Optical imaging of the ventral medullary surface across sleep-wake states

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1Department of Neurobiology and the Brain Research Institute, University of California at Los Angeles, Los Angeles 90095; 2Department of Neurology, Irvine Medical Center, University of California, Orange, California 92868; and 3Biophysics Group, Los Alamos National Laboratory, Los Alamos, New Mexico 87545

Richard, C. A., D. M. Rector, R. K. Harper, and R. M. Harper. Optical imaging of the ventral medullary surface across sleep-wake states. Am. J. Physiol. 277 (Regulatory Integrative Comp. Physiol. 46): R1239–R1245, 1999.—We hypothesized that spontaneous activity declines over widespread areas of the cat ventral medullary surface (VMS) during rapid eye movement (REM) sleep. We assessed neural and hemodynamic activity, measured as changes in reflected 660- and 560-nm wavelength light, from the VMS during sleep and waking states in five adult, unrestrained cats and in two control cats. Relative to quiet sleep, overall activity declined, and variability, assessed by standard deviation, increased by 25% during REM sleep. Variability in activity during waking also increased by 45% over quiet sleep, but mean activity was unchanged. REM sleep onset was preceded by a reduction in the hemodynamic signal from 5 to 60 s before neural activity decline. The activity decline during REM sleep, previously noted in the goat rostral VMS, extends to intermediate VMS areas of the cat and differs from most neural sites, such as the cortex, hippocampus, and thalamus, which increase activity during REM sleep. The activity decline during REM sleep has the potential to modify VMS responsiveness to baroreceptor and chemoreceptor challenges during the REM state.

Differences in level of state-related baseline activity in structures, which have the potential to mediate cardiovascular or respiratory challenges, may contribute to the substantial variation of breathing and cardiovascular patterns between sleep states (22, 25). Among structures that have the potential to modify aspects of cardiovascular and respiratory patterns, neuronal populations on and immediately below the ventral medullary surface (VMS) apparently play a significant role, as indicated by an array of stimulation, cooling, and recording evidence (10, 14, 19).

Rostral VMS responses to pressor challenges in the waking state differ markedly from those in anesthetic and sleep states. Transient elevation of blood pressure elicits a widespread, profound decline in VMS activity during both halothane and barbiturate anesthesia, as indicated by optical studies in cats and goats (13, 14); microelectrode recordings from the nearby subretinal nucleus in the cat also show marked slowing of cell discharge (23). During waking, however, the response to pressor challenge in the goat is a minor increase in rostral VMS activity (14). The relative unresponsiveness to cardiovascular challenges during waking relative to anesthesia suggests that other states, such as sleep, may also modify VMS control of cardiovascular patterns.

Responses of the VMS to ventilatory challenges are also state dependent. Hypoxia increases rostral and intermediate VMS regional activity in the anesthetized goat and cat, respectively; during waking, the rostral response is dramatically accentuated (6). The response to hypercapnia under anesthesia is a decline in both rostral and intermediate area VMS activity; in waking, activity in the rostral VMS markedly increases before declining to the CO2 challenge (9). Cooling a portion of the rostral VMS elicits marked apnea during anesthesia and sleep, but not during waking (7). Application of CO2 to the retrotrapezoid body, sited near the VMS, results in vigorous phrenic activation in waking, but not in sleep (17), and retrotrapezoid lesions diminish phrenic efforts under anesthesia, but much less so during waking (1). Spontaneous activity on the rostral VMS of goats dramatically changes between anesthesia, waking, quiet sleep (QS), and rapid eye movement (REM) sleep (31); these changes in baseline activity may underlie state-dependent VMS responses to challenges.

Altered spontaneous VMS activity during different states has the potential to contribute to the cardiovascular or respiratory failure in particular syndromes associated with sleep. A proportion of victims of sudden infant death syndrome (SIDS), for example, show diminished muscarinic (16) binding in VMS regions projecting to caudal medullary structures that mediate responses to hypotension (36). Although the mechanism(s) of failure in SIDS remain unclear, infants appear to succumb during sleep, and the fatal event may involve an inability to compensate for profound hypotension (12), possibly triggered by a respiratory challenge.

