Spatial Orientation of Caloric Nystagmus in Semicircular Canal-Plugged Monkeys

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INTRODUCTION

Thirty years after the discovery that the sense of angular motion was due to activation of the semicircular canals (Breuer 1874; Crum-Brown 1874; Mach 1875), and shortly after Ewald (Ewald 1892) established the physiological mechanism of canal activation using a “pneumatic hammer” and canal plugging, Robert Bárány discovered that nystagmus could be induced by irrigation of the external auditory canals with cold or warm water (Bárány 1906). With the head tilted up 30° from the supine position so that the lateral canal planes are aligned with the spatial vertical, introduction of cold water into one ear induces horizontal nystagmus with ipsilateral slow phases, presumably coming predominantly from the ipsilateral lateral canal. Irrigation with warm water produces an oppositely directed nystagmus. If the head is placed 30° down from the prone position, the lateral canal is inverted in space from the supine position, and the nystagmus produced by cold and warm stimuli reverses direction. From this, Bárány inferred that the induced nystagmus was the result of utriculopetal or utriculofugal deflection of the cupula in the lateral canals caused by convection currents, which were produced by local cooling or warming of the endolymph moving toward or away from gravity. This resulted in the caloric test, which almost 100 yr later, is still widely used clinically to determine and compare the response of the lateral semicircular canals.

Other factors could also contribute to the caloric response. Direct cooling or warming of the nerves increases or decreases firing rates in the vestibular nerve (Klinke 1992). This is likely to be responsible for the differences in caloric response in the supine and prone positions, in which the induced horizontal eye velocity from cold caloric stimulation is greater in face-up than face-down positions (Clarke et al. 1988; Coats and Smith 1967; Hood 1989; Minor and Goldberg 1990; Paige 1985). It has been estimated that about 75% of the caloric response is the result of convection currents, and about 25–30% of the response is due to warming or cooling of the nerve (Minor and Goldberg 1990). Additionally, since the caloric response is due to stimulation of the semicircular canals, such stimulation should cause activation of central circuits responsible for velocity storage. Velocity storage, which is activated by vestibular stimulation, dominates the normal response to caloric nystagmus, velocity storage alone could not rotate the velocity vector to fit the data. The data could not be modeled simply by a reduction in activity in the ipsilateral vestibular nerve, which would direct the velocity vector along the roll direction. Since there is no cross coupling from roll to yaw, velocity storage alone could not rotate the vector to fit the data. Postulated, therefore, that cooling had caused contraction of the endolymph in the plugged canals. This contraction would deflect the cupula toward the plug, simulating ampullofugal flow of endolymph. Inhibition and excitation induced by such cupula deflection fit the data well in the upright position but not in lateral or prone/supine conditions. Data fits in these positions required the addition of a spatially oriented, velocity storage component. We conclude, therefore, that three factors produce cold caloric nystagmus after canal plugging: inhibition of activity in ampullary nerves, contraction of endolymph in the stimulated canals, and orientation of eye velocity to gravity through velocity storage. Although the response to convection currents dominates the normal response to caloric stimulation, velocity storage probably also contributes to the orientation of eye velocity.
ular, visual, and somatosensory inputs, improves the low frequency characteristics of the angular vestibulo-ocular reflex, sustains eye velocity during prolonged rotation of the subject or the visual surround, and counters vestibular after-reactions (Cohen et al. 1977; Raphan et al. 1979; see Raphan and Cohen 1996, 2002 for review).

A striking feature of velocity storage is its spatial orientation. In the monkey, the duration and gain of the yaw, pitch, and roll components of vestibular and optokinetic nystagmus are altered so that they tend to maintain the eye velocity vector of the nystagmus aligned with gravity or with gravito-inertial acceleration (GIA), the sum of the linear accelerations acting on the head (Dai et al. 1991; Raphan and Sturm 1991; Wearne et al. 1996–1999). The yaw time constant is longest, and the pitch and roll time constants are shortest in the upright position, when the velocity vector of the induced nystagmus is coincident with the earth-vertical, i.e., with gravity (Dai et al. 1991). In side down or prone and supine positions, the time constants of the pitch and roll components, respectively, are maximal, and the yaw axis time constant is reduced (Dai et al. 1991). Pitch and roll eye velocities also appear when yaw axis nystagmus is induced with the head in tilted positions. These velocities arise because of cross-coupling of activity from yaw to pitch or roll. An important assumption in modeling the orientation properties of velocity storage is that there is no cross-coupling from pitch or roll to yaw (Dai et al. 1991; Raphan and Sturm 1991; see Raphan and Cohen 1996 for review).

Because the convection currents that are produced by segmental cooling or warming of the endolymph are oriented with respect to gravity (Bárány 1906), it has been difficult to determine whether the orientation properties of velocity storage contribute to the response. This is primarily because the convection currents in the lateral canals produced by cooling or warming the endolymph are also oriented to gravity and overwhelm other contributions (Aw et al. 1998; Böhm et al. 1992, 1995). A number of studies have demonstrated unknown, nonconvective mechanisms that could tend to orient eye velocity toward gravity (Böhmer et al. 1995; Fetter et al. 1998; Paige 1985; Stahle 1990; Yagi et al. 1992), but there is little information about the degree to which velocity storage might be involved (Arai et al. 1989, 1998; Kawachi 1992; Tsuchiya 1995). Two lines of evidence suggest that velocity storage is activated by caloric stimulation. If caloric nystagmus is suppressed by exposure to a stationary visual surround during its early phases, slow phase velocity recovers slowly, suggesting reactivation of central circuits by afferent input (Raphan and Cohen 1981; Takemori and Cohen 1974). Moreover, if caloric nystagmus is suppressed by light in its terminal stages but that outlasts cupula deflection (Raphan and Cohen 1981).

