Reflections of Efferent Activity in Rotational Responses of Chinchilla Vestibular Afferents

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Plotnik, Meir, Vladimir Marlinski, and Jay M. Goldberg. Reflections of efferent activity in rotational responses of chinchilla vestibular afferents. J Neurophysiol 88: 1234–1244, 2002; 10.1152/jn.00109.2002. To study presumed efferent-mediated responses, we determined if afferents responded to head rotations that stimulated semicircular canals other than the organ being innervated. To minimize stimulation of an afferent’s own canal, its plane was placed nearly orthogonal to the rotation plane. Otolith units were tested in a horizontal head position with the ear placed near the rotation axis to minimize linear forces. Under these circumstances, angular-velocity trapezoids (2-s ramps, 2-s plateau) evoked excitatory responses for both rotation directions. Type III responses were considerably larger in decerebrate than in anesthetized preparations. In addition to their being exclusively excitatory, the responses resembled those obtained with electrical stimulation of efferent pathways in including per-stimulus and more prolonged post-stimulus components and in being larger in irregularly discharging than in regularly discharging units. Responses, which were not seen for rotations <80°/s, grew as velocity increased between 80 and 500°/s but were seldom larger than 20 spikes/s.

Complete section of the VIIIth nerve abolished type III responses, leaving conventional afferent responses intact. To study the separate contributions of canals on the two sides, responses were compared when the labyrinths were intact and when the ipsilateral or contralateral horizontal canal was mechanically inactivated. Both sides contributed to the efferent-mediated responses. That afferents could be influenced from the contralateral labyrinth was confirmed with the use of unilateral galvanic currents. Following inactivation, excitatory responses were produced by rotations exciting or inhibiting the intact horizontal canal with the responses resulting from excitatory rotations being much larger. Such a response asymmetry is consistent with a semicircular-canal origin for the type III responses. A similar asymmetry was seen in the post-stimulus responses to contralateral cathodal (excitatory) and anodal (inhibitory) galvanic currents. We conclude that the efferent system receives a sufficiently powerful vestibular input from both the ipsilateral and contralateral labyrinths to affect afferent discharge.

INTRODUCTION

Vestibular end organs are provided with an efferent innervation originating bilaterally in the brain stem with similar numbers of efferent axons arising from the sides ipsilateral and contralateral to the innervated labyrinth (Gacek and Lyon 1974; Goldberg and Fernández 1980; Marco et al. 1993; Warr 1975). At most a small fraction of efferent neurons may project to both ears (Dechesne et al. 1984; Perachio and Kevetter 1989). A few hundred efferent axons travel in the vestibular nerve (Gacek and Lyon 1974; Goldberg and Fernández 1980; Warr 1975), as compared with >10,000 afferents (Fernández et al. 1995; Gacek and Rasmussen 1961). On reaching the neuroepithelium of each crista or macula, efferent fibers branch profusely so that, despite their small number, they provide a major innervation of hair cells and afferent terminals (Lysakowski and Goldberg 1997; Purcell and Perachio 1997).

The actions exerted by the efferent vestibular system (EVS) on afferent neurons have been studied by electrically stimulating efferent pathways. Unlike the situation in nonvestibular organs, where the predominant efferent effect is inhibitory (for review, see Goldberg et al. 2000), stimulation of the mammalian EVS leads to afferent excitation (Goldberg and Fernández 1980; Marlinski et al. 2000; McCue and Guinan 1994). Such excitatory responses have been observed in afferents from all five vestibular organs in the squirrel monkey (Goldberg and Fernández 1980), from irregularly discharging saccular afferents in the cat (McCue and Guinan 1994), and from afferents of the superior vestibular nerve in the chinchilla (Marlinski et al. 2000). Excitatory responses are large in irregularly discharging afferents and consist of both fast and slow components. Responses are much smaller in regularly discharging afferents and are predominantly slow.

To understand the function of the EVS, we need to know about the response properties of efferent neurons as well as efferent-mediated alterations of afferent activity under conditions more natural than electrical stimulation. In lower vertebrates, efferent neurons receive a convergent input from several vestibular organs on the two sides (Gleisner and Henriksson 1964; Schmidt 1963) and are excited by rotations in either direction (Blanks and Precht 1976; Hartmann and Klinke 1980; Precht et al. 1971). Such responses are termed type III in the classification scheme of Duensing and Schaefer (1958) and may be contrasted with the type I responses of afferents, in which rotations in the two directions lead to oppositely directed effects (Goldberg and Fernández 1971; Lowenstein and Sand 1936). Efferent neurons in lower vertebrates can also respond to somatosensory inputs (Blanks and Precht 1976; Gleisner and Henriksson 1964; Hartmann and Klinke 1980; Precht et al. 1971) and to changes in behavioral state (Highstein and Baker 1985). Although these results provide clues as to the functions of the EVS, it is unclear whether...
the results can be generalized to mammals whose central efferent pathways differ in anatomical organization from those in lower vertebrates (reviewed by Meredith 1988). Efferent neurons in fish (Bell 1981; Highstein and Baker 1986; Meredith and Roberts 1987) and frogs (Pellergrini et al. 1985; Strutz et al. 1981; Will 1982) are predominantly located in the brain stem ipsilateral to the ear innervated, and their dendrites ramify over a large territory. In contrast, efferent neurons in mammals are found on both sides of the brain stem, and their dendrites are restricted to the region immediately surrounding the efferent cell column (Goldberg and Fernández 1980; Warr 1975). In the one study of the discharge properties of mammalian efferent neurons, they were observed to respond to head tilts and somatosensory stimulation (Marlinski 1995).

