Spatial Properties of Central Vestibular Neurons of Monkeys After Bilateral Lateral Canal Nerve Section

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Submitted 22 November 2004; accepted in final form 23 June 2005

INTRODUCTION

Damage to the peripheral labyrinth causes spontaneous nystagmus, postural imbalance, and a reduction in the gain of the angular vestibulo-ocular reflex (aVOR) (Curthoys and Hall-magyi 1995; Igarashi et al. 1970). Over time the symptoms are compensated. Return of function is likely to be due to changes in behavioral strategies (Bockisch et al. 2004; Dichgans et al. 1973; Quinn and Baker 1995; Igarashi et al. 1970). Whether the brain can compensate for the loss of input from specific canals based on information available from the intact canals and what portion of recovery depends on other non-canal-related sensory information is still unclear. In this study, we addressed this question by determining whether central vertical canal-related vestibular neurons changed their spatial properties after all input from the lateral semicircular canals had been lost by section of the lateral canal nerves.

Average canal orientation has been well defined in various mammalian species (cat, monkey, rat, pigeon) (Blanks et al. 1972, 1975, 1985; Curthoys et al. 1975, 1977; Dickman 1996; Reisine et al. 1988). The canal system is an approximately orthogonal set of three pairs of reciprocal semicircular canals, the left and right lateral canals (LLRL), the left anterior and right posterior canals (LARP), and the right anterior and left posterior canals (RALP). Single pairs of reciprocal canals deviate on average ~6° from being parallel in the rhesus monkey (range from 3 to 18°), and maximum activation of individual canal afferents can deviate as much as 10°–12° from the average canal planes (Reisine et al. 1988). The average plane of the lateral canals is tipped up ~30° from the stereotaxic horizontal plane, whereas the planes of the vertical canals are rotated ~100° back from the lateral canal plane. The vertical canal planes are rotated ~45° from the sagittal plane. The difference between orientations of the sensitivity vectors for the canals that comprise a push-pull pair can differ by as much as 13° from the average (Reisine et al. 1988). Therefore the input to central vestibular neurons from individual canals would be expected to vary by about ±15° from the average canal plane, determined anatomically.

Central vestibular neurons have approximately the same amount of variation in their spatial properties (Angelaki et al. 1992; Baker et al. 1983a,b; Brettler and Baker 2001; Graf et al. 1993; Quinn and Baker 1998). Estimating from the data published by these authors, the majority of the units fell within ±15–20° from the average plane, although some units deviated by as much as 45°.

Baker and colleagues have shown that it is possible to determine the spatial properties of central vestibular neurons by rotating cats about a spatial vertical axis while they are tilted at different angles with regard to the axis of rotation. Activation of central semicircular canal-related neurons generally corresponded to the geometrical orientation of the canals (Baker et al. 1984a). That is, lateral canal-related neurons were maximally activated when the lateral canals were in the plane of rotation, and the activity decreased as the animals were tilted forward or back. Vertical canal-related neurons had maximum activation when the animals were tilted ~50° backward from the zero plane. The phase of the modulations was opposite for forward and backward tilts, conforming to geometrical projections of the vertical canals onto the plane of the rotation. In this study, we implemented this approach to study the spatial
properties of vertical canal-related central vestibular neurons. Our hypotheses was that if spatial adaptation had occurred after loss of input from both lateral semicircular canals, it would be reflected in changes in the spatial properties of the central vestibular neurons that receive input from the intact vertical canals. Alternatively, if the spatial characteristics of these neurons were unchanged, we would conclude that no spatial adaptation had occurred.

METHODS

The experiments were performed on two cynomolgus monkeys (Macaca fascicularis, M97050 and M98079) after the lateral canal nerves had been sectioned bilaterally. The studies conformed to the Guide for the Care and Use of Laboratory Animals National Research Council 1996 and were approved by the Institutional Animal Care and Use Committee. Unit recording was begun more than one year after nerve section in M98079 and >2 yr after operation in M97050 at a time when the postural imbalance that followed the nerve section had disappeared in both animals.

The surgical procedures and paradigms for oculomotor testing before and after surgery have been given in detail previously. Briefly, the animals were operated in four stages. In the first stage, a head mount developed by Sirota (Sirota et al. 1988; Yakushin et al. 2000) was implanted to hold the animals’ heads painlessly during the experiment. An aluminum cap protected the head mount between experiments. Two weeks later in the second stage, two search coils were implanted on the left eye. One coil, placed around the iris, was used to record horizontal and vertical components of eye movements (Judge et al. 1980; Robinson 1963). Another coil, placed on top of the eyeball, recorded torsional eye movements (Dai et al. 1994). Wires were led from the orbit under the skin to the top of the head and were soldered to a special connector. About 2 mo later, the lateral canal nerves were sectioned bilaterally in a third stage (Cohen et al. 1983). The labyrinths were approached through the mastoid bone, and the lateral and anterior canals were identified. The area anterior to the ampullae of the anterior and lateral canals was cleared to identify the facial nerve and adjacent anterior and lateral canal nerves. The lateral canal nerve was sectioned with limited visibility behind the ampulla with a No. 11 scalpel blade. The lesions were characterized from the changes in the spatial gains and phases of the aVOR and by the loss of horizontal optokinetic after-nystagmus (OKAN; see RESULTS).

