Carotid and aortic baroreflexes of the rat: II. Open-loop frequency response and the blood pressure spectrum

BARRY R. DWORKIN,1,2 XIAORUI TANG,1 ALAN J. SNYDER,3 AND SUSAN DWORKIN1

1Department of Behavioral Science, 2The Neuroscience Program, 3Department of Surgery, Artificial Organs, Pennsylvania State University College of Medicine, Hershey, Pennsylvania 17033

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MORE THAN 25 YEARS AGO, Cowley et al. (14) reported that compared with normal dogs, the blood pressure (BP) distributions of sinoaortic denervated (SAD) dogs “exhibited curves with twice the 24-h standard deviation.” There have since been similar observations in various species, including humans. One interpretation of these observations is that the baroreflexes normally restrain minute-to-minute BP variability; this contrasts with a view of the baroreflexes as provoked to only occasional action by specific perturbations such as thermal stress, postural shifts, or hemorrhage. The physiology of the postSAD-BP variability is not known. Although the variability is reversed by ganglionic block and, thus probably neurally mediated, in unrestrained rats, brain lesions as extensive as precollicular decerebration do not eliminate it (39). Furthermore, ventilated, intensively maintained, neuromuscular-blocked (NMB) rats show similar ganglionic block-dependent and increased postSAD variability (16), indicating that it does not depend on fluctuating respiration or the skeletal activity of general behavior.

As expected for random data, the SAD variability is independent of the sampling interval (2, 7). However, we found that averaging observations over 30 s (16) also did not attenuate the variance and that suggested that the beat-to-beat variability was not uniformly random. A mean is effectively a time-domain filter, and taking differences between successive 30-s systolic BP (SBP) means (16) amounts to applying a 0.005- to 0.025-Hz band-pass filter to the data (see APPENDIX A). That postSAD variability was unattenuated by this averaging strongly suggested that, although random, its spectral power was concentrated in the very low frequencies (VLF).1

Because a negative feedback element constrains variability, noise increases when it is removed. Furthermore, it is fundamental that an element can op-

1 Although the postSAD increases in VLF power are the prominent result of most spectral studies, the highlighted feature is often the much smaller (see Fig. 2, bar graph ordinates) decrease in the LF (0.3 – 0.5 Hz). Jacob et al. (20) found >10-fold increase in VLF, but their abstract, which does not mention VLF, says “a (0.3 – 0.5 Hz) spectral peak was found in Sham but not SAD animals, suggesting that it is associated with the baroreflex.” Similarly, Cerutti et al. (13) found a greater than sixfold increase in VLF, but their abstract also ignored these effects, and said, “In SAD rats, the power spectral density of MAP, estimated by a fast Fourier transform, was reduced in the low-frequency (LF, 0.27- to 0.74-Hz band).” In their opening sentence, Abu-Amarah et al. (1) citing these studies said, “In rats, arterial baroreflexes operate largely on peripheral resistance within the frequency band of 0.25 to 0.7 Hz.”

Address for reprint requests and other correspondence: B. R. Dworkin, Pennsylvania State Univ. College of Medicine, Hershey, PA 17033 (E-mail: brd1@psu.edu).
pose and neutralize noise only where its transfer function (TF) and the noise spectrum coincide; it is a corollary that the spectral change that occurs, when an element is removed, delineates the closed-loop system’s TF. Dog- and rabbit-baroreflex response curves have corner frequencies at ~0.05 Hz (23, 25, 32), and the spectral effects of SAD in the rat predict a similar TF.

In the companion paper (16), we described the statistical variability of the BP in the NMB preparation, showed that it resembled the patterns of ambulatory rats, and then, by directly activating the carotid sinus (SINUS) and aortic depressor nerve (ADN) with hydraulic and electrical stimuli, measured the steady-state baroreflex responses of arterial (ABP) and venous BP (VBP), interbeat interval (IBI), and the skin (skBF), mesenteric (msBF), and femoral (fmBF) blood flow. In the studies described here, with the same vascular effectors relative to the sensory reference or baroreceptors that reflects the net action of the cardiovascular effectors to the central nervous system (CNS); and feedback is from the BP (ABP), mesenteric (msBF), and femoral (fmBF) blood flow. In the studies described here, with the same stimulus modes, and response measures, we applied both step and periodic stimuli and, with the use of several straightforward methods, determined the open-loop frequency and phase response of the carotid and aortic reflexes, estimated the upper limit of the “central” lag for vagus and peroneal nerve activity, and calculated the absolute gain of the TF.

The diagrams in Fig. 1 represent the cardiovascular system with and without the baroreceptor feedback path $[H(s)]$. In both, the source of variability or noise input $[N_1(s)]$ is the same and located in the central nervous system (CNS); and feedback is from the BP through the baroreceptors. There is a neural summing point $[\Sigma_{\text{CNS}}]$ in the CNS, which combines $N_1(s)$ with the baroafferents’ output, and a hydraulic point at the baroreceptors that reflects the net action of the cardiovascular effectors relative to the sensory reference or adaptation level. The implied hypothesis is that, normally, the baroreflex counteracts endogenous variability propagated from $N_1(s)$ and that the difference between the Pre and Post spectra is due to the action of the baroreflex.