Efferent modification of afferent discharge in response to natural stimulation has only been studied in nonmammalian species. In the toadfish, afferents as well as efferents are activated by behavioral arousal (Boyle and Highstein 1990; Highstein and Baker 1985). Unilateral stimulation of vestibular organs in the pigeon (Dickman and Correia 1993) or the frog (Myers et al. 1997) can alter afferent discharge on the opposite side. Once again, it is difficult to generalize these studies to mammals not only because of differences in central circuitry but also because of differences in the peripheral actions of efferents. While the responses of afferents to electrical stimulation of efferents are similar in the toadfish (Boyle and Highstein 1990) and in mammals (Goldberg and Fernández 1980), responses in the frog can be excitatory or inhibitory (Rossi et al. 1980; Sugai et al. 1991). A similar situation has been found in turtles (Brichta and Goldberg 2000). Electrical stimulation studies have apparently not been done in birds. That the actions in birds might be heterogeneous is suggested by the fact that some afferents are inhibited by mechanical stimulation of the contralateral horizontal canal, while other afferents are excited (Dickman and Correia 1993).

In this study, we investigated whether vestibular-nerve afferents showed presumed efferent-mediated responses to head rotations. Because anesthesia might suppress efferent activity, responses were compared in decerebrate and barbiturate-anesthetized preparations. We were interested in three questions. Does the efferent system in mammals receive a sufficiently powerful vestibular input to affect afferent discharge? Do efferent-mediated responses to natural stimulation resemble those obtained by electrical stimulation of efferent pathways? How are the central pathways conveying vestibular inputs to efferents organized?

So as not to mask efferent-mediated responses, conventional afferent responses to rotations were minimized. For semicircular-canal afferents, this was done by tilting the animal so that the plane of the appropriate canal duct was approximately orthogonal to the rotation plane. In the case of otolith afferents, linear forces were minimized by placing the ear near the rotation axis. While care was taken to minimize conventional afferent responses from the tested organ, the rotations affected other semicircular canals, providing potential inputs to the efferent system. To study the organization of central pathways, we compared responses to horizontal rotations in animals with intact labyrinths and those in which the ipsilateral or contralateral horizontal canal was mechanically inactivated.

METHODS

Individual afferents were recorded in the superior vestibular nerve of adult chinchillas. The animals were of either sex and weighed 400–600 g. Both decerebrate and anesthetized preparations were used. Animal care and experimental procedures were approved by the Institutional Animal Care and Use Committee of the University of Chicago.

Decerebrate preparations

Each chinchilla was injected with atropine sulfate (0.10 mg/kg im) and then anesthetized by inhalation of 2% isoflurane in room air. Body temperature was maintained between 37 and 38°C. The animal was fixed in a head holder. A craniotomy exposed the posterior part of the occipital cortex, which was aspirated to reveal the inferior and superior colliculi. The brain stem was completely transected by suction at an intercollicular level. Anesthesia was discontinued. The superior branch of the left VIIIth nerve was exposed by an extracranial approach (Baird et al. 1988). Stimulating electrodes were implanted on the round window and in the floor of the middle ear on each side.

Anesthetized preparations

Following atropine injection, animals were anesthetized with a solution of 10% 5.5 diethylbarbituric acid, 40% urethane, and 40% monoethyl urea (0.4 mg/kg ip). If needed, additional doses were given to reach and maintain a surgical level of anesthesia. The left vestibular nerve was exposed, and ear electrodes were implanted as in decerebrate preparations.

Physiological testing

The animal was placed on a superstructure attached to a velocity servomotor (Inland Model 823). The superstructure could be pivoted about the animal’s pitch, roll, and yaw axes and could be translated so as not to mask efferent-mediated responses, conventional afferent responses from the tested organ, the rotations produced nonconventional (type III) responses. For semicircular-canal units, rotation-sensitive units innervated either the horizontal (HC) or the superior (SC) canals. Units responding to tilts, but not to rotations were classified as otolith (OTO) units. Most OTO units in the superior nerve might be expected to innervate the utricular macula, although some might supply the anterior part of the saccular macula (Wersäll and Bagger-Sjöbäck 1974). Consistent with a utricular origin, the great majority of OTO afferents responded in opposite ways when the animal was tilted in opposite directions from the horizontal position (Fernández and Goldberg 1976).

