

# Efferent-Mediated Responses in Vestibular Nerve Afferents of the Alert Macaque

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<sup>1</sup>Department of Physiology, McGill University, Montreal, Quebec, Canada; <sup>2</sup>Department of Neurobiology, Pharmacology, and Physiology, University of Chicago, Chicago, Illinois; and <sup>3</sup>Department of Otolaryngology-Head and Neck Surgery, Johns Hopkins University School of Medicine, Baltimore, Maryland

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**Sadeghi SG, Goldberg JM, Minor LB, Cullen KE.** Efferent-mediated responses in vestibular nerve afferents of the alert macaque. *J Neurophysiol* 101: 988–1001, 2009. First published December 17, 2008; doi:10.1152/jn.91112.2008. The peripheral vestibular organs have long been known to receive a bilateral efferent innervation from the brain stem. However, the functional role of the efferent vestibular system has remained elusive. In this study, we investigated efferent-mediated responses in vestibular afferents of alert behaving primates (macaque monkey). We found that efferent-mediated rotational responses could be obtained from vestibular nerve fibers innervating the semicircular canals after conventional afferent responses were nulled by placing the corresponding canal plane orthogonal to the plane of motion. Responses were type III, i.e., excitatory for rotational velocity trapezoids (peak velocity, 320°/s) in both directions of rotation, consistent with those previously reported in the decerebrate chinchilla. Responses consisted of both fast and slow components and were larger in irregular (~10 spikes/s) than in regular afferents (~2 spikes/s). Following unilateral labyrinthectomy (UL) on the side opposite the recording site, similar responses were obtained. To confirm the vestibular source of the efferent-mediated responses, the ipsilateral horizontal and posterior canals were plugged following the UL. Responses to high-velocity rotations were drastically reduced when the superior canal (SC), the only intact canal, was in its null position, compared with when the SC was pitched 50° upward from the null position. Our findings show that vestibular afferents in alert primates show efferent-mediated responses that are related to the discharge regularity of the afferent, are of vestibular origin, and can be the result of both afferent excitation and inhibition.

## INTRODUCTION

The vestibular organs in all vertebrates receive an innervation from the efferent vestibular system (EVS) originating in the brain stem and projecting to hair cells and afferent nerve fibers (Goldberg et al. 2000; Lysakowski and Goldberg 2004; Meredith 1988). The responses of afferents to electrical stimulation of the EVS have been described in fish (Boyle and Highstein 1990; Boyle et al. 1991; Highstein and Baker 1986), frogs (Bernard et al. 1985; Rossi et al. 1980; Sugai et al. 1991), turtles (Brichta and Goldberg 2000; Holt et al. 2006), and mammals (Goldberg and Fernandez 1980; Marlinski et al. 2004; McCue and Guinan 1994). Unlike the situation in non-vestibular organs (Art and Fettiplace 1984; Dawkins et al. 2005; Fuchs and Murrow 1992; Furukawa 1981; Oliver et al. 2000; Sugai et al. 1991), where inhibition is the predominant efferent action, EVS responses can be excitatory or inhibitory.

In amphibia and reptiles, some units are excited, others are inhibited, and still others show a mixed excitatory–inhibitory response (reviewed in Lysakowski and Goldberg 2004). Excitation predominates in fish (Boyle and Highstein 1990; Boyle et al. 1991) and mammals (Goldberg et al. 2000).

In the case of mammals, the efferent innervation is bilateral, with roughly equal numbers of efferent neurons innervating each labyrinth being found on the same or opposite sides of the brain stem (Gacek and Lyon 1974; Goldberg and Fernandez 1980; Marco et al. 1993; Warr 1975). Electrical stimulation on either side in mammals results in comparable excitatory responses (Goldberg and Fernandez 1980; Marlinski et al. 2004; McCue and Guinan 1994). In irregularly discharging afferents, responses are large and consist of a fast response component with a time constant of 10–100 ms and a slow response component with a time constant of 5–20 s. Responses in regular afferents are small and predominantly slow. EVS activation results in a modest reduction in the gain of rotational responses (Goldberg and Fernandez 1980).

Although the afferent responses to electrical stimulation of the EVS provide a foundation for understanding efferent control, knowledge of the discharge properties of efferent neurons and of efferent-mediated responses of afferents under natural conditions is also required. The discharge of efferent neurons has, with one possible exception (Marlinsky 1995), not been studied in mammals. Efferent neurons in lower vertebrates receive a convergent input from several vestibular organs on the two sides (Gleisner and Henriksson 1963; Schmidt 1963) and can be excited by head rotations in either direction (Blanks and Precht 1976; Hartmann and Klinke 1980a; Precht et al. 1971). Such type III responses can be distinguished from the type I response characteristic of peripheral afferents, where responses to rotations in two directions lead to oppositely directed responses (Goldberg and Fernandez 1971; Lowenstein and Sand 1936). Efferent neurons in lower vertebrates can also respond to somatosensory inputs (Blanks and Precht 1976; Gleisner and Henriksson 1963; Hartmann and Klinke 1980b; Precht et al. 1971) and to a variety of sensory stimuli that can change behavioral state (Boyle and Highstein 1990; Highstein and Baker 1985).

To date, few studies have characterized the effect of efferent activation on vestibular afferent responses (reviewed in Goldberg et al. 2000). In the alert toadfish, stimulation evoking escape reactions lead to the activation of efferents and a similar

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excitation of afferents (Boyle and Highstein 1990; Highstein and Baker 1985). Mechanical stimulation of the horizontal canal on one side in the pigeon can affect afferent activity on the other side (Dickman and Correia 1993). Efferent-mediated responses to high-velocity rotations ( $>300$  °/s) have been observed in decerebrate chinchillas (Plotnik et al. 2002). In the latter study, conventional afferent responses were minimized by orienting the head in the null plane for the innervated semicircular canal. Evoked responses resembled those obtained with electrical stimulation of the EVS in that responses were excitatory for both rotation directions, were larger for more irregularly discharging afferents, and consisted of fast and slow response components. Large efferent-mediated fluctuations in background discharge were also observed in irregular units (Plotnik et al. 2005). That both the rotational responses and the fluctuations were efferent mediated was verified by their being abolished when the vestibular nerve was cut between the recording electrode and the brain. At the same time, because comparable fluctuations have never been seen in awake, behaving monkeys (Haque et al. 2004; Keller 1976; Lisberger and Pavelko 1986; Louie and Kimm 1976; Ramachandran and Lisberger 2006; Sadeghi et al. 2007), they are most likely an artifact of decerebration. The presence of the fluctuations raises the possibility that the type III rotational responses were likewise artifactual. Furthermore, in none of the monkey studies was the discharge properties of afferents qualitatively different from those obtained in anesthetized animals, where the EVS is likely to be depressed (Plotnik et al. 2002). Nor were responses related to somatosensory or proprioceptive signals or to active head movements seen in the two studies in alert monkeys where such responses were explicitly sought (Cullen and Minor 2002; Sadeghi et al. 2007). Specifically, afferent responses were similar for passive whole body, passive head-on-body, and active head-on-body rotations, and no responses were seen when the animals attempted to make active head movements while their heads were restrained.

Accordingly, at present, the functional role of the efferent vestibular system in every day life remains unknown. Here, we specifically address the possibility that efferent-mediated responses can be induced via vestibular stimulation. Recordings were made from vestibular afferents of alert behaving primates (macaque monkey) after conventional afferent responses had been nulled by placing the corresponding canal plane orthogonal to the plane of motion. Excitatory efferent-mediated responses of afferents were evoked by high-velocity head rotations in alert monkeys, and were similar to those observed in the decerebrate chinchilla (Plotnik et al. 2002). Comparison of control responses and those evoked after lesions when only one semicircular canal was stimulated provides novel evidence that the observed efferent responses are vestibular in origin.

## METHODS

### *Surgical preparation*

Three monkeys (*Macaca fascicularis*) were prepared for chronic recordings from the vestibular nerve. All procedures were approved by the McGill University Animal Care Committee and the Johns Hopkins School of Medicine Animal Care and Use Committee and were in compliance with the guidelines of the Canadian Council on Animal Care and the National Institutes of Health.

The surgical procedures to prepare animals for vestibular nerve recordings have been described previously (Sadeghi et al. 2007).

Briefly, using aseptic techniques and isoflurane anesthesia (2–3%, to effect), we implanted a recording chamber and a post for head restraint on the skull. In the same procedure, an eye coil was implanted in one eye beneath the conjunctiva (Fuchs and Robinson 1966) and was used in experiments not reported in this paper. Following the surgery, the animals were administered buprenorphine (0.01 mg/kg, im) for postoperative analgesia and the antibiotic cephalosporin (Ancef; 25 mg/kg im, for 5 days).

