Vestibular nucleus neurons respond to hindlimb movement in the decerebrate cat

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Arshian MS, Hobson CE, Catanzaro MF, Miller DJ, Puterbaugh SR, Cotter IA, Yates BJ, McCall AA. Vestibular nucleus neurons respond to hindlimb movement in the decerebrate cat. J Neurophysiol 111: 2423–2432, 2014. First published March 26, 2014; doi:10.1152/jn.00855.2013.—The vestibular nuclei integrate information from vestibular and proprioceptive afferents, which presumably facilitates the maintenance of stable balance and posture. However, little is currently known about the processing of sensory signals from the limbs by vestibular nucleus neurons. This study tested the hypothesis that limb movement is encoded by vestibular nucleus neurons and described the changes in activity of these neurons elicited by limb extension and flexion. In decerebrate cats, we recorded the activity of 70 vestibular nucleus neurons whose activity was modulated by limb movements. Most of these neurons (57/70, 81.4%) encoded information about the direction of hindlimb movement, while the remaining neurons (13/70, 18.6%) encoded the presence of hindlimb movement without signaling the direction of movement. The activity of many vestibular nucleus neurons that responded to limb movement was also modulated by rotating the animal’s body in vertical planes, suggesting that the neurons integrated hindlimb and labyrinthine inputs. Neurons whose firing rate increased during ipsilateral ear-down roll rotations tended to be excited by hindlimb flexion, whereas neurons whose firing rate increased during contralateral ear-down tilts were excited by hindlimb extension. These observations suggest that there is a purposeful mapping of hindlimb inputs onto vestibular nucleus neurons, such that integration of hindlimb and labyrinthine inputs to the neurons is functionally relevant.

multisensory integration; hindlimb; balance; locomotion; proprioceptor

THE VESTIBULAR SYSTEM EXQUISITELY signals head accelerations in space but alone is incapable of providing the information needed to decipher body position and movement in space and to achieve postural stability (Goldberg et al. 2012; Mergner and Rosemeier 1998). A number of studies have focused on the integration of labyrinthine and neck proprioceptive signals in the vestibular nuclei and the deep cerebellar nuclei (Anastasopoulos and Mergner 1982; Boyle and Pompeiano 1981; Brooks and Cullen 2009; Kasper et al. 1988; Luan et al. 2013; Roy and Cullen 2004; 2001; Wilson et al. 1990). The modulation of vestibular signals (carrying information about head-in-space movement) by neck proprioceptive signals (carrying information about head relative to body movement) provides body-in-space information to the central nervous system, which has been hypothesized to serve as an important reference frame for generating motor commands that produce postural adjustments (Brooks and Cullen 2009; Cullen et al. 2011; Goldberg et al. 2012).

Proprioceptive signals from other regions of the body may also modify vestibular system responses that stabilize balance. For example, there is evidence from studies in humans that vestibulospinal reflexes are altered to account for limb position (Anker et al. 2012; Grasso et al. 2011; Marsden et al. 2002; Rosker et al. 2011; Wang and Newell 2012; Welgampola and Colebatch 2001). However, the neural mechanisms responsible for these adjustments in vestibulospinal responses are yet to be elucidated. Anatomical studies demonstrated that neurons at all levels of the spinal cord project to the vestibular nuclei and relay proprioceptive information from the limbs to vestibular nucleus neurons (Grottel and Jakielksbakowska 1993; Hoddevik et al. 1975; Jian et al. 2005; McKelvey-Briggs et al. 1989; Pompeiano 1972; Robbins et al. 1990). Physiological studies showed that vestibular nucleus neurons respond to electrical stimulation of limb nerves, including those innervating muscles (Jian et al. 2002; McCall et al. 2013b). Some vestibular nucleus neurons responded to low stimulus intensities that presumably activated only large afferents from muscle spindles. There is also evidence that the activity of vestibular nucleus neurons is modulated during locomotion (Matsuyama and Drew 2000; Orlovsky 1972), although the studies did not elucidate whether the responses were elicited by limb movements or head translations that occur during walking or were due to efference copy. Passive limb movements were also shown to affect the activity of vestibular nucleus neurons (Fredrickson et al. 1966; Orlovsky 1972), but the studies entailed manual movements of the limbs, such that the dynamic properties of responses of vestibular nucleus neurons to limb motion could not be ascertained.