An alternative hypothesis is that noise is a de novo experimental artifact introduced by random firing of damaged baroreceptors, which, although severed from their receptive endings, remain connected to nucleus of the solitary tract (NTS) target neurons. Primary afferents in other sensory systems, e.g., dorsal root ganglion cells, show spontaneous activity after distal axotomy (9, 29); however, on the basis of several kinds of neurophysiological and statistical evidence, random activity of axiotomized baroreceptors is not a likely source of the postSAD variability (see analysis in APPENDIX B).

### Relationship Between the Pre and PostSAD Models

The baroreflex is characterized by the open-loop TF, $G(s)H(s)$. With the use of electrical modulation of the ADN and volumetric modulation of a carotid sinus as experimental inputs, the phase and relative gain, as a function of frequency, can be directly measured. Combining these data with the pre- and postSAD spectra, which contain independent information of the system’s response to endogenous noise, the absolute gain of the intact system can be estimated as follows. Figure 1: preSAD the system is intact and the reflex is completely normal; thus

$$Pre(s) = N(s) \cdot \frac{G(s)}{1 + G(s) \cdot kH(s)}$$

postSAD the reflex has been obliterated

$$Post(s) = N(s) \cdot G(s)$$

If the pre- and postSAD measurements are consecutive, and within the same subject, though the across-conditions phase is random, the magnitudes are fully comparable. The spectral and open-loop data are thus complimentary. The magnitudes of the Pre and Post spectra give accurate absolute amplitude ratios at each frequency (but with the effects of phase and magnitude confounded), whereas the experimental open-loop measurements, which give only relative amplitudes, provide independent phase data (see APPENDIX A). Thus

$$\frac{|Post(s)|}{|Pre(s)|} = \frac{|N(s)| \cdot |G(s)|}{|N(s)| \cdot |G(s)|}$$

where $k$ is a scaler constant that, depending on the experiment, converts either the normalized stimulation frequency or balloon volume into pressure. Resolving the complex closed-loop term and including a dummy variable, $\varepsilon$, to represent the error between the two kinds of measurement, we obtain

$$\frac{|Post(s)|}{|Pre(s)|} = \sqrt{1 + 2 \cdot |kG(s)H(s)| \cdot \cos(\phi_{\text{ABT}})} + |kG(s)H(s)|^2 + \varepsilon$$

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²We have discussed the limitations of hydraulic pressure stimulation of the rat carotid sinus (16); in contrast, a volumetric balloon imposes accurate and consistent stretch on the receptors, and the receptors have an unaffected independent circulation (27, 28).
which contains, except $k$, only observables, and when solved for $\epsilon$, as a function of $k$, and minimized in the least-squares sense over all frequencies yields the value of $k$ that gives the best fit between the spectral and TF ratios (calculated from the open-loop measurements). Multiplying the relative open-loop gains for each frequency by $k$ will give the absolute gain function of the reflex, as it was, before any surgery took place.

**METHODS**

The subjects, surgery, and general methods are identical to and described in the companion paper (16). All actual surgery or possibly irritating manipulation was done under controlled and carefully monitored, deep isoflurane anesthesia. The protocol is supervised and certified to be in compliance with National Institutes of Health Guidelines by the Pennsylvania State University College of Medicine Institutional Animal Use and Care Committee. The specific protocols and data analysis are as follows.

**Noise Spectra**

**SBP.** For each rat, 50 randomly chosen 90-s postSAD-SBP samples were automatically extracted to binary files from 4-h 6-kHz digital audio tape (DAT) records of undisturbed baseline. Systole was algorithmically detected and backward-step interpolated into a 1-kHz array, and the spectra were obtained by a fast Fourier transform (FFT) (Hanning, 8.3-mHz resolution) of the detrended data. For rat EH, both pre- and postSAD 6-kHz samples (within 24 h at 0.15% isoflurane) were analyzed, and the VLF (0.01–0.2) and low-frequency (LF) (0.2–0.6) power were measured by integration. For all rats, 2.5-s/sample data pre- and postSAD (within 24 h at 0.15% isoflurane) were analyzed for VLF power. Differences due to SAD were evaluated by ANOVA and post hoc t-tests.

**Sympathetic (peroneal) nerve.** Fifty parallel 90-s postSAD sympathetic trials for each rat were extracted from 6-kHz records, and the spectra were calculated.

**Open-Loop Transfer Function**

**Modulation frequency analysis.** ADN. Current levels were selected to activate A or A + C fibers (17, 18; see Ref. 16 for criteria). A fibers were stimulated with the use of a 100-μs pulse width (PW) at 50–60 μA; A + C fibers with 300 μA at 80–100 μA. The test parameters were 110% of the minimum and 90% of the maximum linear range (see Ref. 16): 20–50 impulses/s for A; 3–20 ips for A + C. The periodic stimuli were symmetrical on-off cycles of 0.02–0.4 Hz for 120 s. ADN modulation analysis was done in three rats. SINUS. Stimulation was done by inflating a microballoon in a vasoconstrictor isolated sinus (16); the range was determined as above 1.7–3.2 μl (peak-to-peak) at frequencies of 0.02–0.4 Hz. Complete analyses were done in two rats.