Recordings were made from the left vestibular nerve in control animals (CNTL) and in animals unilaterally labyrinthectomized (UL) on the right side before and after semicircular canals on the left side were inactivated by plugging (UL + PL). For canal plugging, the horizontal (HC) and posterior (PC) canals were mechanically inactivated, whereas the superior (SC) canal remained patent. The surgical approaches for UL (Lasker et al. 2000) and canal plugging (Lasker et al. 1999) have been previously described.

### *Experimental design*

During experiments, the animals were head restrained and were seated in a primate chair placed on a vestibular turntable. The experimental set-up, apparatus, and methods of data acquisition were similar to those previously described (Sadeghi et al. 2007). In addition, in this study, head position with respect to earth-vertical axis (EVA) rotations could be changed by pitching the chair. In the zero-pitch position, the standard horizontal stereotaxic plane was parallel to the earth-horizontal turntable surface so that the plane of the horizontal canal was pitched upward by  $\approx 20^\circ$ . Extracellular recordings were made from vestibular nerve afferents with glass micropipettes filled with 3 M NaCl and having impedances of 20–25 M $\Omega$ .

Three kinds of preparations were used. First, data were obtained from two CNTL animals, allowing comparison with the results previously obtained in the decerebrate chinchilla (Plotnik et al. 2002). Second, recordings were made in two animals contralateral to UL; one animal had served as a CNTL and the other was only studied after UL. The UL recordings allowed us to study responses to rotations that increased or decreased afferent responses confined to the left HC. Third, some time ( $>3$  mo) after UL, the left HC and PC were plugged; these UL+PL preparations were used to determine whether rotational responses were caused by stimulation of the unplugged SC. Recordings began 1 wk after UL or after PL.

### *Identifying vertical canal units*

Once an afferent was isolated, a series of manual rotations and tilts were used to determine which organ it innervated (Goldberg and Fernandez 1975). Given the experimental design, we were particularly interested in vertical canal (VC) units. Otolith units, which responded to static pitches, but not to rotations, were not further studied. Before canal plugging, VC units could be distinguished from HC units by their directional properties when the animal was in the zero-pitch position. Because all recordings were made on the left side, VC units were excited by clockwise (CW) rotations and HC units by counter-clockwise (CCW) rotations, where the rotation direction is viewed from above. SC and PC units could be distinguished because the former were excited by nose-down (ND) and the latter by nose-up (NU) pitch rotations. The same procedures were used after canal plugging to identify SC and otolith units. Units in UL + PL animals not responding to head tilts or rotations were termed non-SC units and may have innervated the HC or PC. In many cases, we were able to determine that the afferent innervated the HC or PC by showing responses at rotational frequencies of 4–8 Hz (Rabbitt et al. 1999). All of the testing for type III responses in this study was done at low frequencies (velocity trapezoids). Afferents innervating plugged canals do not respond to these low-frequency stimuli (Rabbitt et al. 1999).

### Stimulation of vertical canals

We determined the orientation of the superior and posterior canals by recording afferent responses to sinusoidal whole body rotations (0.5 Hz, 50°/s) about an EVA, with the monkey systematically tilted to different positions (Estes et al. 1975; Miles and Braitman 1980). The response of an SC unit is shown in Fig. 1. The unit was excited by downward pitches (Fig. 1A). With the animal at zero pitch, the unit was excited by CW rotations (Fig. 1Ba). Pitching the animal to 20° ND greatly decreased the response (Fig. 1Bb). After pitching to 30° ND, the unit was now excited by CCW rotations (Fig. 1Bc). A similar approach was used for testing afferents that innervate the PC.

To determine the null position, we plotted sensitivities to EVA rotations as the animal was pitched NU and ND (Fig. 1C). Responses were normalized to those at zero pitch. The null position, defined as the pitch where a linear regression crossed zero response, was 21° ND for this particular unit and near 20° ND for all VC units. We used a linear regression rather than a cosine to fit the points because the difference between the two methods was <5% for the  $\pm 30^\circ$  range used. Upward pitches resulted in progressively larger responses. Because of the limited tilt capability of our chair, we were confined to a 30° NU position. The response in this position is calculated to be 50–55% of that obtained when the SC or PC plane coincides with the motion plane.

### Evaluation of efferent-mediated responses

In CNTL and UL animals, the head was maintained in the null position where only HC units should show appreciable conventional afferent responses. We presented trapezoids with peak angular velocities of 320°/s. Ramp and plateau durations were each 2 s. Several trapezoids were given in CW and CCW directions. A poststimulus period of 20 s was interspersed between rotations. Responses to each direction of rotation were averaged. The stimulus protocol was similar to that used to produce efferent-mediated responses in decerebrate chinchillas (Plotnik et al. 2002). In the latter reference, the reasons for using trapezoids, rather than sinusoids, are considered. To minimize linear forces, the ear on the recording side was placed over the axis of rotation. In the UL + PL animals, we compared responses of non-SC units in two orientations: 20° ND (the null SC plane) and 30° NU (a plane that elicits robust, although not maximal, activation of the SC). Comparison between responses in the two conditions was used to address whether SC modulation influenced the response. In this case, a larger response in the NU position is expected.

To maintain alertness, the animal was kept in subdued light. Selected trials were run in the dark in 10 units to assure that the responses did not have a visual component. As has been reported (Keller 1976), responses were not different in the two conditions for either regular or irregular units (paired *t*-test,  $P > 0.3$ ). In addition, no responses were obtained when an optokinetic stimulus consisting of irregularly spaced spots was projected on the walls of the experimental chamber.

Vestibular stimulation and data acquisition were controlled by a real-time data acquisition system (REX) (Hayes et al. 1982). Table velocity and unit activity were recorded on DAT tape for later playback. During playback, head and gaze position and table velocity signals were low-pass filtered at 250 Hz by an eight-pole Bessel filter and sampled at 1 kHz.

### Data analysis

Data were imported into the Matlab (The MathWorks, Natick, MA) programming environment for analysis. Neural discharge was represented using a spike-density function in which a Gaussian (SD = 10 ms) was convolved with the spike train (Cullen et al. 1996). The resting discharge of each unit and CV of the interspike interval were determined from ~10 s of resting discharge collected while the

