INTRODUCTION

Objectives and overview. The primary objective of this laboratory exercise was to introduce the concept of heart rate variability (HRV) to our undergraduate students while testing novel hypotheses. The hypotheses that the students tested include 1) that alternate nostril breathing (ANB) leads to changes in HRV that are suggestive of elevated parasympathetic activity and of a calm and relaxed state. The purpose of this laboratory activity was to introduce the concept of HRV to our students, while having them address a novel question of whether two yogic breathing techniques, namely alternate nostril breathing (ANB) and standard deep breathing (DB), impact the SD of instantaneous heart rate (SDHR), a measure of HRV. Fifty-five undergraduates enrolled in a physiology course designed for non-science majors were tasked with analyzing HR and SDHR from electrocardiograms recorded during normal breathing, DB, and ANB. A repeated-measures ANOVA showed that HR was significantly, albeit slightly, elevated from normal (74.5 ± 13.4 beats/min; means ± SD) during DB (76.5 ± 11.2 beats/min), but not during ANB (75.7 ± 10.1 beats/min). Analysis of SDHR showed significant differences between conditions (normal: 5.5 ± 2.1, DB: 8.6 ± 3.0, ANB: 7.8 ± 2.8 beats/min). The instructors further analyzed the same data set using more robust measures of HRV (SD of sequential N-N intervals, root mean square of successive differences, and high-frequency domain of HRV) to determine whether SDHR during a 2-min epoch is a sufficient measure for HRV in the undergraduate course setting. Statistical analysis for these measures showed a near identical pattern of magnitude and significance among the groups as SDHR. Our students developed a greater appreciation for the effects of breathing patterns on HRV and HR, using the simple measure of SDHR.

Changes in HRV compared with more sophisticated techniques when assessing the effects of different breathing techniques. Students, untrained in these breathing techniques, were given brief instructions on how to perform them. They then performed these techniques while their ECG and respiratory depth and rate were monitored. The students then used a software program to measure their own mean HR and SDHR over a 2-min period to generate a class data set for statistical analysis of each measure in the three different breathing conditions. We as instructors further analyzed the same data set, assessing more common measures of HRV to determine whether changes in SDHR were comparable under those measures under the conditions of the laboratory activity and were, therefore, a suitable substitute of HRV for less advanced classes.

Background. The HR of a healthy human is determined by the depolarization rate, the intrinsic rate, of the sinoatrial node cells. The time between beats, known as the R-R interval based on an ECG tracing, is modulated by both the sympathetic nervous system (SNS), which increases the rate of depolarization, and PNS, which slows the depolarization rate of sinoatrial node cells. The instantaneous HR is the HR that is calculated from each individual R-R interval (see Fig. 1 for an example). At its most basic level, HRV refers to the beat-to-beat variance in the R-R interval during a given time period. HRV is typically quantified using mathematical derivations of the R-R interval during a fixed period. One major contributor to HRV is the change in lung size during a ventilatory cycle: the R-R interval generally decreases during inhalation (that is, a faster instantaneous HR), and increases during exhalation (Fig. 1). The resultant variability in this fluctuating R-R interval is a direct measure of cardiac autonomic tone (2, 7). In general, higher HRV is associated with elevated PNS activity with a resultant “relaxed” phenotype in the individual. Alternately, low HRV is associated with elevated cardiac sympathetic tone.

There are several measures of HRV (1–3), which function as direct measures of cardiac autonomic tone. As a first-order approximation of HRV, we opted to have the undergraduates in the course measure the SDHR. We chose this SDHR measure as opposed to the more sophisticated measures of HRV because of 1) the ease of understanding SDHR in our non-science student population, and 2) the ease of measurement of SDHR in the software program (LabChart from AD Instruments). However, SDHR is not a commonly used measurement, because there is a nonlinear inverse relationship between HR and R-R intervals. The variation in HR at fast HRs will lead to a greater SD compared with the same variation at slow HRs, which could potentially mathematically bias the analysis (11).
Therefore, we (the instructors) wanted to analyze further the
data set using more traditional measures of HRV, including the
SD of R-R intervals (SDNN), root mean square of successive
differences (RMSSD), and high-frequency domain (HF) as a
means to determine whether SDHR is a reasonable measure-
ment of the HRV in our normal student population.

