
Abbreviation: FFT (fast Fourier transform).

the Covariance Window). The timestamps of the QRS complexes derived within the time window are merged during this stage. If the cardiac rhythm is sufficiently regular, the system's Prediction Mode can be used (Figure 1C). In this mode, the seek period for the next peak covariance is determined from the previous QRS timestamp and the average R-R interval obtained from the previous time window, with a user-defined margin (the horizontal yellow lines in Figure 2A and B). If not enough QRS complexes are detected, the slicing method is temporarily used to resume QRS detection within the current time window. Once a threshold for the covariance time course has been established, QRS detection in noisy ECG data can be improved by reducing the noise associated with the template through averaging. If necessary, the user can add missing QRS complexes manually or delete inappropriately detected QRS complexes from the list of QRS timestamps via the application.

3. HRV power spectra

The time course of R-R intervals calculated from the QRS timestamps (Figure 2C), is interpolated by a cubic spline function and resampled at 10 Hz. The HR at any given time can be calculated from the interpolated R-R intervals (shown in the HR Window in Figure 2D). The power spectra associated with the interpolated R-R intervals are obtained by repeated fast Fourier transforms (FFTs) performed every 1 s in a 256-point (25.6 s) sliding window with the Hanning window function. This results in a frequency resolution of approximately 0.039 Hz and a time resolution of 1 s. The LF power is obtained by calculating the mean of the third and fourth components (0.078 and 0.117 Hz) and the HF power by averaging the fifth to eleventh components (0.156, 0.195, 0.234, 0.273, 0.313, 0.352, and 0.391 Hz, respectively). From these, the LF/HF ratio can be obtained (shown in the LF/HF Window in Figure 2D).

The R-R interval analyzer saves data files corresponding to several levels of data processing for external HRV analysis. These are mainly stored in text files and include the list of QRS timestamps, the time course of the interpolated R-R intervals, and the results of the short-term FFTs.

4. Subjects and ECG recording sessions

The feasibility of the application was tested using ECGs obtained from five 21-year-old women. This was part of a study approved by the research ethics committee of Niigata University of Health and Welfare (Number: 17827-170605). All the subjects were fourth-year students at the Department of Orthoptics and Visual Sciences, Niigata University of Health and Welfare, and all provided written informed consent before participating.

Four of the subjects had normal ECGs, but Subject #1 had an asymptomatic abnormal QRS complex (qRS type, perhaps due to a right bundle branch block). No premature beats were observed throughout the whole recording period. The ECG was recorded with the subject sat on a chair. Two electroencephalogram disk electrodes (NE-102A; Nihon-Kohden, Tokyo, Japan) were placed on the left and right wrists. The ground electrode was attached to the forehead. The signal was amplified with an instrumentation amplifier (INA128; Texas Instruments, TX, USA), band pass filtered (0.08–100 Hz) with an oscilloscope (VC-10; Nihon-Kohden, Tokyo, Japan), and sampled at 500 Hz. No further signal conditioning apparatus (such as hum or electromyogram (EMG) filters) were used. The low-cutoff frequency was intently selected to investigate the tolerance of the application to drifts in the ECG recording. The details of the data acquisition system have been described previously [16].

In five out of six recording sessions, the subject sat quietly for 4 min, then answered oral questions by fellow students for 4 min as a light mental load, which was followed by another 4-min rest. The four-minute study periods were rather short but