Background discharge rates of each unit were estimated from 5-s samples collected with the animal in a horizontal (zero-tilt) position. To test for efferent-mediated responses in canal units, we first tilted the animal to a so-called null position (Blanks and Precht 1976) with the canal plane nearly orthogonal to the rotation plane. A null position was recognized because conventional (type I) responses to low-intensity rotations (≤80/°/s) were minimized and also because responses reversed polarity as the animal was tilted through this position. The term “null position” is used even though high-intensity (320°/s) rotations produced nonconventional (type III) responses. For HC units, a null position was achieved by tilting the animal 80–90°
right-ear-down (RED). For SC units, the null position used was 10°–20° RED from the zero-tilt position. Because they do not respond to rotations when the head is centered on the rotation axis (Goldberg and Fernández 1975), OTO units could be studied in any tilt position. In these experiments, we used the zero-tilt position. Clockwise (CW) and counterclockwise (CCW) angular velocity trapezoids were presented. Rotation directions are given as viewed from above. Peak velocities were 80–640°/s with ramp and plateau durations of 2 s. Most units were first tested with 320°/s peak velocities. To minimize linear forces, the ear was placed near the rotation axis. The resulting radial linear forces acting on the ear at 320°/s were calculated to be 0.01–0.02 g for OTO and SC units and <0.005 g for HC units. Such forces would produce only negligible responses (<1 spike/s) in OTO afferents (Goldberg et al. 1990) and even smaller responses in semicircular-canal afferents (Goldberg and Fernández 1975). Tangential linear forces were an order of magnitude smaller.

Normalized measure of discharge irregularity

A normalized coefficient of variation (CV*), appropriate to a mean interval of 15 ms, was calculated from the background discharge of each unit (Baird et al. 1988). In a few units with low rates, ipsilateral cathodal currents were used to elevate the discharge into the normalization range of 8–100 ms. Units were called regular (CV* < 0.05), intermediate (CV* between 0.05 and 0.20), or irregular (CV* > 0.20).

Galvanic currents applied to the contralateral labyrinth

To record efferent-mediated crossed effects, 5-s cathodal (excitatory) and anodal (inhibitory) constant-current steps were applied by way of the contralateral (right) stimulating electrodes. The polarity stated refers to the round-window electrode.

Unilateral inactivation of the horizontal canal

In four decerebrate preparations, the horizontal canal on the side ipsilateral or contralateral to the recording site was inactivated during the initial surgery. The posterior part of the Bulla was removed, and the bony horizontal canal was opened with a dental drill as far as possible from the horizontal ampulla. The membranous horizontal canal was plugged with a mélange of bone dust and bone wax. Plugging was done on the left (ipsilateral) side in two animals and on the right (contralateral) side in the other two animals. SC units were tested in their null position (10°–20° RED). OTO units were tested in a horizontal position, as were HC units following ipsilateral canal plugging. The latter units were identified by their background discharges, their responses to ipsilateral galvanic currents, and their lack of responses to tilts or to moderate-intensity (≤80°/s) rotations. HC units following contralateral plugging could not be tested for efferent-mediated responses to horizontal rotations because such responses were masked by much larger conventional afferent responses.

Severing the VIIIth nerve

In two anesthetized and two decerebrate preparations, the ipsilateral vestibular nerve was acutely sectioned. This was accomplished by removing the bone overlying the anisiform lobule of the cerebellum. After recordings from several vestibular afferents, the dura was incised, and a knife was passed through the cerebellum and into the VIIIth nerve. Several units were recorded postsection. After the experiment, the animal was perfused transcardially with a 2.5% paraformaldehyde–2.5% glutaraldehyde solution in 0.1 M phosphate buffer. Serial sections (40 μm) of the cerebellum, the brain stem, and the vestibular nerve were stained with 1% neutral red and examined microscopically.

Statistical methods

Results were tabulated in EXCEL worksheets. Statistical tests were run using spreadsheet functions or the SYSTAT (Evanston, IL) statistical package. Unless otherwise stated, results are expressed as means ± SE.

RESULTS

Rotational responses were studied in 59 vestibular-nerve fibers from 18 decerebrate preparations and 52 fibers from 16 anesthetized animals. In decerebrate animals, units innervated the HC (n = 16), the SC (n = 20), or OTO organs (n = 23); the corresponding numbers for anesthetized preparations were HC (n = 13), SC (n = 19), and OTO (n = 20). As a standard stimulus set, we used CW and CCW trapezoids with peak velocities of 320°/s in a null position.

Irregular units in decerebrate preparations could show large and sometimes periodic fluctuations in background discharge. Large fluctuations were not seen in regular units in decerebrate animals or in any of the units in anesthetized animals. The fluctuations ranged from 0 to >200 spikes/s. While we suspect that the fluctuations are efferent mediated, we have not excluded other possibilities. Here, we only wish to indicate the steps taken so that the fluctuations did not interfere with rotation responses. We observed that the latter responses were attenuated when the background rate approached zero or became very high. For this reason, we only studied responses when the background was between 10 and 70 spikes/s and did not seem to influence response magnitude. So that the fluctuations did not mask responses, trials were included only when the background, measured before and after the rotation, was stable. It should be emphasized that fluctuations were only observed in irregular units and then only in decerebrate animals. Except for a difference in magnitude, similar responses in the absence of fluctuations were observed in irregular units recorded in anesthetized animals.

Unless otherwise stated, results are from decerebrate preparations.

Efferent-mediated rotational responses

While conventional (type I) responses to moderate-intensity (<80°/s) rotations were almost eliminated when the animal was in a "null" position, high-intensity rotations (320°/s) still elicited responses. The latter responses can be called type III because they were excitatory for rotations in either direction. Such responses resemble the predominantly excitatory responses produced by electrical stimulation of the EVS (Goldberg and Fernández 1980; Marlinski et al. 2000; McCue and Guinan 1994). In addition, like electrical stimulation, high-intensity rotations in the null position produced both per- and post-stimulus responses.

