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Responses of neurons in the rostral ventrolateral medulla to whole body rotations: comparisons in decerebrate and conscious cats

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DeStefino VJ, Reighard DA, Sugiyama Y, Suzuki T, Cotter LA, Larson MG, Gandhi NJ, Barman SM, Yates BJ. Responses of neurons in the rostral ventrolateral medulla to whole body rotations: comparisons in decerebrate and conscious cats. *J Appl Physiol* 110: 1699–1707, 2011. First published April 14, 2011; doi:10.1152/japplphysiol.00180.2011.—The responses to vestibular stimulation of brain stem neurons that regulate sympathetic outflow and blood flow have been studied extensively in decerebrate preparations, but not in conscious animals. In the present study, we compared the responses of neurons in the rostral ventrolateral medulla (RVLM), a principal region of the brain stem involved in the regulation of blood pressure, to whole body rotations of conscious and decerebrate cats. In both preparations, RVLM neurons exhibited similar levels of spontaneous activity (median of ∼17 spikes/s). The firing of about half of the RVLM neurons recorded in decerebrate cats was modulated by rotations; these cells were activated by vertical tilts in a variety of directions, with response characteristics suggesting that their labyrinthine inputs originated in otolith organs. The activity of over one-third of RVLM neurons in decerebrate animals was altered by stimulation of baroreceptors; RVLM units with and without baroreceptor signals had similar responses to rotations. In contrast, only 6% of RVLM neurons studied in conscious cats exhibited cardiac-related activity, and the firing of just 1% of the cells was modulated by rotations. These data suggest that the brain stem circuitry mediating vestibulosympathetic reflexes is highly sensitive to changes in body position in space but that the responses to vestibular stimuli of neurons in the pathway are suppressed by higher brain centers in conscious animals. The findings also raise the possibility that autonomic responses to a variety of inputs, including those from the inner ear, could be gated according to behavioral context and attenuated when they are not necessary.

vestibular system; otolith organ; orthostatic hypotension; sympathetic nervous system

SUBSTANTIAL EVIDENCE SHOWS that the vestibular system contributes to adjustment of sympathetic nervous system activity and blood distribution in the body during movement and changes in posture. In decerebrate or anesthetized cats, stimulation of vestibular afferents by current pulses (23, 41) or moderate-amplitude (10–15°) head tilts (48) produces robust changes in sympathetic nerve activity. These responses are eliminated by placement of lesions in the caudal one-third of the vestibular nucleus complex (23, 41, 48), demonstrating that they were elicited by stimulation of receptors in the inner ear, and not by baroreceptors or other nonlabyrinthine receptors. In conscious cats, surgical elimination of vestibular inputs results in instability in blood pressure during 60° head-up rotations (20) due to a loss of lower body vasoconstriction that ordinarily occurs during such movements (43). Stimulation of the vestibular system through a number of methods has also been shown to alter sympathetic efferent activity and/or blood pressure in rodents (16, 52), rabbits (32), and human subjects (4, 6–8, 17, 19, 22, 37, 42).

The brain stem pathways that produce vestibulosympathetic responses have been established, at least in part. Chemical inactivation of the rostral ventrolateral medulla (RVLM) abolishes the responses of sympathetic nerves to electrical stimulation of vestibular afferents (49); the same region also mediates the changes in sympathetic outflow produced by stimulation of baroreceptors, as well as a number of other afferents (9, 10). In decerebrate cats, the firing of a large fraction of RVLM neurons, including those with projections to the thoracic spinal cord, is altered by activation of vestibular nerve fibers through the use of electrical current pulses (39, 50) or moderate-amplitude rotations of the body in vertical planes (46). These responses are elicited through direct projections from the caudal aspect of the vestibular nuclei to the RVLM (18), as well as indirect relays that include interneurons in the lateral (45) and ventrolateral (38) regions of the caudal medullary reticular formation.

Although the response properties of brain stem neurons mediating vestibulosympathetic responses have been investigated in decerebrate and anesthetized preparations, the effects of vestibular signals on the activity of these cells have not been explored in conscious animals, whose brain stem activity is unaltered by anesthetic agents or elimination of descending influences from higher centers. The responses of neurons mediating other vestibular reflexes differ profoundly between conscious and anesthetized or decerebrate animals, raising the possibility that vestibulosympathetic reflex properties also vary between these preparations. For example, in conscious animals, vestibuloocular reflexes and the excitability of some of the vestibular nucleus neurons that produce these responses are suppressed during a portion of saccadic eye movements (15, 36). In addition, although the firing rate of some other vestibular nucleus neurons is modulated during unexpected changes in body position, these responses are attenuated during voluntary head rotations (28, 33). Such conditional suppression of responses elicited by vestibular stimulation cannot be detected following decerebration or administration of anesthetics. It is thus also possible that complexities of RVLM neuronal responses to whole body rotations could be masked in decerebrate preparations.

The objective of this study was to characterize the firing patterns of RVLM neurons in conscious cats, as well as the modulation of the activity of these cells during whole body

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rotations. A parallel set of experiments was executed using decerebrate cats to allow the responses observed in the two preparations to be directly compared. Because, to our knowledge, this study includes the first recordings from RVLM neurons in conscious animals of any species, it was difficult to predict the outcome. As such, our null hypothesis was that firing patterns of units in the RVLM and their responses to whole body rotations would be similar in decerebrate and conscious cats.

METHODS

All experimental procedures conformed to the American Physiological Society's "Guiding Principles for the Care and Use of Animals," as well as the National Research Council *Guide for the Care and Use of Laboratory Animals*, and were approved by the University of Pittsburgh's Institutional Animal Care and Use Committee. Data were collected from 12 purpose-bred adult cats (Liberty Research, Waverly, NY). Recordings of RVLM neuronal activity were obtained in eight of these animals after a decerebration was performed and anesthesia was removed. The other four cats were instrumented for single-unit recordings when the animals were conscious and unanesthetized.