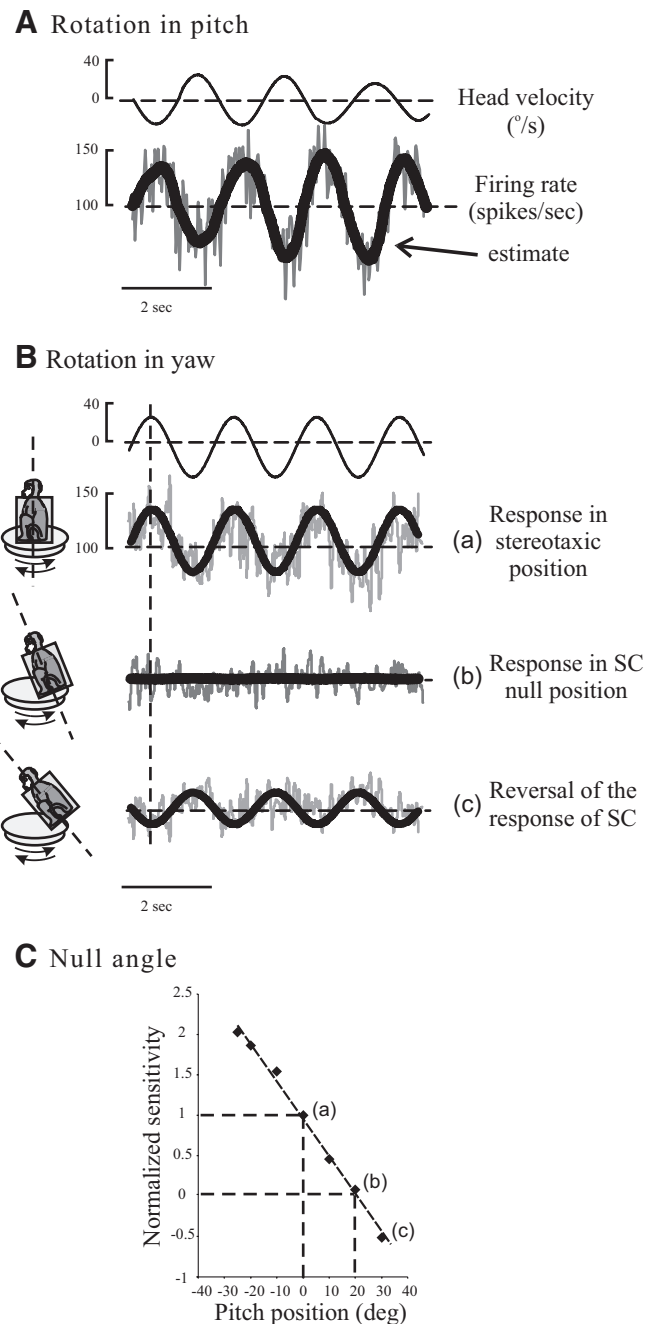


FIG. 1. Finding the null plane of the vertical canals. *A*: response of an afferent to manual pitch rotations. As it is excited by downward pitches, the unit is a superior canal (SC) unit. *B*: response of the same SC unit during rotations about an earth-vertical axis after the animal is placed in 1 of 3 pitch positions. The unit is excited by (a) clockwise (CW) rotations at 0° pitch, defined as the stereotaxic plane being earth horizontal. The unit was (b) nulled near 20° nose-down (ND), and (c) excited by counterclockwise (CCW) rotations at 30° nose-down. In all figures, rotation direction is as viewed from above. *C*: calculating the null angle. The normalized sensitivity, calculated relative to that at 0° pitch, is plotted as a function of pitch angle. A linear regression passes through 0 sensitivity at a pitch angle of 21° ND, which is considered the null position. Letters *a*, *b*, and *c* correspond in *B* and *C*.

animal was in the zero-pitch position. A normalized CV (CV\*) was calculated using the method described previously in the squirrel monkey (Goldberg et al. 1984). Afferents with a CV\* > 0.1 were considered as irregular, and regular afferents were identified as having a CV\* < 0.1.

A least-square regression analysis was used to determine the head velocity sensitivity [(spikes/s)/(°/s)] during sinusoidal rotations (Sadeghi et al. 2007). Responses to trapezoid rotations were quantified by subtracting the resting discharge from the mean firing rate during the 2-s plateau of constant velocity at 320°/s for 5–10 CW and CCW rotations separately. Because they were similar, responses for the two rotation directions were averaged.

### Statistical analysis

Data are described as mean  $\pm$  SE. A two-tailed paired or unpaired Student's *t*-test, corrected when necessary for unequal variances, was used to determine whether the average of two measured parameters differed significantly from each other. Standard linear regressions were used to describe the relation between response magnitude and log(CV\*). To determine whether responses that appeared suspiciously large were outliers, we used a procedure described by Snedecor and Cochran (1967). These outliers were removed to preserve the homogeneity of residual variances between groups when an analysis of covariance (ANCOVA) was used to compare the response magnitudes of the three groups (CNTL, UL, and UL + PL).

## RESULTS

Rotational responses were studied in 81 vestibular nerve fibers (Table 1) in CNTL, UL, and UL + PL conditions. Of these, 34 were regular and 47 were irregular with mean resting discharge of  $99.7 \pm 5.1$  and  $91.7 \pm 5.6$  spikes/s, respectively. Two animals were used in each condition (see METHODS for details). In CNTL and UL animals, we only studied VC units. Most of these innervated the superior canals (16/21 in CNTL and 20/27 in UL). Because there was no statistical difference between them, SC and PC data were combined. In the UL + PL animals, SC and non-SC units were distinguished. Non-SC units presumably innervated plugged canals and could have been PC or HC units.

### CNTL animals

During sinusoidal rotations (0.5 Hz, 50°/s), with the animal in the zero-pitch position, VC units were excited by CW rotations (Fig. 1B, top). This response was almost eliminated when the animal was placed in a null position (Fig. 1B, middle). However, similar to a previous study (Plotnik et al. 2002), we found that high-intensity rotations (trapezoids with peak velocity of 320°/s), when presented in the null position, elicited type III responses, i.e., excitatory responses for either direction of rotation.

Four characteristics of the responses are shown in Fig. 2, which includes data for two SC units: one irregular and the other regular. 1) CW and CCW (directions as viewed from above, see METHODS) rotations lead to similar responses. Because conventional afferent responses would have contributed opposite responses for the two rotation directions, the response similarity suggests that there was little contamination of our presumed efferent-mediated responses. In short, the nulling procedure, which was done at rotation velocities of 50°/s, was effective even for 320°/s rotations. 2) Responses are much larger in the irregular unit. Averaged over the 2-s velocity plateau, discharge increased over the resting rate by 15 spikes/s for the irregular unit compared with 3 spikes/s for the regular unit. 3) The build up of the excitatory response is slower in the regular unit. Discharge rate peaks near the beginning of the velocity plateau in the irregular unit but near the end of the plateau in the regular unit. 4) Although not shown in Fig. 2, excitatory responses in both units persist for  $\sim 15$  s after the rotation ends and presumably reflects the presence of a slow component (see also Plotnik et al. 2002). In irregular units, the discharge during the initial ramp more or less parallels the increase in head velocity and may reflect a fast component. The lack of such a parallel in the regular unit suggests that its response is predominantly slow. Any postrotational response to velocity trapezoids that is of afferent origin must be of opposite sign to the preceding per-rotational response. Hence, a postrotational excitation following a per-rotational excitation is indicative of an efferent-mediated response, as is the similarity of postrotational responses for both rotation directions.

That the response profiles shown in Fig. 2 were typical is shown in Fig. 3, where population responses were calculated for irregular ( $n = 10$ ) and regular units ( $n = 11$ ). As was the case for the individual examples, both rotation directions led to similar excitatory responses. These were considerably larger in the irregular units. Responses took longer to develop in the regular group. Poststimulus excitatory responses were seen in both groups. Statistics for the two groups are summarized in Table 1. Type III responses were statistically significant (*t*-test,  $P < 0.05$ ), as was the difference in response magnitudes for regular and irregular units (*t*-test,  $P < 0.05$ ).

### UL animals

In animals with unilateral (right) labyrinthectomy, when the animal is pitched forward so that the head is oriented in the null

TABLE 1. Efferent-mediated (type III) responses, various preparations

	Regular				Irregular			
	CW	CCW	Both	<i>n</i>	CW	CCW	Both	<i>n</i>
CNTL (VC)	3.0 $\pm$ 0.8	1.2 $\pm$ 0.8	1.9 $\pm$ 0.9	11*	11.9 $\pm$ 3.5	11.0 $\pm$ 3.2	11.1 $\pm$ 3.3	10*
UL (VC)	2.5 $\pm$ 0.4	2.1 $\pm$ 1.2	2.2 $\pm$ 0.7	7*	8.5 $\pm$ 0.9	10.1 $\pm$ 1.2	9.1 $\pm$ 1.1	20*
UL + PL (SC)	0.3 $\pm$ 0.3	0.9 $\pm$ 0.3	0.5 $\pm$ 0.1	5*	2.0 $\pm$ 1.3	2.1 $\pm$ 1.4	2.1 $\pm$ 1.2	6
UL + PL (non-SC)	1.1 $\pm$ 0.3	0.1 $\pm$ 0.1	0.7 $\pm$ 0.2	11*	4.5 $\pm$ 0.8	2.5 $\pm$ 1.2	3.4 $\pm$ 0.7	11*
UL + PL (non-SC)	4.0 $\pm$ 1.2	3.1 $\pm$ 1.0	3.2 $\pm$ 0.8	11*	15.1 $\pm$ 3.0	12.2 $\pm$ 2.3	13.1 $\pm$ 2.1	11*

Responses in spikes/s (mean  $\pm$  SE) of unit populations during 2-s plateaus of velocity trapezoids, peak velocity of 320°/s, clockwise (CW), counterclockwise (CCW), and average of both directions (both). First four rows, animal is in 20° nose down (ND) position where VC responses are nulled. Last row, animal is in 30° nose up (NU) position where SC responses are present. \*Significant (2-tailed *t*-test,  $P < 0.05$ ) difference between the average response to rotations in "both" directions compared to zero. *n*, number of units in each group; CNTL, control animals; UL, unilateral labyrinthectomy; UL + PL, unilateral labyrinthectomy + plugged horizontal and posterior canals; VC, vertical canal units; SC, superior canal unit; PC, posterior canal unit; HC, horizontal canal unit; non-SC, plugged PC or HC units; regular units, CV\* (normalized coefficient of variation)  $< 0.1$ ; irregular units, CV\*  $> 0.1$ .