Results are illustrated in Fig. 1, based on data from an irregularly discharging SC fiber recorded in a decerebrate preparation. This unit showed fluctuations in background discharge, which ranged from 20 to 180 spikes/s during the 100 min the animal was kept in a null position. The unit was only studied during periods when background discharge was between 20 and 60 spikes/s. Rotational responses were optimized when the superior canal and rotation planes were approximately parallel. In the optimal position, 0.2-Hz sinusoids
A small excitation was seen near the CCW peak and a small inhibition near the CW peak. The residual excitation should have reinforced the per-stimulus response to CCW trapezoids (Fig. 1B), while the residual inhibition should have interfered with the CW per-stimulus response (Fig. 1C). Averaging reveals per- and post-stimulus responses each of 10 spikes/s (Fig. 1D).

High-intensity sinusoidal rotations (0.1 Hz, ±320°/s) were introduced with the animal in the null orientation. A double-peaked, type III excitation was observed (Fig. 1E) and was accompanied by a slow upward drift in background activity (not shown). A comparison of the responses to trapezoids and sinusoids in the null position indicates why we preferred the former. Trapezoids separated by 30- to 60-s intervals allowed for a better differentiation of the excitatory responses to CW and CCW rotations and of per- and post-stimulus response components.

In decerebrate preparations almost all afferents tested in null positions (55/59 = 93%) exhibited type III responses to rotational stimuli of 320°/s. Three of the units that did not respond to 320°/s did so at 500 or 640°/s. Many units showed both per- and post-stimulus response components. In other units, post-stimulus responses could predominate. Poststimulus responses typically had durations of 15–30 s but could last as long as 60 s. Qualitatively similar responses were seen in HC, SC, and OTO units.

Although they were seldom equal in magnitude, there was no tendency for responses to CW or CCW rotations to be larger. Paired differences between responses in the two rotation directions did not differ significantly from zero for HC, SC, or OTO units. In a large fraction of canal units (48/55) from decerebrate and anesthetized preparations combined, the rotation direction leading to larger per-stimulus responses was the same as the direction leading to excitation during moderate-intensity sinusoidal rotations in the null position. This last result implies that conventional responses due to misalignment contributed to per-stimulus response asymmetries. We averaged the responses for the two rotation directions. Because of the linearity of conventional responses (Goldberg and Fernández 1971), this should eliminate the influence of imperfect nulling.

**Type III responses and discharge regularity**

Responses to electrical stimulation of the EVS and type III responses recorded in null positions resemble each other in being predominantly excitatory and composed of per- and post-stimulus response components. Another similarity involves differences in responses of regularly and irregularly discharging afferents. In their responses to EVS electrical stimulation, irregular units can have large responses consisting of fast and slow components (Goldberg and Fernández 1980; Marlinski et al. 2000; McCue and Guinan 1994). In contrast, the responses of regular units are small and predominantly slow.

Similar differences are seen in type III rotation responses. Figure 2, A–C, compares type III rotational responses of two otolith units, one regular and the other irregular. The peak of the per-stimulus response is more than 10 times larger in the irregular unit. The latter unit shows a distinguishable per-stimulus component that peaks during the velocity trapezoid.
and a post-stimulus component that lasts >20 s. In the regular unit, there is a gradual buildup of the response during the trapezoid and no break between per- and post-stimulus responses.

Response magnitude was quantified by averaging responses over the 2-s plateau for both CW and CCW rotations. The mean value (± SE) of the averaged excitatory response for all 54 units tested with 320°/s plateaus in decerebrates was 6.1 ± 0.8 spikes/s. Figure 3A presents the relation between the response magnitude and CV* for the 54 units. Data were fit by a power law, \( a(CV^*)^b \), with \( a = 17.4 \pm 2.8 \) spikes/s and \( b = 0.463 \pm 0.094 \) (\( P < 0.001 \)). An analysis of covariance (ANCOVA), done on log-transformed data with CV* as the covariate, indicated that there was no significant difference in the responses of afferents innervating different organs (\( P > 0.2 \)). Given the predominance of post-stimulus responses in regular units, it might be supposed that a power-law relation based on the latter responses would be weaker. This was the case, but there was still a significant relation between the response in the first 10 s of the post-stimulus period and CV*. Data were available for 49 units in decerebrates. A power-law regression gave \( a = 10.3 \pm 2.5 \) and \( b = 0.313 \pm 0.117 \) (\( P < 0.02 \)). An ANCOVA indicated that post-stimulus responses were larger in HC than in SC or OTO units (\( P < 0.001 \)).

**Type III responses in anesthetized preparations**

Rotation responses were smaller in anesthetized than in decerebrate preparations. This is illustrated in Fig. 3B, which compares averaged responses based on the 10 irregular units with the largest type III responses in each kind of preparation. Both samples happened to contain three SC, three HC, and four OTO units. Average responses for the two groups were calculated for the plateau and for 10 s immediately following the trapezoid. On average, plateau responses were four to five times larger in decerebrate preparations and post-stimulus responses were three times larger.