DB has long been known to elevate HRV. Previous work has
demonstrated that DB exercises acutely increase parasympa-
thetic tone, as indicated by increased RMSSD and HF (10, 15).
DB exercises are used to calm individuals, presumably
through activation of the PNS. ANB, a variation of DB often
used in yoga (described below and demonstrated in Supple-
mental Material, available online at https://zenodo.org/record/
2630636), may elevate parasympathetic HRV markers (5, 9,
12–14, 17). One of us (S.J.S.) read in a sports magazine advice
column that ANB should be utilized on the first tee of a golf
round to calm nerves (16). We thus designed the current
laboratory exercise with novice deep breathers (i.e., our non-
yoga-trained undergraduate students) to test the hypotheses as
above.

Learning objectives. At the end of this exercise, the students
should be able to:

1. Understand the role of the sinoatrial node in controlling
HR
2. Understand that HR is a dynamic measure that exhibits
changes on a beat-to-beat scale
3. Explain the role of the SNS and PNS on modulating HR
4. Explain the role of breathing in modulating HR
5. Collect ECG tracings
6. Analyze an ECG tracing to obtain the R-R intervals for a
specific time period, and to calculate mean HR and
SDHR across the 2-min epoch
7. Analyze these data using standard statistical tests, includ-
ing generating SD of mean HR and SD of SDHR, as well
as run repeated-measures ANOVAs with appropriate post
hoc testing.
8. Make appropriate conclusions from these statistical
analysis

Activity level. This activity was used within a nonmajors
biology course at Williams College. Most of the students in
this course only had a background in high school biology and
mathematics. The analysis for our data set could be expanded
on for upper level undergraduate biology courses by expanding
the statistical analyses to include more robust tests (e.g.,
RMSSD) and/or incorporating other variables, such as sex or
fitness state. Alternatively, the target audience could be
adapted to a high school level anatomy and physiology course
by removing statistical work (e.g., SD calculation and statisti-
tests) and having students just calculate the R-R interval
from those intervals that look the largest or smallest by eye.

Prerequisite student knowledge or skills. Before coming to
the laboratory for this exercise, the students in this course had
been exposed in the classroom to the concepts that HR is
controlled typically at the sinoatrial node, as well as sympa-
thetic and parasympathetic influence over these cells. Students
should have some rudimentary database skills, including data
entry, and use of the mathematical functions for means and SD.
Our students acquired these skills, as well as statistical analysis
skills, using the program SPSS from laboratory experiences
earlier in the semester.

Time required. Gathering data during the laboratory took
~1.5 h. The students were able to measure their mean HR and
SDHR in three conditions within just a few minutes. That data
were amassed into a central database file (available in the
Supplemental Material; https://zenodo.org/record/2630636),
which was then distributed to the class, and data analysis was
done in groups outside of the laboratory. This typically took
about an additional 1 h.

Fig. 1. An ECG and respiratory tracing for an individual deep breath. These tracings from the ECG setup and respiratory belt illustrate the shorter R-R interval,
and our own calculated instantaneous heart rate (HR) during inhalation and the protracted R-R interval and slower instantaneous HR during exhalation. bpm,
Beats/min.
METHODS

Equipment and supplies. Our laboratory program uses the AD Instruments four-channel Power Laboratory (26T with a built-in ECG amplifier) and the associated LabChart software (version 8.1.9). For this laboratory, we utilized AD Instruments ECG hardware. However, any ECG collection setup should work if the software has R-wave detection. The ECG was recorded using a single lead (see instructions given to the students for the lead II configuration that we used, although any lead configuration that accurately detects R waves will work). Disposable electrodes (Danlee Medical Products: item no. EG100) were utilized, as were single-use alcohol wipes to clean the sites of electrode placement before and following removal to limit irritation of the skin. The change in chest cavity size was measured with an accelerometer-based respiratory belt (AD Instruments, TN1132/ST). The respiratory belt is optional in this exercise. We found it instructive for students to see HR vary as a function of inhalation/exhalation, visualizing these changes in real time through the spacing between R waves and the amplitude of the wave in the respiration channel (see Fig. 1 for an example). However, the measurement of HRV does not depend on the use of a respiratory belt.

