Rhythmic activity of neurons in the rostral ventrolateral medulla of conscious cats: effect of removal of vestibular inputs

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Barman SM, Sugiyama Y, Suzuki T, Cotter LA, DeStefino VJ, Reighard DA, Cass SP, Yates BJ. Rhythmic activity of neurons in the rostral ventrolateral medulla of conscious cats: effect of removal of vestibular inputs. Am J Physiol Regul Integr Comp Physiol 301: R937–R946, 2011. First published July 6, 2011; doi:10.1152/ajpregu.00265.2011.—Although it is well established that bulbospinal neurons located in the rostral ventrolateral medulla (RVLM) play a pivotal role in regulating sympathetic preganglionic neurons, virtually all neurophysiological studies of this region have been conducted in anesthetized or decerebrate animals. In the present study, we used time- and frequency-domain analyses to characterize the naturally occurring discharges of RVLM neurons in conscious cats. Specifically, we compared their activity to fluctuations in carotid artery blood flow to identify neurons with cardiac-related (CR) activity; we then considered whether neurons with CR activity also had a higher-frequency rhythmic firing pattern. In addition, we ascertained whether the surgical removal of vestibular inputs altered the rhythmic discharge properties of RVLM neurons. Less than 10% of RVLM neurons expressed CR activity, although the likelihood of observing a neuron with CR activity in the RVLM varied between recording sessions, even when tracking occurred in a very limited area and was higher after vestibular inputs were surgically removed. Either a 10-Hz or a 20- to 30-Hz rhythmic discharge pattern coexisted with the CR discharges in some of the RVLM neurons. Additionally, the firing rate of RVLM neurons, including those with CR activity, decreased after vestibular lesions. These findings raise the prospect that RVLM neurons may or may not express rhythmic firing patterns at a particular time due to a variety of influences, including descending projections from higher brain centers and sensory inputs, such as those from the vestibular system.

Rhythm; cardiac-related activity; vestibular system; sympathetic nervous system; baroreceptors; 10-Hz rhythm; cardiac-related activity

There is a consensus that bulbospinal neurons located in the rostral ventrolateral medulla (RVLM) play a pivotal role in regulating the sympathetic preganglionic neurons in the spinal cord that control peripheral vascular resistance and blood pressure (13, 15, 16). Neurophysiological studies conducted in decerebrate or anesthetized animals have characterized the firing patterns and responses to a variety of stimuli of RVLM neurons (e.g., 3, 5–7, 18, 23). A hallmark of sympathetic nerve activity (SNA) and of the activity in brain stem neurons that regulate cardiovascular function is the appearance of a cardiac-related (CR) rhythm, reflecting the influence of the baroreceptor reflex (14, 20, 23, 25, 27). In addition to the CR rhythm, several groups have noted the appearance of a 10-Hz rhythm in SNA of cats (4, 9, 10, 14, 24).

We recently provided the first examples of recordings of RVLM neuronal activity in conscious animals (18). In that study, we compared in decerebrate and awake cats the responses of RVLM neurons to whole-body rotations that activate vestibular receptors. We demonstrated that RVLM neuronal responses to vestibular inputs are ordinarily suppressed in conscious animals and hypothesized that autonomic responses to a variety of inputs, including those from the inner ear, are gated by higher brain centers, according to behavioral context and attenuated when they are not necessary (18).

These findings raise the prospect that CR activity of RVLM neurons, as well as higher-frequency rhythmic discharges of these cells, could differ in awake animals from responses identified in anesthetized and decerebrate animals (3, 5, 7). Both the CR and 10-Hz rhythms in SNA are mediated, at least partly, by the RVLM (4, 5, 7), and in anesthetized animals, bulbospinal RVLM neurons are capable of expressing both CR and 10-Hz activity (7). One goal of the present study was to ascertain whether rhythmic activity of RVLM neurons is similar in conscious cats to that observed in decerebrate and anesthetized preparations. Since evidence supports the view that the CR and 10-Hz rhythms in SNA originate in the brain stem (4, 8, 9, 22), we tested the hypothesis that RVLM neurons in conscious animals would exhibit both of these components in their discharges.

