Interdependence of Spatial Properties and Projection Patterns of Medial Vestibulospinal Tract Neurons in the Cat

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Perlmutter, S. I., Y. Iwamoto, J. F. Baker, and B. W. Peterson. Interdependence of spatial properties and projection patterns of medial vestibulospinal tract neurons in the cat. J. Neurophysiol. 79: 270–284, 1998. Activity of vestibular nucleus neurons with axons in the ipsi- or contralateral medial vestibulospinal tract was studied in decerebrate cats during sinusoidal, whole-body rotations in many planes in three-dimensional space. Antidromic activation of axon collaterals distinguished between neurons projecting only to neck segments from those with collaterals to C6 and/or oculomotor nucleus. Secondary neurons were identified by monosynaptic activation after labyrinth stimulation. A three-dimensional maximum activation direction vector (MAD) summarized the spatial properties of 151 of 169 neurons. The majority of secondary neurons (71%) terminated above the C6 segment. Of these, 43% had ascending collaterals to the oculomotor nucleus (VOC neurons), and 57% did not (VC neurons). The majority of VOC and VC neurons projected contralaterally and ipsilaterally, respectively. Most C6-projecting neurons could not be activated from oculomotor nucleus (V-V6 neurons) and projected primarily ipsilaterally. All VO-C6 neurons projected contralaterally. The distributions of MADs for secondary neurons with different projection patterns were different. Most VOC (84%) and contralaterally projecting VC (91%) neurons had MADs close to the activation vector of a semicircular canal pair, compared with 54% of ipsilaterally projecting VC (i-VC) and 39% of V-C6 neurons. Many i-VC (44%) and V-C6 (48%) neurons had responses suggesting convergent input from horizontal and vertical canal pairs. Horizontal and vertical gains were comparable for some, making it difficult to assign a primary canal input. MADs consistent with vertical-vertical canal pair convergence were less common. Type II yaw or type II roll responses were seen for 22% of the i-VC neurons, 68% of the V-C6 neurons, and no VOC cells. VO-C6 neurons had spatial properties between those of VOC and V-C6 neurons. These results suggest that secondary VOC neurons convey semicircular canal pair signals to both ocular and neck motor centers, perhaps linking eye and head movements. Secondary VC and V-C6 neurons carry more processed signals, possibly to drive neck and forelimb reflexes more selectively. Two groups of secondary i-VC neurons exhibited vertical-horizontal canal convergence similar to that present on neck muscles. The vertical-vertical canal convergence present on many neck muscles, however, was not present on medial vestibulospinal neurons. Spatial transformations achieved by the vestibulocollic reflex may occur in part on secondary neurons but further combination of canal signals must take place to generate compensatory muscle activity.

INTRODUCTION

Activation of vestibular receptors by head rotation evokes compensatory head movements—the vestibulocollic reflex—that help stabilize gaze and keep the head upright on the body. Despite numerous investigations, the neural mechanisms that generate reflex movements in directions opposite to applied stimuli have remained somewhat elusive.

Initial experiments combined individual ampullary or oto-lith nerve stimulation with intracellular motoneuron recordings to identify the morphophysiological characteristics of vestibular input to neck motoneurons. These studies demonstrated that the projections to neck motor nuclei of vestibular nucleus neurons with monosynaptic input from one of the semicircular canals are consistent with a compensatory input/output system (Isu et al. 1988, 1991; Uchino and Hirai 1984; Uchino et al. 1988, 1990; Wilson and Maeda 1974). For example, neurons activated monosynaptically by anterior canal afferents, excited during nose down rotations, produce excitatory postsynaptic potentials (EPSPs) in motoneurons of bilateral dorsal musculature, which act to elevate the head, and inhibitory postsynaptic potentials in motoneurons of bilateral ventral neck muscles, which pull the head down. Recent studies using electrical stimulation of all six ampullary nerves (Shinoda et al. 1993, 1994) revealed a more detailed picture of the canal inputs to motoneurons innervating neck muscles. Each neck muscle has its own characteristic pattern of projections from the six semicircular canals. Polysynaptic projections from saccular and utricular afferents to neck motoneurons (Wilson et al. 1977) also contribute to the spatial properties of reflex output (Schor et al. 1984).

Experiments using natural stimuli have provided additional information on the spatial transformations of vestibular to motor signals. Most neck muscles have maximal vertical responses to rotations about an axis near the animal’s interaural (i.e., pitch) axis and nearly equal responses to horizontal rotation (Baker et al. 1985; Banovetz et al. 1995). To generate these activation patterns, upper cervical motoneurons probably receive inputs from all three semicircular canal pairs (as demonstrated by Shinoda et al. 1993, 1994) and otolith receptors. Although the responses to vertical rotations of many vestibulospinal (Iwamoto et al. 1996; Kasper et al. 1988; Wilson et al. 1990), reticulospinal (Bolton et al. 1992), and cervical spinal (Endo et al. 1994) neurons suggest inputs from both vertical canal pairs and otolith receptors, few neurons respond maximally to pitch rotations. This suggests that the convergence of signals from both vertical canal pairs, which is necessary to produce neck muscles’ preference for pitch, occurs at the neck motoneuron level.

The convergence of horizontal and vertical canal pair signals that occurs in the vestibulocollic pathway, however, may take place within the vestibular nucleus. In a recent study using three-dimensional rotations, we found that the
relative sensitivity to horizontal versus vertical rotations of many secondary medial vestibulospinal tract (MVST) neurons was similar to that of neck muscles (Iwamoto et al. 1996). We suggested that the convergence of vertical and horizontal vestibular signals needed to generate the threedimensional vestibulocollic reflex may occur on the secondary vestibulospinal population.

Unfortunately, the diverse anatomic projections of the MVST made it difficult to make functional comparisons between the responses of neck muscles and those of neurons recorded in Iwamoto et al. (1996). Most vestibulospinal neurons in that study were identified by antidromic activation from a midline electrode in the caudal medulla. MVST axons may descend ipsi- or contralaterally and may terminate in neck segments or project further caudally (Akaike 1983b; Donevan et al. 1990; Nyberg-Hansen 1964; Rapoport et al. 1977b; Wilson and Yoshida 1969). The limited antidromic identification used in Iwamoto et al. prevented us from correlating responses to three-dimensional rotation with the morphological organization described in experiments using intracellular motoneuron recording and electrical stimulation of individual canal nerves (Shinoda et al. 1993; Uchino and Isu 1991; Wilson and Maeda 1974). Information on the projections of physiologically characterized vestibulospinal neurons would significantly enhance the functional interpretation of their spatial activation patterns.

This and the following paper address this issue by examining the morphophysiological characteristics of MVST neurons. This study extends the work of Wilson and Maeda (1974) and Uchino (summarized in Uchino and Isu 1991) by investigating the responses to natural stimulation of vestibular nucleus neurons with identified projection patterns. Substrates of the sensorimotor transformations achieved by the vestibulocollic reflex may be found by simultaneously examining the responses and projections of vestibulospinal pathways. Here, results will show correlations between morphology and physiology that suggest functional specificity of signals sent to different levels of the spinal cord and those sent to both neck and oculomotor centers. A population of vestibulospinal neurons, the projections of which indicate a contribution to the vestibulocollic reflex, have spatial properties that can account for some, but not all, of the responses of neck muscles.

Preliminary results from this study appeared in Perlmutter et al. 1990.

METHODS

Most methods were described in detail in Iwamoto et al. (1996) and are summarized briefly here along with a description of additional procedures. Experiments were performed on 30 cats. Data from 23 second-order and 12 higher-order neurons recorded in 11 animals were included in Iwamoto et al. but will be described more fully here. All procedures conformed to the Principles of Laboratory Animal Care (National Institutes of Health Publication No. 85–23, revised 1985) and were approved by the Northwestern University Animal Care and Use Committee.