About a year later when the postlesion behavioral tests were completed, the animals were anesthetized and a Delrin frame was stereotaxically installed for introduction of microelectrodes (Yakushin et al. 2000).

Experimental protocols and data analysis

EYE-MOVEMENT RECORDINGS. During experiments, the animals sat in the multi-axis stimulator (Neurokinetics, Pittsburgh, PA) with their heads fixed in the stereotaxic horizontal plane (upright position). Movements of the left eye were recorded in three dimensions with the implanted scleral search coils. The techniques of eye movement recording have been described in detail in previous publications (Yakushin et al. 1995, 1998). Yaw and pitch velocities were calibrated by rotating animals in light with a step of velocity at 30°/s about a spatial vertical axis for 30 s in each direction with the animal upright or side down. This rotation evoked nystagmus, and it was assumed that slow phase of eye velocity was close to rotational velocity in this condition (Raphan et al. 1979). Eye rotations to the left and down were positive in accordance with the right-hand rule. The three-dimensional oculomotor responses to vestibular stimuli were extensively tested in both animals prior to this study, and will be the subject of another report. Optokinetic nystagmus (OKN) was induced by movements of an optokinetic cylinder about the yaw axis at 60°/s for 30 s. The lights were then extinguished and OKAN was recorded in darkness. Time constants of OKAN were determined based on fitting the slow phase eye velocities with a single exponential (Cohen et al. 1977). The direction of nystagmus was denoted by the direction of the slow phase eye velocity.

The aVOR was tested while animals were upright and statically tilted forward or backward in 10° increments up to ±90° while being sinusoidally oscillated at 0.5 Hz (60°/s) about a spatial vertical axis in darkness. Desaccaded eye velocities were fit with a sinusoid at the frequency of oscillation using a least mean square algorithm. Temporal gains of the aVOR were determined for each head orientation as the ratio of the amplitude of the sine fit through slow phase eye velocity and amplitude of stimulus velocity.

UNIT RECORDING. Because the head mount developed by Sirota (Beloozerova and Sirota 1986; Correia et al. 1992; Sirota et al. 1988; Yakushin et al. 2000b) was a hollow ring, a special device was built that could fit inside the head mount to record single units in alert animals during rotation around pitch, roll, and yaw axes (Yakushin et al. 1999, 2000a,b). A Delrin block, 80 mm long, 12 mm wide and 5 mm deep, was installed stereotaxically on the top of the monkey’s head, inside the head mount, 1–2 mm above the skin (Yakushin et al. 2000). Holes were drilled orthogonal to the surface of the block at intervals of 1 mm through which electrodes could be introduced. A sterile drill bit (diameter: 0.64 mm) was positioned in one of the premade holes in the Delrin frame, the skin was punctured under local anesthesia, and a small hole was drilled in the skull (Correia et al. 1992). A sterile guide tube, 0.64 mm (23 gauge), sharp on the proximal end with a small stop piece around its distal end was used to guide electrode placement. The tube did not penetrate deeper than 5 mm above the point of interest. Tungsten, varnish-coated wire electrodes (80 μ, ~1–3 ΜΩ at 1 kHz) were introduced into the recording site through the guide tubes that were filled with a mixture of sterile bone wax and petroleum jelly (Vaseline) that stabilized the electrodes during rotation and tilt. The similarity of the internal diameter of the hole in the Delrin frame and in the bone as well as external diameter of the guide tube ensured stable positioning of the guide tube with regard to the skull. The tail end of the electrode was then soldered to a small mechanical micromanipulator (8 × 6 × 40 mm) that moved the electrode over a 30-mm range. The depth of electrode penetration was determined by subtracting the length of the exposed electrode tail from the electrode length.

The location of cells in the medial and superior vestibular nuclei was identified by their position relative to the abducens nuclei and later was confirmed by histological examination. Individual spikes were converted into standard pulses by a spike discriminator (BAK Electronics) with a 2-ms delay and stored in the computer together with parameters of stimulus position and velocity. Analog data were digitized at 600 Hz with 12-bit resolution. The time of the standard pulse occurrence was measured relative to the sampling clock rate (Reisine and Raphan 1992). We assumed that only one spike would occur within the sampling interval of 1.67 ms, which is equal to a peak firing frequency of 600 Hz. Single-spike activity was converted into instantaneous frequency, which was the primary measure of unit activity in this study.