Surgery and Recording Procedures

Decerebrate animals. We employed procedures similar to those used in a recent study (39) to conduct recordings from RVLM neurons in eight decerebrate cats. Animals were anesthetized with isoflurane (5% for induction, 1.5–2.5% for maintenance) vaporized in O_2, and a transducer (Millar Instruments, Houston, TX) was inserted through the femoral artery into the abdominal aorta to record blood pressure. The level of anesthesia was titrated to maintain mean blood pressure at <100 mmHg and to prevent spontaneous and reflexive movements. The trachea was intubated, and both femoral veins were cannulated for drug administration. Rectal temperature was maintained at 37–38°C using a direct current-powered heating lamp and pad. The animals were placed in a stereotaxic frame and supported using hip pins and a clamp placed on the dorsal process of an upper thoracic vertebra. The animals were decerebrated at the midcollicular level after bilateral occlusion of the common carotid arteries. The carotid arteries were dissected free of surrounding tissues, and a ligature was placed around each artery to permit stretch of the carotid sinus. A craniotomy was performed, and the caudalmost aspect of the cerebellum was aspirated to expose the dorsal surface of the medulla.

Anesthesia was removed after all surgical procedures were completed. The animal was then paralyzed using gallamine triethiodide (Sigma, St. Louis, MO; initial dose of 10 mg/kg iv, maintained by hourly injections of 5 mg/kg) and artificially ventilated with room air (20–25 cycles/min). End-tidal CO_2 was maintained at ~4%. If hypotension occurred, mean blood pressure was increased to >90 mmHg by an infusion of phenylephrine in saline (0.005–0.01 mg·kg^{-1}·min^{-1} iv). Recordings from RVLM neurons were performed in decerebrate animals in a fashion similar to that described for conscious cats (see *Conscious animals*), except the landmarks on the exposed surface of the brain stem were used to localize the target area. Neuronal recordings did not commence until after 1 h following the removal of anesthesia; since the recording sessions lasted 8–12 h or longer, most recordings were conducted hours after anesthetic removal, such that anesthesia should have had little effect on neuronal activity.

Conscious animals. Four female cats were instrumented for subsequent recordings from RVLM neurons when animals were conscious and awake by use of procedures we have employed in previous studies (29, 30). Prior to being included in the study, animals were spayed by a veterinarian to prevent cyclic changes in hormonal levels. Animals were handled extensively by laboratory members prior to undergoing surgery and were shown to be compliant for a restraint period of 90 min without vocalizing, attempting to move, or displaying indicators of distress.

A recovery surgery was performed using aseptic procedures in a dedicated operating suite to mount a fixation plate on the skull, perform a craniotomy and attach a recording chamber around the opening in the skull, and implant perivascular probes adjacent to both common carotid arteries. Animals were initially anesthetized with an intramuscular injection of ketamine (20 mg/kg) and acepromazine (0.2 mg/kg). Subsequently, an endotracheal tube and intravenous catheter were inserted. Anesthesia was maintained using 1–2% isoflurane vaporized in O_2, so that limb withdrawal reflexes were absent and heart rate was stable. A saline solution was infused intravenously to replace fluid loss during the surgery. A heating pad and heat lamp were used to maintain core temperature at ~38°C. After surgery, animals received antibiotics (amoxicillin, two 50-mg oral doses per day) for 10 days. For 72 h after the surgery, analgesia was provided through transdermal delivery of fentanyl (25 μg/h; Janssen Pharmaceutical Products, Titusville, NJ).

Perivascular probes (PS series, Transonic Systems, Ithaca, NY) were placed around the common carotid arteries and secured in place using sutures. The animal's head was secured in a stereotaxic frame, and Palacos bone cement (Zimmer, Warsaw, IN) was used to attach a fixation plate to the skull; the plate was secured to screws tapped into the bone. Bone cement was used to attach the connectors from the perivascular probes to the skull, behind the fixation plate. A 1-cm-diameter craniotomy at the midline of the posterior aspect of the skull provided for recordings from RVLM neurons in the caudal medulla. A recording chamber (David Kopf Instruments, Tujunga, CA) was positioned in accordance with stereotaxic coordinates using a micro-drive and attached to the skull adjacent to the craniotomy using bone cement. Animals recovered for 1 mo after the surgery before data collection was initiated. During this time, animals were acclimated for restraint in a cylindrical tube that provided support for the body and ensured that the animal's body position did not change during tilting; the head was immobilized by insertion of a screw into the fixation plate mounted on the skull. Data collection did not commence until animals could be restrained during a recording session of 90 min without vocalizing, attempting to move, or displaying indicators of distress.

All data recordings were conducted in a dimly lit room; the animal was positioned so that its visual field rotated with the body, such that no visual cues were provided regarding body position in space. During recording sessions, an *x-y* positioner was attached to the recording chamber and used to maneuver a ~5-MΩ epoxy-insulated tungsten microelectrode (Frederick Haer, Bowdoin, ME), which was inserted through a 25-gauge guide tube into the cerebellum and lowered into the medulla using a hydraulic microdrive (model 650, David Kopf). Extension cables were used to link the head-mounted perivascular probe connectors to perivascular flowmeter modules (model TS420, Transonic Systems).

During each recording session, a single electrode penetration was made at different locations in the medulla; recording sessions were conducted 4–5 days/wk over a period of 4–6 wk. The most useful landmark in localizing the RVLM was the presence of neurons with respiratory-related activity, since the ventral respiratory group in the cat is positioned just dorsal to the region (26). It was also apparent when the microelectrode tip exited the ventral surface of the brain stem, as there was an abrupt shift in baseline voltage and all neural activity ceased. We focused our recordings in the area ventral to respiratory neurons and within ~1.5 mm of the ventral surface of the brain stem.