Data were obtained from two preparations. MVST neurons were recorded in the brain stem in the first preparation (Fig. 1A). Neurons were identified as second-order by electrical stimulation of the ipsilateral labyrinth and further characterized by antidromic activation from the rostral medial longitudinal fasciculus (MLF), from C1, and from C6. The second preparation (Fig. 1B) involved intraxonal recordings from MVST axons in C1. Electrical stimulation of both labyrinths determined whether the cell body was located in the left or right vestibular nucleus. Antidromic activation from the rostral MLF and from C6 further characterized these axons.

Animal preparation and recording procedures

Surgery was performed under 1% halothane in an 80% nitrous oxide-20% oxygen mixture. Atropine sulfate (0.04 mg/kg) and dexamethasone sodium phosphate (4–5 mg) were administered, the trachea and brachial vein were cannulated, and rectal temperature was maintained close to 38°C by a feedback-controlled heating pad. Blood pressure was monitored from a carotid or femoral artery and maintained with injections of Dextran or metaraminol bitartrate if necessary.

Schematic diagrams of the two recording preparations are shown in Fig. 1. A silver ball electrode was implanted against the round window, and a second electrode was positioned either in the bulla or attached to distant musculature. Bilateral implants were made in animals in which axonal recordings were conducted.

In 10 cats (Fig. 1A), a posterior craniorotomy was opened for access to the left vestibular nucleus. Epoxylite-insulated, tungsten electrodes were used to record neurons extracellularly in regions where VIIIth nerve-evoked field potentials (N1) were found (Shimazu and Precht 1965). Laminectomies were performed at the C1 and C1–C6 levels for placement of three-electrode arrays to stimulate the left MVST, the right MVST, and the left lateral vestibulospinal tract (LVST). The caudal array was positioned in the C6 segment of the spinal cord. The interelectrode distances and relative depths of the medial and lateral electrodes were determined from anatomic studies (Holstege 1988; Nyberg-Hansen 1964). The depth of the array at C1 was chosen to maximize recorded monosynaptic, descending volleys in response to labyrinth stimulation. Depth of the C6 array was adjusted to minimize antidromic thresholds of recorded vestibular units.

In 20 cats (Fig. 1B), laminectomies, but no posterior craniorotomy, were performed. MVST neurons were recorded intraaxonally in the right or left ventral funiculus of the C1 segment, defined by the extent of the first dorsal roots, with micropipettes of 8–40 MΩ impedance. In addition to the physiological characterization described in this paper, the morphology of axonal branching in C1 was studied in some of these animals. Consequently, pipettes were filled with 10% horseradish peroxidase (HRP) or 4% Neurobiotin (Vector Laboratories) in 0.5–1.0 M KCl and Tris(hydroxymethyl)aminomethane buffer (pH 7.6). The morphology of labeled neurons is described in the following paper (Perlmutter et al. 1998). An array of three stimulating electrodes was positioned in the C6 segment for antidromic identification.

After all preparative surgery, a unilateral craniorotomy was made rostral to the tentorium, and a large portion of the left cerebral hemisphere was aspirated. The cat was decerebrated by transecting the brain stem 1–4 mm rostral to the anterior border of the superior colliculus. Anesthesia then was discontinued, and the animal was paralyzed with pancuronium bromide and artificially ventilated. When intraaxonal recordings were made, a bilateral pneumothorax was performed and a clamp secured the spinal processes of the T1 and T2 vertebrae.

After decerebration, a tungsten electrode was placed in the midbrain in all animals for antidromic activation of ascending axon collaterals. It was inserted into the craniotomy opened for decerebration and through the midcaudal portion of the superior colliculus (i.e., 4–8 mm caudal to the level of decerebration). The electrode was positioned with a micromanipulator in the region of the oculomotor nucleus or the nearby ascending medial longitudinal fasciculus. Correct positioning of the electrode tip was achieved by monitor-
FIG. 1. Experimental paradigms. A: extracellular recording from left vestibular nucleus; antidromic stimulating electrodes in oculomotor nucleus (III), C1, and C6 ventral funiculi. Secondary neurons identified with labyrinth stimulation; VIII n, eighth cranial nerve. B: intraaxonal recording in left or right medial vestibulospinal tract (MVST). Vestibular nucleus neurons shown exemplify 2 of several known morphological projection patterns.

Vestibular and electrical stimulation

To antidromically activate axons, cathodal pulses of 100 μs were delivered between single stimulating electrodes in the neuraxis and a distant indifferent electrode. Primary vestibular afferents were activated with 100-μs pulses of 0.1–1.5 mA applied to the labyrinth electrodes.

Neurons recorded extracellularly in the vestibular nucleus were classified as second-order if they were activated by ipsilateral labyrinth shock, at stimulus currents above the intensity which elicited a maximal N1 potential, within 1.3 ms without large (>0.2 ms) latency fluctuations (Wilson and Melvill Jones 1979). One neuron discharged 1.3 ms after some labyrinth shocks, but latency varied widely for repeated stimuli. This cell was classified as a nonsecondary neuron. All other neurons with labyrinth latencies ≤1.3 ms exhibited little fluctuation in latency. About two-thirds of the neurons classified as nonsecondary did not respond to labyrinth stimulation. The remaining cells were activated at latencies as short as 1.3–3.0 ms and latency fluctuated widely from stimulus to stimulus. These cells were classified as nonsecondary because we sought a conservative criterion for monosynaptic responses that would ensure that cells activated disynaptically by labyrinth stimulation were not classified as secondary neurons. It is possible, therefore, that a small percentage of cells classified as nonsecondary were actually secondary neurons.

Neurons recorded intraaxonally in C1 were classified as secondary if they responded to labyrinth shock at latencies <1.5 ms with little fluctuation in latency. Axons that did not meet these criteria were discarded from the material in the present study.

Spinally projecting neurons were identified by recording intraaxonally in C1 or, when recording in the vestibular nucleus, by antidromic activation from at least one C1 stimulating electrode with threshold ≤500 μA. C1 thresholds ranged from 2 to 400 μA (using the lowest threshold from the stimulating array for each neuron; ≤100 μA for 75% of neurons). Neurons identified as vestibulospinal were tested for antidromic activation from oculomotor nucleus and C6 electrodes with ≤1 mA stimulation. Thresholds from the oculomotor nucleus ranged from 18 to 1,000 μA (≤200 μA for 54% of neurons).

The responses of neurons to sinusoidal rotations of 0.5 Hz were recorded. The cat’s head remained fixed to a stereotaxic frame during all recordings. The animal was placed on a turntable mounted on a dual servomotor-controlled apparatus that could produce independent earth-horizontal and -vertical rotations. The frame was fixed to the rotator with the interaural midpoint of the head close to the axes of rotation, and pitched 28° down from earth horizontal to bring the vertical canals to a near vertical position (Blanks et al. 1972). The turntable could be manually rotated about the vertical axis and locked in a fixed position with respect to the apparatus, enabling rotations in many vertical planes relative to the cat (Baker et al. 1985). In our coordinate system, rotation about the 0° earth-horizontal axis is a pitch stimulus and rotation about the 90° axis is roll; the 45° axis is near the plane of the left anterior-right posterior canal pair, and the 135° axis is near the plane of the left posterior-right anterior canal pair. Rotations of 10° peak-to-peak amplitude were applied during extracellular recordings and 5° during intraaxonal recordings. Swaying movements of the body during rotation were minimized with lateral squeeze plates.