Experiments conducted in conscious animals demonstrated that lesions of the inner ear that eliminate vestibular inputs to the central nervous system have a variety of effects on the control of blood pressure. In particular, lower body vasodilation that ordinarily occurs during large (e.g., 60°) head-up rotations was attenuated following the removal of vestibular inputs (39, 42), resulting in lability of blood pressure (31). However, the deficits dissipated within a few days. One possibility is that compensation for the loss of labyrinthine inputs involves an increase in the gain of the baroreceptor reflex, and an accompanying change in the rhythmic discharge properties of RVLM neurons. Thus, we additionally hypothesized that disruption of inputs from the inner ear would increase the fraction of RVLM neurons that have CR and high-frequency rhythmic activity.

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’s 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 four conscious female purpose-bred adult cats (Liberty Research, Waverly, NY) that were instrumented using methods described in previous studies (18, 33, 34) for single-unit recordings. Animals were spayed by a veterinarian prior to being included in this study to prevent cyclic changes in hormonal levels that could have affected cardiovascular regulation. In addition, surgical procedures were only conducted on animals that were highly tractable and that were gradually acclimated over a 1- to 2-mo period for 90 min of restraint during recording sessions (18, 33, 34).

Surgeries

Two surgical procedures were performed aseptically in a dedicated operating suite, as described in detail in prior publications (18, 33, 34). During each surgery, animals were initially anesthetized using an intramuscular injection of ketamine (20 mg/kg) and acepromazine (0.2 mg/kg), an endotracheal tube was inserted, and anesthesia was maintained using 1–2% isoflurane vaporized in O₂. During the first surgery, a 1-cm craniotomy was performed in accordance with stereotaxic coordinates to permit subsequent recordings from the RVLM, and a recording chamber (David Kopf Instruments, Tujunga, CA) was secured to the skull surrounding the opening. Perivascular Probes (PS Series, Transonic Systems, Ithaca, NY) were placed on both carotid arteries, and the connectors were attached to the skull. In addition, a bolt was attached to the skull to permit head fixation during recording sessions. After this surgery, animals received antibiotics (amoxicillin, two 50-mg oral doses per day) for 10 days, and analgesia (fentanyl transdermal system, 25 µg/h; Janssen Pharmaceutical Products, Titusville, NJ) for 72 h.

The second surgery was performed in three of the animals after initial recordings from the RVLM were completed, 3–4 mo following the initial surgery. During this procedure, the tympanic bulla on each side of the skull was opened using a ventrolateral approach to expose the cochlea. A drill was used to remove temporal bone near the base of the cochlea, thereby producing a labyrinthectomy that rendered the vestibular apparatus dysfunctional. This procedure also provided access to the portion of the VIIIth cranial nerve within the internal auditory canal, which was transected under microscopic observation. Thus, two independent lesions affecting the vestibular system were made on both sides to ensure that vestibular inputs were eliminated. In no case did nystagmus or a tonic deviation in eye position occur after the surgery, suggesting that the peripheral lesions were complete. Antibiotics were administered for 10 days following the second surgery; in addition, 3 mg/kg of ketoprofen was administered intramuscularly every 12 h for 3 days to provide analgesia. To ensure that animals received proper hydration and nutrition during the immediate postsurgical period, saline solution was administered intravenously or subcutaneously each day, and animals were fed by hand until the spontaneous consumption of food and water returned to prelesion levels (which required only a few days).

Recording Procedures

For 4–6 wk following the initial surgery, animals were acclimated for restraint in a cylindrical tube that provided support for the body and immobilization of the head by insertion of a screw into the bolt mounted on the skull. This acclimation period was a continuation of training that occurred before surgery. Data collection did not commence until animals could be restrained for a recording session of 90 min without vocalizing, attempting to move, or displaying indicators of distress.

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). Use of this system allowed electrodes to be positioned reproducibly from day to day. 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. Animals were observed during recording sessions to assure they remained awake and calm, while tracking commenced. Both stereotaxic coordinates and physiological landmarks were used to localize the RVLM. One such physiological landmark was the cluster of neurons, observed with respiratory activity, that is positioned just dorsal to the RVLM in the cat (19), which was used to determine when the electrode tip entered the ventrolateral medulla. 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. Our recordings were made in the area ventral to respiratory neurons and within ~1.5 mm of the ventral surface of the brain stem.

