Baroreflexes of the rat:

III. Open-loop gain and electroencephalographic arousal

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ABSTRACT

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 (NMB) 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<.0001), and BP baroreflex gain depended on BP level (r = .496; p<.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<.0001), HP baroreflex gain did not depend on HP level (r = 0.029; NS), and HP baroreflex gain increased with arousal (r = .315; p<.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 (a) with physiological findings showing that arousal attenuates afferent transmission through the NTS, and enhances sympathoinhibition at the RVLM; (b) with observations in humans and animals showing increased cardiac baroreflex sensitivity during sleep, but little if any effect of sleep on blood pressure baroreflex sensitivity. The findings are relevant to all methods of baroreflex gain estimation that use heart period as the index of baroreflex activation.

Keywords: baroreceptor, gain, sleep, peroneal nerve.
INTRODUCTION

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 intact neuromuscular 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 was practically no time-of-day or EEG effects on baroreflex gain. This was not as anticipated\(^1\): In early observations in humans, with the Oxford pressor method that uses a vasoconstrictor to elevate blood pressure and challenge the baroreflex, Pickering, Sleight, and Smyth, \(^{(27)}\) reported clear evidence of greater baroreflex gain during stage III & IV (slow wave; EEG\(_{8}\) ) 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 non-invasively analyzes heart period changes occurring during selected spontaneous blood pressure 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 blood pressure changes on heart period: The baroreflexes are a closed-loop system, and blood pressure 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-elicited peroneal nerve sympathoinhibition during slow wave sleep (corresponding to lower vascular baroreflex sensitivity); whereas, heart period indicated slightly increased sensitivity. That heart period 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% to 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 to the awake resting state, changes in heart period 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 heart period) during slow wave sleep; whereas, others have not. For example, in rats, using a validated sequence method, Oosting (21) found increased gain during “the sleeping period”; but, Zoccoli et al. (33) using a similar heart period 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 heart period, the pressor and sequence methods each have particular shortcomings when compared to 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 (8); (see Table 1, EF & 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
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 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 1244 measurements of arousal state and baroreflex sensitivity in 6 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 (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 assured the following: (a) the EEG was synchronized and dominated by high voltage slow wave activity, (b) mean BP (femoral artery blood pressure) <100mmHg, HR < 420bpm, and (c) there were no evident EEG, BP, or HP (heart period; interbeat interval, or R-R interval) responses to manipulation. During the 3-5 days following 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 re-instituted 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 (250-350g). 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-Ta$_2$O$_5$ capacitance electrode for the ADN; and baroreflex gain and frequency response data, including autonomic nerve and blood flow measurements are in (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 blood pressure (BP), and EEG were continuous and stable for the following durations: EN, 16 days; EO, 9 d; FY, 10 d; GF, 13 d.

During a typical experiment, undisturbed, an NMB rat exhibits relatively normal patterns of sleep and wakefulness (c.f. Figs. 1 & 2 with (6), and with baseline HS1 & HS2 data in Table 1 of (16)). The baroreflex test procedures are automated, and trials are presented on a pseudo-random, pre-determined 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 EF and EH are described in (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 fn. 3). The data from some ADN stimulation frequencies that were not included in the original analyses in (8), were used here (The stimulus strength was above of 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 (8, 9), but all test stimuli were set to a uniform single strength in the A-fiber range (40-75µA; 40 Hz; 100 µs).

The 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 blood pressure (BP) or heart period (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 ips, 20µA ADN. The **gain ratio** statistic was approximately normally distributed with mean ≈1.0 and s.d. ≈ 0.8. The mean and s.d. of the baseline HP, BP and EEGδ were also approximately normally distributed and consistent across stimuli, but different for the two rats; thus, to combine the data for 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 s.d. The derived values for the ratios and baseline z-scored statistics were pooled across all ADN stimuli, and both rats (for EH, N=122; for 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 EEGδ, at the time of the test stimulation. A greater gain ratio implies greater baroreflex sensitivity and a greater EEGδ implies lower arousal.

For 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 change to that rat’s particular ADN stimulus. All stimuli were 40ips, 100µS pulses; the current (40-75 µA) for each rat was set at approximately the median of the A-fiber range, with the particular range, for each rat, determined by the procedure described in (8).

**EEG electrodes:** The electrode placements are based on Friedman (10) and Mistlberger (16): With the rat in an atraumatic head holder, its scalp was incised; holes drilled through the skull, 2 mm right of the midline, at bregma and at lambda; 1 mm x 0-80 screws threaded into each hole, and wire (silver plated 30 g; Teflon insulated) wrapped around each of the screws. The screws were embedded in dental acrylic for stability and insulation.

