Baroreflexes of the rat. III. Open-loop gain and electroencephalographic arousal

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Dworkin, Barry R., and Susan Dworkin. Baroreflexes of the rat. III. Open-loop gain and electroencephalographic arousal. *Am J Physiol Regul Integr Comp Physiol* 286: R597–R605, 2004. First published October 30, 2003; 10.1152/ajpregu.00469.2003.—In early studies of humans, baroreflex sensitivity was found to be higher during sleep; however, subsequent observations in several species, including humans, have been at variance with the original reports. Sleep and arousal are behavioral states, and it is difficult to accurately and repeatedly measure baroreflex sensitivity in behaving animals. However, pharmacologically immobilized (neuromuscularly blocked) rats have apparently normal sleep-wakefulness cycles, and baroreflex gain can be measured directly in this preparation. Using the delta band of the EEG (EEGδ) as an index of sleep and arousal and open-loop aortic depressor nerve (ADN) stimulation as a baroreflex input, we found that blood pressure (BP) level depended on arousal (r = −0.416; P < 0.0001), and BP baroreflex gain depended on BP level (r = 0.496; P < 0.0001), but that BP baroreflex gain was independent of arousal (r = 0.001; NS). Heart period (HP) was different; although HP level depended on arousal (r = 0.352; P < 0.0001), HP baroreflex gain did not depend on HP level (r = 0.029; NS), and HP baroreflex gain increased with arousal (r = 0.315; P < 0.0001). A partial-correlations analysis showed that the presence of the relationship between BP level and BP baroreflex gain probably attenuated the relationship between arousal and BP gain. The results are consistent) 1 with physiological findings showing that arousal attenuates afferent transmission through the nucleus of the solitary tract and enhances sympathoinhibition at the rostral ventrolateral medulla; and 2) with observations in humans and animals showing increased cardiac baroreflex sensitivity during sleep, but little if any effect of sleep on BP baroreflex sensitivity. The findings are relevant to all methods of baroreflex gain estimation that use HP as the index of baroreflex activation.

baroreceptor; gain; sleep; peroneal nerve

IN EARLY STUDIES OF HUMANS, baroreflex sensitivity was found to be higher during sleep (23, 24); however, subsequent observations, using various methods and in several species, including humans, have not consistently confirmed these observations. Sleep and arousal are behavioral states, and measuring baroreflex sensitivity in behaving animals is difficult, but the EEG and the component vascular, cardiac, and neural baroreflex responses of chronically maintained central nervous system (CNS)-intact, neuromuscularly blocked (NMB) rats can be accurately determined (8, 9).

Although NMB rats have distinct diurnal and sleep-wake cycles, our preliminary analyses showed that there were practically no time-of-day or EEG effects on baroreflex gain. This was not as anticipated. In early observations in humans with the Oxford pressor method, which uses a vasoconstrictor to elevate blood pressure (BP) and challenge the baroreflex, Pickering et al. (27) reported clear evidence of greater baroreflex gain during stages III and IV (slow wave; EEGδ) sleep, and recently, with similar methods, Crisostomo et al. (5) found the same for African Americans and Caucasian men but not Caucasian women. Similarly, using a sequence method that noninvasively analyzes heart period (HP) changes occurring during selected spontaneous BP fluctuations, Parati et al. (22) found baroreflex sensitivity elevated during sleep.

Although the pressor and sequence methods have important differences, a common feature of both is that baroreflex gain is estimated entirely from the effects of BP changes on HP. The baroreflexes are a closed-loop system, and BP cannot be simultaneously the independent and dependent variable. To estimate the vascular sympathoinhibitory component, Nakazato et al. (17) used a peroneal nerve recording. They found reduced pressor-elicted peroneal nerve sympathoinhibition during slow-wave sleep (corresponding to lower vascular baroreflex sensitivity), whereas HP indicated slightly increased sensitivity. That HP might not correlate well with the net baroreflex depressor action was first suggested in chronic dog studies using implanted carotid sinus nerve stimulators, which, during sleep, produced 12–22% greater bradycardia but no change in the net BP depressor effect (31). Similarly, in baboons, using carotid occlusion to inhibit the baroreceptors, Combs et al. (3) found that during sleep, compared with the awake resting state, changes in HP increased, in lower abdominal conductance remained the same, and in renal conductance decreased.

