State influences on ventral medullary surface and physiological responses to sodium cyanide challenges

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Macey, Paul M., Christopher A. Richard, David M. Rector, Rebecca K. Harper, and Ronald M. Harper. State influences on ventral medullary surface and physiological responses to sodium cyanide challenges. J Appl Physiol 89: 1919–1927, 2000.—Intravenous sodium cyanide (NaCN) administration lowers ventral medullary surface (VMS) activity in anesthetized cats. Sleep states modify spontaneous and blood pressure-evoked VMS activity and may alter VMS responses to chemoreceptor input. We studied VMS activation during peripheral chemoreceptor stimulation by intravenous NaCN using optical procedures in six cats instrumented for recording sleep physiology during sham saline and control site trials. Images of scattered 660-nm light were collected at 50 frames/s with an optical device after 80–100 μg total bolus intravenous NaCN delivery during waking and sleep states. Cyanide elicited an initial ventilatory decline, followed by large inspiratory efforts and an increase in respiratory rate, except in rapid eye movement sleep, in which an initial breathing increase occurred. NaCN evoked a pronounced decrease in VMS activity in all states; control sites and sham injections showed little effect. The activity decline was faster in rapid eye movement sleep, and the activity nadir occurred later in waking. Sleep states alter the time course but not the extent of decline in VMS activity.

Afferent somatosensory activity from enhanced loading of breathing, rapid blood pressure elevation, sensing of low oxygen, or an accumulation of CO2 (2), although isocapnic hypoxia is a relatively poor arousal stimulus (4). Sleep states apparently modify arousal thresholds to chemoreceptor challenges (24), and, thus, the propensity for arousal from an obstructive event. In addition to the issue of arousal thresholds, other consequences of sleep state modulation of neural mechanisms involved in central responses to chemoreceptor input are of interest. These consequences include responses of neural areas to concomitant blood pressure and somatomotor (e.g., respiratory musculature) changes associated with chemoreceptor stimulation.

The intermediate area of the ventral medullary surface (VMS) is one neural structure that plays a role in mediating the central response to peripheral chemoreceptor stimulation. In the anesthetized cat, activity declines on the intermediate area of the VMS after intravenous sodium cyanide (NaCN) stimulation, a challenge that acts on the peripheral chemoreceptors (3). The activity decline does not appear on cortical control sites and is abolished after carotid sinus nerve transection. The findings from cyanide stimulation studies support evidence from hypoxic challenge data that carotid sinus nerve input is inhibitory to activity on the VMS: carotid sinus nerve transection may “release” the VMS to allow more enhanced responses to hypoxia (8).

The VMS activity associated with chemoreceptor challenges is state dependent. Respiratory responses to acidic stimulation of ventral medullary sites diminish substantially during sleep (16). The extent of rostral VMS responses and even the direction of responses to ventilatory and blood pressure challenges vary substantially between waking and anesthesia (5, 9, 13). Spontaneous activity on the VMS significantly declines during rapid eye movement (REM) sleep in both rostral and intermediate areas, as demonstrated in two species (26, 31). Because ongoing VMS activity is reduced...
during REM sleep, VMS responses to a challenge may be altered during that state. In addition to the potential for modifying response thresholds to chemoreceptor challenges, different breathing patterns occur during the REM state over waking and quiet sleep. A portion of that state-related variation may result from modification of breath-to-breath VMS responses to sleep-induced alterations in afferent activity from the carotid body.

The objective was to examine sleep state influences on VMS activity and on breathing and cardiovascular patterns in response to peripheral chemoreceptor stimulation. Because hypoxia induces a large number of tissue changes that would confound assessment of the study objective, we used intravenous delivery of NaCN to stimulate peripheral chemoreceptors, a technique previously used to avoid the complexities of interpreting gross effects from hypoxia (3). We examined VMS activity in unrestrained, drug-free cats during waking, quiet sleep, and REM sleep states.