Elimination of convection currents is one way to study the contribution of the nonconvective mechanisms, which include velocity storage, to caloric nystagmus. During a Skylab space flight, where the gravitational field was reduced to \(\sim 10^{-6}\) g, robust caloric nystagmus was elicited by bithermal stimulation, despite the absence of convection currents (Clarke and Scherer 1988; Scherer and Clarke 1985, 1987a,b; Scherer et al. 1986). The source of the response to caloric stimulation in microgravity is not entirely clear, but Scherer and Clarke proposed that in addition to its effects on the vestibular nerve, cooling had caused a difference in pressure between the arm of the canal adjacent to the cupula and the endolymph in the vestibule on the opposite side of the cupula, producing hair cell deflection (Clarke et al. 1993a,b; Scherer and Clarke 1985). How the orientation properties of velocity storage might contribute to the spatial orientation of the induced nystagmus could not be inferred from these studies, however, because the linear acceleration of gravity is so weak in orbital flight.

Another way to eliminate convection and maintain the orientation properties of velocity storage is to interrupt the flow of endolymph by plugging the semicircular canals (Ewald 1892). The end organs are histologically intact after canal plugging (Angelaki et al. 1996; Arai et al. 1996; Camis 1930; Ewald 1892; Suzuki et al. 1991; Yakushin et al. 1998), the resting discharge is unaltered in the afferent fibers (Goldberg and Fernandez 1975; Rabbitt et al. 1999), and the orientation properties of velocity storage are unaltered (Raphan et al. 1992b). The canals remain active in detecting angular acceleration after canal plugging (Lasker et al. 1999; Rabbitt et al. 1999; Yakushin et al. 1998), but the dominant time constant of the plugged canals is reduced from 4–5 s (Büttner and Waespe 1981; Correia et al. 1992; Goldberg and Fernandez 1971; Reisine et al. 1988) to about 0.07 s (Yakushin et al. 1998). As a result, the high-pass cutoff characteristics of the canals move to considerably higher values, further reducing the effect of low-frequency stimulation, such as would occur from convection currents. On the other hand, the duration of the induced nystagmus has been shown to be approximately the same in canal-plugged as in normal animals (Arai et al. 2000). Therefore, the duration of the stimulus in the bone must have been the same, despite the fact that the mastoid had been opened to reach the inner ear in the canal-plugged animals. Optokinetic after-nystagmus (OKAN) was also preserved after canal plugging, so that velocity storage was operative. Canal plugging, therefore, presents a unique opportunity to study the characteristics of nonconvective mechanisms and the role of velocity storage in producing the caloric response in three dimensions.

The purpose of this study was to characterize the orientation properties of caloric nystagmus in canal-plugged animals, which could be compared with the orientation of the nystagmus induced by convection currents and other factors in the intact animal. If velocity storage contributes to caloric nystagmus, then caloric stimulation in the upright position would be expected to produce little or no cross-coupling from yaw to pitch and/or roll, and the pitch and roll components would almost entirely be due to direct activation of the lateral canal and the adjacent anterior canal. In side down, prone, and supine positions, however, there should be substantial cross-coupling of the induced horizontal nystagmus to either pitch or roll, depending on the position of the head with regard to gravity.

**METHODS**

Experiments were performed on five cynomolgus monkeys. In two, all six semicircular canals were plugged, and they were tested before and after operation. The responses to rotation before and after oper-
ation in these animals have been reported in detail elsewhere (Yakushin et al. 1998). Three other animals with intact canals were used as controls. The data from the canal-plugged animals form the basis for the quantitative portions of this report. A full analysis of the responses from the normal animals is beyond the scope of this paper and will be considered elsewhere. The experiments 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.

Surgical procedures have been described in detail before (Yakushin et al. 1995, 1998). Briefly, using sterile surgical techniques, head bolts were implanted on the skull in dental acrylic cement under general anesthesia. This provided for painless fixation of the head in stereo-taxic coordinates during testing. Movements of one eye were recorded with two implanted scleral search coils. One coil, in the frontal plane, measured horizontal and vertical eye position (Judge et al. 1980). A second coil, placed approximately orthogonal to the frontal coil, measured roll eye position (Cohen et al. 1992; Dai et al. 1994). The semicircular canals were plugged about 1 mo after coil implantation. The bony and membranous canals were identified and interrupted by grinding across them with a fine diamond burr (Cohen et al. 1964, 1965; Money and Scott 1962; Suzuki and Cohen 1966; Suzuki et al. 1964, 1991, 1995; Yakushin et al. 1995). The orifice of the canals was packed with bone dust, and the animals were allowed adequate time to recover.

At the end of testing, one of the two animals whose canals were plugged (M9308) was perfused through the heart with saline and a paraformaldehyde/formalin solution under deep anesthesia. The temporal bones were removed, decalcified, embedded in celloidin, and processed for anatomical study. On histological examination, the bone had fused to provide an impenetrable block to the flow of endolymph in all six semicircular canals. Examples from the left labyrinth are shown in Fig. 1, A–C. The lateral canal plugs were located in the middle of the limb of the canals. The anterior canal plugs were closer to the ampulla, and the posterior canal plugs were located in the tail close to the common crus of the canal. The approximate position of the plugs in one of the labyrinths is shown in Fig. 4A. The hair cells of the canals (Fig. 1, A–C) and the utricular macula (D), saccular macula (E), and the cochlea (F) were intact.

