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RESEARCH ARTICLE

Responses of caudal vestibular nucleus neurons of conscious cats to rotations in vertical planes, before and after a bilateral vestibular neurectomy

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Abstract Although many previous experiments have considered the responses of vestibular nucleus neurons to rotations and translations of the head, little data are available regarding cells in the caudalmost portions of the vestibular nuclei (CVN), which mediate vestibulo-autonomic responses among other functions. This study examined the responses of CVN neurons of conscious cats to rotations in vertical planes, both before and after a bilateral vestibular neurectomy. None of the units included in the data sample had eye movement-related activity. In labyrinth-intact animals, some CVN neurons (22%) exhibited graviceptive responses consistent with inputs from otolith organs, but most (55%) had dynamic responses with phases synchronized with stimulus velocity. Furthermore, the large majority of CVN neurons had response vector orientations that were aligned either near the roll or vertical canal planes, and only 18% of cells were preferentially activated by pitch rotations. Sustained head-up rotations of the body provide challenges to the cardiovascular system and breathing, and thus the response dynamics of the large majority of CVN neurons were dissimilar to those of posturally-related autonomic reflexes. These data suggest that vestibular influences on autonomic control mediated by the CVN are more complex than previously envisioned, and likely involve considerable processing and integration of signals by brainstem regions involved in cardiovascular and respiratory regulation. Following a bilateral vestibular neurectomy, CVN neurons regained spontaneous activity within 24 h, and a very few neurons (<10%) responded to vertical tilts <15° in amplitude. These findings indicate that nonlabyrinthine inputs are likely important in sustaining the activity of CVN neurons; thus, these inputs may play a role in functional recovery following peripheral vestibular lesions.

Keywords Otolith organ · Vertical semicircular canal · Vestibulo-autonomic responses · Cardiovascular · Respiration

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Introduction

Although a variety of studies have considered the responses of vestibular nucleus neurons to rotations and translations of the head (e.g., Chubb et al. 1984; Kasper et al. 1988; Gdowski and McCrea 1999; Dickman and Angelaki 2002; Zhou et al. 2006), virtually all of these experiments have focused on units in the rostral or middle portions of the nuclear complex. With the exception of a small GABAergic cell population that comprises the parasolitary nucleus (Barmack and Yakhnitsa 2000), the caudal vestibular nuclei (CVN) have not been extensively studied,

particularly in conscious animals. Although the CVN do not play an appreciable direct role in mediating vestibulo-ocular reflexes (Wilson and Melvill Jones 1979), this region has been associated with several other functions. For example, a specialized projection to the upper cervical spinal cord arises from the CVN (Peterson et al. 1978); this projection differs from the classical vestibulospinal tracts in that it terminates mainly in the dorsal horn (Bankoul et al. 1995), and is presumably involved in modulating the processing of sensory signals from neck receptors. The CVN also provide considerable inputs to the cerebellar nodulus and uvula (Epema et al. 1985; Sato et al. 1989; Thunnissen et al. 1989; Barmack et al. 1992). Furthermore, the CVN project to medullary regions that participate in regulation of blood pressure and breathing (Balaban and Beryozkin 1994; Yates et al. 1994, 1995; Ruggiero et al. 1996; Porter and Balaban 1997; Martinelli et al. 2007), and lesions of the CVN abolish cardiovascular and respiratory responses to stimulation of vestibular afferents (Uchino et al. 1970; Yates et al. 1993; Yates and Miller 1994; Rossiter et al. 1996; Kerman and Yates 1998).

In quadrupeds, head-up rotations of the body provide challenges to the maintenance of stable blood pressure and can produce mechanical constraints on breathing (see Yates and Bronstein 2005 for a review). In conscious cats, compensatory adjustments in blood distribution in the body (Jian et al. 1999; Wilson et al. 2006) and respiratory muscle activity (Cotter et al. 2001, 2004) during sustained 60° head-up pitch rotations are attenuated following a bilateral labyrinthectomy. Since the CVN mediate vestibular influences on autonomic regulation, these data suggest that a large fraction of neurons in this region should receive otolith organ inputs elicited by rotations in the pitch plane. Accordingly, anatomical studies have revealed that the otolith organs provide substantial inputs to the CVN (Kevetter and Perachio 1986; Gstoettner et al. 1992; Newlands et al. 2002, 2003; Newlands and Perachio 2003; Yingcharoen et al. 2003). However, recordings in labyrinth-intact decerebrate cats indicated that the activity of virtually no CVN neurons classified as receiving otolith inputs was preferentially modulated by pitch rotations (Endo et al. 1995). It is presently unclear whether this lack of responsiveness is due to a paucity of inputs from the regions of the maculae where receptors are stimulated by pitch rotations, or if the CVN neuronal activity related to the signals was masked in the decerebrate preparation.

In addition to labyrinthine inputs, CVN neurons receive a variety of other sensory signals. Anatomical studies have indicated that this region receives inputs from receptors in the neck, limbs and viscera (Bankoul et al. 1995; Jian et al. 2005). Physiological studies have confirmed that the activity of a majority of CVN neurons is modulated by visceral and limb sensory signals (Jian et al. 2002). In another experiment, recordings were made from CVN neurons of decerebrate cats that had recovered from a bilateral vestibular neurectomy performed 49–103 days previously. Curiously, approximately one-fourth of the units responded to rotations in the pitch plane despite an absence of labyrinthine inputs (Yates et al. 2000), presumably as a consequence of nonlabyrinthine signals to the CVN that were altered in accordance with body position in space. It has been postulated that such "sensory substitution" in the CVN explains why posturally-related disturbances in blood pressure and respiration following a bilateral labyrinthectomy dissipate over time (Yates and Bronstein 2005). It has not yet been ascertained whether CVN neurons in conscious animals regain responsiveness to tilt after the removal of vestibular inputs. Such a determination is important, since the prior observations could be an epiphenomenon limited to acute preparations.

The present study had two goals. First, we determined the dynamic and spatial responses to rotations in vertical planes of CVN neurons in conscious cats, to test the hypothesis that a large fraction of the units have response properties that would be appropriate to trigger vestibulo-autonomic reflexes (Yates and Miller 1994; Rossiter et al. 1996; Woodring et al. 1997; Wilson et al. 2006). In particular, we expected that many CVN neurons would preferentially respond to rotations in the pitch plane, and would have response dynamics consistent with inputs from graviceptors. Second, we determined the effects of a bilateral vestibular neurectomy on the spontaneous activity and responses to vertical rotations of CVN units. We predicted that spontaneous activity would be quickly restored in many neurons following the removal of labyrinthine inputs, and that some of the units would regain responses to tilts in vertical planes.

Methods and materials

All the procedures on animals performed in this study were approved by the University of Pittsburgh's Institutional Animal Care and Use Committee, and conformed to the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Experiments were conducted on four purpose-bred adult female cats obtained from Liberty Research (Waverly, NY, USA). Animals were spayed prior to being included in this study to eliminate cyclic changes in hormonal levels.