The rostral VMS of the goat, cooling of which elicits a profound blood pressure loss, shows a substantial decline in spontaneous activity during the REM sleep state (31). The loss of spontaneous activity suggests a loss of influence from the rostral VMS on breathing or blood pressure during REM sleep. Removal of VMS influences from control of blood pressure and breathing during REM sleep has significant implications for determining the sources of respiratory rate and heart rate variability during the REM state. Breathing and blood pressure activity respond differently to cooling or
chemical stimulation of intermediate and caudal VMS regions from rostral VMS areas (5). We hypothesized that the loss of spontaneous activity during REM sleep, observed in rostral VMS sites in the goat, extends to intermediate and caudal VMS areas of the cat. We explored VMS activity during sleep and waking states in unanesthetized, freely moving cats with optical procedures, which allowed visualization of activity over relatively large VMS areas and provided an indication of regional patterns at relatively high temporal resolution. The optical procedures allowed differentiation of signals from neural and hemodynamic components and thus offered insights into the time course of neural and perfusion changes.

METHODS

Nine adult cats (3.0–4.0 kg) were instrumented for sleep studies and for optical assessment of neural and hemodynamic activity on the VMS. Each animal was anesthetized with pentobarbital sodium (25 mg/kg iv, supplemented as needed with 10 mg/kg) for sterile surgical implantation of recording electrodes. Atropine was administered in conjunct with anesthesia (0.05 mg/kg). Stainless steel screws were placed in the cranium over the sensorimotor cortex and in the bone over the orbit to record electroencephalographic (EEG) and eye movements, respectively. Two sets of insulated, multi-stranded stainless steel wires, bared 8 mm at the tips, were placed in the costal diaphragm from an abdominal approach to record diaphragmatic electromyographic (EMG) activity and the electrocardiogram (ECG). Similar wire electrodes were placed in the neck musculature for nuchal EMG assessment. A carotid artery and jugular vein were cannulated unilaterally for blood pressure measurement and drug delivery, respectively. Leads and cannulas were routed subcutaneously to a headcap placed on the cat’s dorsal cranium.

Neural activity on the VMS was assessed by a miniature optical probe attached to a charge-coupled device (CCD) camera. For access to the ventral surface of the brainstem, the cat was placed supine for a midline incision of the neck. The trachea and surrounding muscles were retracted for access to the basal skull between the foramen magnum and the area just medial to the tympanic bulla. An opening in the skull was made medial to the jugular foramen to accept the fiber optic probe (3.2 mm diameter). The fiber optic probe and attached CCD camera were positioned, and the brain stem surface was imaged to visualize stable contact with the VMS. The probe and camera were cemented in place with dental acrylic and the camera cable led to the headcap. A portion of the right tympanic bulla was removed so that the acrylic bone over the orbit to record electroencephalographic (EEG) activity of large groups of neighboring neurons (18, 34). This technology allows assessment of activity of large groups of neighboring neurons (>7 mm²) with high temporal resolution (50 Hz). Optical assessment offers advantages over multiple microelectrode recordings because the technique avoids the difficulties of ventral medullary access in a freely moving animal and the resulting stability issues for recording over long time periods. In addition, light at 560 (±10) nm was projected onto the VMS and reflected into the light conduit and CCD camera. Reflected light at that wavelength provides an index of hemoglobin concentration and perfusion of the tissue under the optic probe (21). The switching circuit was designed to alternate red and green illumination such that each wavelength was on for 7 ms and then switched off for 3 ms during frame readout, for a total of 10 ms/wavelength or 20 ms for both red and green wavelengths; i.e., 50 frames/s were collected from combined red and green illumination. Switching time was negligible, and there was no overlap of the illumination wavelengths, i.e., no concurrent green and red illumination. There was no illumination during image readout. The intensity of the light projected onto the VMS was monitored with a photodiode and servocontrolled to maintain a constant illumination level.