After sampling RVLM activity over 40–45 days, vestibular inputs were removed, as described above. Recordings resumed the following day in the same region that was sampled previously and continued for 2–3 wk. Subsequently, 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 5–7 days after the procedure (to allow gliosis to occur at the lesion site), when 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 were perfused transcardially with 10% formalin.

Data Analysis Procedures

Spontaneous activity was recorded from neurons in the target area for ~3 min, unless the cell was lost prematurely due to movement of the animal or behaviors such as swallowing. Neuronal activity 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). The output of the Transonic Systems flowmeter, which reflected instantaneous carotid blood flow (CBF), was sampled at 100 Hz. The spike detection and sorting feature of the Spike2 software was subsequently used to delineate the occurrence of neuronal firing. When two or more spikes with distinct shapes that could be readily distinguished were present in a recording field, each was discriminated separately (see Fig. 1 for example), so that the firing patterns of the adjacent located cells could be compared.

CBF-triggered averages and histograms. As a first step in data analysis, averages of neuronal activity were triggered from peak CBF, so we could ascertain whether a neuron likely had CR activity (Fig. 1C). The data were then processed using Datapac software (Run Technologies; Mission Viejo, CA). The event marker used to indicate each spike in an RVLM neuron was converted to a 1-ms square wave pulse. Another 1-ms square wave pulse coincident with the peak of the CBF signal was used to trigger a histogram of RVLM neuronal activity and an average of the CBF signal. This signal was not only used as a trigger for the analysis; it was also used to construct a histogram of CBF peak-to-peak intervals. This was used to show the variability in beat-to-beat cardiac intervals.

A neuron was classified as having CR activity if the histogram of neuronal activity had a peak during the same phase of the CBF signal in the average (and/or the CBF peak-to-peak interval histogram). In many cases, the data were broken into two or more parts to verify that the CR activity was evident in each part. For example, a 180-s data block was broken into three 60-s data blocks, and the CBF-triggered analysis was constructed for each of the smaller data blocks. If this analysis failed to show repetition of the temporal relationship between neuronal activity and CBF, it was considered to be a neuron without CR activity. In addition, we made an estimate of the strength of the
CR activity based on a “signal-to-noise” (peak-to-background) ratio. The “peak counts” was defined as the average of the counts in three or more bins in the “peak” of the histogram of RVLM neuronal activity during the first cardiac cycle (closest to time 0), and “background counts” was defined as the maximum counts in the bins “between peaks” (see example in Fig. 2). The values of peak-to-background were calculated by dividing “peak counts” by “background counts”. For the rare cases in which there were zero counts between peaks, we used a calculation that divided the peak counts by 1. The peak-to-background value had to exceed 1.2 in order for us to consider the neuron as having CR activity.

**Interspike-interval histograms.** Interspike-interval histograms ISIHs (1-ms sample period) were constructed to analyze the spike trains of individual RVLM neurons and to calculate neuronal firing rate (i.e., the reciprocal of the mean ISI). In addition, a prominent peak in this ISIH near 100 ms (or near 30–50 ms) indicates that the activity of a neuron has a 10-Hz (or 20- to 30-Hz) rhythmic component. This was then verified by constructing an autospectrum of RVLM neuronal activity (see below).

**Frequency-domain analyses.** Fast Fourier transform was performed on a minimum of 28.5-s data windows to construct autospectra of the CBF and RVLM neuronal activity and the corresponding coherence function using methods detailed in earlier reports (5, 7, 9). Digital low-pass filtering (cut-off at 250 Hz) of the standardized pulses representing the spikes of individual neurons was performed by convolving the trains with a sinc function having parameters so that the autospectrum reflected the interspike intervals rather than the shape of the pulses. The autospectrum of a signal shows how much power is present at each frequency. Peaks could be detected at a frequency corresponding to the heart beat, at 10 Hz, and in a frequency range of 20 to 30 Hz.

The coherence function (normalized cross spectrum) is a measure of the strength of linear correlation of two signals at each frequency. The squared coherence value (referred to as coherence value) is one in the case of a perfect linear relationship and zero if two signals are unrelated. As in past studies of the relationship between RVLM neuronal activity and the arterial pulse or SNA (7, 23), a coherence value >0.1 was considered to reflect a statistically significant relationship between RVLM neuronal activity and the CBF signal when 32 or more windows were averaged. This is based on an assessment of 95% confidence limits as determined by Benignus (11). Spectral analyses were done over a frequency band of 0 to 100 Hz with a resolution of 0.2 Hz/bin.

