Baroreflexes of the rat. VI. Sleep and responses to aortic nerve stimulation in the dmNTS

Xiaorui Tang and Barry R. Dworkin

Department of Neural and Behavioral Sciences, Pennsylvania State University College of Medicine, Hershey, Pennsylvania

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Baroreflexes of the rat. VI. Sleep and responses to aortic nerve stimulation in the dmNTS. Am J Physiol Regul Integr Comp Physiol 298: R1428–R1434, 2010. First published January 27, 2010; doi:10.1152/ajpregu.00486.2009.—The sensitivity of the baroreflex determines its stability and effectiveness in controlling blood pressure (BP). Sleep and arousal are reported to affect baroreflex sensitivity, but the findings are not consistent across studies. After statistically correcting the effect of sleep on the baselines in chronically neuromuscular-blocked (NMB) rats, we found that sleep affects BP and heart period (HP) baroreflex gain similarly. This finding is consistent with baroreflex modulation of HP and BP before divergence of the sympathetic and parasympathetic pathways. Therefore, we hypothesized that the gain modulation occurs in the dorsal medial nucleus of the solitary tract (dmNTS). The present study used long-term dmNTS recordings in NMB rats and single-pulse aortic depressor nerve stimulation. Under these conditions, the magnitude of A-fiber evoked responses (ERs), recorded from second- or higher-order dmNTS baroreflex neurons, was reliably augmented during high-amplitude low-frequency EEG activity (slow-wave sleep) and reduced during low-amplitude high-frequency EEG activity (arousal; ΔER = 11%, t = 9.49, P < 0.001, degrees of freedom = 1,016). This result has methodological implications for techniques that use changes in HP to estimate baroreflex gain and general implications for understanding the relationship between sleep and cardiovascular control.

baroreflex gain; baroreflex sensitivity; nucleus of the solitary tract; arousal; aortic depressor nerve

THE NEGATIVE-FEEDBACK BAROREFLEXES have a central role in regulating blood pressure (BP), and baroreflex sensitivity or gain determines the stability and effectiveness of BP control. Sleep and arousal are reported to affect baroreflex sensitivity, but the findings are not consistent across studies that differ in experimental methodology and/or species. With the Oxford method, a vasoconstrictive drug elicits BP, and the cardiac baroreflex gain is defined as the heart rate (HR) change (ΔHR) divided by the BP change (ΔBP). Pickering and others (12, 13, 16) reported evidence of evidence of greater cardiac baroreflex gain during stage II and IV slow-wave sleep than during wakefulness in humans. Using a noninvasive correlational method that deduces the baroreflex gain from spontaneous BP and HR sequences, Parati et al. (11) also found that gain increased during sleep, but in chronic animal studies that used a similar sequence method, but with more sophisticated criteria for sleep, Zoccoli et al. (21) found that, for rats, gain was substantially independent of states of wakefulness, active sleep, and quiet sleep.

The Oxford and sequence methods measure BP effects on HR, i.e., the baroreflex cardiac gain, but they do not directly measure the vasomotor baroreflex gain or the total loop gain. This is because measurement of the former requires implanted flowmeters and measurement of the latter requires direct access to the baroreceptors. Thus, in practice, to make inferences to total loop gain, which is the variable of interest, the cardiac reflex is taken as a surrogate for the overall reflex, and it is assumed that changes in cardiac gain proportionally represent changes in loop gain; however, the literature does not consistently support this assumption. For example, in chronic dog studies, implanted carotid sinus nerve stimulators elicit 12–22% greater bradycardia during sleep, but sleep produces no change in the net BP depressor effect (20); in baboons, during sleep, compared with the awake resting state, although the carotid occlusion (baroreceptor inhibition) effect on heart period (HP) is increased, lower abdominal conductance is unaffected, and the renal conductance response to occlusion is attenuated (1). In an earlier study (4), using chronically neuromuscular-blocked (NMB) rats, which have distinct diurnal and sleep-wakefulness cycles, we compared the effects of EEG arousal on the open-loop HP and BP baroreflex gains. Using the power of the δ-band of the EEG (EEGδ) as an index of sleep and arousal and aortic depressor nerve (ADN) electrical stimulation as a baroreflex input, we observed that although the HP baroreflex gain increased with sleep, the open-loop BP baroreflex gain (the decrease in BP in response to the ADN test stimulus) did not. However, on further analysis, we discovered that the raw correlational result was misleading. A partial-correlations analysis, which also took into account the effects of sleep on BP, showed that because BP was directly related to arousal and BP baroreflex gain was directly related to BP, the effect of sleep on BP gain had been “masked.” However, HP gain was different: although, as with BP, HP depended on arousal, HP baroreflex gain did not depend on HP. By mathematically “partialing out” the BP effects on the baroreflex BP gain, the underlying relationship between EEGδ and BP baroreflex gain was shown to be approximately the same as that for HP baroreflex gain. Because the effects of sleep on HP and BP gain were almost exactly the same, we hypothesized that the modulation occurred at a common anatomic locus, before divergence of the parasympathetic and sympathetic pathways. Testing that hypothesis led to the present study.