During testing, the monkeys' heads were fixed in a frame that held two sets of field coils. The axes of the field coils were along the interaural and dorso-ventral axes of the head, establishing a head-fixed reference frame for measuring the orientation of the search coils in front and on top of the eye. Data were recorded with amplifiers having a band-pass of DC to 40 Hz. A computer controlled the equipment and acquired the data. Voltages were digitized at 600 Hz/channel with 12-bit resolution. Coil related voltages were converted to Euler angles. Eye position voltages were smoothed and digitally differentiated by finding the slope of the least-squares linear fit, corresponding to a filter with a 3-dB cutoff above 40 Hz, the cutoff frequency of the filters used for data acquisition. Saccades were eliminated using maximum likelihood ratio criteria (Singh et al. 1981).

Eye velocities were calibrated by rotating the animals in light at 30°/s about the pitch, roll, and yaw axis. It was assumed that horizontal and vertical (VOR) gains were approximately one (Crawford and Vilis 1991; Dai et al. 1991; Raphan et al. 1979; Robinson 1963). Roll gains were assumed to be 0.6 when rotation was around a naso-occipital axis aligned with the spatial vertical (Crawford and Vilis 1991; Henn et al. 1992; Yakushin et al. 1995). Roll gains derived using this assumption agreed with those determined for monkeys.

FIG. 1. Histological sections from left labyrinth of M9308. A–C insets: each canal (A, anterior canal; B, lateral canal, and C, posterior canal) was completely occluded by a dense bone overgrowth after plugging. Arrowheads show the borders of the canals. The hair cells of the crista in each of the canals (A–C) and the utricular macula (D), saccular macula (E), and the cochlea (F) were intact.
using other techniques (Dai et al. 1994; Telford et al. 1996; Yue et al. 1994). After canal plugging, optokinetic nystagmus (OKN) and OKAN were unaltered and acceleration gains for steps of velocity were close to those before plugging (Yakushin et al. 1998). Consequently, it was determined that the eye movement calibrations were unaltered after plugging. Eye velocities to the left, down, and clockwise from the animal’s point of view were positive according to a right-hand rule and are represented by upward deflections in the velocity traces in Figs. 2–4.

During testing, animals sat in a primate chair in a multi-axis vestibular stimulator (Neurokinetics) that provided a light-tight environment and was used for calibration. To receive caloric stimulation, the monkeys were positioned upright, right-side down (RSD), left-side down (LSD), supine, and prone. When animals were upright, the head yaw axis, which was normal to the horizontal stereotaxic plane, was along the earth-vertical, and the lateral canals were approximately 30° above the horizontal stereotaxic plane (Blanks et al. 1985; Reisine et al. 1988). In the supine and prone positions, the lateral canals were tilted approximately 30° from the earth vertical plane, but the ampullae were oppositely directed relative to the spacial vertical when face up or face down. The lateral canals were aligned with the earth-vertical in the RSD and LSD positions, the positions of maximal activation for convection currents, assuming that the dihedral angle between the lateral canals was zero. These head orientations were chosen because they have commonly been used for studying the spatial orientation of velocity storage (Dai et al. 1991; Raphan and Sturm 1991). Time constants would be maximal for yaw when the animals were upright, for pitch when the animals were side-down, and for roll when they were supine or prone.

Caloric stimuli consisted of irrigation of one ear with 10 ml of cold water at approximately 0°C over 15 s. Before stimulation, a 22-gauge plastic tube, calibrated for length, was introduced into the external auditory canal. A shoulder attached to the plastic ensured that the tube delivered water to within 1–3 mm from the drum, but did not puncture it. The external canal was first irrigated with 200 ml of approximately 37°C water to clean out cerumen. The drum was inspected before and after irrigation to ensure that it had not been physically disturbed and that there was no wax in the external canal. At the end of each irrigation, the lights were extinguished, and the response was recorded in total darkness. A 5-min interval in light was allowed to elapse between stimuli to avoid residual cooling in the temporal bones. The order of testing was upright, LSD, RSD, supine, and then prone, because the caloric responses were approximately equal and did not appear to habituate in this order in normal monkeys.

**Computation of orientation vectors**

It was assumed that caloric stimulation had altered activity in the eighth nerve to excite velocity storage (Raphan and Cohen 1981). We, therefore, utilized a method developed for computing the eigenvectors of velocity storage during OKAN and postrotatory nystagmus (Raphan and Sturm 1991; Raphan et al. 1992a) to compute the orientation vectors during caloric stimulation. Briefly, the eigenvector of the three-dimensional eye velocity response is the best fitting tangent line to the eye velocity trajectory as it approaches zero in state space (Raphan and Sturm 1991; Raphan et al. 1992a). At this time, the activity in the vestibular nerve is presumably close or back to its resting level, and the sole contribution to the response is from velocity storage in the central vestibular system.

It was shown in early studies of visual suppression of per- and postrotatory response (Raphan et al. 1979) and OKN/OKAN (Cohen et al. 1977) that velocity storage is dumped with a time constant of approximately 30° above the horizontal stereotaxic plane (Blanks et al. 1985; Reisine et al. 1988). These time constants were assumed to be 30° above the horizontal stereotaxic plane (Blanks et al. 1985; Reisine et al. 1988).

**Caloric responses of normal animals**

In the upright position, cold caloric stimulation of the left ear induced nystagmus with slow phase velocity to the left along the positive z direction (Fig. 3B, bottom). There were also clockwise (+x; Fig. 3B, top) and upward (+y; Fig. 3B, middle) components. Yaw (horizontal) slow phase eye velocity rose to a peak value, culminating at approximately 175°/s about 30 s after the start of irrigation and then declined monotonically to zero. The duration of the response was approximately 120 s. Secondary caloric nystagmus then appeared, but it will not be considered in this paper.