Electrophysiological Procedures

The apparatus to restrain conscious animals and the stereotaxic frame that supported decerebrate animals were mounted on the same

hydraulically controlled tilt table. Experiments on decerebrate and conscious animals were executed over the same time period using the same equipment, albeit on different days, so we were assured that data from the two preparations could be accurately compared. Activity recorded from RVLM neurons was amplified by a factor of 10,000 and filtered with a band pass of 300–10,000 Hz. The output of the amplifier was sampled at 25,000 Hz using a Micro1401 mk 2 data collection system and Spike2 version 6 software (Cambridge Electronic Design, Cambridge, UK). When the responses of a neuron to body rotations were determined, the output of the amplifier was fed into a window discriminator for the delineation of spikes from single units. The discriminator output was sampled at 10,000 Hz as described above. For trials where it was difficult to isolate a single unit using the window discriminator, the spike detection and sorting feature of the Spike2 software was subsequently employed to delineate the occurrence of neuronal firing. We additionally sampled arterial blood pressure (in decerebrate animals) and carotid blood flow (in conscious animals) at 100 Hz. When vestibular stimuli were delivered, table movement was recorded using potentiometers and collected at 100 Hz.

Upon encountering a unit, we recorded its spontaneous activity over a period of 1–3 min, along with blood pressure or carotid blood flow, so we could subsequently determine whether the cell had cardiac-related firing (Fig. 1). The carotid arteries were ligated in decerebrate animals to reduce bleeding at the decerebration site, which also eliminated stimulation of carotid sinus baroreceptors during the cardiac cycle. As an additional test to ascertain whether RVLM neurons received inputs from the carotid sinus in decerebrate cats, we observed the effects of mechanical stretch of the carotid artery. Carotid stretch routinely elicited a decrease in arterial blood pressure (Fig. 1A), indicating that the stimulus was effective.

We next recorded neuronal responses while rotating the entire animal about the pitch (transverse) and roll (longitudinal) axes using a servo-controlled hydraulic tilt table (NeuroKinetics, Pittsburgh, PA). Our procedures for performing vertical vestibular simulation are described in detail elsewhere (21, 31, 47). We first utilized the "wobble" stimulus, a fixed-amplitude tilt, the direction of which moves around the animal at constant speed (35), to determine whether a unit responded to vestibular stimulation. The wobble stimulus was employed for this determination, because it activates vertical semicircular canals and otolith organs (35). We typically first delivered 0.2-Hz wobble stimuli at 5°. If these rotations were ineffective, as determined by an online calculation of the signal-to-noise ratio for responses (see below for definition), we increased the stimulus amplitude to 7.5°. If a neuron did not respond to 0.2-Hz rotations at 7.5° amplitude, 0.1-Hz wobble stimulation at 10° amplitude was provided, although some units were lost before this stimulus could be delivered. We attempted to test the effects of 15° rotations on a few units, but we found that it was difficult to hold stable recordings during the large movements.

For units that responded to rotations, we determined the plane of tilt that produced maximal modulation of the unit's firing rate (response vector orientation). The direction of the response vector orientation lies midway between the maximal response directions to clockwise and counterclockwise wobble stimulation, because the phase differences between stimulus and response are reversed during the two directions of rotation (35). Thus, by considering both responses, we could account for these phase differences. Subsequently, the response vector orientation was confirmed by comparing the gain of responses to rotations in a variety of fixed vertical planes delivered at the same amplitude and frequency as the wobble stimulus. These rotations always included tilts in the roll and pitch planes.

After a unit's response vector orientation was established, planar tilts at or near this orientation were used to study the dynamics of the vestibular response (response gain and phase across stimulus frequencies). Response dynamics were routinely determined over the frequency range of 0.05–0.5 Hz; for some cells, rotations at 0.02 Hz
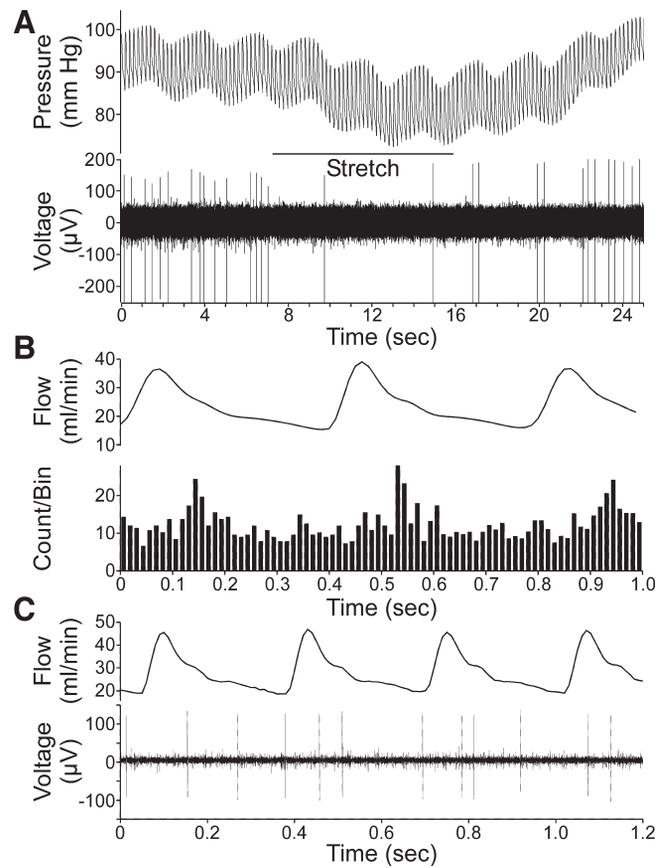