**Response-intensity relations**

In testing canal units in null positions, we saw type III responses to high-intensity velocity plateaus (320°/s) but not to moderate-intensity plateaus (<80°/s). During 2-s velocity ramps to 320°/s, responses were not seen during the first 0.5 s (Figs. 3B and 4A and B). Both observations suggest that type III responses are only seen above a certain velocity because of the presence of a threshold or because of a sigmoidal relation between stimulus and response amplitudes.

To examine these possibilities, we studied type III responses to different stimulus intensities. Responses of an intermediate HC unit are seen in Fig. 4A and of a regular HC unit in Fig. 4B. Per-stimulus responses are present at 160°/s but not at 80°/s. At the latter velocity, post-stimulus responses are still observed. Both the per- and post-stimulus components grow as stimulus intensity increases.

In six units, including the unit in Fig. 4B, peak velocity was raised from 80 to 500°/s. Each unit had a background discharge. Response-intensity curves, based on responses averaged over the 2-s velocity plateau, are plotted for the six units in Fig. 4C. Response amplitudes are small at 80°/s, increase more than twofold at 160°/s, and continue to increase as plateaus reach 500°/s. Responses of each of the six units were...
results for the six units were combined in an average, which is normalized to their response to 320°/s plateaus. Normalized response

normalized to their response to 320°/s plateaus. Normalized results for the six units were combined in an average, which is well fit by a linear relation with a threshold near 60°/s (Fig. 4D, ---) and by a concave upward curve (---), similar to the one relating shock frequency and afferent response when the EVS is electrically stimulated (Goldberg and Fernández 1980; Malminter et al. 2000).

Both of these curves are different from those relating response magnitude to rotation velocity for conventional (type I) afferent responses with background activity. In the latter case, curves do not show thresholds and are linear or concave-downward (Fig. 4D, - - -) (Plotnik and Goldberg 2000).

Unilateral inactivation of the horizontal canal

A simple explanation for type III responses is that each afferent receives a bilateral efferent innervation. In addition, we can assume that the efferent cell groups respond to excitation but not to inhibition of canals. To test this explanation, we compared responses in intact animals and in animals in which the horizontal canal either on the side of recording or on the opposite side was mechanically inactivated by acute plugging (Goldberg and Fernández 1975; Money and Scott 1962). Rotations were delivered with the animal in a horizontal tilt position. According to the proposed scheme, type III responses should become asymmetric after plugging. Yaw rotations toward the intact ear will excite the intact HC and should result in an efferent-mediated excitation of SC and OTO units, while rotations toward the plugged canal, because they inhibit the intact HC, should be ineffective in influencing afferents innervating other organs. In describing the results, the term “ipsilateral” refers to the side of recording, whereas “contralateral” refers to the opposite side. As we always recorded from the left side, CCW rotations are ipsilateral and CW rotations are contralateral.

After unilateral plugging, we observed asymmetries whose directions were consistent with our predictions, i.e., rotations toward the intact labyrinth were more effective than those toward the plugged canal. At the same time, responses to yaw rotations toward the plugged canal were not entirely abolished. Responses from two otolith units are shown in Fig. 5. The irregular unit in the top row was recorded after the ipsilateral HC had been plugged. Contralateral rotations resulted in larger responses (Fig. 5B) than did ipsilateral rotations (Fig. 5A). In the middle row are shown responses from a regular unit. In this case, plugging the contralateral HC resulted in an opposite asymmetry favoring ipsilateral (Fig. 5C) over contralateral rotations (Fig. 5D). Nevertheless, ipsilateral rotations still produced a discernible excitatory response after ipsilateral plugging (Fig. 5A) as did contralateral rotations after contralateral plugging (Fig. 5D). Ipsilateral plugging, by eliminating conventional afferent responses from HC units, also allowed us to test such units with rotations in the horizontal plane. The unit in the bottom row was one such HC unit. A significant response was seen to contralateral (Fig. 5F), but not to ipsilateral (Fig. 5E), rotations. Asymmetries favoring responses to contralateral rotations were seen in three other HC units, yet in two of them, responses were also seen to ipsilateral rotations.

Response magnitudes for ipsilateral (CCW) and contralateral (CW) rotations are compared in Fig. 6 for SC and OTO units in intact animals and in animals with the ipsilateral (left) or contralateral (right) HC plugged. Only plateau responses were quantified. Of the 43 units from intact animals, almost equal numbers had larger responses to ipsilateral (n = 20) or contralateral rotations (n = 23). Asymmetric responses with larger responses to rotations in the expected direction were seen in 12/13 and 13/15 units from ipsi- and contra-plugged animals, respectively. Horizontal rotations toward the plugged HC result in an inhibition of afferent activity in the intact HC. Such rotations led to excitation of 12/15 units in contra-plugged animals and in 10/13 units in ipsi-plugged animals. Asymmetries following canal plugging provide strong evidence that the responses originate in the semicircular canals. The responses seen to rotations toward the plugged canal imply that inhibition of either ipsilateral or contralateral canal afferents can result in an efferent-mediated excitation.