After at least 4 days of recovery from surgery, animals were habituated to a 1-m², sound-attenuated recording chamber constructed to allow recording from freely moving, unrestrained animals. Cats were placed in the recording chamber with food and water ad libitum; cables were attached to the headcap and connected to recording devices with a commutator. Data were collected during a baseline sleep period before any drug delivery or experimental challenge. Scanned CCD pixels (208 × 192), representing all the photons detected by each element of the CCD array during the entire acquisition periods, were digitized with a resolution of 12 bits and stored on a hard disk. Images were digitized continuously, alternating between red and green frames, together with the EEG, ECG, EMG, and eye movement electrophysiological channels (1 kHz/channel). Electrophysiological channels were also written onto polygraph paper (Grass model 7B) for scoring of sleep-wake stages with standard criteria (35). Scored stages were waking (AW), QS, REM sleep, and indeterminate state (IS). To optimize the dynamic range for recording of light signals, illumination intensity was set so that maximal reflectance was two-thirds of the maximum digitizer value, and the black level was set to half of the amplitude of the minimal pixel value.

Heart rate was derived from R–R intervals that, in turn, were determined from R-wave peak detection on the ECG channel. Respiratory rate was similarly derived from the respiratory waveform created by integration of the diaphragmatic EMG channel. Average activity from the imaged area of the VMS was computed for each frame for both 660- and 560-nm illumination (i.e., a single data point for each frame) and displayed as neural activity and hemodynamic traces along with the electrophysiological channels. These data were partitioned by sleep-wake stage and averaged across an epoch from each state. To control for intersubject differences in probe and amplifier aspects, activity in an epoch of REM, AW, or IS was expressed as the ratio to the activity from the nearest QS epoch. Differences in averaged intensity were evaluated with the nonparametric Sign test. In additional analyses, activity changes between states were calculated by averaging images, or a region-of-interest of the frame from a defined state, and subtracting that average from an average of the imaged area from a reference QS state. Quiet sleep was
selected as a reference, because QS consistently exhibited the most stability in activity. ANOVA procedures were used to detect differences in pixels across sleep-wake states and across subregions of an image. These differences were then pseudocolored, with yellow-to-white colors representing activity increases and blue-to-black colors representing neural activity decreases. Significance for differences in individual pixel values was assigned when \( p < 0.05 \).

After experiments were completed, each cat was euthanized with an overdose of pentobarbital sodium. The brain was fixed with a 10% phosphate-buffered Formalin solution and then removed from the skull. The medullary surface was examined to determine probe position and orientation.

**RESULTS**

Of the nine cats used in this study, two had probe placements outside the VMS, and two died before adequate data were collected. Thus data are presented from the VMS of five cats, and the two misplaced probe sites were used for control purposes. Probe placements are represented in Fig. 1 and correspond principally to rostral and intermediate portions of the VMS.

VMS activity declined during REM sleep in all five cats compared with QS (\( p < 0.05 \)). Representative examples of the decrease in activity in REM and the large variability in REM and waking states are given in Fig. 2, A and B. Activity during AW and IS stages did not significantly differ from QS; substantial variability during the waking state contributed to the absence of consistent differences. Figure 2 demonstrates the usual fall in perfusion of the VMS coincident with the fall in neural activity during REM sleep. The mean VMS activity during REM sleep was 98.3 ± 0.01% (SE) of activity during quiet sleep. VMS activity during waking was 99.5 ± 0.03% of QS.

Although hemodynamic changes grossly paralleled those of activity, (i.e., perfusion fell during REM sleep together with neural activity and rose with the transition to waking), the timing and extent of changes differed between the two signals. Hemodynamic changes typically preceded activity changes in the transition from QS to REM sleep by periods of 5–60 s. The initial hemodynamic decline during a QS-REM state transition is shown for an individual cat in Fig. 2B; an averaged perfusion trace from all five experimental animals, aligned by REM onset and overlaid on a similarly-aligned averaged activity trace, is shown in Fig. 3. The decline in VMS activity during REM sleep was not precipitous but typically required 30–60 s to reach a nadir.

