Short communication

Controlled breathing and autonomic rhythms: Influence of auditory versus visual cues

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ARTICLE INFO

Keywords:
- Paced breathing
- Heart rate variability
- Baroreflex sensitivity
- Muscle sympathetic nerve activity

ABSTRACT

We compared standard metrics of autonomic control in 20 humans (10 female) during spontaneous and controlled breathing. Subjects controlled breathing at 0.25 Hz following a metronome (auditory) or scrolling waveforms (visual). Respiratory rates and heart rates were lower during spontaneous breathing compared with auditory and visual. One heart rate variability metric was higher during visual compared with spontaneous breathing, but baroreflex sensitivity and muscle sympathetic nerve activity were not affected by breathing cues. A majority of subjects (86%) perceived that breathing to auditory cues was more difficult compared with visual cues, but this elevated perceived stress did not manifest physiologically.

1. Introduction

Accuracy of human autonomic rhythm assessment is improved when respiratory rate is controlled [1]. Forcing respiratory excursions at a specific frequency is not difficult in human studies because humans can cooperate and follow external cues indicating inspiration and expiration [2]. Over the course of ~25 years, one of the authors (WH Cooke) has used both auditory and visual cues to control breathing frequency. Several reviewers for several different papers have asked whether resultant autonomic regulatory results might have been influenced by the mode of breathing – this question has also been raised of other investigators incorporating controlled breathing protocols (Cooke WH, personal communications). The subjective assessment is that breathing to auditory cues is more difficult for the subject (they must be continuously encouraged to maintain the correct frequency) than breathing to visual cues, and that this increased mental stress might confound the assessment of autonomic rhythms [3]: to our knowledge this notion has not been tested experimentally.

Therefore, the purpose of this study was to compare heart rate variability (HRV), baroreflex sensitivity (BRS) and peripheral muscle sympathetic nerve activity (MSNA) in subjects breathing in time to both a metronome (auditory) and waveforms displayed on a laptop indicating inspiration and expiration (visual). We tested the hypothesis that visual cues would be subjectively less stressful, and that HRV and BRS would be higher in conjunction with lower MSNA during visual compared with auditory cues.

2. Materials and methods

Twenty healthy young adults (10M 10F, age 23 ± 1 yr, height = 173 ± 4 cm and weight = 75 ± 5 kg) participated. Subjects had no history of autonomic dysfunction, hypertension, respiratory disease, diabetes, nicotine use, anxiety disorder, and were not taking any prescription drugs. All female subjects were tested during the early follicular phase of their menstrual cycle. The work was carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans. All participants signed a consent form that had been approved by the Institutional Review Board for Human Subject Research at Michigan Technological University.

2.1. Instrumentation

Experiments were conducted in a quiet, temperature-controlled laboratory with subjects situated in a semi-recumbent position on a cushioned examination table. Beat-to-beat arterial blood pressure was recorded continuously with a NOVA Finometer (Finapres Medical Systems, Amsterdam, The Netherlands). We used a three-lead electrocardiogram, and respiratory rate was continuously measured with a
pneumobelt. Multifiber efferent sympathetic nerve traffic was recorded from the peroneal nerve muscle fascicles at the popliteal fossa by inserting a tungsten microelectrode (Frederick Haer and Co., Bowdon-ningham, ME). A reference electrode was inserted subcutaneously 2 to 3 cm from the recording electrode. Both electrodes were connected to a differential preamplifier and then to an amplifier (total gain 80,000) where the nerve signal was band-pass filtered (700–2000 Hz) and integrated (time constant, 0.1 s) to obtain a mean voltage display of nerve activity. Satisfactory recordings of MSNA were defined by spontaneous pulse-synchronous bursts that did not change during tactile or auditory stimulation, and increased during end-expiratory apnea.

2.2. Experimental design

After instrumentation, all subjects were provided a minimum of 5 min unrecorded rest to confirm hemodynamic and neural stability. Subjects then breathed spontaneously, and to both auditory or visual cues (randomized) for 5 min each. We used a metronome for auditory cues, and a laptop display of oscillating triangular waveforms for visual cues. Under both paced breathing conditions, frequency was maintained at 15 breaths/min (0.25 Hz with a 50% duty cycle). After controlled breathing subjects were asked to report which breathing condition they found most difficult to follow. We did not attempt to scale subjective feelings of “difficulty,” but simply reported subjects’ overall sense of stress associated with the two paced breathing protocols.

2.3. Data analysis

Data were sampled at 500 Hz (WINDAQ, Dataq Instruments, Akron, Ohio) and analyzed with specialized software (WINCPRS, Absolute Aliens, Turku, Finland). R waves generated from the ECG signal were automatically detected and marked. Systolic and diastolic arterial pressures were marked from the Finometer tracings. Muscle sympathetic nerve bursts were automatically detected based on their amplitude and a 1.3 s expected burst peak latency from the previous R wave. All automated detection results were checked manually. Sympathetic bursts of activity were expressed as burst frequency (bursts/min) and burst incidence (bursts/100 heartbeats). Data were averaged from the last 4 min of every 5-minute time period.