TESTING AND ANALYSIS OF UNIT ACTIVITY. Semicircular canal-related units were identified by oscillating the animals in each of three canal planes: left lateral-right lateral, left anterior-right posterior, and right anterior-left posterior. If a unit was found to respond to rotation in any of these planes, two tests were used to establish the unit sensitivity to angular rotation and to characterize its relationship to particular semicircular canals. Because the lateral canal nerves were sectioned, we expected that the central units would respond only to vertical canal activation. Therefore we first determined which vertical canal (canals) provided input to the recorded neurons. For this, the animal was oscillated about a spatial horizontal axis at 0.25 Hz and peak velocity of 26°/s with its head in different orientations in yaw
relative to the axis of rotation (Fig. 1, test 1). According to our coordinate notation, oscillation about the interaural axis, starting with the head pitching forward, corresponded to a head orientation of 0° (360°). The same oscillation, which started with the head pitching backward, corresponded to a head orientation of 180°. Oscillation about a roll axis, which started with the right ear down, was considered 90°, and with the left ear down was 270°. All units were tested about a roll axis, which started with the right ear down, was considered 90°, and with the left ear down was 270°. All units were tested with the head oriented from 180° to 360° in 15° increments.

Unit activity was recorded during 10 cycles of rotation in each head orientation and sensitivity (temporal sensitivity, imp/s·°/s) to rotation was determined as a ratio of the amplitude of the sine fits through the unit activity expressed as instantaneous frequency and stimulus velocity. The temporal sensitivity was considered to be positive if the unit activity was in phase with ipsilateral stimulus velocity and negative if it was 180° out of phase. The unit sensitivity for each head position was plotted as a function of the head orientation and fit with a cosine function \( y = A \cos(x + B) \), to obtain the spatial sensitivity (A) and phase (B) of the response. We assumed that all canal-related inputs to central vestibular neurons are excitatory. Thus if unit activity increased with rotation in a particular direction, we concluded that this unit received a convergent input from the canals that are excited by rotation in that direction. Neurons were assigned to particular vertical semicircular canal activation if their spatial phases (head orientation in which sensitivity was maximal) were within \( \pm 15° \) of the plane of one of the canals that was activated by rotation in the same direction. Outside of these ranges, neurons were considered to have significant canal-canal or canal-otolith convergence. Predicted distributions of sensitivity associated with unit responses in particular canal planes are shown by the idealized curves (Fig. 1, test 1, AC, PC). For a left posterior and anterior canal-related activity, the spatial sensitivity would ideally have a positive peak at 225 and 315°, respectively over the tested range of head orientations. Corresponding right anterior and posterior canal-related activity would have positive peaks at 45 and 135°, respectively.

Once the associated vertical canal was identified, animals were oscillated at 0.2 Hz around a spatial vertical axis at 60°/s either upright or tilted at 15° increments in the fore-aft direction up to 90°. The purpose of this test (test 2) was to determine whether maximal canal-related activity was significantly different from the tilt of the vertical canal plane, which is \( \sim 50° \) tilt backward (Yakushin et al. 1995, 1998). We assumed that the temporal sensitivity of the recorded unit was positive when the activity increased with rotation toward the ipsilateral side and negative if activity increased during rotation toward the contralateral side. Temporal sensitivity was calculated for each head orientation and plotted as a function of the head pitch. The amplitude of modulation of the induced activity varied as the head orientation was changed with regard to the axis of rotation. As before, individual temporal sensitivities were fit with a cosine function \( y = A \cos(x + B) \), to obtain the spatial gain and phase of the response.

Because we assumed that all canal-related inputs to central vestibular neurons are excitatory, activity of neurons related to the lateral canal in test 2, for example, would have its maximal (spatial) sensitivity when the head was pitched forward \( \sim 30° \), the head orientation in which the lateral canals are maximally activated (Blanks et al. 1985; Curthoys et al. 1977; Reisine et al. 1985, 1988). Because the nerves from both lateral canals were cut in these animals, we did not...
expect to find units with this type of activity and none were encountered (see Results).

IDENTIFICATION OF THE NONCANAL-RELATED CONVERGENT INPUTS. The relationship of unit activity to eye position, eye velocity, and activation of otolith organ, neck, and other body proprioceptors was also tested. Because the animals were not trained to watch a head-fixed target, it was not possible to separate vestibular responsiveness in most of the eye-position-related neurons, and they were excluded from this study. To identify the relationship of unit activity to eye position, each unit was recorded for several minutes while the animal looked at objects presented in different parts of the visual field. Horizontal and vertical eye positions were averaged over 25-ms intervals, color-coded according to the unit activity averaged over the same period, and displayed on an x-y plot. Higher firing rates were represented by darker colors. If unit activity was related to eye position, this produced a color gradient. The same was done for horizontal and vertical eye velocities. Using simulations, we determined that it was possible to detect changes in firing rate of \( -3 \text{ imp s}^{-1} \) over a \( \pm 30^\circ \) change in eye position or a \( \pm 30^\circ \) change in eye velocity. Therefore the accuracy of this method was \( \sim 0.05 \text{ imp s}^{-1} \) or \( 0.05 \text{ imp s}^{-1} \), respectively. Five eye-movement-sensitive neurons were not rejected, three in M97050 and two in M98079, because they were strongly modulated by head velocity.

The relation of unit activity to neck proprioceptors was determined by pressing on the neck muscles while the animal had its head fixed by listening to an audio monitor for changes in firing frequency. The relationship of unit activity to afferent activity from body or neck muscles or from skin receptors was studied in a similar fashion. Otolith organ-related activity was determined based on changes in firing frequency on an audio monitor or an oscilloscope during static head tilts in several directions.