**Power spectral analysis (SBP).** The test stimulus modes were SINEUS (2 rats), ADN-A (3 rats), and ADN-A + C (1 rat). The test frequencies for ADN-A were 0.02, 0.03, 0.0375, 0.045, 0.055, 0.0625, 0.0875, 0.1, 0.1125, 0.1375, 0.15, 0.1625, 0.175, 0.2, 0.25, and 0.4 Hz; for ADN-A + C: 0.02, 0.025, 0.0375, 0.05, 0.0625, 0.075, 0.0875, 0.1, 0.1375, 0.175, 0.2, 0.25, and 0.4 Hz; for ADN-A + C: 0.025, 0.03, 0.0375, 0.05, 0.05, 0.0625, 0.075, 0.0875, 0.1, 0.125, 0.1375, 0.175, 0.2, and 0.4 Hz. For each rat, stimulus mode, and test frequency, 5 to 27 spectra were averaged (see Table 1). Each spectrum was obtained by an FFT (8.3-mHz resolution) on the 120-s interpolated, Hanning-windowed responses. The amplitude TF were calculated from the normalized square-root power and interpolated to estimate the −3- and −20-dB frequencies and 0.4-Hz amplitude.

**Sinusoidal fit.** The ensemble-averaged signals were iteratively fit to a sine function. The variables of the fit were the amplitude, phase lag, and frequency. The amplitude TF were directly estimated by calculating the output-to-input amplitude ratio and extrapolated as above.

**Step-frequency analysis.** ADN. The parameters were the maximum used for periodic stimulation. For A fibers, it was 20–50 ips; and, for A + C fibers, it was 9–20 impulses/s. Analyses were completed for five rats. SINEUS. Balloon volume was ∼80% of the linear range maximum (2.5–3.2 μl); analyses were completed for three rats.

**Transient response.** The output variables were systolic BP (SBP), IBI, mesenteric vascular conductance (msVC), and femoral vascular conductance (fmVC). For each rat, for ADN-A, A + C, and SINEUS, 20 stimuli at each of 2–4 strengths were ensemble averaged. With the use of the difference between the stimulation period and mean of the 12-s prestimulus baseline, the initial 50 s of the averaged response was iteratively fit to $y(t) = A(1 − e^{−2t})$, where $A$ is the asymptotic response amplitude, $T$ is the time constant, and the TF of the step is defined as $sY(s)$.

**Transportation lag estimates.** SBP. For each of five rats, SINEUS, ADN-A, and A + C, open-loop transportation lags were measured. Each data set was composed of 50 prestimulus and 80 stimulus-on cardiac cycles. A least-squares line was fit to the prestimulus data, and an exponential was fit to the stimulus data (see Fig. 6); simultaneous solution relative to $t_d$ gave the transportation lag (see Fig. 6). CNS. Fifteen 3.0-μl SINEUS step responses were extracted from 6-kHz DAT; the IBI was measured, back interpolated, and represented as instantaneous frequencies ($f_j$). The balloon volume, heart frequency, and absolute value of the vagus and sympathetic neurograms were ensemble averaged and smoothed by a 10-point second-order Savitzky-Golay algorithm. The stimulus onset was defined with respect to the peak volume rate of change and threshold volume. The response onsets were defined at 3 SD above the baseline.

**Gain-Scaling Factor [k]**

Rat EH. For each of the $n = 20$-modulation test frequencies, $f_j$, the normalized RMS amplitudes, $GHi$, were obtained from FFT modulation TF estimates. Spectral ratio was determined by division of the preSAD by the postSAD amplitude spectra, and the lumped open-loop system lag, $\tau_{lag}$ and estimated first-order phase lag, $\arctan(2\pi f/T)$, were used to calculate the phase. The error, $\epsilon$, between the TF and spectral measurements (Eq. 3) was differentiated with respect to $k$, and the value of $k$, corresponding to the minimum, was determined for each kind of stimulation.
For the other rats in Table 1, a similar procedure was used but with 2.5 s/sample preSAD data, and correspondingly, the calculations were limited to amplitudes at 0.075 Hz.

RESULTS

PostSAD spectra. BLOOD PRESSURE. Figure 2D shows that the normalized high-resolution postSAD-SBP spectra of five undisturbed NMB rats (including rat EH) are very similar. The corresponding postSAD and preSAD spectra for rat EH are shown in the main panel of Fig. 2. The key feature is the large increase in VLF power ($D_{PSD} = 1.2 \times 10^5 \text{ mmHg}^2/\text{Hz}$, df = 49, $t = 3.65$, $P < 0.001$), and a small, but reliable, decrease in LF power ($D_{PSD} = -1.5 \times 10^5$, df = 49, $t = -2.02$, $P < 0.05$) (see Ref. 4). C: PostSAD spectrum in the main panel compared with an identically processed spectrum obtained 3 wk later, but without isoflurane (between-spectra LANOVA: regression $m = 0.995$, $b = 0.1\%$ of peak, $r^2 = 0.965$, $P < 0.0001$, K-S for $=0.1$ Hz, $x^2 = 1.4$, $P > 0.999$). D: highly similar normalized postSAD spectra of 5 different rats (including EH) without isoflurane.