Most chronic animal studies of baroreflex gain and arousal have used behaving subjects and have adapted human methods of baroreflex assessment. Some have found an increased baroreflex sensitivity (based on HP) during slow-wave sleep, whereas others have not. For example, in rats, using a validated sequence method, Oosting et al. (21) found increased gain during “the sleeping period,” but Zoccoli et al. (33) using a similar HP regression measure, with more sophisticated criteria for sleep, reported that baroreflex gain was substantially independent of states of wakefulness, active sleep, and quiet sleep.

Methodology may be responsible for the inconsistencies. In addition to the common limitation of measuring only HP, the

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1However, NMB rats have no respiratory or skeletal muscle activity, and it seemed plausible that these functions might be requisite to the effects of arousal on baroreflex gain.
pressor and sequence methods each have particular shortcomings compared with actual open-loop reflex measurement. The pressor method can be repeated, at most, a few times per hour, and the sequence methods assume stationarity, over time and/or conditions, of the various sources of cardiac variability. Although it is difficult to make open-loop baroreflex sensitivity measurements in ambulatory small animals that are sufficiently well stabilized to have normal sleep patterns, frequent, unobtrusive, and precise open-loop measurements can be made in NMB rats.

In the following we describe and analyze two different extensive sets of gain data. The first set of data is from two of the five NMB rats described in Ref. 8 (see Table 1, rats EF and EH). These rats were selected for the present analysis of the relationship between EEG and baroreflex gain because, for both, the aortic nerve electrode and the sinus balloon were functional and gave stable average baroreflex responses for 18 and 35 days, time sufficient for each kind and intensity of baroreceptor stimulation to have been applied during at least several different EEG-defined arousal levels. These two rats provided 425 open-loop aortic depressor nerve (ADN)-gain vs. EEG-state measurements over a wide range of ADN electrical stimulus intensities. As independent confirmation and replication of the main results from rats EF and EH, we present corresponding data from four additional NMB rats, each of which had multiple gain measurements at a single frequency and electrical intensity of ADN stimulation. This complete replication consists of an additional 819 gain measurements during 49 days; thus the results and conclusions that are presented here are based on 1,244 measurements of arousal state and baroreflex sensitivity in six NMB rats, which were maintained in stable condition for a total of 102 days.

METHODS

The general methods (except as noted) are identical to and described in Ref. 8. All actual surgery or possibly irritating manipulation was done under controlled and carefully monitored deep isoflurane anesthesia. During surgery, the anesthetic level was >1.5% isoflurane, which ensured the following: 1) the EEG was synchronized and dominated by high-voltage slow-wave activity, 2) mean BP (femoral artery BP) was <100mmHg and heart rate (HR) was <420 beats/min, and 3) there were no evident EEG, BP, or HP (interbeat interval or R-R interval) responses to manipulation. During the 3-5 days after completion of surgery, after all wounds healed, anesthesia was gradually withdrawn, and then data collection began. During an NMB experiment, data collection is interrupted and anesthetic levels of isoflurane are reinstituted for any maintenance procedures that might produce discomfort; e.g., suction of the trachea, replacement of a bladder cannula, or removal of feces. The protocol was supervised and certified to be in compliance with NIH guidelines by the Pennsylvania State University College of Medicine Institutional Animal Care and Use Committee. The NMB rats are studied one at a time, monitored, and attended around the clock.

Subjects. All were female Sprague-Dawley rats (250-350 g). The baroreceptors were isolated from the circulation by surgical obliteration or attachment to stimulus input devices. The ADN was not cut distal to the electrode, but affixing the stimulating electrode interrupts the passage of naturally generated impulses, and thus these were baroreflex “open-loop” preparations. For rats EF and EH, specific methods, details of the preparation, surgical isolation and application of Ta-Ta2O3 capacitance electrode for the ADN, and baroreflex gain and frequency response data, including autonomic nerve and blood flow measurements, are in Ref. 8. Four NMB rats (EN, EO, FY, and GF) were similarly prepared, except that no flow transducers or peroneal or vagus nerve recording electrodes were implanted. For these rats, ADN stimulation electrodes and recordings of cardiac interbeat interval (HP), femoral artery BP, and EEG were continuous and stable for the following durations: EN, 16 days; EO, 9 days; FY, 10 days; and GF, 13 days.