Overview of experimental procedures

Single unit recordings were performed from CVN neurons in three labyrinth-intact animals; in two of these cases (animals 2 and 3) unit activity was also sampled following

a bilateral vestibular neurectomy, beginning the day subsequent to the elimination of labyrinthine inputs. In the third case (animal 4), no vestibular lesions were performed. In a fourth animal (animal 1), CVN recordings were not initiated until 2 weeks following the bilateral vestibular neurectomy. Prior to recordings, the animals were trained over a period of 1–2 months to remain sedentary on a tilt table during sinusoidal rotations in vertical planes at frequencies of 0.02–2 Hz and maximal amplitudes ranging from 5° at high frequencies to 15° at low frequencies. The body was enclosed in a cylindrical tube that provided restraint, and straps placed around the animal's body ensured that its position on the table did not change during rotations. The head was immobilized by inserting a screw into a nut fixed to the animal's skull. This restraint of the animals did not permit overt neck rotations to occur. In the three vestibular-intact animals where recordings were performed, the head was pitched 15° down from the stereotaxic plane to bring the vertical canals close to the vertical planes. In the case where only postlesion recordings were conducted, the head was aligned in the stereotaxic plane during data collection.

Surgical procedures

Two recovery surgeries were required for each animal except the case where we did not perform vestibular neurectomies (animal 4). Both surgeries were executed aseptically in a dedicated operating suite utilizing anesthetic and post-surgical procedures that we have employed in many previous studies (e.g., Wilson et al. 2006)

The first surgery involved mounting a fixation plate on the skull, performing a craniotomy and attaching a recording chamber around the opening in the skull, and implanting silver/silver-chloride electrodes lateral to each eye for recording the electrooculogram (EOG) associated with horizontal eye movements. The animal's head was placed in a stereotaxic frame during these procedures. The craniotomy was 1 cm in diameter, and was performed at the midline of the posterior aspect of the skull, which provided for bilateral recordings from the vestibular nuclei. A recording chamber (David Kopf Instruments, Tujunga, CA, USA) was lowered using a microdrive to stereotaxic coordinates that would permit access to the CVN, and attached to the skull adjacent to the craniotomy using Palacos® bone cement (Zimmer, Warsaw, IN, USA). The chamber was tilted at an 8° angle relative to the stereotaxic plane so that electrodes would course slightly rostrally as they were lowered. Leads from the EOG electrodes were soldered to a connector that was attached to the skull in front of the fixation plate. Animals recovered for at least 4 weeks after this surgery before data collection was initiated.

A second surgery was performed on three of the animals to eliminate vestibular inputs. For this purpose, 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 internal auditory canal. The VIIIth cranial nerve was transected under microscopic observation within the internal auditory canal. 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.

Recording procedures

All the recordings were conducted in a dimly lit room; the visual field surrounding the animal was rotated with its body, such that no visual cues regarding body position in space were available. During recording sessions, an *x–y* positioner was attached to the recording chamber and used to maneuver an 8–10 MΩ epoxy-insulated tungsten microelectrode (Frederick Haer, Bowdoin, ME, USA), which was inserted through a 25-gauge guide tube into the cerebellum, and lowered into the medulla using a David Kopf model 650 hydraulic microdrive. Neural activity was amplified by a factor of 1,000 or 10,000, filtered with a bandpass of 300–10,000 Hz, and led into a window discriminator for the delineation of spikes from single units. The output of the window discriminator was led into a 1401-plus data collection system (Cambridge Electronic Design, Cambridge, UK) and Macintosh G4 computer (Apple Computer, Cupertino, CA, USA) running Spike-2 software (Cambridge Electronic Design); the sampling rate was 10,000 Hz.

As the electrode was lowered during daily experimentation, combined roll and pitch tilts were often provided as a search stimulus, to aid in finding neurons that received vertical vestibular inputs. When a unit responsive to rotations was encountered, we recorded its spontaneous activity (in the absence of rotations) along with the horizontal EOG, which was amplified by a factor of 1,000 and sampled at 1,000 Hz. If a unit appeared to have eye movement-related firing, the lighting intensity in the laboratory was increased and a moving object was introduced into the animal's visual field to encourage it to make eye movements, so that a more definitive determination could be made. Not surprisingly, only a paucity of CVN neurons exhibited eye movement-related discharges (Wilson and Melvill Jones 1979), and these units were not studied further.

In labyrinth-intact animals, we then recorded neuronal responses to stimulation of vertical semicircular canals and otolith organs, which was produced by tilting the entire animal about the pitch (transverse) and roll (longitudinal) axes using a servo-controlled hydraulic tilt table (NeuroKinetics, Pittsburgh, PA, USA). Our procedures for performing vertical vestibular simulation have been described in detail previously (e.g., Yates et al. 2000; Jian et al. 2002). We first determined the plane of tilt that produced maximal modulation of the unit's firing rate (response vector orientation). Response vector orientation was calculated from responses to the "wobble" stimulus, a constant-amplitude tilt whose direction moves around the animal at constant speed (Schor et al. 1984). The direction of the response vector orientation lies midway between the maximal response directions to CW and CCW wobble stimulation, because the phase differences between stimulus and response are reversed during the two directions of rotation (Schor et al. 1984). Thus, by consideration of both responses, these phase differences could be accounted for. Wobble stimulation was typically delivered at 0.5 Hz, and sometimes at lower frequencies as well. Subsequently, the response vector orientation was confirmed by comparing the gain of responses to tilts in a variety of fixed vertical planes, typically delivered at 0.5 Hz and at an amplitude of 5°. These tilts always included 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 (i.e., response gain and phase across stimulus frequencies). Response dynamics were routinely determined over the frequency range of 0.1–1 Hz; for some cells rotations at 0.02, 0.05 Hz, and/or 2 Hz were also delivered. The amplitude of these stimuli was usually 2.5–5° at frequencies ≥0.5 Hz, and 5°–15° at frequencies ≤0.2 Hz.

Recording procedures in animals with a bilateral vestibular neurectomy were similar to those in cats with intact labyrinthine inputs, but emphasized low-frequency, large-amplitude tilts that were most likely to stimulate nonlabyrinthine graviceptors. Typically, the stimulus battery included 15° wobble rotations delivered at 0.05–0.1 Hz.

Data analysis

For each unit, spontaneous firing rate and coefficient of variation of firing rate (CV; standard deviation of interval between spikes divided by mean interval between spikes) were determined from recordings performed in the absence of stimulation. Neural activity recorded during rotations in vertical planes was binned (1,000 bins/cycle) and averaged over the sinusoidal stimulus period. Sine waves were fitted to responses with the use of a least-squares minimization technique (Schor et al. 1984). 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). Gain and phase measurements were then corrected for the dynamics of the tilt table, which were ascertained from data provided by potentiometers mounted on the roll and pitch axes. Responses were considered significant if the signal-to-noise ratio (see Schor et al. 1984) for method of calculation) was >0.5 and only the first harmonic was prominent (see Fig. 1 for examples of significant responses). Statistical analyses were performed using Prism 5 software (GraphPad Software, San Diego, CA, USA). Pooled data are presented as means ± one standard error.

Histological analysis

After experimental procedures were completed, electrolytic lesions were made at defined coordinates by passing a 20 μA negative current for 60 s through a 0.5 MΩ tungsten electrode. Approximately 1 week later, the animals were deeply anesthetized using 40 mg/kg pentobarbital sodium injected i.p. and perfused transcardially with 10% formalin. Sections of the brainstem (50 μm thick) were made in the transverse plane and stained with thionine. Locations of recorded neurons were reconstructed on camera lucida drawings of sections with reference to placement of electrolytic lesions, relative *x*–*y* coordinates of electrode tracks, and relative depths of recording sites from the surface of the cerebellum. In two animals (animals 1 and 2), the temporal bone was removed, decalcified using a solution of ethylenediaminetetraacetic acid (EDTA) and hydrochloric acid, embedded in 12% celloidin, cut at 30 μm thickness in the coronal plane, and stained using hemotoxilyn. The temporal bone sections were then inspected histologically to determine the extent of damage to the eighth nerves and vestibular labyrinth.