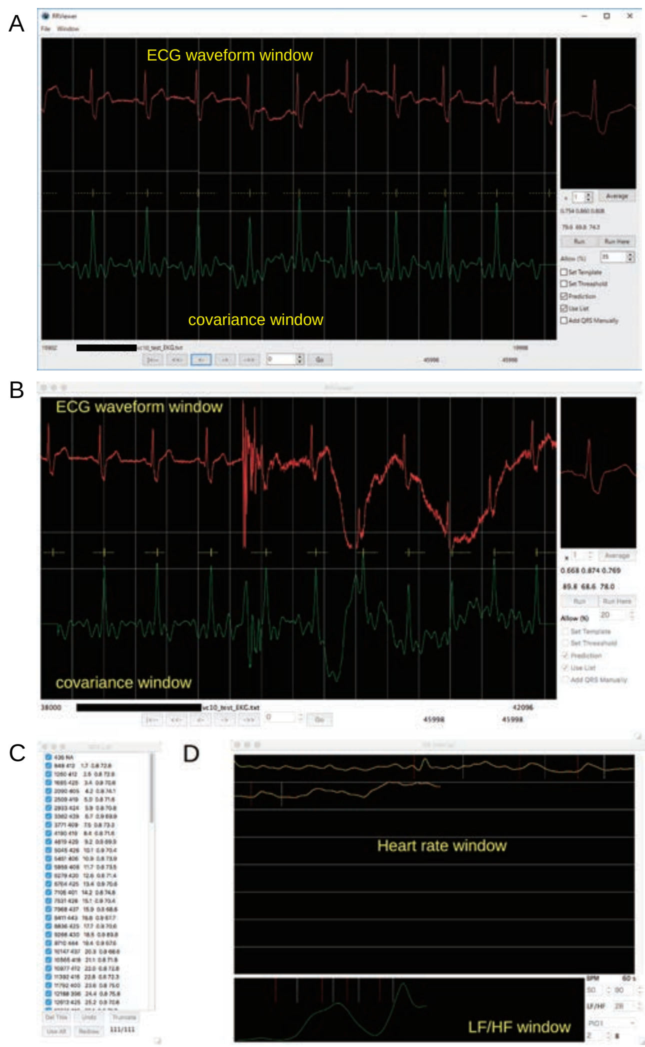


Figure 2. Function and appearance of the analyzer.

A, B: The main window of the R-R interval analyzer running on (A) Windows 10 and (B) Macintosh OS X El Capitan platforms. The upper part of the display (the ECG Waveform Window) shows the raw ECG waveform, the panel on the right shows the QRS template, and the lower part of the display (the Covariance Window) shows the time course of covariance between the ECG and the QRS template. The horizontal white line indicates the threshold for covariance peak time detection. The yellow short vertical lines indicate the QRS timings detected by the application, and the yellow short horizontal lines indicate the search periods for QRS detection in the Prediction Mode.

C: The QRS List Window (Macintosh build).

D: The Heart Rate Window (Macintosh build), with the time course of LF/HF ratios displayed below it. The red and white vertical lines indicate the timing of push-button pressing and releasing, respectively. In this session, the button press and release were recorded only to test the recording system and had no physiological meaning. In A–D, the characters in yellow have been superimposed on the actual screenshots.

Abbreviation: LF/HF (low-frequency to high-frequency ratio).

enough to evaluate the HRV and the short-term transition of LF/HF ratios, because the LF power can be theoretically detected from a 25-s EEG recording and the detection of HF power requires briefer EEG data. The oral enquiries were about their major (mainly from the questions of the national examination they were preparing for. See Table 2 for examples) and the answers were not recorded nor scored. The beginning and end of the enquiry periods were recorded by the experimenter with pressing the push-button. For the sixth of the recording sessions, the ECG was recorded while the subject simply sat quietly for 90 s. In the five sessions with the period of questioning, additional FFT analyses were performed over three 2-min segments (with 1200 samples, using a Hanning window function) at the start of the first rest period, the middle of the period of questioning, and the end of the second rest period. Using the same data segments, the mean R-R intervals and the root mean squares of the second order differentials of QRS timings (RMSSDs) were calculated. The additional calculations of the FFTs, mean R-R intervals, and RMSSDs, as well as the statistical analyses, were performed using GNU R (http://www.r-project.org/) on a Macintosh computer.

5. Availability of the source code

The complete source code for the R-R interval analyzer is freely accessible via Google Drive (https://drive.google.com/file/d/1SaQQh7QwVOq5big3Nd7bFPWnGXHDRIVL/view?usp=sharing) under the GPL license.