A substantial regionalization of cellular activation of the VMS emerged between states, in which one subregion was significantly activated while another portion was significantly deactivated or remained unchanged.

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Fig. 1. Schematic outline of ventral medullary surface (VMS) of cat, showing optical probe positions (○). VI, abducens nerve; XII, hypoglossal nerve exit on VMS. C1 and C2 are control probe placements.

Fig. 2. A: representative example from one cat, illustrating decrease in activity (VMS Act) and in perfusion [indicated by hemodynamic signal (Hem)] during rapid eye movement (REM) sleep and large variability in both signals that occurs during waking (AW). Blood pressure (BP) shows slight elevation during REM in this case. B: example of VMS activity and perfusion decline from transition of quiet sleep (QS) to REM sleep in different cat; the 2 signals grossly paralleled each other, but short-term variation differed. Unlike traces from A, this case illustrates more common finding of earlier decline in perfusion signal over neural activity.

Fig. 3. Averaged trends of hemodynamic (Hem, green) and neural activity (Act, red) signal changes during transition from QS to REM sleep. Traces are aligned to first signs of REM sleep, as indicated by loss of neck muscle tonus. Onset of decline in hemodynamic activity occurs at arrow. Hemodynamic changes preceded neural alterations by 5–60 s.
During waking, activated or deactivated regions would appear, and these patterns would differ from activated or deactivated areas in REM sleep even within a cat. The different patterns between waking and REM sleep suggest unique processes operating within the VMS during different sleep-wake states. Figure 4 includes pseudocolored images of VMS activity during an epoch of waking and REM subtracted from an epoch of QS. In this example, the upper left quadrant (Q1) is activated in waking and deactivated in REM sleep, but the lower quadrant (Q4) is mostly activated in both states. This figure also shows traces of activity and perfusion from two out of the four quadrants, indicating more pronounced declines in activity and perfusion during REM in particular quadrants over others.

Inadvertent misplacement of the optical probe allowed assessment of neural activity in non-VMS areas used as a control. A trace from one such caudal placement (caudal to XII nerve exit) is shown in Fig. 5 to illustrate that the decline in VMS activity during REM is not generalized to other nearby sites. The other control site also lacked a state relationship to activity.

**DISCUSSION**

The study confirms and extends findings from a larger species, the goat, that regions within the VMS show a decline in activity during REM sleep. In addition to rostral VMS areas, intermediate regions also show diminished activity, and with similar substantial variability during REM sleep. The data indicate that, although the VMS hemodynamic changes associated with state generally paralleled the neural patterns, the alterations were not always consonant. Regional patterns of neural activity in AW and REM states indicated local activation of neurons unique to particular states; those local patterns suggest neural participation in processes particular to a single state. Finally, hemodynamic changes appeared to precede the neural activity alterations associated with the onset of REM sleep, with the decline in neural activity proceeding slowly during the course of the state.

**Limitations**

A potential exists for signals emanating from both hemoglobin and activity sources to overlap. However, absorption at the longer, 660-nm, red illumination is reduced by a factor of 400 over the shorter, 560-nm, green light from hemoglobin sources (27). The separation of activity and hemoglobin signal return is supported by the current data, which showed significant differential trends between the two signal sources on occasion, e.g., onset of REM sleep (Fig. 3) and the differential trends of perfusion and activity in particular quadrants of Fig. 4, although the general relationship that increased activity is associated with increased perfusion was typically the case. The pattern of returned light changes associated with red illumination does not depend on hemodynamic components; returned light changes occur in blood-free hippocampal slice preparations associated with neuronal activation (20) and reflectance changes during activation of the cortex even after blockade of blood flow changes (11).