STATISTICAL ANALYSIS. The \( t \)-test was used to compare pairs of data sets. The \( F \) statistic was used to determine the significance of the modulation of unit activity due to vestibular stimulation in each tested head orientation (\( n = 13 \)) (Yakushin et al. 1995). Units were considered unrelated to a particular stimulus if the modulation was not significant in more than half (\( n = 7 \)) of the head orientations.

MORPHOLOGICAL ANALYSIS. At the end of the experiments, 0.3 ml of the neuronal tracer tetramethylrhodamine dextran (TMR; Molecular Probes, 3,000 MW) in 15% 0.1 M acetic acid buffer (pH 3.0) was injected just above the region of the vestibular nuclei where almost all of the vestibular neurons were identified in each monkey. The guide tube for the hypodermic needles was similar to ones used for unit recording. Four days later, the animals were deeply anesthetized and perfused through the heart with 1 l of normal saline followed by 1 l of 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) followed by a 10% sucrose in phosphate buffer (pH 7.4). The brains were removed, immersed for 5 days in 20% sucrose in phosphate buffer (pH 7.4), and sectioned (40 \( \mu \)m) in the frontal stereotaxic plane. The sites of injection were identified by immunohistochemical methods (Kaneko et al. 1996), and the electrode tracks were identified in histological sections stained with neutral red.

RESULTS

VOR and OKAN characteristics after nerve section

To establish that the lateral canal nerves had been sectioned, eye velocity was analyzed during sinusoidal head oscillations and optokinetic stimulation about a yaw axis before and after lesion. Before operation, the spatial properties of the yaw axis aVOR were normal in these animals, with peak gains of \( \sim 0.9 \). Yaw eye velocity was maximal close to the upright position and the yaw gains declined when the animals were tilted forward or back (Angelaki and Hess 1996; Böhrer et al. 1985; Yakushin et al. 1995, 1998). After lateral canal nerve section, the evoked yaw eye velocities were considerably smaller than in the normal animal, with maximal gains of \( \sim 0.2 \). After surgery, yaw gains peaked when the monkeys were tilted back \( \sim 50^\circ \) and were close to zero at tilts close to \( 40^\circ \) forward. The gains reversed phase for forward tilts \( > 40^\circ \). These data are similar to those in previous studies in monkeys in which the lateral canals were plugged or the lateral canal nerves were sectioned (Angelaki and Hess 1996; Böhrer et al. 1985; Lasker et al. 1999; Yakushin et al. 1995). The findings indicate that the lateral canals had been inactivated by the nerve section, and that the yaw eye velocity was being produced by the intact vertical canals.

OKAN was also used as an indication of successful section of the lateral canal nerves. After bilateral labyrinthectomy or when both lateral canal nerves are cut, yaw OKAN in the upright position disappears (Cohen et al. 1973, 1983; Umerra and Cohen 1973; Waespe and Wolfensberger 1985; Waespe et al. 1992), and when one lateral canal nerve is sectioned, ipsilateral OKAN is significantly diminished or lost for \( \pm 2^\circ \) (Arai et al. 1996; Blakley et al. 1989; Brantberg et al. 1996; Fetter and Zee 1988; Hain and Zee 1992; Hain et al. 1994; Ireland and Jell 1982; Lafontaine et al. 1986; Takemori 1997; Tomlinson et al. 1984; Zasorin et al. 1983; Zee et al. 1976).

Before operation, OKAN time constants in one animal (M98079) were 63 and 49 s for left and right slow phase eye velocities, respectively (Fig. 2, A and C). One month after surgery, the OKAN time constants had fallen to 1 and 6 s (Fig. 2C). By 210 days after surgery, OKAN was absent (Fig. 2, B and C) and never recovered (400 days; Fig. 2C). The second animal (M97050) had a similar result; OKAN time constants were 30 and 35 s before surgery and fell to 4 s in the first 2 mo after surgery. The time constants then declined to close to zero and were absent 600 and 1,000 days after nerve section when unit recording began (Fig. 2D). Thus the OKAN time constant measurements were consistent with the conclusion that there was no significant input from the lateral canals in both animals at the time of unit recording.