**Statistical analyses.** Statistical analyses (Wilcoxon test, two-way ANOVA) were performed using Prism 5 software (GraphPad Software, San Diego, CA). Pooled data are presented as means ± SE.

**Histological Procedures.**

A freezing microtome was used to cut the brain stem transversely at 50-μm thickness, and tissue sections were stained with 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. The data from all neuronal recording sites that fell out of the confines of the RVLM were deleted from the analysis.

**RESULTS**

Characterization of CR Activity in RVLM Neurons of Conscious Cats with and without Vestibular Inputs

Activity was recorded from a total of 2,044 neurons whose locations in the RVLM were confirmed by histological reconstructions; 1,167 of the cells were sampled prior to the removal of vestibular inputs, while 877 were examined afterward, as indicated in Table 1. Out of this population, only 162 units (7.9%) had CR activity, including 77 neurons (6.6%) examined when vestibular inputs were intact and 85 (9.7%) sampled after the removal of vestibular inputs (see Table 1). Evidence for CR activity was based on CBF-triggered histograms of neuronal activity for each of these RVLM neurons (see Figs. 2A and 3A, bottom traces). In the example shown in Fig. 2A, the three broad peaks in the CBF-triggered histogram of RVLM neuronal activity were quite similar in contour. In contrast, in the example in Fig. 3A, the first peak in CBF-triggered histogram of RVLM neuronal activity was considerably narrower than the second peak; and by the third cardiac cycle, the peak had waned markedly. The differences in the patterns of CR activity in these two cases parallel the differences in the shapes of the histograms of CBF peak-to-peak intervals that reflect the variability of beat-to-beat intervals (Figs. 2A and 3A, middle traces).

We derived an estimate of the strength of CR activity, which was the ratio of peak-to-background counts in the CBF-triggered histogram of RVLM neuronal activity (see METHODS). The bottom panel of Fig. 2A shows an example of this assessment in which the peak-to-background value was 3.25. The
Fig. 2. Coexistence of CR and 10-Hz activity in an RVLM neuron in a vestibular-intact, conscious cat. A: traces (top to bottom), carotid blood flow (CBF)-triggered average of the CBF signal and CBF-triggered histograms of intervals between consecutive cycles of CBF (CBF Peak-to-Peak Int.) and of RVLM neuronal activity (Pk CBF-to-Unit Int.). Traces were triggered by an event coinciding with the peak of the CBF signal and are based on 426 trials (10-ms bin resolution). In the Pk CBF-to-Unit Int., the asterisks mark the bins used to calculate “peak counts”, and the horizontal line through the histogram is at “background counts”. B: interspike interval histogram (ISIH) of RVLM neuronal activity (Unit-to-Unit Int.; 3-ms bin resolution). C: frequency-domain analyses showing (top to bottom) autospectra of CBF (CBF AS) and RVLM neuronal activity (Unit AS) and the corresponding coherence function relating neuronal activity to the CBF signal (CBF-Unit Coh). Spectra are based on 34 5-s windows; frequency resolution is 0.2 Hz per bin.

Table 1. The number and percentage of neurons located in the rostral ventrolateral medulla with cardiac-related activity

<table>
<thead>
<tr>
<th>Animal</th>
<th>State</th>
<th>Total No. of RVLM Neurons Examined</th>
<th>No. of (%) CR Neurons</th>
<th>No. of CR Neurons with 10-Hz Rhythm</th>
<th>No. of CR Neurons with 20- to 30-Hz Rhythm</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Prelesion</td>
<td>202</td>
<td>27 (13)</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>2</td>
<td>Prelesion</td>
<td>261</td>
<td>23 (9)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Postlesion</td>
<td>296</td>
<td>19 (6)</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Prelesion</td>
<td>404</td>
<td>61 (1)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Postlesion</td>
<td>397</td>
<td>46 (12)</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Prelesion</td>
<td>300</td>
<td>21 (7)</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Postlesion</td>
<td>184</td>
<td>20 (11)</td>
<td>4</td>
<td>10</td>
</tr>
</tbody>
</table>