The primary goal of these experiments was to characterize the effects of ramp-and-hold (trapezoidal) and sinusoidal hindlimb movements on the activity of vestibular nucleus neurons. The decerebrate, paralyzed cat preparation was used to assure that neuronal responses were due to limb movement and were not produced by efference copy. Furthermore, use of this preparation permitted equivalent stimuli to be delivered during each trial, as changes in muscle tone in conscious animals could have altered the sensory inputs elicited by limb movements. No attempts were made to determine whether the neurons sampled projected to the spinal cord, out of concerns that insertion of arrays of stimulating electrodes into the spinal cord could have damaged ascending pathways and altered responses of vestibular nucleus neurons to hindlimb move-
ment. Recordings were focused on the caudal aspect of the vestibular nucleus complex, since prior anatomical studies showed that this region receives projections from all levels of the spinal cord, including the lumbar spinal levels, and physiologic studies showed that caudal vestibular nucleus neurons respond to electrical activation of hindlimb nerves (Jian et al. 2002; McCall et al. 2013b; McKelvey-Briggs et al. 1989). We also determined the responses of neurons with hindlimb inputs to whole body rotations in vertical planes that activate vestibular endorgans and ascertained if there was any correlation between patterns of responses to limb and vestibular stimulation. We hypothesized that the activity of vestibular nucleus neurons was related to limb position in space and that the spatial tuning of neurons to vertical vestibular stimulation would be correlated with the directional responsiveness of those same neurons to hindlimb movement.

**METHODS**

**Materials and methods.** Experiments were performed on 11 purpose-bred (Liberty Research, Waverly, NY) felines of either sex weighing 2–4 kg. All experimental procedures conformed to the Guide for the Care and Use of Laboratory Animals (National Research Council, National Academies Press, Washington, DC, 2011). The methods were prospectively approved by the Institutional Animal Care and Use Committee at the University of Pittsburgh. A portion of the experimental protocol used in these experiments has been described in detail in recent articles (Moy et al. 2012; Sugiyama et al. 2011) and thus will be succinctly discussed below. Novel hindlimb movement experimental paradigms and analytic procedures introduced in these experiments will be discussed in greater detail.

**Surgical procedures.** Animals were anesthetized using isoflurane vaporized in oxygen and tracheotomized. After the tracheotomy, anesthesia was delivered through the intratracheal tube. Blood pressure was recorded using a Millar Instruments transducer (Houston, TX) inserted through the left femoral artery. An intravenous line was introduced into the left femoral vein for drug and fluid delivery. The carotid arteries were ligated bilaterally to halt anterior cerebral circulation, and a midcerebellar decerebration was performed. The head was secured in a stereotaxic frame and the body was secured using a clamp placed on the T1 dorsal spinous process and hip pins. The stereotaxic frame was mounted on a servo-controlled hydraulic tilt device (Neurokinetics, Pittsburgh, PA). A craniotomy was performed overlying the caudal cerebellum and brainstem, and a small portion of the caudal-lateral cerebellum was aspirated to gain access to the caudal aspect of the vestibular nucleus complex. A Velcro strap was wrapped around the right hindlimb immediately proximal to the ankle, ipsilateral to the brainstem recording site, and attached to a servo-controlled rotational motor. A hinged connector between the Velcro strap and the computer-controlled servomotor permitted rotation about the knee and hip joints while precluding motion at other hindlimb joints, including the ankle and metatarsophalangeal joints (see Fig. 1A). Range of motion about the hip and knee was measured and recorded in six animals at the maximum stimulus amplitude applied in these experiments of 60° (stimulus parameters are outlined below). Average range of motion when the hindlimb was moved from maximum extension to maximum flexion was 27.5° about the knee and 40.8° about the knee, which is similar to the range of motion about these joints during the normal ambulatory cycle of the cat (Trank et al. 1996).

Atropine sulfate (0.15 mg/kg) was injected intramuscularly every 6 h to reduce airway secretions, and dexamethasone (1 mg/kg) was injected intravenously every 6 h to minimize brain swelling. Temperature was monitored continuously via a rectal probe and maintained between 37–38°C using a DC-powered heating pad and a heating lamp. Following the completion of surgical procedures, anesthesia was removed and animals were paralyzed using injections of Vecuronium bromide (0.1 mg/kg iv every 20 min). End tidal CO₂ was continuously monitored throughout the experiment and maintained near 4% by adjusting ventilator parameters. Blood pressure was continuously monitored and maintained >100 mmHg by infusion of saline. Phenylephrine (1% solution, titrated to maintain blood pressure >90) was used to support blood pressure in situations where saline infusions were insufficient. At the completion of the recording session, animals were euthanized via intravenous infusion of Euthasol solution (120 mg/kg).