Illumination at longer wavelengths (e.g., red) penetrates tissue deeper than light of shorter wavelengths (e.g., green). Using techniques outlined earlier (32), we found that 560-nm (green) light penetrates to 250 \( \mu m \), whereas 660-nm (red) light penetrates to 500 \( \mu m \). Thus the hemodynamic signal assesses components closer to the surface, whereas the neural activity measures assess light scatter from up to 500 \( \mu m \) below the surface.
Regionalization. The regionalization of activity and the switch in direction of change of local sites between states indicates that the study of VMS action has the potential to reveal a substantially more detailed description than the overall analyses provided here. Further examination of regional areas may indicate underlying groups of neurons that play a substantial role in state-related modulation of cardiovascular and respiratory regulation. Such state-specific topographic organization, in conjunction with anatomic data, may be used to define the processes by which breathing and cardiovascular patterns change during sleep.

Implications for state control. The VMS represents one of the few brain sites that decline in activity during REM sleep. That decline does not occur in nearby areas, such as more-caudal regions of the spinomedullary junction, as demonstrated by the control recordings, and is in contrast to optical studies of other rostral brain areas; the hippocampus, for example, increases activity during REM sleep, as demonstrated by both optical and electrophysiological studies (29, 30). Although the dorsal raphe (24), raphe magnus and obscurus, and locus coerules (33) exhibit neuronal rate slowing during the REM state, most areas within the brain are markedly excited during REM sleep, and especially so during phasic periods of REM sleep. Both medullary raphe and locus coerules regions participate in aspects of blood pressure regulation (3, 4). A high proportion of ventrolateral medulla neurons that project to the locus coerules respond to pressor challenges (15), although the precise cardiovascular relationship between the sites is unclear. Thus neurons in the medullary raphe, locus coerules, and VMS regions (all sites which participate in blood pressure control) show reduced neural activity in REM sleep. The state-related decline in activity for these sites may be only incidental to cardiovascular control aspects. Of theoretical importance is the finding of a region that is actively suppressed or disfacilitated during a period of substantially enhanced variation and redistribution of sympathetic and ventilatory control.

The diminution of VMS activity may participate in mediating the loss of influence from rostral brain areas on breathing and cardiovascular control during REM sleep. Electrical stimulation of certain limbic structures transiently elevates blood pressure in waking and QS, but not in REM sleep (8), and warming of the anterior hypothalamus elicits compensatory increased respiratory rate, but fails to do so during REM sleep (26). The decline in VMS activity during REM sleep suggests a withdrawal of influences over that structure during a state characterized by substantial changes in breathing and cardiovascular patterns.

The reduction in spontaneous activity during a REM sleep period that normally is an excitatory state for other widely-dispersed brain areas, and especially certain respiratory sites, points to a unique role for the VMS during REM sleep. The anatomic relationships between the VMS and other neural sites that share the rare neuronal firing reduction during REM sleep suggest a significant reorganization of cardiovascular control during the REM state.

Perspectives

The neural activity decline on the VMS during REM sleep may participate in the blunting of chemoreceptor and baroreceptor responses during the REM state. The diminished activity contrasts with the substantially
increased neural discharge in virtually every other brain area during REM sleep. The only exceptions to the generalized excitation during REM sleep found thus far are brain structures that, coincidentally, have been implicated in blood pressure regulation; these areas include presumed serotoninergic neurons in the dorsal raphe, cells of the caudal midline medullary raphe, and neurons within the locus coeruleus. We speculate that the REM-related activity decline in the VMS, as well as the other three sites, represents some degree of loss of control in a system that participates in blood pressure and breathing regulation during waking and quiet sleep but that becomes less effective during the REM state. REM sleep is characterized by a significant redistribution of blood flow between splanchnic beds and the skeletal musculature, increased variation in heart and breathing rate and arterial pressure, and phasic activation of breathing. The increased variability and redirection of blood distribution during REM sleep suggest a release of waking and quiet sleep breathing and autonomic control regions and phasic excitation of autonomic and respiratory output neurons by other neural sites. Earlier evidence of reduced forebrain influences on brainstem reflexes during REM sleep suggest that part of the release of VMS control may involve descending influences from rostral sites. The source(s) that phasically activate autonomic and respiratory output remain unknown.

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