Because the VMS is virtually inaccessible to microelectrode techniques in the freely behaving animal, intrinsic optical imaging was used to assess cellular activity (27). This technique captures light differentially scattered by neural activation vs. quiescence and does not employ calcium-activated or voltage-sensitive dyes, the toxic properties of which may compromise neuronal function. The procedure has the additional advantage of evaluating activity over many thousands of neurons, thus allowing assessment of regional activity within the recording area. The techniques have been used to demonstrate altered topographical patterns of neural activity in cortical fields (12), as well as hippocampal and VMS activity changes to hypoxia, hyperoxia, CO₂, hypovolemia, and blood pressure during different states (25).

METHODS

Subjects and procedures. Instrumentation for sleep state assessment and optical measures of neural activity was placed in eight adult cats (3–4 kg) during sterile surgery. Each animal was anesthetized with pentobarbital sodium (25 mg/kg iv, supplemented as needed with 10 mg/kg doses), and atropine (0.05 mg/kg) was administered to minimize secretions. Stainless steel screws were placed in the bone over the sensorimotor cortex and orbital cavity to record the electroencephalogram (EEG) and electrooculogram (EOG) detection of eye movements, respectively. Pairs of insulated, flexible multistranded stainless steel wires, bared at the tips, were led to the headpiece. All animals were treated postoperatively with antibiotics (penicillin G, 2 × 10⁶ units im bid, and chloramphenicol 50 mg/kg iv bid), analgesics (buprenorphine, 0.004 mg/kg im, 4–6 h), and steroids (dexamethasone 0.4 mg·kg⁻¹·day⁻¹ iv, decreasing to 0.05 mg·kg⁻¹·day⁻¹). The cannulae were flushed daily with heparinized saline. All procedures were approved by the Institutional Review Board.

The optical imaging procedure uses the principle that, under illumination, neurons scatter light differentially when activated (18, 19, 27). Neural depolarization is accompanied by cell swelling and membrane conformational changes that diminish light reflection and refraction (17), and the resulting opacity changes have been previously described. Similarly, assessment of reflectance, voltage-sensitive dye, and microelectrode recordings show corresponding measures (10). Very rapid following of optical signals in the hippocampus can be demonstrated by Schaeffer collateral stimulation (28). The rate of following is in excess of what can be expected of perfusion measures.

The VMS was illuminated at 660 (±10) nm (red) to provide an index of activity and at 560 (±10) nm (green) to index perfusion. Groups of neurons within a relatively large area of the VMS, >7 mm², were viewed with high temporal resolution (50 Hz). Reflective light at 560-nm wavelength provided an index of oxygenated hemoglobin concentration and, thus, perfusion of the tissue under the optic probe. Red and green illuminations were alternately switched, such that each wavelength was on for 7 ms and off for 3 ms during frame readout, for a total of 10 ms/wavelength or 20 ms for both red and green wavelengths. Therefore, 50 frames/s were collected for both red and green illumination, with negligible switching time and no overlap of illumination wavelengths, i.e., no concurrent green and red illumination. During image readout, the tissue was not illuminated. Illumination intensity was monitored with a photodiode and was servo-controlled to maintain constant illumination.

Animals were habituated to a 1-m³, sound-attenuated recording chamber, beginning at least 4 days after surgery; the chamber allowed free movement and access to food and water. Signal cables from the animal headcap were attached to a commutator and then led to recording equipment. A baseline sleep cycle was acquired before any cyanide challenge. Signals from the 208 × 192 pixel array of the CCD, representing all photons detected during entire acquisition periods, were digitized with a resolution of 12 bits (range of 0–4,095) and stored on a hard disk. Images were digitized continuously, alternating between red and green frames, together with the EEG, ECG, EMG, blood pressure, and EOG electrophysiological channels (1 kHz/channel). Electrophysiological channels were also written onto polygraph paper (Grass Model 78) for later scoring of sleep-wake stages. To optimize the 12-bit dynamic range for image recording, the device was set so that the brightest pixel intensity was two-thirds of the maximum digitizer value, and the black level was set to half of the amplitude of the minimal pixel value. Such adjustment increased the effective measurement resolution by a factor of 6.7. The recorded 208 × 192 pixel images were reduced by averaging to 80 × 80 pixels in size, matching the effective resolution of the light conduit.