**Recording procedures.** Extracellular recordings of vestibular nucleus neuronal activity were performed using 4–6 MiΩ tungsten microelectrodes (FHC, Bowdoin, ME). Unit activity was captured at 25 kHz while blood pressure, tilt table position, and ipsilateral hindlimb position were sampled at 100 Hz using a Cambridge Electronic Design 1401 data collection system and Spike 2 software (Cambridge, UK). We often used hindlimb movement as a search stimulus and specifically targeted neurons that were responsive to this stimulus. In other instances, whole body movements (vestibular stimulation) were used as a search stimulus, and we tested whether neurons with vestibular inputs responded to hindlimb movement.

To provide hindlimb movement, the servo-controlled motor attached to the hindlimb was controlled via custom programs developed within the Spike2 software. Both ramp-and-hold and sinusoidal hindlimb movements were delivered. In the ramp-and-hold paradigm, the hindlimb was initially maintained in the midline (neutral) position and then brought to 60° extension, back to midline, then to 60° flexion, and again back to midline. Ramps preceding each position segment were presented at three velocities (60, 30, and 15°/s) and the limb was held for 7 s in each position before moving on to the next. The ramp-and-hold stimulus was repeated five times for each velocity tested. These three hindlimb velocities were tested to determine if the magnitude of neuronal responses was dependent on hindlimb velocity.

**Fig. 1.** A: position of the hindlimb and the approximate angles of the knee and hip joints during the midline, extension, and flexion phases of the stimulus. B: method for determining responses of vestibular nucleus neurons to hindlimb movement. Neuronal firing is binned in 0.1-s intervals. The final second of the midline (neutral) position was taken as baseline (bracket from 4 to 5 s) and was used as the basis for comparison. The bin in the subsequent segment (ramp-and-hold movement from midline to flexion in this example) with the highest count was identified (arrow) and the surrounding bins (4 preceding and 5 following) were used as measures of peak firing in response to the hindlimb movement. Ramp velocity: 60°/s. M-F, midline to flexion interval (hindlimb movement to reposition the limb from the midline to flexion positions).
In three of the animals, only one velocity of movement was tested (30°/s) and the limb was held in each position for 30 s. Thus data are not available from these three animals to determine whether hindlimb stimulus velocity affected the responses. Sinusoidal movements were employed to determine responsiveness to frequency and amplitude of hindlimb movements. Frequency trials consisted of sinusoidal hindlimb movements delivered across frequencies of 0.05–1 Hz at 60° amplitude. Amplitude trials consisted of sinusoidal hindlimb movements applied at a constant frequency (0.5 Hz) while varying amplitude from 7.5–60°.

To ascertain if a neuron received vestibular inputs, we first determined its responses to 0.5 Hz wobble stimuli (Schor et al. 1984). The wobble stimulus consists of a constant amplitude tilt that rotates at a constant speed about the animal’s body such that, when viewed from above, the animal appears to wobble. Wobble stimuli were delivered in both the clockwise (CW) and counterclockwise (CCW) directions. To deliver the CW wobble stimulus, two sine waves 90° out of phase with each other were fed to the pitch and roll planes simultaneously, such that the animal’s body sequentially moved through nose down, right ear down, nose up, and left ear down positions. When the sine wave to the pitch axis was inverted, a CCW wobble stimulus was generated. A tilt amplitude of 5–7.5° was employed during the wobble stimuli. With the use of the responses generated from the CW and CCW wobble stimuli, the response vector orientation was determined, which corresponds to the direction of tilt that produced a maximal change in neuronal activity (Schor et al. 1984). The dynamic properties of responses to single vertical plane sinusoidal tilts near the response vector orientation were then ascertained. Single plane tilts were delivered at frequencies from 0.05 to 1 Hz and amplitudes from 2.5–10°.

Data analysis procedures. The spike-sorting feature of Spike 2 software was used to isolate the activity of particular units in the recording field. Trials in which it was difficult to segregate each unit’s activity were discarded.