Modulation time-domain responses. The series of traces in Fig. 4 are examples of the step response (0 Hz) and modulation of the component responses by ADN-A + C stimulation. [The abdominal conductance (msVC) includes the aorta below the superior mesenteric artery.]

Periodic input Power Spectral Analysis. Figure 5 is the SBP-amplitude spectra (EH, ADN-A, ADN-A + C, SINUS) for eight test frequencies. The procedure was the same as for the power spectra shown in Fig. 2, except the absolute value of the amplitude per square root Hertz is plotted; the corresponding normalized FFT amplitudes for all rats are in Table 1. The solid lines are the average spectra during the specified test stimulus (e.g., ADN-A, 0.1 Hz), and the gray areas are average spectra during the baseline periods that immediately preceded the onset of that kind of test stimulus. The averages are across all trials for the specified mode (e.g., ADN-A + C). The figure illustrates the relationship between the noise and the modulation amplitudes at various frequencies, which are both products of $G(s)$. Sinusoidal Fits. An iterative least-squares fit of a sine function to the modulated output is a straightforward measure of the peak-to-peak response amplitude. Table 2 gives the normalized amplitudes for SBP for each rat and stimulus mode; the $-3$- and $-20$-dB frequencies were calculated directly from the power ratios.
Step input. For SBP, the mean 3-dB frequency was 0.035 Hz for ADN-A, 0.046 Hz for A+C, and 0.056 Hz for SINUS; the magnitude at 0.4 Hz was 6–18% of the maximum response. fmVC was similar to SBP, but msVC had a reliably lower and IBI had a reliably higher 3-dB frequency. Table 3 summarizes the results and statistical tests for five rats.

Transportation lags. BARORECEPTOR TO SBP. The mean ADN lag was \(1.07\) s (Table 4 and Fig. 6). After subtracting the transportation lag, cross-correlation analyses of the modulated SBP at each test frequency did not reveal an additional phase shift. However, for \(>0.15\) Hz, the modulation was weak, and at \(<0.05\) Hz, there was considerable VLF noise (see Fig. 2); thus given the close exponential approximation of the response, nonlinear phase effects were assumed to be present.

BARORECEPTOR TO VAGUS. Vagus recordings of 15 SINUS stimulations (rat EH) are shown in Fig. 7. The ensemble average of these, of the corresponding heart rate (HR) traces, and of the
Table 1. FFT fit (SBP)

<table>
<thead>
<tr>
<th>Mode</th>
<th>ADN-A</th>
<th>ADN-A + C</th>
<th>SINUS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat</td>
<td>EH</td>
<td>EC</td>
<td>DY</td>
</tr>
<tr>
<td></td>
<td>Ampl ± SD</td>
<td>n</td>
<td>Ampl ± SD</td>
</tr>
<tr>
<td>0.02</td>
<td>0.88 ± 0.53</td>
<td>10</td>
<td>1 ± 0.53</td>
</tr>
<tr>
<td>0.025</td>
<td>1.00 ± 0.41</td>
<td>5</td>
<td>0.83 ± 0.36</td>
</tr>
<tr>
<td>0.05</td>
<td>0.83 ± 0.43</td>
<td>10</td>
<td>0.71 ± 0.31</td>
</tr>
<tr>
<td>0.075</td>
<td>0.57 ± 0.38</td>
<td>5</td>
<td>0.56 ± 0.24</td>
</tr>
<tr>
<td>0.1</td>
<td>0.48 ± 0.17</td>
<td>5</td>
<td>0.40 ± 0.13</td>
</tr>
<tr>
<td>0.125</td>
<td>0.24 ± 0.17</td>
<td>5</td>
<td>0.36 ± 0.13</td>
</tr>
<tr>
<td>0.15</td>
<td>0.20 ± 0.16</td>
<td>5</td>
<td>0.22 ± 0.12</td>
</tr>
<tr>
<td>0.2</td>
<td>0.14 ± 0.18</td>
<td>5</td>
<td>0.17 ± 0.14</td>
</tr>
<tr>
<td>0.4</td>
<td>0.01 ± 0.03</td>
<td>5</td>
<td>0.02 ± 0.02</td>
</tr>
<tr>
<td>−3 dB Hz</td>
<td>0.053</td>
<td>-</td>
<td>0.052</td>
</tr>
<tr>
<td>−20 dB Hz</td>
<td>0.278</td>
<td>-</td>
<td>0.275</td>
</tr>
<tr>
<td>0.4 Hz</td>
<td>0.008</td>
<td>0.020</td>
<td></td>
</tr>
</tbody>
</table>

Values are expressed as means ± SD. Normalized fast Fourier transform (FFT) systolic blood pressure (SBP) amplitudes (Ampl) as a function of frequency for each rat and stimulus mode. ADN, aortic depressor nerve; SINUS, carotid sinus pressure.