Fig. 1. A: responses of an rostral ventrolateral medulla (RVLM) neuron to stretch of a carotid artery in a decerebrate cat. *Top trace*, blood pressure (sampled at 100 Hz); *bottom trace*, unit activity (sampled at 25,000 Hz). Horizontal line, period during which the artery was stretched. Carotid stretch resulted in a decrease in blood pressure that was preceded by a decline in unit firing. B and C: firing pattern of a RVLM unit with cardiac-related activity recorded in a conscious cat. B and C show results from the same cell. B: averaged changes in carotid blood flow (*top*) and associated changes in RVLM unit firing (*bottom*); 169 sweeps were pooled to generate traces. Averaging was triggered by maximal carotid blood flow at the central peak. The neuron had its highest probability of firing after each blood flow peak, as flow through the carotid artery was declining. C: raw data for a 1.2-s segment of the run used to generate averages in B. Blood flow was sampled at 100 Hz, and unit activity was sampled at 25,000 Hz. Cardiac-related activity of the unit was not obvious until averaging was performed.

and/or 1 Hz were also delivered. The amplitude of these stimuli was 7.5–10° at frequencies ≤0.1 Hz and 5–7.5° at frequencies >0.1 Hz.

Data Analysis Procedures

Spontaneous firing rate was determined for each unit from the initial recording performed before rotational stimuli were delivered. We also triggered averages of neural activity from peak carotid blood flow or blood pressure, so we could ascertain whether the neuron exhibited cardiac-related activity (Fig. 1B). Neural activity recorded during rotations was binned (500 bins/cycle) and averaged over the sinusoidal stimulus period. Sine waves were fitted to responses with the use of a least-squares minimization technique (35); an initial analysis was performed using the Spike2 software while data collection was occurring, and a post hoc final analysis was subsequently executed using MATLAB (MathWorks, Natick, MA). The response sinusoid was characterized by two parameters: phase shift from the stimulus sinusoid (subsequently referred to as phase) and amplitude relative to the stimulus sinusoid (subsequently referred to as gain). We used one primary criterion and two secondary criteria to
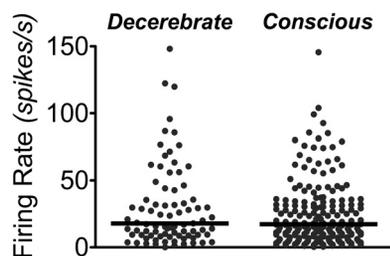
Fig. 2. Spontaneous firing rates of neurons tested for responses to whole body rotations in decerebrate and conscious animals. Each neuron is represented by ●. Horizontal lines, median firing rates in each preparation.

determine if neuronal activity was modulated by rotations, as in many previous studies (21, 29–31, 47). First, responses were considered significant only if the signal-to-noise ratio (calculated as discussed in Ref. 35) was ≥0.5. Data meeting this criterion were considered to represent real modulation of neuronal activity if only the first harmonic was prominent and the responses were consistent from trial to trial. In a small number of cases, rotations were delivered at a frequency similar to that for spontaneous rhythmic activity, such that the averaged activity met the signal-to-noise criterion at that frequency, but not other rotation frequencies. In these instances, the response phases varied tremendously when trials were repeated, allowing spurious trials to be readily detected. Statistical analyses were performed using Prism 5 software (GraphPad Software, San Diego, CA). Pooled data are presented as means ± SE.

Histological Procedures

After recordings were completed in chronic animals, electrolytic lesions were made at defined coordinates by passage of a 100-µA negative current for 60 s through a 0.5-MΩ tungsten electrode. Animals survived for ~1 wk after the procedure (to allow gliosis to occur at the lesion site, which aided in the subsequent identification of the area); then they were deeply anesthetized using an intramuscular injection of 20 mg/kg ketamine and 0.2 mg/kg acepromazine, followed by an intraperitoneal injection of 40 mg/kg pentobarbital sodium, and then perfused transcardially with 10% formalin. The same microelectrode employed for recordings was used for similar placement of a lesion at one or two recording sites in each decerebrate animal. Decerebrate cats were euthanized at the end of the recording session using a 120 mg/kg intravenous injection of pentobarbital sodium, and the brain stem was removed and immersed in formalin for fixation. A freezing microtome was used to cut the brain stem transversely at 50- or 100-µm thickness, and tissue sections were stained using thionine. Photographs of brain stem sections were captured using a digital stereomicroscope, and Motic (Xiamen, China) Images Advanced software and Adobe Illustrator software (Adobe systems, San Jose, CA) were used to generate drawings of the sections. Recording sites were reconstructed on these drawings with reference to the locations of electrolytic lesions, the relative positions of electrode tracks, and microelectrode depths.

RESULTS

Neuronal activity during whole body rotations was recorded from 85 RVLM units in decerebrate cats and 202 cells in conscious cats. Spontaneous firing rates for neurons were determined upon first encountering each cell, before rotations were delivered, and are plotted in Fig. 2. Although the spontaneous firing rates were highly variable in decerebrate and conscious animals, the respective mean (30.9 ± 3.7 vs. 27.7 ± 2.3 spikes/s) and median (17.3 vs. 17.0 spikes/s) values were similar in the two preparations and were not demonstrated to be

significantly different ($P = 0.54$, by 2-tailed Mann-Whitney test).

Responses of RVLM Neurons to Whole Body Rotations of Decerebrate Cats

Eighty-five neurons were tested for responses to whole body rotations in decerebrate cats. The activity of 30 of these units was altered by stretch of the carotid artery; 12 of the cells were inhibited by the stretch (firing rate decreased 56 ± 5%), as illustrated in Fig. 1A, while 18 were excited by the manipulation (firing rate increased 217 ± 5%). The activity of about half (43 of 85) of the neurons was consistently modulated by whole body rotation. Among the 30 cells that responded to carotid stretch, the firing of 19 was modulated by whole body rotations (6 of 12 cells inhibited by carotid stretch and 13 of 18 cells excited by carotid stretch). The activity of most of the neurons that responded to whole body rotations could be modulated by small-amplitude stimuli: 5° for 18 cells, 7.5° for 17 cells, and 10° for 8 cells.