Results

Recordings from CVN neurons in labyrinth-intact animals

The spatial and dynamic properties of the responses of 85 CVN neurons to vertical tilts were fully characterized in three vestibular-intact animals. Figure 1 illustrates averaged responses of one cell to rotations in vertical planes. Figure 1a shows a subset of the traces used to determine the response vector orientation for the unit, which was calculated to be near nose down pitch based on the responses to CW and CCW wobble stimuli. The

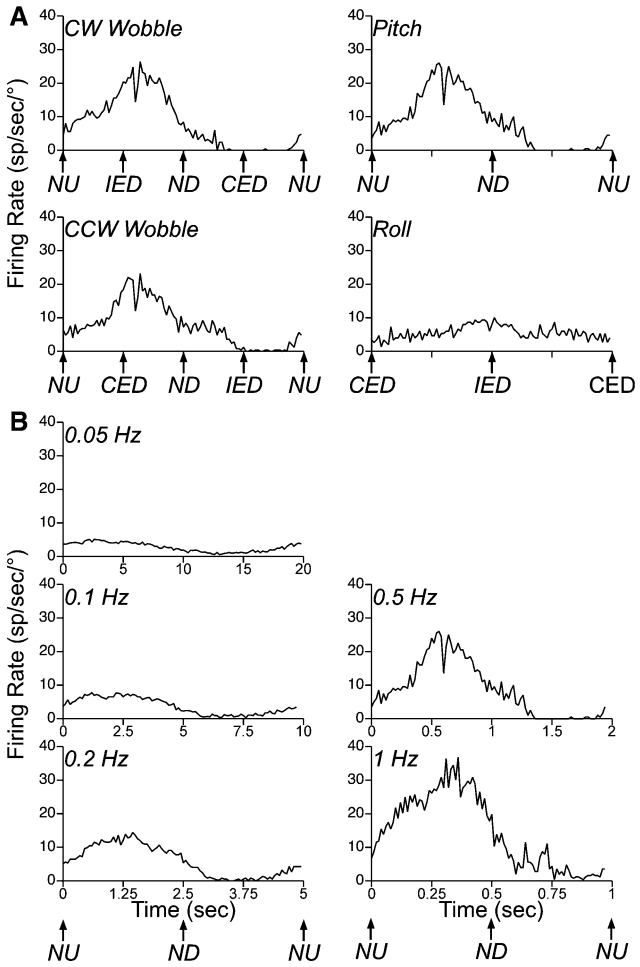

Fig. 1 Examples of averaged responses of one neuron to rotations in vertical planes. **a** Responses to clockwise (CW) and counterclockwise (CCW) wobble stimuli, as well as tilts in the pitch and roll planes, delivered at a frequency of 0.5 Hz and an amplitude of 5°. Traces produced by wobble rotations reflect the average of 25 sweeps, whereas traces elicited by pitch and roll rotations were respectively generated by averaging 20 or 15 sweeps. **b** Responses to rotations in the pitch plane delivered at 0.05–1 Hz. The tilt amplitude was 10° at 0.05 Hz, 7.5° at 0.1 Hz, 5° at 0.2–0.5 Hz, and 2.5° at 1 Hz. The number of sweeps averaged to produce each trace increased with advancing stimulus frequency (e.g., 10 sweeps were averaged at 0.1 Hz and 100 sweeps were averaged 1 Hz). *CED* Contralateral ear down roll; *IED* ipsilateral ear down roll; *ND* nose down pitch; *NU* nose up pitch

response vector orientation was confirmed by considering the relative gains of responses to tilts in single fixed planes. For example, pitch rotations elicited robust responses (signal-to-noise ratio of 1.3; gain of 12.4 spikes/s/°), whereas roll tilts elicited little modulation of the neuron's firing (signal-to-noise ratio of 0.3; gain of 1.9 spikes/sec/°), indicating that the response vector orientation was much closer to the pitch plane than the roll plane. Figure 1b illustrates the responses of the unit to pitch rotations performed at frequencies of 0.05–1 Hz. The response phases led nose down pitch by >90° at all frequencies, and the gain of the response elicited by 1 Hz rotations was seven times larger than the gain of the response to 0.1 Hz tilts.

Figure 2 contains Bode plots that illustrate the dynamic properties of CVN neuronal responses to rotations in fixed planes near the response vector orientation. Both response gain and phase are plotted with respect to stimulus position, such that a response whose phase leads stimulus position by 90° is synchronous with stimulus velocity. By considering differences in the phases of stimuli and responses across frequencies, all units could be subdivided into four types. Neurons were classified as having "graviceptive" properties if the response phase was within 40° of stimulus position at 0.1 Hz, and either remained near or lagged stimulus position as the stimulus frequency increased (see Fig. 2a). "Phase advancing" units exhibited a response phase that was within 40° of stimulus position at 0.1 Hz or lower frequencies, but that advanced >50° as the stimulus frequency was increased to 0.5–1 Hz (see Fig. 2b). "Phase lagging" cells had response phases within 30° of stimulus velocity at 0.1 Hz or lower frequencies, but which lagged this value by >60° as the stimulus frequency was increased to 0.5–1 Hz (see Fig. 2c). Neurons whose response phases lagged stimulus velocity by no more than 30° across the frequency range of 0.1–1 Hz were described as having "velocity" responses (see Fig. 2d). In total, 19/85 of the CVN neurons examined (22%) were classified as having graviceptive responses, 5 or 6% had phase advancing responses, 14 or 16% had phase lagging responses, and 47 or 55% had velocity responses.

Figure 3 indicates the relative response gain per stimulus decade (usually over the frequency range of 0.1–1 Hz, calculated by dividing the gain of the response to the high frequency stimulus by the gain of the response to the low frequency stimulus) for the four types of CVN neurons. On average, the relative gain per stimulus decade for graviceptive neurons was only moderate: a 3.2 ± 0.3-fold increase. However, the relative gain per stimulus decade for phase-lagging and velocity neurons was much larger: 9.9 ± 1.6-fold and 9.0 ± 0.8-fold increases, respectively. A nonparametric one-way ANOVA (Kruskal–Wallis test) combined with Dunn's multiple comparison post-test revealed that the relative gain per decade for graviceptive neurons was significantly smaller than for phase lagging or velocity cells ($P<0.001$). The modest response gain increases with advancing stimulus frequency exhibited by graviceptive neurons is consistent with these cells signaling body position in space.