Identification of MVST neurons

Separate criteria were used to identify MVST neurons in the two preparations (Fig. 1). Units recorded extracellularly in the left vestibular nucleus (Fig. 1A) were identified as projecting in the left or right MVST, or the left LVST, by estimating the location of the node of Ranvier activated with C1 stimulation. These sites
were determined from the relative thresholds for antidromic activation from the three C1 electrodes, histological reconstruction of lesions made with each electrode, and the assumption that threshold is proportional to either the distance or the distance squared between the activation and stimulation site (see Iwamoto et al. 1996 for details). The border between the MVST and the LVST was taken as the medial margin of C1 motor axons as they leave the gray matter (Holstege 1988; Nyberg-Hansen 1964). If the estimated activation site was medial to this border, the cell was classified as an MVST neuron, and if it was lateral to this border, it was classified as an LVST neuron. Left and right MVST axons were distinguished by the location of the estimated activation site relative to the midline. The properties of LVST neurons were described in Iwamoto et al. (1996) and are not considered further here.

Neurons recorded intraxonally (Fig. 1B) were classified as MVST neurons by the location of the electrode penetration and a monosynaptic response to labyrinth stimulation. Fibers considered to be coursing in the MVST were recorded within ~700 µm of the midline and between 2,600 and 4,100 µm from the dorsal surface of the spinal cord, within the MVST as defined anatomically (Holstege 1988; Nyberg-Hansen 1964). Intraxonal recordings were sufficiently far from the midline to localize the recording side. Neurons recorded intraxonally that responded to labyrinth stimulation at latencies that were not clearly monosynaptic could have been higher-order vestibulospinal neurons or reticulospinal neurons and were not included in this study.

Data collection and analysis

Neurons with activity modulated during 0.5-Hz rotations were studied during rotation in the horizontal plane and at least two (typically ≥8) vertical planes. Fifteen neurons, recorded during rotations in at least three planes, had no rotational response and were not included in the data set. Activity of single units during 20–40 cycles of rotation in one plane were averaged as cumulative spike-occurrence histograms. These data were fitted with a 0.5-Hz sine wave using a least-squares error algorithm (Peterson et al. 1980; Schor et al. 1984). The activity of neurons that fell silent during a portion of the rotation cycle were fit with a sine wave clipped at 0 spikes/s using a modified, iterative algorithm. A neuron’s response gain was defined as the peak amplitude of the fitted sine wave divided by the peak turntable excursion, and response phase was expressed relative to turntable position. In our coordinate system, a positive gain for vertical rotations about the 0° (pitch) and 90° (roll) rotation axes represent maximal responses for nose down pitch and left ear down roll, respectively. A positive gain for horizontal rotations represents a maximal response for nose leftward yaw.

The response gain of most neurons varied for different vertical rotation planes (i.e., depending on the orientation of the rotation axis relative to the cat’s head). We described these spatial properties using the traditional “cosine-tuned gain” model. This model assumes that vestibular responses are linear combinations of primary afferent inputs with the same phase and/or spatial orientation (Angelaki 1991; Baker et al. 1984; Schor et al. 1984). In this case, response gain is a cosine function of stimulus orientation and response phase is independent of rotation plane. Responses that are cosine-tuned in this way can be summarized by a vector that defines the rotation plane in which the neuron’s response is maximal. A least-squares error sinusoid (Baker et al. 1984; Schor et al. 1984) was fit to the gain versus orientation angle data for each neuron (Fig. 2). A three-dimensional maximum activation direction vector (MAD) was calculated from the pitch and roll values of the cosine fit and the vectorially averaged gain from several trials of yaw rotation. These vectors were normalized to have a length of one to emphasize response direction (Fig. 2).

Some neurons had responses that could not be accounted for by the cosine-tuned gain model but reflected spatio-temporal convergence of inputs (Angelaki 1991; Baker et al. 1984). Phases were not constant for all vertical rotation planes, and gain was clearly not a cosine function of stimulus orientation. A minimum response ratio (Baker et al. 1985) was used as an estimate of the extent of spatio-temporal convergence exhibited by a neuron. For each cell, gain and phase for all stimulus orientations were plotted in polar coordinates; gain specified the distance and phase the direction from the origin. The data points were connected with line segments, and the resulting figure reflected across the origin to complete a polygon. The minimum response ratio was defined as the ratio of the minimum to maximum radial distances between the origin of the polar plot and the polygon. Extensive spatio-temporal convergence produces ratios near 1; no spatio-temporal convergence produces a ratio of 0. The cosine-tuned gain model was considered to be a sufficient representation of the spatial properties of neurons with minimum response ratios <0.2 (Iwamoto et al. 1996).

To simplify the MAD presentations, the brain is assumed to be symmetrical, and vectors are shown as if the cell bodies of all neurons were on the left side of the brain. Right-to-left vector transformations were performed by inverting the signs of the yaw and roll vector components for right vestibular nucleus neurons.

Estimation of vestibular inputs

The rotational responses of vestibular nucleus neurons are generated by inputs from semicircular canal and/or otolith receptors. The properties of vestibulocollic and vestibulolimb reflexes indicate canal and otolith contributions (Baker et al. 1985; Roberts 1973; Schor and Miller 1981; Wilson and Melvill Jones 1979), and many vestibulospinal neurons have responses with both canal and otolith components (Angelaki et al. 1993; Baker et al. 1984; Bush et al. 1993; Kasper et al. 1988; Schor and Miller 1982; Schor et al. 1984; Wilson and Melvill Jones 1979; Wilson et al. 1990). In the present study, otolith plus canal input is suggested by response phases that drift toward position phase for rotation planes near a neuron’s minimum response plane. Nonetheless, these experiments were not designed to distinguish canal and otolith inputs but rather to identify vestibular neurons with spatial properties that match those of the neck muscles. The responses of neurons with minimum response ratios <0.2 are consistent with inputs from the semicircular canals alone (Baker et al. 1984, 1985). Therefore, for descriptive purposes only, the responses of vestibulospinal neurons were interpreted in terms of the canal inputs that could account for their maximum activation direction vectors. However, it is likely that many of the neurons in this study also received inputs from otolith afferents.

Maximum activation directions of vestibulospinal neurons were compared with those of the semicircular canal pairs, calculated from the anatomic data of Blanks et al. (1972; see Iwamoto et al. 1996 for derivation). MADs for canal pairs with excitation from the left-side canal of the pair are shown in Fig. 3: left horizontal-right horizontal canal pair (lhc/rhc—excitatory input listed first), left anterior-right posterior canal pair (lac.rpc), and left posterior-right anterior canal pair (lpc.rac). Our extracellular recordings could not distinguish excitation from a single canal, inhibition from the contralateral coplanar canal, or paired excitatory-inhibitory input. Angles in three-dimensional space between neuronal MADs and each of the canal pair MADs were calculated. A neuron was considered to receive input from a canal pair if its vector was <70° from that pair’s MAD (Iwamoto et al. 1996). Neurons with convergent inputs from noncoplanar canals were defined as those with MADs <70° from the activation vectors of more than one canal pair. This criterion was chosen over the more common definition of a convergent response as a MAD >20° from a single canal vector so that we could assign unambiguously canal-pair inputs even though the canal pairs are
FIG. 2. Responses of 3 secondary MVST neurons with different projection patterns [A: contralaterally projecting vestibulo-oculo-collic (c-VOC); B: ipsilaterally projecting vestibulo-colic (i-VC); C: ipsilaterally projecting vestibulo-C6 (i-V-C6), see text for description]. a: electrophysiological identification; all neurons recorded intraaxonally. r, stimulation artifacts. b: gain and phase (relative to head position) of responses to 0.5-Hz vertical rotations plotted vs. rotation plane. 0° orientation is pitch, 90° is roll, 45° is near the plane of the left anterior-right posterior canal pair (lacrpc), 135° is near the left posterior-right anterior canal pair (lpcrac). Positive gains and negative phases are associated with peak responses in positive rotation directions: nose down pitch, left ear down roll, nose left yaw. Curves in gain plots are least-squares error fits. Gain and phase in yaw shown to scale to right of plots. c: normalized maximum activation direction vectors (MADs) calculated from pitch and roll values of sine fit to gain data (b), and vectorally averaged gain in yaw. Rotation producing maximal excitation is found using right-hand rule—vector indicates rotation axis. * Neuron in right vestibular nucleus, but vector shown as if it were on the left side.

not exactly orthogonal (Iwamoto et al. 1996). The 70° criterion is considered conservative (Iwamoto et al. 1996), taking into account the weak responses of canal afferents to rotations in ‘‘orthogonal’’ planes (e.g., horizontal canal afferents are modulated by roll rotation), even with the head positioned 28° nose down. These responses cannot be avoided because the semicircular canals are not exactly orthogonal and because their orientation varies from animal to animal.