Responses to the ramp-and-hold hindlimb movement stimulus were analyzed in the manner described below to determine if hindlimb extension and/or flexion movements resulted in an appreciable change in neuronal firing. Unit counts were binned in 0.1-s increments across each repetition of the ramp-and-hold stimulus. Bin counts from each repetition of the ramp-and-hold stimulus were combined (separately for each velocity tested) to generate a composite response histogram. Figure 1B shows an example of one portion of a composite response histogram generated from five trials at 60°/s. Neuronal firing in response to ramp-and-hold movements of the hindlimb often peaked near the end of the ramp movement and then decayed during the hold portion (as shown in Fig. 1B). This response pattern was observed both during hindlimb movements away from midline as well as movements back towards midline. Because of this decay phenomenon, counts from the 10 bins corresponding to the last second of time of the midline hindlimb position were used as independent measures of baseline neuronal activity, rather than considering all bins during the entire midline position. The extension segment of the composite response histogram was then analyzed for a change in activity compared with baseline. In cases where an excitatory response appeared to be present, the bin with peak firing was identified. In cases where an inhibitory response appeared to be present, the bin with minimal firing was identified. Bin counts from the 1-s time interval that surrounded this peak or trough were used as measures of neuronal activity associated with hindlimb extension and/or flexion. Bin counts were compared with baseline and hindlimb movement conditions, respectively. Bin counts were also performed to identify bin counts that represented neuronal activity associated with hindlimb flexion (see Fig. 1B). Bin counts representative of baseline activity (midline hindlimb position) were then compared with bin counts collected as the hindlimb moved to extension or flexion. We determined if a statistically significant difference in firing rate occurred with hindlimb extension or flexion compared with midline using the Mann-Whitney test ($P < 0.05$). We then evaluated if the average bin count with the hindlimb in extension or flexion was $>20\%$ different than the average baseline bin count. Statistically significant responses with a $<20\%$ change in average bin count were excluded to decrease the chances of false positive responses. Finally, only response patterns that were consistent across all velocities of hindlimb movement tested were deemed significant. Units were considered responsive to hindlimb extension or flexion when they met all three criteria: statistically significant change in firing compared with baseline, increase or decrease in firing of $>20\%$ compared with baseline, and consistent responses across the velocities tested. This type of analysis was also used to test for responses when the hindlimb was moved back to the midline position from either extension or flexion positions. Responses to hindlimb extension or flexion movements were then used to categorize vestibular nucleus neuronal responses, as will be detailed in the results section.

Although neuronal responses to flexion or extension of the hindlimb typically dissipated, we additionally considered whether changes in activity were sustained throughout the hold period. For this purpose, the Mann-Whitney test was used to compare cell firing counts from the 10 bins corresponding to the last second of the hold position to counts for the last 10 bins when the limb was in the midline position, as described above.

Neuronal responses to sinusoidal hindlimb movements and whole body tilts were binned at 500 bins/cycle and averaged over the stimulus period. Sine waves were fit to neuronal responses using a least squares minimization technique and the signal-to-noise ratio was calculated (Schor et al. 1984). Responses were considered significant if the signal-to-noise ratio was $>0.5$, only the first harmonic was prominent, and responses were consistent from trial to trial. Response phase and gain were respectively determined by comparing the amplitude and phase shifts of stimulus position and response sine waves. Statistical analyses were performed using Prism 6 software (GraphPad Software, San Diego, CA). Pooled data are represented as means ± SE.

Histological procedures. At the completion of each recording session, a lesion was made at defined stereotaxic coordinates relative to the recording sites by passing a 250-μA negative current through the recording electrode for 60 s. Following euthanasia, the brainstem was removed and fixed in 10% formaldehyde solution. The brainstem was then sectioned transversely (100-μm thickness), mounted serially on slides, stained with 1% thionine, and coverslipped. Recording sites were reconstructed with reference to the location of the electrotylocic lesion, the relative positions of recording tracks, and the depths from the brainstem surface where units were recorded.

RESULTS

Hindlimb movement alters activity of vestibular nucleus neurons. Seventy vestibular nucleus neurons whose activity was modulated by movements of the hindlimb were identified. Most of the neurons sampled were identified by using hindlimb movement as a search stimulus. Out of 57 neurons initially identified as responding to vertical vestibular stimulation, 11 (19%) responded to ipsilateral limb movement. Figure 2 shows activity recorded from one neuron whose firing rate increased with hindlimb flexion and whose firing rate decreased with hindlimb extension.