The response vector orientations for the different types of CVN neurons are shown in Fig. 4, whereas Table 1 summarizes whether the vector orientations were closest to the roll plane, the pitch plane, or the plane of one of the vertical semicircular canals. Table 1 further indicates whether the response vector orientations were directed

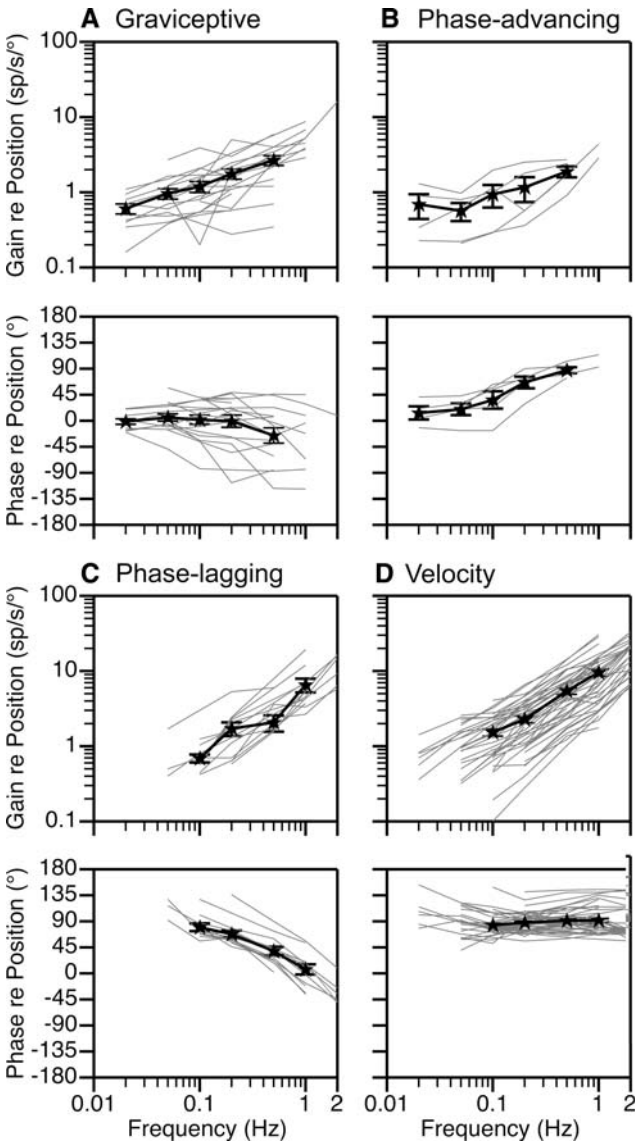

Fig. 2 Bode plots illustrating the dynamic properties of CVN neuronal responses to rotations at multiple frequencies. Response gain and phase are plotted relative to stimulus position. *Thin gray lines* designate responses of individual neurons, whereas *thick black lines* show averaged data for all units. *Error bars* designate one standard error. **a** *Graviceptive* neurons with response phases within 40° of stimulus position at 0.1 Hz, that either remained near or lagged stimulus position as the stimulus frequency increased. **b** *Phase advancing* units whose response phases were within 40° of stimulus position at 0.1 Hz or lower frequencies, but advanced >50° as the stimulus frequency was increased to 0.5–1 Hz. **c** *Phase lagging* cells that had response phases within 30° of stimulus velocity at 0.1 Hz or lower frequencies, but developed phase lags >60° as the stimulus frequency was increased to 0.5–1 Hz. **d** *Velocity* neurons whose response phases lagged stimulus velocity by no more than 30° across the frequency range of 0.1–1 Hz

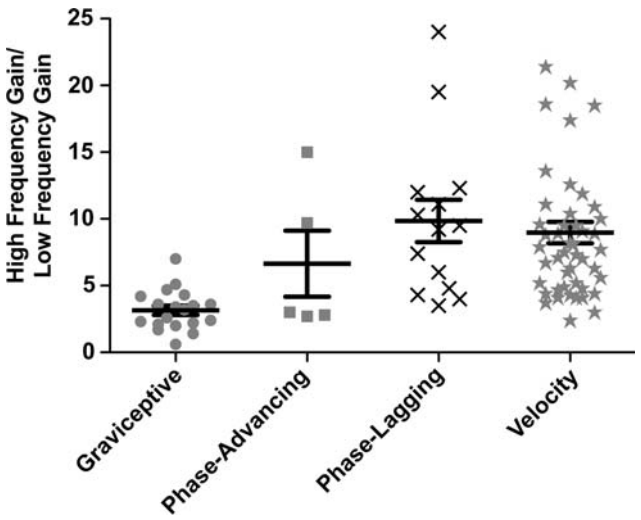

Fig. 3 Relative gain per stimulus decade (usually over the range of 0.1–1 Hz) of different types of CVN neurons. *Symbols* indicate for each neuron the ratio of the gain of the response to the high frequency stimulus to the gain of the response to the low frequency stimulus. *Horizontal lines* indicate the mean relative gain per decade for each neuronal type; *error bars* designate one standard error

ipsilaterally or contralaterally with respect to the side of the brain from which recordings were made. When all neurons were considered together, the response vector orientations were typically closest to the plane of one of the vertical semicircular canals (50 units, 59%) or the roll plane (20/85 units, 24%), although 15 cells (18%) had response vector orientations closest to the pitch plane. Furthermore, 61% (52/85 cells) of the response vector orientations were directed towards the ipsilateral side.

Neurons with graviceptive responses were particularly likely to exhibit response vector orientations that were closest to the vertical canal planes: 13/19 or 68% of the neurons had such a characteristic. Similarly, the response vector orientations of 30/47 or 64% of the velocity neurons were closer to the vertical canal planes than the roll or pitch planes. Moreover, the response vector orientations of many of the velocity neurons (22) were almost directly aligned (within 10°) with the plane of a vertical semicircular canal. In contrast, half (7/14) of the phase lagging neurons had response vector orientations closest to the roll plane.

Some vestibular nucleus neurons, which we refer to as spatiotemporal convergence (STC) units, respond as though they receive vestibular inputs from receptors with differing spatial and frequency components (such as graviceptive and velocity responses with different spatial orientations) (Baker et al. 1984; Kasper et al. 1988; Schor and Angelaki 1992). The response vector orientations for such cells vary as a function of tilt frequency; furthermore, the gains of responses to CW and CCW wobble rotations are usually significantly different (Kasper et al. 1988; Schor and Angelaki 1992). To determine if STC neurons are present in the CVN, the ratio of the gain of the responses to CW and CCW wobble stimulation was determined for the highest frequency rotations employed