Also shown is the number of CR neurons with rhythmic activity at 10 Hz and 20–30 Hz. Data for each animal collected before (prelesion) and after (postlesion) removal of vestibular inputs through a bilateral vestibular neurectomy are shown in separate rows. RVLM, rostral ventrolateral medulla; CR, cardiac-related.

asterisks mark the bins used to calculate “peak counts”, and the horizontal line through the histogram is at “background counts”. The peak-to-background values ranged from 1.25 to 62.50 (mean of 4.99 ± 1.10) for RVLM neurons with CR activity identified in cats with intact vestibular inputs. The corresponding values were from 1.44 to 32 and 5.29 ± 0.71 for neurons studied after removal of vestibular inputs.

The locations in the RVLM of the neurons with CR activity are shown in Fig. 4A, whereas Fig. 4B is a plot of the coordinates of each recording site, based on histological reconstructions, with respect to stereotaxic zero, the midline, and the ventral surface of the brain stem. Activity was recorded from neurons dispersed from 9.1 to 11.1 mm posterior to stereotaxic zero (mean of 10.1 ± 0.01 mm), 2.4 – 4.9 mm lateral to the midline (mean of 3.7 ± 0.01 mm), and 0.03–2.96 mm from the ventral brain stem surface (mean of 1.2 ± 0.1 mm). Neurons with CR activity were distributed over most of this area, and the locations of these units recorded prior and subsequent to lesions were virtually identical: 9.9 ± 0.1 mm posterior to stereotaxic zero, 3.7 ± 0.1 mm lateral to the midline, and 1.2 ± 0.1 mm from the ventral brain stem surface for both populations.

Once a neuron was shown to have CR activity based on CBF-triggered analysis, spectral analysis was used to characterize further its activity pattern and to study the relationship between its activity and the CBF signal as another index of CR activity. This was completed for 48 neurons recorded in cats with intact vestibular inputs and for 50 neurons after removal of vestibular inputs.
vestibular lesions. The autospectra of RVLM neuronal activity in Figs. 2C and 3C (middle) show a peak at the same frequency as the peak in the corresponding autospectra of CBF (top). The coherence functions (Figs. 2C and 3C, bottom) verified the relationship between CBF and the activity of these RVLM neurons. Peak coherence values at the frequency of the heart rate were significantly different than zero in 32 of 48 cases in vestibular intact animals and in 25 of 50 cases in vestibular lesioned animals. These coherence values were 0.34 ± 0.04 and 0.28 ± 0.04, respectively.

Although <10% of RVLM neurons fired in synchrony with the cardiac cycle, the likelihood of recording neurons with CR activity varied over time. Figure 5 shows the percentage of RVLM neurons with CR activity sampled on each recording day. On approximately half of the days, no neurons were observed with CR firing patterns, but on others the fraction was very high. For example, on the 7th day after initiating single-unit recordings in animal 2, 60% of the 24 units sampled had CR activity, which were distributed over an ~1.5-mm dorsal-ventral distance. When tracking was conducted within 0.5 mm of this electrode penetration on subsequent days, just 2/50 neurons located in the RVLM exhibited CR discharges. Furthermore, in cases in which more than one unit could be discriminated in a single recording field, if one of the units had CR activity, it was likely that a second unit did as well. For example, when vestibular inputs were intact, there were 30 instances when a neuron with CR discharges was recorded along with one (n = 27) or two (n = 3) additional cells that could be reliably distinguished from each other through differences in their spike waveforms (as illustrated in Fig. 1). In 16 of these 30 cases, the firing of two of the cells in the recording field was synchronized with the cardiac cycle. Similarly, following the removal of vestibular inputs, 37 recording fields were identified where a cell with CR activity could be isolated along with a second neuron; in 17/37 of these instances, the firing of the second cell was also cardiac-related.

The data provided in Fig. 5 also show that removal of vestibular inputs affects the likelihood of encountering a neuron in the RVLM with CR activity. Following vestibular lesions, CR units were identified during 31/43 recording days, as opposed to 18/57 recording days in labyrinth-intact animals. Figure 6A shows that the percentage of RVLM neurons sampled each day that was determined to have CR activity increased significantly (P < 0.05, Wilcoxon test) subsequent to the surgical elimination of vestibular inputs.