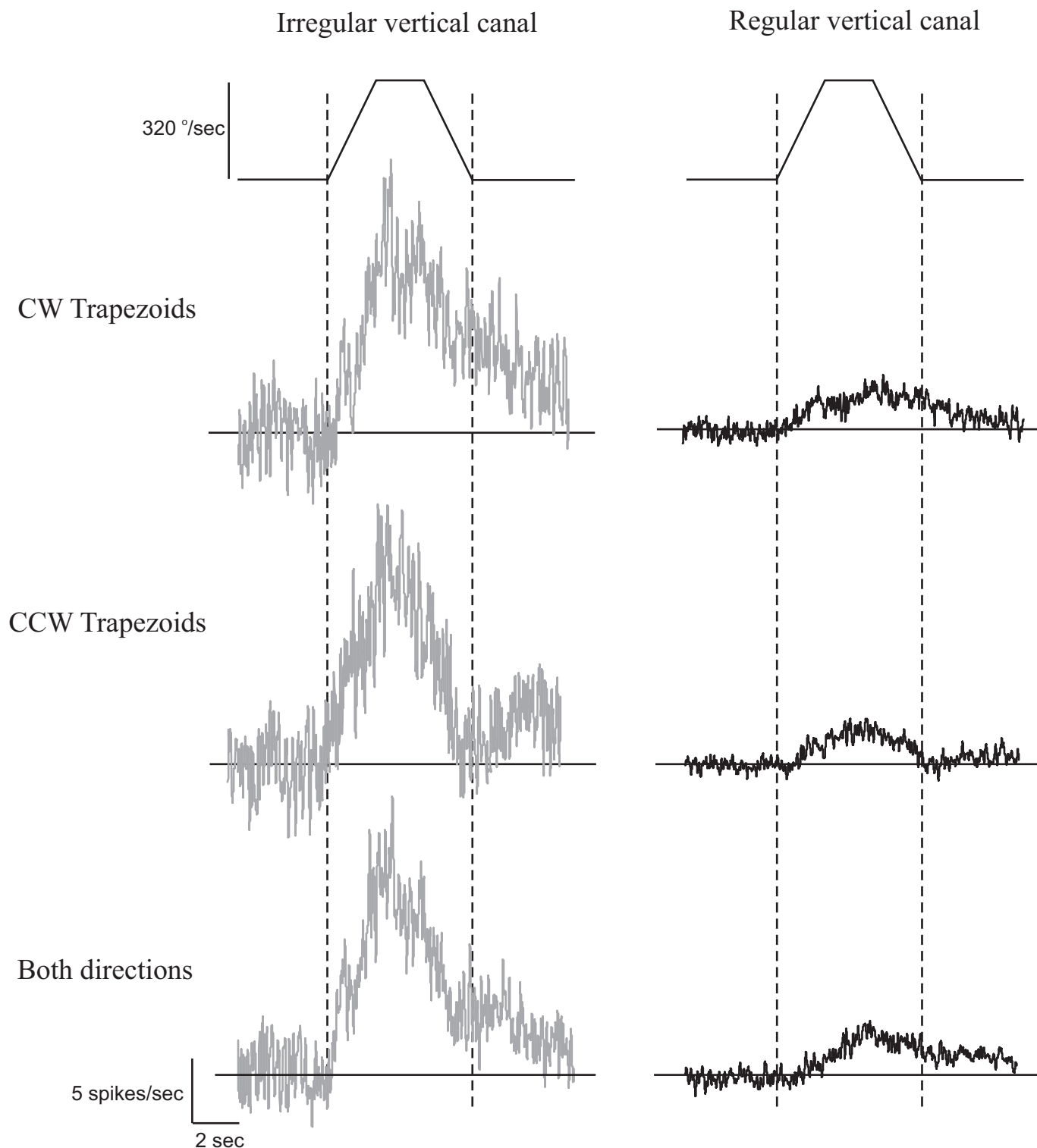


FIG. 2. Efferent-mediated responses in the control (CNTL) condition for an irregular (*left*) and a regular (*right*) vertical canal (VC) unit. The animal was in the VC-null position and rotated using the velocity trapezoid stimulus (top traces). Average responses based on >10 trials are shown for CW and CCW rotations as viewed from above. Traces for both directions are the average of the CW and CCW averages. The horizontal line marks the resting discharge of the afferent. The efferent-mediated response is excitatory and symmetric for both directions of rotation (i.e., type III). The response of the irregular unit is larger and more rapidly developing than that of the regular unit. Note the postrotational excitatory responses in both units. For a conventional afferent response, CW and CCW responses would be in opposite directions and the postrotatory would be in the opposite direction from the per-rotatory response.

position for both the PC and SC canals, the HC on the intact (left) side will be the only canal robustly stimulated by horizontal rotations. In this condition, the HC afferents on the

recording (i.e., left) side will be excited for CCW (i.e., ipsilateral) rotations and inhibited for CW rotations. Thus by recording from left VC afferents following right labyrinthectomy, we

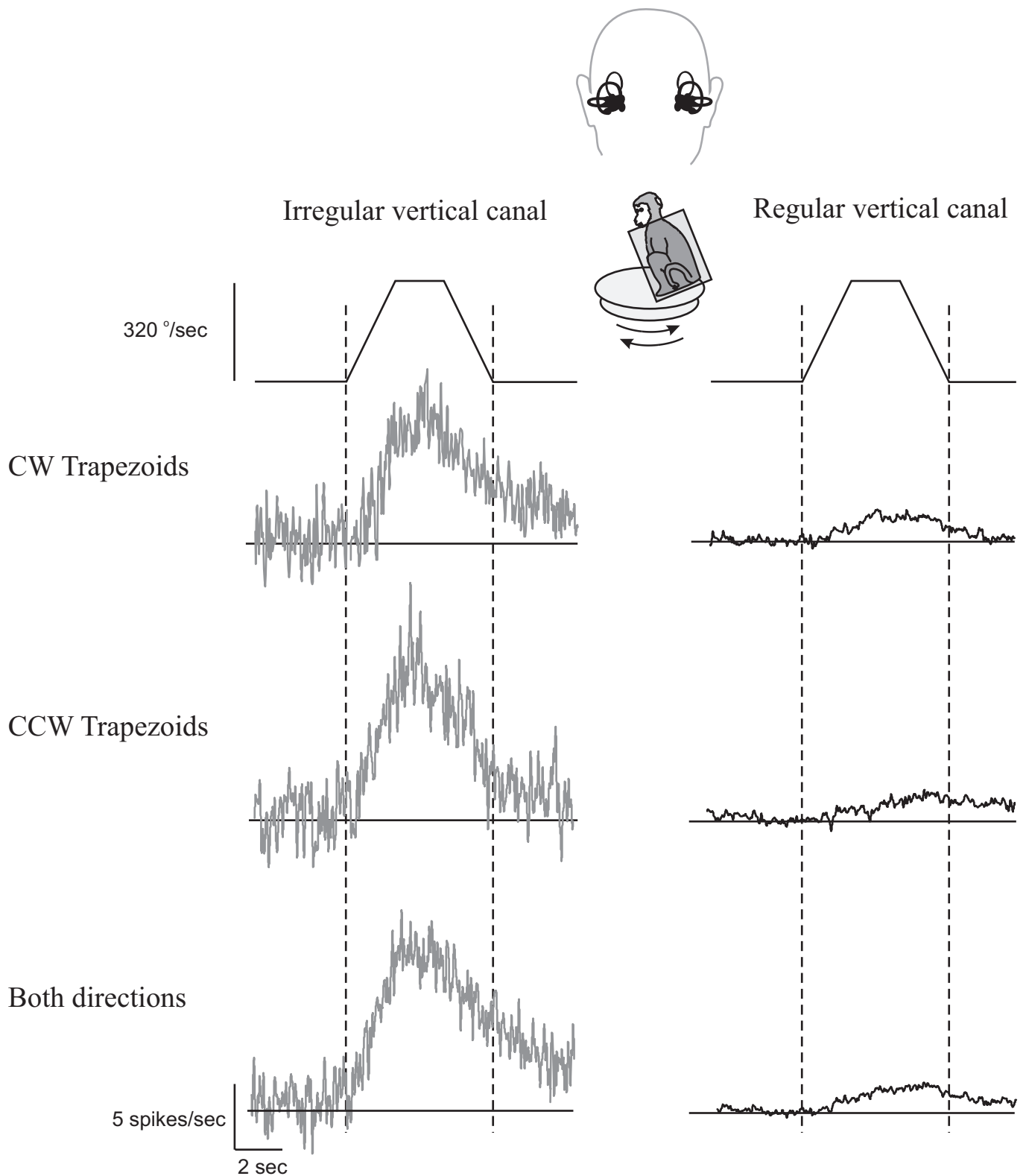


FIG. 3. Efferent-mediated population responses in the control (CNTL) condition averaged for irregular (*left*) and regular (*right*) VC [including SC and posterior canal (PC)] units. The animal was in the VC-null position (*inset*) and rotated using the velocity trapezoid stimulus (top traces). Population responses for CW and CCW rotations and for both directions, based on averaging the average traces, such as seen in Fig. 2 for individual units are shown. The horizontal lines mark the average resting discharge of the afferents. The population responses in the CNTL condition were similar to those shown for the individual afferents in Fig. 2. See Table 1 for sample sizes.