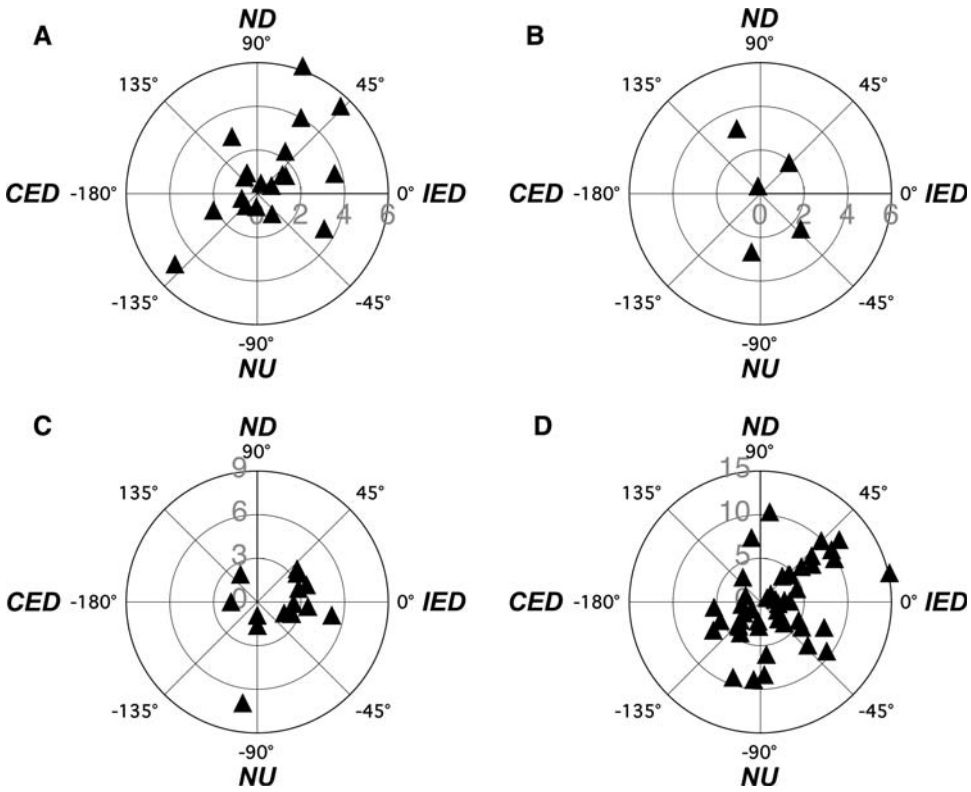

Fig. 4 Polar plots showing the response vector orientations and gains for CVN neurons, determined using wobble stimuli that were usually delivered at 0.5 Hz. Response vector orientations for different neuronal types (**a** *graviceptive*; **b** *phase leading*; **c** *phase lagging*; **d** *velocity*) are indicated in separate panels. *Numbers* along the radius of each plot indicate gain (spikes/s/°). *CED* Contralateral ear down roll; *IED* ipsilateral ear down roll; *ND* nose down pitch; *NU* nose up pitch

Table 1 Number of CVN units of different types with response vector orientations closest to roll, pitch, or vertical canal planes

Unit type	Side	Plane of response vector orientation		
		Roll	Pitch	Vertical canal
Graviceptive	Ipsilateral	1	2	8
	Contralateral	2	1	5
	Total	3	3	13
Phase leading	Ipsilateral	0	0	2
	Contralateral	0	2	1
	Total	0	2	3
Phase lagging	Ipsilateral	6	0	3
	Contralateral	1	3	1
	Total	7	3	4
Velocity	Ipsilateral	7	3	20
	Contralateral	3	4	10
	Total	10	7	30
All types combined	Ipsilateral	14	5	33
	Contralateral	6	10	17
	Total	20	15	50

Neurons with response vector orientations directed ipsilateral or contralateral to the side where recordings were performed are designated separately

for a unit (usually 0.5 Hz), where the STC response is expected to be most evident. For the large majority of cells (79/85), the ratios were <2:1 (i.e., no less than 0.5 and no larger than 2.0); the largest ratio was 2.6:1. In addition, wobble stimuli were delivered at two or more frequencies between 0.05 and 0.5 Hz for 23 neurons. These values changed no more than 23° for any cell, and typically the variability in response vector orientation was much smaller: the median was 8°. Thus, it appears that few CVN neurons exhibit robust STC behavior.

The locations of the CVN neurons that responded to vertical rotations are illustrated in Fig. 5a; the locations are plotted on a horizontal section through the caudal vestibular nuclei. Approximately 34% of the neurons were located in the medial vestibular nucleus, whereas 76% were situated in the inferior vestibular nucleus. Neurons with specific response characteristics did not appear to be clustered in a particular region of the CVN.

Recordings from CVN neurons in animals lacking labyrinthine inputs

Recordings were made from CVN neurons in three animals following a bilateral vestibular neurectomy; the recording locations are shown in Fig. 5b. In one case (animal 1), the recordings were initiated 2 weeks following the elimination of vestibular inputs and continued for 2 months. In the other two cats (animals 2 and 3), data collection started the day after the vestibular neurectomies were performed and continued for 1 month. Every spontaneously-active neuron that was encountered

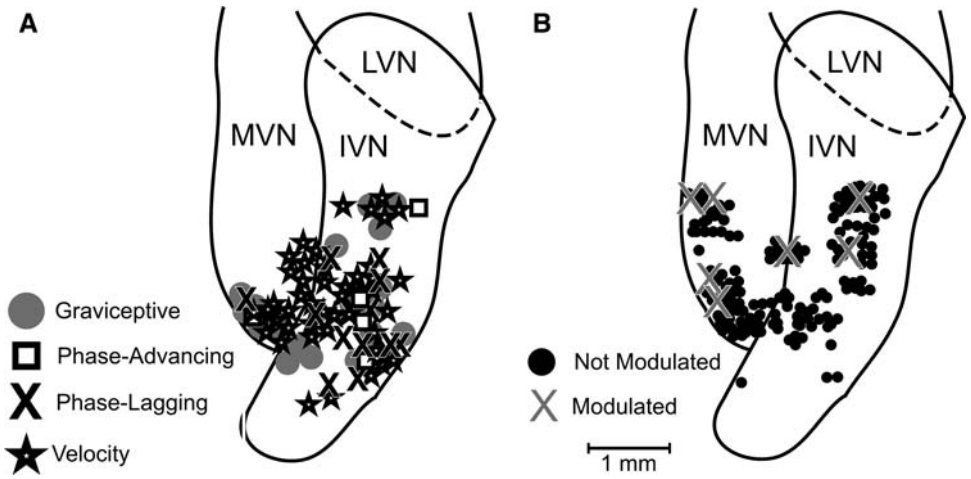

Fig. 5 Locations where activity was recorded from CVN neurons, plotted on a horizontal section through the caudal half of the vestibular nucleus complex. **a** Recording locations in vestibular-intact animals; each unit type is designated by a different symbol. **b** Locations of neurons tested for responses to 15° wobble stimulation following the elimination of vestibular inputs. *Filled circles* designate units whose activity was not modulated by the rotations, whereas *Xs* indicate cells that responded consistently to the tilts. *IVN* Inferior vestibular nucleus; *LVN* lateral vestibular nucleus; *MVN* medial vestibular nucleus

and could be held for extended periods was tested for responses to wobble rotations up to 15° in amplitude delivered at 0.05–0.1 Hz. In animal 2, 6/79 neurons responded to 15° wobble rotations, but the activity of only 1/35 neurons in animal 3 and 0/54 neurons in animal 1 was modulated by 15° wobble stimuli. The signal-to-noise ratios were typically very low for responses of neurons whose activity was not modulated by 15° wobble rotations: the average values were 0.24 ± 0.02 in animal 1, 0.22 ± 0.1 in animal 2, and 0.21 ± 0.03 in animal 3.

The response characteristics of the seven neurons whose activity was consistently modulated by vertical rotations in animals lacking vestibular inputs are shown in Fig. 6. Figure 6a shows examples of the responses of one neuron to 15° pitch rotations; the recordings were performed 3 days following the bilateral vestibular neurectomy. Bode plots indicating the response dynamics of all of the units are provided in Fig. 6b. All of the neurons could be classified as graviceptive, as their response phases were near stimulus position at low stimulus frequencies, and either remained near or lagged position as stimulus frequency increased. In addition, the response gains were relatively consistent across stimulus frequencies; on average, the response gain only increased 1.4 ± 0.2-fold per stimulus decade. The response vector orientations for the units are shown in Fig. 6c. Six of the neurons had response vector orientations that were closer to the pitch plane than the roll or vertical canal planes, although the remaining cell was preferentially activated by roll rotations.