With the animal supine (Fig. 3C), the induced nystagmus had a dominant yaw component to the animal’s left (+z). There was also a roll component, as when upright, but the pitch component was reversed. In the prone position (Fig. 3D), the yaw component of the induced nystagmus was to the animal’s right (−z), the reverse of that when the animal was upright or supine. The pitch and roll components were similar to those in the upright position, but were substantially larger. Since the x axis was aligned with the spatial vertical in the prone position, these increases tended to shift the orientation of eye velocity vector closer to the upward spatial direction, although the increased yaw component limited this shift.

With the left side down (Fig. 3E), irrigation of the left ear produced a strong leftward (+z) yaw component of eye velocity with a clockwise torsional component. A strong upward (−y) pitch component relative to the head was present in this position. Since in this position, the pitch axis was aligned with the spatial vertical, the eye velocity vector was again close to the upward spatial vertical. The peak pitch component of the
FIG. 2. Method for computing orientation vectors in 3 dimensions. A and B: sample roll, pitch, and yaw eye velocities as well as the eye velocity vector magnitude as a function of time induced by left ear (A) and right ear (B) stimulation in the prone position (insert). The horizontal arrows (–——) show the last 37% of the decaying phase of the vector (heavy black line), which was used to calculate the orientation vectors \( \mathbf{e}_{AV_L} \) and \( \mathbf{e}_{AV_R} \) shown in A and B. This calculation was done by determining the optimal linear fit to the trajectory in 3-dimensional eye velocity space (Raphan and Sturm 1991). C: projections of the 3-dimensional representation of data in A and B and the computed orientation vectors, \( \mathbf{e}_{AV_L} \) and \( \mathbf{e}_{AV_R} \). The \( \mathbf{g} \) vector indicates the direction of gravitational force relative in the projected plane in the prone position. The circle with a dot indicates that the acceleration of gravity is coming out of the page toward the reader.

\[
\mathbf{e}_{AV_L} = \begin{bmatrix} 0.80 \\ 0.11 \\ 0.59 \end{bmatrix} \quad \mathbf{e}_{AV_R} = \begin{bmatrix} 0.65 \\ -0.05 \\ -0.76 \end{bmatrix}
\]
nystagmus culminated about 20 s after the culmination of the yaw response (Fig. 3E, middle).

When the animal was right side down (Fig. 3F), the yaw component reversed and was to the right (−z), but the pitch component remained upward, generating a spatially downward component. There was negligible roll. Thus the vector of eye velocity was spatially downward. Average peak values for the individual eye velocity components at the culmination of the nystagmus and the time course of caloric nystagmus induced by stimulation of the left and right ears in five normal animals, listed in Table 1, were generally consistent with the responses shown in Fig. 3.

Caloric responses of animals with all six canals plugged (NC animals)

Despite interruption of endolymph flow after plugging, robust horizontal nystagmus was induced in all head orientations by caloric stimuli (Fig. 4, B–F). Average values of the eye velocities at the points of culmination are given in Table 1. In contrast to the responses before operation, the yaw eye velocity was always to the ipsilateral side. After irrigation of the left ear, the spatial direction of the eye velocity vector was predominantly along the head vertical (z) when upright and supine (Fig. 4, B and C) but moved toward the spatial vertical when prone due to the counter-clockwise roll (x) and leftward yaw (z) components (Fig. 4D). The velocity vector also moved to the spatial vertical when LSD as a result of the leftward yaw (z) and upward pitch (y) components (Fig. 4E). The roll component in the LSD position (Fig. 4E, top, −x) was not of sufficient magnitude to alter the dominant direction of the eye velocity vector. The eye velocity components were different when the animal was right side down (Fig. 3F). As before, the yaw eye velocity was to the left (z), but the direction of the pitch component was reversed (Fig. 4F, middle, down, +y), producing an oppositely directed eye velocity vector.

FIG. 3. A: presentation of head coordinate frame. Curved arrows show the direction of the positive axes of rotation, which are consistent with the right-hand rule. Also shown are the 3 semicircular canals of the left labyrinth and their orientation relative to the head frame, as well as the relationship between the lateral canal plane and the stereotaxic horizontal. B–F: examples of caloric nystagmus induced by irrigation of the left ear in a normal monkey in the upright (B), supine (C), prone (D), left-side down (E), and right-side down (F) positions, shown in the insets. Irrigation was begun at time 0 with the animal upright, and it was continued for 15 s. At the end of stimulation, the animal was moved to the test position, and the lights were extinguished. Positive directions for each of the components of eye velocity were clockwise (CW) relative to the animal for roll, down for pitch, and left for yaw.
Since plugging the canals had eliminated convective endolymph flow, the same activity was transmitted in the vestibular nerve to the CNS after each caloric stimulus, regardless of head position in regard to gravity. As a result, the spatial orientation of the caloric response and the contribution of velocity storage to this orientation could be studied in isolation. Therefore we first analyzed the spatial orientation of the responses in the canal-plugged animals. In the upright position (Fig. 5A), the average vectors for positive and negative yaw stimulation induced by left (gray) and right (black) ear stimulation were close to the yaw axis for both monkeys (+z, left ear stimulation; −z, right ear stimulation), and there was little roll (Fig. 5A1) or pitch (Fig. 5A2). In the supine position, one monkey had a response along the head vertical, similar to the upright condition, while another animal had a strong roll and a weaker pitch component, which brought the average vector closer to the spatial vertical [+x (gray) for left ear stimulation; −x (black) for right ear stimulation; Fig. 5B1 and B2]. The average vector for the two animals had a yaw component of about 77% of the total length of the vector (Fig. 5B3).