During a typical experiment, undisturbed, an NMB rat exhibits relatively normal patterns of sleep and wakefulness (cf. Figs. 1 and 2 with Ref. 6 and with baseline HS1 and HS2 data in Table 1 of Ref. 16). The baroreflex test procedures are automated, and trials are presented on a pseudorandom, predetermined schedule; thus over hours and days, the exogenous barostimulation interacts freely with endogenous sleep cycles and other kinds of periodic and random variability in the physiological state.

Stimuli. The stimulation parameters for the 30-s baroreflex test trials for rats EF and EH are described in Ref. 8. In brief, the kinds of stimuli were high-frequency, low-current ADN (A-fiber optimized); low-frequency, high-current ADN (C-fiber optimized); and carotid sinus balloon. Only the ADN results will be considered in detail (see footnote 3). The data from some ADN stimulation frequencies that

Table 1. Raw correlations of heart period and blood pressure baroreflex responses with EEGs and heart period and blood pressure baselines

<table>
<thead>
<tr>
<th></th>
<th>Heart period Correlations</th>
<th>Blood pressure Correlations</th>
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<tr>
<td></td>
<td>r</td>
<td></td>
</tr>
<tr>
<td>Rat</td>
<td>BRX × EEG</td>
<td>BL × EEG</td>
</tr>
<tr>
<td>EF</td>
<td>↓ 0.310 (+0.313)</td>
<td>↓ 0.024 (0.211)</td>
</tr>
<tr>
<td>EH</td>
<td>↓ 0.405 (+0.211)</td>
<td>↓ 0.271 (0.271)</td>
</tr>
<tr>
<td>EN</td>
<td>↓ 0.363 (+0.507)</td>
<td>↓ 0.397 (0.397)</td>
</tr>
<tr>
<td>EO*</td>
<td>↓ 0.557 (+0.429)</td>
<td>↓ 0.447 (0.447)</td>
</tr>
<tr>
<td>FY</td>
<td>↓ 0.547 (+0.350)</td>
<td>↓ 0.530 (0.530)</td>
</tr>
<tr>
<td>GF</td>
<td>↓ 0.318 (+0.313)</td>
<td>↓ 0.202 (0.202)</td>
</tr>
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Up (↑) and down (↓) arrows give the direction of baroreflex gain (BRX) change with the electroencephalographic measure of sleep/arousal (EEG), or with the corresponding baseline (BL); the absolute value of the correlation coefficient (|r|) gives the strength of the regression relationship in the direction indicated by the associated arrow. The proportion of variance accounted for is r². For rats EF and EH, the baroreflex stimuli were of different strengths; thus the response measure was based on a gain ratio (see METHODS); rats EN, EO, FY, and GF each received only a single stimulus strength, and the calculations were based on the actual changes in physical units. The arrow notation is consistent across all 6 rats, e.g., for BRX × EEG. ↑ indicates that the baroreflex response was larger, for higher EEGs values (i.e., for lower arousal). For rat EO, 24 measurements with baseline blood pressure >170 mmHg were excluded from the calculations in this table, because these were due to a transducer artifact. For the 6 blood pressure BRX × EEG correlations, 5 are effectively zero, and the sixth (EH) is statistically reliable, but in the direction of decreased baroreflex gain with sleep, which is opposite to the usual expectation.

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were not included in the original analyses in Ref. 8 were used here. (The stimulus strength was above the linear stimulus-response range but elicited consistent baroreflex responses.) For each of rats EN, EO, FY, and GF, the trial sequences and procedures were the same as described in Refs. 8 and 9, but all test stimuli were set to a uniform single strength in the A-fiber range (40–75 μA; 40 Hz; 100 μs).