The parasympathetic (cardiac chronotropic) and sympathetic (vascular tone; cardiac inotropic) baroreflex pathways diverge at the dorsal medial nucleus of the solitary tract (dmNTS). For the parasympathetic pathway, the baroreflex dmNTS neurons project to the nucleus ambiguus, and the dorsal motor nucleus of vagus, and then to the heart. For the sympathetic pathway, the dmNTS neurons project to the caudal ventrolateral medulla, the rostral ventrolateral medulla, the sympathetic preganglionic neurons at the intermediolateral column of the spinal cord, and then to the peripheral vascular, renal, and cardiac
sympathetic nerves. The post-dmNTS divergence provides ample opportunity for modulating influences from sleep and arousal to have separate effects on the baroreceptor parasympathetic and sympathetic reflexes; however, our partial-correlations analysis indicated that the effects were, in fact, quite similar (see discussion in Ref. 4 for details and references).

In the present study, we extend the NMB rat preparation to enable direct measurement of the magnitude of single-pulse ADN stimulation-elicited evoked responses (ERs) from second- or higher-order baroreflex neurons in the dmNTS. Sleep and arousal are defined as epochs of high-amplitude low-frequency and low-amplitude high-frequency EEG, respectively. Using these definitions, we found clear evidence that the ER magnitude was reliably augmented during sleep.

**METHODS**

Twelve female Sprague-Dawley rats (230–270 g body wt) were studied one at a time, monitored continuously, and attended around the clock. These same rats were used in our previous studies (17, 18). All acute surgery or any possible irritating manipulation, such as replacement of the bladder cannula or adjustment of the tracheal cannula, was done with accurately controlled and carefully monitored >1.5% deep isoflurane anesthesia. All procedures were approved by the Institutional Care and Use Committee of the Pennsylvania State University College of Medicine and comply with the American Physiological Society’s “Guiding Principles for Research Involving Animals and Human Beings” and National Institutes of Health and US Department of Agriculture regulations.

**General information about the preparation.** The surgical details are described elsewhere (17); briefly, NMB was induced on the first day by injection of α-cobrotoxin (100 μg iv) and maintained by continuous infusion (250 μg/day iv). Mechanical ventilation was at 72 breaths/min at inspiratory-to-expiratory ratio of 1:2, positive end-expiratory pressure of 1–5 cmH2O, minute volume of 180–220 ml, and hyperinflation (antiatelectasis) of 18 cmH2O (2 s/10 min). The EEG electrodes were two 0.80 screws inserted 2.0 mm from the midline in the left parietal bone (1.0 mm anterior to bregma and to lambda). A transurethral bladder cannula, subcutaneous EEG electrodes (no. 30 silver wire), and Teflon femoral artery and silicone vein cannulas were implanted. Core (vaginal) temperature was servoregulated at 37°C. The left ADN was dissected, set on an anodized Ta-Ta2O5 capacitive electrode (17), and imbedded in a silicone compound (Kwik-Sil, WPI, Sarasota, FL). This electrode arrangement enables thousands of stimulations without detectable damage to the ADN. The baroreflex area in the dmNTS was located using atlas coordinates, as well as the somatotopic features of the gracile nucleus, which at the search coordinates is 50–150 μm dorsal to the baroreflex cells of the dmNTS (17). For the multiunit dmNTS recording, 1- to 2-MΩ glass-insulated tungsten microelectrodes (Alpha-Omega, Alpharetta, GA) were mounted in a hydraulic probe drive (model 50-16-1, FHC), which was mounted in a digital stereotaxic carrier. Signals were amplified (gain = 20,000, bandpass = 0.3–3 kHz, XCELL-3X4, 40-#40-8B, FHC) and digitized at 10 kHz. A typical preparation was maintained for an average of 14 days, and the data were acquired on days 4–9. After healing of the surgical incisions and stabilization, which typically required 48–72 h, the isoflurane concentration was set at ±0.5%, which is an analgesic level consistent with patterned sleep.