Table 2. Sinusoidal fit (SBP)

<table>
<thead>
<tr>
<th>Mode</th>
<th>ADN-A</th>
<th>ADN-A + C</th>
<th>SINUS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat</td>
<td>EH</td>
<td>EC</td>
<td>DY</td>
</tr>
<tr>
<td></td>
<td>Freq, Hz</td>
<td>Ampl ± SD</td>
<td>Output Freq</td>
</tr>
<tr>
<td>0.02</td>
<td>0.98 ± 0.01</td>
<td>0.202</td>
<td>10</td>
</tr>
<tr>
<td>0.025</td>
<td>1.00 ± 0.01</td>
<td>0.206</td>
<td>5</td>
</tr>
<tr>
<td>0.05</td>
<td>0.84 ± 0.02</td>
<td>0.050</td>
<td>10</td>
</tr>
<tr>
<td>0.075</td>
<td>0.22 ± 0.01</td>
<td>0.079</td>
<td>5</td>
</tr>
<tr>
<td>0.1</td>
<td>0.20 ± 0.01</td>
<td>0.100</td>
<td>5</td>
</tr>
<tr>
<td>0.125</td>
<td>0.15 ± 0.02</td>
<td>0.126</td>
<td>5</td>
</tr>
<tr>
<td>0.15</td>
<td>0.18 ± 0.02</td>
<td>0.149</td>
<td>5</td>
</tr>
<tr>
<td>0.2</td>
<td>0.11 ± 0.01</td>
<td>0.195</td>
<td>5</td>
</tr>
<tr>
<td>−3 dB Hz</td>
<td>0.035</td>
<td>0.047</td>
<td>0.042</td>
</tr>
<tr>
<td>−20 dB Hz</td>
<td>0.250</td>
<td>0.220</td>
<td>0.300</td>
</tr>
</tbody>
</table>

Normalized sinusoidal fit SBP amplitudes (peak-to-peak) as a function of frequency for each rat and stimulus mode.

Table 3. SBP and component baroreflex mechanisms

<table>
<thead>
<tr>
<th>Mode</th>
<th>ADN-A</th>
<th>ADN-A + C</th>
<th>SINUS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat</td>
<td>EH</td>
<td>EC</td>
<td>DY</td>
</tr>
<tr>
<td></td>
<td>n</td>
<td>A ± SD</td>
<td>T ± SD</td>
</tr>
<tr>
<td>57</td>
<td>45.2</td>
<td>2.99</td>
<td>0.056</td>
</tr>
<tr>
<td>35</td>
<td>10.6</td>
<td>6.82</td>
<td>0.026</td>
</tr>
<tr>
<td>57</td>
<td>3.99</td>
<td>0.041</td>
<td>0.003</td>
</tr>
<tr>
<td>58</td>
<td>9.90</td>
<td>2.10</td>
<td>0.081</td>
</tr>
</tbody>
</table>

Summary of the asymptotic values (A), time constants (T), and −3 dB frequencies for each component response mechanism and mode of baroreflex stimulation for 5 rats. n is the total number of responses analyzed; A is the mean asymptotic response magnitude taken across all test frequencies. The statistics are 2-tail paired t-tests comparing the mean at each frequency for each rat to the corresponding parameter for SBP. *P < 0.05; †P < 0.01; ‡P < 0.001. [Autocorrelation tests gave no evidence of serial dependence in the data (4), and the overall ANOVA was highly reliably.]

balloon volume are shown in Fig. 8. The 3-SD (above baseline) increase in vagus activity occurred in <30 ms, and the time to first peak of vagus activity was ~95 ms; thus the estimated maximum delay from balloon inflation to vagus firing was 30–95 ms, BARORECEPTOR TO SYM-PATHETIC. The peroneal nerve data in Fig. 8 parallel the vagus data. With the use of the same stimulus onset definition, the 3-SD change was at ~20 ms, and the time to the first peak was ~84 ms.