We designed the experimental protocol to parse the effects of hindlimb velocity and position on the activity of vestibular nucleus neurons by considering responses to both ramp-and-hold and sinusoidal stimuli. During the ramp portion of the ramp-and-hold paradigm, hindlimb velocity was constant with a corresponding changing hindlimb position. During the hold portion of the stimulus, hindlimb velocity was zero and the position signal was constant. During sinusoidal stimuli, stimulus position and velocity were constantly changing, but the
peak velocity of the movement increased as stimulus frequency increased. Consequently, delivery of sinusoidal stimuli at multiple frequencies allowed a determination of the effects of stimulus velocity on response magnitude.

The vast majority of vestibular nucleus neurons developed peak responses near the junction of the ramp and the hold segments of hindlimb motion (see Fig. 3, A–C, for examples). For example, the average latency of peak activity of vestibular nucleus neurons following the onset of a 1-s hindlimb ramp movement (60°/s) from midline to extension was 0.8 ± 0.1 s (median 0.8 s). Following this peak in activity, while the limb was maintained in the new position there was typically a slow decay in activity. However, the activity remained well above baseline activity measured when the hindlimb was in the midline position (see Fig. 3, A–C, for examples). This sustained increase in activity, determined at the end of the hold segment, was statistically significant (Mann-Whitney, \(P < 0.05\)) in the majority of neurons (60 of 70, 85.7%). To summarize, vestibular nucleus neuronal peak activity occurred near the end of the ramp segment of the stimulus and slowly decayed during the hold segment. This pattern of response restricted our ability to parse whether responses were related to the velocity or position components of hindlimb movement. Furthermore, the interpretation of the findings was also confounded by the fact that vestibular nucleus neurons can respond nonlinearly to stimuli (Massot et al. 2012; Newlands et al. 2009).

Comparisons of magnitudes of responses were also made across the three different stimulus velocities tested (60, 30, and 15°/s). Use of a 30°/s velocity ramp produced a peak response that was only 6.2 ± 2.6% larger than the response to a 15°/s velocity ramp. Similarly, responses to a 60°/s velocity ramp were just 11 ± 2.7% larger than responses to a 15°/s velocity ramp, despite the fact that the stimulus velocity was quadrupled. These small increases in response magnitude with large increases in hindlimb velocity argue against hindlimb velocity being the major determinant of the alterations in vestibular nucleus neuronal activity in response to hindlimb movement. Delivery of sinusoidal hindlimb movements, discussed in detail below, was additionally used to discriminate if hindlimb velocity or position signals were responsible for generating the responses of vestibular nucleus neurons during hindlimb movement. A caveat, as noted above, is that vestibular nucleus neurons can respond nonlinearly to stimuli (Massot et al. 2012; Newlands et al. 2009), which complicates the interpretation of responses to rotations at different velocities or amplitudes.

There were three potential responses for each hindlimb movement: excitation response, inhibition response, or nonresponse. Response outcomes were analyzed independently for each of the four hindlimb movements (from midline to extension, from extension to midline, from midline to flexion, and from flexion to midline). Responses to hindlimb movements away from midline (from midline to extension and from midline to flexion) were used as the primary criterion in the categorization schema outlined in detail below. Responses to hindlimb movements toward midline (from extension to mid-
line and from flexion to midline) were used to augment the categorization schema. Neural responses to hindlimb motion ultimately fell into four categories: unidirectional, reciprocal, bidirectional, and omnidirectional (Fig. 3).

The most common response pattern of vestibular nucleus neurons (52.9%, 37 neurons) to hindlimb ramp-and-hold movements was the reciprocal classification (Fig. 3A). Neurons in the reciprocal category responded with an increase in firing rate during one direction of hindlimb movement away from midline and with a decrease in firing rate during the opposite direction of hindlimb movement away from midline (in Fig. 3A, the reciprocal neuron exhibits an excitation response to hindlimb extension and an inhibitory response to hindlimb flexion). Neurons with this response type were subcategorized by the direction of movement that resulted in the excitation response. Twenty neurons exhibited excitation responses to hindlimb extension and seventeen to hindlimb flexion.

The activity of another group of neurons was modulated in response to one direction of hindlimb movement away from midline (such as midline to extension, as is the case for the neuron in Fig. 3B) but was nonresponsive to the other direction of hindlimb movement away from midline (midline to flexion in this example). Neurons that exhibited this response pattern were termed unidirectional. Twenty vestibular nucleus neurons (28.6%) had unidirectional patterns of response activity to hindlimb movement. The direction of hindlimb movement away from midline that generated the response was nearly equally split between flexion (11 neurons) and extension (9 neurons). Excitation responses were present for the majority of neurons (16 neurons, 7 responding to flexion, and 9 responding to extension), with inhibitory responses present for the remaining neurons (4 neurons, all responding to flexion).