Results

1. Multiple builds using the same source code

The same source code based on Lazarus was used to build the GUI R-R interval analyzer for both Macintosh and Windows computers (Figure 2A and B). A list of the detected QRSs and the time course of HRV were displayed in discrete windows (Figure 2C and D). The whole process took less than 4 s for 12-min data, even when using an old Macintosh computer (Mac Pro, early 2008 model, Intel Xeon, 2.8 GHz, quad-core × 2) or a small 2-in-1 Windows tablet (T101HA, Intel Atom Z8350, 1.44 GHz, quad-core × 1). The processing time would be shorter with faster hard disk drives or solid-state drives, or with suppression of the refreshing of the windows. Using computers with lower performance (iMac, mid 2007 model, Intel Core2Duo, 2.0 GHz, dual-core × 1; W3-810fp, Intel Atom Z2760, 1.5 GHz, dual-core × 1), the run times for the same data were approximately 14 and 12 s, respectively.

2. Accuracy of QRS detection

The combination of the covariance-based algorithm and the Prediction Mode meant the application had a high tolerance to contamination by surface EMG and baseline fluctuations caused by body movements (Figure 3). In addition, the covariance calculation acted as a high-cut filter that eliminated the surface EMGs from the raw

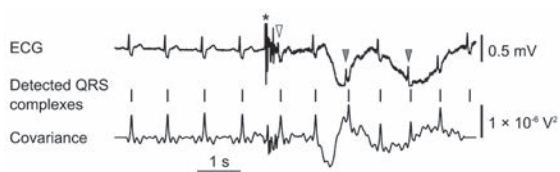


Figure 3. Tolerance of the analyzer to baseline drifts.
An example of the tolerance of the R-R interval analyzer to baseline drifts during QRS detection. Upper trace: An ECG recorded from Subject #1. Lower trace: The associated time course of covariance. Despite saturation (asterisk), electromyogram contamination (white arrow), and baseline drifts (gray arrows), the QRS complexes were detected appropriately (vertical lines) by the combination of the covariance-based algorithm and the application's Prediction Mode.

data, as well as providing greater immunity to ECG drifts than the correlation coefficients (Figure 4). After removing the DC offset, the early and late halves of the waveforms with drift added (Figure 4A, (b) and (c)) tended to have different signs, canceling each other out in the covariance calculation. Thus, the effect of drift on the covariance values was limited (Figure 4B). Conversely, the correlation coefficients were small because the variances of the waveforms with drift added were larger than that of the waveform without drift (Figure 4C), requiring a lower slice level not to miss QRS complex (the horizontal dotted line in Figure 4C), which resulted in lower noise tolerance.

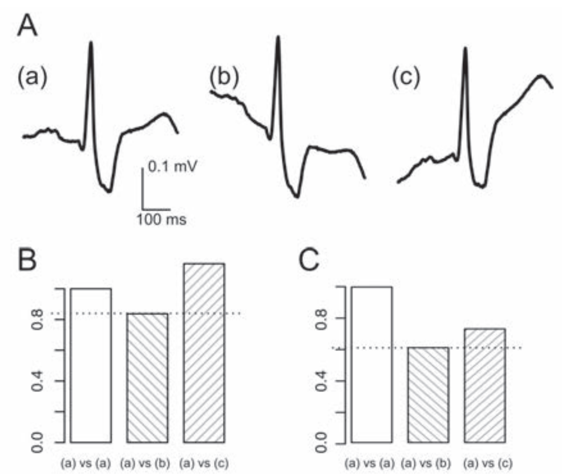


Figure 4. Advantage of covariance-based QRS complex detection.

A: (a) An example of a QRS complex with P and T waves. As shown in Figure 1B(1), the DC-offset is eliminated already. (b) and (c) The same complex as (a) except that a steep (b) negative- or (c) positive-going drift (0.4 mV/s) is artificially added, respectively.