In sum, the central (neural) component of the baroreflex is <100 ms or <10% of the overall transportation lag.
Estimation of the gain-scaling factor \(k\). The values of \(k\) that gave the minimum summed squared error between the spectrum-determined pre-to-postSAD ratio and the experimentally determined open-to-closed-loop TF ratio (see equations 1 and 2) are the maximum entries in Table 5. Figure 9 shows the \(k\) corrected \(rhs\) (TF ratio; right-hand side) and the \(lhs\) (spectral ratio; left-hand side) of equation 1 plotted against frequency (left panels) and one another (right panels). The plots and correlation analyses show a close correspondence between the theoretically equivalent functions derived from entirely different kinds of data in the same rat (ADN-A: \(n = 20, r = 0.95, P < 0.0001\); ADN-A + C: \(n = 19, r = 0.89, P < 0.0001\); and SINUS: \(n = 21, r = 0.92, P < 0.0001\)). It should be particularly noted that, in scaling, the same value of \(k\) was used at each frequency; the procedure was thus a linear transformation and did not change the correlation coefficient or its statistical reliability. The conventional correlation coefficients, \(r\), in Fig. 9 are for the best-fitting regression lines. The theoretical absolute identity lines are drawn to show the relationship to the actual data, and the correlations were also calculated with the fit constrained to these (\(m = 1; b = 0\)) lines with a result (ADN-A: \(n = 20, r_1 = 0.94, P < 0.0001\); ADN-A + C: \(n = 19, r_1 = 0.87, P < 0.0001\); and SINUS: \(n = 21, r_1 = 0.90, P < 0.0001\)) that is very similar to the unconstrained “best-fit” line. Applying the derived \(k\) values to the normalized open-loop TF gives estimates of absolute gain at each frequency (Table 5). At >0.3 Hz, the left-hand (\(lhs\)) and right-hand side (\(rhs\)) ratios of equation 1 conform to one another better for the SINUS than for the ADN (Fig. 9). This is consistent with the established adaptation characteristics of barosensitive stretch endings (24) and is supported by Table 5 and Fig. 10, which show that, for SINUS, the open-loop gain and the feedback gain \((|GH||N|^{-1}|Post|^{-1} = |GH||G|^{-1} = |H|)\) are comparatively larger at higher frequencies. In the closed-loop, i.e., preSAD, the net phase shift becomes 180° at ~0.28 Hz, thus for the SINUS, which has increasing sensitivity in this range, the positive feedback is enhanced and endogenous noise is correspondingly amplified. In that the preSAD spectral estimates (which are the same for all stimulus modes) include stretch endings, the SINUS open-loop measurements, which (unlike the ADN measurements) also include stretch endings, should more authentically emulate the detailed properties of the natural intact system; hence, the better fit at the higher frequencies. RATS DY, EC, AND EF. With the use of the 2.5-s/sample preSAD data for rat EH, the absolute gain was 1.39 ± 0.35 (compared with 1.71 ± 0.52 for the high-resolution estimate); taken overall, on the basis of the 2.5-s data, the mean absolute gain for rats DY, EC, EF, and EH was 1.47 (3 df, 95% CI = ±0.48). DISCUSSION To gauge the absolute gain of the intact baroreflex, the open-loop TF was used as a template by constraining the explicitly measured open-loop lag and relative magnitude at each frequency to the ratio of the pre- to postSAD endogenous noise spectra. In contrast to the periodic and step inputs of the open-loop analysis, the properties of the endogenous noise input are not explicitly defined. However, because most postSAD variability is blocked by chlorisondamine, the sympathetic nerve firing rate spectrum (Fig. 3) probably approximates \(N(s)\), and if the noise is wide band, because the noise terms cancel, the actual spectrum is not critical (see APPENDIX B). The gain estimates obtained for four NMB rats, including the detailed data from rat EH, were similar to one another (1.47 ± 0.48) and to published values for other preparations, including those from the unanesthetized and reversibly isolated-sinus dog (35, 36), the open-loop baroreflex preparation that is most nearly physiological (Ref. 5 gives 1.19 ± 0.24, and Ref. 19 gives 1.36 ± 0.25). Differences between hydraulic and electrical stimulation. The gain calculation assumed that numerators and denominators on both sides of equation 1 were identical. In principle, this is correct for the SINUS, but not for the ADN, where nerve stimulation bypasses natural stretch endings. If these have a frequency-dependent TF, the \(lhs\) and \(rhs\) quotients will not multiplicatively scale to superimposable curves; this defect is evident in Fig. 9 for higher frequencies of the ADN-A and A + C.

![Fig. 6. Transportation lag measurement example. The data consist of SBP samples from 50 prestimulus and 80 stimulus-on cardiac cycles. A line was fit to the prestimulus and an exponential to the stimulus-on data. The simultaneous equations were solved for the intersection, and the time from the stimulus onset \(t_\text{on}\) to the calculated intersection was taken as the transportation lag. The data for 5 rats are in Table 4.](http://ajpregu.physiology.org/)
Anatomically, $H$ is the feedback path from the baroreceptor stretch endings ($\Sigma_{BR}$) to $\Sigma_{CNS}$ (Fig. 1, preSAD). Functionally, $H$ transforms BP into neural activity; but, the TF cannot be accounted within a purely physical framework. The best that can be done is to indirectly estimate the relative attenuation. Normally, the postganglionic sympathetic outflow [Fig. 1, preSAD: between $\Sigma_{CNS}$ and $G(s)$] is a function of both $H$ and the endogenous variability ($N_1$); however, in the open-loop, i.e., postSAD, $H$ is eliminated. If the sympathetic activity has a level spectrum (Fig. 3) and is the principal input to $G$, $u_G$ is the ratio of sympathetic and SBP spectra, and $|H| = |GH|/|G|$. The result (Fig. 10) is consistent with $|H|$ being relatively flat in the VLF, and for the SINUS, which includes the stretch endings, having increasing gain at $>0.3$ Hz (see also Figs. 3 and 6 in Ref. 22).