Human subjects. All work was approved by the Williams College Institutional Review Board. Adopters of this activity are responsible for obtaining permission for human or animal research from their home institution. For a summary of Guiding Principles for Research Involving Animals and Human Beings, please see https://www.physiology.org/author-info.animal-and-human-research. Fifty-five male and female Williams College undergraduate students (ages 18–22 yr) enrolled in a nonmajors physiology course voluntarily participated in this experiment as part of the laboratory component for the course. The laboratory activity consisted of a within-subjects design, where subjects would breathe at their normal rate, perform DB at a comfortable rate, and perform ANB at a comfortable rate.

For DB, the students were instructed to take deep, slow breaths, without taking pauses between breaths. For ANB, the students were instructed to use the thumb and ring finger of their dominant hand to alternate between blocking their right and left nostrils. The pattern of breathing is 1) in through the right nostril, 2) out through the left nostril, 3) in through the left nostril, and 4) out through the right nostril. The process is then repeated. Please see the Supplemental Material (https://zenodo.org/record/2630636) for a video of a demonstration of both DB and ANB by a local yoga instructor (Mary Edgerton). Students were encouraged to utilize deep breaths during ANB. Subjects performed each of these conditions for 3 min with 5 min of rest in between each condition. The order of these conditions was randomized across subjects to control for ordering effects.

Instructions given to the volunteers. The following instructions were given to the volunteers:

1. At the onset of the laboratory program, the students were given instructions by a local yoga instructor for DB and ANB. In all conditions (normal, DB, ANB), the volunteer was instructed to sit quietly, upright with their head facing forward, facing away from the monitor, and without talking, joking, or smiling.

2. The volunteer rolled down socks to the ankles. The electrode placement sites were wiped down with an alcohol pad and allowed to dry before placement. The electrodes were applied to the inner forearms (midway between wrist and elbow), and to the inside of the left leg, just above the ankle, but not directly over bone. The electrodes were attached for a single-lead ECG tracing, positive electrode (black with our equipment; please note that the color scheme of the electrodes may vary with different vendors) on the left leg, negative electrode (white) on the right arm, and ground (green) on the left arm. The volunteer

![Fig. 2. Typical ECG tracings and respiratory belt tracings are shown. Fifteen-second clips of the ECG signal (A), instantaneous heart rate (HR) calculated from the ECG tracing (B), and respiratory belt tracing (C) student is shown during normal breathing (left), deep breathing (DB: middle), and alternate nostril breathing (ANB: right). The respiratory sinus arrhythmia is notable in this student in DB, as the instantaneous HR is fastest at maximum inhalation and slowest during maximum exhalation. This particular student also achieved deeper breaths during DB than during ANB. BPM, beats/min.](http://advan.physiology.org)
2. “The variability in heart rate that we are seeing here is driven by neural input into the heart (we learned of two nerves that innervate the heart). Which nerve do you think is influencing the neural input into the heart (we learned of two nerves that innervate the heart)?”

Troubleshooting. Three issues that we ran into during this exercise were as follows. 1) A noisy ECG tracing. This was typically fixed by either having the student cease extraneous movement (i.e., tapping their foot), or by moving the ECG electrode slightly. 2) Some students would giggle at the onset of the breathing exercises. We waited a full 1 min of the 3-min data collection period before analyzing the data. 3) Some students had a deviated septum, making the use of ANB nearly impossible with this small subset of students. The data from those students are not included in the data set.