Characteristics of unit activity after section of the lateral canal nerves

All of the 37 neurons included in this study were activated by rotation about a spatial vertical axis in darkness and hence were considered to receive input from the semicircular canals. Twenty-one units were obtained from M97050 and 16 units from M98079 (Table 1). Otolith and/or neck related cells that did not have significant convergent inputs from the semicircular canals were excluded (see STATISTICAL ANALYSES). Three of 21 units in M97050 and 3 of 16 units in M98079 were solely related to vertical canal activation. All of the other vertical canal-related units (31) had an additional convergent input from the otolith organs, the neck and/or from body proprioceptors or were eye-movement-related. Only three non-eye-movement-related units (3/18) in M97050 and one unit (1/14) in M98079 were vestibular-only (VO) neurons. None of these were the pure vertical canal-related cells mentioned in the preceding text. The majority of the remaining non-eye-movement-related units had very different discharge characteristics from VO neurons in normal animals (Yakushin et al. 2005).
The number of convergent inputs is consistent with recent findings (Dickman and Angelaki 2002; Jian et al. 2002; Uchino 2001; Uchino et al. 2000; Zhang et al. 2001, 2002). Spontaneous activity varied from 16.3 to 135.3 imp*s$^{-1}$ in all recorded cells in the two animals, with eight of the recorded neurons having spontaneous activity >80 imp*s$^{-1}$. Only one neuron with a high spontaneous rate was purely vestibular. Its activity was not related to any of the other modalities, and therefore it could have been a primary afferent. Mean spontaneous activity was not significantly different in the two animals ($P=0.1$) and on average was 55.8 ± 29.4 imp*s$^{-1}$, similar to data obtained from normal monkeys [48.5 ± 21.2 imp*s$^{-1}$ (Zhang et al. 1993), 66–124 imp*s$^{-1}$ (Zhang et al. 1995), 36–83 imp*s$^{-1}$ (Tomlinson and Robinson 1984), 37 imp*s$^{-1}$ (Chubb et al. 1984)]. This implies that the recorded neurons were in a normal state and had recovered from any adverse effects of the lateral canal nerve section.

We searched the rostral medial vestibular nuclei (MVN) and SVN for units that had lateral and/or vertical canal-related activity. No canal-related unit activity was found in MVN although many units that did not respond to semicircular canal activation were encountered. Consequently, all canal-related

![FIG. 2. Optokinetic nystagmus (OKN) and after nystagmus (OKAN) of animal M98079, before (A), and 7 mo after surgery (B). C and D: time constants of OKAN for animals M98079 (C) and M97050 (D) at different times after surgery. OKAN was lost in both directions after bilateral lateral canal nerve section at the time recording began in the vestibular nuclei.](http://jn.physiology.org/)

### TABLE 1. Summary table

<table>
<thead>
<tr>
<th>Type of Convergence</th>
<th>M97050</th>
<th>M98079</th>
<th>Both animals, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vertical canal only</td>
<td>3</td>
<td>2</td>
<td>14</td>
</tr>
<tr>
<td>Vertical canal + otolith</td>
<td>7</td>
<td>4</td>
<td>30</td>
</tr>
<tr>
<td>Vertical canal + neck</td>
<td>3</td>
<td>2</td>
<td>14</td>
</tr>
<tr>
<td>Vertical canal + otolith + neck</td>
<td>8</td>
<td>7</td>
<td>42</td>
</tr>
</tbody>
</table>

One unit was excluded from this summary table because its otolith convergent input was not determined. Two units (1 for each animal), which were identified as only vertical canal-related neurons, had convergent input.
units reported in this study were located in SVN. Injection of TMR on the left in M98079 labeled the area in the vestibular nuclei where the majority of vertical canal-related neurons were recorded (Fig. 3). These lay in SVN. We also searched through areas of MVN where vestibular-only (VO) neurons are typically located (Chubb et al. 1984; Dickman and Angelaki 2002; Fuchs and Kimm 1975; Keller and Kamath 1975; McCrea et al. 1987; Reisine and Raphan 1992; Scudder and Fuchs 1992; Yakushin et al. 2005). No horizontal canal-related neurons were encountered in this region. Consistent with the earlier study of Waespe et al. (1992), light microscopic examination of MVN did not show evidence of significant cell loss in rostral MVN.

Oscillation about a spatial horizontal axis

Animals were sinusoidally oscillated about a spatial horizontal axis in different head orientations in yaw to determine whether the ipsi- or contralateral anterior or posterior canals activated the recorded units and whether the neurons had otolith input. For the unit shown in Fig. 4A, the activity was modulated in-phase with the stimulus (Fig. 4A, dashed vertical line, 1st–3rd traces) when the animal was oscillated with head orientations from 180 to 270° (Fig. 4A, inset). The amplitude of modulation was negligible when the head was oriented at 315°. Activity was 180° out of phase when the animal was oriented at angles greater than 330° (Fig. 4C). Activity of other canal-related neurons also followed predicted curves (Fig. 1).

The spatial sensitivity and phase of all units recorded from each animal are shown in Fig. 5. Units activated close to canal planes, i.e., in the shaded area (±15°), are shown by ■ and ▲, whereas cells outside this region that were close to the fore-aft or side-down directions of tilt are shown by ◯ and △. Slightly over half of the units (58%) lay within ±15° of vertical canal planes. Deviation of the spatial response from this range indicates that there was convergent input from the other vertical canals or from the otolith organs. The amount of convergence in this test was similar to that obtained from normal animals (Yakushin et al. 2005). The average sensitivities of the vertical canal neurons were the same in M97050 and M98079 (P = 0.54, 0.57 ± 0.49 and 0.49 ± 0.25 imp*s⁻¹/°s⁻¹, respectively), and were not different from average sensitivities in two normal animals (0.50 ± 0.32 and 0.73 ± 0.54 imp*s⁻¹/°s⁻¹) (Yakushin et al. 2005) (P = 0.209, ANOVA). Thus the spatial sensitivity of the vertical canal neurons from the two animals with bilateral lateral canal nerve section was within the normal range.