B: Covariances between waveform (a) and itself, waveform (b) and waveform (c). The data were normalized by the covariance between waveform (a) and itself.

C: As for B, for the correlation coefficients. The correlation coefficient between (a) and (b) decreases to 56% of the correlation coefficient between (a) and (a). On the other hand, covariance between (a) and (b) are approximately 90% of that between (a) and (a).

During the ECG tests, the ECG signals occasionally scaled out for a few seconds as a result of body movements, mainly right after the start of the period of questioning. This required manual corrections in one or two of the 8-s time windows before the application automatically resumed further QRS detection. The percentages of missing QRS complexes (i.e., those the application failed to detect) and incorrectly detected QRS complexes (i.e., those detected at the incorrect time) were 0%–4.4% and 0%–2.6%, respectively (Table 1).

The R-R interval analyzer utilizes a user-selected template as the reference waveform for the covariance calculation. Because the waveforms of the P, QRS, and T waves depend on the subject and the positions of the electrodes, different

Figure 5. Compatibility across the QRS templates. Time courses of covariances between the ECG waveform (upper trace) and three QRS templates. The ECG waveform and template (a) were obtained from the recording of Subject #3. Templates (b) and (c) were obtained from Subjects #2 and #1, respectively. The horizontal scale bar is common to all panels. The vertical scale bar is common to the ECG waveform and templates (a)–(c). The covariance waveforms using the QRS templates derived from the other subjects ((b) and (c)) are yet enough spiky for peak detection.

templates were used for each of the five subjects in this study (Table 3). To investigate whether a template from one subject would be compatible for analyzing the data for a different subject, covariance time courses were calculated using the templates from three of subjects in this study (Figure 5). Template (a) was obtained by averaging twelve QRS complexes from Subject #2. Template (a) and the ECG recorded from the same subject gave sharp peaks in the covariance time course ((a) in Covariance). Templates (b) and (c) were obtained from Subjects #3 and #1, respectively. Although template (c) exhibited a wider and steeper S-wave than those of (a) and (b), it nevertheless provided sharp peaks in the covariance (Figure 5, (c)), similar to those using templates (a) and (b). These findings demonstrated that the R-R interval analyzer did not necessarily require a template taken from the data of the subject under analysis.

3. Evaluation of the effect on HRV of a light mental load

Time courses of LF/HF ratios are shown in the upper part of Figure 6A. This demonstrates the increased LF/HF ratios during the period the subject was answering questions, while the changes in HR (Figure 6A, lower part) were not apparent except for subject #3 at the beginning of the questioning. To confirm this finding, additional FFT calculations were performed using a longer time window (2 min) to give a greater frequency resolution (Figure 6B). The LF-band powers within the period of questioning were greater than that in the two rest periods for all the subjects. The pooled data demonstrated a significant increase in LF/HF ratio in the period of questioning, even though the mental load was light (Figure 6C). In contrast, the changes in the R-R intervals varied inconsistently across the subjects (Figure 6D and E). As shown in Figure 6F, RMSSD, an index of parasympathetic tone [17], decreased in subject #4 during the questioning period but there was no consistency across the subjects. Therefore these two measures did not differ significantly between the three study periods in the pooled data. These data suggest that the LF/HF ratio is sufficiently sensitive to detect the effects of light mental stress on the autonomic nervous system and that the R-R interval analyzer can be used in physiological studies.

Discussion

In this study, an open-source, multi-platform R-R interval analyzer with a GUI was developed using Lazarus, an open-source RAD (Figure 2). To test the application, HRVs of five participants were evaluated while resting and under a light mental load. Even under difficult experimental conditions, with the ECG electrodes placed on the subject’s wrists, a low cutoff frequency, and no EMG or hum filters (Figure 3), more than 95% of the QRS complexes were detected automatically with their appropriate timings (Table 1); this was achieved because of the EMG- and drift-canceling effects of covariance calculation (Figure 4). Because the developing environment and complete source code are freely available, users can modify and improve the application without the need for reverse engineering such as disassembling. For example, some users may wish to re-write the file input methods (procedures ReadData and ReadPIOData in the class TForm1) to fit their own file structure because there are no standard data formats in electrophysiological recordings.