Safety considerations. Because this laboratory only manipulates breathing patterns, there were minimal safety concerns associated with it. However, there are safety considerations around application of the electrodes and interpretation of ECG tracings. Skin irritation may occur in response to the electrode adhesive. Students were provided with alcohol wipes to remove the adhesive after electrode removal or during the experiment, if necessary. Students should be made aware of possible skin irritation before the experiment. Students may also perceive an electrical anomaly as a cardiac defect and become distressed. It should be made clear to students before the experiment that these measurements are not diagnostic in nature, and that a healthcare provider may be consulted if there is significant concern. In the event that a cardiac abnormality, either in rhythm or in waveform, appears as a possibility to the instructor, the student should be informed of such privately and instructed to consult a healthcare provider.

Table 1. Instructor-performed additional analysis of physiological parameters

|                         | Normal Breathing | Deep Breathing | Alternate Nostril Breathing |
|-------------------------|------------------|----------------|-----------------------------|
| RMSSD, ms               | 48.9 ± 35.2      | 68.7 ± 40.0*   | 55.1 ± 35.8*†               |
| SDNN, ms               | 64.3 ± 28.8      | 99.2 ± 43.3*   | 86.7 ± 37.4*†               |
| HF, ms²               | 1,541 ± 2,355    | 2,468 ± 3,178* | 1,653 ± 2,918†              |

Values are means ± SD; n = 55 students. The instructors analyzed the same heart rate data set for three different measures of heart rate variability. RMSSD is the root mean square of successive differences. SDNN is the standard deviation of the beat-to-beat (N-N) intervals. HF is the high frequency (0.15–0.4 Hz) domain using power spectral analysis. *P < 0.05 vs. normal breathing. †P < 0.05 vs. deep breathing.
Table 2. Ratio of measurements taken during the different conditions compared with during normal breathing

|                  | Deep Breathing: Normal Breathing | Alternate Nostril Breathing: Normal Breathing |
|------------------|---------------------------------|---------------------------------------------|
| HR, beats/min    | 1.04 ± 0.09 (38)                | 1.03 ± 0.07 (34)                            |
| SDHR, beats/min  | 1.66 ± 0.56 (50)                | 1.53 ± 0.65 (47)                            |
| SDNN, ms         | 1.62 ± 0.66 (50)                | 1.44 ± 0.49 (46)                            |
| RMSSD, ms        | 1.61 ± 0.71 (43)                | 1.27 ± 0.51 (34)                            |
| HF, ms²          | 3.69 ± 5.59 (42)                | 1.53 ± 1.39 (28)                            |

Values are means ± SD; n = 55 students. The no. of students who exhibited an increase in the measurement relative to normal breathing is shown in parentheses.

Table 3. Anecdotal quotes from students concerning the laboratory

| Student No. | Quote                                                                                           |
|-------------|-------------------------------------------------------------------------------------------------|
| 1           | "The lab reinforces topics from the course. I enjoyed the lab. I think that the alternate nostril breathing seemed funny to people at first, then we realized that it had a real effect, and we found it very cool." |
| 2           | "For me and my partner, alternate nostril breathing did in fact have an effect on heart rate variability. This reinforced concepts from the course, especially because we were able to see some of those concepts in practice, so that helped me make sense of the material. I would totally recommend this to any and all people who are interested in out-of-the-box experiments like this." |
| 3           | "I remember alternate nostril breathing did raise the heart rate variability. It must’ve reinforced something because I still remember it [a year later]." |
asked to identify which parameters of HRV are useful indicators of parasympathetic tone, and whether or not HR alone is an effective measure of this. Students may present their findings in a brief two- to three-page laboratory report, or with a PowerPoint presentation, depending on the level of the class.