Effects of contralateral galvanic polarization

The results with canal plugging imply that both labyrinths contribute to type III responses and that both afferent excitation and afferent inhibition can lead to an efferent-mediated excitation. Galvanic polarization confirmed these inferences for the contralateral labyrinth. As has been shown previously (Baird et
al. 1988; Goldberg et al. 1984), anodal currents applied via the round window decrease the discharge of all afferents, while cathodal currents increase it. There is one difficulty in interpreting the results from contralateral polarization. Currents can spread to the opposite (ipsilateral) labyrinth and directly affect afferent discharge. Responses to current spread can be distinguished from efferent-mediated responses because the former effects appear promptly at the start of the current step and stop immediately at stimulus arrest and also because responses reverse direction when current polarity is reversed. To minimize spread, we used a 5-s current of $10\mu A$ as our standard stimulus even though this was near the threshold for contralateral efferent-mediated effects. At this current strength, the currents reaching the ipsilateral ear are so small that responses to currents of opposite polarity are mirror images and will cancel when averaged. Hence, averaging anodal and cathodal responses provides a measure of contralateral responses uncontaminated by spread. To estimate the separate effects of anodal and cathodal currents, we used post-stimulus responses. Small but definite responses were typically seen for both polarities of current. This is illustrated for an HC unit responding to contralateral cathodal (Fig. 7A) and anodal currents (Fig. 7B). In total, 37 units were tested in decerebrate preparations. Excitation was seen in the averaged per-stimulus responses of 33 units. There was a weak but statistically significant relation between per-stimulus response amplitude and $CV^*$ (Fig. 7C, $P < 0.01$), but this only held during the first 2.5 s of the 5-s stimulus. Figure 7D compares post-stimulus anodal and cathodal responses for the population. Increased discharge was
and cathodal currents. D: for contralateral stimulation, the magnitude of excitation seen in response to cathodal (excitatory) and anodal (inhibitory) currents are compared; responses are average response during the first 10 s of the post-stimulus period. Each point represents a different unit. —, unity line. Most points fall above the unity line, indicating stronger responses to cathodal than to anodal stimulation of the contralateral ear.

Severing the vestibular nerve

The results of canal plugging demonstrate that type III rotational responses require labyrinthine inputs. Responses remaining after ipsilateral plugging imply that contralateral organs can affect vestibular-nerve discharge. This would only seem possible were the responses efferent mediated. To verify this conclusion, we cut the vestibular nerve in four animals (2 anesthetized, 2 decerebrate). Histological examination indicated that the nerve was completely sectioned in two animals (1 anesthetized, 1 decerebrate), while 10–30% of the nerve remained intact in the other two animals.

All units were tested in null positions with 320°/s trapezoids. Combining data for all four animals, normal type III responses were seen in 16/19 units before the lesion. No type III responses were seen in the seven units recorded in the two cases after complete nerve section. In the other two animals, nine responses were seen in 16/19 units before the lesion. No type III responses were seen in the remaining three units tested after partial section.

In all four animals, the lesion did not lead to a depression of afferent activity. For example, the background discharge had a mean of 52 ± 5 spikes/s postlesion (n = 23) compared with 43 ± 4 spikes/s prelesion (n = 43); the increase was not statistically significant (t-test, P > 0.10) After the lesion, canal afferents had normal rotation responses and otolith afferents responded to tilts. All units had normal responses to cathodal and anodal galvanic currents applied to the ipsilateral ear. It can be concluded that injury caused by the sectioning of the nerve did not alter the peripheral function of the afferents.

From these results it would appear that the type III responses are mediated by efferent vestibular pathways.

**DISCUSSION**

The efferent vestibular system can use vestibular inputs to modify afferent discharge. Although such modifications have been described in other vertebrates (Dickman and Correia 1993; Myers et al. 1997), this is their first demonstration in mammals. In the next section, we compare type III rotational responses with the afferent responses to electrical stimulation of efferent pathways. We then discuss the organization of central efferent pathways. Finally, we consider possible functional implications of our findings.

**Efferent-mediated rotation responses and electrical stimulation of efferent pathways**

Our type III rotational responses resemble those obtained with electrical stimulation (Goldberg and Fernández 1980; Marlinski et al. 2000; McCue and Guinan 1994). Both kinds of responses are predominantly excitatory, consist of per- and post-stimulus response components, and are larger and faster in irregular than in regular afferents. Responses to electrical stimulation require relatively high shock rates (Goldberg and Fernández 1980; Marlinski et al. 2000). This has two implications for our results. First, even though our type III afferent responses seldom exceeded 20 spikes/s, the underlying responses of efferent neurons need not be small. This conjecture is based on the fact that low shock rates are ineffective, particularly in producing fast efferent-mediated responses. Using a published relation between afferent response and efferent shock rate (Fig. 17C in Goldberg and Fernández 1980) as an indication of efferent response rates, we estimate that efferent responses would have to be near 100 spikes/s to produce type III afferent responses of 20 spikes/s in irregular units. Second, the relation between type III response magnitude and rotation velocity indicated either the presence of a threshold or a concave-upward relation (Fig. 4D). A similar relation is implicit in the concave-upward growth of responses with time (see, for example, Figs. 2C and 3B). The ineffectiveness of low shock rates and, by inference, low efferent discharge rates may contribute to a similar ineffectiveness of small head velocities (<80°/s) in producing efferent-mediated afferent responses.