Histological procedures

Near the conclusion of each experiment, positions of C1 and oculomotor nucleus stimulating electrodes, and some extracellular recording sites, were marked electrolytically (electrode tip negative, 20–30 μA for 20 s). The animals then were anesthetized deeply with pentobarbital sodium (50 mg/kg iv) and perfused with 10% formalin. After fixation, the brain stem and spinal cord were sectioned at 100 μm on a freezing microtome. Rhombencephalic sections were stained by the Kluver-Barrera method, spinal cord and midbrain sections with thionin. Stimulating positions and locations at which neurons were recorded extracellularly were reconstructed. Processing of the spinal cord in experiments where HRP or Neurobiotin was injected is described in the following paper (Perlmutter et al. 1998).

RESULTS

Vestibulospinal neurons (154) were classified as second order on the basis of response latency after labyrinth shock. Responses to 0.5-Hz rotations suggested that 139 of these could be described using the cosine-tuned gain model (see METHODS). Firing rate was modulated sinusoidally, except for rotations in a well-defined ‘‘null’’ response plane, and...
gain during vertical rotations was fit as a cosine function of head orientation with respect to the rotation axis. Response phases were constant for rotation planes far from the null plane and were closer to velocity phase (±90°) than position phase (±0°). For some neurons, phases varied near the null (Fig. 2), but all 139 neurons had minimum response ratios <0.2. MADs were calculated for these neurons. The remaining 15 neurons had spatio-temporal convergent behavior (Baker et al. 1984; Iwamoto et al. 1996) during 0.5-Hz rotations, suggesting strong otolith input, and a maximum activation direction was not calculated. Two of them, however, were also examined during 2.0-Hz rotations, at which they exhibited cosine-tuned gain behavior. MADs derived from 2.0-Hz trials were similar to those of other neurons with the same projection pattern and were included in the data set. The 141 secondary neurons for which maximum activation directions were calculated included 23 recorded in the vestibular nucleus, and 118 recorded intraaxonally in the C1 ventral funiculus.

Fifteen spinally projecting neurons were recorded in the vestibular nucleus but were driven polysynaptically or not at all by labyrinth stimuli. MADs for 12 of these higher-order units were calculated for 0.5-Hz rotations, and the other three exhibited spatio-temporal convergent properties.

**Electrophysiological identification of projection pattern**

Antidromic responses (Fig. 2Aa) were identified by short, constant latency activation with sharply defined threshold. Many neurons were tested for following of high-frequency stimuli or collision with orthodromically elicited or spontaneous spikes. A collision test always was performed when antidromic latencies were ≥1.0 ms. Antidromic latencies after C1 stimulation for neurons recorded in the vestibular nucleus ranged from 0.4 to 1.7 ms (≈0.8 ms for 76% of neurons). Latencies from the oculomotor nucleus electrode ranged from 0.3 to 1.5 ms (≈0.8 ms for 82% of neurons).

MVST neurons were classified into three main groups according to projection pattern, following the nomenclature of Uchino et al. (1988). All neurons were either activated antidromically from C1 or recorded intraaxonally in C1. Vestibulo-oculocollic (VOC) neurons were activated antidromically from the oculomotor nucleus but not from the C6 spinal segment. Vestibulo-collic (VC) neurons were not activated from either oculomotor nucleus or C6. Cc-projecting neurons were activated from the C6 segment. Some Cc-projecting neurons also could be activated antidromically from the oculomotor nucleus. Ipsi- and contralateral projections were defined relative to the side from which labyrinth shock evoked a monosynaptic response, or, for nonsecondary neurons, from which the cell body was recorded.

The projection patterns of the 141 secondary neurons are detailed in Table 1. The axons of most VOC neurons, which comprised 31% of secondary neurons, descended in the MVST contralateral to their cell bodies (c-VOC neurons). The majority of VC neurons (40% of secondary neurons) descended ipsilaterally (i-VC neurons). Cc-projecting neu-
TABLE 1. Secondary MVST neurons with responses suggesting convergent semicircular canal pair input

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<th></th>
<th>c-VOC</th>
<th>i-VOC</th>
<th>c-VC</th>
<th>i-VC</th>
<th>c-VOC(_a)</th>
<th>c-VOC(_b)</th>
<th>i-VOC(_a)</th>
<th>i-VOC(_b)</th>
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<td>46</td>
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<td>5</td>
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<td>141</td>
<td>84</td>
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<td>No. of convergent cells</td>
<td>70° criterion‡</td>
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<td>1 (20)</td>
<td>1 (9)</td>
<td>21 (46)</td>
<td>6 (60)</td>
<td>3 (60)</td>
<td>16 (62)</td>
<td>54 (38)</td>
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<tr>
<td>No. of convergent cells</td>
<td>10° criterion§</td>
<td>20 (53)</td>
<td>1 (20)</td>
<td>4 (36)</td>
<td>34 (74)</td>
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<td>4 (80)</td>
<td>23 (88)</td>
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<td>0</td>
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<td>2 (20)</td>
<td>4 (80)</td>
<td>17 (65)</td>
<td>33 (23)</td>
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Values in parentheses are in percent. MVST, medial vestibulospinal tract; c- and i-VOC, contra- and ipsilaterally projecting vestibulo-oculocollic; c- and i-VC, contra- and ipsilaterally projecting vestibulo-collic; c-VOC\(_a\), contratermally projecting vestibulo-oculocollic; c-VOC\(_b\), contra- and ipsilaterally projecting vestibulo-C\(_6\); VON, vestibuloocular neurons. * Total, total secondary MVST population. † VON, population of vestibuloocular neurons from Fukushima et al. (1990). See text for description of projection classes. ‡ Convergence criterion from this study: maximum activation direction vector (MAD) of neuron <70° from MADs of two canal pairs. § Convergence criterion from Fukushima et al. (1990): MAD of neuron >10° from MADs of both the nearest single canal and canal pair.

Neurons (29% of secondary neurons) that also had an ascending branch had axons in the contralateral MVST (c-VOC\(_b\) neurons), and most with no ascending branch had axons that descended ipsilaterally (i-VOC\(_a\) neurons).

The 12 vestibulospinal neurons that were not monosynaptically activated from the labyrinth included four VC neurons (3 i-VC, 1 c-VC), four C\(_6\)-projecting neurons (3 i-VOC\(_a\), 1 i-VOC\(_b\)), and four ipsilaterally projecting neurons for which antidromic activation from the oculomotor nucleus was not tested (2 projected to C\(_6\) and 2 did not).