**Inquiry applications.** The level of inquiry we opted for in this laboratory exercise was “Method” inquiry, where the instructors generated the question and designed the experiment. We took this approach because of the lack of experience in experimental science from most of our students in this population. However, we intend to adapt this laboratory exercise to our major’s course, “Animal Physiology,” for which we will take a “Guided Inquiry” approach. Groups at other institutions can use many other questions and modifications of experimental design. For example, one could monitor the HR and HRV during inhalation and compare them with those measures during exhalation. Furthermore, other groups may want to examine whether the effect of these different breathing exercises is impacted by sex, or by cardiovascular-trained state, or by previous experience with yoga-style breathing. Instructors can easily calculate respiration rate (which is in our Supplemental Material data set, but not summarized here) and look for associations between this measure and HRV. This laboratory could be easily adapted for more rigorous data analysis. For example, advanced students may be able to export the interbeat intervals of the selected data set and generate their own SDNN or RMSSD analyses.

**Additional resources.** For additional information on this topic, please see Ghiya and Lee (4), Kleger et al. (6), and Mohanty and Saoji (8).

Our Supplemental Material, which are sample videos and a sample data set, is available in Zenodo (at https://zenodo.org/record/2630636; DOI:10.5281/zenodo.2630636).

**Additional information (advanced measures of HRV).** While SDHR showed profound and consistent changes during the different breathing conditions, the exact autonomic contributions to this measure have not been fully established. It is likely that SDHR is influenced by both the SNS and PNS, as opposed to purely the PNS. SDNN is a more common measure of HRV, but, like SDHR, it is influenced by both the SNS and PNS. More sophisticated measures of HRV that measure PNS activity are RMSSD and HF. The calculations for RMSSD and SDNN are straightforward. SDNN is simply the SD of all R-R intervals in a given period, whereas RMSSD is calculated by taking the sum of all squared differences between consecutive beats for a given period and taking the square root of that sum. Calculating HF is a more complicated endeavor and requires a fast Fourier transformation. While these are among the more commonly utilized measures of HRV, a more exhaustive list of commonly utilized measures of HRV, which showed a significant effect for both RMSSD ($P < 0.001$) and HF ($P < 0.01$). Comparing the individual conditions also indicates that, while DB increased both RMSSD ($P < 0.01$) and HF ($P < 0.01$), ANB only produces significant increases in RMSSD ($P < 0.01$), but not HF ($P = 0.70$). When directly comparing the two techniques, the magnitude of increase in HRV from normal breathing is significantly higher during DB than ANB when using either HF ($P < 0.01$) or RMSSD ($P < 0.01$). SDNN also varied significantly as a function of condition ($P < 0.01$). Like RMSSD, SDNN was elevated during both DB ($P < 0.01$) and ANB ($P < 0.01$) relative to control. SDNN was also higher during DB than ANB ($P < 0.01$). These findings corroborate the shift in sympathovagal balance caused by the breathing exercises toward vagal dominance, as suggested by SDHR. While SDHR is not utilized as frequently as RMSSD, HF, or SDNN, we were able to observe differences in SDHR, indicating that this value may be as sensitive to RMSSD, HF, and SDNN to different breathing exercises.

**Additional information (summary of references).** See Refs. 1, 2, 6, 7, 10, 13. These papers function as reviews of HRV, its derivations, their clinical significance, the interpretive caveats associated with them, and their prognostic value.

See Ref. 5. This paper discusses the educational value of using cardiovascular parameters in introducing underlying physiological concepts to undergraduate students.

See Refs. 3, 4, 8, 9, 11, 12, 14, 17. These publications discuss the interplay between respiration and HRV, and how various respiratory techniques may affect HRV and by extension autonomic tone.

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**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the authors.

**AUTHOR CONTRIBUTIONS**

C.J.L. and S.J.S. performed experiments; C.J.L. analyzed data; C.J.L. and S.J.S. interpreted results of experiments; C.J.L. and S.J.S. prepared figures; C.J.L. drafted manuscript; C.J.L. and S.J.S. edited and revised manuscript; C.J.L. and S.J.S. approved final version of manuscript; S.J.S. conceived and designed research.