Oscillation about the spatial vertical axis

Typical examples of the vertical canal-related neurons that were encountered in this study are given in Fig. 6. The cells were tested with rotation about a spatial vertical axis with the head in tilted positions in pitch. The activity of the cell shown in Fig. 6A increased with rotation toward the contralateral side.
in the upright position, i.e., it had type II behavior, and the modulation in activity increased as the animal was tilted back (Fig. 6A, 0 to −60°). The phase of the modulation was the same for all tilts backward. For forward tilts, there was little modulation in unit activity as the animal was reoriented 30° tilt forward (Fig. 6A), but significant modulation reappeared for forward tilts > 30° (Fig. 6A). In the forward tilt positions, however, the activity increased for ipsilateral rotation, which was type I behavior. The spatial properties of this unit are summarized in Fig. 6B, in which sensitivity is plotted as positive when the activity increased for ipsilateral rotation (Fig. 6C) and as negative when the activity increased for contralateral rotations. We classified this unit as Type II, based on its activity in the upright position.

For each unit, the spatial sensitivity (maximal sensitivity) and phase (head orientation at which the unit was maximally activated) was obtained from the sinusoidal fit of the temporal sensitivities plotted as a function of head orientation (Fig. 6B). The spatial phase of the units shown in Fig. 6 was ~60° back, with a spatial sensitivity of ~0.2 imp·s⁻¹·°⁻¹. This type of activation indicates that the unit was activated by the vertical semicircular canals. In the head back positions, type I activation indicates contralateral vertical canal input, whereas Type II activation indicates input from the ipsilateral vertical canals.

All of the units recorded in both animals were maximally activated with the head tilted back between 30–60° (M97050, Fig. 7A; M98079, Fig. 7B), and the distribution of neurons with type 1 and type 2 behavior were similar (○ and ▲, respectively). The average spatial phase was −47 ± 17° for M97050 and −50 ± 12° for M98079. There was no difference between these planes (P = 0.5), and they lay close to the plane of maximal activation of the vertical canals, which is ~50° (Blanks et al. 1985; Curthoys et al. 1977; Dickman 1996; Reisine et al. 1985, 1988; Yakushin et al. 1995). Therefore based on our rejection criteria (Fig. 1, bottom), we conclude that no spatial adaptation had occurred. Slight differences in the spatial phases from the planes of the vertical canals of the VO and VPS neurons in the normal animals (−59 ± 6°, P = 0.005) (Yakushin, et al. 2005)
could be attributed to individual variations between animals (Reisine et al. 1988). There was no difference in spatial sensitivities in monkeys M97050 and M989079 as determined by this test ($P = 0.75; 0.19 \pm 0.10$ and $0.20 \pm 0.12$ imp/s°s$^{-1}$, respectively), but these sensitivities were smaller than in the normal animals ($P = 2 \times 10^{-9}$) (Yakushin et al. 2005). These sensitivities were also smaller than would have been predicted in the nerve-sectioned animals from oscillation about a spatial horizontal axis. Possible explanations for this difference in sensitivity are considered in the next section.

**Non-canal-related convergent inputs**

Convergent inputs from the otolith organs and neck muscle proprioceptors were determined with static tilt and compression of neck muscles (see METHODS). Eighty-six percent of vertical canal-related units were activated by an additional convergent input. Of these, 72% were activated by static head tilt, 56% received convergent projections from the neck and other body muscles, and 40% of the cells received input from both the otolith organs and the somatic musculature (Table 1). Thus a large number of vertical canal-related neurons were sensitive to head tilt and neck and body proprioceptors. For the units without neck proprioceptive inputs, 31% were vertical canal-only (5/16) and 69% had convergent inputs from both vertical canal and the otolith organs (11/16). We compared the convergence of these cells to that of the VO and VPS neurons of normal animals (Yakushin et al. 2005). Excluding lateral canal-related neurons, there were 36% (9/25) that were only vertical canal-related and 64% (16/25) that had convergent inputs from both vertical canal and the otolith organs. Thus the neurons that received convergent otolith inputs were comparable in both studies. Half of the neurons in the present study received convergent neck proprioceptive input, which, to our knowledge, has not been reported before for neurons in SVN.

**FIG. 6.** Activity of the same neuron as in Fig. 4 recorded during oscillation about a spatial vertical axis with the head in different angles of pitch relative to the axis of rotation. A: Neuronal response to oscillation about a spatial vertical axis while the head was tilted from $-90^\circ$ (backward, top trace) to $+90^\circ$ (forward). The corresponding head orientation is marked on the right side of each trace. The positive direction of the primate axis (bottom trace) corresponds to rotation to the right (contralateral). The activity of this unit was out-of-phase with the stimulus while the animal was upright or tilted backward, but in-phase when the animal was tilted more than 30° forward. Since the recorded unit was located on the left side of the brain stem, its out-of-phase modulation with a rightward head velocity in the upright corresponds to Type II activity (See Methods). B: Unit sensitivity and phase (C) in each of the head orientations is shown in A. The sensitivity was plotted as negative or positive according to its relation to the phase of stimulus velocity and the side of the brain stem where this unit was recorded.