Despite the fact that few CVN neurons responded to moderate-amplitude vertical tilts in animals lacking vestibular inputs, many cells quickly regained spontaneous activity following a bilateral vestibular neurectomy. A caveat is that we were not able to detect silent neurons, and thus could not ascertain whether the fraction of neurons that lacked spontaneous discharges changed following the peripheral vestibular lesions. Recordings resumed the day after the bilateral vestibular neurectomy in animals 2 and 3, when considerable spontaneous activity could be detected in the vestibular nuclei. For animal 2, the mean firing rates for all units examined were 31 ± 2 spikes/s when the labyrinths were intact, 47 ± 6 spikes/s in the first week after the bilateral vestibular neurectomy, and 29 ± 2 spikes/s subsequently. In animal 3, the spontaneous firing rates were as follows: 20 ± 1 spikes/s before the vestibular neurectomies, 25 ± 4 spikes/s in the first week after the surgery, and 33 ± 4 spikes/s during the following 3 weeks. Recordings were not initiated in animal 1 until 2 weeks subsequent to the peripheral vestibular lesions, when the mean firing rate of CVN units was 17 ± 2 spikes/s. It is unclear why the firing rates of CVN units varied between animals, although this could be due to differences in the subregions of the CVN that were sampled. Removal of vestibular inputs did not result in any significant changes ($P > 0.4$, Mann Whitney test) in the regularity of neuronal firing. In animal 2, the mean CV of spontaneous firing rate was 0.77 ± 0.06 before the removal of vestibular inputs and 0.76 ± 0.05 subsequently. In animal 3, the average CV of firing rate was 0.86 ± 0.05 when the labyrinths were

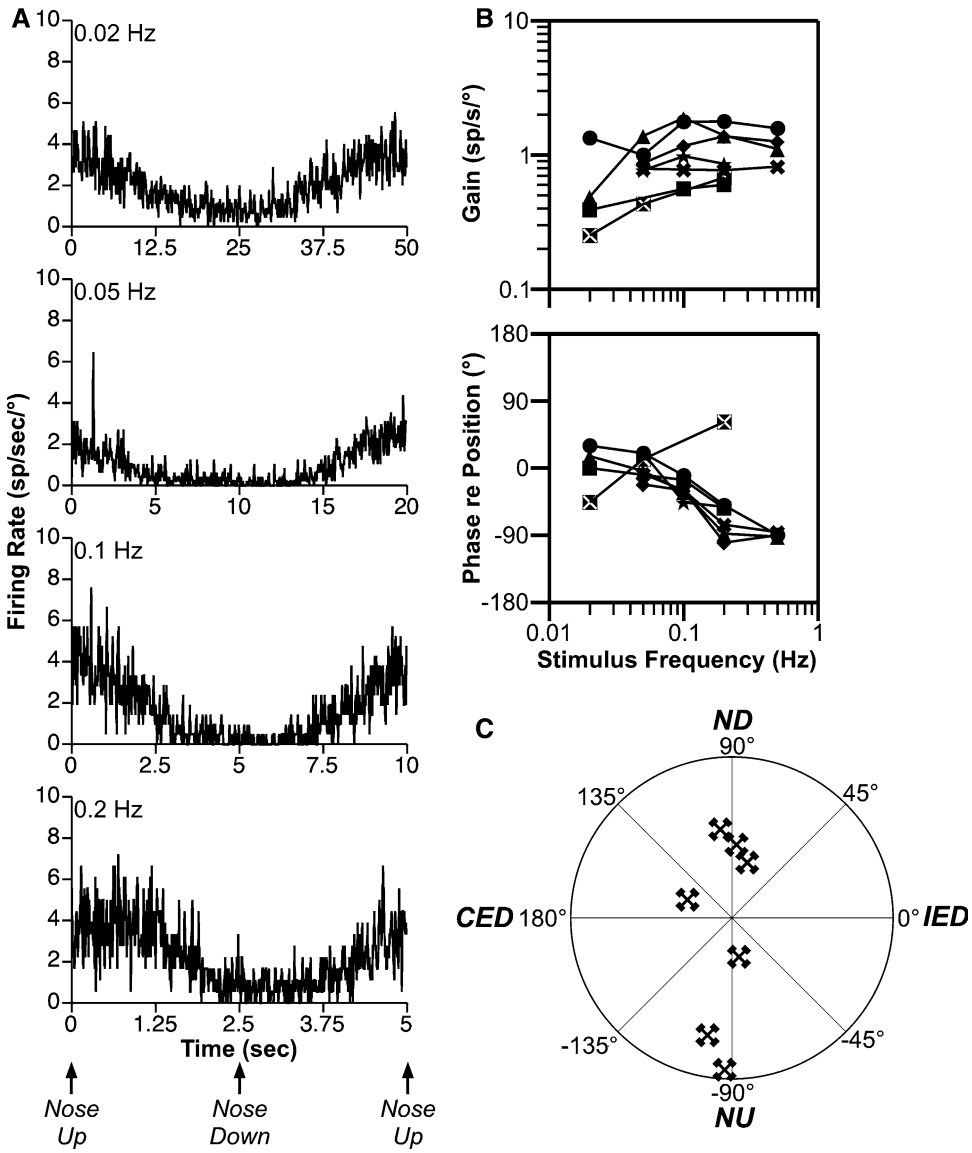

Fig. 6 Characteristics of neuronal responses to vertical rotations following a bilateral vestibular neurectomy. **a** Examples of the responses of one neuron to 15° pitch rotations at frequencies of 0.02–0.2 Hz. The number of sweeps averaged to generate each sweep are as follow: 0.02 Hz, 3; 0.05 Hz, 8; 0.1 Hz, 7; 0.2 Hz, 12. **b** Bode plots indicating the response dynamics of neurons in animals lacking labyrinthine inputs. Response gain and phase are plotted with respect to stimulus position. **c** Polar plots showing the response vector gains and orientations of CVN neurons following a bilateral vestibular neurectomy, determined using wobble stimuli delivered at 0.05 Hz. The outer radius of the plot designates a gain of 2 spikes/s/°. *CED* Contralateral ear down roll; *IED* ipsilateral ear down roll; *ND* nose down pitch; *NU* nose up pitch

intact and 0.91 ± 0.07 following the bilateral vestibular neurectomy.

Histological confirmation of peripheral vestibular lesions

A histological analysis of temporal bone sections was performed for animals 1 and 2 to determine whether the labyrinthectomy and eighth cranial nerve transections were complete. In both of the cases, we confirmed that the vestibular labyrinth had been opened, thereby producing a functional lesion by permitting the perilymph and endolymph to escape. Furthermore, both eighth cranial nerves were completely severed; the cut ends of the nerves were degenerated and surrounded with glial scars, and all vestibular endorgans appeared to be necrotic.