The application is based on a template-matching method that uses the covariance between the ECG waveform and a user-defined template. The template is currently a 0.54-s segment derived from the ECG waveform of the subject under examination; this is long enough to include the P wave, QRS complex, and ST junction. Of these ECG components, the QRS complex may be the most crucial for the covariance calculation for the QRS detection (Figure 5). It would therefore be beneficial for the template to be obtained

Table 1. Accuracy of QRS detection.

Subject	Recording duration (min)	Manually detected QRS complexes	Automatically detected QRS complexes	Missing (%)	Errors (%)
#1a	1.5	111	111	0	0
#1	13.0	888	887	10 (1.13)	9 (1.01)
#2	11.8	848	833	37 (4.36)	22 (2.59)
#3	12.7	887	869	23 (2.59)	5 (0.56)
#4	12.7	899	891	13 (1.45)	5 (0.56)
#5	12.5	896	896	0	0

Table 2. Example questions in the oral enquiries.

What neurotransmitter do the postganglionic sympathetic nerve fibers release?
What organ secretes insulin?
What symptoms are the hyperthyroidism accompanied by?
Where is the primary visual area?
What hole does the ophthalmic nerve pass through from the brain to the orbit?

Table 3. Correlation coefficients between the QRS templates for the five subjects.

Subject	#2	#3	#4	#5
#1	0.81	0.77	0.71	0.77
#2	—	0.93	0.77	0.90
#3	—	—	0.72	0.89
#4	—	—	—	0.84