The effects of vestibular lesions on the spontaneous firing rates of RVLM neurons with and without CR activity are shown in Fig. 6B. A two-way ANOVA confirmed that the
firing rates of both groups decreased significantly after the removal of vestibular inputs \((P < 0.01)\) and that units with CR activity fired more slowly than the general population of RVLM neurons \((P < 0.01)\).

**10-Hz and 20- to 30-Hz Rhythmic Activity Coexists with CR Activity in RVLM Neurons of Conscious Cats**

A small fraction of the cells with CR activity (24/162, 15%) had additional rhythmic discharges at 10 Hz (7–14 Hz); 12 of the neurons were identified in vestibular intact animals, and 12 were studied following vestibular lesions, as indicated in Table 1. The data in Fig. 2 are for a neuron with both CR and 10-Hz activity in a vestibular intact cat. The 10-Hz rhythm is evident in both the ISIH with a peak at \(\approx 100\) ms (Fig. 2B) and the wide peak in the autospectrum of neuronal activity at 10-Hz (Fig. 2C, middle).

A 20-to 30-Hz rhythm coexisted with CR activity in 20 RVLM neurons from animals 1 and 4, including 10 neurons studied before and 10 neurons recorded after vestibular lesions. The data in Fig. 3 are for a neuron with both CR and 20-Hz activity in a vestibular lesioned cat. The 20-Hz rhythm is evident in both the ISIH with a peak at \(\approx 60\) ms (Fig. 3B), and the wide peak in the autospectrum of neuronal activity at \(\approx 20\)-Hz (Fig. 3C).

Not surprisingly, evidence for 10-Hz or 20- to 30-Hz rhythmic activity in an RVLM neuron was not restricted to units with CR activity. On occasions when a neuron without CR activity was in the same recording field as one with CR activity, they were included in the time-domain and frequency-domain analyses that revealed these rhythmic firing patterns. No attempt was made to assess the percent of the general population of RVLM neurons that expressed these high-frequency rhythmic discharge patterns.
have been responsible for the variability in CR activity of RVLM neurons noted between recording sessions. The environmental factors and state-dependent influences that lead to modulation of SNA and CR firing patterns of RVLM neurons are yet to be determined, but they may include such parameters as alertness or anxiety. For example, if an animal is alerted by a perceived danger, the activity of neurons in regions of the medulla that are responsible for generating sympathetic rhythms could be altered by descending projections, and as a result, CR firing patterns would be expressed by a higher fraction of RVLM neurons.

The spontaneous firing rates of RVLM neurons, including those with CR activity, were lower in animals studied after the removal of vestibular inputs compared with recordings made in labyrinth-intact animals. The vestibular nuclei provide direct inputs to the RVLM (28, 35), as well as indirect inputs relayed through brain stem regions that mediate the baroreceptor reflex and participate in generating rhythmic SNA, including nucleus tractus solitarius (1, 37, 41), the lateral tegmental field (40), and the caudal ventrolateral medulla (38). One explanation for the reduced RVLM neuronal activity after the elimination of labyrinthine inputs is a loss of excitatory monosynaptic and polysynaptic excitatory drive to RVLM neurons from the vestibular nuclei. A caveat, however, is that a previous experiment showed that the average firing rate of spontaneously active neurons in the vestibular nuclei returned to prelesion levels within a day after bilateral transection of the VIIIth nerves (33), although this study did not address whether the activity of the subset of neurons that contribute to autonomic regulation was restored. Another possibility is that loss of labyrinthine inputs elicits a compensatory increase in the gain

Fig. 6. A: percentage of RVLM neurons with CR activity recorded on each day, prior (prelesion) and subsequent (postlesion) to the surgical elimination of vestibular inputs. B: average spontaneous firing rates for RVLM neurons with and without CR activity. Solid bars designate prelesion firing rates, while open bars indicate postlesion firing rates. Error bars designate one standard error.