Spatial response properties of secondary MVST neurons

Neurons with different projection patterns had different distributions of response vectors. This is exemplified by the three intraxonally recorded neurons in Fig. 2.

The neuron in Fig. 2A, recorded in the right C\(_1\) ventral funiculus, fired a double discharge 1.2 ms after stimulation of the left labyrinth and was driven antidromically 0.5 ms after a pulse delivered to the oculomotor nucleus electrode (Fig. 2Ab). There was no response to C\(_6\) stimulation as high as 1.0 mA. This neuron was classified as a c-VOC neuron. It responded strongly to rotations in the plane of the lpc/rac (large gain at 135°, Fig. 2Ab) and response phase lagged head velocity by 23°. There was little modulation of activity in yaw (gain shown to scale to right of plot, Fig. 2Ab). For rotations in vertical planes, the minimum response ratio was 0.06, and gain was fit as a cosine function of orientation. The neuron’s MAD (Fig. 2Ac) was aligned closely with that of the lpc/rac vector (see Fig. 3). Spatial properties that resembled those of a semicircular canal pair were typical of neurons with axons that branched and projected to the spinal cord and to the oculomotor nucleus.

The axon shown in Fig. 2B, recorded in the right ventral funiculus, was activated 1.2 ms after right labyrinth shock (Fig. 2Ba) and was not driven by oculomotor or C\(_6\) stimulation with currents as large as 1.0 mA; it was classified as an i-VC neuron. As in Fig. 2A, this neuron had a brisk response in the plane of the ipsilateral (right) posterior–contralateral anterior canal pair (45° orientation). Response gain was smaller than for the neuron of Fig. 2A, and response phase lagged head velocity by 20° (Fig. 2Bb). The minimum response ratio was 0.04. Unlike the VOC neuron in Fig. 2A, this cell was activated by horizontal rotation to the ipsilateral side with a gain that was one-third the maximum gain for vertical rotation. Its MAD was aligned with the ipsilateral posterior–contralateral anterior canal pair axis in the top view and was shifted toward the horizontal canal pair axis in the front view (Fig. 2Bc). Responses that were consistent with convergent inputs from the horizontal canal pair and one vertical canal pair were typical of many neurons that projected neither to the oculomotor nucleus nor the C\(_6\) spinal level.

The neuron in Fig. 2C, recorded in the right ventral funiculus, responded to right labyrinth shock with a double, or occasionally triple, discharge with onset latency of 1.1 ms (Ca; amplitude of second and third spikes decreased). The neuron fired antidromically 0.8 ms after a pulse applied to the right medial C\(_6\) electrode but could not be activated with oculomotor stimuli ≥1.0 mA. It was classified as an i-VOC\(_a\) neuron. It exhibited a strong increase in firing during horizontal rotation to the contralateral side with peak firing lagging peak head velocity by 8°. It responded to vertical rotations with the same spatial activation pattern seen in Fig. 2, A and B, but the depth of modulation was much smaller (note smaller scale in Fig. 2Cb). The minimum response ratio for vertical rotations was 0.13; phase shifted toward position phase for rotation planes near the minimum response plane. This neuron’s strong sensitivity to contralateral yaw (type II yaw response) is seen as a MAD pointing downward in the front view (Fig. 2Cc). Type II yaw and/or type II roll (activation during contralateral ear down rotation) responses (Duensing and Schaefer 1958; Markham and Curthoys 1972; Shimazu and Precht 1966) were common for secondary vestibulospinal neurons that projected to the C\(_6\) segment.

MADs for the entire population of MVST neurons are shown in Fig. 3. Table 1 shows the percentage of cells with responses suggesting convergent input from more than one semicircular canal pair using two convergence criteria. The 10° criterion was used by Fukushima et al. (1990) in a study on vestibuloocular neurons and is shown here for comparison. The following description uses only the 70° criterion described in METHODS.

VOC neurons. There was no clear difference between the spatial properties of ipsilaterally and contratermally projecting VOC neurons. The vast majority had maximal responses in rotation planes near those of a semicircular canal pair and in directions that excite the ipsilateral canal of the pair. MADs of 36 of 43 (84%) secondary VOC neurons (32/38 c-VOC neurons, 4 of 5 i-VOC neurons) were <70° from...
one of the canal pair axes shown in Fig. 3 and >70° from all other canal pair vectors. About equal numbers of cells had responses consistent with input from lhc/rhc (11), lac/rpc (13), and lpc/rac (12).

Seven VOC neurons had responses suggesting convergent input from noncoplanar canals based on the 70° criterion. Five of these were modulated during both horizontal and vertical rotations and had MADs consistent with input from the horizontal canal pair and one vertical canal pair (4 from lac/rpc and 1 from lpc/rac). All five of these neurons were activated by horizontal rotation to the ipsilateral side. Two c-VOC neurons had responses suggesting input from both vertical canal pairs. They were activated maximally by ipsilateral ear down rotation in a plane near the cats’ longitudinal (i.e., roll) axis, suggesting convergent input from lpc/rac and lac/rpc.

**VC NEURONS.** The spatial properties of c-VC neurons resembled those of VOC neurons. Ten of 11 (91%) cells had MADs aligned with a canal pair vector with excitation from the ipsilateral member of the pair. The majority of c-VC neurons, however, were activated by horizontal rotation (7 MADs near the lhc/rhc vector, 3 near lpc/rac). One c-VC neuron had responses suggesting lhc/rhc and lpc/rac input.

I-VC neurons had a different distribution of response vectors. About one-half of them (25/46, 54%) had MADs near a canal pair axis (9 near lhc/rhc, 8 near lac/rpc, 8 near lpc/rac). The other one-half of the i-VC population (21/46 neurons, 46%) appeared to receive convergent horizontal and vertical canal pair input.

The convergent i-VC cells included 15 neurons that were activated by horizontal rotation to the ipsilateral side. Of these, 13 had MADs suggesting input from one vertical canal pair (8 from lpc/rac, 3 from lac/rpc, 2 from rpc/lac), and two exhibited a type II roll response (consistent with input from rpc/lac and rac/lpc). Six i-VC neurons were activated by horizontal rotation to the contralateral side. Five of these had responses suggesting input from one vertical canal pair (4 from lpc/rac, 1 lac/rpc), and one had a maximal vertical response to nose up rotation in a plane near the cats’ inferotemporal (i.e., pitch) axis (consistent with excitation from bilateral posterior canal afferents).

The most common pattern of convergence for both VC and VOC neurons was horizontal-vertical canal pair input. Unlike any VOC neuron, however, several i-VC neurons had comparable vertical and horizontal response gains, making it difficult to assign a primary canal input. In addition, type II yaw or type II roll responses were exhibited by some VC neurons, but no VOC cells. Ten (22%) VC neurons had MADs consistent with excitatory input from the contralateral member of at least one semicircular canal pair.

**V-C6 NEURONS.** The spatial properties of the V-C6 population were dominated by type II yaw or roll responses. Ipsilaterally and contralaterally projecting cells had similar properties. Twenty-one of 31 V-C6 neurons (68%) exhibited type II responses (10 yaw, 5 roll, 6 both). Four other V-C6 neurons that did not exhibit type II responses had responses consistent with convergent input from more than one canal pair. Only six (19%) V-C6 neurons had MADs near one of the canal pair vectors shown in Fig. 3 (1 near lac/rpc, 1 near lhc/rhc, 4 near lpc/rac).

Fifteen (48%) V-C6 neurons were modulated in both horizontal and vertical rotation planes. Four of these (all i-V-C6 neurons) were activated by yaw to the ipsilateral side and 11 (8 i-V-C6, 3 c-V-C6) by yaw to the contralateral side.