**Organization of efferent pathways**

In lower vertebrates, efferent neurons receive a convergent input from several vestibular organs (Blanks and Precht 1976;
Gleisner and Henrikkson 1964; Hartmann and Klinke 1980; Highstein and Baker 1986; Precht et al. 1971) and may respond almost equally to rotations in both directions (Blanks and Precht 1976; Hartmann and Klinke 1980; Precht et al. 1971). In anurans, where such type III responses have been most extensively studied, efferent neurons have their somas and dendrites predominantly on the ipsilateral side (Pellergrini et al. 1985; Strutz et al. 1981). Because they reach the ipsilateral vestibular nuclei, the dendrites may be directly contacted by ipsilateral vestibular-nerve fibers. A disynaptic excitatory input from the contralateral vestibular nuclei seems plausible as a source of contralateral excitation. Commissural fibers interconnecting the two vestibular nuclei are excitatory in the frog (Dieringer and Precht 1979; Ozawa et al. 1974). Conceivably such fibers could send collateral projections to the efferent cell group.

The situation may be different in mammals. As was noted in the introduction, efferent neurons have a distinctive location and much more restricted dendritic fields in mammals as compared with lower vertebrates (Meredith 1988). Moreover, antidromically identified efferent neurons in the guinea pig did not show type III responses (Marlinski 1995). In the presumed absence of such responses, type III responses in afferents might still be expected. To see this, consider Fig. 8, where potential pathways are indicated by numerals. An obvious basis for type III responses are the bilateral excitatory projections from efferent neurons to each labyrinth (6 and 7 in Fig. 8) (Gacek and Fernández 1980; Marco et al. 1993; Warr 1975), coupled with an excitatory input from each labyrinth to the efferent group on the same side (3) (Marlinski 1995; White 1985). Figure 8 shows the vestibular-nerve input to efferents being reinforced by pathways through the ipsilateral (2) and contralateral (4) vestibular nuclei. The first possibility needs experimental confirmation; the second has been described (Marlinski 1995). In short, a convergence in the labyrinth of efferent neurons excited directly or indirectly from ipsilateral and contralateral semicircular canals could give rise to type III responses.

Canal plugging allowed us to distinguish the separate efferent-mediated responses resulting from an increase or decrease in afferent discharge of the horizontal canals on the two sides. Consistent with the scheme presented in Fig. 8, excitation of either the ipsilateral or contralateral canals resulted in an efferent-mediated excitation. In addition, inhibition of either canal produced an efferent-mediated excitation, albeit one that was considerably smaller than that resulting from afferent excitation. Without a detailed analysis of central mechanisms, we can only speculate about the basis of the responses based on afferent inhibition. The simplest way to convert afferent inhibition into an efferent excitation is to include inhibitory relays in the pathways leading to the efferent neurons with the latter being disinhibited by a reduction in afferent discharge. Crossed and uncrossed disinhibitory pathways could explain efferent-mediated responses based on contralateral and ipsilateral afferent inhibition, respectively. Only a crossed pathway is included in Fig. 8 (5). A feature of disinhibition is that the increase in discharge of the inhibited neurons is easily saturated, presumably as a result of afferent silencing (Abend 1978). Such a saturation could contribute to the asymmetry in efferent-mediated responses produced by afferent excitation and inhibition.

A simple explanation for type III responses is provided by the bilateral efferent innervation of each labyrinth. Implicit in this proposal is the assumption that individual afferents receive a bilateral innervation. This would seem to be contradicted by anatomical findings in the gerbil (Purcell and Perchio 1997) in which the preponderance of efferent fibers destined for the central and peripheral zones of the cristae came from the ipsilateral and contralateral efferent groups, respectively. Given the functional organization of the cristae (Baird et al. 1988), this would suggest that ipsilateral efferents supply irregular afferents, while contralateral efferents innervate regular afferents. In an attempt to confirm this arrangement physiologically, we have compared the responses to electrical stimulation of ipsilateral and contralateral efferent cell groups in the chinchilla (Marlinski et al. 2000). Consistent with previous results in the squirrel monkey (Goldberg and Fernández 1980), contralateral stimulation was quite effective in exciting both regular and irregular afferents. Although we were unable to confirm Purcell and Perchio’s findings, the slowly developing time course of efferent responses makes it difficult, if not impossible, to distinguish between responses to direct electrical stimulation of contralateral efferents and transynaptic activation of ipsilateral efferents. To settle the issue, it would be well to repeat Purcell and Perchio’s study in the chinchilla. If their results are correct, we would have to conclude that both type I and II responses are commonly found in different neurons within the efferent group on each side or else, as has been found in lower vertebrates (Blanks and Precht 1976; Hartmann and Klinke 1980; Precht et al. 1971), that efferent neurons themselves respond in a type III manner. Either alternative could be the result of a convergence in each efferent cell group of ipsilateral (Fig. 8, 2 and 3) and contralateral excitatory pathways (Fig. 8, 4).