Gain ratio statistic and compilation of data. Because for rats EF and EH there were many different stimulation parameters, for each rat, we defined a relative open-loop gain ratio on an individual trial as the systolic BP or HP change from baseline, during the stimulus, divided by the mean change for all responses to that kind and amplitude of stimulus, e.g., 35 impulses/s, 20 μA ADN. The gain ratio statistic was approximately normally distributed with mean ≈ 1.0 and SD ≈ 0.8. The mean and SD of the baseline HP, BP, and EEGs were also approximately normally distributed and consistent across stimuli, but different for the two rats; thus, to combine the data for rats EF and EH, these variables were standardized as z-scores by subtracting the individual rat’s mean from each measure and dividing by the corresponding SD. The derived values for the ratios and baseline z-scored statistics were pooled across all ADN stimuli, and both rats (for rat EH, n = 122; for rat EF, n = 303), giving a total of 425 sets of measurements for the ADN trials. These sets were used to relate the open-loop baroreflex sensitivity (HP or BP gain ratio) to the arousal state, i.e., z-scored EEGs, at the time of the test stimulation. A greater gain ratio implies greater baroreflex sensitivity, and a greater EEGs implies lower arousal.

For rats EN, EO, FY, and GF, all of the ADN test stimuli were of the same uniform level; thus calculation of a gain ratio statistic was...
not necessary, and the gain analysis for these rats was based on the magnitude of the raw BP or HP change to that rat’s particular ADN stimulus. All stimuli were 40 impulses/s, 100–262 μs pulses; the current (40–75 μA) for each rat was set at approximately the median of the A-fiber range, with the particular value for each rat determined by the procedure described in Ref. 8.

EEG electrodes. The electrode placements are based on Friedman et al. (10) and Mistlberger et al. (16). With the rat in an atraumatic head holder, its scalp was incised; holes were drilled through the skull, 2 mm right of the midline, at bregma, and at lambda; 1 mm screws were threaded into each hole, and wire (silver plated 30 g; Teflon insulated) was wrapped around each of the screws. The screws were embedded in dental acrylic for stability and insulation.

EEG signal processing. With the use of online hardware, –24 dB/octave digital filters (no. 79–78–5 FHC), the ×108 preamplified (differential between the screws) EEG was analyzed into the following bands, and each band was separately rectified and integrated at the indicated time constant ($t_c$): δ (0.5–3 Hz; $t_c = 1.0$ s), θ (6.5–7.5 Hz; 0.5 s), α (8.5–18 Hz; 0.5 s), and β (20–45 Hz; 0.1 s). The resulting units of measurement are microvolt (RMS) seconds per second. All four signals were recorded continuously during the entire experiment. Only the results for the δ-band will be described in detail (see footnote 3). The typical range of raw values for EEGδ is $25–200 \mu V_{RMS} \cdot s^{-1}$.

Peroneal nerve recordings. The peroneal nerve was dissected minimally and suspended on a pair of silver wire (Medwire Ag7/40T) hook (0.5-mm diameter) electrodes ($\approx$10 kΩ), with a spacing of 2 mm. A silicone casting compound was injected from beneath. Peroneal nerve data are impulses per second for spikes of $>10 \mu V_{RMS}$. For each point in Fig. 3, impulse rates were accumulated over three respiratory cycles, and $\sim$1,445 such values were averaged in each hour.

Fig. 2. Plot of hour 11 of day 13. The horizontal bar on the abscissa of Fig. 1 shows the time interval of this graph; the variables are the same as in Fig. 1. The 2 stimulation trials shown are carotid sinus balloon inflations. The structures of 3 sleep cycles (the typical sleep period is 20 min) are evident in the 4 EEG bands, and the relationship between sleep and vagus or peroneal nerve activity can be seen to be more intricate than the simple reciprocity suggested in the 24-h plot; additionally, the cardiovascular effects of 2 sinus balloon step stimuli (black markers at top) are reflected in the blood pressure (BP), heart rate, and systolic femoral blood flow traces.
Based on the relationship between EEG₃ and the responses to the ADN test stimuli².

For rats EN, EO, FY, and GF, the BP and HP responses to ADN stimulation were similar to those reported in Refs. 8 and 9. The BP decrease for each rat was 28.9 ± 8.6, 28.8 ± 13.9, 32.7 ± 9.0, 16.3 ± 15.6, with a group mean of 26.7 ± 11.8 mmHg. The HP increase was 12 ± 9.5 ± 6, 19 ± 1.1 ± 3, with a group mean of 9 ± 5 ms.