Eight (26%) V-C6 neurons appeared to receive input from both vertical canal pairs (including 4 that also responded to horizontal rotations). Four of these eight neurons had MADs shifted toward the roll axis (3 ipsilateral ear down, 1 contralateral ear down), and four had MADs shifted toward the pitch axis (3 nose up, 1 nose down).

**VOC-C6 NEURONS.** The VO-C6 population had spatial properties that were intermediate between those of VOC and V-C6 neurons. Four of 10 (40%) had MADs near those of a single canal pair (1 near lac/rpc, 3 near lpc/rac), and 2 (20%) had type II responses. Three c-VO-C6 neurons had responses consistent with input from the horizontal and one vertical canal pair (1 lhc/rhc plus lac/rpc, 1 lhc/rhc plus rpc/lac, 1 rpc/lhc plus lac/rpc), and three were excited maximally by ipsilateral ear down roll rotations.

**Spatial response properties of nonsecondary vestibulospinal neurons**

MADs for 12 higher-order vestibulospinal neurons are shown in Fig. 4. Four neurons were not tested for antidromic activation from the oculomotor nucleus electrode, and their projection pattern could not be completely identified. Although the population is small, most higher-order neurons exhibited spatial properties like those of C6-projecting secondary neurons. Only two neurons (17%) had MADs close to one of the canal pair axes shown in Fig. 3 (1 near lhc/rhc, 1 near lpc/rac), and seven (58%) neurons had type II responses. Two other neurons had MADs suggesting equally strong inputs from lhc/rhc and lac/rpc, and the last fired maximally to ipsilateral ear down roll. It does not appear that C6 and non-C6 projecting higher-order vestibulospinal neurons have different spatial properties—both groups appear to receive highly convergent canal inputs.

**Resting discharges, gains, and phases**

Resting discharge rate and the coefficient of variation of interspike intervals were measured for each neuron when
the head was held fixed in space (28° nose down, see METHODS). The mean spontaneous firing rate of secondary MVST neurons was 21 ± 17 (SD) spikes/s, and ranged from 1 to 83 spikes/s. The coefficients of variation were distributed unimodally about a median of 0.66. Neurons with higher spontaneous firing rates had a tendency to fire more regularly (correlation coefficient of linear regression was statistically significant, \( P < 0.01 \)).

Mean maximal response gain (estimated as the length of unnormalized response vectors) of secondary neurons was \( 6.0 \pm 3.6 \text{ spikes} \cdot \text{s}^{-1} \cdot \text{deg}^{-1} \). Mean response phase for horizontal rotations was calculated for neurons that received horizontal canal pair input based on MAD orientation. For these neurons, peak firing during yaw led maximum horizontal excursion of the head by a mean of 93 ± 19°. Mean vertical phase was calculated for responses in the rotation plane that was closest to the predicted maximal activation plane. For neurons with vertical canal pair input, peak firing led maximum vertical excursion by a mean of 77 ± 16°. The difference between mean horizontal and vertical phases was statistically significant (\( P < 0.05 \)) and exhibited by individual neurons. Neurons with responses suggesting both horizontal and vertical canal pair input had response phases for yaw that were on average 13° more advanced than response phases for vertical rotations. Neither gain nor phase was correlated with resting discharge rate or coefficient of variation. Phase differences for horizontal and vertical rotations may be due to inputs from otolith receptors, which are activated for earth-vertical, but not earth-horizontal, rotations and have response phases that are on average closer to head position phase than canal afferents. The firing properties of the 12 nonsecondary neurons were not qualitatively different from those of secondary neurons. The mean spontaneous firing rate was 31 spikes/s, the median coefficient of variation was 0.67, and the mean maximal gain was \( 6.0 \text{ spikes} \cdot \text{s}^{-1} \cdot \text{deg}^{-1} \). Responses during horizontal rotations were on average more advanced than those during vertical rotations; mean phase leads were 84° and 62°, respectively.

**Relation between firing properties, spatial preferences, and projection pattern**

The correlation between spatial properties and axonal projection pattern suggests a functional specialization among vestibular neurons. An analysis of variance (ANOVA) model was used to test for correlations between other response properties and projection pattern and spatial properties. Spontaneous discharge rate, coefficient of variation, response gain, and phase were examined individually for correlations with projection type (VOC, VC, or V-C_6), projection side (ipsilateral or contralateral), and convergent canal input (based on MAD orientation). Three-way, univariate ANOVAs were performed using an unbalanced, factorial model with two-way interactions.

The ANOVA for maximal response gain revealed lower gains for secondary neurons with MADs suggesting convergent canal pair input than neurons with MADs close to a single canal pair vector (statistically significant \( F \) ratio, \( P < 0.05 \)). Figure 5A plots the maximal gains of neurons with convergent (■) and nonconvergent (○) inputs as the endpoints of their unnormalized MADs. End points of vectors aligned with the canal pair axes (Fig. 3) tend to lie further from the origin, although there is substantial overlap. The mean maximal gains for nonconvergent and convergent neurons were 6.6 and 4.7 spikes \cdot \text{s}^{-1} \cdot \text{deg}^{-1} \), respectively. This difference was seen for neurons of each projection type (Fig. 5B). There was no significant difference in gain between ipsilaterally and contralaterally projecting neurons.

A similar dependence was found for response phase in yaw. The ANOVA for phase during horizontal rotation was calculated for neurons with horizontal canal pair input based on MAD orientation. Neurons with MADs suggesting convergent input from another canal pair had phases that were less advanced relative to head position (mean phase = 86°) than those with only horizontal canal pair input (mean phase = 101°). Figure 6A plots the mean phase leads of secondary neurons with convergent and nonconvergent inputs separated into different projection types. The difference in response phase between convergent and nonconvergent neurons is not present in the V-C_6 population.

ANOVA showed no correlations between projection type...
or spatial properties and vertical response phase (considering phase for rotation in the plane closest to the predicted maximal activation plane of neurons with MADs suggesting input from at least one vertical canal pair). All interaction terms had significant F ratios. The analysis did reveal one notable distinction, however. The phases of neurons with convergent and nonconvergent input were significantly different only for the V-Vo population (Fig. 6B), which is opposite the finding for horizontal response phase (Fig. 6A). Neurons that project to the C6 segment seem to have a different distribution of response phases than other vestibulospinal neurons, in addition to different spatial properties.

Similar univariate analyses of variance were performed for spontaneous discharge rate and coefficient of variation. No significant correlations were found with projection pattern, projection side or spatial properties.

**Location of recorded neurons**

Recording sites of the 35 neurons recorded extracellularly in the vestibular nucleus were estimated from the relative positions of electrolytic lesions made at the end of each experiment. Neurons were located in the medial, lateral, and descending nuclei. Many were near the border between the medial and lateral nuclei at the level of the acoustic stria. No relation was found between a neuron’s spatial properties and its location within the vestibular nucleus.

**Discussion**

**Organization of secondary vestibulospinal neurons**

The majority (71%) of second-order MVST neurons terminated rostral to the C6 segment, consistent with previous anatomic (e.g., Nyberg-Hansen 1964) and electrophysiological (e.g., Akaike 1983b; Rapoport et al. 1977b; Wilson and Maeda 1974) evidence. Donevan et al. (1990) demonstrated with anterograde transport of Phaseolus vulgaris leucoagglutinin that vestibulospinal projections are heaviest to C1, and that the density of axonal branching and terminal labeling decreases steadily through the lower cervical segments.

Thirty-eight percent of the neurons in this study were antidromically activated from the oculomotor nucleus. The percentage of MVST neurons that innervate oculomotor neurons may actually be higher. Isu and Yokota (1983) studied secondary vestibular neurons that were activated by horizontal rotation and projected to the contralateral abducens nucleus and found that only one-third could be antidromically activated from the oculomotor nucleus. Some neurons classified as VC in the present study, probably responsive to yaw, may have had undetected collaterals to the abducens nucleus (Isu et al. 1991; Oghaki et al. 1988).