Response vector orientations were established for 36 of the 43 neurons that responded to whole body rotations (Fig. 3). Data for neurons whose activity was affected by stretch of the carotid artery are depicted on the outer ring of the polar plot; the distribution of values was similar for units that were inhibited and excited by carotid stretch. Units were classified as having response vector orientations near (within 45° of) ipsilateral ear-down roll, contralateral ear-down roll, nose-up pitch, or nose-down pitch. Just over half (20 of 36) of the units could be better activated by roll than by pitch rotations; among these cells, half were excited by ipsilateral ear-down roll, while the other half of the units were excited by contralateral ear-down roll. Sixteen of the 36 neurons were better activated by pitch than by roll rotations; most (13 of 16) of these units were excited by nose-up rotations, whereas only 3 of 16 were excited by nose-down rotations.

We additionally determined responses to rotations in a plane near the response vector orientation (usually roll or pitch) at frequencies ranging from 0.02 to 1 Hz for units that could be
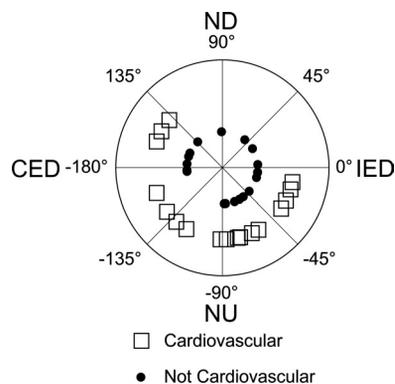

Fig. 3. Polar plot showing response vector orientations determined for RVLM neurons in decerebrate cats. *Outer ring*, data for neurons whose activity was altered by carotid artery stretch ("cardiovascular" neurons). *Inner ring*, data for neurons that were either not tested for responses to carotid stretch or failed to respond to the stimulus ("not cardiovascular" neurons). Response vector orientations were determined using wobble stimuli, usually at 0.2 Hz (26 of 35 neurons), but at 0.1 Hz for 9 cells and 0.05 Hz for 1 unit. CED, contralateral ear-down roll; IED, ipsilateral ear-down roll; ND, nose-down pitch; NU, nose-up pitch.

held for a sufficient period of time. Figure 4 illustrates the responses of a neuron with a response vector orientation of $-27.5°$ to roll rotations at 0.05–0.5 Hz (Fig. 4, A–D) and to pitch rotations at 0.2 Hz (Fig. 4E). Dynamic properties of responses to rotations were characterized for 23 neurons at three or more frequencies over a stimulus decade and are shown in Fig. 5. The response gains for most units increased moderately as frequency increased to ~0.2 Hz but then remained the same or decreased as the stimulus frequency was increased to ≥0.5 Hz. Response gains increased >5-fold per stimulus decade for only 4 of 23 neurons and increased >10-fold per stimulus decade for just 2 of 23 units. The responses were synchronized with body location in space (i.e., the response phase deviated <45° from tilt table position) at low stimulus frequencies for all but one cell, where the response lagged stimulus position considerably. As stimulus frequency was increased, the responses of five cells lagged stimulus position >45°, although the responses of most neurons remained in phase with stimulus position across the range of frequencies tested.

The locations of the RVLM neurons tested for responses to whole body rotations are indicated in Fig. 6A. Most of the units were located within 1 mm of the ventrolateral surface of the medulla and spanned from the rostral tip of the inferior olivary nucleus to ~2 mm caudal to this level. Neurons that responded to carotid stretch and those that did not respond and neurons that did respond to moderate-amplitude rotations and those that did not respond were intermixed in the RVLM.

Responses of RVLM Neurons to Whole Body Rotations of Conscious Cats

We additionally tested the effects of whole body rotation on the firing rate of 202 neurons in 4 conscious cats instrumented for chronic recordings. Only 12 of 202 units exhibited any indication of cardiac-related activity; the locations of these cells, as well as those whose firing was unrelated to the cardiac cycle, are shown in Fig. 6B. Among the 202 units tested for responses to whole body rotations, the activity of only 2 was modulated: 1 cell responded to ipsilateral ear-down rotations, and 1 cell had a response vector orientation near contralateral ear-down roll. In most cases, large wobble rotations were employed before a neuron response to rotations was discounted: 10° for 111 cells and 7.5° for 48 neurons. However, 41 cells were lost before large-amplitude rotations could be delivered and were tested using only 5° stimuli.