Discussion

The major finding of this study is that the majority of CVN neurons in labyrinth-intact conscious cats have relatively simple responses to tilts in vertical planes, which are similar to those in decerebrate animals (Endo et al. 1995). In both preparations, most CVN neurons exhibited either graviceptive responses consistent with inputs from otolith organs, or dynamic responses with phases synchronized with stimulus velocity. Many of these velocity units had response vector orientations aligned with the plane of a vertical semicircular canal, suggesting that the cells received predominantly canal inputs. In general, the large majority of CVN neurons in both decerebrate and awake cats had response vector orientations that were near the roll or vertical canal planes, although 18% of the cells were

preferentially activated by pitch rotations. In particular, only three neurons with graviceptive responses that were best activated by pitch rotations were detected in this study. This observation is curious considering that the CVN participate in correcting disturbances in blood pressure and breathing elicited by sustained head-up pitch of the body (Uchino et al. 1970; Yates et al. 1993; Yates and Miller 1994; Rossiter et al. 1996; Rossiter and Yates 1996; Woodring et al. 1997; Kerman and Yates 1998; Jian et al. 1999; Cotter et al. 2001, 2004; Wilson et al. 2006).

Following the removal of vestibular inputs, spontaneous activity could be observed in at least a subset of CVN neurons within 24 h, and during the first week after the lesions the firing rate of spontaneously active cells was at least as high as in labyrinth-intact animals. This finding is in agreement with prior observations in guinea pigs (Ris and Godaux 1998), but contradicts a previous study in cats indicating that normal spontaneous activity did not return to the vestibular nuclei until a week after a bilateral labyrinthectomy (Ryu and McCabe 1976). Moderate-amplitude tilts (15°) produced consistent modulation of the activity of a small subset of CVN neurons in two of three animals. Although the fraction of units whose activity was synchronized with the tilts was low, 8% in one cat and 3% in the other, it seems likely that larger changes in body orientation would produce responses in a greater number of cells. The inputs that provided for these responses are unknown; however, since the restraint employed for the animals did not permit overt neck rotation, it seems unlikely (although not impossible) that the signals originated from neck mucles.

The present data support the findings of a previous study conducted in decerebrate cats that had undergone a bilateral vestibular neurectomy, which also showed that the firing of a small percentage of CVN neurons is altered by vertical tilts following the elimination of vestibular inputs (Yates et al. 2000). In both decerebrate and conscious animals lacking labyrinthine inputs, most CVN neurons whose activity was modulated by vertical rotations were preferentially activated by pitch tilts, despite the fact that few units in this region responded best to pitch rotations in labyrinth-intact cats. It is thus unclear whether these alterations in vestibular nucleus neuronal activity elicited by postural changes in animals lacking labyrinthine inputs have functional significance. Previous studies showed that lesions of the CVN result in long-lasting or permanent impairments in adjusting blood pressure during postural alterations (Mori et al. 2005), whereas the effects of a bilateral vestibular neurectomy on cardiovascular control dissipate within a week (Jian et al. 1999), suggesting that integrity of the CVN is essential for adequate autonomic responses during movement. However, the present data do not address whether modulation of the activity of CVN neurons during changes in body position is essential for these responses to occur. An alternate possibility is that spontaneous firing of CVN neurons is adequate to support the activity of cells in other regions that adjust blood pressure during movement in accordance with signals they receive from nonlabyrinthine receptors.

Since a preponderance of CVN neurons in labyrinth-intact animals, particularly those with graviceptive responses, were not preferentially activated by tilts near the pitch plane, it is presently unclear how this region of the vestibular nuclei signals the presence of head-up body rotations that affect homeostasis to brainstem areas involved in cardiovascular and respiratory regulation. One possibility is that convergence of inputs from CVN neurons to downstream regions provides for the detection of changes in body orientation in the pitch plane. For example, a downstream neuron that receives convergent inputs from cells with response vector orientations near the planes of the left and right anterior canals would be best activated by pitch rotations. However, an alternate prospect raised by this study is that the function of the vestibular system in autonomic regulation is limited to adjusting the baseline excitability of brainstem neurons that control blood pressure or breathing. For example, an increase in head movements and the general level of activity in the CVN could induce an increase in excitability of cells in the autonomic regulatory areas, such that they are more responsive to signals such as those from baroreceptors indicating a disturbance in blood pressure or blood oxygenation. As such, the role of the vestibular system in autonomic regulation might simply be to indicate when movements resulting in sudden cardiovascular and respiratory challenges are likely. Further studies will be needed to differentiate between these alternatives.

The CVN have functions in addition to autonomic regulation, as this region projects to the cerebellar nodulus and uvula (Epema et al. 1985; Sato et al. 1989; Thunnissen et al. 1989; Barmack et al. 1992) as well as to the dorsal horn of the upper cervical spinal cord (Peterson et al. 1978; Bankoul et al. 1995). The present data indicate that these areas mainly receive from the CVN relatively simple signals regarding head position or head velocity, at least during rotations in vertical planes. STC behavior and other complex responses were not obvious in a large majority of CVN neurons, despite the fact that this region receives considerable inputs from the uvula and nodulus in addition to projecting to these regions (Shojaku et al. 1987; Patton et al. 1991). An exception is the group of neurons that exhibited a large augmentation in response gain with increasing stimulus frequency, and which had response phases near stimulus velocity when low-frequency tilts were performed that lagged to near stimulus position during high-frequency rotations. The functional significance of this population of cells, as well as the

combination of inputs that produce their responses to vertical rotations, remains to be determined.

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References

Baker J, Goldberg J, Hermann G, Peterson BW (1984) Spatial and temporal response properties of secondary neurons that receive convergent input in vestibular nuclei of adult cats. Brain Res 294:138–143

Balaban CD, Beryozkin G (1994) Vestibular nucleus projections to nucleus tractus solitarius and the dorsal motor nucleus of the vagus nerve: potential substrates for vestibulo-autonomic interactions. Exp Brain Res 98:200–212

Bankoul S, Goto T, Yates BJ, Wilson VJ (1995) Cervical primary afferent input to vestibulospinal neurons projecting to the cervical dorsal horn: an anterograde and retrograde tracing study in the cat. J Comp Neurol 353:529–538

Barmack NH, Yakhnitsa V (2000) Vestibular signals in the parasolitary nucleus. J Neurophysiol 83:3559–3569

Barmack NH, Baughman RW, Eckenstein FP, Shojaku H (1992) Secondary vestibular cholinergic projection to the cerebellum of rabbit and rat as revealed by choline acetyltransferase immunohistochemistry, retrograde and orthograde tracers. J Comp Neurol 317:250–270

Chubb MC, Fuchs AF, Scudder CA (1984) Neuron activity in monkey vestibular nuclei during vertical vestibular stimulation and eye movements. J Neurophysiol 52:724–742

Cotter LA, Arendt HE, Jasko JG, Sprando C, Cass SP, Yates BJ (2001) Effects of postural changes and vestibular lesions on diaphragm and rectus abdominis activity in awake cats. J Appl Physiol 91:137–144

Cotter LA, Arendt HE, Cass SP, Jian BJ, Mays DF, 2nd, Olsheski CJ, Wilkinson KA, Yates BJ (2004) Effects of postural changes and vestibular lesions on genioglossal muscle activity in conscious cats. J Appl Physiol 96:923–930

Dickman JD, Angelaki DE (2002) Vestibular convergence patterns in vestibular nuclei neurons of alert primates. J Neurophysiol 88:3518–3533

Endo K, Thomson DB, Wilson VJ, Yamaguchi T, Yates BJ (1995) Vertical vestibular input to and projections from the caudal parts of the vestibular nuclei of the decerebrate cat. J Neurophysiol 74:428–436