**EEG sleep/arousal identification.** From the bandpass-filtered (0.1–100 Hz) EEG, two kinds of sleep-arousal cycles were identified (Fig. 1): from sleep to wakefulness (S-W) and from wakefulness to sleep (W-S). The S-W cycles were identified as approximately symmetrical periods of high-amplitude low-frequency EEG activity followed by low-amplitude high-frequency activity; correspondingly, the W-S cycles were symmetrical periods of low-amplitude high-frequency EEG activity followed by high-amplitude low-frequency activity. Because of the NMB, EMG or electrooculogram criteria could not be used to distinguish arousal from rapid eye movement (REM) sleep; thus, to ensure accurate identification of sleep-arousal cycles, we imposed several additional constraints. 1) Cycle start points were set ≥2 min after an EEG transition was complete, and cycle stop points were set ≥2 min before the next transition. To eliminate the need to interpret ambiguous midcycle EEG transitions (dashed oval in Fig. 1), we used ERs from only the first 25% of the cycle after the cycle start point and the last 25% before the cycle stop point (i.e., Q1 and Q4 in Fig. A1). 2) For a cycle to be valid, it must not have had any abrupt (>25%) change in BP or HP within the cycle, except at the midcycle transition. 3) Complete cycles had durations of >10 and <40 min (see details in the Appendix). Figure 1 shows a typical recording of EEG, HP, arterial BP, and venous BP from an individual rat. Six sleep-wakefulness sequences, three S-W and three W-S, are identified in this example record.

**Average EEG power.** Average EEG power is the root mean square (RMS) of the bandpass-filtered (0.1–100 Hz) EEG signal.

**Magnitude of the A-fiber ERs.** Figure 2 shows a stimulus-triggered ensemble-averaged A-fiber ER trace from a dmNTS recording site. Using electrophysiology, pharmacology, and histology, we verified that the recordings were from second- or higher-order dmNTS baroreflex neurons, and not from ADN fibers in the solitary tract (17, 18). The A-fiber ER is the activity in the poststimulus latency range of 4–20 ms. Because the Pythagorean distance between the dmNTS recording and ADN stimulation sites is >11.5 mm and only A-fibers
Table 1. The cycle sequences of s-w and w-s have no influence on sleep/arousal effects on the magnitude of dmNTS A-fiber ERs and other measured cardiovascular variables.

<table>
<thead>
<tr>
<th></th>
<th>High-Amplitude Low-Frequency EEG Periods (Sleep)</th>
<th>Low-Amplitude High-Frequency EEG Periods (Arousal)</th>
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<tr>
<td></td>
<td>ERs, %</td>
<td>sBP, mmHg</td>
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<tr>
<td>S-W</td>
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<td></td>
<td>106 ± 0.93</td>
<td>131 ± 0.74</td>
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<tr>
<td>W-S</td>
<td>105 ± 1.02</td>
<td>129 ± 0.89</td>
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*t*-test (S-W vs. W-S)

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<th></th>
<th>t</th>
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<td>0.87</td>
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<td>(S-W vs. W-S)</td>
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Values are means ± SE (n = 11 rats). ERs, (ADN A-fiber dmNTS) evoked responses; sBP, systolic blood pressure; dBP, diastolic blood pressure; HP, heart period; df, degrees of freedom; NS, not significant. The magnitude of A-fiber ERs during a quarter period of sleep or arousal is percentage of the mean magnitude of A-fiber ERs for the 4 quarters in that sleep-wakefulness (S-W) or wakefulness-sleep (W-S) cycle (see detailed calculation in appendix).
sBP or HP change from baseline during the ADN stimulus divided by the mean change for all responses to that kind and amplitude of stimulus, e.g., 35 impulses/s at 20 μA. Variables were converted to “z-scores” by subtracting the individual rat’s mean from each measure and dividing by the corresponding standard deviation (4). Each point in Fig. 4C represents the EEG activity of each quarter period and the magnitude of A-fiber dmNTS ERs of that same quarter period, and all sleep and arousal periods, as well as the midcycle transitional quarter periods, are included. Figure 4 shows that HP baroreflex gain increased with sleep (A; $r = 0.315$, $P < 0.0001$, df = 424), the uncorrected BP baroreflex gain was independent of sleep/arousal (B; $r = 0.001$, $P$ not significant, df = 424), and, as predicted in an earlier study (4), the magnitude of A-fiber dmNTS ERs was increased with sleep (C; $r = 0.2$, $P < 0.0001$, df = 4,067).