**EEG signal processing:** Using on-line, hardware, -24 dB/octave, digital filters (#79-78-5 FHC), the x10⁴ preamplified (differential between the screws) EEG was analyzed into the following bands, and each band separately rectified and integrated at the indicated time constant: δ (0.5-3 Hz; t_c=1.0s), θ (6.5-7.5 Hz; 0.5s), α (8.5-18 Hz; 0.5s), and β (20-45 Hz; 0.1s). The resulting units of measurement are µV(\text{RMS})•s•s⁻¹. All four signals were recorded continuously
during the entire experiment. Only the results for the δ-band will be described in detail (see fn. 3). The typical range of raw values for EEG_δ \approx 25-200 \mu V_{\text{RMS}}\cdot s\cdot s^{-1}

Peroneal nerve recordings: The peroneal nerve was dissected minimally, and suspended on a pair of silver wire (Medwire Ag7/40T) hook (.5 mm dia.) electrodes (≈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_p-p. For each point in Fig. 3 impulse rates were accumulated over three respiratory cycles and ~1445 such values averaged in each hour.

Measurement and recording of cardiovascular variables and description of materials and their sources is in (8). The data resolution was at least one systolic and diastolic sample per heart beat, continuous; increased to 6000 samples per second (6 KS/s) during stimulations.

RESULTS

Properties of the preparation: The general physiological features of the preparation are illustrated by the traces in Figs.1 & 2. Because of NMB, the circulation is unaffected by muscle contraction or changes in posture. The NMB rats have diurnal, ultradian, and sleep cycles superimposed on randomly varying (9), but, on average over days, stable baselines. The cardiovascular variables reflect only artificially elicited open-loop baroreflex responses (see Figs. 1 & 6 in 8)), and centrally generated autonomic activity, which, because all endogenous baroreceptor input has been eliminated, is unattenuated by baroreflexes. The general relationship between the major cardiovascular variables and the four EEG bands is shown in Figs.1 & 2. During periods of increased slow wave activity (e.g. at hours 2, 10 and 18), heart rate, BP, systolic femoral flow, vagus nerve activity, and peroneal nerve activity all dip to lower average levels.

Fig. 3 is a plot of EEG_δ vs. peroneal nerve firing rate during 92 consecutive hours for rat EH. It shows that baseline muscle sympathetic nerve
activity is directly related to the EEGδ measure of arousal (r=0.500; df=91, p<0.001). For rat EF, the relationship was similar (r=.684; df=175; p<.0001).

Elicitation of the baroreflex and the index of arousal: ADN electrical stimulation gave the most robust, consistent baroreflex activation, and EEGδ is a commonly accepted index of “slow-wave” or “quiet” sleep2; given this, our analyses were based on the relationship between EEGδ and the responses to the ADN test stimuli3.

For rats EN, EO, FY and GF, the BP and HP responses to ADN stimulation were similar to those reported in (8, 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 (EEGδ) effects on baseline blood pressure and heart period: For rats EF and EH, Fig. 4 (top) shows the correlation scatter plot (r = 0.352; df = 424; p<.0001) for EEGδ and heart period (HP), and Fig. 4 (bottom) is the correlation (r = -0.416; df = 424; p<.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-EEGδ correlations were: -.624, -.544, -.537, and -.550. The HP-EEGδ correlations were: .507, .429, .350, and .313. With the exception of the GF HP, for which p<.01, all of the correlations were reliable at
p<.0001; confirming the generality of the relationships apparent in the traces of Figs. 1 & 2.

<Figure 5 Goes Here>

Effects of baseline blood pressure, and heart period on corresponding baroreflex gain: Fig. 5 (top) shows that, for EF and EH, the ADN produced baroreflex bradycardia is unrelated (r = 0.029; df = 424; NS) to the baseline heart period at the time of stimulus application. In contrast, Fig. 5 (bottom) shows a strong relationship (r = .496; df = 424; p<.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.