The activity of 10 neurons (14.3%) was modulated in response to hindlimb movements away from midline with the same response type (excitation or inhibition) to both directions of movement. Neurons in this response category were termed bidirectional (Fig. 3C). For example, a neuron would be categorized as bidirectional if it exhibited an excitation response during hindlimb movement from midline to extension and it also exhibited an excitation response during hindlimb movement from midline to flexion (as in Fig. 3C). Five of the neurons within the bidirectional category exhibited excitation responses and five exhibited inhibition responses.

Neurons within the unidirectional, reciprocal, or bidirectional categories all responded to hindlimb movements from the extension or flexion positions back to midline in the same manner: the response pattern was opposite to that elicited by the movement from midline (see Fig. 3, A–C) or there was no detectable response. For example, if a neuron with a unidirectional response pattern exhibited an excitation response to hindlimb extension, it either would show an inhibition response with hindlimb movement back to the midline position (as in Fig. 3B) or would not exhibit a response. One group of neurons had a distinctly different response pattern with hindlimb movements from extension or flexion back to midline. These neurons exhibited the same responses when the hindlimb was moved away from and back to midline (for example, the firing of the neuron would increase as the hindlimb was moved from midline to extension, as well as from the extended position back to midline, as illustrated in Fig. 3D). This response type was termed omnidirectional.

Fig. 3. Vestibular nucleus neurons exhibited one of four categories of responses to ramp-and-hold movements of the hindlimb: reciprocal (A), unidirectional (B), bidirectional (C), or omnidirectional (D). *Responses that met the criteria outlined in the methods section and Fig. 1. Bins are in 0.1-s intervals. Ramp speed: 60°/s.
because neurons with such responses had similar changes in activity regardless of the direction of hindlimb movement that elicited the response. Three neurons exhibited omnidirectional response patterns.

The dynamic properties of responses of vestibular nucleus neurons to sinusoidal movements of the hindlimb were ascertained for 51 of the 57 reciprocal and unidirectional neurons (Fig. 4). Bidirectional and omnidirectional neurons did not exhibit a sinusoidal response to sinusoidal movements of the hindlimb because their activity was modulated during multiple phases of the hindlimb movement. As such, neurons with bidirectional and omnidirectional responses to hindlimb movement were excluded from this analysis. The gain of responses of vestibular nucleus neurons to sinusoidal hindlimb movement increased by a factor of $1.64 \pm 0.09$ per stimulus decade as stimulus frequency was advanced. Responses led hindlimb position slightly, and the advances in response phases increased modestly as stimulus frequency became higher, with the average phase advance increasing from $29 \pm 4^\circ$ at 0.05 Hz to $70 \pm 3^\circ$ at 1 Hz. These values were significantly different (Wilcoxon matched pairs signed rank test, $P = 0.001$). Sensitivity of vestibular nucleus neurons to hindlimb movement (firing rate per degree of rotation) increased when small-amplitude sinusoidal hindlimb movements were provided: from $0.5 \pm 0.1$ spikes s$^{-1}$ deg$^{-1}$ when 60$^\circ$ sinusoidal movements were delivered to $1.4 \pm 0.2$ spikes s$^{-1}$ deg$^{-1}$ when 7.5$^\circ$ sinusoidal movements were delivered (Wilcoxon matched pairs signed rank test, $P < 0.0001$), as indicated in Fig. 5.