Arousal (EEGs) effects on baseline BP and HP. For rats EF and EH, Fig. 4, top, shows the correlation scatterplot \((r = 0.352; df = 424; P < 0.0001)\) for EEG₃ and HP, and Fig. 4, bottom, is the correlation \((r = -0.416; df = 424; P < 0.0001)\) for systolic BP. The data are the mean values during the 2-min interval before each of the 425 baroreflex test stimuli; these relationships are consistent with observations made in human sleep laboratories, as well as baseline data of many previously studied NMB rats. For rats EN, EO, FY, and GF, the BP-EEG₃ correlations were -0.624, -0.544, -0.537, and -0.550. The HP-EEG₃ correlations were 0.507, 0.429, 0.350, and 0.313. With the exception of the rat GF HP, for which \(P < 0.01\), all of the correlations were reliable at \(P < 0.0001\), confirming the generality of the relationships apparent in the traces of Figs. 1 and 2.

Effects of baseline BP and HP on corresponding baroreflex gain. Figure 5, top, shows that for rats EF and EH the ADN-produced baroreflex bradycardia is unrelated \((r = 0.029; df = 424; NS)\) to the baseline HP at the time of stimulus application. In contrast, Fig. 5, bottom, shows a strong relationship \((r = 0.496; df = 424; P < 0.0001)\) between baseline BP and the ADN-produced baroreflex depressor response. These correlations will be further considered in analyzing the effects of arousal on the gain ratios.

Arousal (EEGs) effects on the BP and HP baroreflex gain ratios. Figure 6, top, shows a moderate relationship between EEG₃ and HP \((r = 0.315; df = 424; P < 0.0001)\); this result is similar to, and consistent with, evidence, previously reported for humans and other species, of increased HP baroreflex sensitivity with sleep. Figure 6, bottom, shows the complete absence of a relationship between the EEG₃-defined arousal level and BP baroreflex sensitivity \((r = 0.001; df = 424; NS)\). These findings were replicated, and individually reliable, for each of the rats (EN, EO, FY, and GF). For rat EN the of atonia and rapid eye movements; however, the identification of “quiet” sleep can be made entirely on the basis of the EEG₃. NMB rats have no skeletal muscle activity; however, because the activity in skeletal nerve is not directly affected by the pharmacological block, it is possible to monitor efferent nerve activity attendant on skeletal outflow. We did not do this in the same subjects that were used for measurements of baroreflex gain, but data from other long-term NMB rats show the expected relationship between EEG₃ and skeletal nerve activity (see Figs. 1, 6 and 11 of Ref.7).

Having included the results of sinus stimulation, other EEG measures, or the three additional rats (DU, EC, and EG) described in Refs. 8 and 9 would not have substantially changed any of the results: for all five rats, for both the ADN electrode and sinus balloon stimulation, neither HP nor BP measures of the baroreflex gain ratio were dependent on the EEG₃ or EEG₆ amplitude. Although the HP \((r = 0.18; P < 0.01)\) and BP \((r = 0.17; P < 0.005)\) gain ratios increased at higher EEG₆, compared with EEG₃, these effects were weak, and unlike EEG₃, EEG₆ lacks a generally accepted relationship to sleep and arousal. For rats DU, EC, and EH, the sinus-elicited HP was completely unaffected by EEG₆, but, in fact, the sinus HP effects are very small (Table 1 and Fig. 6 of Ref. 9), and EEG dependence, if present, would have been difficult to detect.