That efferent-mediated rotation responses are always excitatory can be related to the excitatory action of efferents. But this is not sufficient to produce type III responses. To see this, recall that rotations exciting one or more canals inhibit (disfacilitate) coplanar canals on the opposite side. If we assume that the system is linear and that the pathways leading back to the labyrinth from the two ears are balanced, excitatory and inhibitory inputs should cancel (Fig. 9A). Again assuming linearity, an imbalance in binaural inputs should result in an increase in discharge for one rotation direction and a decrease for the other direction (Fig. 9B). Clearly nonlinearities are needed to produce type III responses. An obvious nonlinearity is discharge silencing by inhibitory inputs, either peripherally or centrally.

**FIG. 8.** A bilaterally symmetric model of central efferent vestibular pathways. Labyrinth (Lab) sends direct projections (3) to ipsilateral efferent group (e) and indirect projections via the ipsilateral (1, 2) and contralateral (4) vestibular nuclei (Vn). The Vn on the 2 sides are interconnected by inhibitory fibers (5). All other connections are excitatory. Efferent neurons to each labyrinth come from the ipsilateral (6) and contralateral (7) efferent groups.
Were the background discharge of efferent neurons low, for example, the potential reduction in discharge would be restricted compared with their potential increase (Fig. 9C). Because inhibitory silencing requires stimulation beyond a certain size, a need for silencing might contribute to the ineffectiveness of small-magnitude head rotations in producing type III responses in afferents. A second nonlinearity results from the concave-upward relation between afferent response and efferent rate, which results in an increase in efferent discharge being more effective than a decrease (Fig. 9D). These arguments are based on the presumption that separate efferent neurons are excited by CW and CCW rotations. Were the efferent neurons themselves type III, comparable nonlinearities would have to be present in their input pathways.

Regardless of the pathways involved, our results demonstrate that the efferent system allows the labyrinth to be influenced from the contralateral ear. This finding was anticipated in studies of the pigeon (Dickman and Correia 1993) and frog (Myers et al. 1997). In both of these studies, excitation of the contralateral ear exerted a predominantly inhibitory effect, whereas our crossed responses were excitatory. The difference can presumably be related to differences in the peripheral actions of efferent activation between some lower vertebrates (Brichta and Goldberg 2000; Rossi et al. 1980; Sugai et al. 1991) and mammals (Goldberg and Fernández 1980; Marlinski et al. 2000; McCue and Guinan 1994).

**Functional implications**

Efferent-mediated type III rotational responses were small, seldom exceeding 20 spikes/s. Moreover, such responses required relatively large rotations, typically 320°/s. In contrast, rotations of this magnitude can result in conventional (type I) responses of 100–300 spikes/s. Not only are the type III responses small, it is unclear how they would influence gains and phases, for example, during sinusoidal stimulation. In the latter circumstance, efferent responses consisted of a double-peaked excitation (Fig. 1E) superimposed on a steady upward drift of the background discharge. While the double-peaked excitation is fast enough to affect response dynamics, its main effect would be to increase the mean discharge rate during stimulation. We assume that the double-peaked excitation corresponds to the per-stimulus responses seen during trapezoidal rotations or electrical stimulation of efferent pathways (Goldberg and Fernández 1980). Based on the latter study, there may also be a slight reduction in afferent gain. The upward drift in discharge rate, which probably corresponds to the post-stimulus response, would appear too slow to affect response dynamics but could influence the background discharge both during and after stimulation.

These considerations suggest that the efferent-mediated responses are of questionable functional importance because they would, at most, cause a modest increase in background discharge and, possibly, a small reduction in afferent gain. Before reaching this conclusion, however, we need to review several issues related to the function of a vestibular input to the efferent system. The most obvious comment is that acute preparations, while they are convenient for demonstrating a vestibular input to efferent pathways, are less suited in evaluating the functional significance of such an input. This is especially so as there are likely to be inputs from several sources, including possibly efference copy commands from motor centers (Goldberg et al. 2000; Highstein 1991). Three other comments can be made.

First, as already noted, the need for large rotations is consistent with studies of the responses of afferent fibers to electrical stimulation of efferent pathways. Second, there may be a need to limit the size of efferent-mediated rotation responses. An inspection of Fig. 8 shows that there is a potential positive feedback loop involving each labyrinth and the ipsilateral efferent cell group. The presence of crossed excitatory connections (Fig. 8, 4) would also result in positive feedback. Were the loop gains relatively high, the system would become unstable. We suspect, in fact, that the oscillations we have seen in irregular afferents in decerebrate preparations may be a result of such feedback. Third, confining ourselves to changes in discharge rate may provide too restrictive a view of efferent function. Here we can consider regular afferents. Their responses to either electrical stimulation of efferent pathways (Goldberg and Fernández 1980; Marlinski et al. 2000) or the presumed efferent-mediated rotation responses of the present study are so small as to be of dubious significance. Yet the peripheral regions of the crista, which are the territories supplied by regular afferents (Baird et al. 1988), are richly endowed with an efferent innervation (Lysakowski and Goldberg 1997). The efferent responses of regular afferents and one component of the responses of irregular units are slow, which leads to the suspicion that metabotropic receptors linked to second-messenger systems may be involved. A similar situation may exist in lateral lines, where a slow, excitatory response persists after the fast inhibitory efferent response is blocked by nicotinic antagonists (Flock and Russell 1973; Sewell and Starr 1991). Once the possibility of second-messenger systems is opened, changes in discharge rate become only one facet of efferent control.

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