DISCUSSION

This study is one of the first in any species to describe the activity patterns of RVLM neurons in conscious animals. Furthermore, we studied the effects on the firing characteristics of these neurons of a procedure that alters the regulation of peripheral blood flow and blood pressure: the elimination of vestibular inputs (31, 39, 42). One major finding was that the activity of $<$10% of RVLM neurons in conscious cats had a CR rhythm. Our recordings included the entirety of the rostral portion of the ventrolateral medulla that has been defined as containing neurons that regulate blood pressure and that have CR activity (3, 6, 15, 16, 23), such that it is unlikely that the findings were biased by sampling only a limited portion the RVLM. Furthermore, we found that the likelihood of identifying a neuron with CR activity in the RVLM varied between recording sessions, even when tracking occurred in a very limited area, and the likelihood was higher after vestibular inputs were surgically removed. In addition, when two RVLM neurons could be discriminated in a single recording field, and one had CR activity, the other did as well in approximately half of the instances. This observation further raises the prospect that firing patterns related to the cardiac cycle were more likely to be observed at particular times.

These novel findings based on recordings from presumed "cardiovascular neurons" in conscious animals imply that the brain stem circuitry responsible for generating the CR rhythm is affected by a variety of extrinsic influences, including higher centers of the brain. Because the animals were conscious and the baroreceptors were intact, we assume there were not large shifts in blood pressure from day to day that affected the excitability of RVLM neurons and our likelihood of identifying neurons with CR activity. Furthermore, modest changes in blood pressure would not be expected to suppress the CR rhythm (20, 21).

Studies in anesthetized animals have shown that hypothalamic neurons can influence the rhythmic activity of sympathetic nerves through their projections to the brain stem (2). Furthermore, lesion studies indicated that both the forebrain and medial thalamus contribute to regulating SNA, presumably through their connections in the brain stem (29, 30). Differences among the influences of any one of these areas could
of the baroreceptor reflex, which fits with observations that instability in blood pressure during postural alterations dissipates within a week following a loss of labyrinthine inputs and vestibulo-sympathetic responses (31). Because most RVLM neurons that regulate SNA are inhibited via activation of the baroreceptor reflex arc (3, 17), an increase in the baroreceptor reflex gain after elimination of signals from the inner ear could explain our findings that the likelihood of detecting an RVLM neuron with CR activity increased after vestibular lesions and that the overall activity in the RVLM was depressed.

There is evidence that “central command” signals that occur during voluntary movement alter the baroreceptor reflex gain (32). This raises the possibility that daily variability in the number of neurons with CR activity encountered was due to higher centers modulating the integration of baroreceptor signals in the brainstem. A third possibility is that vestibular lesions produced anxiety or an alternation in the alertness of the animals, which led to a change in the excitability of RVLM neurons and the likelihood that they exhibited CR activity. Further work in conscious animals is, thus, needed to examine the influences of vestibular signals, as well as cognitive state, on the processing of inputs from baroreceptors.

The relationships between RVLM neuronal activity and the CBF signal in these conscious cats were remarkably similar to the relationship between RVLM neuronal activity and the arterial pulse in anesthetized cats (7, 23). Specifically, the coherence values relating RVLM neuronal activity to the CBF at the frequency of the heart beat reported here are similar to values relating RVLM neuronal activity to the arterial pulse in these earlier studies. In the prior studies, it was also not uncommon to find cases in which the CR activity demonstrated with time domain analysis (e.g., CBF-triggered histogram) could not be confirmed with frequency domain analysis.

A major difference between RVLM neuronal activity in conscious cats vs. anesthetized cats was related to their spontaneous firing rates. In anesthetized cats, RVLM neurons with CR activity typically have firing rates that average 2 to 3 spikes/s as opposed to an average that is 3–7 times greater in conscious animals. This likely contributed to the high level of “background” activity evident in the CBF-triggered histograms of RVLM neuronal activity. Another difference between these conscious animals and anesthetized cats is the greater variability in heart rate in the former group. This contributed to the waning of the magnitude of peaks in the second and subsequent cycles of the CBF-triggered histograms of RVLM neuronal activity.

This study further determined whether neurons with CR activity also had rhythmic activity at higher frequencies, since a number of groups have reported that both CR and 10-Hz discharges can coexist in SNA (4, 9, 10, 14, 24). As expected, we identified RVLM neurons with a mixture of CR and 10-Hz activity in every animal. The functional relevance of the 10-Hz rhythm is indicated by the observation that its sudden appearance in SNA is accompanied by a rise in arterial pressure (4). Moreover, there is evidence that the mechanism responsible for generation of the 10-Hz rhythm serves the purpose of coordinating the discharges of sympathetic nerves with different targets, such as the heart and the vasculature of the viscera and skeletal muscle (4, 24).