DISCUSSION

Studies of modulation by sleep and arousal of baroreflex sensitivity across different species and/or experimental methodologies (10, 19, 21) do not agree. To understand why, it is necessary to know where and how the modulation occurs. In the present study, we have, for the first time, directly measured the effects of naturally occurring cycles of sleep and arousal on the open-loop baroreflex sensitivity at the dmNTS and found clear evidence that sensitivity increases during slow-wave sleep.

Slow-wave sleep is distinguishable from wakefulness by EEG criteria; however, distinguishing wakefulness from desynchronized REM sleep is more problematic. Conventionally, EMG and/or electromyogram signals are used to help distinguish the states, but paralysis by NMB precluded those criteria; thus, to minimize possible misidentification, we restricted sampling to only stable, nontransitional periods of sleep and wakefulness. It is known that, in the cat, the end of a desynchronized episode in one cycle through the end of a desynchronized episode in the subsequent sleep cycle includes a regular progression of component states: arousal, slow-wave sleep, transition state, and desynchronized sleep (8). A similar pattern, including slow-wave sleep, REM sleep, and arousal

Table 2. Sleep/arousal effects on A-fiber ERs in the dmNTS

<table>
<thead>
<tr>
<th>Rat No.</th>
<th>Cycles</th>
<th>Cycle Length, min</th>
<th>Sleep, %</th>
<th>Wake, %</th>
<th>sBP, mmHg</th>
<th>sBP, Wake</th>
<th>dBP, mmHg</th>
<th>dBP, Wake</th>
<th>HP, ms</th>
<th>Sleep, %</th>
<th>Wake, %</th>
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<td>1.33</td>
<td>1.32</td>
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Individually, compared with arousal, the magnitude of A-fiber dmNTS ERs was significantly increased during sleep in rats 1–9 and decreased (not significantly) in rats 10 and 11. Over all 11 rats, sleep and arousal had significant effects on every variable. *$P \leq 0.0001$. 

Fig. 3. Summary of sleep-wakefulness differences in EEG activity and A-fiber dmNTS ERs (see APPENDIX for procedure used to calculate percentages; A, systolic and diastolic blood pressure (sBP and dBP); B, and HP (C) for the 11 rats. Magnitude of EEG activity and ERs were higher, sBP and dBP were lower, and HP was longer during sleep than during wakefulness. Values are means ± SE. *$P \leq 0.0001$. 

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Between EEG 424, root-mean-squared amplitude of 1-min separately and, finding no reliable difference, pooled the S-W order effects, we first analyzed the S-W and W-S sequences. To control for possible sequence arousal patterns, within which we were able to identify clear postsynaptic potentials (9). These data led us to hypothesize modulation, and, indeed, other physiological and neurophysiological evidence suggests that the dmNTS is the common site. Hypothalamic defense area stimuli inhibit carotid- or ADN-elicited baroreflex bradycardia and depressor responses (2, 6, 7), and dmNTS cells with excitatory inputs from the baroreceptor sinus nerve and inhibitory inputs from the hypothalamic defense area are hyperpolarized by hypothalamic defense area stimulation in a way that shunts sinus nerve-evoked excitatory postsynaptic potentials (9). These data led us to hypothesize that arousal attenuates baroafferent transmission through the dmNTS (4).

Because the baroreflex is a closed loop, the overall loop gain, which is the decrease in BP per unit increase in receptor pressure (mmHg/mmHg), cannot be directly measured without opening the loop and isolating the receptors. An open loop requires surgical interventions, which are technically difficult and, in humans, ethically unacceptable. Instead, most studies have estimated relative baroreflex gain from HR, either by manipulating receptor pressure and measuring corresponding changes in rate (12, 13) or by statistically analyzing the sequential relationship of natural pressure and rate fluctuations (11). Both of these methods assume that the cardiac rate response is representative of, and affected in parallel with, the overall baroreflex depressor response; however, some studies suggest that this assumption may not be correct (20).