When prone, the vectors in both monkeys were approximately the same, having a significant roll component, which again brought the average vector closer to the spatial vertical (−x for left ear stimulation; +x for right ear stimulation; Fig. 5C3). This was opposite to the direction when the animals were supine, since the spatial vertical had been inverted relative to the head. The average contribution of the yaw component to the orientation vector was reduced to about 38% of the total length of the vector (Fig. 5C1), and the contribution of the roll component was increased from about 15% to about 90% of the total length of the vector (Fig. 5C3). The increase in the pitch component from the upright to the supine-prone position was small (about 5%). Thus the dominant orientation changes in

**Characteristics of the three-dimensional orientation in plugged animals**

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TABLE 1. Average eye velocity components induced by caloric stimulation in normal animals and in animals with all six semicircular canals plugged

<table>
<thead>
<tr>
<th>Response Plane</th>
<th>Upright (°/s)</th>
<th>Supine (°/s)</th>
<th>Prone (°/s)</th>
<th>Ipsi Ear Down (°/s)</th>
<th>Contra Ear Down (°/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normals Roll</td>
<td>53 ± 47</td>
<td>46 ± 18</td>
<td>111 ± 57</td>
<td>43 ± 31</td>
<td>63 ± 58</td>
</tr>
<tr>
<td>Pitch</td>
<td>81 ± 60</td>
<td>116 ± 63</td>
<td>87 ± 65</td>
<td>100 ± 58</td>
<td>62 ± 60</td>
</tr>
<tr>
<td>Yaw</td>
<td>113 ± 881</td>
<td>159 ± 60</td>
<td>213 ± 71</td>
<td>109 ± 79</td>
<td>117 ± 10</td>
</tr>
<tr>
<td>All six canals plugged Roll</td>
<td>25 ± 6</td>
<td>72 ± 70</td>
<td>48 ± 11</td>
<td>28 ± 16</td>
<td>34 ± 28</td>
</tr>
<tr>
<td>Pitch</td>
<td>18 ± 14</td>
<td>35 ± 36</td>
<td>18 ± 4</td>
<td>113 ± 39</td>
<td>87 ± 65</td>
</tr>
<tr>
<td>Yaw</td>
<td>160 ± 93</td>
<td>134 ± 79</td>
<td>83 ± 6</td>
<td>118 ± 55</td>
<td>93 ± 47</td>
</tr>
</tbody>
</table>

Values are expressed in °/s as mean ± SD. In the Normals group: animals = 5, n = 10; canal-plugged group: animals = 2, n = 4.

both the supine and prone positions were in roll, toward the spatial vertical.

In each instance, as for supine and prone, a pitch component was induced along the spatial vertical in the same direction as the yaw component in the head frame. Thus for left ear down, the pitch was negative (−y) for left ear irrigation and positive (+y) for right ear irrigation. These vectors were reversed when the right ear was down. The average roll components were small in the side down positions. Thus the dominant effect of unilateral caloric stimulation in all head positions was to induce spatial components of slow phase eye velocity components whose direction in space was the same as the direction of the yaw component in the head coordinate frame.

Characteristics of the three-dimensional orientation in normal animals

In normal animals, convection currents toward or away from gravity in the individual canals substantially enhanced or modified the responses of the canal-plugged condition. In upright and supine positions, the yaw component of eye velocity was toward the irrigated ear as in the plugged animals (Fig. 6, A and B). In addition, when the yaw component was positive with the animal supine, the roll component was also positive, as in the plugged condition and consistent with the organizational scheme inherent in velocity storage (Dai et al. 1991; Raphan and Cohen 1988; Raphan and Sturmi 1991; Raphan et al. 1992a). In prone position, the response was toward the contralateral ear (Fig. 6, C1 and C2), opposite to the canal-plugged response pattern (note changes in the direction of yaw in Fig. 6, C1–C3). This indicates that the convection currents were strong enough to reverse any nerve-induced cooling response. In LSD position (Fig. 6, D1–D3), convection currents induced eye velocities whose vector was along the +z direction for both right and left ear stimulation. Similarly, while in the RSD position, the yaw component of the eye velocity was along the −z direction, regardless of the ear that had been stimulated (Fig. 6, E1–E3). The generated +z component was associated with a −y component for left ear down, and −z component was associated with a −y component for right ear down (Fig. 6, D3 and E3). Thus regardless of head orientation during stimulation, the associated pitch component was in the same spatial direction as the induced yaw component relative to the head coordinate frame. The components of the cross-coupled nystagmus had magnitudes, which rotated the orientation vector consistent with the up-down asymmetries in time constant observed for vertically induced vestibular nystagmus and OKAN (Matsuo and Cohen 1984; Raphan and Cohen 1988).