Barman and Gebber (7) showed that, with few exceptions, the axons of RVLM neurons, whose discharges were correlated to both the CR and 10-Hz rhythms in SNA, projected to the intermediolateral region of the thoracolumbar spinal cord. Furthermore, they reported that the bulbospinal sympathetic pathway emanating from the RVLM is composed almost exclusively of neurons capable of expressing both a CR and a 10-Hz rhythmic firing pattern. Thus, it is reasonable to propose that RVLM neurons with this characteristic identified in the current study were bulbospinal sympathetic neurons.

The fraction of RVLM neurons with CR activity that also expressed a 10-Hz rhythm was rather low in the current study. This is not surprising since, at least in anesthetized animals, the appearance of the 10-Hz rhythm in SNA is most apt to occur under the conditions of reduced baroreceptor nerve activity induced by either baroreceptor denervation or by lowering arterial pressure (4, 7, 9). Because we did not monitor arterial pressure in these animals, we were not able to determine whether the firing pattern of an RVLM neuron could be altered by varying blood pressure and thus the level of baroreceptor activity. It is also possible that the 10-Hz and CR rhythms are independently gated by higher centers; further experiments in conscious animals are needed to test this hypothesis.

In two of the four animals, we identified a few RVLM neurons that had a mixture of CR and 20- to 30-Hz rhythmic activity. Gootman and Cohen (26, 27) have reported this high-frequency rhythm in SNA; they indicated that it was episodic and not present in every animal. Furthermore, they were not able to determine which areas of the nervous system participate in generating the 20- to 30-Hz activity. Additional studies are needed to establish the factors that gate the 20- to 30-Hz rhythm, as well as the functional relevance of this rhythm in terms of autonomic control. Curiously, none of the RVLM neurons encountered in this study had a combination of CR, 10-Hz, and 20- to 30-Hz activity, suggesting that when the pattern generator for one of the higher-frequency rhythms was active, the other was suppressed.

Not surprisingly, we encountered RVLM neurons that had 10-Hz or 20- to 30-Hz rhythmic discharges but not CR activity. An assessment of the rhythmic firing patterns of neurons without CR activity was done only for neurons that were in the same recording field as a neuron with CR activity. Because we did not do this analysis for many such RVLM neurons, we cannot determine the percentage of the general population of RVLM neurons that express one of these higher-frequency firing patterns in the absence of CR activity. It is well established that rhythmic activity in these ranges is common in many neural systems (4, 10, 36). Importantly, Barman et al. (10) showed that the 10-Hz rhythm in SNA was not correlated to the 10-Hz rhythm in either the inferior olive or neocortical spindles of urethane-anesthetized cats. They proposed that the 10-Hz rhythm in SNA reflects the organization of a brain stem network that specifically governs sympathetic outflow rather than a common oscillator that provides input to functionally diverse neural networks.

**Perspectives and Significance**

The present findings in conscious animals raise the prospect that regulation of SNA may be more multifaceted than previously established by studies in decerebrate or anesthetized preparations. Our data support the view that brain stem networks that generate rhythmic SNA, including those responsible for the CR rhythm in SNA, are not simple circuits whose...
activity is entrained only by baroreceptor inputs. Instead, RVLM neurons may or may not express rhythmic firing patterns at a particular time due to a variety of influences, including descending projections from the hypothalamus and cerebral cortex and sensory inputs, such as those from the vestibular system. Furthermore, these data support previous findings in conscious animals that the vestibular system contributes to the regulation of blood pressure (31, 39, 42) and show that vestibular influences on cardiovascular regulation are mediated in part by the RVLM. Our data suggest that further examination of the activity of neurons that participate in cardiovascular regulation is warranted in conscious animals and that recordings should be conducted during the execution of a variety of different behaviors. In particular, the effects of such parameters as alertness, anxiety, and exercise on the expression of rhythmic activity by RVLM neurons should be explored and, when feasible, should include recordings of SNA.

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