were able to compare the type III, presumed efferent-mediated responses evoked by excitation and inhibition of the intact HC. As shown in Fig. 4, based on population averages, excitatory

response magnitudes in VC units were similar for HC excitation and inhibition and were of comparable magnitude to those seen in CNTL animals (Table 1; *t*-test,  $P > 0.8$  for regular units

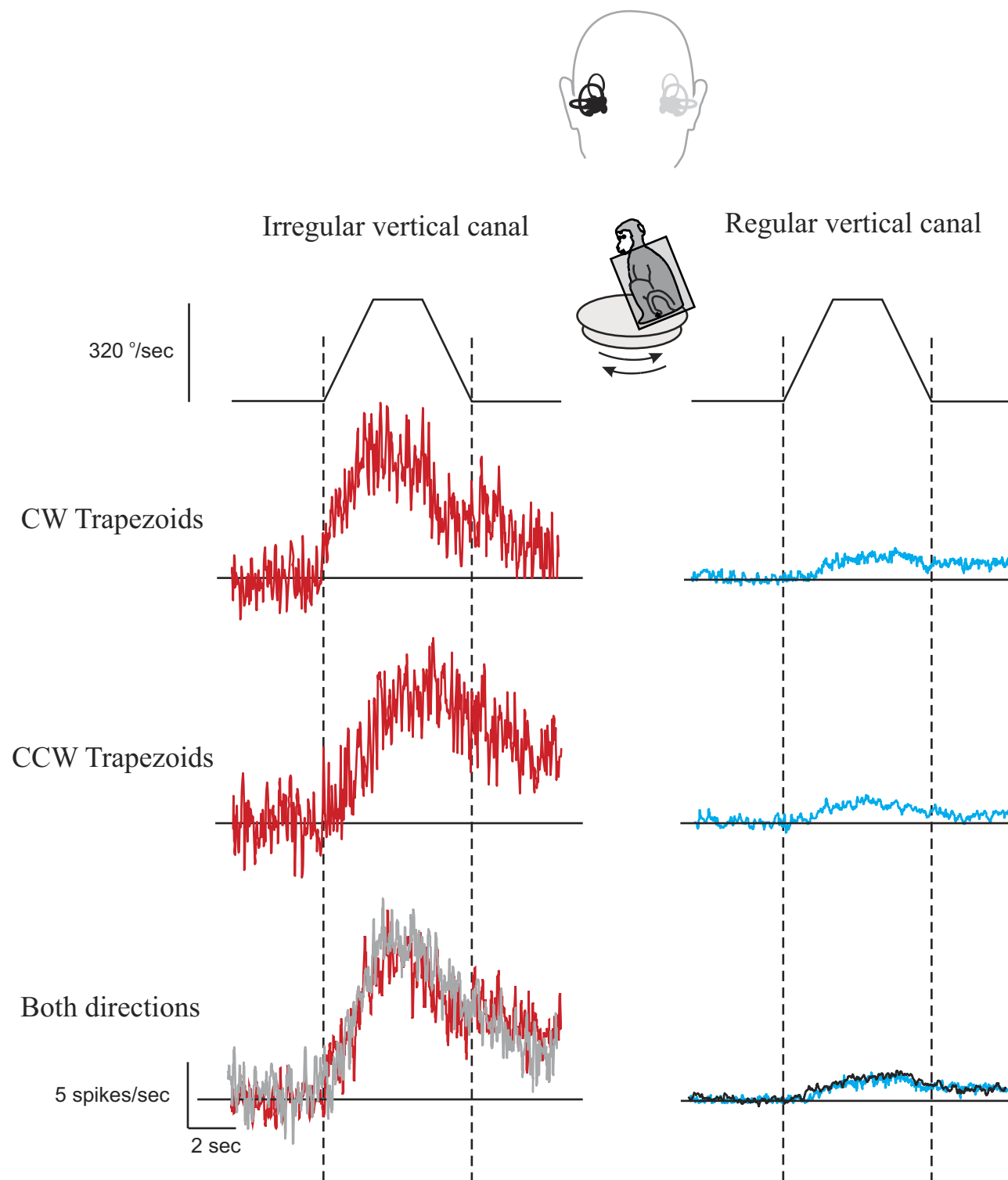


FIG. 4. Efferent-mediated responses following unilateral labyrinthectomy (UL) on right side, i.e., contralateral to the recording side, averaged for irregular (*left*) and regular (*right*) VC units. See Table 1 for sample sizes. *A*: the animal was in the VC-null position (*inset*) and rotated using the velocity trapezoid stimulus (top traces). In this position, only the horizontal canal (HC) on the intact side should be responsive. Population responses are shown for CW and CCW rotations, as well as the average population responses for rotations in both directions (bottom traces). The horizontal lines mark the average resting discharge of the afferents. Rotations that were excitatory (CCW) or inhibitory (CW) for the HC both resulted in excitatory efferent-mediated response in the VC units. The dynamics and magnitudes of the responses were similar to the CNTL condition for either irregular and regular units [compare uncolored (CNTL) and colored (UL) traces].

and  $P > 0.4$  for irregular units). A paired  $t$ -test indicated that the directional (CW vs. CCW) difference in VC units was not significant ( $P > 0.05$ ).

#### UL + PL animals

To test whether type III rotational responses depended on vestibular input from canals other than the one innervated by the afferent being studied, we next recorded afferent responses from animals that had first undergone UL on the right side and then had the horizontal and posterior canals plugged on the left side (UL + PL). In this experiment, only the afferents innervating the one remaining functional canal (i.e., the intact SC on the left side) remained responsive to rotations. As a result, we could control canal signals by moving the animal from 20° ND, where the SC signal was nulled, to 30° NU. In the latter position, the SC plane should be 60° from the horizontal plane of motion, and the canal's response should be >50% of maximum. Thus by comparing the evoked responses with those observed in the preceding experiments, we were able to address to what extent the type III responses were the result of canal inputs to central efferent pathways.

Overall, we found that, when the head was pitched forward from an effective to a null position, we could reduce the type III response of SC canal afferents to an almost negligible magnitude. This is shown in the SC afferents in Fig. 5, where responses of SC afferents recorded in the SC-null position are markedly smaller in UL + PL (blue and red traces indicate regular and irregular units, respectively) than in CNTL (gray traces) or UL animals (data not shown). In addition, the responses of non-SC units following UL + PL were considerably smaller in the 20° ND, SC-null position (Fig. 5, non-SC afferents) than in the 30° NU position (see also Fig. 6B), again consistent with our hypothesis that much of the type III responses result from canal inputs to central efferent pathways. Note that, as expected, the responses of SC and non-SC units were similar in the 20° ND position ( $t$ -test,  $P > 0.5$  for regular units and  $P > 0.3$  for irregular units).

To quantify these results, we first compared the response of SC units in the SC-null position for UL + PL versus UL animals. In the UL + PL condition (Fig. 6A, black circles), no vestibular organ is stimulated and hence no type III response is expected, whereas in the UL condition (Fig. 6A, green squares), the left HC is stimulated and can result in an efferent-mediated response. Figure 6A shows type III response magnitudes plotted versus  $\log(\text{CV}^*)$  for the two preparations. Although the responses in both conditions showed an increase with increasing  $\text{CV}^*$ , the responses were significantly smaller in the UL + PL condition. Both the slope and the intercept of the regression line in the responses were about four times smaller in the absence of a functional left HC (i.e., UL + PL). The difference in the regression slopes is significant ( $P < 0.01$ ). Note that, as mentioned above, in the UL + PL animals, there was little or no input from any vestibular organ, whereas in the UL animals, there was input from the left HC.