Implications of the TF for the SBP variability spectrum. The physiological function of the baroreflex is to attenuate BP variability, and its direct manifestation is a trough in the spectrum that corresponds to the passband of the intact reflex. In the VLF ($<0.1$ Hz) region, the attenuation due to feedback is approximately uniform; this is because the phase is effectively constant, and the feedback TF, $H$, is flat. Our open-loop estimates of the $-3$-dB frequency of the NMB rat baroreflex of $0.03–0.07$ Hz agree with determinations for the anesthetized dog and rabbit of $0.04–0.05$ Hz (23, 25, 32); and, our spectral measurements agree with previous studies in freely moving rats (13, 20). Figure 9 compares the actual attenuation of BP variability, by the baroreflex, with what was calculated from the open-loop determinations of $GH$. Given that the estimated absolute gain also agrees with applicable published values, the overall correspondence is quite good.

The VLF trough is the “business end” of the baroreflex; by comparison, the LF peak is a minor feature, but because it has been repeatedly noted (1, 10, 13, 20, 21, 31) and it appears to depend on an intact baroreflex, it should be predictable from a correct TF. For phase shifts of 90 to 270° in a closed-loop negative feedback system, the signal, arriving back at the summing point, augments, rather than offsets, the input signal; resulting in its amplification rather than attenuation. At precisely 180°, the signal remains coherent as it repeatedly circulates the loop; thus the system can display resonant behavior, i.e., oscillate. For a system of frequency $f$, the phase lag for a transport delay, $\tau_{lag}$, is $\phi = 2\pi \tau_{lag}/f$; the system resonates when $\phi = \pi$. Thus, e.g., $\tau_{lag} = 1.05 \text{s} \rightarrow f_{res} = 0.48$ Hz. In addition to $\tau_{lag}$ for first-order linear systems (see Fig. 6), the phase lag,
Absolute gain as a function of frequency for rat EH. The relative gains from the open-loop measurements were multiplied by the appropriate gain-scaling factor \( k \) (see equations 2 and 3).

\[
\phi(f) = \arctan(2\pi f T), \quad \text{where } T, \text{ the time constant, is equivalent to a delay of } \arctan(2\pi f T) / 2\pi.
\]

Burgess et al. (10, 11) modeled the rat baroreflex with the use of a combination of transport and first-order delays, and they concluded that the LF peak is a resonance. The data of their most thoroughly analyzed rat (B: \( f_{\text{res}} = 0.35 \pm 0.05, \tau_{\text{lag}} = 0.8 \pm 0.1, T = 3 \pm 1 \)) largely overlaps that of ours (EH: \( f_{\text{res}} \approx 0.33 \) (Fig. 2), \( \tau_{\text{lag}} = 1.05 \pm 0.03 \) (Table 4), \( T = 2.8 \pm 0.1 \)), and both are in accord with their analysis, given that the frequency reported for the LF peak, in fact, encompasses a broad range (1, 10, 13, 20, 21, 31). Finally, although the open-loop LF gain is very low and the LF resonance is not a major component of BP variability (in terms of noise power, the VLF-SAD increase is 100 times the LF decrease), if the LF frequency depends on the delay between neural efferent and circulatory events, it is potentially a useful and noninvasive index of sympathetic vascular kinetics and status (12).

Calculating the gain from the spectra. For rats, the relative gain, lag, and time constant estimates from Tables 1, 3, and 4 can be combined in equation 3 with empirically determined pre- and postSAD amplitudes and \( \varepsilon \) minimized with the use of a least-squares algorithm. In each subject, preSAD measurements can be made with several different treatments; then, after SAD, the baseline spectrum under each treatment determined and the ratios calculated. [Conservatively, to assume that \( N(s) \) is stationary, the postSAD treatment effects must be small.] This method can substitute for pharmacological determinations (37), and if recent evidence that HR does not uniformly sample general baroreflex function is correct (6, 16), it might prove to be more valid.

Guided by the rat analysis, gain can be estimated in species where long-term BP recordings are feasible, but TF measurements are not, for example, in a mouse strain. The relative gains can be first determined from the spectra: the net lag estimated from the LF resonance peak, and for each frequency, the normalized gain calculated.

In practice, the spectral gain estimates are robust and depend chiefly on the ratio of VLF amplitudes.
Thus, theoretically, comparison with normalized gains over many frequencies is preferable; but the <0.075-Hz amplitudes alone are sufficiently accurate for many purposes [see APPENDIX A, Averaging TF (b)].

**Perspectives**

In this and the companion paper (16), we examined the properties of the BP variability spectra and the baroreflex TF in the same chronic unanesthetized NMB rats. Our measurements were in accord with those from other species and preparations. Furthermore, we showed that when algebraically combined and mutually constrained, the spectra and TF could together gauge the absolute gain of the baroreflex. A form of this method may be useful in evaluating the effects of genetics, drugs, or other manipulations on baroreflex function.

All in all, statistical analysis, computational models, and the experimental findings support the assertion that postSAD-increased variability is caused by removing the restraint of the baroreflex on endogenous sources of noise. This underscores that, rather than being only occasionally exercised, the baroreflex is constantly active, probably making adjustments equivalent to 10–20 mmHg, at least, every few minutes. The purpose, if any, of such ceaseless interplay between endogenous noise and the reflex remains to be elucidated (15, p. 79–84).