**FIG. 7.** Polar plot of sensitivity and phase of all of the canal-activated units that were recorded from M97050 (A) and M989079 (B). The heavy lines show the means. Insets in A indicate head orientation that would have maximally activated the lateral (30°) and vertical (-50°) semicircular canals. The emboldened lines show canals that were maximally activated in each head orientation.
We determined whether the non-canal related convergent inputs had significantly altered the spatial gains and temporal phases of the vertical canal neurons. Theoretically, if a unit received an input only from a single vertical canal, the temporal phases and spatial sensitivity obtained by rotation about spatial horizontal and vertical axes should be comparable if the geometrical orientation of the canal to the plane of rotation is considered [(sensitivity vertical axis) = (sensitivity horizontal axis) *cos45°]. If a unit received significant additional input from other sources, then the temporal and spatial gains and phases could be affected and therefore the results of the two tests might not be comparable. Two potential sources were identified in this study. One was proprioceptive input from the neck; the second was input related to dynamic otolith activation induced by rotation about a horizontal axis.

Temporal phases obtained by rotation about each of the two axes in which spatial gains were maximal were compared. The study of neurons from normal animals did not contain units that were significantly modulated by neck proprioceptive input. The temporal phases obtained from horizontal and vertical axis rotation were correlated in these neurons (P < 0.05; Fig. 8A, ○) (Yakushin et al. 2005). In contrast, there was no correlation between these parameters in the vertical canal neurons of the present study (P > 0.05; Fig. 8A, ●, ▪). Similarly, the spatial gains of the neurons from horizontal and vertical axis rotation were well correlated in the normal animals (Fig. 8B, ○), but there was no correlation between two parameters in the present study (Fig. 8B, ●, ▪). It is likely that neck proprioceptive and dynamic otolith inputs to the vertical canal-related cells were responsible for the changes in the spatial properties of these neurons.

DISCUSSION

Data in this study support the hypothesis that vertical canal-recipient, central vestibular neurons do not alter their spatial phases after loss of input from the lateral canal. The average planes of the head orientation in which vertical canal-related central vestibular neurons were maximally activated by rotation about a spatial vertical axis after loss of input from the lateral canal were −47 ± 17 and −50 ± 12°. These planes were close to the maximal activation planes of central vertical canal-related neurons recorded in normal monkeys [−57 ± 7 and −63 ± 5°; (Yakushin et al. 2005)], and in other studies [monkeys (Dickman and Angelaki 2002), cats (Baker et al. 1984a; Graf et al. 1993)]. These planes were also close to the optimal response planes of the vertical canals (−50°) derived from morphological studies (Blanks et al. 1985; Curthoys et al. 1977; Dickman 1996; Reisine et al. 1985, 1988) by recording primary afferent activity from the vertical canals (Haque et al. 2004; Reisine et al. 1985, 1988) or from analysis of oculomotor responses after canal plugging (Angelaki and Hess 1996; Angelaki et al. 1996; Böhmer et al. 1985; Yakushin et al. 1995). The oculomotor responses in the nerve-cut animals also conformed to these average response planes. Deviation of the spatial responses for individual neurons about the average value (±15°) is consistent with the deviation of the primary afferents about the plane of the functional vertical canal-pairs (Reisine et al. 1988). Based on these findings, we conclude that central vertical canal-related neurons did not change the spatial properties of their response to compensate for the loss of the lateral canals.

The percentage of central vestibular neurons that received convergent otolith inputs in the present study was not significantly different from those in the normal animals (Yakushin et al. 2005). Neither was the amplitude (spatial sensitivity) and direction (spatial phase) of the response vector of the neurons, determined by oscillation about a spatial horizontal axis different from normal. This indicates that the vertical canal neurons in the normal and nerve-sectioned animals responded to convergent dynamic otolith input similarly.

There was a significant difference in the temporal phase and spatial gains of the responses to oscillation about a spatial vertical axis between the normal and nerve-sectioned animals. The extensive (56%) neck proprioceptive input to SVN units, which has not been reported before, is likely to have been responsible for at least a part of this difference. This could have come about because only the head was fixed in our animals while the body was free to move relative to the axis of rotation.
We speculate that when animals were oscillated about a spatial vertical axis, the neck and body moved relative to the head due to inertia, activating neck proprioceptive input and that this, in turn, affected the firing rates of vertical canal-related neurons. This is in agreement with reduction in modulation of the unit discharge rate in response to combined neck and vestibular stimulation in normal monkeys (Gdowski and McCrea 2000).