**REFERENCES**

1. Billman GE. The LF/HF ratio does not accurately measure cardiac sympathovagal balance. *Front Physiol* 4; 26, 2013. doi:10.3389/fphys.2013.00026.

2. Billman GE, Huikuri HV, Sacha J, Trimmel K. An introduction to heart rate variability: methodological considerations and clinical applications. *Front Physiol* 6: 55, 2015. doi:10.3389/fphys.2015.00055.

3. Elstad M, O’Callaghan EL, Smith AJ, Ben-Tal A, Ramchandra R. Cardiorespiratory interactions in humans and animals: rhythms for life. *Am J Physiol Heart Circ Physiol* 315: H6–H17, 2018. doi:10.1152/ajpheart.00701.2017.

4. Ghiya S, Lee CM. Influence of alternate nostril breathing on heart rate variability in non-practitioners of yogic breathing. *Int J Yoga* 5: 66–69, 2012. doi:10.4103/0973-6131.91717.

5. Hodgson Y, Choate J. Continuous and noninvasive recording of cardiovascular parameters with the Finapres finger cuff enhances undergraduate student understanding of physiology. *Adv Physiol Educ* 36: 20–26, 2012. doi:10.1152/advan.00097.2011.

6. Kleger RE, Stein PK, Bigger JT Jr. Heart rate variability: measurement and clinical utility. *Ann Noninvasive Electrocardiol* 10: 88–101, 2005. doi:10.1111/j.1542-474X.2005.10101.x.

7. Malpas SC. Neural influences on cardiovascular variability: possibilities and pitfalls. *Am J Physiol Heart Circ Physiol* 282: H6–H20, 2002. doi:10.1152/ajpheart.2002.282.1.H6.

8. Mohanty S, Saoji AA. Comments on “Alternate Nostril Breathing at Different Rates and Its Influence on Heart Rate Variability in Non Practitioners of Yoga”. *J Clin Diagn Res* 10: CLO1, 2016. doi:10.7860/JCDR/2016/20276.8145.

9. Muralikrishnan K, Balakrishnan B, Balasubramanian K, Visnegarwala F. Measurement of the effect of Isha Yoga on cardiac autonomic...
nervous system using short-term heart rate variability. *J Ayurveda Integr Med* 3: 91–96, 2012. doi:10.4103/0975-9476.96528.

10. Sacha J. Interplay between heart rate and its variability: a prognostic game. *Front Physiol* 5: 347, 2014. doi:10.3389/fphys.2014.00347.

11. Srivastava RD, Jain N, Singhal A. Influence of alternate nostril breathing on cardiorespiratory and autonomic functions in healthy young adults. *Indian J Physiol Pharmacol* 49: 475–483, 2005.

12. Subramanian RK, P R D, P S. Alternate nostril breathing at different rates and its influence on heart rate variability in non practitioners of yoga. *J Clin Diagn Res* 10: CM01–CM02, 2016. doi:10.7860/JCDR/2016/15287.7094.

13. 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* 93: 1043–1065, 1996. doi:10.1161/01.CIR.93.5.1043.

14. Telles S, Sharma SK, Balkrishna A. Blood pressure and heart rate variability during yoga-based alternate nostril breathing practice and breath awareness. *Med Sci Monit Basic Res* 20: 184–193, 2014. doi:10.12659/MSMBR.892063.

15. Tharion E, Samuel P, Rajalakshmi R, Gnanasenthil G, Subramanian RK. Influence of deep breathing exercise on spontaneous respiratory rate and heart rate variability: a randomised controlled trial in healthy subjects. *Indian J Physiol Pharmacol* 56: 80–87, 2012.

16. Tomasi T. ‘Air’ it Out with Your Driver. Golf Magazine, October, 2015, p. 50.

17. Tyagi A, Cohen M. Yoga and heart rate variability: A comprehensive review of the literature. *Int J Yoga* 9: 97–113, 2016. doi:10.4103/0973-6131.183712.