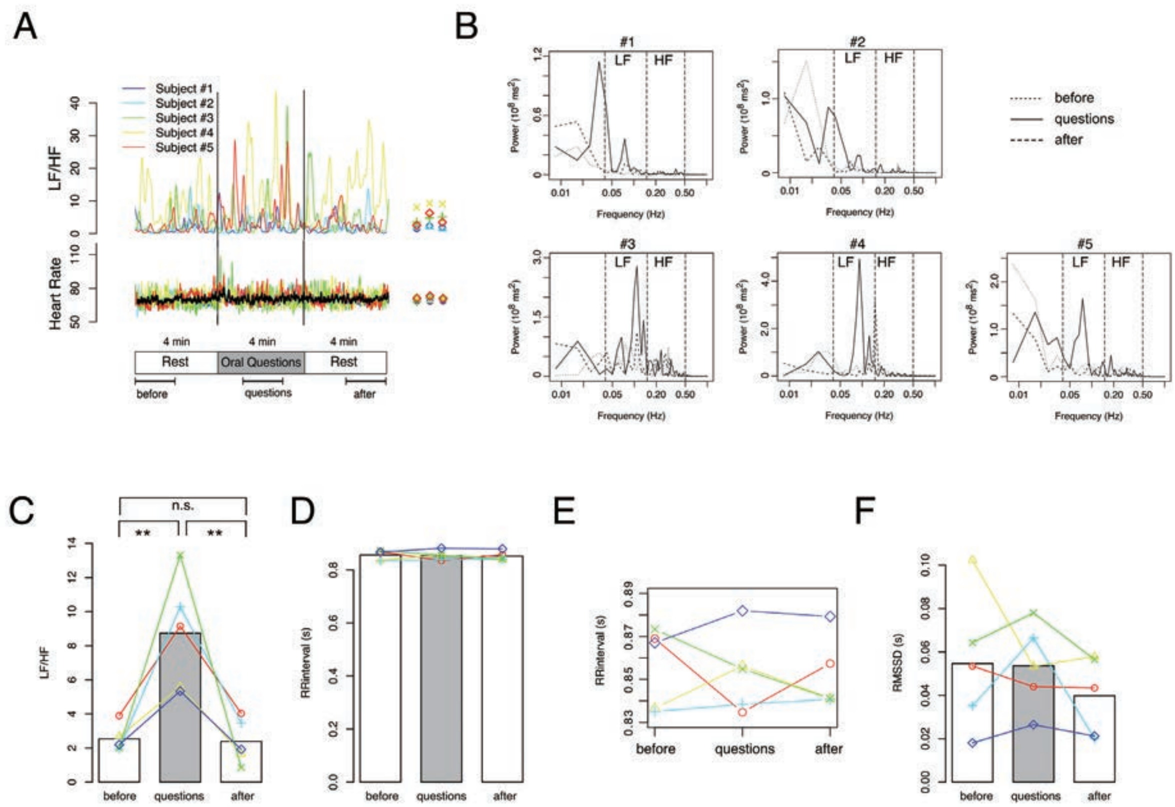


Figure 6. Increase in LF/HF ratios during the mild mental load.

The increase in LF/HF ratios during the period the subject was subjected to a mild mental load by answering questions. A: Time courses of the LF/HF ratios (upper), based on the results of a short-term (25.6 s) FFT calculated by the application and immediate heart rates (lower). The subjects are indicated by color. The boxes at the top indicate the three study periods, and the horizontal bars beneath them indicate the time windows for longer (2 min) FFTs in the three periods. The black line in the lower panel represents the averaged heart data. The symbols at the right of both panels indicate the data averaged across before (left), within (mid), and after (right) questioning for every subject. B: Power spectra of the interpolated R-R intervals in 2-min time windows before (dotted), within (solid), and after (dashed) the period of questioning for every subject (shown above the panel). C: LF/HF ratios before, during, and after the period of questioning, averaged over the five subjects. D: Same as for C, except for the mean R-R intervals. E: Same as for D, except that only the line plots are shown in magnified scale. F: Same as for C, except for the RMSSDs. In C–F, the subjects are indicated by colors and symbols in the same manner as A.

Abbreviations: FFT (fast Fourier transform), HF (high-frequency), LF (low-frequency), LF/HF (low-frequency to high-frequency ratio), RMSSDs (root mean squares of the second order differentials of QRS timings).

automatically from the subject's ECG data based on previously defined characteristic QRS templates for typical electrode positions (such as for limb lead I) and QRS axes. Currently, the application scans the ECG data only in the forward direction (from the beginning to the end of the file). In future implementations, scanning in the reverse direction may be helpful for allowing QRS detection to resume more quickly when the ECG scales out. In addition, a subroutine that checks whether the detected R-R intervals are within the possible range for a human ECG would improve QRS detection.

Using Lazarus, programmers can develop multithread applications without a detailed knowledge of the APIs for handling threads in the target OS. However, multithreading was not used in this application because multithread applications can sometimes be difficult to debug, and the covariance calculation is simple (Figure 2B) and requires only small computing resources. Despite the simple, single-thread, monolithic design of the application, the message queue was frequently processed in the main QRS detection loop to avoid disturbing the performance of the whole system. Nevertheless, it may be beneficial to increase the speed of the cubic spline interpolation and graphic coordinate calculations for the interpolated R-R intervals, up or to run these in a separate thread, because they take several seconds in computers with lower performance. In these feasibility tests, significant increases in LF/HF ratios were observed within the period of questioning (Figure 6A, B, and C), with no significant changes in the mean R-R intervals or RMSSDs (Figure 6D, E, and F). The peaks found in the LF/HF ratios during the questioning period (Figure 6A) might reflect the short term changes in autonomic nervous function elicited by the questions that the subject felt difficult. These findings might suggest that oral questions on the national examination resulted in sufficient stress to affect the students' cardiovascular nervous functions, despite an over six-month of preparation and training period. Further experiments with the other stress markers (thermography, the blood cortisol level, the amylase secretion, for examples), under a strictly controlled conditions are required for consideration of the mental health of students preparing for serious examinations in medicine and related fields.

Conflicts of interest

No potential conflicts of interest are disclosed.