We assessed cardiac vagal modulation in the time domain with R-R intervals (RRI), R-R interval standard deviations (RRISD) and the percentage of RRI that varied by 50 ms or more (pNN50). For frequency-domain assessment, non-equidistant beat-to-beat RRI data were interpolated linearly, and then resampled at a frequency of 5 Hz. We incorporated a low-pass response filter with a cut-off frequency 0.4 Hz and used Fourier analysis to calculate power spectrums. Frequency bands were separated into low (0.04–0.15 Hz) and high (0.15–0.4 Hz) frequencies. To reduce variability between subjects, we normalized power by dividing high and low frequencies by total power (0.04–0.4 Hz) and then multiplied by 100 to express high- and low-frequency domains as percentages of total power.

We calculated BRS with the sequence method by identifying three or more continuously increasing systolic pressures and accompanying RRI increases (up sequences) and three or more continuously decreasing systolic pressures and accompanying RRI decreases (down sequences). To be included as a valid sequence, we required that systolic pressure increases be at least 1 mmHg change/breath, with at least 4 ms change/breathe for RRI. Up and down baroreflex gain was calculated using linear regression with the requirement that correlation coefficients be ≥0.8.

Statistical analyses were performed with SigmaPlot 14.0 (Systat Software, San Jose, California). Dependent variables of interest were assessed with a one-way repeated measures ANOVA. Significant interactions were assessed using a Bonferroni post-hoc test. Data are presented as mean ± SE. A probability value ≤0.05 was considered statistically significant.

3. Results and discussion

No sex differences were identified for any dependent variable, and so all data represent pooled results from both males and females. Subjects maintained their respiratory frequencies at almost exactly 15 breaths/min during both auditory and visual cues (14.7 ± 0.1 auditory and 14.9 ± 0.1 visual; p = 1.0), but breathed at a slower pace when allowed to breathe spontaneously (12.7 ± 0.8 breaths/min). Breathing rate during spontaneous breathing was statistically lower than both auditory (p = 0.018) and visual (p = 0.007), although four individuals averaged greater than 15 breaths/min during spontaneous breathing.

Heart rate was lower, and RRI higher when subjects breathed spontaneously compared to both auditory and visual cues. Heart rate variability expressed as the percentage of normal inter-beat intervals that varied by 50 ms or more (pNN50) was higher (but RRISD was not) when participants paced their breathing to visual cues compared with normal, spontaneous breathing. Beat-to-beat arterial pressures and BRS (both up and down sequences) were not different between conditions. During spontaneous breathing, RRI and systolic pressure normalized spectral power at the high frequency was lower, and both were higher at the low frequency compared with both auditory and visual protocols. It is not always possible to obtain adequate, analyzable nerve recordings from all research participants. From our sample of 20 subjects, we report nerve data for 15. Burst incidence (bursts/100 heart beats) and frequency (bursts/min) of MSNA decreased compared with spontaneous breathing when subjects controlled their breathing using either auditory or visual cues. These data are shown in Table 1.

The figure shows RRI and SAP power spectrums for one subject during all three breathing protocols. Enhanced low frequency power is evident for both RRI and SAP at the low frequency during spontaneous breathing, and then the spectrums tighten around the respiratory frequency of 0.25 Hz when breathing to both auditory and visual cues (Fig. 1).

Controlled (or paced) breathing ≥0.2 Hz has also been suggested for BRS assessment to avoid the over-estimation that can occur when participants breathe at or around the resonance frequency of 0.1 Hz. [5,6]. Baroreflex-MSNA interactions are present as means ± SE. n = 20 for all variables except MSNA (n = 15). Heart rate, (HR); R-R interval (RRI); systolic arterial pressure (SAP); diastolic arterial pressure (DAP); mean arterial pressure (MAP); RRI high and low frequency spectral power normalized to total power (RRHIHFnu and RRILFnu); systolic pressure high and low frequency spectral power normalized to total power (SAPHFnu and SAPLFnu); baroreflex up sequences (BRSup); baroreflex down sequences (BRSDown); muscle sympathetic nerve activity (MSNA); variables that do not share the same superscript letter are different with a p value ≤0.05.

![Table 1](image-url)
is likely not an indicator of stress, but rather the consequence of a slower breathing rate and a likely prolonged expiratory phase [15]. Mental stress increases peripheral sympathetic activity in some subjects [3], and therefore could confound assessment of autonomic rhythms during a stressful breathing protocol. A majority of subjects in our current experiment (86%) indicated that auditory cues were more difficult (stressful) to follow than visual cues, but MSNA was not different between controlled breathing protocols.

In this simple study we provide subjective evidence that breathing to visual cues is less stressful than auditory cues, but we cannot recommend one method over the other. Our results should be useful for others as they consider potential confounding effects of experimental controlled breathing techniques.

**Funding source(s)**

Summer Undergraduate Research Fellowship (SR Jewell), Michigan Technological University, Houghton, MI; Portage Health Foundation, Houghton, MI (WH Cooke). Neither funding source contributed to experimental design, analysis or interpretation.

**Declaration of competing interest**

None.

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