Heart rate was determined from the ECG signal using R-wave peak detection. Respiratory rate was assessed from the integrated diaphragmatic EMG signal. Average activity from the imaged area of the VMS was calculated over each frame for 660-nm illumination (i.e., a single data point for the VMS surface was visualized during probe placement to ensure appropriate contact. The probe system was cemented to the ventral skull with dental acrylic, and attached cables were led to the headpiece. All animals were treated postoperatively with antibiotics (penicillin G, 2 × 10⁶ units im bid, and chloramphenicol 50 mg/kg iv bid), analgesics (buprenorphine, 0.004 mg/kg im, 4–6 h), and steroids (dexamethasone 0.4 mg·kg⁻¹·day⁻¹ iv, decreasing to 0.05 mg·kg⁻¹·day⁻¹). The cannulae were flushed daily with heparinized saline. All procedures were approved by the Institutional Review Board.

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each frame) and displayed as neural activity along with the electrophysiological channels.

Each challenge consisted of an 80–100 μg (20–25 μg/kg) NaCN bolus administered intravenously; challenges were delivered only once within any one state in a recording period and were repeated on subsequent epochs of states (up to eight challenges per state per animal). A sham dose of an equivalent volume of saline served as a control in each state.

Analysis. Sleep-state periods were classified as quiet sleep, REM sleep, and waking according to standard criteria for the cat (33). Image processing, including subtraction of experimental from baseline conditions, image averaging, gray scale adjustment, pseudocoloring, and display of results were performed on an Intel Pentium-based computer. Successive images from 50-s baseline periods before each challenge were used to derive image reflectance intensity for red illumination, against which image values in the 200-s challenge period were subtracted. ANOVA was used to statistically evaluate significant changes of the image frames between conditions ($P < 0.05$). The resulting difference images were pseudocolored to convey statistically significant changes in activation such that yellow-to-red-to-white colors represent increased cellular activation (increased reflectance) and blue-to-purple-to-black colors represent decreased cellular activation (increased reflectance). Green pixels represent no statistically significant change from baseline conditions. The availability of two illumination wavelengths assisted determination of movement artifacts, because signal components contributed by motion affect reflectance of both illumination wavelengths in the same manner. Recordings were extensive (near daily for 3–9 wk), with multiple collections of trials from each state condition. Recordings were continued until sufficient representation from each state was obtained, an advantage offered by the chronic optical procedure. Because of nonnormal distributions in measures, nonparametric Mann-Whitney (paired values) and Kruskal-Wallis (unpaired values) tests were used for overall physiological and activity assessment between states.

Probe localization. After experiments were completed, the cats were euthanized with an overdose of pentobarbital sodium. The medullae were preserved with 10% phosphate buffered formaldehyde and examined to determine probe position and orientation.

RESULTS

Surgery. Two of the eight cats succumbed immediately after surgery, before recordings were performed. The remaining six cats recovered and experienced normal sleep-wake patterns by the fourth day after surgery.

Probe. The locations of the probes are illustrated in Fig. 1. Experimental sites were localized to the rostral and intermediate areas of the VMS, 1–3 mm lateral to the midline. A control site in the pons is shown, rostral to the exit of cranial nerve V. All probes functioned appropriately for the duration of the studies. Challenges. A total of 62 challenges was delivered to the five cats with probes on the VMS, as shown in Table 1.

Arousals. Cyanide elicited an arousal in 43% of challenges during sleep (39% during quiet sleep and 45% during REM), as indicated by an increase in EEG frequency and restoration of nuchal tone; typically, arousals began 30 s after challenge onset, late into the breathing, cardiovascular, and VMS activity changes. Half of these arousals were sustained, whereas the remainder lasted <20 s. Cats differed in arousal patterns in response to challenges, ranging from no arousals to arousals after every challenge. Most cats showed some cyanide responses with arousals and some responses without arousals.