Vestibular nucleus neurons that responded to hindlimb motion were tested for responses to whole body rotations in vertical planes that activated labyrinthine receptors. Nineteen neurons were lost after hindlimb movement testing and before completion of the vestibular stimulation protocol. Thus the responsiveness of these neurons to vestibular inputs is uncertain. Of the remaining 51 neurons, 29 (56.9%) exhibited responses to whole body rotations in vertical planes. Response vector orientations could be determined for 26 of these neurons (Fig. 6), and the remaining three units exhibited spatial-temporal convergence behavior wherein a response vector cannot be determined because there is convergence of vestibular inputs with different spatial and temporal properties (McCall et al. 2013a; Moy et al. 2012; Schor and Angelaki 1992). Neurons were classified as having spatial-temporal convergence behavior if their activity was modulated more robustly by one direction of the wobble stimulus (CW or CCW) than the other (gain to one direction of wobble stimulus more than two times that to the opposite direction of wobble stimulus), and the results were repeatable from trial to trial. The vast majority of hindlimb movement responsive neurons that exhibited responses to vestibular stimulation in vertical planes had response vector orientations within 45$^\circ$ of the roll plane (24 neurons, 92%), which were most often near the contralateral ear down rather than the ipsilateral ear down direction (16 vs. 8 neurons, respectively). These response vector orientations were similar to those reported in a recent study from our laboratory that considered responses to vertical tilts of neurons.
in the same region of the vestibular nucleus complex (Arshian et al. 2013). For neurons that responded to hindlimb motion with reciprocal or unidirectional responses, we considered whether there was a relationship between the direction of limb movement and the direction of vestibular stimulation that increased neuronal activity. Neurons with reciprocal or unidirectional responses were considered because their firing rate increased during a particular direction of movement of the hindlimb. In contrast, neurons with bidirectional and omnidirectional responses did not explicitly encode information about the direction the hindlimb was moving. Six of seven (86%) reciprocal and unidirectional neurons with response vector orientations within 90° of ipsilateral ear-down roll had excitatory responses to hindlimb flexion (Fig. 6). The opposite was true for reciprocal and unidirectional neurons with response vector orientations within 90° of contralateral ear down roll, with 9 of 12 (75%) neurons having excitatory responses to hindlimb extension. This relationship between directions of body rotation and hindlimb movement that elicited increased activity of vestibular nucleus neurons was statistically significant (P < 0.02, Fisher’s exact test).

Sufficient data were obtained for 21 neurons that responded to both hindlimb movement and whole body rotations to construct Bode plots illustrating the dynamic properties of their responses to vertical plane vestibular stimulation. The average Bode plot for these neurons is shown in Fig. 7. Response gains for most neurons were similar across the range of tilt frequencies delivered, increasing from $2.6 \pm 0.8$ spikes·s$^{-1}$·deg$^{-1}$ at 0.05 Hz to $6.6 \pm 1.5$ spikes·s$^{-1}$·deg$^{-1}$ at 1.0 Hz. On average, response phases led stimulus position by $20^\circ \pm 19^\circ$ at 0.05 Hz and $45^\circ \pm 16^\circ$ at 1.0 Hz. Eighteen neurons exhibited response dynamics resembling those of otolith organ afferents: response gains increased only slightly with advancing stimulus velocity and response phases were near stimulus position (Anderson et al. 1978; Fernández and Goldberg 1976). Three neurons had response dynamics resembling those of primary semicircular canal afferents: steep gain advances with increasing stimulus frequency and phase leads approximating stimulus velocity (Anderson et al. 1978; Fernández and Goldberg 1976). Such responses to vestibular stimulation are similar to those that have previously been described for neurons in the caudal aspect of the vestibular nucleus complex of decerebrate cats (Arshian et al. 2013; Endo et al. 1995; Jian et al. 2002).

Hindlimb movement modifies the activity of vestibular nucleus neurons within the lateral, medial, and inferior vestibular nuclei. Fifty-five of the 70 neurons identified with responses to hindlimb movement were located within the caudal portion of vestibular nucleus complex (medial and inferior vestibular nuclei); the remaining 15 neurons were located within the dorsal division of the lateral vestibular nucleus (Fig. 8). No neurons were tested for responsiveness to hindlimb motion within the superior vestibular nucleus. No relationships were apparent between the location of a neuron in the vestibular nucleus complex and its hindlimb movement response category or the dynamics of its responses to vestibular stimulation.

DISCUSSION

Previous studies that utilized electrical stimulation of hindlimb nerves (Jian et al. 2002; McCall et al. 2013b) or manual positioning of the hindlimbs (Fredrickson et al. 1966; Orlovsky 1972) demonstrated that hindlimb inputs affect the activity of vestibular nucleus neurons. The present study extended these results by showing that most vestibular nucleus neurons that
responded to hindlimb movement encoded the direction of the movement, with approximately half of the neurons responding to hindlimb extension and the other half responding to hindlimb flexion. A small proportion of vestibular nucleus neurons encoded limb movement but not the direction of movement. These findings suggest that the responses of some vestibular nucleus neurons to stimulation of labyrinthine receptors would be altered either in accordance with the position of the limbs in space, or whether the limbs were stationary or in motion. The activity of only a subset (19%) of vestibular nucleus neurons was modulated by movement of the ipsilateral hindlimb that included rotation of the knee and hip joints, but not more distal joints. It is yet to be determined whether the activity of additional neurons would have been affected by movements that included rotation of distal hindlimb joints or by movement of the contralateral limb.