Modeling canal activations as a function of head orientation

To gain insight into the component contributions to the caloric response, the model of three-dimensional eye velocity generation developed by Yakushin et al. (1995, 1998) was utilized to infer how activations related to specific canal planes would induce the observed eye velocities. These planes are consistent with the planes determined anatomically and from physiological recordings (Blanks et al. 1985; Reisine et al. 1988). It was assumed that excitation of the canal nerves corresponded to positive directions of activation of the canal planes, which would be induced by equivalent positive direction of head rotations. For the left labyrinth, the positive directions of movement would be a head movement to the left for left lateral canal stimulation, 45° down-left for left anterior canal stimulation, and 45° up-left for left posterior canal stimulation (Fig. 7A, z′, x′, and y′). Compensatory eye rotations would be opposite to these excitations, in accordance with the eye movements produced by electrical stimulation of the ampullary nerves on the left side (Cohen et al. 1964; Suzuki et al. 1964). We further assumed that cooling of the nerve would produce equal inhibition of the canal nerves on the left side, and that this would be approximately 25–30% of the total response (Minor and Goldberg 1990). Although the operations on the temporal bone had eliminated the mastoid and other cavities that existed before, conduction through the temporal bone was considered to be the major source of the cooling (Feldmann et al. 1991). It was therefore assumed that the cooling equally inhibited all canal nerves. The eye velocity orientation vector was alsochosen as 1/3 of the total unit vector, since it was assumed that the nerve cooling contributed about 1/3 to the total vector. Based on these constraints, the canal vector during cooling of the nerve alone was chosen as (−0.3, −0.3, −0.3).

When this vector was inserted into the model, it generated an eye velocity vector close to the roll (−x) direction, having components (−1, 0, 0.02) in head coordinates (Fig. 7A, “Eye Velocity 1†”). This eye velocity did not correspond to the observed eye velocity vector (Fig. 7A, “Actual Eye Velocity”), which had its dominant component along the z axis (−0.09, 0.19, 0.98). There was no set of negative canal activations that could generate an eye velocity vector outside the sector defined by the canal vectors to predict the actual direction of eye velocity. Therefore inhibition of the canal nerves alone could not have produced the observed eye velocity.

To correct for this deficiency, the canal activations were modified on the assumption that the cooling had caused contraction of the endolymph between the plug and the cupula. This would cause deviation of the hair cells in the direction of the plug in all three canals, similar to the utriculofugal (ampullofugal) deflections produced in normals by convection.
Ampullofugal flow of endolymph produces inhibition of the lateral canal nerves (−zc) and excitation of the anterior (+xc) and posterior (+yc) canal nerves, as shown by the curved arrows in Fig. 4A (Ewald 1892). Therefore we modified the canal activation vectors to increase the inhibition of the lateral canal afferent activity and to generate excitation of the anterior and posterior canal nerves. We assumed that the equal inhibition of the canal nerves added to the inhibition and excitation produced by this ampullofugal deflection. Consequently, the vector was chosen as 0.5, 0.5, −0.75. This produced a resultant
eye velocity direction that closely approximated the eye velocity vector in the canal-plugged animal when upright (Fig. 7B, “Eye Velocity 2”). It should be noted that the explicit values used to model the data are not unique. The critical constraint is that the anterior and posterior canal activation values should be the same and positive, and the lateral canal activation should be negative. The reverse, inhibition of the anterior and posterior canal nerves and excitation of the lateral canal nerve, would have produced an eye velocity in the opposite direction and could not have fit the data. The approximation of the observed response, while close to the “Actual Eye Velocity” in the upright position, may have arisen due to lateral canal inhibition alone, with no contribution from the vertical canals, and velocity storage, which is oriented toward the spatial vertical, could then have provided additional rotation toward the spatial vertical. However, this would still have required the proposed contraction mechanism, which countered the inhibition due to cooling of the nerves of the vertical canals, effectively zeroing their responses, while increasing the inhibition of the lateral canal nerve.

We tested the hypothesis that velocity storage contributed to the orientation of the caloric response by considering the model predictions for tilted head positions. Because the nature of the cooling was the same in every head orientation, it would be expected that eye velocity would be the same in all positions (Fig. 7, C–F, “Eye Velocity 2”), if no other factors were involved. This did not occur. For example, the “Actual Eye Velocity” vectors were significantly different from the direction of “Eye Velocity 2” for side down (LSD and RSD), supine, and prone positions (Fig. 7, C–F). In LSD and RSD, the actual eye velocity vector had a large pitch component, while in supine and prone, and there was a large roll component that shifted the eye velocity vector toward the spatial vertical. Because of the absence of convection currents in the canal-plugged animals, the most likely cause for this substantial shift in the direction of the eye velocity vector is the spatial orientation of velocity storage. Velocity storage also probably contributed to orienting the eye velocity in the upright position toward the spatial vertical.

Because of the substantial contributions of convection currents in the normal animal, and the lack of understanding of how convection currents might be induced in all canals in the various head positions, it was premature to speculate on the basis of the evidence presented here how to model the normal response.

**DISCUSSION**

This study shows that caloric nystagmus is readily induced in monkeys after plugging all six canals had eliminated endolymph flow due to convection. Primary evidence for the absence of convective flow comes from the demonstration that the canals were occluded by bone, leaving only a small space between the plug and the ampulla. This precluded fluid flow that would significantly deflect the cupula and activate the nerve. Supporting this, we have previously shown in these same animals that this occlusion reduced the time constant of the canals by almost two orders of magnitude (Yakushin et al. 1995, 1998). As a result, the gain of the angular vestibulo-ocular reflex (aVOR) fell to zero at low frequency head rotations (0.2 Hz; Yakushin et al. 1995, 1998), requiring high frequencies of acceleration to induce enough flow to produce significant eye velocity (Lasker et al. 1999; Rabbitt et al. 1999, 2001; Yakushin et al. 1998). These frequencies are well out of the range of the frequency content of any convection current that might be produced by the caloric stimulus.