Respiratory responses. A typical example of a response to a cyanide challenge during quiet sleep is shown in Fig. 2; averaged breathing rates within each state are shown in Fig. 3. The initial respiratory response in waking and quiet sleep was a reduction in breathing rate. After ~20 s, a large inspiratory effort or sequence of augmented inspiratory efforts developed. During and after the large inspiratory efforts, breathing rates increased rapidly. Four characteristics were observed in the response to challenges across states: 1) large inspiratory efforts emerged in response to all challenges, typically developing 15–20 s after the onset of the challenge (mean 20 s, range 7–60 s); 2) breathing rates initially declined (mean 22%) in wak-

Table 1. Numbers of challenges by state and by cat

<table>
<thead>
<tr>
<th>Cat</th>
<th>REM</th>
<th>QS</th>
<th>Awake</th>
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<td>1</td>
<td>7</td>
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<td>11</td>
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<td>All</td>
<td>12</td>
<td>21</td>
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<td>62</td>
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REM, rapid eye movement sleep; QS, quiet sleep.
and quiet sleep until the large inspiratory effort, and then increased rapidly; 3) a subset of cats experienced a decreased breathing rate after the onset of augmented inspiratory efforts (up to 20 s after the inspiratory onset); and 4) in REM sleep, the initial decline in breathing rate did not appear. During quiet sleep, a short (5–9 s) apnea occasionally followed the large inspiratory effort (29% of quiet sleep challenges). These breathing pauses were not observed in waking or in REM sleep.

Cardiovascular responses. A typical heart rate response to a challenge during quiet sleep is illustrated in Fig. 2, and averaged heart rate responses within each state are shown in Fig. 4. An initial heart rate decrease developed shortly after challenge onset, but only in quiet sleep. With the large inspiratory effort, in quiet sleep, heart rate increased sharply to a higher level than baseline and then gradually returned toward baseline. An increase in heart rate after the challenge developed across all states ($P < 0.05$); the extent of increase, however, did not significantly differ between states. Although heart rate initially declined in quiet sleep, beginning ~5 s after the challenge, in waking and REM sleep heart rate increased during this early phase ($P < 0.05$, Fig. 4). Blood pressure increased with the initial heart rate decline and decreased with the large inspiratory effort for all challenges, typically falling rapidly for 5–10 s. After reaching a nadir, the blood pressure returned to baseline over a period of 2–3 min.

VMS activity responses. The VMS response to a cyanide challenge was a decline in activity in all states...
Responses from one cat across waking and sleep states are shown in Fig. 5, and averaged responses across all cats and all challenges in each state are illustrated in Fig. 6. The latency to the nadir was maximal in waking compared with in REM and quiet sleep ($P < 0.05$). The maximal extent of the activity decline did not significantly differ between states. However, the initial rate of decline in REM sleep was significantly faster than in quiet sleep ($P < 0.05$).

Control responses. A sham infusion of an equivalent bolus of saline (Fig. 7A) resulted in no appreciable change in VMS activity or heart rate. Neural activity at the control site in response to 50-$\mu$g doses on the pontine ventral surface is shown in Fig. 7B. Despite a physiological response, activity changes on the control site were not significant.

Topographic changes. The activity changes over the field of view of the probe were not uniform. Regionalized activity changes were found in each subject in response to the cyanide challenges. A variety of regional patterns emerged between states and during the course of responses to challenges. Images from three challenges in one cat during waking, quiet sleep, and REM are shown in Fig. 8, with image changes shown relative to a baseline period. In all cases, the activity decline was not uniform, beginning either laterally (waking and REM examples) or caudally (quiet sleep example). Similarly, subregions with greater activity decline were seen in all sites. A second regionalization pattern that occurred in four of the five VMS sites was a spreading of activity change, as in the quiet sleep example, with the decline starting in a caudal subregion and spreading in a rostral direction. The recovery pattern usually differed from the decline, with regions showing an activity increase relative to baseline, as shown by warm colors in medial regions of waking and quiet sleep images in Fig. 8. Two common patterns were a caudal decline during the response followed by a rostral increase during recovery, as with the quiet sleep example in Fig. 8, and a lateral decline during the response followed by a medial increase during recovery, as in the waking and REM examples in Fig. 8.