Previously (4), using NMB rats, with ADN electrodes, but without dmNTS brain stem recordings, we found that the HP gain was greater during periods of greater EEG (Fig. 4A) that the BP gain was unaffected by the EEG (Fig. 4B); this result appeared to show differential effects of sleep on the two efferent baroreflex mechanisms. A true differential effect would imply that HP gain was not a good surrogate for BP gain. Although the raw variables gave different results, sleep also lowers the HR and BP baseline, and a partial-correlations analysis showed that the apparent difference could be explained by a dependence of the BP gain, but not the HP gain, on the corresponding baseline level (4). The difference between the HP and BP gains was an artifact of measurement, and not a physiologically relevant effect: the lowered baseline BP is closer to the hydrostatic “floor,” and responses at lowered baseline pressure have a higher probability of being “clipped” or limited by the floor. In a previous study (4), after partial-correlations correction for BP baseline, the relationship between BP gain and “EEG slow-wave activity” was 0.26 (Fig. 7 in Ref. 4), which is comparable to the correlation between EEG and the nucleus of the solitary tract (NTS) neural ER of 0.20 in the present study. The partial-correlations analysis thus showed that the HP and BP gains were affected approximately in parallel.

If the underlying effect of sleep on HP and BP gain is similar, this would be consistent with a common site of modulation, and, indeed, other physiological and neurophysiological evidence suggests that the dmNTS is the common site. Hypothalamic defense area stimuli inhibit carotid- or ADN-elicited baroreflex bradycardia and depressor responses (2, 6, 7), and dmNTS cells with excitatory inputs from the baroreceptor sinus nerve and inhibitory inputs from the hypothalamic defense area are hyperpolarized by hypothalamic defense area stimulation in a way that shunts sinus nerve-evoked excitatory postsynaptic potentials (9). These data led us to hypothesize that arousal attenuates baroafferent transmission through the dmNTS (4).

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**Fig. 4.** Effect of sleep/wakefulness on relative baroreflex gain for HP (A), “uncorrected” relative baroreflex gain for blood pressure (B; see Ref. 4), and single-pulse ERs in the dmNTS (C). Each point in A and B represents root-mean-squared amplitude of 1-min δ-band of the EEG (EEGδ) and the averaged evoked cardiovascular responses to a standard stimulus test train during the same 1-min period: in A, $r = 0.315$, degrees of freedom (df) = 424, $P < 0.0001$; in B, gray dashed line shows slope of the partial correlations-corrected relationship between EEGδ and blood pressure gain ($r = 0.26$, df = 424, $P < 0.0005$), and black solid line shows slope of uncorrected relationship between EEGδ and blood pressure gain ($r = 0.001$, df = 424, $P = 0.1$ significant). Each point in C represents magnitude of EEG activity for each quarter period of a sleep-arousal cycle and ER magnitude of the same quarter period in the cycle ($r = 0.2$, df = 3,387, $P < 0.0001$). A is from Fig. 7 of Dworkin and Dworkin (4). B is modified (with gray dashed line to indicate difference in relationship between EEGδ and blood pressure gain before and after partial-correlations correction) from Fig. 7 of Dworkin and Dworkin (4): after partial-correlations correction for blood pressure baseline, the relationship between blood pressure gain and “EEG slow-wave activity” was 0.26, which is comparable to the correlation of 0.20 between EEG and the nucleus of the solitary tract neural ER in the present study.

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states, is seen in freely moving rats (15). Similarly, over days or weeks, NMB rats have stable regular alternating sleep-arousal patterns, within which we were able to identify clear S-W and W-S sequences. To control for possible sequence order effects, we first analyzed the S-W and W-S sequences separately and, finding no reliable difference, pooled the S-W and W-S data. Irrespective of absolute cycle length, each sequence was divided into quarters: the first and last quarters were the S or W phases used in the analyses, and the two midcycle quarters were designated transitional, and, except for Fig. 4C, the transitional quarters were excluded from the analysis. Although our criteria possibly might be seen as too stringent, they offered, in exchange for a loss of half of the data, a high degree of confidence that the identification of the states was as objective as possible.

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To examine that hypothesis, we added dmNTS recordings to the cardiovascular and EEG measures and found that natural sleep modulates transmission of baroreceptor activity in the dmNTS. As predicted, the magnitude of A-fiber ERs from the second- or higher-order dmNTS baroreflex neurons, elicited by single-pulse ADN A-fiber stimulation, was reliably augmented during high-amplitude low-frequency EEG activity and reduced during low-amplitude high-frequency EEG activity (Fig. 4C). We have thus confirmed the conjecture of the partial-correlations analysis (4) by direct physiological measurement.