To further examine whether whole body rotations differentially affected the firing rates of RVLM neurons in conscious and decerebrate animals, we determined the mean signal-to-noise ratios for the largest-amplitude wobble rotations performed for cells that failed to respond to the stimuli, as well as the smallest-amplitude wobble rotations that were effective for the responsive units. We then compared the representative signal-to-noise ratio established for each cell in decerebrate and conscious cats. Since the firing of many RVLM neurons was modulated by whole body rotations in decerebrate cats, but not conscious animals, the median amplitude of the stimuli delivered during the representative runs was lower in the former animals (7.5°) than in the latter (10°). Nonetheless, the
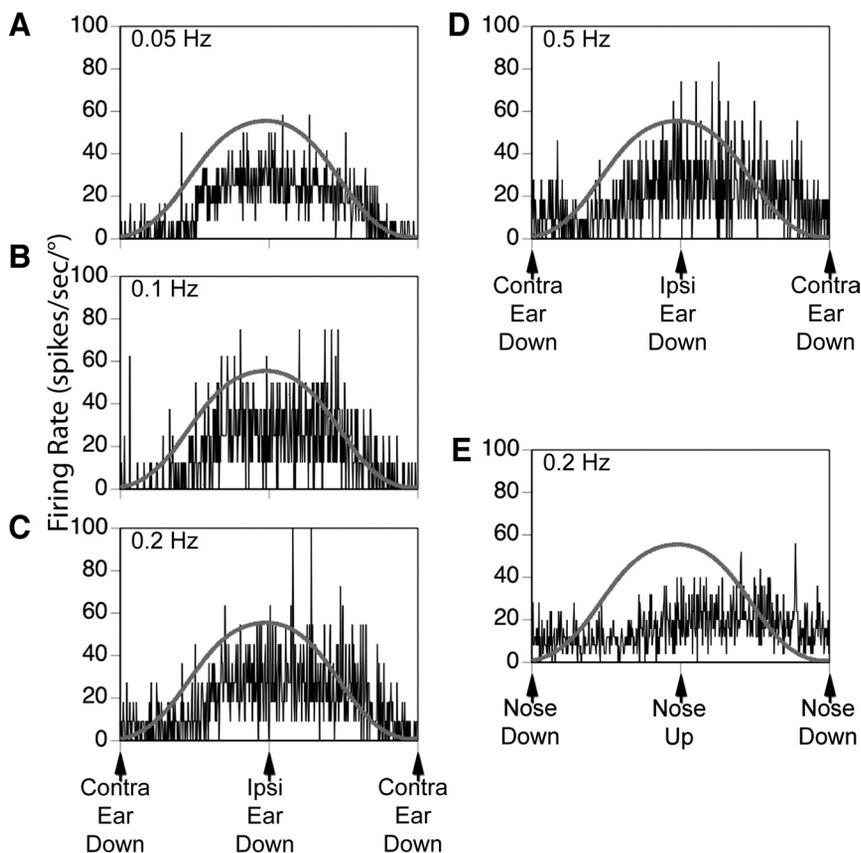

Fig. 4. Averaged responses of a RVLM neuron to sinusoidal rotations of a decerebrate cat. A–D: responses to ear-down (roll) rotations at different frequencies. E: response to pitch (sagittal plane) rotations at 0.2 Hz. Rotation amplitudes were 10° at 0.05–0.1 Hz, 7.5° at 0.2 Hz, and 5° at 0.5 Hz. Numbers of sweeps averaged for each trace are as follows: 3 in A, 4 in B, 10 in C, 27 in D, and 25 in E. Each histogram contains 500 bins (bin width varies from 40 ms at 0.05 Hz to 4 ms at 0.5 Hz). A sine wave superimposed on each trace shows table movement. Response to roll rotations was in phase with table position at low stimulus frequencies but lagged table position slightly at higher frequencies. Response to 0.2-Hz pitch (E) is much smaller than response to 0.2-Hz roll (C), in accordance with response vector orientation calculated using the wobble stimulus (−28°). Contra, contralateral; Ipsi, ipsilateral.
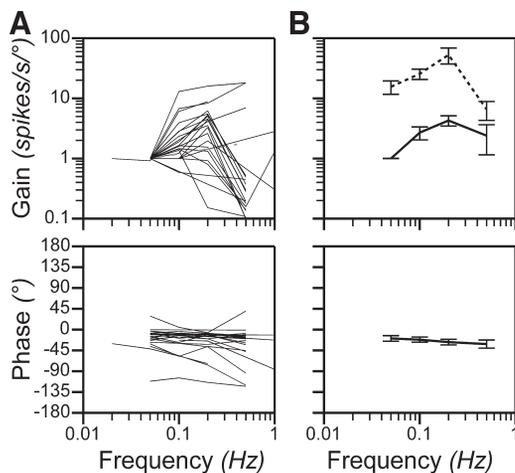
Fig. 5. Bode plots illustrating responses of RVLM neurons to fixed-plane rotations at multiple frequencies; response gain and phase are plotted in relation to stimulus position. *A*: individual Bode plots for each cell where responses to rotations were measured at ≥3 frequencies over a stimulus decade (e.g., 0.05–0.5 Hz). To facilitate comparisons, gain at the lowest frequency tested for a neuron (usually 0.05 Hz, but 0.02 Hz for 1 cell and 0.1 Hz for 3 units) was standardized at 1 spike·s⁻¹·degree⁻¹. *B*: average data for all neurons. Solid lines, average of standardized data in *A*; dashed lines, unstandardized raw data. Only 1 average is shown for phase, as no standardization occurred.

average representative signal-to noise ratios were much higher in decerebrate animals (0.67 ± 0.04 vs. 0.20 ± 0.01 in conscious cats). The median representative signal-to-noise ratios also diverged considerably in the two preparations (0.74 in decerebrate cats and 0.17 in conscious animals) and were confirmed to be significantly different ($P < 0.0001$, by 2-tailed Mann-Whitney test). When the neurons in conscious animals that were tested using small (5°) maximal stimuli were eliminated from the sample, the mean (0.20 ± 0.01) and median (0.16) signal-to-noise ratios were similar to those for the whole population and remained significantly lower than the signal-to-noise ratios for decerebrate cats ($P < 0.0001$, by 2-tailed Mann-Whitney test).