**APPENDIX A**

**Equation 1**

We can accurately measure both GH and the spectra; by comparison, the estimate of G is rough, thus the algebraic aim is a pair of expressions relating the spectral ratio to the experimental TF ratio.

Regardless of phase, the magnitude of the product (quotient) equals the product (quotient) of the magnitudes, thus

\[
\frac{\left|\text{Post}(s)\right|}{\left|\text{Pre}(s)\right|} = \frac{|N(s) \cdot G(s)|}{|N(s)| \cdot |G(s)|} = \frac{G(s)}{1 + G(s) \cdot H(s)}
\]

Because the phase of \(G(s)H(s)\) represents the lag that occurs in transit of the signal through the loop, \(|1 + G(s)H(s)|\) involves addition at \(\Sigma_{\text{CNS}}\); in resolving it, the phase needs to be considered [by substituting \(j\omega\) for \(s\) and representing \(G(j\omega)\) and \(H(j\omega)\) in magnitude-phase form]

\[
\frac{\left|\text{Post}(s)\right|}{\left|\text{Pre}(s)\right|} = |1 + G(s) \cdot e^{-j\phi_G} \cdot H(s) | \cdot e^{-j\phi_H} = |1 + G(s)H(s) | \cdot e^{-j\phi_GH}
\]

then, changing to trigonometric form and applying the usual definition of the magnitude gives

\[
\frac{\left|\text{Post}(s)\right|}{\left|\text{Pre}(s)\right|} = \sqrt{1 + 2 \cdot |G(s)H(s)| \cdot \cos(\phi_{GH}) + |G(s)H(s)|^2}
\]

The actual experimental input to \(G(s)H(s)\) is microliters or impulses per second, not millimeters Hg. Because the peak-to-peak stimulus amplitudes were constrained to the linear stimulus-response range (16), a frequency-independent scaler \([k]\) with units of millimeters Hg per impulses per second or millimeters Hg per microliters converts the test stimuli to equivalent pressures (see equations 2 and 3) in the intact system.

**Averaging TF**

(a) A “sliding block” average of \(N\) points is equivalent to convolution of the original data with

\[
f(t) = \begin{cases} 
\frac{1}{N} & \text{for } 0 < t \leq \frac{N}{\text{fr}} \\
0 & \text{for } \frac{N}{\text{fr}} < t
\end{cases}
\]

which has the Fourier transform,

\[
|F(f)| = \frac{2}{2\pi f} \sin \left(\frac{2\pi f \cdot N}{2}\right)
\]

or for \(N = 20\)

\[
= \frac{1}{20\pi f} \sin (20\pi f)
\]

(b) A difference between successive samples of \(N\) points, as might be used to assess the effects of a stimulus against baseline, is equivalent to convolution with

\[
F(t) = \begin{cases} 
0 & \text{for } 0 < t \leq N \\
\frac{1}{N} & \text{for } 1 < t \leq 2N \\
0 & \text{for } 2N < t
\end{cases}
\]

The transform of this is

\[
F(f) = \frac{-2}{f^2 \pi f} \sin^2 \left(\frac{2\pi f \cdot 2N}{4}\right)
\]
APPENDIX B

Evaluation of the Random Noise Hypothesis

Neuroanatomic. On the basis of lesion studies, the baroreceptors themselves are probably not the noise source: lesions of the NTS that destroy both the presynaptic terminals and the second-order neurons appear to produce at least as much variability as peripheral anatomy (7, 8, 33, 38).

General statistical properties. Assume that ABP is inversely proportional to the output of the second-order neuron pool, which is proportional to the sum of the firing rates of n baroreceptors. Each carotid sinus nerve and ADN has ~625 fibers (3, 26); thus a conservative (the postSAD variability is quantitatively consistent across the literature, and fewer cells favor the random hypothesis), but tenable, assumption is that of ~2,000 baroreceptors, at least 100 are spontaneously active, and that their combined output is the system input, N_2(s) (Fig. 11, diagram).

Saturation stimulation of one ADN at 1 impulse/s produces an ~5-mmHg SBP change (17), which is ~0.5σ of the postSAD ABP. Thus the spontaneous output of each baroreceptor is modeled as a Poisson variate with λ ~ 1; and, for n = 100, such baroreceptors firing together, nλ = 100, which is well approximated by the normal variate (μ = 100; σ^2 = 100). Thus the probability that the firing rate of the ensemble increases for 1 s by 1 σ (10 impulses/s) is ~0.16. However, nearly all of the spectral power of the postSAD variability is at <0.05 Hz, and convolution of the firing rate time series with a low-pass filter having this characteristic is equivalent to requiring that this rate be sustained for ~20 s, which is an event with a probability ~10^-8 (see Fig. 2 and Ref. 30).

Finally, in view of the above information, random activity predicts that with partial, in contrast to total, SAD, a smaller number of damaged cells and smaller λ would lead to greater variability; however, rats without any baroreflex have significantly more variability than those with partial function (34).

In sum, it is highly unlikely that postSAD BP variability could be the product of summed independent random activity of damaged neurons.

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