Perspectives

Doba and Reis (3) found that lesions in the NTS produced “fulminating hypertension,” which was “abolished by midcolicular decerebration.” Reis (14) initially thought that he had found an important locus of integration of excitatory and inhibitory influences on BP, but as evidence of a more extensive and anatomically distributed baroreflex began to emerge, his supposition appeared to be wrong. With time, the NTS came to be regarded by most physiologists as a “relay,” rather than an important integrative center. However, in concordance with studies in acute and slice preparations, our recent report in NMB rats of long-term potentiation of the baroreflex input and, now, of sleep/wakefulness dependence of ADN-elicited ERs in the NTS affirms that there is functionally important modulation of the baroreceptor input at or before the NTS.

APPENDIX

Identification and analysis of sleep cycles. Because we did not find a reliable automated procedure for paralyzed rats, individual sleep cycles were identified by manual scanning of the EEG record. Our specific goal was limited to comparison of sleep and wakefulness; thus, by rigorously defining a procedure for parsing the cycles into sleep and wakefulness periods, we sought to eliminate systematic bias that could influence the results. When a putative EEG sleep cycle was identified, to minimize ambiguity at the transitions, the start and end points, designated as $t_0$ and $t_{end}$, were set 120 s later and earlier than the visually apparent start and end points of the cycle; i.e., the first and last 2 min of data were discarded (Fig. A1). Sleep cycles have variable durations (Fig. 1), and the cycle duration was defined as $(t_{end} - t_0)$. Somewhere in the midsection of a cycle, there is a transition from sleep to wakefulness or wakefulness to sleep, but the precise point of transition is often not distinct; thus, to eliminate the need for a judgment, we divided the cycle into quarters (i.e., $Q_1$–$Q_4$), discarded the data from $Q_2$ and $Q_3$, and took the data for analysis only from $Q_1$ and $Q_4$. Thus, given that the duration of a quarter cycle was $(t_{end} - t_0)/4$, the data were taken from the intervals $[t_0]$ to $[t_0 + (t_{end} - t_0)/4]$ and $[t_{end} - (t_{end} - t_0)/4]$. The corresponding EEG activity was calculated as follows

$$EEG_{Qn} = \sqrt{\frac{4}{t_{end} - t_0}} \int_{t_0}^{t_{end} - t_0/4} [V_{EEG}]^2 \, dt$$

(for the first quarter, $Q_1$)

and

$$EEG_{Q4} = \sqrt{\frac{4}{t_{end} - t_0}} \int_{t_{end} - t_0/4}^{t_{end} - t_0/4} [V_{EEG}]^2 \, dt$$

(for the fourth quarter, $Q_4$)

where $V_{EEG}$ is the instantaneous EEG amplitude.

To enable pooling of EEG data across cycles and rats, the EEG activity for each quarter was converted to a percentage using the mean of the four quarters as the base

$$EEG_{Qn} = \left( \frac{EEG_{Qn}}{\sum_{n=1}^{4} EEG_{Qn}} \right) \times 100\% \quad (n = 1, 2, 3, \text{ and } 4)$$

Thus, $EEG_{Qn}\%$ is a measure of the relative magnitude of EEG activity during $Q_n$ compared with the average EEG magnitude of the four quarters and is a random variable that is >100% when the magnitude of EEG activity during $Q_n$ is greater than the average EEG magnitude of the four quarters and <100% when the magnitude of EEG activity during $Q_n$ is less than the average EEG magnitude of the four quarters.

Analysis of ERs. Similar to the EEG analysis, to pool ER data across cycles and rats, we converted the magnitude of ER for each quarter to a percentage using the mean of the 4 quarters as the base

$$Mag_{ERQn} = \left( \frac{Mag_{ERQn}}{\sum_{n=1}^{4} Mag_{ERQn}} \right) \times 100\% \quad (n = 1, 2, 3, \text{ and } 4)$$

Thus $Mag_{ERQn}\%$ is a measure of the relative magnitude of ER activity during $Q_n$ compared with the average ER magnitude of the four quarters and is >100% when the magnitude of ER during $Q_n$ is greater than the average ER magnitude of the four quarters and <100% when the magnitude of ER during $Q_n$ is less than the average ER magnitude of the four quarters.

The only exception to restricting the analysis to $Q_1$ and $Q_4$ was for the correlational analysis in Fig. 4, which compares the neural ERs of the present study with the cardiovascular ERs in an earlier study (4). Because in the earlier study we did not classify the sleep state, using all four quarters for Fig. 4 better aligned the ER with the cardiovascular data.

GRANTS

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