An enhanced response of neck proprioceptors after canal nerve section is consistent with the changes in the cervicoocular reflex (COR) after semicircular canal lesions and could provide the basis for the recovery in posture and balance after partial or complete labyrinthine damage. The gain of the COR, which is small in the normal animals (Roy and Cullen 2002), is significantly increased after either lateral canal plugging or nerve section (Baker et al. 1982; Böhmer and Henn 1983; Bronstein and Hood 1986; Dichgans et al. 1973). What is not clear from the present results is whether this enhancement was due to new synaptic input from the neck or to disinhibition of synaptic connections that were already present. Although there is no information as to the projections of neck proprioceptors onto SVN neurons, there is substantial convergence on other canal-recipient central vestibular neurons (Abend 1977; Anastasopoulos and Merger 1982; Angelaki et al. 2002; Brettler and Baker 2001; Dickman and Angelaki 2002; Endo et al. 1995; Gdowski and McCrea 1999, 2000; Kasper and Thoden 1981; Kasper et al. 1988; Markham and Curthoys 1972; McCrea et al. 1996; Pompeiano et al. 1987; Rubin et al. 1977; Sato et al. 2000, 2002; Wilson et al. 1990; Yakushin et al. 1999; Zhang et al. 2001; Yakushin et al. 2005), and the latter hypothesis seems more likely.

A striking finding was that after bilateral section of the lateral canals, no lateral or vertical canal-related neurons were encountered in rostral medial vestibular nuclei (MVN). The inability to find canal-related neurons in MVN was not due to technical problems or to location or extent of the search because successful recordings were made over the same period in a normal animal in another study (Yakushin et al. 2005). The lack of lateral canal-related neurons in MVN was not unexpected because the was no lateral canal input. The cells also could not have been activated by velocity storage because storage disappears after lateral canal nerves had been sectioned (Cohen et al. 1973; Uemura and Cohen 1973). What was unexpected was that we failed to find vertical canal-related neurons in this area. Most units recorded in the rostral part of the MVN are related to the horizontal aVOR in the normal animal (Cheron et al. 1996; Chubb et al. 1984; Fuchs and Kimm 1975; Furuya and Markham 1981; Ishizuka et al. 1980; Keller and Kamath 1975; McCrea et al. 1980, 1987; Newlands and Perachio 2003; Reisine and Raphan 1992; Scudder and Fuchs 1992; Searles and Barnes 1977) but also have a convergent input from at least one vertical semicircular canal or the otolith organs (Baker et al. 1984a,b; Brettler and Baker 2001; Curthoys and Markham 1971; Dickman and Angelaki 2002; Fukushima et al. 1990; Markham and Curthoys 1972; Sato et al. 2002; Yakushin et al. 2005). Microscopic inspection of MVN did not reveal a loss of neurons nor was there heavy gliosis that might have accompanied neuronal death. More likely, vertical canal input converge onto cells related to the lateral canals in MVN but was not strong enough to modulate the firing rate of these cells after the loss of the lateral canal input. This is consistent with the idea that MVN essentially processes information related to the horizontal aVOR (Straka et al. 2002; Tokumasu et al. 1969).

Of note was the absence of VO neural activity in MVN. It has been postulated that these cells are responsible for generation of horizontal velocity storage in the vestibular system (Reisine and Raphan 1992; Yokota et al. 1992; Yakushin et al. 2005). Only the yaw component of velocity storage disappears when the lateral canal nerves are sectioned with the animals tested in an upright position, and it reappears when the animals are tilted forward or back (Fig. 3 of Cohen et al. 1983). Whether the VO cells disappeared or were disfacilitated is not known, but the data support the idea that activity of horizontal VO neurons is dependent on intact input from the lateral canals (Reisine and Raphan 1992).

The average spontaneous activity recorded in this study (57.3 imp s−1) was slightly higher than the range reported by others from normal animals (Chubb et al. 1984; Fuchs and Kimm 1975; Tomlinson and Robinson 1984; Zhang et al. 1993, 1995). The average spontaneous activity of various types of central vestibular neurons is different, being highest for velocity-plus-saccade cells (~100 imp s−1) (Chubb et al. 1984; Zhang et al. 1993). Horizontal aVOR-related, VO neurons, which are likely to be responsible for producing OKAN and the dominant time constant of the horizontal aVOR (Reisine and Raphan 1992) and which were not encountered in the present study, have the lowest spontaneous rate (~35–45 imp s−1) of the canal-related central vestibular neurons (Chubb et al. 1984; Yakushin et al. 2005; Zhang et al. 1993). Elimination of the VO neurons from the population of the sampled neurons, which have a lower mean frequency, could have been responsible for the increase in the average spontaneous firing rate.

In summary data in this study, obtained long after the surgery when all possible adaptive processes should have been completed, indicate that central vertical canal-related vestibular neurons do not change their spatial properties after loss of canal input due to nerve section. Sensory signals from the otoliths and neck could contribute to the restoration of head and postural instability after surgery.

ACKNOWLEDGMENTS

We thank Dr. Evgeny Baharin and D. Ogorodnikov for implementing the program for data analyzes. We also acknowledge the technical assistance of V. Rodriguez and J. Maruta.

GRANTS

This work was supported by National Institutes of Health Grants DC-04996, DC-03787, DC-05204, EY-04148, EY-11812, and EY-01867.

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