DISCUSSION

The major finding of this study is that the activity of about half of RVLM neurons is robustly modulated by moderate-amplitude (≤10°) whole body rotations of decerebrate cats, but not conscious animals. The disparity between the two preparations in responses of RVLM neurons to rotations was evident from the number of units meeting our significance criteria, as well as the mean signal-to-noise ratios for activity recorded during stimuli. These data suggest that the brain stem circuitry mediating vestibulosympathetic reflexes is highly sensitive to changes in body position in space but that the responses of neurons in the pathway to vestibular inputs are ordinarily suppressed by higher brain centers. This finding raises the possibility that the magnitude of autonomic responses to vestibular stimulation could be gated according to behavioral context and attenuated when they are not necessary or physiologically appropriate. For example, vestibulosympathetic responses may be masked when an animal is executing voluntary movements or experiencing routine passive movements that are expected to activate vestibular receptors but are unlikely to generate fluctuations in blood pressure. However, the gain of

the responses could increase when physiological challenges are more likely, such as when a cat is traversing a tree branch and is at risk of experiencing a sudden change in body position that affects fluid distribution in its body. Further experiments are needed to test this hypothesis.

A recent study in human subjects also raised the possibility that the gain of vestibulosympathetic responses is modified in accordance with physiological requirements. This study demonstrated that the magnitude of forearm vasoconstriction during vestibular stimulation in women varies between phases of the menstrual cycle (24). Furthermore, the differences between conscious and decerebrate cats in the sensitivity of RVLM neurons to labyrinthine stimulation fit well with previous data. In decerebrate cats, activation of vestibular receptors by 10–15° head-up rotations produced strong modulation of sympathetic nervous system activity (48). However, in conscious
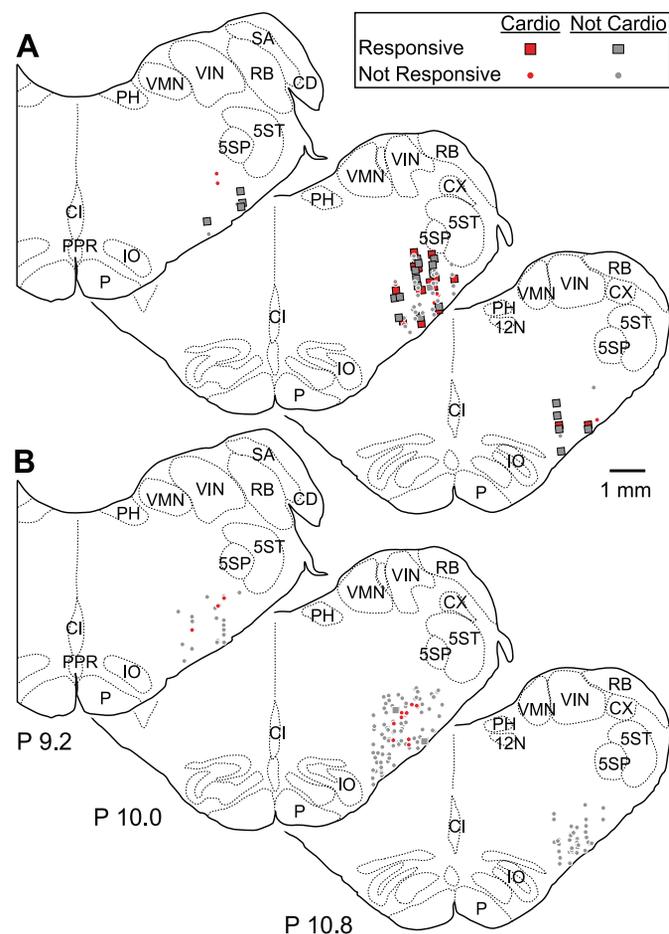

Fig. 6. Locations of RVLM neurons whose responses to whole body rotations were examined in decerebrate (*A*) and conscious (*B*) animals. Red symbols designate neurons that were activated by cardiovascular (cardio) stimuli; these neurons exhibited cardiac-related firing in conscious animals, or their firing rate was altered by stretch of the carotid artery in decerebrate cats. Large squares, neurons whose firing was modulated by whole body rotations; smaller circles, cells that failed to respond to vestibular stimulation. Numbers below the bottom set of sections indicate the level posterior (P) to stereotaxic zero, in accordance with Berman's atlas (5). 5SP, spinal trigeminal nucleus; 5ST, spinal trigeminal tract; 12N, hypoglossal nerve; CD, dorsal cochlear nucleus; CI, inferior central nucleus; CX, external cuneate nucleus; IO, inferior olive; P, pyramid; PH, nucleus prepositus hypoglossi; PPR, postpyramidal nucleus of the raphe; RB, restiform body; SA, stria acustica; VIN, inferior vestibular nucleus; VMN, medial vestibular nucleus.

cats, 20° head-up static tilts had little effect on blood distribution in the body (44, 51) and elicited no appreciable vasoconstriction (44). In awake human subjects, large-amplitude head tilts were also required to elicit changes in sympathetic nervous system activity (19). Large-amplitude body movements would presumably modulate the firing of an appreciable fraction of RVLM units in conscious animals, although we were unable to test this premise during the current study: it was difficult to maintain stable recordings from these cells when rotations >10° were delivered.

Vestibulosympathetic responses are unlike other vestibular-elicited reflexes, in that they are ordinarily not required during small-amplitude movements. For example, a <5° deviation in head position would degrade visual acuity unless the vestibuloocular reflex produced a compensatory eye movement (12, 40). In contrast, a 5° movement does not affect blood distribution in the body and blood pressure and, thus, does not require adjustments in sympathetic nervous system activity (44, 51). Many neurons located at the caudal aspect of the vestibular nuclei, the region that mediates vestibuloautonomic responses (18, 23, 41, 48), are powerfully responsive to head movements that are only a few degrees in magnitude in decerebrate (13, 21) and conscious (29) cats. Furthermore, the characteristics of the responses of caudal vestibular nucleus neurons to whole body rotations were similar in the two preparations (13, 21, 29). As such, the sensitivity of caudal vestibular nucleus neurons to head movements is far greater than warranted to adjust blood distribution in the body during postural alterations. These findings suggest that attenuation of the responsiveness to labyrinthine stimuli of neurons in the vestibulosympathetic reflex pathway occurs in conscious cats, but at a site other than the vestibular nuclei, perhaps at the level of the RVLM or in regions such as the caudal ventrolateral medulla (38) that participate in conveying vestibular signals to the RVLM.