A large VOC population is consistent with Uchino’s finding that 107 of 202 MVST neurons activated with anterior or posterior canal nerve stimulation were activated antidromically from the oculomotor nucleus (Isu et al. 1988; Uchino et al. 1988, 1990). In the cat, between 43 and 90% of contralaterally projecting vestibulocular neurons are reported to have descending collaterals (Isu and Yokota 1983; Iwamoto et al. 1990; McCrea et al. 1981; Ohgaki et al. 1988). Fewer VOC neurons appear to exist in the squirrel monkey (Minor et al. 1990).

The projection of VOC neurons to the ipsilateral or contralateral side was identified by antidromic activation from the C1 spinal cord in these experiments. Previous electrophysiological and morphological evidence found that the ascending and descending collaterals of vestibulo-oculospinal neurons project to the same side of the neuraxis (Graf and Ezure 1986; Isu and Yokota 1983; Isu et al. 1988; McCrea et al. 1981; Minor et al. 1990; Ohgaki et al. 1988; Uchino and Hirai 1984). Thus the c-VOC neurons described here probably projected to the contralateral oculomotor nucleus, and the i-VOC neurons projected to the ipsilateral oculomotor nucleus. The VOC projection was predominantly contralateral (48/53 neurons) and terminated above C6 (43/53 neurons), in agreement with previous reports.

VOC neurons that project to the ipsilateral oculomotor nucleus appear to be rare (5/141 neurons in the present study). Uchino and colleagues found i-VOC neurons only in the horizontal canal system (Isu et al. 1991; Uchino and Hirai 1984; Minor et al. 1990; but c.f. Iwamoto et al. 1990). An HRP study of 21 second-order vestibular neurons that terminated in the ipsilateral abducens nucleus found none with axon collaterals ascending in the MLF (Ohgaki et al. 1988).

It has been suggested (Graf and Ezure 1986; Isu and Yokota 1983; Isu et al. 1988; McCrea et al. 1981) that VOC neurons participate in the coordination of eye and head movements, which frequently are coupled (Bizzi et al. 1971; Denise et al. 1987; Vidal et al. 1982; Wilson et al. 1983). The relatively large size of the VOC projection suggests that this may be an important function of secondary MVST pathways in the cat.

Sixty-two percent of the population of vestibulospinal neurons were not antidromically driven from the oculomotor nucleus. Most of these (72/88) descended in the ipsilateral MVST, as reported by Uchino and colleagues (Isu et al. 1988; Uchino et al. 1988, 1990).
Many secondary vestibulospinal neurons exhibited type II responses to natural, rotational vestibular stimuli. This is somewhat surprising given the monosynaptic excitation from the ipsilateral labyrinth. However, type II responses in secondary neurons have been observed by several investigators since originally reported by Shimazu and Precht (1966). The present study extends their finding by demonstrating that type II responses in the secondary MVST population are exhibited primarily by neurons projecting to C6 and not by VOC neurons.

**Excitatory and inhibitory vestibulospinal projections**

Secondary vestibular neurons that project to contralateral extraocular motoneurons are all excitatory (in cat: Baker et al. 1973; Cohen et al. 1964; Uchino et al. 1979, 1980), whereas those ascending ipsilaterally are inhibitory (in cat: Baker et al. 1969, 1973; Schwindt et al. 1973; Uchino et al. 1979, 1980). This suggests that c-VOC neurons are excitatory and that i-VOC neurons are inhibitory. Intracellular recordings from motoneurons have demonstrated directly excitatory inputs from small numbers of c-VOC neurons. c-VOC neurons activated by anterior or posterior canal nerve stimulation produced monosynaptic EPSPs in motoneurons of contralateral C1 neck extensors (Uchino and Hirai 1984) or longus capitis motoneurons (Isu et al. 1988), respectively. Both pathways descend via the MVST (Uchino and Isu 1991; Wilson and Maeda 1974). c-VOC neurons with MADs near the plane of the horizontal canal pair may be excitatory vestibulobulbocervical neurons with ascending collaterals to the oculomotor nucleus (Baker et al. 1969; Isu and Yokota 1983) or excitatory interneurons in the inhibitory pathway to medial rectus motoneurons (Baker and Highstein 1978). The excitatory/inhibitory nature of neurons without ascending collaterals is more difficult to assess. For highly convergent neurons, it is not clear which canal provides monosynaptic input and comparisons cannot be made with studies using stimulation of individual canal nerves. Comparisons may be valid for secondary neurons with MADs near those of the horizontal canal pair because it is reasonable to assume that they receive their monosynaptic input from the ipsilateral horizontal canal. i-VC and c-VC neurons with MADs aligned with the lhc/rhc vector are probably inhibitory and excitatory, respectively, by analogy with those activated by ipsilateral horizontal canal nerve stimulation (Isu et al. 1991; Uchino and Isu 1989). Motoneuron recording experiments have described both excitatory and inhibitory secondary i-VC and c-VC neurons that are activated monosynaptically by vertical canal nerve stimulation (Isu et al. 1988, 1990; Uchino et al. 1988, 1990). Thus i-VC and c-VC neurons with MADs aligned with a vertical canal pair vector could have been either excitatory or inhibitory.

**Morphophysiological specialization**

The correlation between spatial properties and projection patterns of secondary vestibulospinal neurons constitutes a functional specialization of vestibulolugal pathways to structures involved in different motor systems. Signals distributed to both the oculomotor nucleus and upper cervical spinal cord, and those projecting only to the oculomotor nucleus (Fukushima et al. 1990), seem to reflect limited spatial processing of primary afferent activity. Responses of 84% of the secondary VOC neurons resembled those of ipsilateral semicircular canal afferents. VOC neurons may relay stimulus direction information to extraocular and neck motoneurons that acts to link eye and head movements. Vestibulospinal neurons that do not have ascending collaterals carry signals that reflect additional spatial processing of afferent information. Only 54% of secondary i-VC neurons had MADs aligned with a canal pair axis. Many had responses suggesting extensive convergence of input from vertical and horizontal canal pairs, and a small group exhibited type II yaw responses. These signals may drive specific neck muscles or muscle groups, which have similar response properties (Baker et al. 1985; Banovetz et al. 1995). Only 24% of the C6-projecting neurons, which probably innervate the cervical enlargement, had properties that resembled ipsilateral canal afferents.

Further specialization of the vestibulospinal projection is seen in the difference between signals sent to ipsilateral and contralateral sides of the spinal cord. VOC neurons conveying single canal pair signals project predominantly to the contralateral side and are excitatory. VC and V-C6 neurons convey more convergent signals primarily to the ipsilateral side, and neither excitatory nor inhibitory signals appear to dominate (see previous text).

Differences in both gain and phase also were found for different groups of secondary neurons (Figs. 5 and 6). Bimodal distributions of response dynamics have been reported previously for vestibular neurons (Shimazu and Precht 1965; Shinoda and Yoshida 1974). Selective projections of otolith afferents (Fernandez and Goldberg 1976) or of primary canal afferents with different dynamics (Fernandez and Goldberg 1971; Tomko et al. 1981) could account for the observed phase differences (Fig. 6). Canal afferents with different dynamics appear to be involved in generating the different frequency responses of eye and neck reflexes (Bilցotto et al. 1982; Highstein et al. 1987). The relative phase behavior of convergent and nonconvergent neurons may represent different levels in the transformation of sensory to motor signals. Response phases of convergent neurons were on average closer to position phase and hence to neck muscle behavior (Bilցotto et al. 1982; Ezure and Sasaki 1978). The significance, if any, of the differences in phase behavior between C6-projecting and other vestibulospinal neurons (Fig. 6) is not clear.