References

1. Task Force of the European Society of Cardiology and The North American Society of Pacing and Electrophysiology. Heart rate variability: Standards of measurement, physiological interpretation, and clinical use. Circulation. 1996; 93: 1043-1065.
2. Perini R, Veicsteinas A. Heart rate variability and autonomic activity at rest and during exercise in various physiological conditions. European Journal of Applied Physiology. 2003; 90: 317-325.
3. Miki Y, Suzuki Y, Watanabe E, Hayano J, Yamamoto Y, Nomura T, et al. Long-range correlations in amplitude variability of HF and LF components of heart rate variability. 38th Annual International Conference of the IEEE Engineering in Medicine and Biology Society (EMBC). 2016; 6218-6221, DOI: 10.1109/EMBC.2016.7592149.
4. Pakarinen S, Korpela J, Torniainen J, Laarni J, Karvonen H. Cardiac measures of nuclear power plant operator stress during simulated incident and accident scenarios. Psychophysiology. 2018; e13071.
5. Elisabeth M, Goffeng EM, Nordby KC, Tarvainen MP, Järvelin-Pasanen S, Wagstaff A, et al. Fluctuations in heart rate variability of health care workers during four consecutive extended work shifts and recovery during rest and sleep. Industrial Health. 2018; 56: 122-131.

6. Hjortskov N, Rissén D, Blangsted AK, Fallentin N, Lundberg U, Søgaard K. The effect of mental stress on heart rate variability and blood pressure during computer work. European Journal of Applied Physiology. 2004; 92: 84-89.
7. da Silva RMFL, de Souza Miranda CE, Tonidandel Barbosa MT, Bicalho MAC. Heart rate and its variability assessed by spectral analysis in elderly subjects with orthostatic hypotension: a case-control study. Arquivos Brasileiros de Cardiologia. 2018; DOI: 10.5935/abc.20180043.
8. van Leeuwen WMA, Sallinen M, Virkkala J, Lindholm H, Hirvonen A, Hublin C, et al. Physiological and autonomic stress responses after prolonged sleep restriction and subsequent recovery sleep in healthy young men. Sleep and Biological Rhythms. 2018; 16: 45-54.
9. Hoogerwerf MD, Veldhuizen, IJT, Tarvainen MP, Merz EM, Huis in 't Veld EMJ, de Kort WLAM, et al. Physiological stress response patterns during a blood donation.ox Sanguinis. 2018; DOI: 10.1111/vox.12646.
10. Ikei H, Song C, Miyazaki Y. Physiological effects of touching wood. International Journal of Environmental Research and Public Health. 2017; 14: 801;DOI:10.3390/ijerph14070801.
11. Kubota Y, Chen LY, Whitsel EA, Folsom AR. Heart rate variability and lifetime risk of cardiovascular disease: the Atherosclerosis Risk in Communities Study. Annals of Epidemiology. 2017; 27: 619-625.
12. Physionet. WFDB Software Package. https://physionet.org/physiotools/manuals.shtml (accessed on April 18, 2018).
13. The Free Software Foundation. What is free software? http://www.gnu.org/philosophy/free-sw.html (accessed on April 18, 2018).
14. Centre for computing history. Richard Stallman. http://www.computinghistory.org.uk/det/1794/Richard-Stallman/ (accessed on April 18, 2018).
15. Raymond ES. The Cathedral and the Bazaar. http://www.catb.org/~esr/writings/cathedral-bazaar/cathedral-bazaar/index.html (accessed on April 18, 2018).
16. Toda H, Tokai, S, Nishimura E, Komatsu T, Ubukata H. Application of omni-purpose electric devices to electrophysiological student practices at the Department of Orthoptics and Visual Sciences, Niigata University of Health and Welfare. Niigata Journal of Health and Welfare. 2017; 17: 32-40.
17. Shaffer F, Ginsberg JP. An overview of heart rate variability metrics and norms. Frontiers in Public Health. 2017; 5: 258; DOI: 10.3389/fpubh.2017.00258.