Other evidence for the lack of convective flow after plugging comes from a comparison of the horizontal component of the nystagmus in the normal and canal-plugged animals. The slow phase velocity of the yaw component in the normal animal varied according to head position in regard to gravity. The velocities were always toward the left (+z) when either ear was stimulated in the LSD positions and to the right (–z) in the RSD positions (Fig. 3, E and F, and Fig. 6, D and E). Similarly, the yaw component of the response was reversed in the normal animal in the prone and supine positions (Fig. 3, C and D, and Fig. 6, B and C). Left ear stimulation produced eye velocity to the left when supine and to the right when prone. These direction changes were presumably due to changes in direction of convection flow in the lateral canal. In contrast, the yaw component of eye velocity in the plugged canal animals was always toward the ipsilateral ear regardless of head orientation (Fig. 4). Similar results have been reported after lateral canal plugging by Paige (1985).

With convection flow eliminated, the following question arises: what other factors were responsible for the caloric responses in the canal-plugged animals? There is evidence that thermal effects on the nerve play a role in generating the caloric response (Coats and Smith 1967; Minor and Goldberg 1990; Paige 1985). Minor and Goldberg (1990), using a model of Paige (1985), concluded that nerve cooling contributes about 30% of the eye movement response. In our model of the caloric response in canal-plugged animals, we first assumed that cooling of the vestibular nerve reduced the firing rates about 30% equally in all branches of the vestibular nerve (Fig. 7A, “Eye Velocity 1”). It was not possible, however, using only nerve cooling to predict the caloric eye velocity responses in the canal-plugged animal in any of the head positions. The reason for this was that equivalent inhibition of the activity from each canal would produce a predominant response along

![Fig. 5](http://jn.physiology.org/)

**FIG. 5.** Two-dimensional projections in the yaw/roll (J) and the yaw/pitch (2) planes showing the orientation vectors of caloric nystagmus in the upright (A), supine (B), prone (C), left-side down (D), and right-side down position (E) in the 6-canal plugged animals. Trajectories of the decaying phases of the caloric vectors were plotted in gray for left ear stimulation and in black for right ear stimulation. Insets on the left show head positions and head coordinate axes in the spatial frame, and the g in each graph indicates the direction of gravitational force. The caloric vectors were normalized so that the length of the vector at the culmination of the caloric response was 1. The 3 × 1 matrices (J) of the averaged orientation vector for the left ear (êL) and the right ear (êR) for data shown in J and 2. Note that responses from the left and right ear stimulation were symmetrical, and yaw components were dominant in the upright position (A), roll components in the supine (B) and prone (C) positions, and pitch in the left-side down (D) and right-side down positions (E). A circle with a dot indicates that the acceleration of gravity is coming out of the page while a circle with an x indicates the acceleration of gravity is into the page.
the roll direction, or about 90° from the observed response in the upright condition.

Even if the change in the bone temperature were not distributed uniformly, the cooling would still induce inhibition of the canal nerve activity in all canals. This would maintain the eye velocity vector within the solid angle defined by the normals to FIG. 6. 1 and 2: 2-dimensional projections and the orientation vectors of caloric nystagmus in 5 normal animals in the upright (A), supine (B), prone (C), left-side down (D), and right-side down (E) positions. Scheme as in Fig. 5. The $3 \times 1$ matrices ($\mathbf{J}$) of the averaged orientation vector for the left ear ($\mathbf{e}_{avL}$) and the right ear ($\mathbf{e}_{avR}$) for data shown in 1 and 2. Predominant velocity was along the z (head-vertical or yaw) axis and earth-vertical axis as in all canal-plugged animals.
the canal planes. The experimentally observed eye velocity orientation vector moved outside this solid angle. Since velocity storage does not orient from roll to yaw, but only from yaw to roll (Dai et al. 1992; Raphan and Cohen 1996; Raphan and Sturm 1991; Raphan et al. 1992a; Wearne et al. 1999), reverse cross-coupling could not have contributed to the observed orientation of eye velocity which was predominantly along the yaw axis. Therefore the direction of eye velocity in the upright condition could not be explained by nerve cooling alone, and other factors must be present to account for the caloric response after canal plugging.

Based on their experiments on caloric nystagmus in microgravity, Scherer and Clarke (1985) proposed that unequal fluid contraction on the two sides of the cupula in the lateral canals was largely responsible for the caloric nystagmus that was observed in space in the absence of convection currents. Minor and Goldberg (1990) concluded that thermal expansion of the endolymph fluid would not contribute significantly to the caloric response in normal subjects because induced pressure effects would be rapidly normalized to both sides of the cupula. Furthermore, they argued that there would be no sustained activation of canal afferents and the activity would decay with the cupula-endolymph time constant of 5 s. This controversy is still unsettled, but fluid contraction could play a significant role in generating the three-dimensional eye velocity response in the canal plugged animals due to differential pressure on the cupula created by contraction of different column lengths on either side of the plug. Scherer and Clarke (1985) estimated the pressure differential across the cupula \( P \) at about \( 10^{-7} \) dynes/cm², which is an order of magnitude beyond the threshold for activating the hair cells innervating the canal ampulla (Oman and Young 1969; Steer et al. 1968). The net effect of this
deflection would be to mimic utriculofugal (ampullofugal) endolymph flow resulting from cooling that produced convection currents, thereby producing inhibition in the lateral canal and excitation in the anterior and posterior canals.

We reasoned that it was likely that contraction of endolymph between the cupula and the plug had occurred in our experiments because the plugging did not allow fluid to circulate freely and therefore gave a more sustained response than in normal canals. With the introduction of the predicted excitation of the anterior and posterior canals and inhibition of the lateral canals, the model almost exactly predicted the eye velocity that was observed in the upright condition (Fig. 7, “Eye Velocity 2”). We would emphasize that although the exact weights for canal activations were unknown in our simulations, the constraint that the anterior and posterior activations must be equal or close to each other, imposes a structure on the signs of the canal activations that we have postulated. This strongly suggests that a pressure differential across the cupula may have been present in the canal-plugged animals to contribute to the observed response. The finding that horizontal caloric nystagmus could not have been produced simply by inhibition and excitation due to equivalent cooling or heating of the canal nerves on one side in our monkeys, supports the conclusion of Scherer and Clarke that a pressure differential across the cupula may have been operative to produce horizontal (±z axis) caloric nystagmus in microgravity.