A comparable result was found when we analyzed the responses of non-SC afferents in the UL + PL animals (Fig. 6B). Again responses were recorded either in the presence (30° NU) or absence (20° ND) of stimulation of the remaining SC. Responses of the non-SC units were compared in the two positions. As expected, the response was smaller by 4–5 times

(regression slope =  $0.22 \pm 0.04$ ,  $P \ll 0.01$  compared with slope of 1) in the SC-null orientation (i.e., no vestibular input) versus the 30° NU condition (i.e., >50% maximum response from SC). Notably, the regression was significant ( $P \ll 0.01$ ), implying that type III responses were not abolished when the one remaining functional canal was nulled. In short, eliminating canal signals greatly reduced but did not eliminate the responses.

#### Comparing groups

Overall, we saw excitatory responses to both rotation directions in all units recorded in the three conditions (CNTL, UL, UL + PL). A previous study in decerebrate chinchilla reported that type III responses grow supralinearly as rotation velocity and, hence, canal signals increase (Plotnik et al. 2002). Thus in this study, one might have expected a reduction in type III responses as canal signals were successively reduced in UL and UL + PL animals. One half the canal input was removed by UL. When UL + PL animals are tested in the 30° NU position, the remaining SC input is calculated from the cosine angle between the canal and rotation planes to be 54% of the HC input in UL animals. The expected reduction between CNTL and UL + PL animals should be about fourfold at the very least. There is, however, no hint of such a reduction in Table 1. In fact, the mean type III response among irregular afferents is 20% higher in UL + PL animals (30° NU) than in CNTL animals.

That the expected reduction did not occur was confirmed by an ANCOVA, which compared the response magnitudes (RMs) for the three groups (i.e., VC units in CNTL and UL animals in the 20° ND and non-SC units in UL + PL animals in the 30° NU position) with  $\log(\text{CV}^*)$  as the covariate (Fig. 6C). Two units with unusually large type III responses were discarded as outliers (Fig. 6C, arrows). The ANCOVA confirmed that the slopes of the regressions,  $\text{RM} = a + b \times \log(\text{CV}^*)$ , were homogeneous for the three groups ( $P > 0.7$ ). The pooled slope was  $b = 10.3 \pm 1.1$  spikes/s and was highly significant ( $P \ll 0.001$ ). Intercepts were  $a = 15.5 \pm 1.0$  (CNTL),  $13.5 \pm 0.7$  (UL), and  $16.7 \pm 0.6$  (UL + PL). Although the ANCOVA indicated a significant intercept difference ( $P < 0.01$ ), this was small and not always in the predicted direction. Therefore, the CNTL-UL difference was 15%, and not the predicted 100%, and UL + PL animals had the largest intercept.

#### DISCUSSION

This is the first demonstration that angular rotations can produce efferent-mediated, type III responses in vestibular afferents of alert, behaving primates. Responses were similar to those previously obtained in the decerebrate chinchilla (Plotnik et al. 2002). Results obtained after UL confirmed that excitation and inhibition of afferent discharge both resulted in similar excitatory efferent-mediated responses. UL + PL animals showed that the type III responses depended on canal inputs. In this section, we review these results and discuss their implications for the organization of central efferent pathways and for the function of the efferent vestibular system (EVS).



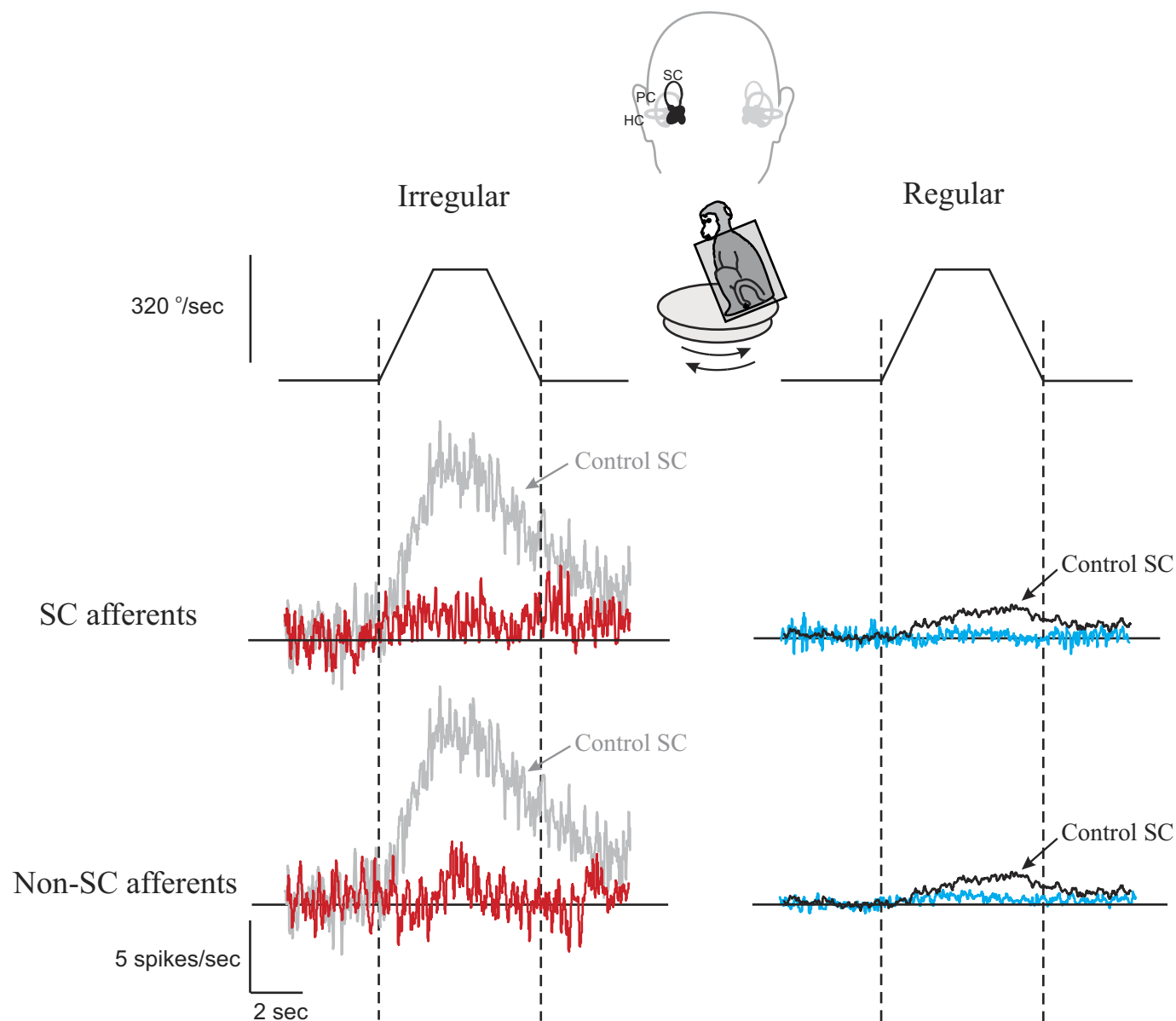


FIG. 5. Following contralateral UL followed by unilateral plugging (PL) of the ipsilateral HC and PC, only the ipsilateral SC is responsive to rotations. By placing the animal in the SC-null position, canal signals should be minimized in UL + PC animals compared with UL animals in which the ipsilateral HC was nearly maximally sensitive. Population responses in the SC-null position (cartoon) averaged for both rotation directions in the UL + PL condition are compared with the responses in the UL condition. See Table 1 for sample sizes. As expected, efferent-mediated responses were greatly reduced in SC or non-SC (HC + PC) units in UL + PL animals (colored traces) compared with SC units in CNTL animals (uncolored traces). The horizontal lines mark the average resting discharge of the afferents. In the UL + PL condition, only the ipsilateral SC was potentially responsive to rotations; because this canal was nulled, no vestibular signals were available. The large reduction in UL + PL compared with UL animals is consistent with vestibular signals making a large contribution to efferent-mediated responses.

#### *Efferent-mediated responses in alert macaques are similar to those observed in decerebrate animals*

Previous studies have shown a convergence of inputs from different vestibular organs to vestibular efferent cells (Gleisner and Henriksson 1963; Schmidt 1963). As a result, stimulation of one sensory organ can result in efferent-mediated responses in other sensory organs. The arrangement was exploited in the decerebrate chinchilla by recording from afferent fibers whose conventional afferent responses were minimized by placing the innervated canal orthogonal to the plane of motion (Plotnik et al. 2002). High-velocity rotations that stimulated other canals resulted in type III responses, i.e., responses that were

excitatory for rotations in both CW and CCW directions. These findings suggested that the rotational responses were efferent mediated in that they resembled those obtained by electrical stimulation of the EVS (Goldberg and Fernandez 1980; Marlinski et al. 2004; McCue and Guinan 1994) and were abolished when the recording site in the vestibular nerve was surgically separated from the brain.