DISCUSSION

Stimulation of carotid chemoreceptors by NaCN elicited a sequence of respiratory and cardiovascular patterns and a decline in VMS activity in all sleep-waking states. The decline in VMS activity in response to NaCN administration had been previously observed in anesthetized preparations. However, relative to anesthetized conditions, sleep states have the potential to modify the direction or extent of evoked changes in VMS activity to other challenges (14), necessitating the current studies. Sleep states significantly modified responses of both VMS activity and physiological mea-
sures. During REM sleep, VMS activity declined more rapidly than during any other state. The VMS activity pattern showed a lag in the nadir of the response in waking relative to sleep states. Moreover, respiratory rate initially increased in response to the challenge during REM before declining, unlike quiet sleep and waking patterns. An early heart rate decline specific to quiet sleep emerged.

**Stimulation considerations.** NaCN rather than systemic hypoxia was used because hypoxia exerts widespread effects on neural tissue, the cardiovascular system, and afferent transducer mechanisms, complicating interpretation of VMS neural changes to the stimulus. Hypercapnia challenges were given as part of a different study; these challenges also resulted in a decline of VMS activity but with different state effects (30). A considerable body of evidence from whole nerve and single fiber carotid sinus nerve recordings, concurrent with respiratory measures, demonstrates that carotid chemoreceptors respond to NaCN in a dose-dependent fashion (20, 32) and that these challenges elicit dose-dependent ventilatory changes, with the largest doses eliciting gasping. The dosage range employed in this study resulted in a range of breathing effects, from an initial slowing to deep inspiratory efforts and a breathing rate increase. However, no gasping occurred, suggesting that the dose levels did not result in extreme respiratory distress and did not elicit a “ceiling effect” in the response.

There is a possibility that hypocapnia results from the hyperventilation induced by NaCN. Such a possibility might represent a confounding influence on the VMS activity responses. However, we found that increased CO2 results in a fall in VMS neuronal activity in the same sites studied here (30). If cyanide leads to hypocapnia in the current experiments, then VMS activity should not fall; hypocapnia might reduce the magnitude, but not the direction, of the VMS activity response.

Earlier studies of NaCN effects on the carotid body suggest a more prominent effect on chemoreceptors than on baroreceptors (20). Unanesthetized rats show a lag in blood pressure responses of 3 s after respiratory responses (6); both the respiratory and blood pressure changes are abolished by carotid sinus nerve transection. We found modest blood pressure changes to NaCN challenge. The largest blood pressure alterations were associated with the deep inspiratory efforts and were likely secondary to such efforts. Thus the most prominent effects of cyanide appear to be medi-
ated by carotid chemoreceptors, possibly interacting with aortic chemoreceptors.

Response mechanisms. The state-related changes in the VMS activity during cyanide challenges have significant implications for determining the role of the VMS in modulating breathing and cardiovascular control during sleep and waking conditions. As was found in studies of anesthetized animals (3), NaCN elicited an activity decline to peripheral chemoreceptor stimulation in all sleep-waking states. That decline was faster in REM sleep than in other states, and the nadir of this decline during REM sleep occurred earlier than during waking.

“Spontaneous” activity on the VMS is significantly lower during REM sleep than during other states, as shown in two species (26, 31). In REM sleep, the lower spontaneous activity has the potential to interact with the decline resulting from a NaCN challenge. However, challenges during REM sleep did not result in a further net decline of VMS activity; instead, the activity decline was accelerated. A comparable, more rapid fall is found in response to a pressor challenge in REM sleep (29). We speculate that the relative loss of regulation during the REM state, and the low background level of activity, provide a set of circumstances during which activity cannot be sustained and falls rapidly. Although breathing patterns and arousals from chemoreceptor challenges do not significantly change between waking and quiet sleep, arousal thresholds are typically raised during nonphasic portions of REM sleep (24). Hypoxia is a relatively weak stimulus for arousal from sleep (4); the rapid decline in VMS activity may further diminish arousal potential for hypoxia in REM sleep.