Addition of these two components did not predict the orientation of eye velocity in side down, prone and supine positions in the canal-plugged animals, however, and the eye velocity of the caloric nystagmus was not maintained along the z axis, as when the animals were in the upright position. Rather, the predicted eye velocities would have remained along the head z axis and would have pointed along the spatial horizontal in the side down and prone/supine positions. Instead, the cross-coupled pitch components in side-down positions and roll components in supine/prone positions tended to align the eye velocity vector with the spatial vertical, respectively. Moreover, the velocity vectors were directed along the spatial vertical with the same polarity as the yaw velocity in the head frame. Thus if yaw eye velocity was to the left (+z), then the pitch and roll components for side-down and prone/supine positions were along the positive direction in space. These were (+x) for supine, (−x) for prone, (−y) for LSD, and (+y) for RSD positions. The reverse would be true if yaw eye velocity was to the right (−z). These orientations of the velocity vectors to the spatial vertical are similar to the orientations of OKN/OKAN and postrotatory nystagmus in tilted positions (Dai et al. 1991, 1992; Raphan and Sturm 1991; Raphan et al. 1992a). We have previously shown that the orientation properties of velocity storage are unaltered after canal plugging (Raphan et al. 1992b). Therefore we conclude that velocity storage and its inherent orientation properties play a significant role in orienting the velocity vector of caloric nystagmus toward the spatial vertical.

Although the trajectories of the caloric response were curved in velocity space, it should be understood that they represent the dynamic responses of a system that includes a number of responses in its early portions. The actual orientation vectors, i.e., the eigenvectors of the velocity storage system, are fixed in the head and can be estimated only from the data over the time period during which eye velocity was <37% of the culmination or when cupular activation had presumably declined to zero. Although velocity storage is likely to play a significant role in orienting eye velocity toward the spatial vertical in tilted positions of the head, previous work has demonstrated that velocity storage only orients responses from yaw to other directions and not from roll or pitch to yaw (Dai et al. 1991, 1992; Raphan and Sturm 1991; Raphan et al. 1992a; Wearne et al. 1999). Since the response from nerve cooling was along the roll direction, there could have been no cross-coupling from roll to yaw, and this possibility was rejected. Thus velocity storage stands as an independent mechanism for orienting caloric responses, separate from the contraction due to cooling of the endolymph. While velocity storage is weaker in humans than in monkeys, the relative cross-coupling is sufficient to orient eye velocity toward the acceleration of gravity, as in the monkey (Gizzi et al. 1994). Thus the three-dimensional caloric responses in humans may be directly extrapolated from the responses in the monkey.

The evidence presented in this paper cannot be used to explain the normal caloric response, because there is insufficient information about whether and how convection currents are induced in the three canals in the various head positions. There is data on this (Aw et al. 1998, 2000; Böhmer et al. 1992, 1995, 1996; Fetter et al. 1998), but it is inadequate to make quantitative predictions as to the nature of this activity. Some global observations can be made, however. The induced eye velocities produced by cold caloric stimulation of the normal animal were consistent in each position with the convective flow of endolymph in the lateral canals toward gravity, which appears to be a dominant factor in producing the various responses (Fig. 6). Thus in the upright and supine positions, cold irrigation produced yaw eye velocity to the left (+z) for left ear irrigation and to the right (−z) for right ear irrigation, consistent with ampullofugal convective flow in the lateral canal on the irrigated side. These directions were reversed in the prone position, consistent with an ampullopetal flow. Left ear down produced leftward eye velocity for both left ear and right ear irrigation, consistent with ampullopetal flow in the left lateral canal and ampullofugal flow in the right lateral canal. Right ear down produced the opposite effect generating rightward eye velocity (−z) for both right ear and left ear irrigation. The downward, counterclockwise components in the upright position most likely originated from ampullopetal flow in the anterior canal, which caused inhibition of anterior canal activity. The downward and clockwise components in the supine position probably originated from ampullofugal flow in the anterior and posterior canals. Reversal of these currents in the prone position would produce the upward component.

As in the canal-plugged condition, the orientation of the eye velocity was also consistent with the orientation properties of velocity storage. This produced a positive roll component (+x) for positive yaw eye velocity, induced when the animal was supine, and a negative roll component when negative yaw eye velocity was induced. When the induced yaw eye velocity was reversed for prone position, the direction of the induced roll component was maintained the same in head coordinates since the positive roll direction was reversed in space (+x is pointing down in space). Right ear and left ear down produced corresponding spatial pitch components consistent with the direction of the induced yaw component in head coordinates. Thus velocity storage is likely to play an important role in orienting
the caloric response in normal animals as well as in monkeys with plugged canals.

In summary, we have considered four dominant mechanisms that could have contributed to caloric responses in the normal animal: convections currents, nerve cooling, pressure gradients across the cupula due to endolymph contractions as a result of cooling, and velocity storage. Canal plugging, which eliminated convection, was useful in demonstrating that contraction of the endolymph by cold, and presumably expansion by hot, stimulation can also contribute to the caloric response. In addition, the role of the orientation properties of velocity storage were particularly evident after canal plugging and are likely to play an important role in the orienting the caloric responses in normal animals.

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