<Figure 6 Goes Here>

Arousal (EEG\(\delta\)) effects on the BP and HP baroreflex gain ratios: Fig. 6 (top) shows a moderate relationship between EEG\(\delta\) and heart period (r = .315; df = 424; p<.0001); this result, is similar to, and consistent with, evidence, previously reported for humans and other species, of increased heart period baroreflex sensitivity with sleep. Fig. 6 (bottom) shows the complete absence of a relationship between the EEG\(\delta\) defined arousal level and blood pressure 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 EN the HP-EEG\(\delta\) regression was r = .363 (df = 180; p<.0001), and the BP-EEG\(\delta\) regression was r = .063 (df = 180 NS). For EO the HP-EEG\(\delta\) regression was r = .557 (df = 230; p<.0001), and the BP-EEG\(\delta\) regression was r = .001 (df = 230; NS). For FY the HP-EEG\(\delta\) regression was r = .547 (df = 341; p<.0001), and the BP-EEG\(\delta\) regression was r = .079 (df = 341 NS). For GF the HP-EEG\(\delta\) the regression was r = .318 (df = 180; p<.01), and the BP-EEG\(\delta\) regression was r = .028 (df = 67; NS).

In a parallel analysis to that used with EF & EH (but based on raw HP and BP changes rather than gain ratios), the data from EN, EO, FY and GF was
z-scored and combined into a single set: The overall HP-EEG$_\delta$ regression was $r = .491$ (df = 819; $p<.0001$), and the BP-EEG$_\delta$ regression was $r = .027$ (df =819; NS).

Thus, the analysis of each of the 6 NMB rats shows a strong, statistically reliable, enhancement, at higher EEG$_\delta$ levels (i.e., lower arousal) of HP baroreflex sensitivity, but no enhancement of BP baroreflex sensitivity.

**DISCUSSION**

The heart period 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 sympathoinhibition (17), implying reduced, not increased, baroreflex sensitivity, with sleep.

Why does arousal affect baroreflex heart period responses differently than blood pressure responses? The partial correlations analysis, for rats EF and EH,((28); Appendix) shown in Fig. 7 addresses this question by using the EEG$_\delta$-baseline (Fig.4), and the baseline-gain relationships (Fig.5) to dissect the EEG$_\delta$-gain relationships (Fig. 6) for HP and BP: Fig. 4 shows that both baselines depend on EEG$_\delta$: the heart period becomes longer and the blood pressure lower during slow wave sleep. Fig. 5 shows that the BP gain ratio is strongly coupled to the BP baseline (cf. Fig. 2 in (19)); this means that changes in EEG$_\delta$ will be transmitted through the blood pressure baseline relationship to the gain-ratio (Fig. 7 bottom). Because, the BP baseline decreases with EEG$_\delta$, the predicted effect of sleep (increased EEG$_\delta$) 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 EEGδ; 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-EEGδ correlation from .027 to -.125 (df=819, p<.0005), and the HP-EEGδ correlation from .491 to .388 (df=819, p<.0005). Thus, removing the EEGδ-BP baseline effect increased the effect of arousal on BP gain, and removing the EEGδ-HP baseline effect decreased the effect of arousal on HP gain, consistent with the results and analysis of EF & EH shown in Fig. 7 (See Table 1).

In sum, using direct open-loop baroreflex measurements, we found that, during periods of higher EEGδ, 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 grey electrical stimulation: Hilton (4, 11, 12) showed that hypothalamic defense area (HDA) stimuli effectively block, either carotid sinus or ADN elicited, baroreflex bradycardia and depressor responses. In sino-aortic denervated rats with an ADN electrode, stimulation of the posterior hypothalamus attenuates ADN elicited bradycardia, but does not alter either the baroreflex blood pressure 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 NTS where baroreceptor afferent and HDA fibers converge. Mifflin (15) identified cells with excitatory inputs from the sinus nerve and inhibitory inputs from the HDA, and showed that HDA stimulation produces hyperpolarizing IPSP's, which shunt sinus nerve evoked EPSP's 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 sino-atrial (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 brainstem mechanism, would lower cardiac baroreflex gain at higher arousal levels (13, 18, 20). Conversely, lower arousal and reduced sympathetic activity, via higher EEG\text{\textgreek{d}}, would be expected to augment cardio-vagal 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\text{\textgreek{d}}; thus, it is probable that RVLM presympathetic activity (the substrate of baroreflex inhibition) is also lower. In early studies with anesthetized rats, fictive arousal via hypothalamic stimulation excited kynurenate sensitive presympathetic 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$\delta$, would be expected to diminish sympatho-vascular (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$\delta$ power), and heart period (HP) and blood pressure (BP) baroreflex gain: With higher levels of EEG$\delta$, 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, vago-cardiac mechanisms. It will be necessary to directly measure sleep and arousal related changes in baroreceptor elicited, and baseline neural activity, at the key relays in the reflex to verify this hypothesis.

**PERSPECTIVE**

The relationship between blood pressure 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 (SIDS).