Comparison of our data with that of Fukushima et al. (1990) suggests specialization among vestibulococular projections, as well. In that study, we investigated the responses to three-dimensional rotations of vestibular nucleus neurons antidromically activated from the oculomotor nucleus, but we did not test for descending collaterals. Thus the population of neurons reported in Fukushima et al. includes VOC neurons and VO neurons (axons projecting to the oculomotor nucleus but not the spinal cord, not studied in the present experiments). The overall degree of convergence among secondary oculomotor-projecting neurons in Fukushima et al. was similar to that of the VOC neurons reported here (14 vs. 16%, 70° criterion; Table 1). However, most convergent VOC neurons were activated by horizontal rotation to
the ipsilateral side (Fig. 4, front view), whereas similar numbers of neurons in Fukushima et al. responded to ipsilateral and contralateral yaw. This suggests that convergent VO neurons are activated primarily by horizontal rotation to the contralateral side.

The conclusion that efferent pathways from the vestibular nucleus do not transmit a homogeneous signal is becoming widespread. Neurons involved in vestibulocaudal and vestibulospinal reflexes have different spontaneous firing properties, dynamics, and perhaps different vestibular sensitivities (Highstein et al. 1987; Iwamoto et al. 1990). Cerebellar inputs onto vestibulofugal neurons also are discriminated (Akaike 1983a; Hirai 1988; Sato et al. 1990). VO, but not VOC neurons, receive floccular inhibition; neurons projecting to neck segments are inhibited by the anterior vermis, but not by lobules I and II, which inhibit neurons projecting to the thoracic and lumbar cord. Specialized spatial properties of VO, VC, and C6-projecting neurons probably reflect different requirements for eye, neck, and limb control.

Role of secondary vestibulospinal neurons in spatial transformation of vestibulocollic reflex

Neurons that terminate rostral to the C6 segment are likely to make the most significant vestibulospinal contribution to the vestibulocollic reflex (Akaike 1983b; Donevan et al. 1990; Isu and Yokota 1983; Nyberg-Hansen 1964; Wilson and Maeda 1974). The spatial properties of neck motoneurons could be generated by inputs from vestibulocollic neurons that carry appropriate combinations of canal and otolith signals. To explore this possibility, the MADs of VOC and VC neurons from this study are redrawn in Fig. 7, B and C, to estimate the vectors of neurons which terminate on the left side of the spinal cord. The contralaterally projecting population was produced by reflecting the c-VC and c-VOC vectors about the midline (assuming that the brain stem is symmetrical). Figure 7A plots the MADs of seven left-side neck muscles calculated from electromyographic responses in alert cats (dorsal extensors from Banovetz et al. 1995; longus capitis, a ventral flexor, measured for this study). For comparison, if a neuron provided a spatially tuned excitatory input to a muscle, its MAD and that of the muscle would be aligned; the vector of a spatially tuned inhibitory input would point in the opposite direction.

The front views in Fig. 7 demonstrate that the convergence of vertical and horizontal vestibular signals that characterize neck muscle responses is present in the population of secondary vestibulospinal neurons. The relative sensitivity of two groups of ipsilaterally projecting neurons to vertical and horizontal rotations resembles that of some neck muscles. First, excitatory inputs to longus capitis may originate from signals present on ipsilaterally projecting neurons with MADs suggesting inputs from lpc/rac and rhc/lhc (front view Fig. 7C, vectors marked with *). Second, ipsilaterally projecting neurons with MADs in the shaded region of the front view of Fig. 7C could provide the appropriate inhibitory input to drive the dorsal extensor muscles occipitonasalis, rectus capitis posterior major, splenius, obliquus capitis caudalis, and complexus.

These two groups of neurons both had MADs suggesting excitatory input from the ipsilateral posterior canal. Few convergent neurons in Fig. 7C have MADs suggesting excitatory input from the ipsilateral anterior canal. This bias for ipsilateral posterior over anterior canal input among MVST neurons with horizontal-vertical convergence may be related to an anatomic difference between vestibulospinal neurons monosynaptically activated from the posterior and anterior canal nerves. i-VC neurons that relay anterior canal signals to neck motoneurons are known to descend in the LVST (Uchino et al. 1988; Wilson and Maeda 1974), whereas those activated from the posterior canal descend exclusively in the MVST (Uchino and Isu 1991). Lateral tract neurons with excitatory input from ipsilateral anterior canal afferents could carry signals appropriate for excitation of neck extensor muscles or inhibition of flexor muscles if they also are activated by contralateral or ipsilateral horizontal rotation, respectively. We did not record from LVST cells in these experiments.

The top views in Fig. 7 show that vertical responses tuned about the pitch axis are present on only very few secondary MVST neurons. Few vectors are either aligned with or 180° away from the muscle vectors in the top view. Although our sample was small, higher-order vestibulospinal neurons (Fig. 4) also do not appear to have appropriate spatial properties. This result is in agreement with previous
studies (Iwamoto et al. 1996; Kasper et al. 1988; Wilson et al. 1990) that have shown that the responses of vestibulospinal neurons do not reflect the vertical-vertical canal pair convergence needed to account for the maximal vertical responses of neck muscles to pitch rotation. Pitch or roll signals may be formulated in pathways other than the vestibulospinal tracts. However, muscle-like spatial properties have not been found on populations of pontomedullary reticulospinal (Bolton et al. 1992) and cervical spinal (Endo et al. 1994) neurons either. It is possible that the direct projections of vestibulospinal axons onto neck motoneurons generate the appropriate canal convergence. For example, the near pitch preference of the left biventer cervicis muscle may be produced by properly weighted combinations of excitatory signals from contralaterally and ipsilaterally projecting neurons with MADs aligned with the rac/lpc and lac/rpc axes, respectively (Fig. 7, B and C). As discussed earlier (Excitatory and inhibitory vestibulospinal projections), the substrates for this convergence exist in the bilateral excitatory and inhibitory projections of vestibulospinal neurons with MADs aligned with the canal pair vectors.

It is difficult to speculate to what extent \( C_e \)-projecting MVST neurons contribute to the vestibulocollic reflex. Although neurons projecting below neck segments send collaterals to upper cervical motor nuclei (Perlmutter et al. 1998; Rapoport et al. 1977a; Shinoda et al. 1988), their spatial properties argue strongly for a functional role different from that of VOC and VC neurons. \( V_e \)-type neurons have MADs further away from the canal pair MADs, and some have roll preferences like those of LVST neurons (Iwamoto et al. 1996) and forelimb muscles (Wilson et al. 1986). Perhaps \( C_e \)-projecting neurons are involved in the coordination of neck, back, and forelimb movements or are specialized to compensate for disturbances of posture.

In conclusion, our results suggest that part of the spatial transformation performed by the vestibulocollic reflex can occur by convergence of vertical and horizontal semicircular canal signals onto secondary VC neurons. This convergence could represent the mechanism by which reflex pathways execute part of the spatial transformation achieved by the reflex. This hypothesis predicts that these secondary neurons would have specific projections to motoneurons with responses reflecting similar horizontal-vertical vestibular convergence. Additional mechanisms are necessary, however, to generate motor commands with appropriate vertical-vertical convergence. This may be accomplished by appropriate distribution of vestibulospinal signals to neck muscle motor nuclei. The following paper describes our initial investigation of these possibilities, in which we studied the axonal morphology in \( C_v \) of individual, physiologically characterized vestibulospinal neurons.

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