We were concerned that the type III responses were peculiar to the decerebrate preparation, especially because afferents in this preparation showed large, periodic fluctuations in background discharge (Plotnik et al. 2005), which have not been observed in alert animals (Sadeghi et al. 2007; this study). Here

we found that similar responses can be obtained in alert monkeys in the absence of such fluctuations. As was the case in the chinchilla (Plotnik et al. 2002), responses resembled those obtained with electrical stimulation of the EVS (Goldberg and Fernandez 1980; Marlinski et al. 2004; McCue and Guinan 1994). They were entirely excitatory for either rotation direction, much larger in irregular units, and consisted of fast and slow response components. Electrical stimulation is ideal for the delineation of fast and slow responses because of the rapid onset of shock trains. Because of the gradual build-up of the rotations used in the monkey and the decerebrate chin-

chilla, a precise separation of the two components was not possible. Nevertheless the responses in irregular units seemed to consist of a mixture of fast and slow responses, whereas the responses in regular units were predominantly slow.

*Efferent-mediated responses are excitatory for both inhibitory and excitatory vestibular stimuli*

We found that type III efferent-mediated responses recorded from vestibular nerve afferents following contralateral UL were similar to those in CNTL animals. It should be noted that in the 20° ND position exclusively used to test for type III responses in UL animals, both SC and PC were in a null position, whereas the HC was close to its maximal plane of stimulation. In this position, rotations should have affected only the ipsilateral (left) HC with CCW rotations being excitatory and CW rotations being inhibitory. Interestingly, the efferent-mediated responses were excitatory for both directions of rotation. Similar results had been found in chinchillas following unilateral plugging of either the ipsilateral or contralateral HC (Plotnik et al. 2002). The presence of type III responses after ipsilateral plugging shows that stimulation of one labyrinth can affect vestibular organs on the other side by way of the EVS, a conclusion anticipated in the pigeon (Dickman and Correia 1993).

In our UL experiments, efferent-mediated type III responses were symmetric for the two rotation directions, implying that afferent excitation and inhibition were equally effective in activating the EVS. Our results were similar to those obtained in decerebrate chinchilla where high-velocity rotations in either direction led to excitatory responses after plugging the contralateral or ipsilateral HC (Plotnik et al. 2002). The one notable difference was that the excitatory responses after plugging were asymmetric, with afferent excitation yielding larger efferent-mediated responses than inhibition. Asymmetries were larger after ipsilateral plugging. The large rotational velocities used in these experiments should have silenced many peripheral and central vestibular neurons but would be less likely to drive the neurons into excitatory saturation (Sadeghi et al. 2007). This provides a basis for asymmetries in the efferent-mediated type III responses. We can offer two explanations as to why asymmetries were smaller in the monkey. First, the mean resting discharge of the vestibular nerve afferents is higher in macaques (Sadeghi et al. 2007) compared with chinchillas (Hullar et al. 2005), whereas rotational sensitivities are similar in the two species. Hence, inhibitory cut-off should be more

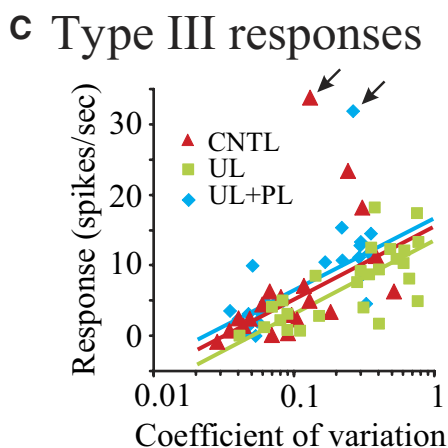
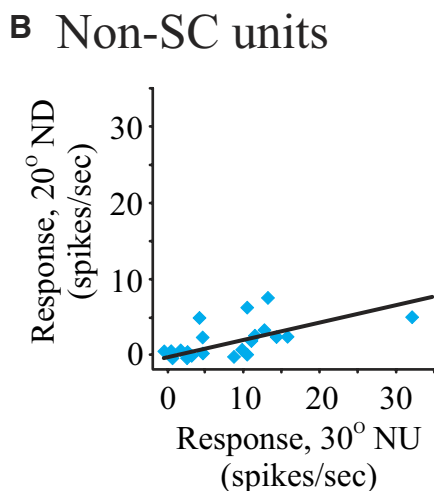
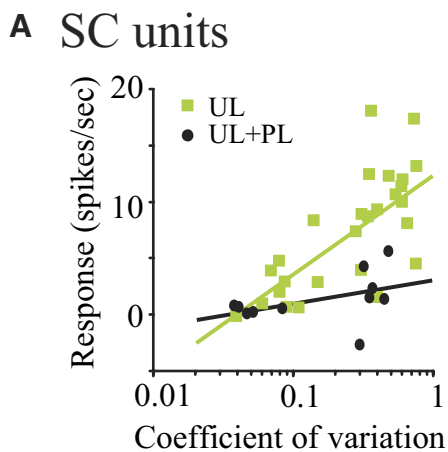


FIG. 6. Comparisons of efferent-mediated, type III responses under various conditions. Each point represents the average response for CW and CCW rotations in individual units. *A*: responses vs. normalized coefficient of variation or CV\* for SC units following UL in comparison to SC units following UL + PL. These responses were recorded with the animal pitched to 20° ND (SC null) condition. Straight lines are linear regressions between type III response magnitudes and log (CV\*) for the 2 preparations. *B*: responses in non-SC units in UL + PL animals are compared in 20° ND (SC null) (ordinate) and 30° NU (abscissa) positions. Straight line is linear regression through the origin. *C*: responses vs. normalized coefficient of variation (CV\*) for VC units in CNTL (red triangles) and UL (green squares) animals pitched to 20° ND and for non-SC units in UL + PL animals pitched to 30° NU (blue diamonds). Straight lines for the 3 groups determined from an ANCOVA that compared response magnitudes with log (CV\*) as the covariate. Two points (arrows) had to be left out of ANCOVA to preserve the homogeneity of residual variances among the 3 groups. Response magnitude as a function of log (CV\*) was similar for the 3 groups.

conspicuous in the chinchilla. Second, plugging in the chinchilla experiments was done acutely, whereas recordings in the monkey UL experiments were done after a 1-wk recovery period. Although compensation for UL is to be expected (Newlands and Perachio 1990a,b; Ris and Godaux 1998; Ris et al. 1995; Sadeghi et al. 2006, 2007), none is likely in the acute, plugging experiments. Conceivably, some form of compensation might explain the similarity in type III response magnitudes in CNTL, UL, and UL + PL animals of this study.

#### *Efferent-mediated responses depend on vestibular stimulation*

Vestibular nerve section central to the recording electrode was used in the chinchilla experiments to show that type III rotational responses were efferent mediated (Plotnik et al. 2002). That the responses depended on vestibular input was shown in our UL + PC preparation, where only one SC could respond to our rotations. By moving the SC from an effective to a null position, we could reversibly reduce the type III response to an almost negligible magnitude. The etiology of the small response that remained in the null position was not studied. It seems unlikely that it was caused by an error in achieving the SC-null position because this should have produced a small type I response, which was not evident. Centrifugal linear forces acting on otolith organs could produce type III responses, but these were minimized by placing the ear over the rotation axis such that any remaining forces would be very small. It is also possible that the high rotational speeds may have resulted in somatosensory/proprioceptive-related stimulation or may have aroused the animal. However, afferent responses do not seem to be affected by somatosensory and/or proprioceptive inputs (Cullen and Minor 2002; Sadeghi et al. 2007), and we found no evidence for arousal-related effects in this study. The main point, nevertheless, is that moving to the null position reduced the response by 75–80%, a percentage that has to be attributed to vestibular stimulation.

#### *Implications for the organization of central efferent pathways*

Anatomical studies show that each labyrinth receives a bilateral projection from efferent cell groups, originating almost equally from the ipsilateral and contralateral sides (Gacek and Lyon 1974; Goldberg and Fernandez 1980; Marco et al. 1993; Warr 1975). The bilateral projection has been confirmed physiologically by comparing the afferent responses to electrical stimulation of efferent neurons on the two sides (Goldberg and Fernandez 1980; Marlinski et al. 2004). There is evidence that the main efferent group receives direct inputs from the ipsilateral vestibular nerve (Highstein and Baker 1986; Korte 1979; Li et al. 2005; White 1985) and bilaterally from the vestibular nuclei (Chi et al. 2007).