An implicit assumption in human baroreflex-sleep research has been that baroreflex heart period (HP) effects are useful indicators of blood pressure (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 heart period baroreflex is not always a reliable estimate of the blood pressure 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 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 \( r_{ab} \) is the partial correlation of \( a \) with \( b \), when \( c \) is held constant,

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

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 regressions showed decreased gain at higher arousal, and for two, though N was 107 instead of 425, the correlation was reliable at p<.0001. (Using 10 BP baseline “deciles”, all 10 of the conventional regression coefficients were in the predicted direction; binomial: p<.001)

FIGURE and TABLE LEGENDS

Table 1. The raw correlations of the heart period and blood pressure baroreflex responses with EEGδ and the heart period and blood pressure baselines. 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. For Rats EF and EH, the baroreflex stimuli were of different strengths; thus the response measure was based on a gain ratio (see Methods); 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 EEGδ values (i.e., for lower arousal). For rat EO, 24 measurements with baseline BP>170mmHg were excluded from the calculations in this table, because these were due to a transducer artifact, and probably erroneous. N.B. for the six blood pressure BRX×EEG correlations, five are effectively zero, the sixth (EH) is statistically reliable, but in the direction of decreased baroreflex gain with sleep, which is opposite to the usual expectation.

Figure 1. A plot of 24 hours of data for the 13th of 35 days for NMB rat EH. The top trace markers (black bars) show when various baroreflex test stimuli were
administered (bar height indicates stimulus strength and type). “ECG RMS Power” is the rectified integrated voltage squared of the QRS-wave of each heartbeat. The “caudal flow” probe is on the aorta, immediately caudal the superior mesenteric artery. This location, which included the femoral, inferior mesenteric, iliolumbar, and caudal arteries, was a broad sample of skeletal and visceral abdominal flow. The upper and lower femoral and caudal pulse transit time flow traces are the systolic and diastolic values. PIP is the peak airway pressure. The vagus and peroneal nerve traces are impulses per second for spikes of >10\(\mu\)V\(_{p-p}\). Ultradian cycles of \(\approx 8\) h are evident, especially in the EEG and nerve activity traces. The horizontal bar on the abscissa shows the time interval of this record that is expanded in the record shown in Fig. 2.

Figure 2. A 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 two 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 CV effects of two sinus balloon step stimuli (black markers at top) are reflected in the BP, HR and systolic fmBF traces.

Figure 3. Baseline sympathetic (peroneal) nerve firing rate vs. EEG\(\delta\) for Hours 117-212 (Days 5 – 8) for rat EH (r = -0.500; df = 91, p<.001). The data are impulses per second for spikes of >10\(\mu\)V\(_{p-p}\). For each point impulse rates were accumulated over three respiratory cycles and ~1445 such values averaged in each hour. Each point is a one hour average. See Fig. 6 in (8) for technical details. For rat EF, the relationship was somewhat stronger (r = -.684; df = 175; p<.0001).

Figure 4. The relationship between (top) heart period (HP) (r = 0.352; df = 424; p<.001) or (bottom) systolic blood pressure (BP) (r = -0.416; df = 424; p<.001) and the EEG\(\delta\) measure of arousal for rats EF and EH. The data are from the
120 s baseline prior to each test stimulus. The scales are z-scores in order to equate the mean and s.d. across rats. See Figs. 1 & 2 and (8) for examples of the raw physiological units.

**Figure 5.** The relationship between (top) HP gain ratio ($r = 0.029; df = 424; NS$) or (bottom) BP gain ratio ($r = .496; df = 424; p<.0001$) and the corresponding pre-stimulus baseline for rats EF and EH. The scales are z-scores to equate the mean and s.d. across rats and different ADN test stimulus strengths.

**Figure 6.** The relationship between (top) HP gain ratio ($r = .315; df = 424; p<.0001$) or (bottom) BP gain ratio ($r = 0.001; df = 424; NS$) and EEG$_\delta$ for rats EF and EH. The scales are z-scores to equate the mean and s.d. across rats and different ADN test stimulus strengths.