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²In classifying sleep stages, the distinction between wakefulness and rapid-eye-movement sleep involves measures of skeletal muscle activity in the form of atonia and rapid eye movements; however, the identification of “quiet” sleep can be made entirely on the basis of the EEG₃. NMB rats have no skeletal muscle activity; however, because the activity in skeletal nerve is not directly affected by the pharmacological block, it is possible to monitor efferent nerve activity attendant on skeletal outflow. We did not do this in the same subjects that were used for measurements of baroreflex gain, but data from other long-term NMB rats show the expected relationship between EEG₃ and skeletal nerve activity (see Figs. 1, 6 and 11 of Ref.7).
HP-EEG$_5$ regression was $r = 0.363$ (df = 180; $P < 0.0001$), and the BP-EEG$_5$ regression was $r = 0.063$ (df = 180; NS). For rat EO the HP-EEG$_5$ regression was $r = 0.557$ (df = 230; $P < 0.0001$), and the BP-EEG$_5$ regression was $r = 0.001$ (df = 230; NS). For rat FY the HP-EEG$_5$ regression was $r = 0.547$ (df = 341; $P < 0.0001$), and the BP-EEG$_5$ regression was $r = 0.079$ (df = 341, NS). For rat GF the HP-EEG$_5$ regression was $r = 0.318$ (df = 180; $P = 0.01$), and the BP-EEG$_5$ regression was $r = 0.028$ (df = 67; NS).

In a parallel analysis to that used with rats EF and EH (but based on raw HP and BP changes rather than gain ratios), the data from rats EN, EO, FY, and GF were z-scored and combined into a single set. The overall HP-EEG$_5$ regression was $r = 0.491$ (df = 819; $P < 0.0001$), and the BP-EEG$_5$ regression was $r = 0.027$ (df = 819; NS).

Thus the analysis of each of the six NMB rats shows a strong, statistically reliable enhancement at higher EEG$_5$ levels (i.e., lower arousal) of HP baroreflex sensitivity but no enhancement of BP baroreflex sensitivity.

**DISCUSSION**

The HP result (Fig. 6, top) is consistent with evidence that baroreflex-elicited bradycardia is greater during sleep (23, 27), and the absence of a similar relationship for the BP response (Fig. 6, bottom) is also in accord with previous studies. Observations in animals of vascular baroreflex responses (3, 31) have not shown sleep effects, and observations in humans of peroneal nerve activity have shown decreased sympathoinhi-

HP-EEG$_5$ regression was $r = 0.352$ (df = 424; $P < 0.0001$) or systolic BP (bottom) ($r = -0.416$; df = 424; $P < 0.0001$) and the EEG$_5$ measure of arousal for rats EF and EH. The data are from the 120 s baseline before each test stimulus. The scales are z-scores to equate the mean and SD across rats. See Figs. 1 and 2 and Ref. 8 for examples of the raw physiological units.

Fig. 4. Relationship between heart period (HP; top) ($r = 0.352; df = 424; P < 0.0001$) or systolic BP (bottom) ($r = -0.416; df = 424; P < 0.0001$) and the EEG$_5$ measure of arousal for rats EF and EH. The data are from the 120 s baseline before each test stimulus. The scales are z-scores to equate the mean and SD across rats. See Figs. 1 and 2 and Ref. 8 for examples of the raw physiological units.

Fig. 5. Relationship between HP gain ratio (top; $r = 0.029; df = 424; NS$) or BP gain ratio (bottom; $r = 0.496; df = 424; P < 0.0001$) and the corresponding prestimulus baseline for rats EF and EH. The scales are z-scores to equate the mean and SD across rats and different aortic depressor nerve (ADN) test stimulus strengths.

Fig. 6. Relationship between HP gain ratio (top; $r = 0.315; df = 424; P < 0.0001$) or BP gain ratio (bottom; $r = 0.001; df = 424; NS$) and EEG$_5$ for rats EF and EH. The scales are z-scores to equate the mean and SD across rats and different ADN test stimulus strengths.
bition (17), implying reduced, not increased, baroreflex sensitivity, with sleep.

Why does arousal affect baroreflex HP responses differently than BP responses? The partial-correlations analysis for rats EF and EH (Ref. 28; APPENDIX) shown in Fig. 7 addresses this question by using the EEG/HP-gain relationships (Fig. 5) to dissect the EEG/HP-gain relationships (Fig. 6) for HP and BP. Figure 4 shows that both baselines depend on EEG6: the HP becomes longer and the BP lower during slow-wave sleep. Figure 5 shows that the BP gain ratio is strongly coupled to the BP baseline (cf. Fig. 2 in Ref. 19); this means that changes in EEG6 will be transmitted through the BP baseline relationship to the gain ratio (Fig. 7, bottom). Because the BP baseline decreases with EEG6, the predicted effect of sleep (increased EEG6) through this compound path is to reduce the gain ratio. However, Fig. 6 shows that the BP gain ratio, in fact, does not change with EEG6; consequently, by implication, another mechanism, one that increases gain with sleep, is also operating.