The bilateral organization of the EVS is easily seen as leading to type III rotational responses in CNTL animals (Fig. 7). Consider the situation where the animal is pitched into the null position for the VCs. A rightward rotation will only affect the HCs, causing excitation and inhibition of the type I neurons in the right and left vestibular nuclei, respectively. Thus rotations in either direction will activate the efferents bilaterally with a possible preference for the efferent neurons on the side of the rotation. How afferent inhibition results in an efferent-mediated excitation, as was seen after canal plugging (Plotnik et al. 2002) or UL, is less clear. Disinhibition is an obvious mechanism and provides the motivation for including inhibitory (dashed line) connections in Fig. 7. This last suggestion must be considered conjectural. In particular, there is no information as to how the inhibitory neurons fit into the overall circuitry of efferent pathways.

#### *Functional considerations in mammals*

There are relatively few efferent neurons innervating each labyrinth, perhaps 300 on each side (Goldberg and Fernandez 1980; Marco et al. 1993). Despite the small number of parent neurons, each efferent axon, on reaching the neuroepithelium, branches profusely (Purcell and Perachio 1997) to provide a major innervation (Lysakowski and Goldberg 1997, 2008),

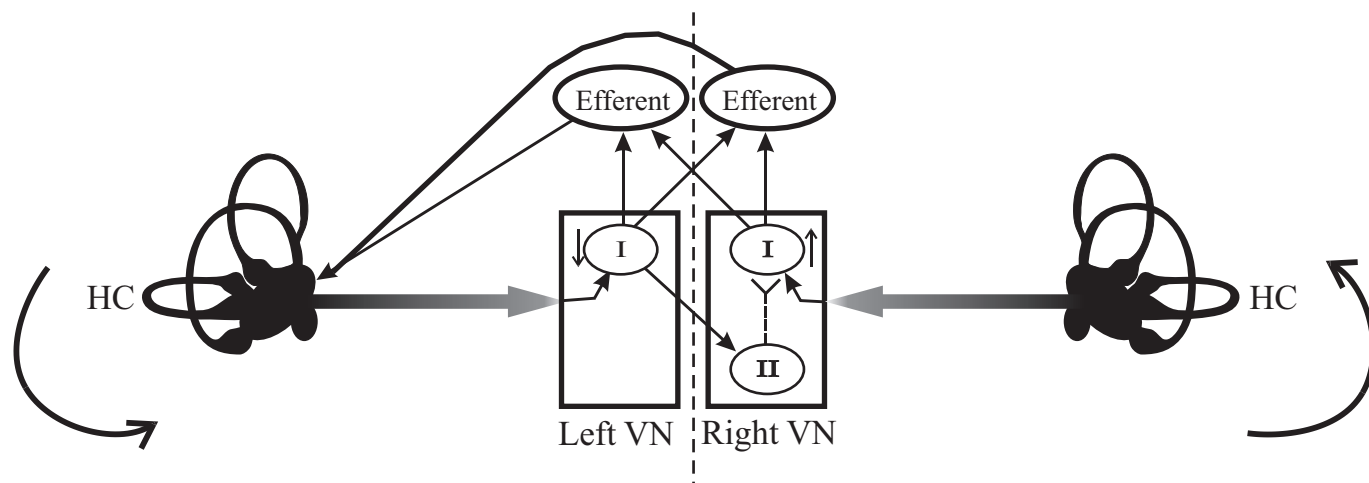


FIG. 7. Schematic of the connectivity of the central efferent vestibular pathways. Type I cells in the vestibular nuclei (VN) are excited by ipsilateral rotations (e.g., rightward rotations shown by curved arrows) and are inhibited by contralateral rotations (response direction denoted by small arrows). Type II neurons in each VN make inhibitory synapses with type I neurons on the same side (dashed line). Following UL, type I neurons on the ipsilesional side are modulated by ipsilesional rotations, as a result of disinhibition through ipsilesional type II cells. Signals from type I cells on the 2 sides can lead to excitatory responses from efferent cells during rotations in both directions, thus resulting in type III responses in the periphery.

with afferent boutons outnumbering efferent boutons by only a 3:1 ratio. Reflecting the heavy efferent innervation, high-frequency (300/s) electrical stimulation of central EVS pathways can give rise to large responses (>100 spikes/s) in irregular afferents but smaller responses in regular afferents (10 spikes/s) (Goldberg and Fernandez 1980; Marlinski et al. 2004; McCue and Guinan 1994). However, it has proved difficult to find more natural circumstances that lead to large efferent-mediated responses in the mammalian vestibular nerve, even in irregular units.

Several potential functions have been suggested for the EVS, including a modulation of afferent discharge in anticipation of active head movements and the maintenance of balance between the background discharge of the two labyrinths (Goldberg and Fernandez 1980; Goldberg et al. 2000; Highstein 1991; Sadeghi et al. 2007). Neither suggestion has been confirmed. Afferent discharge is similar during active and passive head movements, does not respond to neck proprioception or active neck motor torques, and does not serve to compensate for the imbalance in resting activity produced by UL (Cullen and Minor 2002; Jamali et al. 2008; Sadeghi et al. 2007). In this study, as in a previous study in the decerebrate chinchilla (Plotnik et al. 2002), we were able to use high-velocity angular rotations to modify afferent discharge by way of the EVS. Although the type III responses seen in alert monkeys show that efferent pathways are functional in a behaving animal, it must be recognized that the responses are small ( $\approx 10$  spikes/s) compared with those produced by conventional afferent stimulation (>200 spikes/s) (Sadeghi et al. 2007).

In considering the function of the EVS, the following points may be relevant. First, efferent pathways are not highly organized. This is indicated by the observation that type III, efferent-mediated responses can be evoked in the afferents innervating one organ by stimulation of several organs on the same or opposite sides and by rotations that increase or decrease discharge (Plotnik et al. 2002; this study). In the frog, multiorgan interactions may be the result of individual efferent neurons branching to two or more organs (Prigioni et al. 1983; Rossi et al. 1980; Sugai et al. 1991). It is unclear the extent to which such interactions in mammals are a result of the peripheral branching of efferent neurons or the organization of central efferent pathways. Second, the EVS may be involved in functions other than the short-term alteration of afferent discharge. To cite an example, it has been suggested that efferents are crucial for the maturation of the peripheral organs (Simmons 2002). The possibility of functions other than discharge modulation is most clearly suggested by the small and slow efferent responses of regularly discharging afferents to electrical stimulation (Goldberg and Fernandez 1980; Marlinski et al. 2004; McCue and Guinan 1994) and to high-velocity rotations (Plotnik et al. 2002; this study). Were the only function of the efferents to modify spike discharge on a relatively fast time scale, it would be difficult to understand why the peripheral/extrastriolar zones of the cristae and maculae, where regular fibers reside (Baird et al. 1988; Goldberg et al. 1990), receive as rich or richer an efferent innervation as central/striolar zones (Lysakowski and Goldberg 1997, 2008; Nomura et al. 1965). Third, experiments in the decerebrate chinchilla have shown efferent-mediated fluctuations in background that can exceed 100 spikes/s (Plotnik et al. 2005). Although such large fluctuations are not seen in anesthetized (Goldberg and Fernandez

1971; Lysakowski et al. 1995; Tomko et al. 1981) or alert preparations (Sadeghi et al. 2007; this study), their existence shows that the EVS does not need high-frequency electrical stimulation to exert a powerful control of the periphery. A possible interpretation is that the efferent cell groups are regulated by descending inhibition that is disabled by decerebration. Finally, it is notable that large afferent responses to electrical stimulation of the EVS require several closely spaced shocks (Goldberg and Fernandez 1980; Marlinski et al. 2004; McCue and Guinan 1994). Because single shocks are relatively ineffective, this would imply that large responses require potentiation at the peripheral efferent synapse, which could reflect the presynaptic facilitation of neurotransmitter release and/or the amplification of postsynaptic effects. Regardless of the mechanisms involved, the efferent synapse may act as a filter, maximizing the effects of high-frequency activity in central efferent neurons and minimizing the influence of lower, tonic discharge rates. As a result, an input may be quite effective in exciting efferents, yet have only a small or no influence on afferent discharge. Thus experiments aimed at directly characterizing efferent neurons may be needed to provide further insights into the contribution of vestibular and nonvestibular influences on efferent control.

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