**Figure 7.** A partial correlations analysis for the baroreflex (top) bradycardia (HP) and (bottom) depressor (BP) responses for rats EF and EH. The difference between the partial correlations analysis and the raw correlation for each of the rats EF and EH was similar to the combined data shown in the figure. The analysis shows two “opposing” paths between EEG$_\delta$ and the depressor response, but only a single path from EEG$_\delta$ to bradycardia. The opposing paths cancel one another. See the discussion for an explanation and interpretation of the analysis.
### Table 1

**Raw Correlations**

<table>
<thead>
<tr>
<th>RAT</th>
<th>Heart Period</th>
<th>Blood Pressure</th>
<th>N Responses</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(</td>
<td>r</td>
<td>_{BRX \times EEG})</td>
</tr>
<tr>
<td>EF</td>
<td>↑.310</td>
<td>+.313</td>
<td>↓.024</td>
</tr>
<tr>
<td>EH</td>
<td>↑.405</td>
<td>+.211</td>
<td>↑.271</td>
</tr>
<tr>
<td>EN</td>
<td>↑.363</td>
<td>+.507</td>
<td>↑.397</td>
</tr>
<tr>
<td>EO*</td>
<td>↑.557</td>
<td>+.429</td>
<td>↑.447</td>
</tr>
<tr>
<td>FY</td>
<td>↑.547</td>
<td>+.350</td>
<td>↑.530</td>
</tr>
<tr>
<td>GF</td>
<td>↑.318</td>
<td>+.313</td>
<td>↓.202</td>
</tr>
</tbody>
</table>

*24 values with baseline BP>170mmHg were excluded from the calculations in this table.

↑↓arrows give the direction of baroreflex gain (BRX) change with sleep (EEG) or baseline (BL); the absolute value of the correlation coefficient (|r|) gives the strength of the regression relationship in the indicated direction. The proportion of variance accounted for is $r^2$. 
The authors thank V. Chinchilli for reviewing the partial correlations procedures, and X. Tang for help in data collection for rats EF & EH.

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References


**Footnotes**

1 However, 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.

2 In classifying sleep stages the distinction between wakefulness and REM 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 EEGδ. NMB rats have no skeletal muscle activity; however, because the activity in skeletal nerve is not directly affected by the α-ctx pharmacological block, it is possible to monitor efferent nerve activity attendant upon 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 shows the expected relationship between EEGδ and skeletal nerve activity (see Fig. 1, 6 & 11 of (7)).

Having included the results of sinus stimulation, other EEG measures, or the three additional rats (DU, EC and EG) described in (8); (9) would not have substantially changed any of the results: For all five rats, for both the ADN electrode and sinus balloon stimulation, neither heart period (HP) or blood pressure (BP) measures of the baroreflex gain ratio were dependent on the EEGθ or EEGβ amplitude. Although the HP (r = 0.18; p<001) and BP (r = 0.17; p< .005) gain ratios increased at higher EEGα; compared to EEGδ, these effects were weak, and unlike EEGδ, EEGα lacks a generally accepted relationship to sleep and arousal. For rats DU, EF and EH, the sinus elicited HP was completely unaffected by EEGδ, but, in fact, the sinus HP effects are very small (see Table 1 & Fig 6 (9)), and EEG dependence, if present, would have been difficult to detect.

Measurement of the VLF noise spectrum is a non-invasive method of directly assessing changes in vascular (BP) baroreflex gain. (see page R1931 of (9)).

For EF the correlation between the BP gain ratio and EEGδ increased from .057 to .293 (df=303; p<.0005), and for EH the correlation increased from -.262 to .127 (df=122; p<.0005); whereas, the change in the HP gain ratio correlation was not reliable for either rat.
Figure 1
Figure 3

Peroneal Nerve Activity

EEG(δ)

r = .500

Arousal / Sleep (z-units)
Heart Period Baseline

Blood Pressure Baseline

EEG(δ)

(z-units)

Arousal / Sleep

Figure 4
Heart Period Baseline (z-units) 

**Heart Period Gain Ratio**

![Heart Period Baseline Chart](chart)

- $r = 0.029$

Blood Pressure Baseline (z-units)

**Blood Pressure Gain Ratio**

![Blood Pressure Baseline Chart](chart)

- $r = 0.496$

Figure 5
Heart Period
Gain Ratio

Blood Pressure
Gain Ratio

EEG(δ)

Figure 6
Arousal /Sleep (EEG δ)

Heart Period Baseline

Baroreflex Bradycardia (gain-ratio)

HP Partial Correlations

<table>
<thead>
<tr>
<th>HP Ratio</th>
<th>EEGδ</th>
</tr>
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<tbody>
<tr>
<td>EEG (δ)</td>
<td>.331</td>
</tr>
<tr>
<td>HP baseline</td>
<td>-.077</td>
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</tbody>
</table>

BP Partial Correlations

<table>
<thead>
<tr>
<th>BP Ratio</th>
<th>EEGδ</th>
</tr>
</thead>
<tbody>
<tr>
<td>EEG (δ)</td>
<td>.261</td>
</tr>
<tr>
<td>BP baseline</td>
<td>.539</td>
</tr>
</tbody>
</table>

Figure 7