The HP result buttresses the BP analysis. Similar to BP, the HP baseline depends on arousal (Fig. 4); however, in contrast to BP, the HP gain ratio does not increase with the HP baseline (Fig. 5). Thus arousal does not increase HP gain through this path (Fig. 7, top), and the direct relationship between arousal and HP gain (Fig. 6) is unopposed.

The result of the partial-correlations analysis for each of the rats, EN, EO, FY, and GF, was similar. For the combined data, removing the effect of the baselines on gain changed the BP-EEG correlation from 0.027 to 0.125 (df = 303; \( P < 0.0005 \)) and the HP-EEG correlation from 0.491 to 0.388 (df = 819; \( P < 0.0005 \)). Thus removing the EEG-HP baseline effect decreased the effect of arousal on HP gain, consistent with the results and analysis of EF and EH shown in Fig. 7 (see Table 1).

In sum, using direct open-loop baroreflex measurements, we found that during periods of higher EEG6 an ADN stimulus produced a larger bradycardia but not a larger BP depressor response. The partial-correlations analysis deciphers this result and suggests a possible heuristic to the physiological mechanisms.

Fictive arousal can be produced in anesthetized animals by hypothalamic and periaqueductal gray electrical stimulation: Hilton and colleagues (4, 11, 12) showed that hypothalamic defense area (HDA) stimuli effectively block either carotid sinus or ADN-elicited baroreflex bradycardia and depressor
responses. In sinoaortic denervated rats with an ADN electrode, stimulation of the posterior hypothalimus attenuates ADN-elicited bradycardia but does not alter either the baroreflex BP or peripheral vascular resistance responses (19, 26).

The vagal and sympathetic baroreflex pathways probably share the same first-order (sensory) neurons, and synaptic inhibition can occur in the nucleus of the solitary tract (NTS) where baroreceptor afferent and HDA fibers converge. Mifflin et al. (15) identified cells with excitatory inputs from the sinus nerve and inhibitory inputs from the HDA and showed that HDA stimulation produces hyperpolarizing inhibitory postsynaptic potentials that shunt sinus nerve-evoked excitatory postsynaptic potentials in the NTS. From the NTS, the vagal and sympathetic pathways diverge to the nucleus ambiguous (NA) and caudal ventrolateral medulla (CVLM).

NA vagal motor neurons project directly to the cardiac parasympathetic ganglia (25) and from there to the sinoatrial (SA) node. In unanesthetized rats, the initial (<20 s) baroreflex bradycardia is predominantly vagal (2, 32), via the release of ACh at the SA node; however, sympathetic endings release norepinephrine and neuropeptide Y, which together can reduce parasympathetic output by blocking release of ACh from adjacent postganglionic vagal terminals; this peripheral reciprocal inhibition, or a functionally similar brain stem mechanism, would lower cardiac baroreflex gain at higher arousal levels (13, 18, 20). Conversely, lower arousal and reduced sympathetic activity, via higher EEG, would be expected to augment cardiovagal baroreflex sensitivity. Thus vagal outflow or cardiac plexus effects of arousal on baroreflex gain, if any, are likely to be similar to those in the NTS.

CVLM inhibitory interneurons (GABA) project to sympathetic premotor neurons in the rostral ventrolateral medulla (RVLM). These RVLM neurons have tonic activity, which may, or may not, depend on obligatory exogenous sources of excitation (1, cf. 30), for example, from the hypothalamus. In NMB rats (Fig. 3), sympathetic (peroneal) nerve activity is lower at higher EEG; thus it is probable that RVLM presym- pathetic activity (the substrate of baroreflex inhibition) is also lower. In early studies with anesthetized rats, fictive arousal via hypothalamic stimulation excited kynurenate-sensitive presym pathetic neurons in the RVLM and increased lumbar sympathetic nerve discharge but did not affect sympathetic nerve baroreflex gain (29); however, recent studies in unanesthetized rabbits have shown that kynurenate in the RVLM decreases the renal sympathetic baroreflex gain (14). Given these findings in the RVLM, lower arousal and reduced sympathetic activity, via higher EEG, would be expected to diminish sympathovascular (BP) baroreflex sensitivity. Thus RVLM effects of arousal on baroreflex gain are likely to be opposite to those in the NTS.