In addition, the response vector orientations of vestibular nucleus neurons to vertical plane whole body rotations correlated with the directional responses to hindlimb movement. Neurons with response vector orientations near ipsilateral ear down were preferentially excited by hindlimb flexion. Conversely, neurons with vector orientations near contralateral ear down roll were preferentially excited by hindlimb extension. These observations suggest that there is a purposeful mapping of hindlimb inputs onto vestibular nucleus neurons, such that integration of hindlimb and labyrinthine inputs to the neurons is functionally relevant.

Previous studies examined the effects of proprioceptive inputs from the neck on the activity of vestibular nucleus neurons (Cullen et al. 2011; McCrea et al. 1999; Roy and Cullen 2001, 2004). In nonhuman primates, vestibular nucleus neurons respond to the velocity component of neck rotation but do not change their firing rate when the body is oriented in different positions relative to an Earth-stationary head. In contrast, the present study showed that the firing rate of some vestibular nucleus neurons is altered in accordance with hindlimb position in space. These findings suggest that the functional significance of proprioceptive signals from different regions of the body to the vestibular nuclei may vary. Whereas neck and vestibular signals are integrated by the vestibular system to decipher body movement in space, hindlimb signals to the vestibular system could play a vastly different role. Our working hypothesis is that these signals serve to adjust vestibulospinal reflexes to account for limb position in space when a balance perturbation occurs.

This study did not explicitly test how hindlimb signals affect the processing of labyrinthine inputs by vestibular nucleus neurons, although it was evident that many neurons received convergent limb and vestibular signals. There is limited and contradictory information in the literature regarding the modulation of labyrinthine signals by hindlimb inputs. In decerebrate cats, dorsiflexion or ventroflexion of the wrist affects the magnitude of responses of Purkinje cells in the cerebellar vermis to whole body rotations (Buschini et al. 2006). In the anesthetized rat model, varying limb position altered the gain and dynamics of responses to vestibular stimulation of cerebellar cortex neurons but not vestibular nucleus neurons (Barresi et al. 2012). In contrast, in decerebrate guinea pigs the magnitude of responses to roll tilts of vestibular nucleus neurons was modulated during locomotion (Marlinsky 1992). It is unclear whether the discrepancies in the results were due to differences in species, or to differences in preparations (anesthetized vs. decerebrate). Thus additional studies will be needed to determine the effects of hindlimb movement on the processing of labyrinthine inputs by vestibular nucleus neurons.

Locomotion entails both head translations and limb movements, and thus deciphering the integration of vestibular and hindlimb inputs by vestibular nucleus neurons could be complicated in conscious animals. It is possible that the influences of limb inputs on the processing of labyrinthine inputs is only relevant when these inputs deviate from the pattern expected during locomotion, as during stumbling. Treadmill walking in head-fixed animals could result in combination of labyrinthine and proprioceptive signals that is unexpected (since locomotion-related labyrinthine inputs are suppressed), thereby eliciting responses of vestibular nucleus neurons that are not

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**Fig. 7.** Average dynamics of responses to whole body rotations of vestibular nucleus neurons whose activity was modulated by hindlimb motion. Bars indicate standard error.
physiologically relevant. Studies employing electrical stimulation of hindlimb nerves suggested that hindlimb proprioceptive inputs to the vestibular nuclei are amplified following a bilateral labyrinthectomy (Jian et al. 2002; McCall et al. 2013b). Thus it may be insightful to compare the responses of vestibular nucleus neurons to hindlimb movement before and after removal of labyrinthine inputs. Amplification of these responses after damage to the labyrinths as animals regain postural stability would support the notion that hindlimb inputs to the vestibular nuclei complement labyrinthine signals in achieving postural stability.

Conclusions. Vestibular nucleus neuronal activity is altered in response to hindlimb movement with a variety of response patterns. In particular, the majority of vestibular neurons that respond to hindlimb movement encode the direction of that movement. These findings suggest that integration of afferent signals from the hindlimbs and labyrinth by vestibular nucleus neurons is important for postural control and maintenance of balance.

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DISCLOSURES

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