Although the principal difference between the preparations used in this study was decerebration, other minor differences must be considered as potentially contributing to the disparity in findings observed in decerebrate and conscious cats. First, the carotid arteries were ligated in the decerebrate animals, to prevent bleeding at the transection through the brain stem, which unloaded carotid sinus baroreceptors. Since the activity of some feline RVLM neurons is inhibited by baroreceptor inputs (2, 11, 27), a consideration is whether removal of baroreceptor inputs caused these cells to become hyperexcitable and more responsive to vestibular stimulation. This prospect seems unlikely to account for the differences between conscious and decerebrate animals, since the spontaneous firing rates of RVLM neurons were virtually identical in the two preparations. For the same reason, it is not probable that paralysis of decerebrate animals resulted in increased responsiveness of RVLM neurons to labyrinthine inputs. The descending inhibition from higher centers of the brain to neurons in the brain stem circuitry mediating vestibulosympathetic responses is the most likely explanation for the response of RVLM neurons to vestibular stimulation in decerebrate cats, but not conscious animals.

The characteristics of RVLM neuronal responses to whole body rotations of decerebrate cats were similar to those reported in a previous study (46), which additionally demonstrated that the effects of rotations were due to inputs from the

inner ear, and not receptors located elsewhere. For the vast majority of neurons, the response gain did not increase appreciably when high-frequency rotations were delivered, and the response phase remained near stimulus position at all frequencies of rotation or lagged stimulus position slightly. These response properties indicate that RVLM neurons principally received graviceptive inputs from the otolith organs, as opposed to signals from semicircular canals (1, 14, 34). The prior study of RVLM neuronal responses to rotations (46) did not discern whether the units examined received baroreceptor inputs. We found that neurons whose activity was modified by carotid sinus stretch, and presumably received baroreceptor signals, had responses to rotations similar to the general population of RVLM cells. Some carotid stretch-responsive neurons were excited by this stimulus and others were inhibited, whereas previous studies mainly focused on RVLM bulbospinal neurons that were inhibited by baroreceptor inputs (2, 11, 27). In addition to these cells, the RVLM of cats has been reported to contain interneurons whose firing increases with baroreceptor stimulation (3). It thus seems likely that our sample included baroreceptor-sensitive interneurons and bulbospinal neurons. The direction of tilt that increased the firing of RVLM neurons, including those that responded to carotid stretch, was highly variable between cells, with some units being activated by ear-down rotations and others responding to head-up rotations. In contrast, sympathetic nerve activity selectively increases during head-up rotations (48). These findings show that the responses of sympathetic preganglionic neurons to changes in body position in space are not a simple reflection of the activity of RVLM neurons. Instead, spinal cord neurons regulating sympathetic outflow likely receive convergent inputs from multiple cells in the RVLM and, perhaps, other brain stem regions that contribute to controlling vasomotor activity (25); integration of these signals generates sympathetic nerve responses to whole body rotations with properties that deviate from those of individual bulbospinal neurons.

A caveat is that we did not ascertain whether the RVLM neurons studied provided inputs to sympathetic preganglionic neurons. Antidromic stimulation in the thoracic spinal cord would have been extremely difficult in conscious animals and likely would have resulted in distress of the animals. Since the experiments in decerebrate animals were designed to parallel those in conscious cats, we did not deliver antidromic stimulation in either preparation. Nonetheless, we previously established that a majority of bulbospinal RVLM neurons respond to labyrinthine inputs in decerebrate cats (39). In addition, the region of the RVLM from which we recorded has been shown to contain a large number of presympathetic neurons (9–11, 27). On the basis of the locations of the recorded neurons and the fact that the population contained neurons whose activity was synchronized to the phases of the cardiac cycle (a hallmark of a sympathetic premotor neuron), we are certain that we sampled in the same area identified by Dampney and McAllen as regulating sympathetic outflow (9–11, 27).

Since this study included the first recordings from RVLM neurons in conscious animals of any species, some of the general characteristics of the firing patterns of the units are also relevant for understanding the control of blood pressure. First, the spontaneous firing rate of RVLM neurons in conscious animals was similar to that in decerebrate cats with carotid

sinus baroreceptors unloaded and was moderately high (median of ~17 spikes/s). In addition, only a small fraction (~6%) of these units exhibited cardiac-related activity, indicating that most RVLM neurons are not highly sensitive to baroreceptor inputs in conscious animals. Since responses of RVLM units to vestibular stimulation appear to be attenuated by higher centers in conscious cats, changes in neuronal activity elicited by baroreceptor and, perhaps, other inputs could also be masked. Further studies are needed to explore this possibility, as well as whether responsiveness of RVLM neurons to baroreceptor signals can be adjusted based on context. For example, it is feasible that when an animal is alerted or stressed, the magnitude of the blood pressure fluctuations required to modulate RVLM neuronal firing would decrease.

In summary, this study showed that the sensitivity of RVLM neurons to vestibular stimulation is lower in conscious than in decerebrate cats, presumably because the responses are actively suppressed. This attenuation of vestibulosympathetic responses is physiologically appropriate, since the vestibular system responds robustly to changes in body position in space that are too small to affect fluid distribution in the body and require an adjustment in peripheral blood flow. In effect, vestibular system influences on sympathetic nervous system activity appear to be exaggerated in decerebrate cats. These findings raise the possibility that responses of RVLM neurons to other signals are artificially accentuated in decerebrate and, perhaps, also anesthetized animals. As such, replacement of "reduced preparations" with awake and behaving animal models is prudent in future neurophysiological studies investigating the neural control of blood pressure.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

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