In conclusion, we have used open-loop direct stimulation of the baroreceptor afferents in two groups of CNS-intact NMB rats to determine the relationship between natural variations in arousal (EEG, power), and HP and BP baroreflex gain. With higher levels of EEG, the HP gain was strongly enhanced, but the BP gain was unaffected. A partial-correlations analysis showed that the difference was probably due to a coexisting inverse dependence of the BP, but not the HP, gain on baseline levels. The results are consistent with physiological findings showing that arousal attenuates the common afferent transmission through the NTS, enhances sympathoinhibition at the RVLM, and attenuates, or does not affect, vagocardiac mech-


gains. It will be necessary to directly measure sleep and arousal-related changes in baroreceptor-elicted and baseline neural activity at the key relays in the reflex to verify this hypothesis.

Perspectives

The relationship between BP regulation by the baroreflex, and sleep and arousal, has long been of basic scientific interest and could be pertinent to a number of clinical problems, including hypertension, sleep apnea, and sudden infant death syndrome.

An implicit assumption in human baroreflex-sleep research has been that baroreflex HP effects are useful indicators of BP effects, and the HP data show that baroreflex gain increases in sleep. The experimental animal literature does not support either the assumption or the conclusion: Although for methodological reasons, the focus of animal neurophysiological studies has been the heightened arousal of “fight or flight,” rather than the lowered arousal of sleep, an early study in chronic dogs, using sinus nerve stimulation (31), found that HP but not BP gain was increased in sleep. Our observations of CNS-intact NMB rats confirm and extend these earlier findings and underline that the HP baroreflex is not always a reliable estimate of the BP baroreflex.

APPENDIX

Partial correlation is a statistical method for explaining part of the observed relationship between two variables by a third variable. For example, although there is a correlation between body weight and reading level in school children, it can be accounted almost entirely by chronological age. Using partial correlation we remove the effect of age and reveal that there is either no direct relationship between weight and reading scores or a much weaker one, possibly due to nutritional status.

The concepts and procedures used to decipher the relationship between arousal and baroreflex gain parallel the reading example, but the data have a somewhat different twist. Correlations can be either positive or negative; thus a third variable can diminish the relationship between a pair of variables, as well as increase it, and exactly that appears to be what the BP baseline does to the relationship between arousal and BP gain. The partial-correlations procedure removes this influence and reveals the underlying relationship; however, it is important to keep in mind that this does not change the empirical relationship between arousal and BP gain; it only suggests hypotheses about the mechanism of the relationship. In general, where \( r_{abc} \) is the partial correlation of \( a \) with \( b \), when \( c \) is held constant

\[
r_{abc} = \frac{r_{ab} - (r_{ac}r_{bc})}{\sqrt{(1 - r_{ac}^2)(1 - r_{bc}^2)}}
\]

A statistically less efficient but more intuitively transparent way of achieving a similar analysis is by partitioning the data into subsets that are homogeneous in levels of \( c \). In the reading score example, this would mean doing the correlations within each individual grade (where age would be relatively constant), instead of over the entire school population. Similarly, sorting the 425 gain-ratio measurements (Fig. 6) into BP baseline “quartiles” and doing a separate regression analysis within each quartile would reduce the effect of BP baseline on the arousal-gain correlation. Using this procedure, all four regres-

\(^*\)Measurement of the very low frequency noise spectrum is a noninvasive method of directly assessing changes in vascular (BP) baroreflex gain. (see page R1931 of Ref. 9).
sions showed decreased gain at higher arousal, and for two, although \( n \) was 107 instead of 425, the correlation was reliable at \( P < 0.0001 \).

(Using 10 BP baseline “deciles,” all 10 of the conventional regression coefficients were in the predicted direction; binomial: \( P < 0.001 \).)

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GRANTS

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