Epema AH, Guldemond JM, Voogd J (1985) Reciprocal connections between the caudal vermis and the vestibular nuclei in the rabbit. Neurosci Lett 57:273–278

Gdowski GT, McCrea RA (1999) Integration of vestibular and head movement signals in the vestibular nuclei during whole-body rotation. J Neurophysiol 82:436–449

Gstoettner W, Burian M, Cartellieri M (1992) Central projections from singular parts of the vestibular labyrinth in the guinea pig. Acta Otolaryngol 112:486–495

Jian BJ, Cotter LA, Emanuel BA, Cass SP, Yates BJ (1999) Effects of bilateral vestibular lesions on orthostatic tolerance in awake cats. J Appl Physiol 86:1552–1560

Jian BJ, Shintani T, Emanuel BA, Yates BJ (2002) Convergence of limb, visceral, and vertical semicircular canal or otolith inputs onto vestibular nucleus neurons. Exp Brain Res 144:247–257

Jian BJ, Acernese AW, Lorenzo J, Card JP, Yates BJ (2005) Afferent pathways to the region of the vestibular nuclei that participates in cardiovascular and respiratory control. Brain Res 1044:241–250

Kasper J, Schor RH, Wilson VJ (1988) Response of vestibular neurons to head rotations in vertical planes. I. Response to vestibular stimulation. J Neurophysiol 60:1753–1764

Kerman IA, Yates BJ (1998) Regional and functional differences in the distribution of vestibulosympathetic reflexes. Am J Physiol Regul Integr Comp Physiol 275:R824–R835

Kevetter GA, Perachio AA (1986) Distribution of vestibular afferents that innervate the sacculus and posterior canal in the gerbil. J Comp Neurol 254:410–424

Martinelli GP, Friedrich VL Jr, Prell GD, Holstein GR (2007) Vestibular neurons in the rat contain imidazoleacetic acid-ribotide, a putative neurotransmitter involved in blood pressure regulation. J Comp Neurol 501:568–581

Mori RL, Cotter LA, Arendt HE, Olsheski CJ, Yates BJ (2005) Effects of bilateral vestibular nucleus lesions on cardiovascular regulation in conscious cats. J Appl Physiol 98:526–533

Newlands SD, Perachio AA (2003) Central projections of the vestibular nerve: a review and single fiber study in the Mongolian gerbil. Brain Res Bull 60:475–495

Newlands SD, Purcell IM, Kevetter GA, Perachio AA (2002) Central projections of the utricular nerve in the gerbil. J Comp Neurol 452:11–23

Newlands SD, Vrabec JT, Purcell IM, Stewart CM, Zimmerman BE, Perachio AA (2003) Central projections of the saccular and utricular nerves in macaques. J Comp Neurol 466:31–47

Patton JFR, La Noce A, Sykes RM, Sebastiani L, Bagnoli P, Ghelarducci B, Bradley DJ (1991) Efferent connections of lobule IX of the posterior cerebellar cortex in the rabbit—some functional considerations. J Auton Nerv Syst 36:209–223

Peterson BW, Maunz RA, Fukushima K (1978) Properties of a new vestibulospinal projection, the caudal vestibulospinal tract. Exp Brain Res 32:287–292

Porter JD, Balaban CD (1997) Connections between the vestibular nuclei and brain stem regions that mediate autonomic function in the rat. J Vestib Res 7:63–76

Ris L, Godaux E (1998) Neuronal activity in the vestibular nuclei after contralateral or bilateral labyrinthectomy in the alert guinea pig. J Neurophysiol 80:2352–2367

Rossiter CD, Yates BJ (1996) Vestibular influences on hypoglossal nerve activity in the cat. Neurosci Lett 211:25–28

Rossiter CD, Hayden NL, Stocker SD, Yates BJ (1996) Changes in outflow to respiratory pump muscles produced by natural vestibular stimulation. J Neurophysiol 76:3274–3284

Ruggiero DA, Mtui EP, Otake K, Anwar M (1996) Vestibular afferents to the dorsal vagal complex: substrate for vestibular-autonomic interactions in the rat. Brain Res 743:294–302

Ryu JH, McCabe BF (1976) Central vestibular compensation. Effect of the bilateral labyrinthectomy on neural activity in the medial vestibular nucleus. Arch Otolaryngol 102:71–76

Sato Y, Kanda K, Ikarashi K, Kawasaki T (1989) Differential mossy fiber projections to the dorsal and ventral uvula in the cat. J Comp Neurol 279:149–164

Schor RH, Angelaki DE (1992) The algebra of neural response vectors. Ann N Y Acad Sci 656:190–204

Schor RH, Miller AD, Tomko DL (1984) Responses to head tilt in cat central vestibular neurons. I. Direction of maximum sensitivity. J Neurophysiol 51:136–146

Shojaku H, Sato Y, Ikarashi K, Kawasaki T (1987) Topographical distribution of Purkinje cells in the uvula and the nodulus projecting to the vestibular nuclei in cats. Brain Res 416:100–112

Thunnissen IE, Epema AH, Gerrits NM (1989) Secondary vestibulocerebellar mossy fiber projection to the caudal vermis in the rabbit. J Comp Neurol 290:262–277

Uchino Y, Kudo N, Tsuda K, Iwamura Y (1970) Vestibular inhibition of sympathetic nerve activities. Brain Res 22:195–206

Wilson VJ, Melvill Jones G (1979) Mammalian vestibular physiology. Plenum Press, New York

Wilson TD, Cotter LA, Draper JA, Misra SP, Rice CD, Cass SP, Yates BJ (2006) Vestibular inputs elicit patterned changes in limb blood flow in conscious cats. J Physiol 575:671–684

Woodring SF, Rossiter CD, Yates BJ (1997) Pressor response elicited by nose-up vestibular stimulation in cats. Exp Brain Res 113:165–168

Yates BJ, Miller AD (1994) Properties of sympathetic reflexes elicited by natural vestibular stimulation: implications for cardiovascular control. J Neurophysiol 71:2087–2092

Yates BJ, Bronstein AM (2005) The effects of vestibular system lesions on autonomic regulation: observations, mechanisms, and clinical implications. J Vestib Res 15:119–129

Yates BJ, Jakus J, Miller AD (1993) Vestibular effects on respiratory outflow in the decerebrate cat. Brain Res 629:209–217

Yates BJ, Grélot L, Kerman IA, Balaban CD, Jakus J, Miller AD (1994) Organization of vestibular inputs to nucleus tractus solitarius and adjacent structures in cat brain stem. Am J Physiol 267:R974–R983

Yates BJ, Balaban CD, Miller AD, Endo K, Yamaguchi Y (1995) Vestibular inputs to the lateral tegmental field of the cat: potential role in autonomic control. Brain Res 689:197–206

Yates BJ, Jian BJ, Cotter LA, Cass SP (2000) Responses of vestibular nucleus neurons to tilt following chronic bilateral removal of vestibular inputs. Exp Brain Res 130:151–158

Yingcharoen K, Siegborn J, Grant G (2003) Brainstem projections of different branches of the vestibular nerve: an experimental study by transganglionic transport of horseradish peroxidase in the cat. III. The saccular nerve. Exp Brain Res 151:190–196

Zhou W, Tang BF, Newlands SD, King WM (2006) Responses of monkey vestibular-only neurons to translation and angular rotation. J Neurophysiol 96:2915–2930