The activity decline to a cyanide challenge suggests a particular role for the VMS during peripheral chemoreceptor stimulation. In the anesthetized cat, hypoxia elicits activation of the intermediate area VMS, which is enhanced by carotid sinus nerve transection; i.e., the carotid sinus nerve traffic exerts an inhibitory or disfacilitatory effect on VMS responses to hypoxia (8). Bilateral vagotomy, however, reduces VMS activation to either short-lasting or more prolonged hypoxia (1). After carotid sinus nerve transection, the enhanced VMS activation response to systemic hypoxia indicates that the activation comes from sites other than the carotid chemoreceptors and most likely arises from vagal afferents or is at least facilitated by vagal input, because bilateral vagotomy substantially reduces the increase in VMS activity. Carotid sinus nerve transection abolishes the VMS activity decline to NaCN in anesthetized preparations (3); such transection enhances the VMS response to a pressor challenge. Vagotomy substantially reduces the VMS response to a pressor challenge (13). Afferent activity from the carotid body travels by way of the carotid sinus nerves to the nucleus of the solitary tract, which projects, in turn, to the VMS. Collectively, the systemic hypoxia, NaCN, and pressor data, with carotid sinus nerve and vagal transection, suggest that the carotid sinus...
nerves act as a disfacilitatory or inhibitory input to the VMS, whereas vagal input exerts an excitatory influence. The implication of the more rapid decline in VMS activity during REM sleep is that there are common state-related mechanisms operating both on the integration of carotid sinus nerve activity in the case of cyanide challenges and on vagal activity in the case of pressor challenges.

Breathing. The initial respiratory response to intravenous NaCN administration was a decline in breathing rate, appearing before large inspiratory efforts and an increase in breathing rate; in quiet sleep, apnea frequently followed the large inspiratory efforts. NaCN, administered to anesthetized preparations via the vertebral artery, also elicits a decline in phrenic nerve output; with increasing doses, apnea ensues (21). Similarly, injection of NaCN into the intermediate area of the ventral medulla (22) or application into the intermediate area VMS (15) also depresses phrenic nerve output. A remarkable aspect of the breathing findings was an increase in breathing rate to initial cyanide challenge during REM sleep, rather than a decline as in other states. We speculate that the breathing rate increase derives from the more rapid decline in VMS activity to the challenge. The breathing pattern during REM paralyses that found earlier in anesthetized preparations, i.e., an initial small, then a large increase in ventilation. The response patterns elicited during anesthetized conditions may be analogous to the REM state, especially if forebrain influences on cardiorespiratory control during anesthesia are comparable to REM sleep (7).

Limitations. A portion of the response of the CCD camera to scattered light may derive from hemodynamic rather than activity sources. The contributions from perfusion were minimized, however, by a nearly 400-fold differential sensitivity of 660 nm (red) illumination vs. the 560 nm (green) illumination to hemoglobin components (23). The index of perfusion, i.e., reflected and scattered green light, grossly declined concurrently with VMS activity and thus should not represent hyperperfusion. Because blood-free hippocampal slice preparations also show transmitted light changes on activation (18) or during cortical activation after blood vessel blockade (11), changes in reflected red light cannot be attributed solely to hemodynamic aspects.

The demands of sampling responses across three sleep and waking states limited the dose levels that could be used. Only one dose per state could be delivered, because arousal frequently accompanied cyanide administration, requiring another sleep cycle sequence of waking, quiet sleep, and REM sleep, with a finite time period in each state. Because few REM periods could be reliably recorded on any one day, the number of sleep states required for a full dose response determination could not be achieved over the duration of the studies. The dose level was comparable with the most effective dose found in a previous anesthetized preparation (3).

Regionalization. The regionalization of activity demonstrates that the overall signal changes observed during the challenges did not result from a uniform level of activity over the area under the probe. Determination of the regional activation associated with the cyanide challenges has the potential to indicate the local sites involved in mediating responses to hypoxia. Specific topographic organization may be used to define the processes by which breathing and cardiovascular patterns change during chemoreceptor challenge.

In summary, the primary rostral and intermediate area VMS response to a NaCN challenge during waking, quiet sleep, and REM sleep states was a decline in neural activity; the decline in activity was faster in REM sleep, and the nadir was delayed in waking, but the magnitude of change did not significantly differ between states. Unique breathing and cardiovascular patterns were found between states, including heart rate declines specific to quiet sleep and initial increases, rather than declines, in breathing rate during REM sleep. Carotid body input to the VMS appears to be inhibitory or disfacilitatory across all sleep-waking states, and this input interacts with spontaneous VMS activity levels to cause state-dependent activity timing changes.

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